Efficacy and Safety of Single-Agent Pertuzumab, a Human Epidermal Receptor Dimerization Inhibitor, in Patients with Non–Small Cell Lung Cancer

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Abstract

Purpose: Pertuzumab, a first-in-class human epidermal receptor 2 (HER2) dimerization inhibitor, is a humanized monoclonal anti-HER2 antibody that binds HER2's dimerization domain and inhibits HER2 signaling. Based on supporting preclinical studies, we undertook a Phase II trial of pertuzumab in patients with recurrent non–small cell lung cancer (NSCLC).

Experimental Design: Patients with previously treated NSCLC accessible for core biopsy and naive to HER pathway inhibitors were treated with pertuzumab i.v. once every 3 weeks. Tumor assessments were done at 6 and 12 weeks and then every 3 months thereafter. The primary efficacy end point was overall response rate by Response Evaluation Criteria in Solid Tumors. Measurement of tumor glucose metabolism (SUV_{max}) by F-18-fluorodeoxyglucose positron emission tomography was used as an exploratory pharmacodynamic marker of drug activity.

Results: Of 43 patients treated with pertuzumab, no responses were seen; 18 of 43 (41.9%) and 9 of 43 (20.9%) patients had stable disease at 6 and 12 weeks, respectively. The median and 3-month progression-free survival rates (PFS) were 6.1 weeks (95% confidence interval, 5.3-11.3 weeks) and 28.4% (95% confidence interval, 14.4-44.2%), respectively. Of 22 patients who underwent F-18-fluorodeoxyglucose positron emission tomography, six (27.3%) had a metabolic response to pertuzumab as evidenced by decreased SUV_{max}. These patients had prolonged PFS (HR = 0.11, log-rank P value = 0.018) compared with the 16 patients who had no metabolic response. Four patients (9.3%) experienced a grade 3/grade 4 adverse event judged related to pertuzumab; none exhibited grade 3/grade 4 cardiac toxicity.

Conclusions: Pertuzumab is well tolerated as monotherapy. Pharmacodynamic activity correlated with prolonged PFS was detected in a moderate percentage of patients (27.3%). Further clinical development of pertuzumab should focus on rational combinations of pertuzumab with other drugs active in NSCLC.

Lung cancer remains the leading cause of cancer-related deaths in the United States. It is estimated that in 2006 over 162,000 people will die from lung cancer and over 174,000 new cases will be diagnosed (1). Eighty-five percent of patients with lung cancer have non–small cell lung cancer (NSCLC; ref. 2), and of those, ~40% present with metastatic and incurable disease (3). Cytotoxic chemotherapy has historically been the mainstay of treatment for advanced NSCLC, but treatments have reached a therapeutic plateau (4, 5). More effective treatment is clearly needed.

Therapies that inhibit growth stimulatory pathways in tumors hold great promise in the treatment of NSCLC, and those directed at human epidermal growth factor receptor (HER) family receptor signaling have been the most intensively investigated. The HER family receptor tyrosine kinases, which includes HER1/epidermal growth factor receptor (EGFR), HER2, HER3, and HER4, have been implicated in the development and progression of multiple types of cancer, including NSCLC (6–8). The strongest evidence for a direct role of HER family members in NSCLC tumorigenesis comes from studies demonstrating that *EGFR* mutations are found in 2% to 37% of NSCLC tumors (8–11), and that tumors harboring such mutations commonly respond to treatment with EGFR tyrosine kinase inhibitors gefitinib and erlotinib (12–14). Approximately, one-third of NSCLC tumors also contain increased *EGFR* gene copy number as assessed by

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Note: This manuscript is dedicated to the memory of Dr. John R. Murren.

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fluorescence *in situ* hybridization (15). This correlates partially with the presence of *EGFR* mutations (15). Patients with relapsed NSCLC treated with erlotinib versus placebo have prolonged progression-free survival (PFS) and overall survival (16). Thus, EGFR is a validated target for NSCLC therapeutics.

The contribution of other HER family members to NSCLC tumorigenesis is less certain. After stimulation by agonist ligands, the activated forms of these receptors form either homodimers or heterodimers with other HER receptors, leading to the transduction of proliferative and antiapoptotic signals. HER2, although ligandless, is capable of forming active homodimers only when highly overexpressed (as is the case in HER2+ breast cancer; ref. 17). Approximately 5% of NSCLCs have such elevated HER2 expression (18). Mutation in the *HER2* coding region, similar to those identified in *EGFR*, have also been described in lung adenocarcinomas, but are rare (10, 11, 19).

A more prevalent role for HER2 in NSCLC tumorigenesis may be through its ability to form heterodimers with other HER family receptors once they are activated through ligand binding. Due to its constitutively "open" conformation, HER2 is the preferred binding partner for activated HER receptors, including EGFR and HER3 (20). In various experimental systems, heterodimers containing HER2 exhibit a slower rate of internalization, a relatively slow rate of ligand dissociation, and relaxed ligand specificity (21) and, thus, generate stronger mitogenic signals than other homodimer or heterodimer pairs (21, 22). Recent studies suggest that increased *HER2* gene copy number in NSCLC is associated with an increased response rate to the EGFR tyrosine kinase inhibitor gefitinib (23). Therefore, therapies that inhibit the dimerization of (*HER2*) with activated HER family members have potential for significant clinical utility in the treatment of NSCLC (24).

Pertuzumab (Omnitarg, formerly rhuMAb 2C4; Genentech, Inc.) is first in the new class of targeted therapeutic agents known as HER dimerization inhibitors (25). Pertuzumab is a recombinant, humanized monoclonal antibody that recognizes an epitope in the dimerization domain of HER2 (26). Binding of pertuzumab to HER2 prevents both HER2 homodimerization and heterodimerization with other HER receptors (27). Consistent with this mechanism of action, pertuzumab antitumor activity in NSCLC xenograft models is not restricted to tumors with HER2 overexpression and therefore differs from the therapeutic monoclonal antibody trastuzumab (Herceptin), which binds a nonoverlapping region of extracellular HER2 (26) and requires HER2 overexpression for activity (28). A Phase I trial of pertuzumab given i.v. every 3 weeks showed its overall safety in patients with a diverse range of tumors with the most common adverse events being mild (grade 1 or grade 2) gastrointestinal toxicity (nausea, vomiting, diarrhea, and abdominal pain), asthenia, and rash (29). At doses of 2.0 to 15.0 mg/kg, pharmacokinetics of pertuzumab followed a two-compartment model; doses above 5 mg/kg yielded plasma trough levels that exceeded the target of 25 μg/mL found to be efficacious in preclinical models (29). Based on data from the Phase I study (29), fixed doses of pertuzumab were used in Phase II trials. This dosing approach has been further substantiated by additional pharmacokinetic analysis (30). Recently, a Phase II study of pertuzumab in ovarian cancer comparing two every 3-week fixed dosing regimens (420 mg with an 840 mg loading dose and 1050 mg) showed that the two regimens exhibit similar clinical activity. In unselected patients with recurrent epithelial ovarian cancer, 14.5% of patients had signs of clinical activity (4.3% PR, 6.8% stable disease at >6 months, and 3.4% with CA-125 reduction ≥50%; ref. 31). In that study, prolonged PFS correlated positively with the presence of phosphorylated HER2 (pHER2), a marker of activated HER2 (31).

The objective of this study was to characterize the safety and antitumor activity of pertuzumab in patients with NSCLC who had previously been treated with chemotherapy. Core tumor biopsies were required before treatment to assess potential predictive markers for antitumor activity including total HER2 levels and pHER2 by immunohistochemistry. Because pertuzumab inhibits AKT (a regulator of glucose uptake) in prostate and breast cancer cell lines (27) and enhanced glucose metabolism is a characteristic of NSCLC tumors that can be measured noninvasively (32), we also investigated, as an exploratory pharmacodynamic marker in a subset of prospectively identified patients, changes in tumor glucose uptake as measured by F-18-fluorodeoxyglucose positron emission tomography (FDG-PET).

Patients and Methods

Patient population. Eligible patients were ≥18 years of age with histologically documented locally advanced or metastatic NSCLC (excluding bronchioloalveolar carcinoma); had previously been treated with a platinum-containing regimen that included either a taxane or a *Vinca* alkaloid with no limit to the number of prior chemotherapy regimens; had an Eastern Cooperative Oncology Group performance status of 0 or 1; had adequate cardiac, hematologic, hepatic, and renal function; and had tumor accessible for core needle biopsy and were willing to consent to the procedure. Patients were still eligible if they underwent a biopsy to obtain tumor and the biopsy did not provide adequate tumor tissue for analyses. Patients were ineligible if they had a history or radiographic evidence of central nervous system or brain metastases, a history of significant cardiac disease, or any prior treatment with a HER pathway inhibitor (e.g., erlotinib and gefitinib).

Study design. This was an open-label, multicenter Phase II study of pertuzumab given as a single agent. After patient eligibility was confirmed and a tumor biopsy obtained, pertuzumab was given i.v. with an 840-mg loading dose followed by 420 mg every 3 weeks. Patients were evaluated for response by radiographic imaging and for cardiac toxicity by echocardiography at 6 and 12 weeks and then every 3 months, and finally at the treatment termination. Responses required confirmatory assessment at least 4 weeks later. Patients with nonprogressing disease and acceptable toxicity were eligible to receive pertuzumab every 3 weeks for up to 1 year (17 cycles).

Study assessments and end points. The best overall response at any time during the study was assessed by Response Evaluation Criteria in Solid Tumors (33). Assessment of safety and tolerability was based on the incidence, nature, severity, and relatedness of adverse events graded according to the National Cancer Institute-CTC, Version 2.0.[11] Cardiac safety was specifically assessed by measurements of serum cardiac troponin T levels and left ventricular ejection fraction (LVEF) by echocardiogram. For patients with an asymptomatic decrease in LVEF, specific rules based on a decrease in LVEF of ≥10% from baseline to an absolute LVEF of ≤45% were incorporated for stopping or holding treatment. All echocardiograms were reviewed by a central facility (Gentiae).

[11] www.jastro.jp/guideline/nci/nci-ctc.doc

FDG-PET studies. At six preselected clinical study sites, FDG-PET was done at baseline and after two cycles of pertuzumab (between days 8 and 21 after the second dose). Scanner calibration was verified using scans of a uniform cylindrical phantom containing an aqueous solution of FDG. After fasting for at least 4 h, patients were injected with 5 to 20 mCi (185-740 MBq) of FDG and scanned from the base of the skull to proximal thighs at ~45 to 60 min postinjection. Images were reconstructed with iterative methods and corrections for attenuation, scatter, random coincidences, dead time, and decay.

PET scans were reviewed centrally at the Dana-Farber Cancer Institute by blinded reviewers. Results of these FDG-PET imaging studies were not used for trial decision-making purposes. Based on FDG avidity, size, and anatomic location, up to four lesions per patient were chosen from the baseline scan; the choice of lesions was made independent of CT scan appearance or knowledge of the target lesions used for assessing response by Response Evaluation Criteria in Solid Tumors at the study sites. For each lesion, the image slice with the highest FDG uptake was identified and a region-of-interest boundary was determined using a threshold of 70% of the maximum. With pixel values representing radioactivity concentration, the standardized uptake value (SUV_{max}) was calculated by normalizing for injected dose and body weight. For each region of interest, the percentage change in maximum SUV between time points was determined. The cutoff for metabolic response used (≥25% average decrease in SUV_{max}) was based on European Organization for Research and Treatment of Cancer criteria for a single region of interest (34).

Immunohistochemistry. Tumor biopsies were embedded in optimal cutting temperature compound and flash frozen in liquid nitrogen within 30 min after biopsy. Frozen sections at 5 μm in thickness were immediately fixed on glass slides using acetone containing 1 mmol/L sodium orthovanadate. Sections were then treated with glucose oxidase to quench endogenous peroxidase activity, blocked using avidin/biotin blocking kit reagents (Vector Laboratories) and blocked with 10% normal horse serum and 3% bovine serum albumin. Sections were then incubated for 1 h at room temperature with mouse monoclonal antibodies directed against HER2 phosphotyrosine 1248 (clone PN2A, Neomarkers; 5 μg/mL), total HER2 (clone TAB250, Zymed Laboratories; dilution, 1:200), or nonspecific mouse IgG1 as a control (clone mineral oil plasmacytoma 21, PharMingen; 5 μg/mL). The bound antibody was detected using a biotinylated horse anti-mouse IgG and visualized using the avidin-biotin peroxidase complex method (Vectastain Elite, Vector Laboratories) using metal-enhanced 3,3′-diaminobenzidine (Pierce) as chromogen. HER2 immunoreactivity associated with tumor cell membranes was scored as 0 (absent), 1+ (weak), 2+ (moderate), or 3+ (strong). Immunohistochemistry for pHER2 (monoclonal antibody PN2A) was considered positive if membrane-associated immunoreactivity was present in any tumor cells. SKBR3 cells (with *HER2* gene amplification, HER2 protein overexpression, and constitutive HER2 phosphorylation) and MCF-7 cells (*HER2* gene nonamplified, normal EGFR2 expression levels) with and without heregulin stimulation and MDA-MB-468 cells, which do not express HER2, were included as controls in all assays. In the TAB250 assay for total HER2, SKBR3 had strong (3+) immunostaining, MCF-7 cells had moderate (2+) staining, and MDA-MB-468 cells were negative (0). In the PN2A assay, SKBR3 cells had moderate (2+) staining, heregulin-stimulated MCF-7 cells had focal weak (1+) staining, and unstimulated MCF-7 cells and MDA-MB-468 cells were negative. Nonspecific IgG1 was included as a negative antibody control in all assays.

EGFR *mutation detection.* DNA isolated from frozen or fixed tumor samples was screened for mutations in *EGFR* exons 19 and 21 according to Jänne and colleagues (35). In samples with sufficient available tumor, mutation status was confirmed by bidirectional sequencing using the Sanger method as previously described (35).

Statistical methods. The primary efficacy end point for this study was best overall response at any time during the study after initiation of treatment with pertuzumab. A sample of 40 treated subjects would allow the rate of best response to be estimated with SE of ≤8%. The 95% Blythe-Still-Casella exact confidence interval (36) was calculated for response rate; all patients who received the study drug were included. Patients who discontinued therapy for any reason before undergoing a post-baseline tumor response evaluation were considered nonresponders in the modified intent-to-treat analysis of best overall response rate. Median PFS was calculated using Kaplan-Meier methodology.

Results

Patients and demographics. Between August 2003 and October 2004, 51 patients were enrolled. Eight patients were not treated due to either worsening performance status (four patients), newly diagnosed brain metastases (three patients), or withdrawal from the study by patient choice (one patient). Forty-three patients were treated with at least one dose of pertuzumab (Table 1). At baseline, the median patient age was 62 years old. The majority were Caucasian, had a history of smoking (91%), and had an Eastern Cooperative Oncology Group performance status of 1 (70%). Patients were most frequently diagnosed with adenocarcinoma (46%) and 49% had received >1 prior chemotherapy regimen.

Efficacy. The best response associated with pertuzumab therapy was stable disease. Eighteen of forty-three [41.9%; 95% confidence interval (95% CI), 27.0-57.7%] patients had stable disease at the 6-week evaluation point and 9 of 43 (20.9%) patients had stable disease at the 12-week evaluation point. The median PFS was 6.1 weeks (95% CI, 5.3-11.3 weeks),

Table 1. Patient baseline characteristics

Variable	(*N* = 43)
Age, y	
Median	62
Range	39-77
Gender	
Male	26 (60)
Female	17 (40)
Race	
White	42 (98)
Black	1 (2)
Eastern Cooperative Oncology Group performance status	
0	12 (28)
1	30 (70)
Unknown	1 (2)
History of smoking	
Yes	39 (91)
No	4 (9)
Histologic subtypes	
Adenocarcinoma	20 (46)
Squamous cell carcinoma	12 (28)
Other	11 (26)
No. previous treatment regimens	
Mean	
1	22 (51)
2	16 (37)
3	4 (9)
4	1 (2)

NOTE: Values are *n* (%) unless otherwise noted.

and 3-month PFS was 28.4% (95% CI, 14.4-44.2%; Fig. 1). There was no correlation between PFS and either gender, tumor histology, or history of tobacco use (data not shown).

Predictive tumor diagnostics. Of 43 treated patients, 38 had frozen tumor biopsies available that were suitable for analysis. Immunohistochemical staining of tumors using a sensitive procedure which detects relatively low levels of HER2 expression in *HER2* nonamplified cells showed that most patients (34 of 38) had detectable HER2 expression. There was no relationship between level of HER2 expression and PFS (data not shown). Immunohistochemical staining for pHER2 showed that 4 of the 38 tumors had detectable pHER2. There was no relationship between detectable pHER2 and PFS (data not shown).

Exploratory FDG-PET scanning. A total of 22 patients had exploratory FDG-PET scanning done at baseline and in the third week of cycle 2. In Fig. 2, data are presented for each patient as the percentage change in SUV_{max} for each target lesion. Of the 22 patients, six (27%) had an average decline of ≥25% in SUV_{max} across all target lesions (identified by an asterisk in Fig. 2). This degree of change in SUV_{max} is consistent with European Organization for Research and Treatment of Cancer criteria for a partial metabolic response (34). Figure 3 depicts PFS based on metabolic response for the 22 patients who had a PET scan and the 13 patients without PET scans who were treated with at least two doses of pertuzumab. Patients with a metabolic response had a median PFS of 35.4 weeks (95% CI, 21.1-35.4 weeks) versus 6.1 weeks (95% CI, 5.3-13.0 weeks) in those without a metabolic response (HR = 0.11, $P = 0.018$). The demographics and results of predictive marker analyses (HER2, pHER2, and *EGFR* mutational status) for the six patients with a metabolic response are shown in Table 2.

Safety and tolerability. The most common study drug-related adverse events (occurring in ≥10% of patients) were diarrhea, nausea, and fatigue (Table 3). Four (9%) patients experienced a grade 3 or grade 4 adverse event that was judged by the investigator to be related to study drug (lung infiltrate, dyspnea and acute respiratory distress syndrome, hypersensitivity reaction, and diarrhea). Consistent with Phase I studies with pertuzumab (29), the most common drug-related adverse events were mild diarrhea (21%), nausea (14%), and fatigue (14%). No patients exhibited grade 3 or grade 4 cardiotoxicity, elevated cardiac troponin, or had a confirmed decrease in LVEF that met prespecified criteria for cardiac toxicity in asymptomatic individuals.

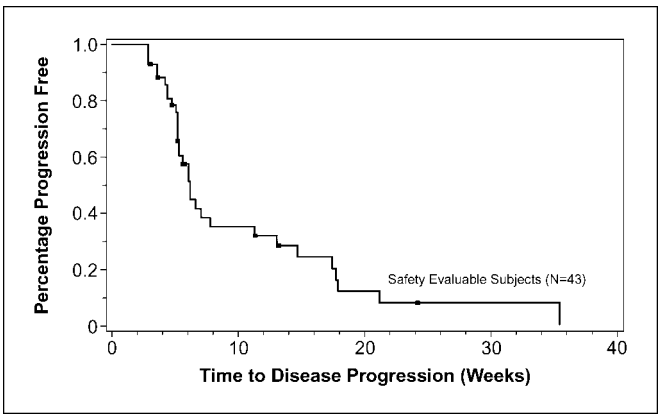

Fig. 1. PFS. Kaplan-Meier graph for the 43 patients treated with pertuzumab. The median PFS was 6.1 weeks (95% CI, 5.3-11.3 weeks) and 3-month PFS was 28.4% (95% CI, 14.4-44.2%).

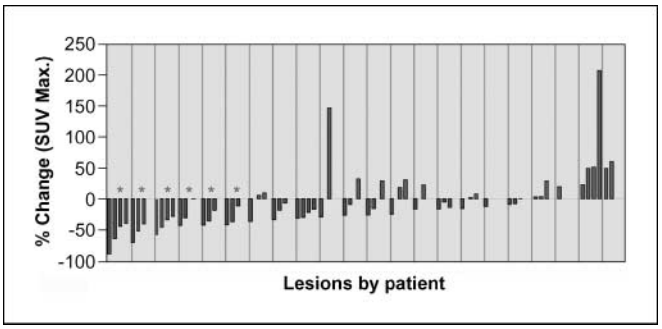

Fig. 2. The percentage change in SUV_{max} as determined by FDG-PET. The percentage change in SUV_{max} for all measured lesions was grouped by patient and was determined for up to four lesions per patient. Each lesion is represented by a vertical bar. *, patients with an average decrease in SUV_{max} of ≥25% from baseline.

Discussion

The primary objective of this Phase II study was to assess the best overall response at any time to single-agent pertuzumab in patients with recurrent NSCLC. In this study, the best overall response observed was stable disease by Response Evaluation Criteria in Solid Tumors with 18 of 43 (42%) and 9 of 43 (21%) having stable disease at the 6-week and 12-week evaluation points, respectively. No partial responses were reported. Because preclinical models showed single-agent pertuzumab slows or halts the growth of NSCLC xenografts without causing regression of established tumors (24), the lack of responses in this study was not unexpected. Pertuzumab did not have evidence of clinically significant therapeutic activity in this group of patients with NSCLC selected for good performance status (Eastern Cooperative Oncology Group performance status, 0-1) and no brain metastases. Most of these

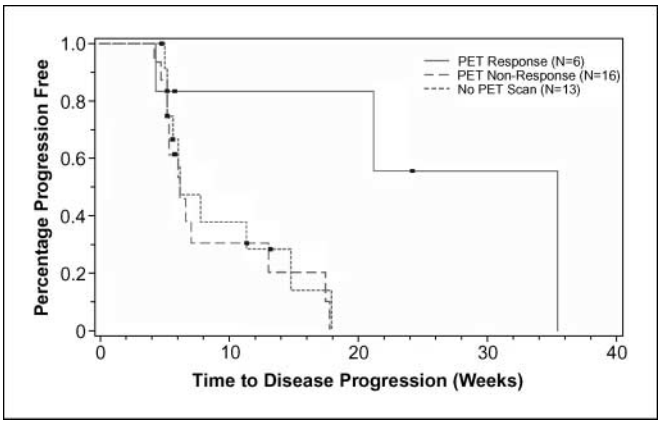

Fig. 3. PFS as a function of pharmacodynamic response by FDG-PET. Kaplan-Meier graph for the 22 patients treated with pertuzumab and who received FDG-PET scanning. Six patients had a response by PET, which was defined as an average decrease in SUV_{max} of ≥25%. Sixteen patients did not respond by PET according to that criterion. The 13 patients who similarly received two doses of pertuzumab, but did not undergo FDG-PET scanning, are included for comparison. PFS HR = 0.11; $P_{log\text{-}rank} = 0.018$ for pharmacodynamic response versus no response.

Table 2. Demographics and results of predictive marker analyses for patients with pharmacodynamic evidence of drug activity

Patient	Sex/age	Histologic type	Smoker	HER2/pHER2	EGFR	Average change in SUV_{max}
a	M/74	Adenocarcinoma	Yes	3+/neg	WT	-56.4
b	M/75	NOS	Yes	unknown	WT	-56.2
c	M/58	Squamous	No	unknown	unknown	-44.3
d	F/72	Adenocarcinoma	Yes	2+/neg	E21 L858R	-34.4
e	M/63	NOS	Yes	3+/pos	WT	-34.2
f	F/72	Adenocarcinoma	Yes	1+/neg	WT	-31.4

Abbreviations: NOS, not otherwise specified; WT, wild type; neg, negative; pos, positive.

patients were not heavily treated before starting pertuzumab because previous treatment of 38 of the 43 patients (88%) was limited to one or two chemotherapy regimens. The median PFS in this clinical study was 6.1 weeks (95% CI, 5.3-11.3 weeks), which is shorter than that seen with single-agent chemotherapy (docetaxel or pemetrexed) or erlotinib in NSCLC patients who have previously received chemotherapy (37, 38). The comparison across trials is limited by differences in trial design and trial demographics; thus, it does not allow for a true comparison of efficacy between these agents. The primary end point of this trial was PFS; thus, data was not collected on the subsequent treatments after completion of pertuzumab therapy. Therefore, information on the efficacy of salvage therapy is not available.

This trial also prospectively evaluated pHER2 in fresh tumor tissue before treatment. Previously, based on preclinical experimental evidence showing a relationship between efficacy of pertuzumab and activated HER2 in xenograft models, pHER2 was suggested to be a potential predictive efficacy marker (24). Consistent with that, a recently completed clinical trial of pertuzumab in patients with platinum-resistant ovarian cancer found that pHER2 was predictive for prolonged PFS (31). That study used a highly sensitive ELISA method suitable for large tumor samples, such as those obtained through laparoscopy. Because this study used core needle biopsies, which provide smaller tumor samples, it was not possible to analyze pHER2 by ELISA. Using a less sensitive immunohistochemistry assay for pHER2, 4 of 38 samples (10.5%) were classified as pHER2 positive. This was lower than the roughly 30% predicted from preclinical studies (data not shown). Although PFS was not increased in this subset, the small sample size limits any firm conclusions. However, the finding that several of the tumors that exhibited a metabolic response to pertuzumab treatment (as assessed by FDG-PET) had been classified as pHER2 negative by the immunohistochemistry assay (Table 2) suggests that the assay for pHER2 used in this study may not have been sufficiently sensitive or that an alternative predictive marker should be considered for patients with lung cancer. HER2 expression was also evaluated using an assay that detects HER2 expression in *HER2*-nonamplified cells and is more sensitive than the Herceptest assay, which was designed to detect HER2 overexpression associated with *HER2* amplification in breast cancer. This assay was selected because pertuzumab showed activity against *HER2* nonamplified tumor cells, which did not overexpress HER2 in preclinical models (24). The majority of patients in our study had tumors that had detectable HER2 expression using this assay, whereas in a prior study with trastuzumab, ~17% of NSCLC patients screened were HER2-positive using the Herceptest (39). In the present study, HER2 expression levels were found not to correlate with PFS. This result was anticipated because levels of HER2 expression are not associated with pertuzumab sensitivity in preclinical models (24). Other potential predictive efficacy markers include *HER2* coding region gene mutations (19) and increased *HER2* gene copy number (23). Given the low frequency of *HER2* mutations in NSCLC patients, however, this alone would be unlikely to account for the nearly 25% of patients with pharmacodynamic evidence of drug activity in the current study. Since at the time this study was initiated this genetic data linking *HER2* and lung cancer was not yet available and tumor tissue obtained was prioritized for immunohistochemical analysis of HER2 activation (pHER2), we did not determine whether any patients' tumors had *HER2* mutations. The lack of sufficient sample material to conduct additional analyses is likely to be an issue confronted in other trials in NSCLC wherein suitable archival material may not be readily available.

Measurement of glucose metabolism through FDG-PET imaging was evaluated as a potential pharmacodynamic marker in this study. This approach has been successfully used in clinical trials with other targeted agents (e.g., imatinib in GIST tumors), for which tumor shrinkage is not a reliable marker of drug activity (40). In the present study, an average decrease in SUV_{max} of ≥25% across measured lesions was used as the response cutoff and was based on European Organization for Research

Table 3. Adverse events in 43 treated patients

	n (%)
Drug-related adverse events (≥10% of patients)	
Diarrhea	9 (21)
Nausea	6 (14)
Fatigue	6 (14)
Drug-related grade 3/grade 4 adverse events, any incidence	
Any grade 3 or grade 4 adverse event	4 (9)
Lung infiltration	1 (2)
Hypersensitivity	1 (2)
Dyspnea and acute respiratory distress syndrome	1 (2)
Diarrhea	1 (2)
Cardiac toxicity or confirmed LVEF decrease ≥10% and post-baseline LVEF <50%	0 (0)

and Treatment of Cancer criteria for a single region-of-interest (34). Using this threshold, 6 of 22 patients (27.3%) had a metabolic response, and this response correlated with prolonged PFS (Figs. 2 and 3). Although limited by the lack of a control group, these data suggest that pertuzumab may have clinical activity in a subset of NSCLC patients. Similar to the findings in this study, a decrease in SUV_{max} after one cycle of platinum-based chemotherapy also predicts for prolonged PFS in chemonaive NSCLC (41). The results of these pharmacodynamic studies, together with the specificity of pertuzumab for its target (HER2), also provide direct clinical evidence that HER2 signaling is present in a moderate percentage of NSCLC tumors.

Although anecdotal in nature, an *EGFR* kinase region mutation was found in a tumor that had been prospectively obtained from one of the patients with pharmacodynamic evidence for pertuzumab activity (Table 3). Such mutations have been shown to increase sensitivity to EGFR tyrosine kinase inhibitors (12, 13). This finding suggests that there may be some overlap in the spectrum of tumors responsive to these two different HER pathway inhibitors. Consistent with this hypothesis, increased *HER2* copy number (and presumably increased HER2 signaling) has been described in patients with clinical response to EGFR kinases inhibitors (23). A recent preclinical study that included two NSCLC tumor xenografts found that activity of the combination of erlotinib and pertuzumab was additive in one xenograft (QG56) and greater than additive in the other (Calu3; ref. 42), suggesting that combining the two classes of HER pathway inhibitors may improve efficacy in the clinic. Further mechanistic studies are needed to explore this possibility.

In conclusion, the data presented show that pertuzumab has pharmacodynamic activity in a subgroup of NSCLC patients. Further clinical development of pertuzumab should focus on rational combinations of pertuzumab with other drugs that are active in NSCLC. Furthermore, efforts to develop a predictive marker and assay suitable for selecting patients with NSCLC for treatment with pertuzumab should continue in parallel.

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