Phase II Study of Celecoxib and Trastuzumab in Metastatic Breast Cancer Patients Who Have Progressed after Prior Trastuzumab-Based Treatments

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ABSTRACT

Purpose: **Preclinical studies demonstrate a link between overexpression of HER-2/*neu* and cyclooxygenase-2 (COX-2) activity. To explore the possibility that COX-2 is a therapeutic target, we conducted a phase II study of celecoxib, a selective COX-2 inhibitor, and trastuzumab in patients with HER-2/*neu*-overexpressing metastatic breast cancer that had progressed while receiving trastuzumab.**

Experimental Design: **Eligible patients had bi-dimensionally measurable or evaluable HER-2/*neu*-overexpressing metastatic breast cancer. HER-2/*neu* overexpression, defined as 2+ or 3+ by the HercepTest, was required. Patients had to have progressed despite prior trastuzumab-based therapy. Treatment consisted of celecoxib (400 mg twice daily) and trastuzumab.**

Results: **Twelve patients were enrolled (42% status post 1 regimen for metastatic disease 58% status post > 2 prior regimens (range of 2–6). Eleven patients were evaluable. There were no responses. Median duration of treatment was 9 weeks. One patient had stable disease at 3 months but progressed at 6 months. A second patient stopped treatment at 3 months because of unresolved grade 2 rash, felt to be related to celecoxib. Toxicities were generally grade 1 or 2. One patient (8%) experienced grade 3 toxicity (abdominal pain).**

Conclusions: **Celecoxib combined with trastuzumab is well tolerated. However, this combination in patients with HER2/neu-overexpressing, trastuzumab-refractory disease, was not active.**

INTRODUCTION

The *human epidermal growth factor receptor 2* (HER-2/*neu*) gene encodes a 185-kDa transmembrane receptor with tyrosine kinase activity that is a member of the epidermal growth factor receptor family (1) and is amplified in 20–30% of human breast cancers (2, 3). Overexpression of HER-2/*neu* has been correlated with shorter disease-free and overall survival in breast cancer patients (2, 4). Trastuzumab is a humanized anti-HER-2/*neu* monoclonal antibody (Herceptin; Genentech, South San Francisco, CA) that is active in women with HER-2/*neu*-overexpressing metastatic breast cancer. Response rates to trastuzumab in pretreated patients ranged from 12 to 15% (5, 6) and 26% overall as first line metastatic treatment (7). A randomized Phase III trial reported that patients who received the combination of trastuzumab and chemotherapy had a statistically significantly longer time to progression, greater overall response rate, and increased median overall survival rate compared with those receiving chemotherapy alone (8). Several other trials reported promising results using the combination of trastuzumab with chemotherapy (9, 10).

To increase the therapeutic benefit of trastuzumab, new approaches are still needed. It is important, therefore, to explore treatment strategies that might improve the management of patients with HER-2/*neu*-overexpressing tumors. There is considerable evidence that cyclooxygenase-2 (COX-2), an inducible enzyme that catalyzes the synthesis of prostaglandins, represents a potential target for inhibiting tumor growth. Prostaglandins can enhance carcinogenesis by inhibiting immune surveillance, inducing cell proliferation, suppressing apoptosis, and stimulating angiogenesis and invasiveness (11–14). COX-2 is overexpressed in multiple human premalignant and malignant conditions, including tumors of the breast (15–17). The most specific support for a cause-and-effect relationship between COX-2 and tumorigenesis come from preclinical studies. Multiparous female transgenic mice that are engineered to overexpress human COX-2 in mammary glands develop focal mammary gland hyperplasia, dysplasia, and metastatic tumors (18). Importantly, the formation and growth of tumors are reduced in mice engineered to be COX-2 deficient (19–21).

In addition to this genetic evidence, numerous pharmacological studies suggest that COX-2 is a therapeutic target. Treatment with selective COX-2 inhibitors reduces the formation, growth, and metastases of tumors in experimental animals (15, 22–26). In a proof of principle clinical trial, celecoxib, a selective COX-2 inhibitor, reduced the number of intestinal tumors in patients with familial adenomatous polyposis (27).

COX-2 may be important for mediating HER-2/*neu*-induced mammary tumorigenesis. Recently, Subbaramaiah

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et al. (28) demonstrated a clear link between the overexpression of HER-2/*neu* and the up-regulation of COX-2 in human breast cancer, mediated by the Ras pathway. Ristimaki *et al.* (29) analyzed COX-2 expression in a tissue array of 1576 human breast cancers and showed that overexpression of COX-2 was associated with poor prognostic factors, including the presence of *HER-2/neu* gene amplification, and was correlated with poor distant disease-free survival. Howe *et al.* (30) demonstrated that treatment with celecoxib reduced the incidence of mammary tumors in Neu-transgenic mice. Taken together, these results raise the possibility that treatment with a selective COX-2 inhibitor could be beneficial to patients with HER-2/*neu*-overexpressing breast cancer. To explore this possibility, we conducted a clinical trial to evaluate whether the addition of celecoxib to trastuzumab is beneficial to patients with HER-2/*neu*-overexpressing breast cancer who had already progressed while receiving trastuzumab-based regimens.

PATIENTS AND METHODS

Patient Eligibility. Patients >18 years of age with bi-dimensionally measurable or evaluable metastatic breast cancer, that is HER-2/*neu* overexpressing as defined by a score of 2+ or 3+ by the HercepTest were eligible. HER-2/*neu* testing was done on all primary tumor samples. Progression after prior trastuzumab treatment for at least 12 weeks either as a single agent or in combination with chemotherapy was required. Any number or type of prior cytotoxic therapy was allowed in either the adjuvant/neoadjuvant or metastatic setting. Any number or type of prior hormonal therapies, either for stage IV disease and/or as adjuvant therapy, was allowed but had to be discontinued at least 3 weeks before entry. Eligible patients had to have Karnofsky performance status of >70%; adequate hematological, renal, and hepatic functions (granulocytes >1500/μl, hemoglobin >8.0 g/dl, platelets >100,000/μl, serum creatinine <1.5 mg/dl, aspartate aminotransferase and alanine aminotransferase <2 × upper limit of normal, and bilirubin <1.5 × upper limit of normal); and left ventricular ejection fraction >50%. A life expectancy of >3 months was required, and menstruating females of child-bearing potential were required to use contraception while on study and to have a negative pregnancy test before study enrollment. Patients with a history of brain metastases had to be off steroids for at least a three-month interval after brain irradiation and to have no evidence of progression of brain metastases. Patients with other malignancies, except adequately treated non-melanoma skin cancers or *in situ* cervical cancer, must have been disease-free for at least five years to be eligible. Patients were excluded if they had leptomeningeal disease or allergic reactions to sulfonamides. This study was reviewed and approved by the institutional review board of Memorial Sloan-Kettering Cancer Center, and all patients signed the informed consent before study enrollment.

Study Design. This was a phase II study. The primary objective was to evaluate the efficacy of celecoxib (400 mg, orally twice a day) and trastuzumab in the treatment of patients with HER-2/*neu*-overexpressing metastatic breast cancer that had progressed while receiving trastuzumab. The secondary objective was to evaluate the safety of celecoxib in this patient population.

Intervention. Baseline evaluations within 2 weeks of entering the study included a complete medical history and physical examination, assessment of functional status, complete blood count with differential, platelet count, comprehensive serum chemistries, tumor markers (CA15–3 or BR 27–29, carcinoembryonic antigen), and β-human chorionic gonadotropin in premenopausal patients. Within 4 weeks of entering the study, bone scans and computed tomography imaging were performed as appropriate for disease assessment. Electrocardiogram and multi-gated acquisition scan or echocardiogram were required within 3 months of enrolling in the study. HER-2/*neu* status was determined at study entry using DAKO HercepTest. Subsequently, fluorescent *in situ* hybridization (FISH) analysis was carried out on a subset of specimens that were available.

Patients on trastuzumab continued this therapy while those who had stopped the antibody were restarted on it. In either case, celecoxib was initiated 3 weeks after the last dose of chemotherapy if administered previously. Trastuzumab was given as dosed previously, either as 2 mg/kg i.v. over 30 min weekly or 6 mg/kg i.v. over 90 min every 3 weeks. If at least 2 weeks had elapsed since prior weekly trastuzumab treatment, then it was recommended, but not required, that a reloading dose of trastuzumab at 4 mg/kg i.v. over 90 min be administered, followed by 2 mg/kg i.v. over 30 min weekly. If at least 6 weeks had elapsed since the last dose of trastuzumab (given on a once every 3 week basis), then it was recommended that a reloading dose be given at 8 mg/kg i.v. over 90 min, followed by 6 mg/kg i.v. over 90 min every 3 weeks.

Response and Toxicity Assessment. Every 4 weeks from the initiation of celecoxib/trastuzumab, patients were assessed clinically by history and physical examination, toxicity assessment, tumor measurements (if measurable by physical examination), hematological and chemistry profiles, and tumor markers. Every 3 months from the initiation of therapy, radiographic studies were performed as indicated for disease assessment. Every 3 months, a multi-gated acquisition scan or echocardiogram was performed to evaluate cardiac function, given the potential cardiotoxicity with trastuzumab (31).

A complete response was defined as the disappearance of all clinical and radiographic signs of tumor for at least 4 weeks; a partial response was defined as a >50% reduction in the sum of products of the bi-perpendicular diameters of all measurable lesions with no increase in size of any lesion and no new lesions; a minor response was defined as a 25–49% reduction in the sum of the products of the bi-perpendicular diameters of all measurable lesions; stable disease was a <25% reduction in the sum of the products of the bi-perpendicular diameters of all measurable lesions; and disease progression was defined as a ≥25% increase in size of any lesion or the appearance of any new lesion.

Toxicities were graded according to National Cancer Institute common toxicity scale. If the patient experienced grade 3 toxicity, celecoxib was held for ≤2 weeks until ≤grade 1 toxicity was achieved and was restarted at 50% dose reduction or 200 mg (twice daily). Trastuzumab could be held at the physician's discretion for ≤2 weeks and resumed at full dose. If treatment interruption was >2 weeks, the patient was removed from the study. Therapy was continued until there was evidence of disease progression, unacceptable toxicity, death, major pro-

tocol violations, extraordinary medical circumstances, or patient's withdrawal from study.

Analysis of HER-2 Expression. The HER-2/*neu* status of all specimens was assessed by immunohistochemistry on formalin-embedded tissue. Immunohistochemistry was performed using the DAKO HercepTest (rabbit antihuman HER-2/*neu* polyclonal antibody; DAKO Corp., Carpinteria, CA) according to the manufacturer's instructions. The intensity of membrane staining was evaluated according to the following criteria set forth by the DAKO HercepTest: score 0, no or up to 10% membrane staining; score 1+, partial and/or faint membrane staining present in >10% of tumor cells; score 2+, weak to moderate complete membrane staining present in >10% of tumor cells; and score 3+, strong complete membrane staining present in >10% of tumor cells. Scores 0 and 1+ were considered normal (negative for overexpression) and scores 2+ and 3+ were considered positive for HER-2/*neu* overexpression.

FISH analysis was performed using the PathVysion HER-2/*neu* probe kit (Vysis Inc., Downers Grove, IL) according to the manufacturer's instructions. This system uses a spectrum orange fluorophore-labeled DNA probe for chromosome 17, and 4′,6-diamidino-2-phenylindole counterstain for nuclei. Spectrum orange and green signals were counterstained on 60 cells/case, with a ratio of orange:green signals >2 considered positive.

Statistical Methods. This study used a Simon two-stage design to test the efficacy of the celecoxib/trastuzumab regimen. This plan called for enrollment of 12 patients in the first stage and termination of the study if no patient responded. If at least one response was observed, then an additional 25 patients would be enrolled (for a total of 37). After completion of the trial, if four or more responses were observed (>11%), then the treatment would be declared worthy of further testing in this disease. This design yielded a >0.9 probability of a positive result if the true response rate was >20%. It yielded a >0.9 of a probability of a negative result if the true response rate was <5%. These low estimates of response were selected because the eligible patients were heavily pretreated.

RESULTS

From November 2000 to August 2002, a total of 12 patients were enrolled. Patient characteristics are listed in Table 1. The median number of prior treatments in the metastatic setting was 2 (range 1–6). Five of 12 (42%) patients were HER-2/*neu* 2+ on HercepTest, and 7 of 12 (58%) patients were HER-2/*neu* 3+ on HercepTest. When the study was initiated, FISH analysis was not a requirement for study entry. Nonetheless, FISH analysis was subsequently carried out on the samples that were available. Of the five patients with 2+ HER-2/*neu* overexpression, two had FISH analyses that revealed no evidence of amplification, one had an inconclusive result, and two did not have FISH analysis. Of the seven patients with 3+ HER-2/*neu* overexpression, three had FISH analyses that revealed HER-2/*neu* amplification, one had FISH analysis that was inconclusive, and three did not have FISH analyses.

Of the 12 patients who enrolled, 11 were evaluable for response. Ten of 12 patients were treated with weekly trastuzumab. A total of 107 weekly trastuzumab infusions were delivered, with patients receiving a median of 11 weekly trastuzumab treatments (range of 4–23 treatments). Two patients received trastuzumab treatments every 3 weeks for a total of 10 treatments (5 doses/patient). All patients received celecoxib (400 mg twice daily) as intended except for 1 patient who developed a rash (see below). A pill count was done at each visit to ensure compliance; 98.6% of prescribed doses of celecoxib were taken. There were no clinical responses. The median duration of treatment with trastuzumab/celecoxib was 9 weeks (range of 4–24 weeks). One patient had stable disease at 3 months but had disease progression at 6 months. Another patient was removed from the study after 3 months of treatment, before she was evaluable, because of an unresolved grade 2-maculopapular rash, felt to be related to celecoxib. This patient had a biopsy of the rash that showed spongiotic dermatitis suggesting a drug eruption. This patient continued to receive trastuzumab and the rash resolved, consistent with the conclusion that it was secondary to celecoxib.

Table 1 Patient Characteristics ($N = 12$)

Patient characteristics	No.	%
Age (years)		
Median	55	
Range	40–70	
Karnofsky performance status, %		
Median	90	
Range	80–90	
No. of metastatic sites		
1	3	25
2	8	67
3+	1	8
Prior chemotherapy		
Adjuvant chemotherapy	8	67
Metastatic chemotherapy		
1 prior regimen	5	42
2+ prior regimens	7	58
HER-2/*neu*		
2+ on HercepTest	5	42
3+ on HercepTest	7	58

Therapy was generally well tolerated (Table 2). Serious toxicity was infrequent, with one patient experiencing grade 3 abdominal pain (8%). This patient was removed from the study because of progression of disease at 10 weeks. The majority of toxicities were grade 1 or 2. Grade 1 toxicities included elevated alkaline phosphatase (3 of 12, 25%), fatigue (4 of 12, 33%), mucositis (3 of 12, 25%), diarrhea (2 of 12, 17%), rash (1 of 12, 8%), dry skin (1 of 12, 8%), pruritus (1 of 12, 8%), insomnia (1 of 12, 8%), dyspepsia (1 of 12, 8%), elevated aspartate aminotransferase (1 of 12, 8%), and elevated alanine aminotransferase (1 of 12, 8%). Grade 2 toxicities included rash (2 of 12, 17%), edema (2 of 12, 17%), and bone pain (1 of 12, 8%). There were no deaths on study.

DISCUSSION

Extensive preclinical evidence points to a role for COX-2 in tumor formation, growth, and metastasis. Because a link has been established between the overexpression of HER-2/*neu* and COX-2 in human breast cancer (28–30), this trial was carried out to explore whether adding a selective COX-2 inhibitor to

Table 2 Toxicity profile of trastuzumab and celecoxib ($N = 12$)

Toxicity	NCI[a] Grade (% of patients)				
	1	2	3	4	5
Rash	1 of 12 (8%)	2 of 12 (17%)			
Dry skin	1 of 12 (8%)				
Pruritus	1 of 12 (8%)				
Fatigue	4 of 12 (33%)				
Insomnia	1 of 12 (8%)				
Mucositis	3 of 12 (25%)				
Dyspepsia	1 of 12 (8%)				
Abdominal pain			1 of 12 (8%)		
Bone pain		1 of 12 (8%)			
Diarrhea	2 of 12 (17%)				
Increased AST	1 of 12 (8%)				
Increased ALT	1 of 12 (8%)				
Increased Alk phos	3 of 12 (35%)				
Edema		2 of 12 (17%)			

[a] NCI, National Cancer Institute; AST, aspartate aminotransferase; ALT, alanine aminotransferase; alk phos, alkaline phosphatase.

trastuzumab would be of benefit to patients whose disease had already progressed while receiving trastuzumab. In this context, any observed therapeutic response would be presumed to be secondary to the addition of the selective COX-2 inhibitor. Because of this strong link and because clinical testing for COX-2 is neither reliable nor standardized, we did not test for COX-2 expression on the pathology specimens of patients. Recognizing the potential to miss modest but clinically important activity in this pilot study, any activity in this refractory setting would have provided significant support for larger trials.

In this heavily pretreated patient population, we found no evidence that treatment with celecoxib was beneficial. One potential limitation of this study was the inclusion of patients who had tumors with 2+ staining using the HercepTest and who had no evidence of amplification of HER-2/*neu* on FISH analysis. By 2001, there was strong evidence that patients with HER-2/*neu* 2+ overexpressing tumors were unlikely to benefit from trastuzumab without evidence of amplification by FISH analysis (32–36). However, by then most of the patients had already been enrolled. We did subsequently attempt to do FISH analysis on patient tissue samples that were available. Unfortunately, only three patient samples with HER-2/*neu* 3+ overexpression showed HER-2/*neu* amplification via FISH technique. The remaining patients either had negative or inconclusive results, or samples were not available because HER-2/*neu* testing was done on the primary tissues. Regardless, there was no evidence of response in seven patients who had tumors with HER-2/*neu* 3+ overexpression, including three of seven with proven HER-2/*neu* amplification.

There are other potential explanations for the lack of clinical efficacy. One possibility is that selective COX-2 inhibitors are inactive in the treatment of human breast cancer. However, as discussed below, it is premature to draw this conclusion. Another potential explanation for the lack of efficacy in this trial could be that COX-2 inhibition is cytostatic, not cytocidal. In most preclinical studies, selective COX-2 inhibitors reduce the growth rate of established tumors rather than induce tumor regression (15, 22–24, 26, 37). The design of the current study coupled with the known Gompertzian kinetics of tumor growth would make a modest decrease in the rate of tumor growth undetectable (38). Furthermore, because COX-2 is a downstream effector of the HER-2/*neu* oncogene, inhibiting COX-2 and HER-2/*neu* could be inhibiting the same linear pathway and may not result in any additive or synergistic effects. Another important issue concerns the treatment background of patients who participated in this trial. The likelihood of a therapeutic response is diminished in heavily pretreated patients. Because 58% of the patients in our study had received $\geq$2 regimens for metastatic disease before enrollment, the likelihood of response to subsequent therapy was markedly reduced. Additionally, all patients had to have progressed during prior trastuzumab-based treatments, and thus trastuzumab resistance was a strong possibility. However, preclinical data suggest that resistance to trastuzumab may not be complete and may be reversible (39). Mackey *et al.* (40) demonstrated continued response to trastuzumab-based treatments in patients who had progressed during prior trastuzumab-based regimens, suggesting that clinical resistance to trastuzumab may not be absolute. Despite evidence of resistance to trastuzumab, clinicians frequently continue trastuzumab with a series of chemotherapeutic agents. Given this practice, we elected to continue trastuzumab while testing celecoxib. At the time that the trial was conducted, the standard of care in treating patients with HER-2/*neu*-overexpressing metastatic breast cancer included the combination of chemotherapy and trastuzumab, because a large pivotal trial demonstrated that patients receiving trastuzumab and chemotherapy had a statistically significantly longer time to progression, greater overall response rate, and increased median overall survival rate compared with those patients receiving chemotherapy alone (8). As a consequence, there was a limited number of patients receiving only trastuzumab absent chemotherapy. Thus, we could not successfully accrue to a first line trial of celecoxib and trastuzumab.

It is possible that the results would be different in treatment naïve patients. In support of this idea, Chow *et al.* (41) reported results of a pilot trial of 31 patients who received preoperative celecoxib and 5-fluorouracil, epirubicin, and cyclophosphamide *versus* and 5-fluorouracil, epirubicin, and cyclophosphamide

alone in locally advanced breast cancer. This study suggests that the combination arm could be superior to and 5-fluorouracil, epirubicin, and cyclophosphamide alone in terms of clinical and pathological response rates. Several other trials are currently ongoing to evaluate the combination of celecoxib and an aromatase inhibitor in the neoadjuvant and advanced breast cancer settings. The preliminary results appear promising with an acceptable toxicity profile (42–44). The potential importance of evaluating whether selective COX-2 inhibitors augment the antitumor activity of other agents is underscored by the findings of another recent study involving patients with non-small cell lung cancer. In this study, the addition of celecoxib to neoadjuvant carboplatin/paclitaxel was suggested to enhance overall response (45).

The secondary end point of our trial was safety. Toxicity was acceptable consistent with other reports (45, 46). Grade 3 toxicity (*i.e.,* abdominal pain) was observed in 1 of 12 patients. A second patient developed a rash that resolved with discontinuation of the drug.

On the basis of the results of this study, the combination of celecoxib (400 mg twice daily) plus trastuzumab cannot be recommended for use in women with heavily pretreated breast cancer. Moreover, to our knowledge there are no preclinical breast cancer studies in which the combination of a COX-2 inhibitor and trastuzumab have been found to be more effective than either agent alone. Whether celecoxib will be useful in treatment-naïve patients or in combination with other agents remains to be defined. Interestingly, because COX-2 is expressed in ductal carcinoma *in situ* (16, 17), studies to evaluate the efficacy of a COX-2 inhibitor in the adjuvant treatment of ductal carcinoma *in situ* could also be considered. Although disappointing, the results of the current trial do not diminish the need for, or appropriateness of, additional studies to evaluate whether selective COX-2 inhibitors may have a role in the prevention or treatment of breast cancer.

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