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A Phase I-II Study of Combined Blockade of the ErbB Receptor Network with Trastuzumab and Gefitinib in Patients with HER2 (ErbB2)-Overexpressing Metastatic Breast Cancer

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Abstract

Purpose—To determine the safety, and efficacy of the epidermal growth factor receptor tyrosine kinase inhibitor gefitinib in combination with trastuzumab in patients with metastatic HER2-positive metastatic breast cancer.

Experimental Design—Patients with HER2-overexpressing breast cancer were treated with trastuzumab 2 mg/kg/week and gefitinib 250 to 500 mg/day. The primary end point of the study was to increase the proportion progression-free from 50% to 65% at 6 months in chemotherapy-naïve patients and from 50% to 70% at 3 months in patients previously treated with chemotherapy in the metastatic setting.

Results—In the phase I study, all patients treated with gefitinib 500 mg/day developed grade 3 diarrhea. The phase II study was conducted using trastuzumab and gefitinib 250 mg/day. One patient achieved a complete response, 2 had a partial response, and 6 had stable disease for an overall response rate of 9% and a clinical benefit rate of 28% (9 of 32). Median time to progression (TTP) was 3 months (95% confidence interval, 2.3-4.1) in patients with no prior systemic therapy in the metastatic setting ($n = 23$). In patients treated with prior systemic therapy ($n = 9$), the median TTP of 5.3 months (95% confidence interval, 2.8-8.1). Overall median survival was 27 months. TTP was similar in EGFR-positive compared with EGFR-negative patients.

Conclusions—Gefitinib 250 mg/day was the maximal dose that can be safely administered with weekly trastuzumab. Interim analysis of the efficacy suggested that the combination was unlikely

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to result in clinical benefit compared with trastuzumab alone. These results do not support the use of this combination in patients with HER2-positive breast cancer.

HER2/neu (ErbB2) is a member of the erbB family of transmembrane receptor tyrosine kinases, which also includes the epidermal growth factor (EGF) receptor (EGFR, ErbB1), HER3 (ErbB3), and HER4 (ErbB4). Binding of ligands to the extracellular domain of EGFR, HER3, and HER4 induces the formation of tyrosine kinase-active homodimers and heterodimers to which activated HER2 is recruited as a preferred partner (1). Although HER2 does not bind any of the ErbB ligands directly, its catalytic activity can potentially amplify signaling by ErbB-containing heterodimers via increasing ligand binding affinity and/or receptor recycling and stability (2-5).

Amplification of the *HER2/Neu (ERBB2)* gene occurs in ~25% of invasive breast cancers and is associated with poor patient outcome (6). Trastuzumab (Herceptin), a humanized monoclonal IgG1 that binds to the juxtamembrane region of the HER2 receptor, induces clinical responses in HER2-overexpressing breast cancers and prolongs patient survival when combined with chemotherapy (7-14). The clinical efficacy of trastuzumab seems limited to breast cancers that overexpress HER2 as measured by intense membrane staining in the majority of tumor cells with HER2 antibodies [3+ by immunohistochemistry (IHC)] or excess copies of the *HER2* gene determined by fluorescence *in situ* hybridization (FISH). However, many patients with *HER2* gene-amplified metastatic breast cancers do not respond or eventually escape trastuzumab, suggesting both *de novo* and acquired mechanisms of therapeutic resistance.

Translational Relevance

In this paper, we tested the safety, tolerability, and efficacy of the combination of gefitinib and trastuzumab in patient with HER2-overexpressing breast cancer. The combination of 250 mg/day of gefitinib with weekly trastuzumab was feasible, although it was associated with a higher toxicity than that associated with the same dose of gefitinib alone. Analysis of the efficacy data strongly suggested that the combination was unlikely to result in greater clinical benefit than that reported with trastuzumab alone in similar patients with HER2-positive metastatic breast cancer. We propose that in light of these results and the plethora of novel drugs targeted against other elements of the ErbB receptor network, combinations of trastuzumab with epidermal growth factor receptor – specific inhibitors are not warranted. Additional therapies such as those targeting the HER2 tyrosine kinase directly and/or the interaction between HER2 and HER3 are likely to be more synergistic than gefitinib when combined with trastuzumab.

Several studies have already reported on potential mechanisms of resistance to trastuzumab (reviewed in ref. 15). In addition, structural and cellular data using ErbB receptor ectodomains and different HER2 monoclonal antibodies have shown that trastuzumab is unable to block ligand-induced EGFR/HER2 and HER2/HER3 heterodimers (16,17). These data coupled with the ability of HER2 to be transactivated by ErbB coreceptors, such as EGFR and HER3, provide tumor cells with other potential mechanisms of drug resistance. For example, the ErbB ligands EGF, transforming growth factor α , β cellulin, and heregulin have been shown to rescue from or attenuate the antiproliferative effect of HER2 antibodies (18-21). Furthermore, we recently generated trastuzumab-resistant BT-474 cells *in vivo* (15); the resistant cells exhibited higher levels of P-EGFR and EGFR/HER2 heterodimers as well as overexpression of EGFR, transforming growth factor α , heparin-binding EGF, and heregulin RNAs compared with the parental trastuzumab-sensitive cells. The small molecule EGFR tyrosine kinase inhibitors gefitinib and erlotinib were effective against the antibody-resistant cells, suggesting that a combined blockade of the EGFR and HER2 can delay and/

or abrogate the emergence of drug resistance. Indeed, several studies have previously shown synergy of EGFR antagonists in combination with trastuzumab against HER2-overexpressing breast cancer cells and xenografts (19,22-24).

Based on these data, we proposed that a combined inhibition of the ErbB receptor network with the EGFR tyrosine kinase inhibitor gefitinib and the HER2 antibody trastuzumab would be synergistic against HER2-overexpressing breast cancers. We report herein a phase I to II trial of this combination in patients with HER2-positive metastatic breast cancer.

Materials and Methods

Patient eligibility

Patients with histologically-confirmed HER2-positive metastatic breast cancer with an Eastern Cooperative Oncology Group performance status of 0 to 2, measurable tumor, adequate renal, hepatic, and hematologic function and a left ventricular ejection fraction of $\geq 50\%$ by MUGA scan (4 wk before registration), and who had received 0 to 2 prior chemotherapy or hormonal regimens for metastatic disease were eligible. Patients that had received a cumulative dose of doxorubicin that was $>360 \text{ mg/m}^2$ or with brain metastases as the sole site of clinical disease were ineligible. Patients were also excluded if they required the following medications: carbamazepine, dexamethasone, ethosuximide, glucocorticoids, griseofulvin, nafcillin, nelfinavir, nevirapine, oxcarbazepine, phenobarbital, phenylbutazone, phenytoin, primidone, progesterone, rifabutin, rifampin, rofecoxib, St. John's Wort, sulfadiazine, sulfapyrazole, or troglitazone. Concurrent use of hormonal or radiation therapy was not permitted. HER2 positivity was defined as 3+ score based on IHC using the DAKO HercepTest or ≥ 2 copies of the *HER2* gene by FISH using the Vysis method. EGFR levels in tumor sections were not required for study entry. In all evaluable tumors, HER2 status by IHC and/or FISH was reviewed and confirmed at the Eastern Cooperative Oncology Group Pathology Coordinating Center (M.P.).

Treatment plan and dose escalation

All patients were treated with trastuzumab 4 mg/kg IV over 90 min on day 1 only followed by 2 mg/kg IV over 30 min on subsequent weekly doses until disease progression. Gefitinib was administered as a single oral dose daily until disease progression or until grade 3/4 toxicity was observed. The phase I portion of the study included two dosing cohorts of gefitinib, 250 and 500 mg/d. Patients were treated based on a standard 3+3 study design. Dose-limiting toxicity was defined as any of the following toxicities, if they occurred within the first cycle of treatment and were attributed to protocol therapy: grade 3 to 4 thrombocytopenia and/or neutropenia lasting >7 d, or neutropenia associated with fever of $\geq 38^\circ\text{C}$ or 100.5°F ; grade 3 to 4 skin toxicity; grade 3 to 4 central nervous system, cardiac [including prolongation of progesterone receptor (PR) interval of >217 ms], lung, hepatic, or renal toxicity; significant ocular toxicity (corneal punctuate staining with symptoms, >1 quadrant of conjunctival hyperemia, or unanticipated significant ocular toxicity consistent with corneal changes of translucencies, opacities, or significant unexplained hyperemia); and grade 4 diarrhea and/or vomiting, or grade 3 diarrhea and/or vomiting that persist >24 h despite maximum treatment.

For the phase II portion of the trial, patients were treated with the dose of gefitinib found to be safe in the phase I cohort in combination with full-dose trastuzumab. The primary efficacy end point was percent progression-free compared with the published experience with single-agent trastuzumab. Patients were prospectively stratified into two cohorts based on prior therapy of metastatic disease; treatment was continued until disease progression or

the occurrence of clinically unacceptable toxicity. Planned interim analyses of treatment efficacy were to be done as outlined in the Statistical Methods section below.

Safety and efficacy

Clinical status, liver and renal function tests, urinalysis, and a complete blood count were assessed weekly during the first 4 wk of treatment and every 4 wk thereafter. Treatment-related toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. Disease status was assessed according to the Response Evaluation Criteria in Solid Tumors at baseline, every 12 wk up to 2 y postregistration, and then every 6 mo until 3 y postregistration. Patients with an apparent complete or partial response had repeat tumor measurements at >4 wk after the apparent clinical response to confirm it.

Statistical design and methods

The primary end points of the phase I portion of this study were to determine the dose of gefitinib that can be used in combination with trastuzumab and to evaluate the toxicity of the combination. The primary end point of the phase II part of the trial was (a) to evaluate the proportion that were progression free at 3 mo (from 50-70%) of treatment with the combination of gefitinib and trastuzumab in patients who had progressed on treatment for metastatic breast cancer, and (b) at 6 mo (from 50-65%) in patients who had not been treated in the metastatic setting.

E1100 was a nonrandomized phase II trial where patients were prospectively stratified into two groups based on prior therapy in the metastatic setting. A two-stage phase II design was used for each group. For group “a”, if ≥ 26 of 42 eligible patients were progression free at 3 mo, the combination would be considered promising (with ≥ 6 progression free among the first 15 eligible patients entered in the first stage). With this design, there was 0.90 probability of concluding that the combination was effective if the true progression-free rate at 3 mo was 70%. For group “b”, if ≥ 45 of 78 eligible patients were progression free at 6 mo, the combination of trastuzumab and gefitinib would be considered promising (with ≥ 9 progression free among the first 19 eligible patients entered in the first stage). With this design, there was 0.90 probability of concluding that the combination was effective if the true progression-free rate at 6 mo was 65%. After the first-stage accrual goal was met within an arm, accrual was required to be suspended for planned interim efficacy analysis. Time to progression (TTP) was defined as time from date of registration to date of progression. Patients without documented progression were censored at the date last known progression free. Survival was defined as time from date of registration to date of death from any cause. The Kaplan-Meier method was used to estimate progression and survival distributions (25). Summaries of patient demographics and disease characteristics and analyses of outcome excluded patients classified as ineligible. Toxicity summaries included all patients that received protocol treatment and experienced toxicities. Eligible patients treated at the maximum tolerated dose during the phase I portion were included in analyses with group “b” based on receiving no prior treatment for metastatic disease.

EGFR protein levels and gene copy number

EGFR protein was measured by IHC in formalin-fixed tumor sections using the DAKO method (monoclonal antibody H11) and a dextran polymer conjugate visualization system. When >10% of tumor cells showed membranous (partial or complete) staining of any intensity, the tumor was considered EGFR positive. *EGFR* gene copy number was evaluated by FISH using the Abbott Molecular Vysis DNA probe according to published protocols (26). Tumors were considered gene amplified or FISH positive if they contained ≥ 2 copies of the *EGFR* gene by FISH.

Results

Patient population

A total of 41 patients were registered in this trial. Six patients were classified as ineligible. Demographics of the 35 eligible patients are summarized in Table 1. Patients ranged in age from 30 to 83 with a median of 56. HER2 FISH was done in 25 of 35 evaluable patients; 23 of 25 tumors exhibited ≥ 2 copies of *HER2* and were considered FISH positive. Fifty-four percent had an Eastern Cooperative Oncology Group PS of 0% and 49% of the tumors were estrogen receptor (ER) and/or PR positive. Six of 28 patients with evaluable tumor samples were EGFR positive (1 to 3+) by IHC (Dako) and none exhibited *EGFR* gene amplification as measured by FISH.

Phase I: maximally tolerated dose

The phase I portion opened at six institutions on September 2001 (Vanderbilt University, Rush-Presbyterian/St. Luke's Medical Center, Indiana University Medical Center, Johns Hopkins University, Montefiore Medical Center). The first 3 patients treated with trastuzumab and 250 mg gefitinib did not experience dose-limiting toxicity. The next 3 patients were treated with full-dose trastuzumab plus 500 mg gefitinib. All 3 patients experienced grade 3 treatment-related diarrhea and/or vomiting despite optimal medical therapy. Therefore, the 250-mg dose of gefitinib was chosen for the phase II study of the combination.

Phase II

This portion of the study opened group-wide in August 2002.

Toxicities

For patients treated with prior systemic therapy in the metastatic setting, the median number of days on treatment was 69 (range, 7-594; including temporary treatment interruptions). For therapy-naïve patients, the median number of days on treatment was 76 (range, 9-723; including temporary treatment interruptions). Treatment was stopped primarily for disease progression and toxicity. Among the 14 patients who stopped treatment due to toxicity, 6 experienced grade 3 treatment-related diarrhea. The most common side effects were skin rash (76%) and diarrhea (89%); of these, 13% and 32%, respectively were classified as grade 3. No patients in the phase I portion of E1100 receiving gefitinib 250 mg/day experienced grade 3 diarrhea. Table 2 summarizes all treatment-related toxicities (where related is defined as possibly, probably, or definitely related). Toxicity data for patients classified as ineligible were included in this table if the patient received protocol treatment and therapy-related toxicities were reported. Of note, up to 50% of the patients receiving trastuzumab plus 250 mg of gefitinib in the phase I and II trials experienced grade 3 toxicities consisting mostly of diarrhea. One patient experienced grade 4 decrease in neutrophils. Four patients had $>10\%$ reductions in left ventricular ejection fraction at either week 4 and or week 12 from start of treatment, and only in one was this value below the lower normal limit. In all cases, this was not associated with signs or symptoms of heart failure and it was promptly reversible. There were no treatment-related deaths.

Clinical efficacy

At the planned interim efficacy analysis for patients with no prior systemic treatment for metastatic disease, lack of promising results led to early suspension of this arm and to a review of the available efficacy data among patients who had received prior treatment for metastatic breast cancer. Both arms were closed August 2004 because neither arm met the benchmark necessary for continuation to the planned second stage of accrual. At the current

analysis reported here, median follow up was 43 months. Among nine patients with prior systemic therapy, four had received prior hormonal therapy but not chemotherapy in the metastatic setting. There was 1 partial response (disease progressed 5 months later) and 1 patient exhibited disease stabilization for 3 months. Median TTP in this group was 5.3 months with a 95% confidence interval on that estimate of 2.8 to 8.1 months (Fig. 1). Median survival was 27 months with a 95% confidence interval lower limit on that estimate of 24.1 months. The upper limit is currently beyond the observed data (beyond 45 months; Fig. 2).

Twenty-three patients with no prior systemic therapy for metastatic disease were treated with the combination. There was 1 complete response and 1 partial response (disease progressed 16.5 and 27 months later) with 5 patients exhibiting disease stabilization for 3 months. Median TTP was 3 months (95% confidence interval, 2.3-4.1 months; Fig. 1). Median overall survival was 25 months with a 95% confidence interval of 20 to 37 months (Fig. 2). A subanalysis of all eligible patients regardless of prior therapy treated with gefitinib 250 mg and with EGFR IHC showed a median TTP (95% CI) of 3.8 (1.6 to 8.9) months in the EGFR+ group ($n = 5$) and 4 (2.3 to 6.6) months in the EGFR-negative group ($n = 20$). TTP was 2.7 (2.2 to 3.8) months in the ER/PR negative group ($n = 14$) and 7.3 (2.8 to 19) months in the ER+ and/or PR+ group ($n = 17$).

Discussion

This phase I/II trial tested the tolerability and efficacy of the combination of the anti-HER2 antibody trastuzumab and the EGFR tyrosine kinase inhibitor gefitinib. The expectation was that the combination, by blocking two receptor tyrosine kinases in the ErbB network, will induce a longer TTP than that reported in HER2-overexpressing tumors treated with trastuzumab alone. Two doses of gefitinib, 250 and 500 mg/day, together with full-dose trastuzumab were evaluated in the phase I portion of the trial. All patients treated with the 500 mg/day dose of gefitinib developed grade 3/4 gastrointestinal toxicity despite optimal medical therapy, thus resulting in the selection of the 250 mg/day dose for the part of the trial addressing therapeutic efficacy.

The severe diarrhea observed in all patients treated with the 500-mg dose of gefitinib was somewhat unanticipated. In phase I studies of gefitinib, grade 3/4 diarrhea was the dose-limiting toxicity, but it occurred at doses ≥ 700 mg/day (27-29). In phase II studies, the 500-mg dose has been associated with grade 3 diarrhea in $\leq 10\%$ of patients (30-33). To our knowledge, the only study where 500 mg of gefitinib was not tolerated is a trial in colorectal cancer in which gefitinib was combined with the anti-EGFR antibody cetuximab (34). In this trial, a more complete inhibition of the EGFR induced by the combination would be a viable explanation for the enhanced diarrhea. Furthermore, severe gastrointestinal toxicity is not a prominent side effect of trastuzumab, with a reported frequency of $\leq 5\%$ (8,14). This frequency contrasts with the higher incidence of diarrhea in several phase I to II trials pertuzumab (35-38), a HER2 antibody that blocks heterodimerization of HER2 with other ErbB receptors such as EGFR and HER3 (16,39,40). Similar toxicity in excess frequency over rash has been observed with the dual irreversible HER2/EGFR inhibitor HKI-272 (41). Taken together, these results suggest a basal level of EGFR-HER2 crosstalk in the gut that may compensate for the inhibition of the EGFR tyrosine kinase by gefitinib. The combined use of trastuzumab may have prevented such compensation and, therefore, sensitized to gefitinib-induced toxicity in the gut. This speculation is further supported by the 32% (12 of 38 patients) incidence of grade 3 diarrhea observed in the phase II portion of the trial (Table 2), where 250 mg of gefitinib were used. We should note that grade 3 gastrointestinal toxicity with this lower dose of gefitinib has been exceedingly rare in single-agent trials (30,31,33).

A planned interim analysis of the proportion of patients that were free of tumor progression at 3 and 6 months showed that the combination of full-dose trastuzumab with 250 mg gefitinib did not merit further testing. The median TTP for patients that had or had not had prior therapy for metastatic disease was consistent with that of 3.1 to 3.8 months reported for trastuzumab alone in 3 previous single agent trials (8,12,14). Of note, the overall response rate (9%) was lower than that reported in these three larger phase II trials with trastuzumab alone (8,12,14). The reasons for this apparent difference are unclear, although the overall survival of the patients in our study was comparable with that in single-agent trials with trastuzumab.

The mean steady-state plasma concentration of gefitinib at the 250-mg dose is reported to be $<1 \mu\text{mol/L}$ or 447 ng/mL (27,29), a concentration below the one required to optimally inhibit EGFR activity and tumor cell growth in culture (19,22). These data coupled with the negative clinical outcome of the phase II study raise the question whether the dose of gefitinib used was appropriate for optimal inhibition of the EGFR tyrosine kinase in tumors. Although the trial reported here cannot address this question directly, several pharmacodynamic and clinical studies suggest 250 mg of gefitinib might be appropriate to inhibit EGFR function. At doses $\geq 150 \text{ mg/day}$, gefitinib inhibits EGFR phosphorylation as measured by IHC with site-specific phospho-antibodies in receptor-expressing cells in the skin (42). In a cohort of gastric cancers that were rebiopsied 28 days after daily administration of 250 or 500 mg of gefitinib, EGFR phosphorylation in tumor cells was markedly inhibited regardless of the dose level (43), although the HER2 status of those patients was not reported. The presence and severity of a skin rash have been positively associated with a better response and outcome in trials with EGFR antagonists (31,44,45). In this trial, 76% of patients (29 of 38) developed a grade 1 to 3 rash. This is comparable with the frequencies reported in the trials of gefitinib in patients with non-small cell lung cancer. Therefore, based on these cumulative data, we believe that the dose of 250 mg of gefitinib used in the trial reported here was adequate to inhibit the EGFR in tumor cells.

A subanalysis of all eligible patients showed no differences in TTP in EGFR-positive versus EGFR-negative patients as measured by IHC. This is not surprising if we consider that activity of EGFR inhibitors has been shown in cancers with undetectable receptor levels (46) underscoring the weakness of currently available EGFR antibodies to detect low levels of activated receptors. In this trial, however, a more viable interpretation of the lack of difference in TTP between receptor-positive and receptor-negative patients may be that blockade of the EGFR alone in HER2-positive patients does not add to the antitumor effect of trastuzumab.

There are several molecular mechanisms that might explain the lack of benefit of the combination of gefitinib and trastuzumab even in the setting of optimal inhibition of the EGFR and HER2. In a recent report, Sergina et al. (47) showed that, in HER2-overexpressing breast cancer cells, the inhibition of HER2 phosphorylation induced by gefitinib is followed by feedback up-regulation of activated HER3 (ErbB3) and Akt, thus limiting the inhibitory effect of the EGFR tyrosine kinase inhibitor. Furthermore, in mice bearing breast cancer xenografts with high levels of HER2, Arpino et al. (16,40) added pertuzumab, an antibody that blocks HER2-HER3 heterodimerization, to gefitinib and trastuzumab. Only the triple therapy was able to completely eradicate the established xenografts (48). In a recent trial, the addition of pertuzumab to trastuzumab in patients with HER2-overexpressing breast cancers who had progressed in trastuzumab resulted in a 40% clinical response rate (35), further implying that HER3 signaling, as a result of HER2-HER3 crosstalk, is a possible mechanism of escape from trastuzumab therapy. Inhibition of HER2-mediated transactivation of HER3 would also explain the antitumor effect of lapatinib, a small-molecule direct inhibitor of the HER2 and EGFR tyrosine kinases (49), observed

against HER2-positive tumors that had escaped trastuzumab (50,51). In the phase II study reported here, coupling between HER2 and HER3 and activation of HER3 would not be completely inhibited by the combination of gefitinib and trastuzumab and are, therefore, plausible explanations for the negative signal of clinical activity observed.

Forty-nine percent of the patients enrolled were ER and/or PR positive, but concurrent hormonal therapy was not permitted in the phase II study. The TTP was 7.3 months in the hormone receptor–positive groups versus 2.7 months among ER/PR-negative tumors. Whether the omission of concurrent hormonal therapy affected the response to gefitinib and trastuzumab was not addressed by this study. Some reports suggest that ER/PR function may compensate for blockade of the ErbB receptor network. Treatment of HER2-overexpressing cells with ErbB receptor or mitogen-activated protein kinase signaling inhibitors up-regulates ER function and PR levels (52,53). Treatment with trastuzumab has been shown to restore detectable ER levels in some ER-negative tumors that subsequently responded to aromatase inhibitors (54). Furthermore, a randomized Phase III study (TANDEM) was done to evaluate the efficacy of trastuzumab plus anastrozole compared with anastrozole alone in postmenopausal women with HER2+ and ER+ and/or PgR+ metastatic breast cancer (Kaufman et al. ESMO 2006). Patients in the combination arm had significant improvement in progression-free survival and clinical benefit rate compared with patients in the anastrozole arm. These data could suggest that omission of concurrent hormonal therapy was unwarranted. On the other hand, a retrospective analysis of 805 patients with metastatic breast cancer in 3 large clinical trials showed that hormone receptor status did not affect the clinical benefit of trastuzumab given as a single agent or in combination with chemotherapy (55).

In summary, the combination of 250 mg/day of gefitinib with weekly trastuzumab was feasible, although it was associated with a higher toxicity than that associated with the same dose of gefitinib alone. Analysis of the efficacy data strongly suggested that the combination was unlikely to result in greater clinical benefit than that reported with trastuzumab alone in similar patients with HER2-overexpressing metastatic breast cancer. We propose that in light of these results and the plethora of novel drugs targeted against other elements of the ErbB receptor network, combinations of trastuzumab with EGFR-specific inhibitors are not warranted. Additional therapies such as those targeting the HER2 tyrosine kinase directly and/or the interaction between HER2 and HER3 are likely to be more synergistic than gefitinib when combined with trastuzumab.

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Fig. 1. TTP for patients who had been treated with prior systemic therapy for metastatic disease (*top*; $n = 9$) or without prior therapy (*bottom*; $n = 23$). The Kaplan-Meier method was used to estimate patient outcome.

Fig. 2. Overall survival for patients who had been treated with prior systemic therapy for metastatic disease (*top*; $n = 9$) or without prior therapy (*bottom*; $n = 23$). Median follow-up for all eligible patients was 43 mo. The Kaplan-Meier method was used to estimate patient outcome.

Table 1

Patients characteristics

	No. (%)
Evaluable patients	35*
Age, y	
Median	56
Range	30-83
ECOG PS	
0	19 (54)
1	15 (43)
2	1 (3)
Menopausal status	
Pre	7 (20)
Post	22 (63)
Peri	6 (17)
Hormone receptors	
ER-negative/PR-negative	16 (46)
ER+ and/or PR+	17 (49)
Unknown	2 (5)
Visceral metastases	28 (80)
Prior systemic therapy (metastatic)	
Chemotherapy	3 (9)
Hormonal therapy	4 (11)
Both	2 (6)
Prior adjuvant therapy	
Chemotherapy	20 (57)
Chemotherapy and hormonal therapy	9 (26)
HER2 status (Dako HercepTest)	
2+	1 (3) [†]
3+	34 (97)
HER2 FISH (Vysis)	
Positive	23/25 (92) [‡]
Negative	2/25 (8)
EGFR+ (IHC) [§]	6/28 (21)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group PS.

* Includes 3 patients from the phase I portion treated with combination trastuzumab and gefitinib 500 mg/d.

[†] Patient was HER2 FISH-positive.

[‡] FISH done in 25 of 35 evaluable patients.

[§] Includes 1 to 3+ by DAKO; done in 28 of 35 evaluable patients. One of 6 EGFR+ patients received gefitinib 500 mg/d.

Table 2

Select toxicities experienced by patients treated with the combination of trastuzumab and gefitinib 250 mg/d ($n = 38$)

Adverse event	Grade 1/2	Grade 3/4
	<i>n</i> (%)	<i>n</i> (%)
Diarrhea	22 (58)	12* (32)
Rash	24 (63)	5 (13)
Nausea, vomiting	10 (26)	1 (3)
Stomatitis	12 (32)	
Fatigue	20 (53)	
Anorexia	14 (37)	
Dyspepsia	7 (18)	
Pruritus	4 (11)	1 (3)
Dry skin	14 (37)	
Conjunctivitis	3 (8)	1 (3)
Photophobia		1 (3)
Myalgias	6 (16)	
Arthralgias	6 (16)	
Neutropenia	4 (11)	1 (3)
Hemoglobin	19 (50)	
Transaminitis	19 (50)	
Alk phosphatase	5 (13)	
Hyperbilirubinemia	5 (13)	1 (3)
Hypokalemia	3 (8)	1 (3)
Pneumonitis, dyspnea	1 (3)	1 (3)

NOTE: Toxicity summary includes all patients (eligible or ineligible) that received protocol treatment and experienced side effects. Toxicities graded according to CTC version 2.0.

* Grade 3 diarrhea experienced only by patients receiving gefitinib 250 mg/d in phase II portion of the trial.