Cardiac Toxicity and Efficacy of Trastuzumab Combined with Pertuzumab in Patients with Trastuzumab-Insensitive Human Epidermal Growth Factor Receptor 2–Positive Metastatic Breast Cancer

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Abstract

Purpose: To evaluate safety and efficacy of trastuzumab with pertuzumab in patients with human epidermal growth factor receptor 2 (HER2) – positive metastatic breast cancer who had progressive disease on trastuzumab-based therapy.

Experimental Design: Patients with measurable HER2$^+$ metastatic breast cancer, ≤3 trastuzumab-based regimens, and left ventricular ejection fraction (LVEF) ≥55% received 8 or 6 mg/kg trastuzumab and 840 mg pertuzumab i.v. 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 done. With the lower limit of normal LVEF 55%, left ventricular systolic dysfunction was observed in six patients, three grade 1, two grade 2, and one grade 3 according to the National Cancer Institute Common Terminology Criteria for Adverse Events. The objective response rate was 18%. Two patients had partial responses, three had stable disease, and six had progressive disease. The median time to progression was 6 weeks. In baseline tumors from formalin-fixed paraffin-embedded primary and/or metastatic tumor biopsies, pHER2-Y1248 trended toward an increase in patients with partial response compared with those with stable disease/progressive disease ($P = 0.095$).

Conclusion: Trastuzumab plus pertuzumab may have clinical benefit in selected patients who have previously been 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.

The humanized monoclonal antibody trastuzumab (Herceptin, Genentech, Inc.) targets the extracellular domain of the human epidermal growth factor receptor 2 (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 multifactorial 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 heterodimerization with other HER family receptors through regions of extracellular domains II and IV (8, 9). Trastuzumab binds to the extracellular domain IV 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 G_1 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

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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), a humanized monoclonal antibody against a region of HER2 extracellular domain domain II, blocks HER2 heterodimerization 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 disruption of HER2 heterodimerization and downstream signaling (18). Phase I study of pertuzumab showed 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 $HER2^+$ metastatic breast cancer who progressed through trastuzumab-based therapies. Based on the results of preclinical 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.

Table 1. Patient demographics

Characteristic	*n* (%)
No. patients	11 (100)
Age, y	
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, progestin receptor; IHC, immunohistochemistry; FISH, fluorescent *in situ* hybridization.

Materials and Methods

Patient population. Patient eligibility criteria included measurable metastatic breast cancer; ≤3 prior trastuzumab-based treatments; age ≥18 y; HER2 overexpression (3+ by immunohistochemistry or gene amplification by fluorescent *in situ* hybridization); an Eastern Cooperative Oncology Group performance status ≤1; recovery from prior chemotherapy- and/or radiation-related toxicities to grade ≤1 (excluding alopecia); left ventricular ejection fraction (LVEF) ≥55% with no clinical signs or symptoms of congestive heart failure; 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 neurologic symptoms. Patients were excluded if they had uncontrolled hypertension, significant valvular disease, uncontrolled cardiac arrhythmias, prior myocardial infarction or angina pectoris requiring antianginal medication, poorly controlled diabetes mellitus, or prior exposure to >360 mg/m^2 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 directly to the Pharmaceutical Development Service of NCI for trial purposes only. Patients received 8 mg/kg trastuzumab i.v. on cycle 1 day 1 if they had not had trastuzumab within 1 mo, or 6 mg/kg trastuzumab if they received trastuzumab within 1 mo. On cycle 1 day 2, patients received 840 mg pertuzumab i.v. On day 1 of each of the subsequent 3-wk cycles, patients received 6 mg/kg trastuzumab i.v. plus 420 mg pertuzumab i.v. If patients developed hypersensitivity during any treatment cycle, they received antihistamine before 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 and bone scans at baseline and computed tomography 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 (25).

Safety assessments and cardiac evaluation. Clinical examination and laboratory assessments were done on day 1 of each cycle. Adverse events were graded according to the NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 3.0.[7]

Cardiac function was monitored with electrocardiogram and echocardiogram at the baseline and every 3 wk before treatment. Cardiac magnetic resonance imaging was done at the baseline for patients who enrolled later in the trial and for those who had a reduction in LVEF by echocardiogram. All cardiac studies during the trial were done at the NCI facilities. Efforts were made to have the same technician carry out the echocardiogram studies for each patient using the same machine throughout the trial. Each echocardiogram and electrocardiogram were read by one of three cardiologists. The lower limit of normal LVEF is 55% for our institution. Left ventricular systolic dysfunction (LVSD) was graded based on changes in LVEF according to the NCI-CTCAE version 3.0: grade 1 (LVEF 55-50%), grade 2 (LVEF 50-40%), and grade 3 (LVEF 40-20%). Trastuzumab and pertuzumab

[7] http://ctep.cancer.gov

Table 2. Observed toxicity events for patients receiving trastuzumab plus pertuzumab

Toxicity	CTC grade			
	1	2	3	4
	n (%)			
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)
LVSD	3 (27)	2 (18)	1 (9)	0 (0)

NOTE: *n*, number of patients.

were discontinued for symptomatic cardiac toxicities. For asymptomatic cardiac toxicities, trastuzumab and pertuzumab were held and cardiac function was reevaluated. Treatment was resumed if the LVEF was ≥55% within 1 to 3 wk. If the LVEF was <55% after 3 wk of holding therapy, trastuzumab and pertuzumab were permanently discontinued.

Correlative studies. Immunohistochemistry for HER2, pHER2-Y877, pHER2-Y1248, phoshorylated mitogen-activated protein kinase (pMAPK), pAkt, and Ki67 was done 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 posttreatment, 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), pHER2-Y877 (rabbit polyclonal; Cell Signaling Technology), pHER2-Y1248 (rabbit polyclonal; Cell Signaling Technology), Ki67 (MIB-1, mouse monoclonal; DAKO Corp.), pMAPK (pTEpY, rabbit polyclonal; Promega), and pAkt (S473, rabbit polyclonal; Cell Signaling Technology; refs. 26–30). These antibodies were validated with proper positive and negative controls before applying to the tumor samples. Immunohistochemistry was done 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; ref. 29). The immunohistochemical 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 of the combination treatment. The secondary end points were time to progression and correlative studies of HER2 signaling. Time to progression 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 ($P = 0.05$; ref. 31). The trial would stop accrual early in the event that 2 or more of the initial 12 patients developed symptomatic congestive heart failure 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 immunohistochemical staining of the tumors from patients who had a partial response (PR) compared with stable disease (SD) or progressive disease (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 fluorescent *in situ* hybridization in 10 patients. One patient had a tumor with HER2 3+ by immunohistochemistry but was negative by fluorescent *in situ* hybridization. Nine patients had received anthracyclines (range, 240-360; median, 240 mg/m^2), and four 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, 22-136 weeks). The median time from last cycle of trastuzumab to initiation of trastuzumab and pertuzumab was 13 weeks (range, 4-96 weeks).

Toxicities. A total of 64 cycles of trastuzumab and pertuzumab were given. The major adverse events are summarized in

Table 3. Characteristics of patients experiencing cardiac toxicity on trastuzumab plus pertuzumab

Characteristic	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 (3 wk)	Yes (3 mo)	Yes (3 mo)	No (4 mo)	No*	Yes (1 wk)

Abbreviations: Pt, patient; EF, ejection fraction; T, trastuzumab; P, pertuzumab; HTN, hypertension; XRT, radiation treatment; Echo, echocardiogram; MRI, magnetic resonance imaging.
*The patient died 2 mo after the onset of LVSD.

Table 2. Grade 2 diarrhea was seen in 3 (27%) patients. Two patients had grade 1 and one patient had grade 3 allergic reaction related to infusion and symptoms resolved after antihistamine treatment. These patients received antihistamine before subsequent monoclonal antibody infusion.

Cardiac toxicities. A total of 92 echocardiogram and 8 cardiac magnetic resonance imaging studies were done. Six (54%) patients experienced a reduction in LVEF. Three patients had grade 1, two had grade 2, and one had grade 3 LVSD. The patient with the grade 3 LVSD experienced symptomatic congestive heart failure and required medical management. Grade 2/3 LVSD were associated with global hypokinesis. Two patients had a LVEF reduction of ≥15%. No specific electrocardiogram changes related to acute cardiac dysfunction were observed when cardiac toxicities occurred 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 rechallenge with trastuzumab for treatment of metastatic disease before receiving trastuzumab and pertuzumab. A reduced LVEF appeared within one to two 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 congestive heart failure 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 congestive heart failure 2 months after treatment termination.

Efficacy. Two (18%) patients achieved a PR, 3 (27%) had SD for ≥18 weeks (range, 18-36 weeks), and 6 (55%) had PD while on treatment for ≤6 weeks. The median time to

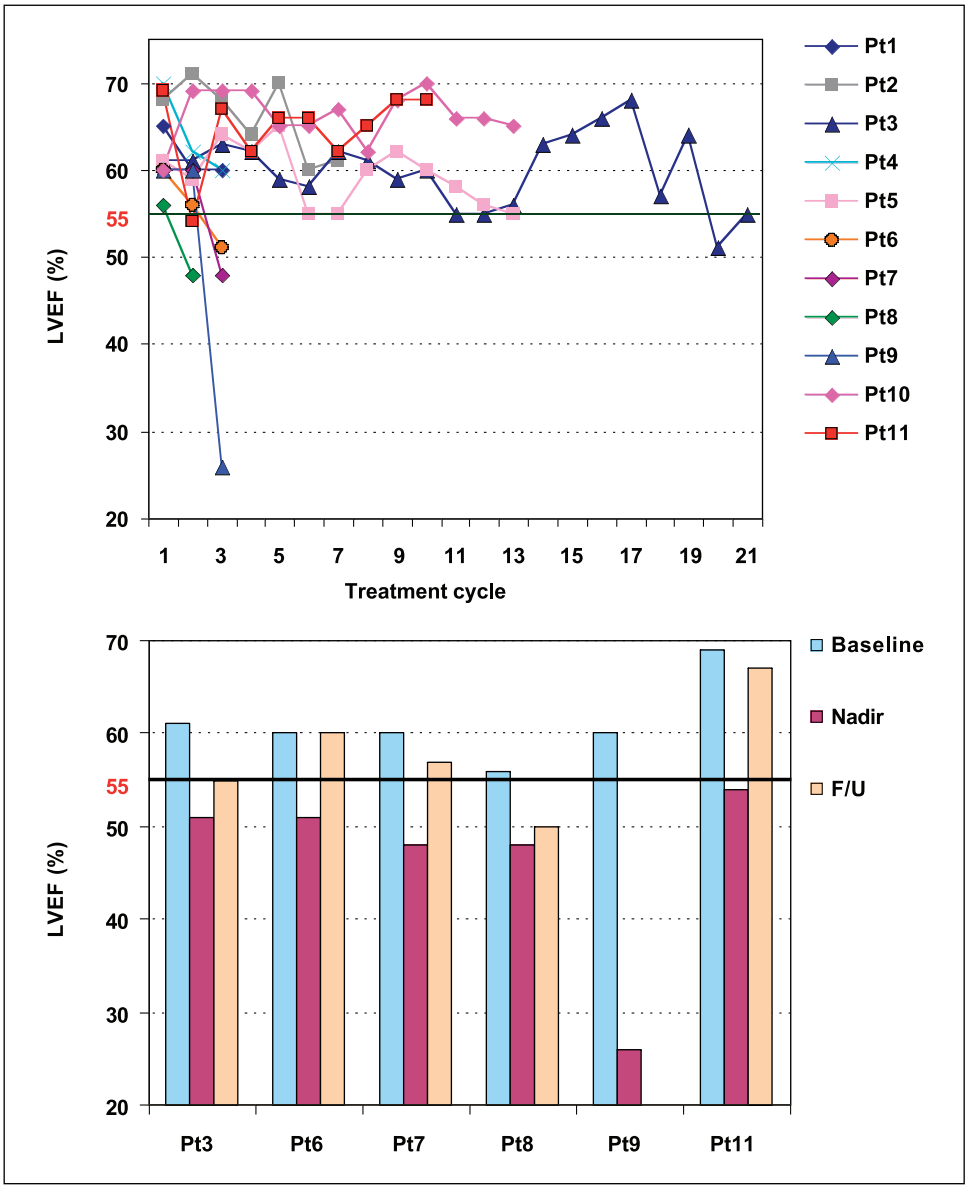

Fig. 1. *A,* LVEF in all patients treated with trastuzumab combined with pertuzumab. *B,* LVEF in patients treated with LVSD. Pt, patient; C, cycle of treatment; F/U, follow-up.

Table 4. Immunohistochemical 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)	
pHER2-Y1248	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)

NOTE: Immunohistochemical staining index = (staining intensity of a sample - staining intensity of negative control) × percentage of stained cells / 100 (ref. 30).
*P = 0.095.
† Percentages of immunohistochemically stained cells.

progression was 6 weeks (range, 3-54 weeks). The two patients who achieved PR had nonvisceral 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 of treatment. Her disease progressed after 36 weeks.

Tumor proliferative state and HER2 pathway evaluation. Correlative studies were conducted on baseline tumors and tumors after trastuzumab and pertuzumab treatment. Eight baseline patient tumor samples (three from the metastatic and five 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 immunohistochemistry. Ki67-positive tumor cells ranged from 18.6% to 95.3% in the baseline tumors. Table 4 summarizes the immunohistochemical 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 with those with SD or PD as determined by an exact Wilcoxon rank sum test. However, there was a trend toward 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 pHER2-Y1248, pHER2-Y877, pMAPK, pAkt, and Ki67 increased, whereas HER2 levels decreased, after one cycle of trastuzumab and pertuzumab in a patient who had PD (Fig. 2).

Discussion

In this study, the response rate 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 cannot 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 congestive heart failure 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

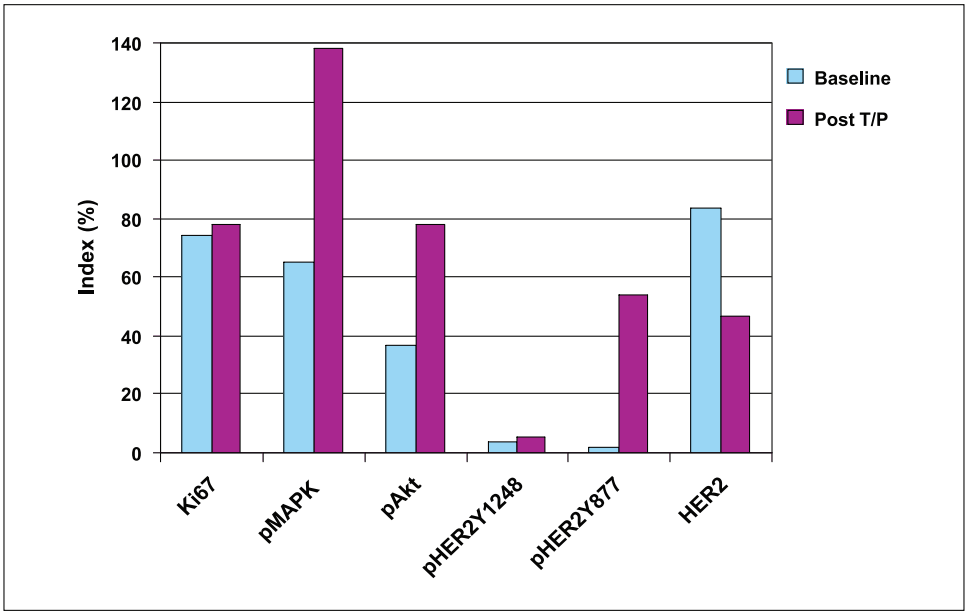

Fig. 2. Immunohistochemical staining indices of HER2 pathway markers and the percentage of Ki67-positive cells of a patient with progressive disease. IHC, immunohistochemistry; T/P, trastuzumab/pertuzumab.

chemotherapy for treatment of HER2$^+$ metastatic breast cancer. 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 of ≥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 seemed to be lower than what was reported in metastatic breast cancer patients treated with trastuzumab. In the pivotal phase III clinical trial of the metastatic breast cancer 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 pretreated 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 that 2 of the 21 patients had reduced LVEF of ≤50% from normal baseline values and one of them developed congestive heart failure 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 of >10% to a 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 congestive heart failure (38). Based on the tumor types, it is 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 toward 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, although not statistically significant, in ovarian cancer patients (36). More studies are needed to confirm these observations because 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 on HER2 receptor activation. Whereas 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 preclinical models (10, 18). We observed increased levels of pHER2Y877, pHER2Y1248, pMAPK, and pAkt, suggesting activation of the HER2 pathway in one patient with PD after trastuzumab/pertuzumab treatment. It has been reported that monoclonal antibodies against HER2 can either be growth inhibitory or stimulatory depending on the intracellular 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 showed 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 type I receptor levels increased in trastuzumab-resistant tumor cells, and antibody to insulin-like growth factor type I receptor 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 homodimerization and heterodimerization 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 mammalian target of rapamycin 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 an issue of concern. 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.

Disclosure of Potential Conflicts of Interest

S.M. Swain: travel costs defrayed by Genentech. The other authors disclosed no potential conflicts of interest.

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