

Loss of Phosphatase and Tensin Homolog or Phosphoinositol-3 Kinase Activation and Response to Trastuzumab or Lapatinib in Human Epidermal Growth Factor Receptor 2–Overexpressing Locally Advanced Breast Cancers

Bhuvanesh Dave, Ilenia Migliaccio, M. Carolina Gutierrez, Meng-Fen Wu, Gary C. Chamness, Helen Wong, Archana Narasanna, Anindita Chakrabarty, Susan G. Hilsenbeck, Jian Huang, Mothaffar Rimawi, Rachel Schiff, Carlos Arteaga, C. Kent Osborne, and Jenny C. Chang

A B S T R A C T

Purpose

Phosphatase and tensin homolog (PTEN) loss or activating mutations of phosphoinositol-3 (PI3) kinase (*PIK3CA*) may be associated with trastuzumab resistance. Trastuzumab, the humanized human epidermal growth factor receptor 2 (HER2) monoclonal antibody, and lapatinib, an epidermal growth factor receptor/HER2 tyrosine kinase inhibitor, are both established treatments for HER2-overexpressing breast cancers. Understanding of the cellular response to HER2-targeted therapies is needed to tailor treatments and to identify patients less likely to benefit.

Methods

We evaluated the effect of trastuzumab or lapatinib in three HER2-overexpressing cell lines. We confirmed the *in vitro* observations in two neoadjuvant clinical trials in patients with HER2 overexpression; 35 patients received trastuzumab as a single agent for the first 3 weeks, then docetaxel every 3 weeks for 12 weeks (trastuzumab regimen), whereas 49 patients received lapatinib as a single agent for 6 weeks, followed by trastuzumab/docetaxel for 12 weeks before primary surgery (lapatinib regimen). Apoptosis, Ki67, p-MAPK, p-AKT, and PTEN were assessed by immunohistochemistry. Genomic DNA was sequenced for *PIK3CA* mutations.

Results

Under low PTEN conditions, *in vitro* data indicate that lapatinib alone and in combination with trastuzumab was effective in decreasing p-MAPK and p-AKT levels, whereas trastuzumab was ineffective. In the clinical trials, we confirmed that low PTEN or activating mutation in *PIK3CA* conferred resistance to the trastuzumab regimen ($P = .015$), whereas low PTEN tumors were associated with a high pathologic complete response rate ($P = .007$).

Conclusion

Activation of PI3 kinase pathway is associated with trastuzumab resistance, whereas low PTEN predicted for response to lapatinib. These observations support clinical trials with the combination of both agents.

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INTRODUCTION

The epidermal growth factor receptor (EGFR)/human epidermal growth factor receptor (HER) family of transmembrane type I receptor tyrosine kinases plays an important role in processes such as cell proliferation, differentiation, and survival. Conformational changes after receptor dimerization lead to autophosphorylation and initiation of divergent signal transduction cascades.¹ These type I receptors signal through the MAPK/ERK pathway, stimulating cell division.² Cell line evidence also suggests that these receptors also modulate cell survival

through activation of the AKT/phosphoinositol-3 kinase (PI3K) pathway.³ Aberrant HER1 and HER2 signaling contributes to cancer cell proliferation and survival.

The HER2 monoclonal antibody, trastuzumab, has been approved as adjuvant treatment for patients with breast cancer with HER2 overexpression, reducing both the recurrence rate and mortality.⁴ Lapatinib, a reversible dual kinase inhibitor against EGFR and HER2,⁵ has activity in patients with HER2 overexpression when given either as first-line therapy or after treatment failure with trastuzumab⁶⁻⁸ and has been approved in combination

From the Lester and Sue Smith Breast Center, Dan L. Duncan Cancer Center, Baylor College of Medicine; Methodist Hospital, Houston, TX; and Vanderbilt University, Nashville, TN.

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B.D. and I.M. contributed equally to this work as first authors. C.A., C.K.O., and J.C.C. contributed equally to this work as senior authors.

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Corresponding author: Jenny C. Chang, MD, The Methodist Hospital, Department of Medicine, 6550 Fannin St, SM383, Houston, TX 77030; e-mail: jcchang@tmhs.org.

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with capecitabine in patients with metastatic disease, with significantly improved progression-free survival.⁹ Recent data have also shown that the addition of lapatinib to letrozole almost tripled progression-free survival rates in patients with breast cancer whose tumors coexpressed steroid receptors and HER2.¹⁰

The antitumor effects of HER2 inhibitors require the modulation of key signaling pathways and cell cycle/apoptosis regulatory molecules that mediate the transforming effects of HER2.¹¹⁻¹³ Activation of the PI3K pathway, as a result of loss or low levels of the phosphatase and tensin homolog (*PTEN*), is associated with resistance to trastuzumab. Recent data further support the observation that activation of the PI3K pathway by *PIK3CA* mutation or loss of *PTEN* is associated with resistance to trastuzumab.¹⁴ Mechanisms for lapatinib are less well established. Recent *in vitro* data suggest that, unlike trastuzumab, loss of *PTEN* function is not associated with lapatinib resistance.¹⁵ Previously, we observed in repeat biopsies of human primary breast cancers that blocking activation of the PI3K/AKT survival pathway is the main mechanism of action of trastuzumab.¹⁶ The objectives of this study were to expand on these earlier observations and, first, to define cellular mechanisms of action of trastuzumab and lapatinib in cell lines and in clinical human biopsy samples and, second, to define possible predictive markers of response, especially in the PI3K/AKT pathway. As such, we evaluated the effect of lapatinib or trastuzumab given alone or in combination in HER2-overexpressing cell lines. We confirmed our *in vitro* observations in two sequential neoadjuvant clinical trials in patients with HER2-overexpressing locally advanced breast cancer, for which repeat biopsies were analyzed for involvement of either the MAPK/ERK or PI3K/AKT pathway. The results of the first neoadjuvant trastuzumab study have been reported¹⁶ and are reiterated in this current analysis for comparison with the results from the lapatinib trial.

METHODS

In Vitro Cell Culture Transfections and Treatments

SKBR3 cells were grown in McCoy's Media (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Cellgro, Manassas, VA) and 1× penicillin/streptomycin, and BT474 cells were grown in DMEM Glutamax Media (Invitrogen) with 10% fetal bovine serum and 1× penicillin/streptomycin. The cells were then transfected with 50 nmol/L of mock or *PTEN* short interfering RNA (siRNA; Dharmacon, Lafayette, CO) for 24 hours using Dharmafect (Dharmacon), according to the manufacturer's instruction, and treated in the following four groups: vehicle control; lapatinib alone (1 μM); trastuzumab alone (10 mg/mL); or lapatinib (1 μM) and trastuzumab (10 mg/mL). The cells were harvested 48 hours after treatment, and Western analysis for p-MAPK and p-AKT (Cell Signaling Technologies, Danvers, MA) was performed.

Next, we transfected MDA361 cells, which contain E545K *PIK3CA* mutation, with *PTEN* short hairpin RNA (shRNA) using Lipofectamine reagent (Invitrogen) or with mock shRNA. After 24 hours, Western analysis on a portion of cells for *PTEN*, p-AKT, and estrogen receptor-α was performed to demonstrate effectiveness of the shRNA. The rest of the cells were then divided into four groups and treated with control (dimethyl sulfoxide), lapatinib (1 μM), trastuzumab (10 mg/mL), or a combination of lapatinib (1 μM) and trastuzumab (10 mg/mL) and allowed to grow in six-well plates for a period of a month. The cells were then stained with crystal violet, counted for colony formation, and photographed.

Patients and Clinical Samples

Two sequential neoadjuvant studies (NCT00133796 and NCT00206427) were conducted in patients with HER2-overexpressing (defined as HercepTest [DAKO, Copenhagen, Denmark] score of 3+ or HER2 amplified by fluorescent *in situ* hybridization) breast cancers (primary cancers > 4 cm). These

studies were approved by the Institutional Review Board of Baylor College of Medicine. In brief, the inclusion criteria were age greater than 18 years, a diagnosis of breast cancer confirmed by core needle biopsy, and adequate liver and kidney function tests (within 1.5× the institution's upper limit of normal). Exclusion criteria included severe underlying chronic illness or disease and prior systemic treatments or treatment with other chemotherapeutic drugs.

In the first study (trastuzumab regimen), trastuzumab was administered initially as an intravenous loading dose of 4 mg/m² and then weekly at 2 mg/m². After the initial 3 weeks of single-agent trastuzumab, docetaxel (100 mg/m² every 3 weeks) was administered for a total of four cycles, with weekly trastuzumab. After completion of neoadjuvant treatment, primary surgery was performed, if the tumor was operable. The second trial (lapatinib regimen) was a sequential study where lapatinib 1,500 mg orally was administered for 6 weeks, followed by docetaxel (100 mg/m² every 3 weeks) for a total of four cycles, with weekly trastuzumab (Fig 1). Core biopsies of the primary cancers were performed at the following time points. In the first study (trastuzumab regimen), biopsies were performed at baseline, week 1, and week 3, whereas in the second study (lapatinib regimen), core biopsies were performed at baseline and weeks 2, 4, and 6, while the patients were on targeted therapy alone. Sample sizes were altered as a result of unforeseen loss of tissue because of tropical storm Allison.

Clinical response was assessed in all patients by physical examination before and after therapy by two experienced breast specialists (J.C.C. and M.R.). **Pathologic complete response** (pCR) after preoperative therapy was prospectively defined as complete disappearance of all invasive cancer or only residual minute foci (< 0.1 cm in diameter), as previously defined.^{17,18}

Laboratory Methods

The details for the immunohistochemistry assays have been described elsewhere¹⁹ (Appendix Table A1 and Appendix Fig A2, online only). Ki67 staining for proliferation index, cleaved caspase-3 (CC3), and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) staining were scored by point counting of at least 500 cancer cells, and results were presented as percentage of positive tumor cells. All the slides were scored (M.C.G. and I.M.) without knowledge of the clinical parameters. Genomic DNA was isolated (10 to 100 ng) and sequenced using the BigDye Terminator Cycle Sequencing Kit and an ABI 3730 automated capillary sequencer (Applied Biosystems, Foster City, CA).

Statistical Methods

Patient and tumor characteristics were summarized by descriptive statistics. Changes in tumor sizes from the initial to post-treatment measurements and changes in next week biomarker levels between the initial and subsequent

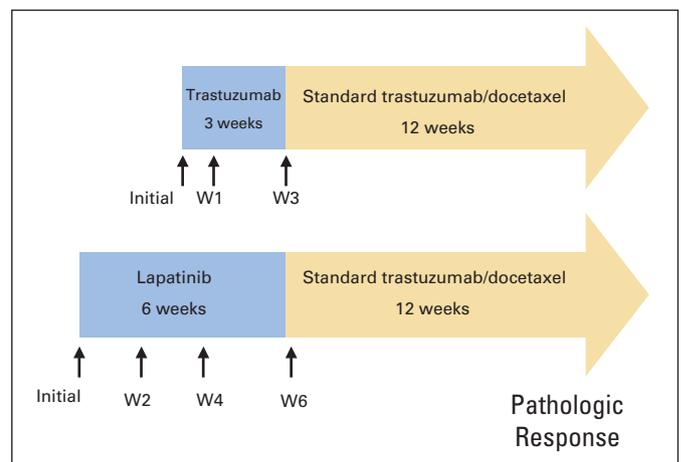


Fig 1. Schema of two sequential neoadjuvant studies in patients with human epidermal growth factor receptor 2-overexpressing locally advanced breast cancers. Biopsies were obtained in the neoadjuvant trastuzumab study at baseline, week (W) 1, and W3. In the second study (lapatinib), biopsies were obtained at baseline, W2, W4, and W6. Pathologic response was assessed after completion of all neoadjuvant treatment.

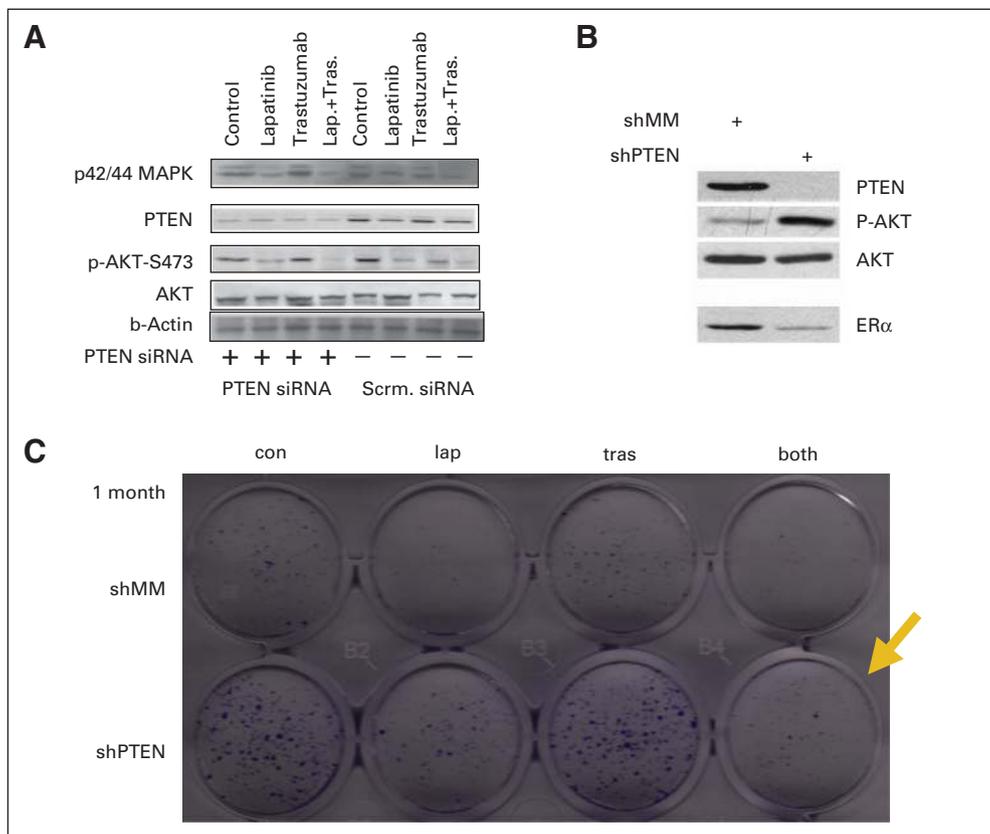


Fig 2. Effect of PTEN on human epidermal growth factor receptor 2-overexpressing breast cancer cell lines. (A) Western blot analysis of SKBR3 breast cancer cell lines transfected with PTEN short interfering RNA (siRNA) and, after 24 hours, treated with control, lapatinib (Lap.; 1 μ M), trastuzumab (Tras.; 10 mg/mL), and lapatinib (1 μ M) plus trastuzumab (10 mg/mL). (B) Western blot analysis of MDA361 cells containing E545K *PIK3CA* mutation treated with PTEN short hairpin RNA. (C) Colony formation assay of MDA361 cells treated with control (con), lapatinib (lap; 1 μ M), trastuzumab (tras; 10 mg/mL), and lapatinib (1 μ M) plus trastuzumab (10 mg/mL).

biopsy samples were compared using the Wilcoxon signed rank test, for which parallel comparisons were made in the previously published report for the trastuzumab study.²⁰ In the current study, we also adopted linear mixed models to model the changes in biomarker levels over time. Separate models were constructed to account for the fact that patients were enrolled onto the two sequential neoadjuvant studies. Time was treated as a continuous fixed effect with both linear and quadratic effects, and subject was treated as a random effect in the models. *P* values were computed by model-based contrasts. Means of predicted values and 95% CIs as twice the SEM were plotted to visualize the changes over time. Response rates were calculated along with exact 95% CIs. Association between activation of PI3K/AKT pathway and treatment response for each study was assessed using the Fisher's exact test. Odds ratios and corresponding 95% CIs were computed for all comparisons. In addition, activation of PI3K/AKT pathway in response to trastuzumab or lapatinib on pCR was examined simultaneously by testing the interaction term between activation of PI3K/AKT pathway and treatment in the logistic regression model. Odds ratios and corresponding 95% CIs for comparing treatments on pCR by activation of PI3K/AKT pathway were calculated. Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

In Vitro Effects of Lapatinib or Trastuzumab in SKBR3 and BT474 Cells

Under normal PTEN conditions (scrambled siRNA), both lapatinib and trastuzumab, alone and in combination, reduced p-MAPK or p-AKT levels in SKBR3 and BT474 cells. Under low PTEN condi-

tions (PTEN siRNA), lapatinib alone or in combination with trastuzumab decreased p-MAPK and p-AKT levels, whereas trastuzumab alone did not (Fig 2A, representative data for SKBR3 cells; similar results with BT474 cells are not shown). Thus, with low PTEN conditions, lapatinib alone or in combination was effective in decreasing p-MAPK and p-AKT, whereas trastuzumab alone did not change these levels, suggesting that under low PTEN conditions, breast cancer cells may be resistant to trastuzumab but not to lapatinib-containing treatments.

In Vitro Effects of PTEN in Presence of *PIK3CA* Mutation

MDA361 cells possess the E545K mutation in *PIK3CA*. Under normal PTEN conditions, trastuzumab and lapatinib, alone and in combination, were both effective in decreasing proliferation. Consistent with earlier results, under low PTEN conditions (PTEN shRNA), these cells were resistant to trastuzumab, whereas lapatinib, alone and in combination, was associated with a decrease in the proliferation assay (Fig 2B). These data further support the observation that under low PTEN conditions, breast cancer cells with *PIK3CA* mutations were resistant to trastuzumab but remained sensitive to lapatinib-containing treatments.

Patient Characteristics and Clinical and Pathologic Responses

The clinical characteristics of the patients enrolled onto these phase II neoadjuvant studies are listed in Table 1 and Appendix Table

Table 1. Patient Demographics and Clinical Characteristics

Characteristic	Trastuzumab (n = 35)		Lapatinib (n = 49)	
	No. of Patients	%	No. of Patients	%
Age, years				
Median	53.5		53.7	
Range	33-69		29-83.5	
Tumor size, cm				
Median	10		8	
Range	4-25		2.5-30	
Biomarkers				
ER positive	6	17	20	41
PR positive	4	11	15	31
Pathologic complete response	32*		34*	
Yes	11	34	23	68
No	21	66	11	32

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

*Three patients who received trastuzumab and 15 patients who received lapatinib did not have data for pathologic complete response.

A2 (online only). In the trastuzumab study, where trastuzumab was administered for 3 weeks, the median tumor size at presentation was $10 \times 10 \text{ cm}^2$ (range, 4×4 to $25 \times 25 \text{ cm}^2$). As previously reported, regression in the product of bidimensional tumor measurements with a median decrease of -20.0% (range, -78.6% to 0% ; $P < .001$) was observed in primary tumors after only 3 weeks of single-agent trastuzumab. In the lapatinib study, where lapatinib was administered for 6 weeks, the initial median tumor size was $8 \times 8 \text{ cm}$ (range, 2.5×2.5 to $25 \times 30 \text{ cm}$), and the median decrease in size was -73% (range, -100% to 92.9% ; $P < .001$, Wilcoxon signed rank test) after 6 weeks of lapatinib. Regarding pCR after completion of all neoadjuvant therapy, 11 (34.4%) of 32 patients achieved pCR with the trastuzumab regimen, 24 (63.1%) of 38 patients achieved pCR with the lapatinib regimen.

Serial Changes in Biomarkers With Trastuzumab or Lapatinib Treatment

Survival pathways (p-AKT, CC3). Consistent with previously reported data of trastuzumab, the apoptosis index (CC3) was significantly increased in week 1 ($P = .004$, model-based contrast) and had a significant time effect on overall change in CC3 ($P = .001$, model-based contrast; Fig 3A; Appendix Table A3, online only).¹⁶ Current thinking suggests that reductions in activated AKT, a major signaling molecule in the cell survival pathways, should lead to induction of apoptosis. Again, as previously published, there was no significant change in expression of either nuclear or cytoplasmic p-AKT with trastuzumab treatment.

With lapatinib, there was no significant change in CC3 at any time point ($P = .41$, model-based contrast; Fig 3A). There was no significant change in expression of p-AKT with lapatinib treatment. To explore the possibility of caspase-independent apoptosis, we examined a subset of the lapatinib-treated patient samples with TUNEL, as well as downstream effects of the cell survival pathway by a pan-Foxo (Foxo1/Foxo3/Foxo4) antibody (Abcam, Cambridge, MA), and demonstrated no statistically significant change with lapatinib treatment (data not shown).

These data suggest that trastuzumab exerts its effects by inducing apoptosis.¹⁶ However, we were not able to demonstrate apoptosis or regulation of the AKT pathway with lapatinib treatment.

Cell cycle and proliferation markers (Ki67, cytoplasmic and nuclear p-MAPK). As previously reported,¹⁶ contrary to in vitro data, trastuzumab did not decrease Ki67 ($P = .66$, model-based contrast) or p-MAPK ($P = .20$, model-based contrast; Figs 3B and 4). With lapatinib, Ki67 was significantly reduced in week 2, and this decrease in proliferation continued through weeks 4 and 6 ($P < .001$, model-based contrast). Consistent with this decrease, p-MAPK showed a significant decrease in week 2 and continued through week 4 ($P = .02$, model-based contrast; Fig 4).

Activation of PI3K Pathway As Pretreatment Predictive Markers of Response to Trastuzumab or Lapatinib

Evaluating biomarkers in the pretreatment specimens that may be predictive indicators of response and resistance, we primarily examined activation of the PI3K/AKT pathway because this has been reported in trastuzumab resistance.¹⁶ Expression of PTEN by immunohistochemistry and mutation in PI3K (*PIK3CA* mutations) were correlated with pathologic responses (Table 2).

With trastuzumab, low PTEN expression was associated with resistance to treatment, with 15.4% of patients (two of 13 patients; 95% CI, 1.9% to 45.5%) with low PTEN achieving pCR; in contrast 44.4% of patients (eight of 18 patients; 95% CI, 21.5% to 69.2%) with high PTEN achieved pCR ($P = .13$, Fisher's exact test). *PIK3CA* mutations also exhibited numerically lower response to trastuzumab, with 20% of patients (two of 10 patients; 95% CI, 2.5% to 55.6%) with mutations achieving pCR and 38.1% of patients (eight of 21 patients; 95% CI, 18.1% to 61.6%) without mutations achieving pCR ($P = .43$, Fisher's exact test). Considering activation of this pathway in tumors with low PTEN or *PIK3CA* mutations, 18.2% of patients (four of 22 patients; 95% CI, 5.2% to 40.3%) with low PTEN or *PIK3CA* mutations achieved pCR, whereas 66.7% of patients (six of nine patients; 95% CI, 29.9% to 92.5%) without low PTEN or *PIK3CA* mutations achieved pCR ($P = .015$, Fisher's exact test). Thus, activation of the PI3K pathway was statistically significantly associated with trastuzumab resistance, consistent with our in vitro results and the published literature.^{14,21}

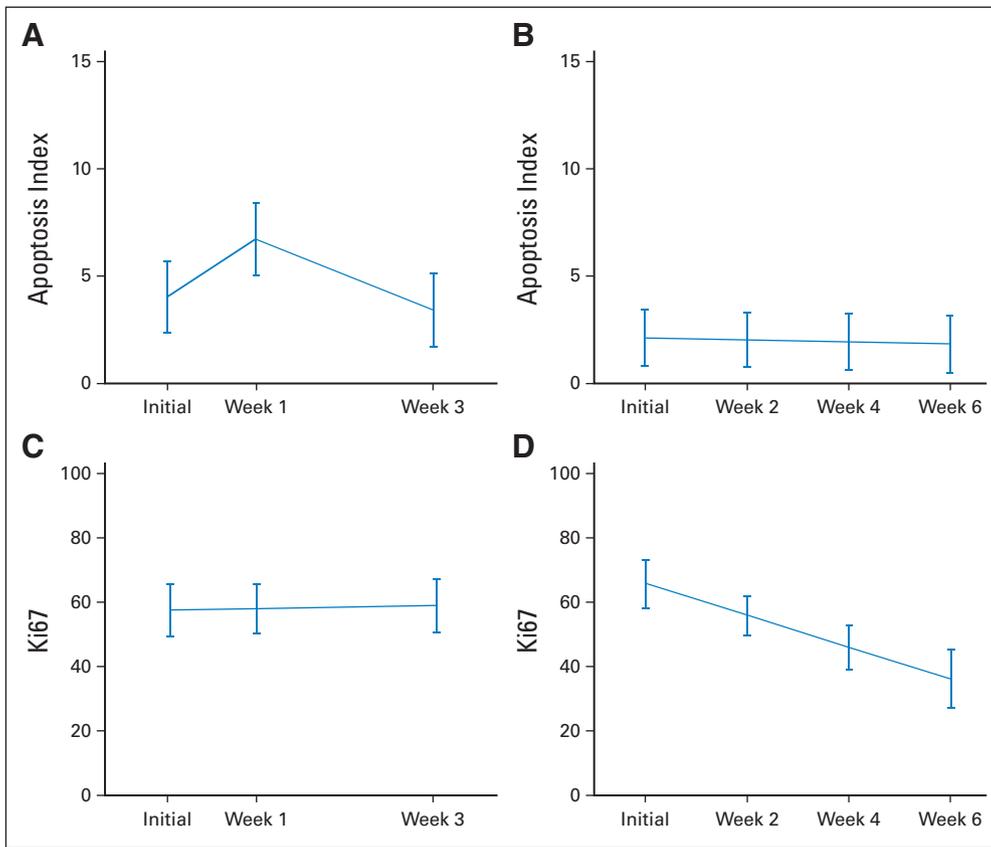


Fig 3. (A-B) Changes in apoptosis as measured by cleaved caspase-3 (CC3). With trastuzumab, there was a significant increase in CC3 found in week 1 ($P = .02$). With lapatinib, no significant change in CC3 was observed. (C-D) Changes in proliferation as measured by Ki67. With trastuzumab, there was no significant change in Ki67. With lapatinib, Ki67 decreased significantly by week 2, and this decrease was continued in weeks 4 and 6 ($P < .001$).

With the lapatinib regimen, the opposite effect was observed, with 92.3% of patients (12 of 13 patients; 95% CI, 64% to 99.8%) with low PTEN achieving pCR compared with 41.2% of patients (seven of 17 patients; 95% CI, 18.4% to 67.1%) with normal PTEN ($P = .007$, Fisher's exact test). *PIK3CA* mutations were not associated with response or resistance. Considering activation of this pathway in tumors with low PTEN or *PIK3CA* mutations, 81.3% of patients (13 of 16 patients; 95% CI, 54.4% to 96%) with low PTEN or *PIK3CA* mutations achieved pCR, compared with 41.7% of patients (five of 12 patients; 95% CI, 15.2% to 72.3%)

without low PTEN or *PIK3CA* mutations ($P = .05$, Fisher's exact test). Thus, low PTEN expression was associated with pathologic response to this lapatinib-containing regimen. Furthermore, in a combined analysis of response rates, there was a statistically significant interaction between low PTEN/*PIK3CA* mutations and treatment (trastuzumab/lapatinib; $P = .001$, logistic regression analysis). Thus, the odds of achieving pCR were significantly greater in patients with low PTEN or *PIK3CA* mutations treated with lapatinib than in those patients treated with trastuzumab (odds ratio, 19.5; 95% CI, 3.7 to 102.4).

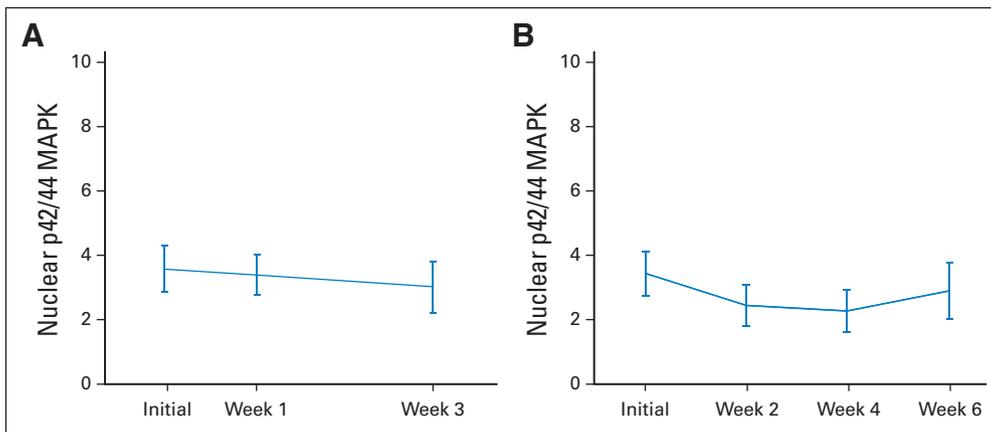


Fig 4. Changes in p-MAPK. (A) Trastuzumab showed no significant change in p-MAPK. (B) Lapatinib showed a significant decrease in p-MAPK by week 2, which was continued through week 4 ($P = .02$).

Table 2. Activation of PI3K Pathway As Pretreatment Predictive Markers of Response to Trastuzumab or Lapatinib

Marker	pCR		No pCR		OR	95% CI	P*
	No. of Patients	%	No. of Patients	%			
Trastuzumab	11		21				
Nuclear PTEN							.13
Low (≤ 3)	2	15.4	11	84.6	0.23	0.04 to 1.34	
High (> 3)	8	44.4	10	55.6	1.00	—	
PIK3CA							.43
WT	8	38.1	13	61.9	2.46	0.41 to 14.63	
Mutation	2	20	8	80	1.00	—	
Nuclear PTEN low/PIK3CA mutation							.02
Yes	4	18.2	18	81.8	0.11	0.02 to 0.65	
No	6	66.7	3	33.3	1.00	—	
Lapatinib	24		14				
Nuclear PTEN							.007
Low (≤ 3)	12	92.3	1	7.7	17.14	1.79 to 163.8	
High (> 3)	7	41.2	10	58.8	1.00	—	
PIK3CA							1.00
WT	16	61.5	10	38.5	1.07	0.15 to 7.54	
Mutation	3	60	2	40	1.00	—	
Nuclear PTEN low/PIK3CA mutation							.05
Yes	13	81.3	3	18.8	6.07	1.11 to 33.24	
No	5	41.7	7	58.3	1.00	—	

Abbreviations: PI3K, phosphoinositol-3 kinase; pCR, pathologic complete response; OR, odds ratio; PTEN, phosphatase and tensin homolog; WT, wild type.
*Fisher's exact test.

DISCUSSION

We describe differential effects of trastuzumab and lapatinib under low and normal PTEN conditions in HER2-overexpressing breast cancer cell lines and confirm these observations in two neoadjuvant trials. These *in vitro* data with breast cancer cell lines (SKRB3, BT474, and MDA361) indicate that under low PTEN conditions, the PI3K/AKT pathway may not be constitutively activated, and thus, trastuzumab treatment did not decrease p-MAPK and p-AKT. Conversely, lapatinib was effective and decreased both p-MAPK and p-AKT under low PTEN conditions.

The clinical trials were designed to answer several questions, including the mechanism of action of trastuzumab and lapatinib on the PI3K/AKT or RAS/MAPK pathways and the role of low PTEN and *PIK3CA* mutations in relation to trastuzumab and lapatinib response. Trastuzumab resulted in tumor regression by induction of apoptosis. Because reductions in p-AKT induced by trastuzumab would be expected to precede apoptosis, it is possible that the peak reduction occurred early, before the first repeat biopsy, and was therefore not detected in this study. Consistent with this observation, we confirm the earlier results of Berns et al¹⁴ that activating mutations in PI3K and PTEN loss were associated with trastuzumab resistance.

In the laboratory, blockade of EGFR and HER2 receptors by monoclonal antibodies inhibited cell proliferation.²²⁻²⁴ With trastuzumab, our results did not show a significant change in cell proliferation as measured by Ki67 or p-MAPK.¹⁷ However, with lapatinib, both Ki67 and p-MAPK showed a significant decrease during treatment. These data support the assertion that, unlike trastuzumab, lapatinib affects proliferation through RAS/MAPK,

as one of several possible pathways. Hence, these data suggest that in human breast cancers, trastuzumab seems to primarily affect cell survival and has less effect on cell cycle kinetics, at least after a short duration of treatment, whereas lapatinib seems to affect cell cycle kinetics through RAS/MAPK and has less effect on cell survival.¹⁷ Thus, these two therapeutic agents have different cellular mechanisms and may therefore have different baseline predictive markers of response.

As mentioned previously, it has been established that PTEN loss and mutations in PI3K have been described in trastuzumab resistance.^{14,21} PTEN is a dual phosphatase that negatively regulates AKT. Patients with PTEN-deficient tumors were found to have a poorer response to trastuzumab-containing therapy.²¹ Consistent with these findings, Berns et al¹⁴ used a large-scale unbiased RNA interference screen and identified PTEN as the only modulator of trastuzumab sensitivity, and patients with activation of PI3K pathway had worse clinical outcome.¹⁴ Conversely, data with lapatinib have been limited primarily to cell lines and are conflicting. In one study, with selective knockdown of PTEN using RNA interference in HER2-overexpressing lines, the activity of lapatinib was not affected, thus suggesting that these cell lines remained sensitive to lapatinib despite loss of PTEN.²⁵ Conversely, in a recent publication using an unbiased genetic approach in cell lines, PTEN was identified as a modulator of lapatinib sensitivity.²⁶ Here, we describe that low PTEN selects for trastuzumab resistance but lapatinib sensitivity in these small clinical trials. These results suggest the potential utility of PTEN as a biomarker, especially if these results are confirmed by several large, ongoing, multicenter, phase III clinical trials.

In conclusion, these prospective in vivo studies demonstrate that activation of PI3K/AKT pathway predicts for resistance to trastuzumab but not to lapatinib. Our data support the design of ongoing clinical studies of the combination of both HER2-targeted agents. On the basis of these and other data, such clinical trials are currently underway.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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REFERENCES

- Riese DJ 2nd, Stern DF: Specificity within the EGF family/ErbB receptor family signaling network. *Bioessays* 20:41-48, 1998
- Amundadottir LT, Leder P: Signal transduction pathways activated and required for mammary carcinogenesis in response to specific oncogenes. *Oncogene* 16:737-746, 1998
- Okano J, Gaslightwala I, Birnbaum MJ, et al: Akt/protein kinase B isoforms are differentially regulated by epidermal growth factor stimulation. *J Biol Chem* 275:30934-30942, 2000
- Romond EH, Perez EA, Bryant J, et al: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 353:1673-1684, 2005
- Rusnak DW, Affleck K, Cockerill SG, et al: The characterization of novel, dual ErbB-2/EGFR, tyrosine kinase inhibitors: Potential therapy for cancer. *Cancer Res* 61:7196-7203, 2001
- DeSimone PA, Bence AK, Anderson EB, et al: A phase I study to investigate the safety, tolerability, and pharmacokinetics of single oral escalating doses of GW572016 in healthy volunteers. *Proc Am Soc Clin Oncol* 21:34a, 2002 (abstr 375)
- Blackwell K, Kaplan E, Franco S, et al: A phase II, open-label, multicenter study of GW572016 in patients with trastuzumab-refractory metastatic breast cancer. *J Clin Oncol* 22:196, 2004 (suppl 14S; abstr 3006)
- Dees EC, Burris H, Hurwitz H, et al: Clinical summary of 67 heavily pre-treated patients with metastatic carcinomas treated with GW572016 in a phase Ib study. *J Clin Oncol* 22:241, 2004 (suppl 14S; abstr 3188)

- Geyer CE, Forster J, Lindquist D, et al: Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med* 355:2733-2743, 2006
- Johnston S, Pippen J Jr, Pivov X, et al: Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. *J Clin Oncol* 27:5538-5546, 2009
- Cho HS, Mason K, Ramyar KX, et al: Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* 421:756-760, 2003
- Smith KL, Robbins PD, Dawkins HJS, et al: Detection of c-erbB-2 amplification in breast cancer by in situ hybridization. *Breast* 2:234-238, 1993
- Nahta R, Yuan LX, Zhang B, et al: Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. *Cancer Res* 65:11118-11128, 2005
- Berns K, Horlings HM, Hennessy BT, et al: A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* 12:395-402, 2007
- Xia W, Mullin RJ, Keith BR, et al: Anti-tumor activity of GW572016: A dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. *Oncogene* 21:6255-6263, 2002
- Mohsin SK, Weiss HL, Gutierrez MC, et al: Neoadjuvant trastuzumab induces apoptosis in primary breast cancers. *J Clin Oncol* 23:2460-2468, 2005
- Kuerer HM, Newman LA, Buzdar AU, et al: Residual metastatic axillary lymph nodes following neoadjuvant chemotherapy predict disease-free survival in patients with locally advanced breast cancer. *Am J Surg* 176:502-509, 1998
- Li X, Lewis MT, Huang J, et al: Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J Natl Cancer Inst* 100:672-679, 2008

AUTHOR CONTRIBUTIONS

Conception and design: Bhuvanesh Dave, M. Carolina Gutierrez, Gary C. Chamness, Anindita Chakrabarty, Susan G. Hilsenbeck, Rachel Schiff, Carlos Arteaga, C. Kent Osborne, Jenny C. Chang
Financial support: Jenny C. Chang
Administrative support: Helen Wong, Mothaffar Rimawi, Jenny C. Chang
Provision of study materials or patients: Archana Narasanna, Mothaffar Rimawi, Jenny C. Chang
Collection and assembly of data: Bhuvanesh Dave, Ilenia Migliaccio, M. Carolina Gutierrez, Gary C. Chamness, Helen Wong, Archana Narasanna, Anindita Chakrabarty, Jian Huang, Mothaffar Rimawi, Carlos Arteaga, Jenny C. Chang
Data analysis and interpretation: Bhuvanesh Dave, Ilenia Migliaccio, M. Carolina Gutierrez, Meng-Fen Wu, Gary C. Chamness, Archana Narasanna, Anindita Chakrabarty, Susan G. Hilsenbeck, Jian Huang, Mothaffar Rimawi, Rachel Schiff, Carlos Arteaga, C. Kent Osborne, Jenny C. Chang
Manuscript writing: Bhuvanesh Dave, Meng-Fen Wu, Gary C. Chamness, Carlos Arteaga, C. Kent Osborne, Jenny C. Chang
Final approval of manuscript: Bhuvanesh Dave, Ilenia Migliaccio, M. Carolina Gutierrez, Meng-Fen Wu, Gary C. Chamness, Helen Wong, Archana Narasanna, Anindita Chakrabarty, Susan G. Hilsenbeck, Jian Huang, Mothaffar Rimawi, Rachel Schiff, Carlos Arteaga, C. Kent Osborne, Jenny C. Chang

- Allred DC, Harvey JM, Berardo M, et al: Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 11:155-168, 1998
- Clopper CJ, Pearson ES: The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 26:404-413, 1934
- Nagata Y, Lan KH, Zhou X, et al: PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 6:117-127, 2004
- Bacus SS, Stancovski I, Huberman E, et al: Tumor-inhibitory monoclonal antibodies to the HER-2/Neu receptor induce differentiation of human breast cancer cells. *Cancer Res* 52:2580-2589, 1992
- Peng D, Fan Z, Lu Y, et al: Anti-epidermal growth factor receptor monoclonal antibody 225 up-regulates p27KIP1 and induces G1 arrest in prostatic cancer cell line DU145. *Cancer Res* 56:3666-3669, 1996
- Ben-Bassat H, Rosenbaum-Mitrani S, Hartzstark Z, et al: Inhibitors of epidermal growth factor receptor kinase and of cyclin-dependent kinase 2 activation induce growth arrest, differentiation, and apoptosis of human papilloma virus 16-immortalized human keratinocytes. *Cancer Res* 57:3741-3750, 1997
- Xia W, Husain I, Liu L, et al: Lapatinib antitumor activity is not dependent upon phosphatase and tensin homologue deleted on chromosome 10 in ErbB2-overexpressing breast cancers. *Cancer Res* 67:1170-1175, 2007
- Eichhorn PJ, Gili M, Scaltriti M, et al: Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BE225. *Cancer Res* 68:9221-9230, 2008

Glossary Terms

AKT: A transforming serine-threonine kinase involved in cell survival.

HER2: The number of copies of the HER2 gene divided by the number of copies of chromosome 17 (strictly the number of copies of the pericentric region of chromosome 17 to which the CEP17 FISH probe hybridizes).

Lapatinib: A dual tyrosine kinase inhibitor, lapatinib has been developed as an inhibitor of the tyrosine kinase activities of ErbB1 (EGFR) and ErbB2. Like other tyrosine kinase inhibitors, it competes with ATP binding to the intracellular regions of the receptors that are activated following tyrosine phosphorylation.

MAPK (mitogen-activated protein kinase): MAPKs are a family of enzymes that form an integrated network influencing cellular functions such as differentiation, proliferation, and cell death. These cytoplasmic proteins modulate the activities of other intracellular proteins by adding phosphate groups to their serine/threonine amino acids.

Pathologic complete response (pCR): The absence of any residual tumor cells in a histologic evaluation of a tumor specimen is defined as a complete pathologic response.

PI3K: Phosphatidylinositol-3 phosphate kinase (PI3K) adds a phosphate group to PI3, which is a downstream signaling molecule involved in survival/proliferative pathways mediated by growth factors such as the EGF and the PDGFs.

PTEN (phosphatase and tensin homolog): PTEN is a tumor suppressor gene with a gamut of regulatory activities. The gene product is a multifunctional molecule. The predominant activity identified for PTEN is its lipid phosphatase activity that converts inositol trisphosphates into inositol bisphosphates, thus inhibiting survival and proliferative pathways that are activated by inositol trisphosphates. PTEN acts to maintain arrest in the G1 phase of the cell cycle and enable apoptosis through an AKT-dependent mechanism.