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Cardiac toxicity and efficacy of trastuzumab combined with pertuzumab in patients with trastuzumab-insensitive HER2-positive metastatic breast cancer

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Abstract

Purpose—To evaluate safety and efficacy of trastuzumab with pertuzumab in patients with HER2+ metastatic breast cancer (MBC) who had progressive disease on trastuzumab-based therapy.

Experimental Design—Patients with measurable HER2+ MBC, 3 trastuzumab-based regimens, left ventricular ejection fraction (LVEF) ≥ 55% received 8 mg/kg or 6 mg/kg trastuzumab and 840 mg pertuzumab intravenously followed by 6 mg/kg trastuzumab and 420 mg pertuzumab every 3 weeks. Cardiac evaluation and tumor response were assessed every 3 and 6 weeks, respectively.

Results—Eleven patients received 64 cycles of trastuzumab plus pertuzumab. A total of 92 echocardiograms and 8 cardiac magnetic resonance imaging studies were performed. With the lower limit of normal LVEF 55%, left ventricular systolic dysfunction (LVSD) was observed in six patients, three grade 1, two grade 2 and one grade 3 according to the NCI-Common Terminology Criteria for Adverse Events (CTCAE). The objective response rate was 18%. Two patients had partial responses (PR), three stable disease (SD), and six progressive disease (PD). The median time to progression (TTP) was 6 weeks. In baseline tumors from formalin-fixed paraffin-embedded primary and/or metastatic tumor biopsies, pHER2-Y1248 trended towards an increase in patients with PR compared to those with SD/PD (p=0.095).

Conclusion—Trastuzumab plus pertuzumab may have clinical benefit in selected patients who have been previously treated with trastuzumab. Cardiac toxicity, although asymptomatic in most

cases, was associated with this treatment. Further evaluation of efficacy of this combination is required to define the overall risks and benefits.

Introduction

The humanized monoclonal antibody, trastuzumab (Herceptin®, Genentech Inc. South San Francisco, CA) targets the extracellular domain of the HER2 protein. Trastuzumab in combination with chemotherapy improves survival by 50% in patients with early and metastatic HER2+ breast cancer (1–3). Although some patients with HER2+ metastatic breast cancers benefit from the initial trastuzumab treatment alone, tumor progression eventually occurs (4–6). Resistance to trastuzumab is a challenging problem in management of HER2+ breast cancer.

Mechanisms of resistance to trastuzumab are likely multi-factorial as recently reviewed (7). Unlike other HER family receptors that require ligand binding for activation, HER2 always presents an active open conformation and is the preferred receptor for hetero-dimerization with other HER family receptors through regions of II and IV extracellular domains (ECD) (8, 9). Trastuzumab binds to domain IV ECD of HER2 but does not significantly block the heterodimer formation between HER2 and HER3 (10). It has been shown that HER receptor ligands can overcome trastuzumab-induced G1 cell cycle arrest and inhibition of tyrosine phosphorylation of the HER receptors (11). These results suggest that trastuzumab alone is not sufficient to abolish HER2 signaling due to the complex nature of the HER family receptor/ligand interactions. Several preclinical studies have shown that combination of antibodies to various epitopes of HER2 were more effective than individual antibodies in inducing antibody-dependent cytotoxicity, accelerating receptor down-regulation, interfering HER family receptor signaling, and inhibiting tumor cell growth (12–15).

Pertuzumab (Genentech Inc.), a humanized monoclonal antibody against a region of domain II of HER2 ECD, blocks HER2 hetero-dimerization and ligand-activated signaling from HER2/HER1 and HER2/HER3 heterodimers (10, 16, 17). Pertuzumab in combination with trastuzumab synergistically inhibited growth and induced apoptosis of tumor cells in vitro through disrupting HER2 heterodimerization and downstream signaling (18). Phase I study of pertuzumab demonstrated acceptable toxicity profiles in treatment of solid tumors (19).

Among the toxicities of HER2 targeted therapy, cardiac dysfunction has a potential serious consequence. HER family members play a crucial role in normal cardiac development. Mice with HER2 or HER4 knockout had malformation of cardiac ventricles which were embryonically lethal (20, 21). Cardiac-restricted deletion of HER2 led to dilated cardiomyopathy and reduced tolerance to fluid overload in mice (22, 23). It has been shown that heregulin stimulated proliferation and inhibited apoptosis of neonatal and adult cardiac myocytes that persistently expressed HER2 and HER4 (24). Thus, targeting HER2 may alter the signals necessary for survival of cardiac myocytes leading to cardiac toxicity.

We conducted a clinical study evaluating the toxicity and efficacy of trastuzumab plus pertuzumab in the treatment of patients with HER2+ MBC who progressed through trastuzumab-based therapies. Based on the results of pre-clinical studies, we hypothesized

that combination of the two anti-HER2 antibodies would be more efficient in controlling tumor growth and in overcoming trastuzumab resistance. We focused on the cardiac evaluation to ensure safety of the patients taking this combination treatment.

Materials and Methods

Patient population

Patient eligibility criteria included measurable MBC, ≥ 3 prior trastuzumab-based treatments, age ≥ 18 years, HER2 over-expression (3+ by immunohistochemistry (IHC) or gene amplification by fluorescent in-situ hybridization (FISH)), an Eastern Cooperative Oncology Group performance status ≤ 1 , recovered from prior chemo- and/or radiation-related toxicities to grade ≤ 1 (excluding alopecia), LVEF $\geq 55\%$ with no clinical signs or symptoms of congestive heart failure (CHF), and adequate hematologic, renal and hepatic organ functions. Patients with brain and/or leptomeningeal metastases were required to have asymptomatic stable disease after standard surgery and/or radiation and could not be receiving corticosteroids for management of neurological symptoms. Patients were excluded if they had uncontrolled hypertension, significant valvular disease, uncontrolled cardiac arrhythmias, prior myocardial infarction or angina pectoris requiring anti-anginal medication, poorly controlled diabetes mellitus, or prior exposure to >360 mg/m² doxorubicin and/or liposomal doxorubicin.

All patients gave written informed consent. The National Cancer Institute (NCI) Institutional Review Board approved the protocol and consent form.

Treatment schedule

Trastuzumab and pertuzumab were provided by Genentech Inc. directly to the Pharmaceutical Development Service of the NCI for trial purposes only. Patients received 8 mg/kg trastuzumab intravenously (IV) on cycle 1 day 1 if they had not had trastuzumab within one month; or 6 mg/kg trastuzumab if they received trastuzumab within 1 month. On cycle 1 day 2, patients received 840 mg pertuzumab IV. On day 1 of each of the subsequent 3-week cycles, patients received 6 mg/kg trastuzumab IV plus 420 mg pertuzumab IV. If patients developed hypersensitivity during any treatment cycle, they received anti-histamine prior to subsequent cycles of monoclonal antibody infusion.

Tumor response

Tumor burden was evaluated with physical examination at baseline and before each cycle of treatment and imaging studies including computed tomography (CT) and bone scans at baseline and CT after every 2 cycles plus bone scan if bone metastases were present. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) (25).

Safety assessments and cardiac evaluation

Clinical examination and laboratory assessments were performed on day 1 of each cycle. Adverse events were graded according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) v3.0.⁷ (⁷ <http://ctep.cancer.gov>)

Cardiac function was monitored with electrocardiogram (ECG) and echocardiogram (ECHO) at the baseline and every 3 weeks before treatment. Cardiac magnetic resonance imaging (MRI) was performed at the baseline for patients who enrolled later in the trial and for those who had a reduction in LVEF by ECHO. All cardiac studies during the trial were performed at the NCI facilities. Efforts were made to have the same technician perform the ECHO studies for each patient using the same machine throughout the trial. Each ECHO and ECG was read by one of three cardiologists. The lower limit of normal LVEF is 55% for our institution. LVSD was graded based on changes in LVEF according to the NCI-CTCAE v3.0, grade 1 (LVEF 55–50%), grade 2 (LVEF 50–40%) and grade 3 (LVEF 40–20%). Trastuzumab and pertuzumab were discontinued for symptomatic cardiac toxicities. For asymptomatic cardiac toxicities, trastuzumab and pertuzumab were held and cardiac function was re-evaluated. Treatment was resumed if the LVEF was $\geq$ 55% within 1 to 3 weeks. If the LVEF was $<$ 55% after 3 weeks of holding therapy, trastuzumab and pertuzumab were permanently discontinued.

Correlative studies

IHC for HER2, pHER2-Y877, pHER2-Y1248, pMAPK, pAkt and Ki67 were performed on formalin-fixed paraffin-embedded tumors. Core or punch biopsies of tumors were obtained before and after one cycle of trastuzumab plus pertuzumab as baseline and post treatment, respectively. If a tumor biopsy was not feasible before treatment, tissue blocks from prior tumor biopsies were obtained and used as baseline. Antibodies used in this study were HER2 (CB11, mouse monoclonal, Biogenex, San Ramon, CA), pHER2-Y877 (rabbit polyclonal, Cell Signaling Technology, Beverly, MA), pHER2-Y1248 (rabbit polyclonal, Cell Signaling Technology, Beverly, MA), Ki67 (MIB-1, mouse monoclonal, DAKO Corp, Carpinteria, CA), pMAPK (pTEpY, rabbit polyclonal, Promega, Madison, WI), and pAkt (S473, rabbit polyclonal, Cell Signaling Technology, Beverly, MA) (26–30). These antibodies were validated with proper positive and negative controls prior to applying to the tumor samples. IHC was performed according to the methods and conditions established in our laboratory (29, 30). The HER2 pathway markers were scored quantitatively with the Automated Cellular Imaging System (DAKO, Carpinteria, CA) (29). The IHC staining indices of HER2, pHER2-Y877, pHER2-Y1248, pMAPK and pAkt, and Ki67 labeling percentage were determined as described in our previous publications (29, 30).

Statistical considerations

The primary end points of this study were safety and response rate (RR) of the combination treatment. The secondary end points were time to progression (TTP) and correlative studies of HER2 signaling. TTP is defined as time from registration date to the date of documented disease progression or death on study, whichever occurs first. The planned sample size was 37 based on Simon two-stage optimal design target a 20% response rate and to rule out an unacceptably low 5% response rate ($p=0.05$) (31). The trial would stop accrual early in the event that 2 or more of the initial 12 patients developed symptomatic CHF or any other serious toxicity.

An exact Wilcoxon rank sum test was used to determine the statistical significance of the difference in the levels of various proteins determined with IHC staining of the tumors from patients who had PR compared to SD or PD.

Results

Patients

A total of 11 patients were enrolled in this study. The study did not reach the planned accrual because of safety concerns as discussed in the toxicities section and logistical factors. Patient characteristics are summarized in Table 1. HER2 amplification was confirmed by FISH in 10 patients. One patient had a tumor with HER2 3+ by IHC but was negative by FISH. Nine patients had received anthracyclines (range: 240 to 360, median 240 mg/m²), and 4 received trastuzumab as part of their adjuvant therapy. All patients had received trastuzumab for treatment of metastatic disease. The median cumulative duration of prior trastuzumab was 82 weeks (range: 22 to 136 weeks). The median time from last cycle of trastuzumab to initiation of trastuzumab and pertuzumab was 13 weeks (range: 4 to 96 weeks).

Toxicities

A total of 64 cycles of trastuzumab and pertuzumab were given. The major adverse events are summarized in Table 2. Grade 2 diarrhea was seen in 3 patients (27%). Two patients had grade 1 and one patient grade 3 allergic reaction related to infusion and symptoms resolved after anti-histamine treatment. These patients received anti-histamine prior to subsequent monoclonal antibody infusion.

Cardiac toxicities

A total of 92 ECHO and 8 cardiac MRI studies were performed. Six patients (54%) experienced a reduction in LVEF. Three patients had grade 1, two grade 2 and one grade 3 LVSD. The patient with the grade 3 LVSD experienced symptomatic CHF and required medical management. Grade 2/3 LVSD were associated with global hypokinesis. Two patients had a LVEF reduction 15%. No specific ECG changes related to acute cardiac dysfunction were observed when cardiac toxicities occurred or throughout the trial.

Table 3 lists the characteristics of patients who experienced cardiac toxicity. Two patients had a history of reduced LVEF during prior adjuvant trastuzumab-based treatment but tolerated subsequent re-challenge with trastuzumab for treatment of metastatic disease before receiving trastuzumab and pertuzumab. A reduced LVEF appeared within 1–2 cycles in most patients. Figure 1 shows LVEF in all patients and in patients who experienced cardiac toxicity. LVEF returned to normal in four patients within 1 week to 3 months after withholding treatment. One patient had persistent grade 2 LVSD 4 months after treatment discontinuation. The patient with CHF had a history of left chest wall radiation, baseline tachycardia and extensive chest wall disease including an 8 cm mass extending through the chest wall. She had the greatest decrease in and the lowest absolute LVEF. She died of PD with CHF 2 months after treatment termination.

Efficacy

Two patients (18%) achieved a PR, three (27%) SD for 18 weeks (range: 18 to 36 weeks), and six (55%) PD while on treatment for 6 weeks. The median TTP was 6 weeks (range: 3 to 54 weeks). The two patients who achieved PR had non-visceral disease only. One patient had received prior trastuzumab for 136 weeks, started trastuzumab and pertuzumab 96 weeks from the prior trastuzumab treatment and achieved PR after 32 weeks. She remained in PR at 54 weeks and elected to discontinue treatment. The second responder had prior trastuzumab for 120 weeks, came on study 4 weeks from the prior trastuzumab treatment and achieved PR after 12 weeks treatment. Her disease progressed after 36 weeks.

Tumor proliferative state and HER2 pathway evaluation

Correlative studies were conducted in baseline tumors and tumors after trastuzumab and pertuzumab treatment. Eight baseline patient tumor samples (3 from the metastatic and 5 from the primary tumors) and one paired pre- and post-trastuzumab/pertuzumab samples were evaluated for Ki67, HER2, pHER2-Y1248, pHER2-Y877, pMAPK, and pAkt by IHC. Ki67-positive tumor cells ranged from 18.6 to 95.3% in the baseline tumors. Table 4 summarizes the IHC staining indices of the markers and the percentages of Ki67-positive cells. There were no statistical differences in the levels of Ki67 or HER2 pathway markers in the baseline tumors for patients with PR compared to those with SD or PD as determined by an exact Wilcoxon rank sum test. However, there was a trend towards higher levels of pHER2-Y1248 in the baseline tumors from patients with PR (54.3 and 57.7) compared with tumors from patients with SD or PD (median 3.7; range 0–44; $p=0.095$). Interestingly, the levels of the pHER2-Y1248, pHER2-Y877, pMAPK, pAkt and Ki67 increased, while HER2 levels decreased post one cycle of trastuzumab and pertuzumab in a patient who had PD (Figure 2).

Discussion

In this study, the RR to trastuzumab plus pertuzumab was 18% which was consistent with an ongoing study of similar patient population with the same combination treatment (32). Due to the small size of this study, the definitive clinical efficacy of trastuzumab plus pertuzumab in the studied patient population can not be determined. Overall, the combination trastuzumab and pertuzumab was well tolerated. However, a higher incidence of cardiac toxicity was observed.

Because of the known cardiac toxicity of trastuzumab and unknown cardiac effects of trastuzumab plus pertuzumab, we applied stringent cardiac evaluation criteria during this trial. In our study, 6 of 11 patients had a reduction in LVEF $<55\%$, 3 had LVEF $<50\%$ and 1 had symptomatic CHF with an LVEF of 26%. All patients had prior exposure to anthracyclines, the majority had exposure to adjuvant trastuzumab, and all of them had trastuzumab for treatment of metastatic disease. As cardiac safety was a major concern, accrual of this study was stopped before the planned enrollment of 37 patients.

Cardiac toxicity of trastuzumab was reported in clinical trials when trastuzumab was used alone or in combination with chemotherapy for treatment of HER2+ MBC. Cardiac

dysfunction occurred in 4.7% patients in one clinical trial and 2% in another when patients were treated with weekly trastuzumab alone (4, 5). When trastuzumab was given every 3 weeks, 16.4% patients had a reduction in LVEF $\geq 15\%$ and 13.4% had LVEF $<50\%$. Prior anthracycline exposure was associated with more frequent and worse cardiac dysfunction (6). It is known that anthracyclines cause direct damage to cardiac myocytes and long-term clinical adverse cardiac consequences (33, 34). It is conceivable that prior anthracycline exposure leads to a compromised myocardium and a reduced adaptive capacity to damages caused by trastuzumab and pertuzumab.

Interestingly, the cardiac events of trastuzumab-based treatment in the adjuvant setting ranged from 0 to 4.1%, as recently reviewed (35). These rates appeared to be lower than what was reported in MBC patients treated with trastuzumab. In the pivotal phase III clinical trial of the MBC patients, 27% and 13% had cardiac dysfunction after treatment with concurrent trastuzumab and anthracycline or trastuzumab plus paclitaxel, respectively (1). These observations support the hypothesis that trastuzumab-related cardiac toxicity may increase in the heavily pre-treated population with more advanced disease, which could also account for the observed high incidences of cardiac toxicity in our study.

Cardiac toxicity due to pertuzumab has been documented in several studies. The phase I clinical study of pertuzumab reported 2 of the 21 patients had reduced LVEF $\geq 50\%$ from normal baseline values and one of them developed CHF after two cycles of pertuzumab. It was not clear if these patients had prior exposure to trastuzumab or anthracyclines (19). In the study of pertuzumab for treatment of advanced ovarian cancer patients, 5 of 123 patients had a reduction in LVEF to $<50\%$ but only one was centrally confirmed (36). After two cycles of pertuzumab, 6 of the 67 hormone-refractory prostate patients experienced a decrease $>10\%$ to an LVEF $<50\%$ (37). A recently published phase II study of pertuzumab in treatment of 41 advanced prostate cancer patients observed a 26.8% asymptomatic grade 1/2 reduction in LVEF, 4.9% asymptomatic LVEF $<50\%$ and no CHF (38). Based on the tumor types, it's likely that few patients on the above discussed studies had prior exposure to anthracyclines or trastuzumab.

The majority of the cardiac toxicity observed in our study was asymptomatic and reversible. Although trastuzumab-induced cardiac dysfunction is reversible in most patients, the long-term cardiac effects of trastuzumab are still unknown (39). Similarly, the long-term cardiac effects of trastuzumab plus pertuzumab are yet to be determined. Furthermore, the NCI-CTCAE criteria have not been uniformly used for all the clinical trials. Therefore, the differences in the criteria used to evaluate cardiac toxicity must be taken into consideration.

The evaluation of HER2 signaling was exploratory and was limited due to the small numbers of tumor samples and yet yielded some interesting results. There was a trend towards increased levels of pHER2-Y1248 in baseline tumors from patients with PR after trastuzumab and pertuzumab treatment. Increased levels of pHER2 were associated with increased response to pertuzumab and improved progression-free survival, though not statistically significant, in ovarian cancer patients (36). More studies are needed to confirm these observations as pHER2 may have the potential to be used to select patients who would respond to pertuzumab-based treatment.

The tyrosine residues Y877 and Y1248 of HER2, residing in the activation loop and cytoplasmic tail, respectively, are autophosphorylated upon HER2 receptor activation. While pHER2Y877 regulates HER2 kinase activity, pHER2Y1248 serves as a docking site for cytoplasmic proteins of HER2 signal transduction cascade (40, 41). Both trastuzumab and pertuzumab reduced phosphorylated receptor tyrosine levels and inhibited HER2 signaling in the pre-clinical models (10, 18). We observed increased levels of the pHER2Y877, pHER2Y1248, pMAPK and pAkt, suggesting activation of HER2 pathway in one patient with PD post trastuzumab/pertuzumab treatment. It has been reported that monoclonal antibodies against HER2 can either be growth inhibitory or stimulatory depending on intra- or extracellular localization of the monoclonal antibodies (42). Further clinical and laboratory studies are required to confirm this observation and to elucidate the potential mechanisms.

This observation is also consistent with the in vitro studies that demonstrated incomplete blockade of the HER2 signaling and/or compensatory activation of other pathways accounting for the failure of HER2-targeted treatment. It has been shown that insulin-like growth factor-I receptor (IGF-IR) levels increased in trastuzumab-resistant tumor cells, and antibody to IGF-IR restored the sensitivity to trastuzumab (43, 44). A recent study showed that the combination of lapatinib, a HER1/HER2 tyrosine kinase inhibitor, with trastuzumab or with a polyclonal antibody against HER2 significantly down-regulated survivin and enhanced apoptosis of the tumor cells in vitro (45). Triple combination of trastuzumab, pertuzumab and gefitinib effectively blocked signals from HER homo- and hetero-dimerization and inhibited the growth of HER2+ breast cancer xenografts more effectively than single agents or dual combinations (46). Targeting other HER2 downstream kinases such as mTOR is also being tested in clinical trials (47). Based on the available information, it is apparent that complete blockade of HER receptor signaling at multiple levels may be required for effective growth inhibition of HER2+ tumors.

In conclusion, more studies are needed to define the efficacy of trastuzumab plus pertuzumab and to identify patients who could benefit from this treatment with minimal cardiac toxicity. Although stringent criteria for evaluation of cardiac toxicity and advanced disease may have contributed to the observed high rate of cardiac toxicity, cardiac safety is a concerning issue. Prior exposure to anthracyclines and prior left ventricular dysfunction with trastuzumab treatment may be potential risk factors of cardiac toxicity for patients who are treated with trastuzumab plus pertuzumab. Cardiac status should be closely monitored in future clinical trials using this combination treatment.

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References

1. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*. 2001(344):783–92. [PubMed: 11248153]
2. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med*. 2005(353):1673–84. [PubMed: 16236738]
3. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med*. 2005; 353:1659–72. [PubMed: 16236737]
4. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol*. 1999; 17:2639–48. [PubMed: 10561337]
5. Vogel CL, Cobleigh MA, Tripathy D, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol*. 2002; 20:719–26. [PubMed: 11821453]
6. Baselga J, Carbonell X, Castaneda-Soto NJ, et al. Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule. *J Clin Oncol*. 2005; 23:2162–71. [PubMed: 15800309]
7. Lan KH, Lu CH, Yu D. Mechanisms of trastuzumab resistance and their clinical implications. *Ann N Y Acad Sci*. 2005; 1059:70–5. [PubMed: 16382045]
8. Garrett TP, McKern NM, Lou M, et al. The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Mol Cell*. 11:495–505. [PubMed: 12620236]
9. Cho HS, Mason K, Ramyar KX, et al. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature*. 2003; 421:756–60. [PubMed: 12610629]
10. Agus DB, Akita RW, Fox WD, et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell*. 2002; 2:127–37. [PubMed: 12204533]
11. Motoyama AB, Hynes NE, Lane HA. The efficacy of ErbB receptor-targeted anticancer therapeutics is influenced by the availability of epidermal growth factor-related peptides. *Cancer Res*. 2002; 62:3151–8. [PubMed: 12036928]
12. Kasprzyk PG, Song SU, Di Fiore PP, King CR. Therapy of an animal model of human gastric cancer using a combination of anti-erbB-2 monoclonal antibodies. *Cancer Res*. 1992; 52:2771–6. [PubMed: 1349849]
13. Spiridon CI, Ghetie MA, Uhr J, et al. Targeting multiple Her-2 epitopes with monoclonal antibodies results in improved antigrowth activity of a human breast cancer cell line in vitro and in vivo. *Clin Cancer Res*. 2002; 8:1720–30. [PubMed: 12060609]
14. Friedman LM, Rinon A, Schechter B, et al. Synergistic down-regulation of receptor tyrosine kinases by combinations of mAbs: implications for cancer immunotherapy. *Proc Natl Acad Sci U S A*. 2005; 102:1915–20. [PubMed: 15684082]
15. Drebin JA, Link VC, Greene MI. Monoclonal antibodies reactive with distinct domains of the neu oncogene-encoded p185 molecule exert synergistic anti-tumor effects in vivo. *Oncogene*. 1988; 2:273–7. [PubMed: 2451200]
16. Jackson JG, St Clair P, Sliwkowski MX, Brattain MG. Blockade of epidermal growth factor- or heregulin-dependent ErbB2 activation with the anti-ErbB2 monoclonal antibody 2C4 has divergent downstream signaling and growth effects. *Cancer Res*. 2004; 64:2601–9. [PubMed: 15059917]
17. Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell*. 2004; 5:317–28. [PubMed: 15093539]
18. Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. *Cancer Res*. 2004; 64:2343–6. [PubMed: 15059883]

19. Agus DB, Gordon MS, Taylor C, et al. Phase I clinical study of pertuzumab, a novel HER dimerization inhibitor, in patients with advanced cancer. *J Clin Oncol.* 2005; 23:2534–43. [PubMed: 15699478]
20. Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature.* 1995; 378:394–8. [PubMed: 7477377]
21. Gassmann M, Casagrande F, Orioli D, et al. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature.* 1995; 378:390–4. [PubMed: 7477376]
22. Crone SA, Zhao YY, Fan L, et al. ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nat Med.* 2002; 8:459–65. [PubMed: 11984589]
23. Ozcelik C, Erdmann B, Pilz B, et al. Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy. *Proc Natl Acad Sci U S A.* 2002; 99:8880–5. [PubMed: 12072561]
24. Zhao YY, Sawyer DR, Baliga RR, et al. Neuregulins promote survival and growth of cardiac myocytes. Persistence of ErbB2 and ErbB4 expression in neonatal and adult ventricular myocytes. *J Biol Chem.* 1998; 273:10261–9. [PubMed: 9553078]
25. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst.* 2000; 92:205–16. [PubMed: 10655437]
26. Qian X, LeVeae CM, Freeman JK, Dougall WC, Greene MI. Heterodimerization of epidermal growth factor receptor and wild-type or kinase-deficient Neu: a mechanism of interreceptor kinase activation and transphosphorylation. *Proc Natl Acad Sci U S A.* 1994; 91:1500–4. [PubMed: 7509075]
27. Muthuswamy SK, Gilman M, Brugge JS. Controlled dimerization of ErbB receptors provides evidence for differential signaling by homo- and heterodimers. *Mol Cell Biol.* 1999; 19:6845–57. [PubMed: 10490623]
28. Kwon YK, Bhattacharyya A, Alberta JA, et al. Activation of ErbB2 during wallerian degeneration of sciatic nerve. *J Neurosci.* 1997; 17:8293–9. [PubMed: 9334404]
29. Tan AR, Yang X, Hewitt SM, et al. Evaluation of biologic end points and pharmacokinetics in patients with metastatic breast cancer after treatment with erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor. *J Clin Oncol.* 2004; 22:3080–90. [PubMed: 15284258]
30. Wedam SB, Low JA, Yang SX, et al. Antiangiogenic and antitumor effects of bevacizumab in patients with inflammatory and locally advanced breast cancer. *J Clin Oncol.* 2006; 24:769–77. [PubMed: 16391297]
31. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials.* 1989; 10:1–10. [PubMed: 2702835]
32. Baselga J, Cameron D, Miles D, Verma S, Climent M, Ross G, Gimenez V, Gelmon K. Objective response rate in a phase II multicenter trial of pertuzumab (P), a HER2 dimerization inhibiting monoclonal antibody, in combination with trastuzumab (T) in patients with HER2-positive metastatic breast cancer (MBC) which has progressed during treatment with T. 2007 ASCO Annual Meeting Proceedings Part I. 2007; 25:18S. (June 20 Supplement). Abstract # 1004.
33. Billingham ME, Mason JW, Bristow MR, Daniels JR. Anthracycline cardiomyopathy monitored by morphologic changes. *Cancer Treat Rep.* 1978; 62:865–72. [PubMed: 667860]
34. Pinder MC, Duan Z, Goodwin JS, Hortobagyi GN, Giordano SH. Congestive heart failure in older women treated with adjuvant anthracycline chemotherapy for breast cancer. *J Clin Oncol.* 2007; 25:3808–15. [PubMed: 17664460]
35. Smith KL, Dang C, Seidman AD. Cardiac dysfunction associated with trastuzumab. *Expert Opin Drug Saf.* 2006; 5:619–29. [PubMed: 16907652]
36. Gordon MS, Matei D, Aghajanian C, et al. Clinical activity of pertuzumab (rhuMAb 2C4), a HER dimerization inhibitor, in advanced ovarian cancer: potential predictive relationship with tumor HER2 activation status. *J Clin Oncol.* 2006; 24:4324–32. [PubMed: 16896006]
37. de Bono JS, Bellmunt J, Attard G, et al. Open-label phase II study evaluating the efficacy and safety of two doses of pertuzumab in castrate chemotherapy-naïve patients with hormone-refractory prostate cancer. *J Clin Oncol.* 2007; 25:257–62. [PubMed: 17235043]

38. Agus DB, Sweeney CJ, Morris MJ, et al. Efficacy and safety of single-agent pertuzumab (rhuMAB 2C4), a human epidermal growth factor receptor dimerization inhibitor, in castration-resistant prostate cancer after progression from taxane-based therapy. *J Clin Oncol.* 2007; 25:675–81. [PubMed: 17308272]
39. Ewer MS, Vooletich MT, Durand JB, et al. Reversibility of trastuzumab-related cardiotoxicity: new insights based on clinical course and response to medical treatment. *J Clin Oncol.* 2005; 23:7820–6. [PubMed: 16258084]
40. Dankort D, Jeyabalan N, Jones N, Dumont DJ, Muller WJ. Multiple ErbB-2/Neu Phosphorylation Sites Mediate Transformation through Distinct Effector Proteins. *J Biol Chem.* 2001; 276:38921–8. [PubMed: 11500516]
41. Bose R, Molina H, Patterson AS, et al. Phosphoproteomic analysis of Her2/neu signaling and inhibition. *Proc Natl Acad Sci U S A.* 2006; 103:9773–8. [PubMed: 16785428]
42. Hurwitz E, Stancovski I, Sela M, Yarden Y. Suppression and promotion of tumor growth by monoclonal antibodies to ErbB-2 differentially correlate with cellular uptake. *Proc Natl Acad Sci U S A.* 1995; 92:3353–7. [PubMed: 7724565]
43. Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J Natl Cancer Inst.* 2001; 93:1852–7. [PubMed: 11752009]
44. Nahta R, Yuan LX, Zhang B, Kobayashi R, Esteva FJ. Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. *Cancer Res.* 2005; 65:11118–28. [PubMed: 16322262]
45. Xia W, Gerard CM, Liu L, Baudson NM, Ory TL, Spector NL. Combining lapatinib (GW572016), a small molecule inhibitor of ErbB1 and ErbB2 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances apoptosis of ErbB2-overexpressing breast cancer cells. *Oncogene.* 2005; 24:6213–21. [PubMed: 16091755]
46. Arpino G, Gutierrez C, Weiss H, et al. Treatment of human epidermal growth factor receptor 2-overexpressing breast cancer xenografts with multiagent HER-targeted therapy. *J Natl Cancer Inst.* 2007; 99:694–705. [PubMed: 17470737]
47. Chan S, Scheulen ME, Johnston S, et al. Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. *J Clin Oncol.* 2005; 23:5314–22. [PubMed: 15955899]

Figure 1.

LVEF in all patients treated with trastuzumab combined with pertuzumab (A); LVEF in patients with LVSD (B)

Abbreviations: LVEF, left ventricular ejection fraction; LVSD, left ventricular systolic dysfunction; Pt, patient; C, cycle of treatment; F/U, follow up.

Figure 2.

IHC staining indices of HER2 pathway markers and the percentage of Ki67-positive cells of a patient with progressive disease

Abbreviations: IHC, immunohistochemistry; T/P, trastuzumab/pertuzumab.

Table 1**Patient Demographics**

Characteristic	No (%)
No. of patients	11 (100)
Age, years	
Median (Range)	53 (36–68)
Race / ethnicity	
White	6 (54.5)
Black	3 (27.3)
Asian	1 (9)
Hispanic	1 (9)
Stage of disease	
Metastatic	11 (100)
Visceral only	3 (27.3)
Visceral and nonvisceral	3 (27.3)
Nonvisceral only	5 (45.4)
Hormone receptor status	
ER, PR or both positive	3 (27.3)
ER and PR negative	8 (72.7)
HER2 positive (IHC combined with FISH)	11 (100)
Previous therapy	
Anthracyclines	9 (81.8)
Taxanes	10 (91)
Trastuzumab as adjuvant therapy	4 (36.4)
Trastuzumab for metastatic disease	11 (100)
Cumulative duration of prior trastuzumab (wk)	
Median (Range)	82 (22–136)
Time from last cycle trastuzumab to initiation of trastuzumab and pertuzumab (wk)	
Median (Range)	13 (4–96)

Abbreviations: ER, estrogen receptor; PR, progesterin receptor; IHC, immunohistochemistry; FISH, fluorescence in-situ hybridization; wk, week.

Table 2

Observed toxicity events for patients receiving trastuzumab plus pertuzumab

Toxicity	<u>CTC Grade</u>							
	<u>1</u>		<u>2</u>		<u>3</u>		<u>4</u>	
	<u>N</u>	<u>%</u>	<u>N</u>	<u>%</u>	<u>N</u>	<u>%</u>	<u>N</u>	<u>%</u>
Hematologic								
Neutropenia	0	0	0	0	0	0	0	0
Hemoglobin	2	18	0	0	0	0	0	0
Thrombocytopenia	2	18	0	0	0	0	0	0
Nonhematologic								
Diarrhea	2	18	3	27	0	0	0	0
Allergic reaction	2	18	0	0	1	9	0	0
Rash	1	9	0	0	0	0	0	0
Nail changes	2	18	1	9	0	0	0	0
Left ventricular systolic Dysfunction	3	27	2	18	1	9	0	0

N: number of patients.

Table 3

Characteristics of patients experiencing cardiac toxicity on trastuzumab plus pertuzumab

Patient	Pt3	Pt6	Pt7	Pt8	Pt9	Pt11
Age	52	46	53	58	54	36
Baseline EF (%) (method)	61 (ECHO)	60 (ECHO)	60 (ECHO)	56 (ECHO)	60 (ECHO)	69 (ECHO)
Lowest EF (%) (method)	51 (ECHO)	51 (ECHO)	48 (MRI)	48 (ECHO)	26 (MRI)	54 (ECHO)
Absolute change in EF (%)	10	9	12	8	34	15
LVSD (Grade)	1	1	2	2	3	1
Cycles of T/P when LVSD occurred	18	2	2	1	2	1
Cumulative duration of prior T (wk)	136	52	82	22	112	92
LSVD while on prior T	No	Yes	Yes	No	No	No
Cumulative dose of doxorubicin (mg/m ²)	240	240	240	240	240	360
History of HTN	Yes	Yes	No	No	No	No
XRT to chest wall (side)	Yes (left)	No	No	Yes (right)	Yes (left)	Yes (right)
Extensive chest wall disease	No	No	No	No	Yes	No
Resolution of LVSD (time of follow-up)	Yes (3wk)	Yes (3 mo)	Yes (3 mo)	No (4 mo)	No *	Yes (1 wk)

Abbreviations: Pt, patient; EF, ejection fraction; LVSD, left ventricular systolic dysfunction; T, trastuzumab; P, pertuzumab; HTN, hypertension; XRT, radiation treatment; wk, week; mo, month.

* The patient died 2 months after the onset of LVSD.

Table 4

IHC staining indices of the HER2 pathway markers and percentages of the Ki67-positive cells in the baseline tumors*

Markers	PR (N=2)		SD/PD (N=6)	
	Median	Range	Median	Range
pHER2-Y1248	56.0	54.3–57.7	3.7	0–44.0**
pHER2-Y877	38.8	33.4–44.1	3.3	0–44.5
pMAPK	56.6	42.4–70.8	33.7	12.9–90.1
pAkt	13.5	4.2–22.7	1.6	0–36.8
HER2	64.3	61.6–67.1	74.6	18.7–91.5
Ki67 [†]	49.4	37.2–61.6	57.4	18.6–95.3

Abbreviations: IHC, immunohistochemistry; PR, partial response; SD, stable disease; PD, progressive disease.

* IHC staining index = (staining intensity of a sample – staining intensity of negative control) × percentage of stained cells /100. (30)

** p-value = 0.095

[†] Percentages of IHC stained cells.