Phase II Study of Weekly Intravenous Trastuzumab (Herceptin) in Patients With HER2/*neu*-Overexpressing Metastatic Breast Cancer

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The HER2/*neu* proto-oncogene is overexpressed in 25% to 30% of patients with breast cancer. Trastuzumab (Herceptin; Genentech, San Francisco, CA), a recombinant humanized monoclonal antibody with high affinity for the HER2 protein, inhibits the growth of breast cancer cells overexpressing HER2. In this phase II study the efficacy and toxicity of weekly administration of trastuzumab was evaluated in 46 patients with metastatic breast cancer whose tumors overexpressed HER2. A loading dose of 250 mg trastuzumab was administered intravenously, which was followed by 10 weekly doses of 100 mg each. Upon completion of this treatment period, patients with no disease progression could receive a weekly maintenance dose of 100 mg. Patients in this trial had extensive metastatic disease, and most had received prior anticancer therapy. Ninety percent of patients achieved adequate serum levels of trastuzumab. Toxicity was minimal, and no antibodies against trastuzumab could be detected. Objective responses were observed in 5 of the 43 evaluable patients, which included 1 complete remission and 4 partial remissions, for an overall response rate of 11.6%. Responses were seen in mediastinum, lymph nodes, liver, and chest wall lesions. Minor responses (seen in 2 patients) and stable disease (14 patients) lasted for a median of 5.1 months. These results demonstrate that trastuzumab is well tolerated and clinically active in patients with HER2-overexpressing metastatic breast cancers who have received extensive prior therapy. The regression of human cancer through the targeting of putative growth factor receptors such as HER2 warrants further evaluation ot trastuzumab in the treatment of breast cancer.

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DURING the last decade, proto-oncogenes encoding growth factors and growth factor receptors have been found to play important roles in the pathogenesis of several human malignancies, including breast cancer.[1] The HER2 gene (also known as *neu* and c-*erb*B-2) encodes a 185-kd transmembrane glycoprotein receptor ($p185^{HER2}$) that has partial homology with the epidermal growth factor receptor. Both receptor molecules have intrinsic tyrosine kinase activity.[2-4] Overexpression of HER2, which occurs in 25% to 30% of human breast cancers,[5,6] is an independent predictor of poor prognosis in patients with primary disease involving axillary lymph nodes.[5,7,8] Several lines of evidence support a direct role for HER2 in the pathogenesis and clinical aggressiveness of HER2-overexpressing tumors: (1) the introduction of HER2 into nonneoplastic cells causes their malignant transformation,[9,10] (2) transgenic mice expressing HER2 develop mammary tumors,[11] (3) HER2 overexpression is common in ductal carcinoma in situ and in associated invasive cancers,[12,13] and (4) antibodies directed at $p185^{HER2}$ can inhibit the growth of tumors and transform cells that express high levels of this receptor.[14-18] The latter observation suggests that $p185^{HER2}$ may be a potential target for the treatment of breast cancer or preinvasive breast lesions because these cells commonly overexpress HER2.

The murine monoclonal antibody (MoAb) 4D5, directed against the extracellular domain of $p185^{HER2}$ (ECD^{HER2}), is a potent inhibitor of growth in vitro and in xenograft models of human breast cancer cells overexpressing HER2.[19-21] Murine antibodies, however, are limited clinically because they are immunogenic. To facilitate further clinical investigations, MoAb 4D5 was humanized. Monoclonal antibody 4D5 was initially derived by immunizing mice with cells expressing high levels of the HER2 gene product, $p185^{HER2}$.[19] Recombinant humanized anti-$p185^{HER2}$ MoAb

trastuzumab (Herceptin; Genentech, San Francisco, CA) was engineered by inserting the complementarity-determining regions of MoAb 4D5 into the framework of a consensus human IgG_1.[22] The resulting MoAb has high affinity for $p185^{HER2}$ (K_d = 0.1 nmol/L), markedly inhibits in vitro and in human xenografts the growth of breast cancer cells containing high levels of $p185^{HER2}$ (Fig 1), and induces antibody-dependent cellular cytotoxicity.[22,23] Trastuzumab was found to be safe and to have dose-dependent pharmacokinetics in two prior phase I clinical trials.

We conducted a phase II study of trastuzumab in patients with metastatic breast cancer. The objectives of the trial were to determine the antitumor activity of trastuzumab in this patient population as well as to further define the toxicity profile and the pharmacokinetics of trastuzumab.

PATIENTS AND METHODS

Patients

Patients eligible for this study were adult women whose metastatic breast carcinomas overexpressed HER2 (see below). All patients had measurable disease, a Karnofsky performance status of at least 60%, and preserved hematologic, liver, renal, and pulmonary function. Patients with lymphangitic pulmonary metastases, history of brain metastases, or bone metastases as the only site of measurable disease were excluded. Chemotherapy or additive hormonal therapy within 3 weeks before study entry (6 weeks for mitomycin C or nitrosoureas) was not permitted. Informed consent was obtained and documented in writing before patient entry into the study.

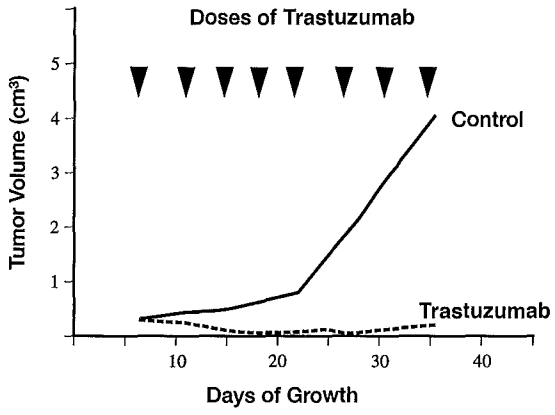

Fig 1. Activity of trastuzumab against well-established BT-474 tumor xenografts. Trastuzumab was given intraperitoneally twice a week for 4 weeks at doses of 1, 10, and 30 mg/kg. The data from the group treated with a dose level of 10 mg/kg is shown here. The control group was treated with a nonspecific humanized immunoglobulin G MoAb at a dose of 30 mg/kg. Trastuzumab doses ≥1 mg/kg markedly suppressed the growth of the HER2-overexpressing BT-474 xenografts.

Tumor expression of HER2 was determined by immunohistochemical analysis, as previously described,[5,6] using a set of thin sections prepared from the patient's paraffin-archived tumor blocks. The primary detecting antibody used was murine MoAb 4D5, which has the same complementarity-determining regions as trastuzumab. Tumors were considered to overexpress HER2 if at least 25% of the tumor cells exhibited characteristic membrane staining for $p185^{HER2}$.

Antibody Administration

The pharmacokinetic goal was to achieve trastuzumab trough serum concentrations exceeding 10 μg/mL, a level associated with optimal inhibition of cell growth in a preclinical model.[22] The optimal dose and schedule of trastuzumab was based on two prior phase I clinical trials, conducted at the University of California, Los Angeles, and at Memorial Sloan-Kettering Cancer Center (New York, NY), which had documented dose-dependent pharmacokinetics. In the current trial, trastuzumab was given intravenously over a period of 90 minutes in the outpatient setting. Each patient received a loading dose of 250 mg trastuzumab on day 0, and beginning on day 7, 100 mg of trastuzumab weekly for a total of 10 doses. At the completion of this treatment period, patients having stable disease or minor, partial, or complete responses were entered on a maintenance phase of weekly trastuzumab administration until disease progression.

Pharmacokinetics, Determination of Extracellular Domain of $p185^{HER2}$ Levels, and Antibodies Directed Against Recombinant Human Monoclonal Antibody HER2

Blood samples for pharmacokinetic analysis were collected just before each treatment with trastuzumab and within the first hour following the end of each trastuzumab infusion. Serum concentrations of trastuzumab were determined in an assay that detects binding to ECD^{HER2}. The nominal limit of detection for trastuzumab in serum samples was 156 ng/mL. The presence of antibodies to trastuzumab was determined with a bridging-type titer enzyme-linked immunoassay. Circulating concentrations of ECD^{HER2} shed by the patient's tumors were also determined using enzyme-linked immunoassay.[24] The pair of antibodies used for the assay were 7C2, as capture antibody, and 2C4, as horseradish peroxidase-conjugated antibody to detect the bound complex; the lower limit of detection for this assay ranged from 2.8 to 8.3 ng/mL.

Serum levels of trastuzumab as a function of time were analyzed for each patient using a one-compartment model. Model parameters (volume and the elimination rate constant, K_e) were estimated for each patient using a maximum likelihood estimation procedure.[25] Trastuzumab half-life ($t_{1/2}$) was calculated by dividing ln2 by K_e.

Evaluation of Toxicity and Response

Toxicity was scored based on modified National Cancer Institute Common Toxicity Criteria. Complete blood cell count, urinalysis, coagulation profile, and hepatic enzyme, renal, and electrolyte studies were performed weekly while on the study.

Tumor response was determined at the completion of the initial 11-week treatment period. All responses were confirmed

by an independent extramural evaluation committee composed of an oncologist and a radiologist. A complete response was defined as disappearance of all radiographically and/or visually apparent tumor; partial response as a reduction of at least 50% in the sum of the products of the perpendicular diameters of all measurable lesions; minimal response as a reduction of at least 25% and less than 50% in the diameters; stable disease as no change of greater than 25% in the size of measurable lesions; and progressive disease as an increase of 25% or more in any measurable lesion or the appearance of any new lesion. Although bone metastases were not considered measurable for response, patients were required to have at least stable bone lesions to be considered responders. Patients who had entered the maintenance phase of the study had their tumor responses evaluated every 11 weeks or earlier if clinically indicated. Time to tumor progression was calculated from the beginning of therapy to disease progression. The confidence limits for response rates were calculated using the exact method for a single proportion.[26]

RESULTS

Forty-six patients were enrolled in the study. Patient characteristics are shown in Table 1. Their level of tumor overexpression of HER2 was relatively high, with more than 80% of the tumors exhibiting positive membrane staining in more than half of the cells. Our patient population had extensive metastatic disease: 34.5% of the patients had three or more metastatic sites. Dominant sites of metastases were visceral in 80% of cases (lung in 18 cases, liver in 13 cases, both liver and lung in five cases, and ovary in one case). Only 17.4% of cases had dominant metastases in soft tissues (skin, lymph nodes), and one patient had bone as the dominant site of disease. The total number of patients with bone disease was 18 (39%). All but one of the patients had received prior chemotherapy, with 82.6% having received at least one regimen for metastatic disease and 63% having received two or more regimens. Of this latter group, four patients had previously received high-dose chemotherapy with hematopoietic stem cell support.

Data on trastuzumab pharmacokinetics are available from 45 patients (Table 2). Over 90% of the examined population (41 patients) had trastuzumab trough levels above the targeted 10 μg/mL level. The mean serum half-life of trastuzumab was 8.3 $\pm$ 5.0 days (mean $\pm$ S.D.). As shown in Table 2, trastuzumab serum half-life was found to depend on the presence of circulating ECD^{HER2} released from the tumor into the serum. Antibodies against trastuzumab (human antihuman antibodies) were not detected in any patients.

Treatment with trastuzumab was remarkably well tolerated (Table 3). Of 768 administrations of trastuzumab, only 11 events occurred that were considered to be related to the use of the antibody. Fever and chills occurred on five occasions after administration of the first dose of trastuzumab. The fever lasted less than 8 hours in all cases and did not recur on subsequent administrations of the antibody. Three patients experienced chest pain in areas of tumor involvement shortly after infusion of the first dose of trastuzumab, requiring an overnight admission to the hospital for pain control in one case. The pain did not recur on successive administrations of the antibody. None of the patients whose cancer regression met the formal criteria for complete or partial response had pain at a tumor site after administration of trastuzumab.

The number of patients evaluable for treatment response on evaluation day 77 was 43 (Table 4).

Table 1. Patient Characteristics

	Patients, n = 46 (%)
Level of HER2 expression*	
25% to 50% of cells	7 (15.2)
>50% of cells	39 (84.8)
Receptor status	
Estrogen receptor-positive (n = 40)	17 (42.5)
Progesterone receptor-positive (n = 39)	15 (38.5)
No. of metastatic sites	
1	16 (34.5)
2	14 (30.4)
≥3	16 (34.5)
Dominant site of metastasis	
Viscera	37 (80.4)
Skeleton	1 (2.2)
Soft tissues	8 (17.4)
Prior therapy	
Chemotherapy	45 (97.8)
Adjuvant chemotherapy	26 (56.5)
Neoadjuvant chemotherapy	4 (8.7)
Metastatic disease†	
None	8 (17.4)
1 regimen	9 (19.6)
2 regimens	9 (19.6)
>2 regimens	20 (43.5)
Hormonal therapy	
Adjuvant tamoxifen	7 (15.2)
Metastatic disease	21 (45.6)

NOTE. The median patient age was 50 years (range, 30 to 65 years). The median Karnofsky performance status was 90 (range, 60 to 100).

* Percent of tumor cells with cytoplasmic membrane staining.

† Median, 2; range, 0 to 7.

Table 2. ECD^{HER2}-Dependent Pharmacokinetics of Trastuzumab

N	Patient Group	Herceptin $t_{1/2}$ (d), Mean ± SD
45	All patients	8.3 ± 5.0
40	Circulating ECD^{HER2} <500 ng/mL	9.1 ± 4.7
5	Circulating ECD^{HER2} >500 ng/mL	1.8 ± 1.0

Abbreviations: $t_{1/2}$, serum half-life; ECD, extracellular domain.

Among the 43 evaluable patients, five had tumor responses: one patient had a complete remission and four had partial remissions. Therefore, the overall response rate (complete plus partial remissions) for evaluable patients was 11.6% (95% confidence interval, 4.36% to 25.9%). Two patients had minor responses and 14 patients had stable disease at day 77. These patients entered a maintenance phase of weekly antibody administration until progression of disease. The median time to progression for patients with either minor or stable disease was 5.1 months. After 2 weeks of treatment, an additional patient had a greater than 50% reduction in the size of metastatic disease on the mediastinum and chest wall. While the duration of response was greater than 4 weeks, by evaluation day 77, the lesion had begun to regrow from the size of maximal response to therapy. Per protocol guidelines, this patient was therefore considered not to have responded to therapy but rather to have had progression of disease.

DISCUSSION

During the last decade, overexpression of the HER2 gene has been shown to play an important role in the pathogenesis and poor prognosis of breast cancer. As a consequence, strategies directed at interfering with HER2 expression or the function of its protein, $p185^{HER2}$, have been anticipated to have therapeutic value. Extensive preclinical studies have shown that certain monoclonal antibodies directed against $p185^{HER2}$ can inhibit growth of HER2-overexpressing tumor cells.[15-19] This study provides the first clinical evidence of the antitumor activity of one of these agents, trastuzumab.

Of 43 patients with $p185^{HER2}$-positive tumors evaluable for response after treatment with trastuzumab, five experienced a complete or partial remission, for an overall response rate of 11.6%. One additional patient had a greater than 50% reduction of cancer lasting over 1 month, but was not considered a responder by our protocol definition. The objective antitumor responses observed were of clinical importance since two patients had regression of cancers in their livers and one patient achieved a pathologically proven complete response of her chest-wall disease, which has persisted for over 48 months. Our patients had many sites of metastatic involvement, which is one of the worst prognostic characteristics regarding response to therapy. This selection was a consequence of entry criteria, which specified that patients with disease involving only their bones were ineligible for accrual because bone is the solitary site of initial metastatic involvement in up to 60% of cases.[27] It will be of interest to determine in follow-up studies whether the response rate to trastuzumab will be higher if patients with less-extensive breast cancer are treated, since laboratory studies have shown that the response to antireceptor antibodies is greater with lower tumor burden.[28] Another relevant question is whether the response rate to trastuzumab will be higher in

Table 3. Trastuzumab-Related Toxicity

	Moderate (Grade 2)	Severe (Grade 3)
Fever and chills	5	
Pain at tumor site	2	1
Diarrhea	2	
Nausea and emesis	1	

NOTE: Administrations of trastuzumab = 768.

Table 4. Response Rate Obtained With Trastuzumab in 43 Evaluable Patients

	N (%)
Complete response	1 (2.3)
Partial response	4 (9.3)
Overall response	5 (11.6)
Minor response	2 (4.6)
Stable disease	14 (32.6)
Progression of disease	22 (51.2)

a patient population with less prior chemotherapy for stage IV disease, since prior experience has shown that untreated patients usually respond better to new anticancer drugs.[29] This issue is currently being addressed in an ongoing clinical trial in which trastuzumab is being given as first-line therapy for metastatic disease (see report by Shak et al in this supplement, pp 71-77).

It is important to note that while not achieving a complete or partial response, 37% of patients in this trial achieved minimal responses or stable disease. In clinical trials of many anticancer drugs, particularly chemotherapy, the achievement of stable disease is not considered a reliable measure of anticancer activity. With trastuzumab, however, stable disease may be an authentic reflection of the biological action of the drug, which differs markedly from conventional anticancer agents. In the laboratory, trastuzumab has been noted to be cytostatic, causing growth arrest, rather than cytocidal, causing cell death. The unusually long durations of minimal responses and stable disease seen in our trial may relate to this distinction. These data are especially interesting in light of the absence of significant toxicity observed here, since in a setting in which palliation is a main objective, quality of life while on treatment should be a main end point.

The dose and schedule of trastuzumab administration used in this protocol provided adequate serum concentrations in all patients, except in those with circulating levels of tumor-shed ECD^{HER2} at serum concentrations greater than 500 ng/mL. ECD^{HER2} is known to be released by some breast cancer cells that overexpress $HER2$[30-32] and elevated ECD^{HER2} serum levels have been reported previously in patients with breast cancer.[31,33,34] Our initial explanation for the short serum half-lives and subtherapeutic trough levels of trastuzumab in this group of patients was that in the presence of ECD^{HER2} in the serum, antigen-antibody complexes form and are rapidly cleared from the circulation. An alternative explanation is that a higher level of ECD^{HER2} in the serum may correlate with a higher tumor burden. However, it is important to notice when analyzing the data presented that our sample population was small and patients had large tumor burdens. Hence, the effects of elevated serum ECD^{HER2} levels on trastuzumab pharmacokinetics and response to therapy must be further defined in larger follow-up studies.

There are several possible mechanisms, which are not mutually exclusive, that could explain the clinical results observed. The first is based on the observation that trastuzumab induces a marked downregulation of $p185^{HER2}$.[19] Antibody-induced downregulation of $p185^{HER2}$ has been shown to induce reversion of the transformed phenotype in HER2-transformed cells.[14] By a similar mechanism, the continuous exposure to trastuzumab at adequate concentrations achieved in our trial could be reversing the malignant phenotype of the clinical cancers by downregulating their level of $p185^{HER2}$. Another possible mechanism of action concerns the observation that trastuzumab is a potent inducer of antibody-dependent cellular cytotoxicity.[22] However, while this immune-mediated mechanism might play a role in the observed clinical responses, antibody-dependent cellular cytotoxicity is obviously not involved in the pronounced growth-inhibitory effects of the antibody in vitro.

The observed activity of trastuzumab against advanced breast cancers overexpressing HER2 provides the first clinical evidence that anti-growth factor receptor-directed strategies may be useful in the treatment of human breast cancer. Therefore, continued research with this agent and other HER2-targeted treatment strategies appears warranted. In preclinical studies both in vitro and in human tumor xenografts, trastuzumab markedly potentiates the antitumor effects of several chemotherapeutic agents, including cisplatin, doxorubicin, and paclitaxel,[23,35] without increasing their toxicity. A recently completed phase III trial has confirmed clinically that the addition of trastuzumab markedly enhances the antitumor activity of doxorubicin and paclitaxel compared with the activity of these agents alone (see Shak et al, pp 71-77). Taken together, these results support the concept that trastuzumab, given alone or in combination with chemotherapy, will be a useful tool for the treatment of patients with HER2-overexpressing breast cancer. Further studies will be needed to establish whether trastuzumab also may be of therapeutic benefit in the adjuvant setting or in patients with a lower degree of HER2 overexpression.

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