Phase II Study of Weekly Intravenous Recombinant Humanized Anti-p185^{HER2} Monoclonal Antibody in Patients With HER2/*neu*-Overexpressing Metastatic Breast Cancer

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Purpose: **Breast cancer frequently overexpresses the product of the HER2 proto-oncogene, a 185-kd growth factor receptor (p185^{HER2}). The recombinant humanized monoclonal antibody (rhuMAb) HER2 has high affinity for p185^{HER2} and inhibits the growth of breast cancer cells that overexpress HER2. We evaluated the efficacy and toxicity of weekly intravenous administration of rhuMAb HER2 in patients with HER2-overexpressing metastatic breast cancer.**

Patients and Methods: **We treated 46 patients with metastatic breast carcinomas that overexpressed HER2. Patients received a loading dose of 250 mg of intravenous rhuMAb HER2, then 10 weekly doses of 100 mg each. Patients with no disease progression at the completion of this treatment period were offered a maintenance phase of 100 mg/wk.**

Results: **Study patients had extensive metastatic disease, and most had received extensive prior anticancer therapy. Adequate pharmacokinetic levels of rhuMAb HER2 were obtained in 90% of the patients. Toxicity was minimal and no antibodies against rhuMAb HER2 were detected in any patients. Objective responses were seen in five of 43 assessable patients, and included one complete remission and four partial remissions (overall response rate, 11.6%; 95% confidence interval, 4.36 to 25.9). Responses were observed in liver, mediastinum, lymph nodes, and chest wall lesions. Minor responses, seen in two patients, and stable disease, which occurred in 14 patients, lasted for a median of 5.1 months.**

Conclusion: **rhuMAb HER2 is well tolerated and clinically active in patients with HER2-overexpressing metastatic breast cancers that had received extensive prior therapy. This is evidence that targeting growth factor receptors can cause regression of human cancer and justifies further evaluation of this agent.**

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DURING THE LAST DECADE, proto-oncogenes that encode 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 as *c-erbB*-2) encodes a 185-kd transmembrane glycoprotein receptor (p185^{HER2}) that has partial homology with the epidermal growth factor receptor, and that shares with that receptor intrinsic tyrosine kinase activity.[2-4] HER2 is overexpressed in 25% to 30% of human breast cancers[5,6] and predicts for a worse prognosis in patients with primary disease that involves 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: The introduction of HER2 into non-neoplastic cells causes their malignant transformation.[9,10] Transgenic mice that express HER2 develop mammary tumors.[11] HER2 overexpression is common in ductal carcinomas in situ and in their associated invasive cancers.[12,13] Antibodies directed at p185^{HER2} can inhibit the growth of tumors and of transformed 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 (MAb) 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 that overexpress HER2.[19-21] However, murine antibodies are limited clinically because they are immunogenic. To facilitate further clinical investigations, therefore, MAb 4D5 was humanized. The resulting recombinant humanized anti-p185^{HER2} monoclonal antibody (rhuMAb HER2) was found to be safe and to have dose-dependent pharmacokinetics in two prior phase I clinical trials.

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We now report the results of a phase II study of multiple-dose intravenous administration of rhuMAb HER2 in patients with metastatic breast cancer. The objectives of this trial were to determine the antitumor activity of rhuMAb HER2 in this patient population, as well as to define further the toxicity profile and pharmacokinetics of rhuMAb HER2.

PATIENTS AND METHODS

Preparation and Humanization of rhuMAb HER2 Antibody

MAb 4D5 was initially derived by immunizing mice with cells that expressed high levels of the HER2 gene product, $p185^{HER2}$.[19] MAb 4D5, directed at the extracellular domain of $p185^{HER2}$ (ECD^{HER2}), inhibits the in vitro growth of breast cancer cells that contain high levels of $p185^{HER2}$.[19,20] rhuMAb HER2 was engineered by inserting the complementarity determining regions of MAb 4D5 into the framework of a consensus human immunoglobulin G_1 (IgG_1).[22] The resulting rhuMAb HER2 has high affinity for $p185^{HER2}$ (Dillohiation constant [K_d] = 0.1 nmol/L), markedly inhibits, in vitro and in human xenografts, the growth of breast cancer cells that contain high levels of $p185^{HER2}$, and induces antibody-dependent cellular cytotoxicity (ADCC).[22,23] rhuMAb HER2 is produced by a genetically engineered Chinese hamster ovary (CHO) cell line, grown in large scale, that secretes rhuMAb HER2 into the culture medium. Antibody is purified from the CHO culture media using standard chromatographic and filtration methods. Each lot of antibody used in this study was assayed to verify identity, purity, and potency, as well as to meet Food and Drug Administration requirements for sterility and safety.

Selection of Patients

Patients eligible for this study were adult women whose metastatic breast carcinomas overexpressed HER2 (see later). All patients had measurable disease, a Karnofsky's performance status of at least 60%, and preserved hematologic, liver, renal, and pulmonary function. Patients with lymphangitic pulmonary metastasis, history of brain metastasis, 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 or nitrosureas) was not permitted. Informed consent was obtained and documented in writing before study entry.

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

Antibody Administration

The pharmacokinetic goal was to achieve rhuMAb HER2 trough serum concentrations greater than 10 μg/mL, a level associated with optimal inhibition of cell growth in the preclinical model.[22] The optimal dose and schedule of rhuMAb HER2 was based on two prior phase I clinical trials, conducted at University of California, Los Angeles, and Memorial Sloan-Kettering Cancer Center, which had documented dose-dependent pharmacokinetics. In this current trial, rhuMAb HER2 was administered intravenously over a period of 90 minutes in the outpatient setting. Each patient received a loading dose of 250 mg of rhuMAb HER2 on day 0, and beginning on day 7,100 mg weekly for a total of 10 doses. At the completion of this treatment period, patients with stable disease or minor, partial, or complete responses were entered onto a maintenance phase of weekly rhuMAb HER2 administration until disease progression.

Evaluation of Toxicity

Toxicity was scored based on a modified National Cancer Institute common toxicity criteria. Complete blood cell counts, urinalysis, coagulation profile, and hepatic enzyme, renal, and electrolyte studies were performed weekly while on the study.

Pharmacokinetics, Determination of Extracellular Domain of $p185^{HER2}$ Levels, and Antibodies Directed Against rhuMAb HER2

Blood samples for pharmacokinetic analysis were collected just before each treatment with rhuMAb HER2 and within the first hour following the end of each rhuMAb HER2 infusion. Serum concentrations of rhuMAb HER2 were determined in a receptor binding assay that detects binding with ECD^{HER2}. The nominal limit of detection for rhuMAb HER2 in serum samples was 156 ng/mL. The presence of antibodies to rhuMAb HER2 was determined with a bridging-type titer enzyme-linked immunosorbent assay (ELISA). Circulating concentrations of ECD^{HER2} shed by patients' tumors were also determined using an ELISA.[24] The pair of antibodies used for the assay were 7C2 as coat and 2C4 as horseradish peroxidase–conjugated antibody; the lower limit of detection for this assay ranged from 2.8 to 8.3 ng/mL (Baly D, Wong WL, unpublished data, November 1994).

Serum levels of rhuMAb HER2 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] rhuMAb HER2 half-life ($t_{1/2}$) was calculated by dividing ln 2 by K_e.

Tumor Response

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. Complete response was defined as the disappearance of all radiographically and/or visually apparent tumor, partial response as a $\geq$ 50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions, minimal response as a $\geq$ 25% and less than 50% reduction in the diameters, stable disease as no change greater than 25% in the size of measurable lesions, and progressive disease as a $\geq$ 25% increase in any measurable lesion or the appearance of any new lesion. Although bone metastases were considered not measurable for response, patients had to have at least stability of bone lesions to be considered responders. Patients who had entered the maintenance phase of the study had tumor responses evaluated every 11 weeks, or earlier if clinically indicated. Time to tumor progression was calculated from the beginning of therapy to progression. Confidence limits for response rates were calculated using the exact method for a single proportion.[26]

Table 1. Patient Characteristics

Characteristic	Patients (N = 46) No.	%
Age, years		
Median	50	
Range	30-65	
Karnofsky performance status		
Median	90	
Range	60-100	
Level of HER2 expression*		
25%-50% cells	7	15.2
> 50% 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 (no. of regimens)		
None	8	17.4
1	9	19.6
2	9	19.6
> 2	20	43.5
Median	2	
Range	0-7	
Hormonal therapy		
Adjuvant tamoxifen	7	15.2
Metastatic disease	21	45.6

*In percent of tumor cells with cytoplasmic membrane staining.

RESULTS

Patients characteristics are listed in Table 1. A total of 46 patients were enrolled onto the study. Their level of tumor overexpression of HER2 was relatively high, with more than 80% of the tumors having more than half of their cells exhibit positive membrane staining. Our patient population had extensive metastatic disease: 34.5% of patients had three or more metastatic sites. Dominant sites of metastases were visceral in 80% of cases (lung in 18, liver in 13, both liver and lung in five, and ovary in one). Only 17.4% of cases had dominant metastases in soft tissues (skin and lymph nodes) and only 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 rhuMAb HER2 pharmacokinetics are available from 45 patients (Table 2). More than 90% of the examined population (41 patients) had rhuMAb HER2 trough levels above the targeted 10-μg/mL level. The mean serum $t_{1/2}$ of rhuMAb HER2 was 8.3 ± 5.0 days. The rhuMAb HER2 serum $t_{1/2}$ was found to be dependent on the presence of circulating ECD^{HER2} released from the tumor into the serum (Table 2). Representative examples of pharmacokinetics profiles are shown in Fig 1. Figure 1A shows the serum levels of rhuMAb HER2 in a patient with undetectable level of circulating ECD^{HER2}; stable, therapeutic serum levels of the drug were maintained in this patient for more than 1 year. Figure 1B shows the serum levels of rhuMAb HER2 in a patient with high levels of circulating ECD^{HER2}; trough levels of rhuMAb HER2 were consistently below detectable levels throughout the treatment course and until disease progression. Antibodies against rhuMAb HER2 (human antihuman antibodies [HAHA]) were not detected in any patients.

Treatment with rhuMAb HER2 was remarkably well tolerated. Of a total of 768 administrations of rhuMAb HER2, only 11 events occurred that were considered to be related to the use of the antibody (Table 3). Fever and chills occurred on five occasions after the first administration of rhuMAb HER2. 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 the infusion of the first dose of rhuMAb HER2; in one case this required an overnight hospital admission for pain control. 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 rhuMAb HER2.

The number of patients assessable for treatment response on evaluation day 77 was 43. Three patients were not assessable for response. One had a bacteremic infection of an intravenous catheter that required prolonged administration of antibiotics, which precluded treatment

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

N	Patient Group	rhuMAb HER2 $t_{1/2}$ (days)
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

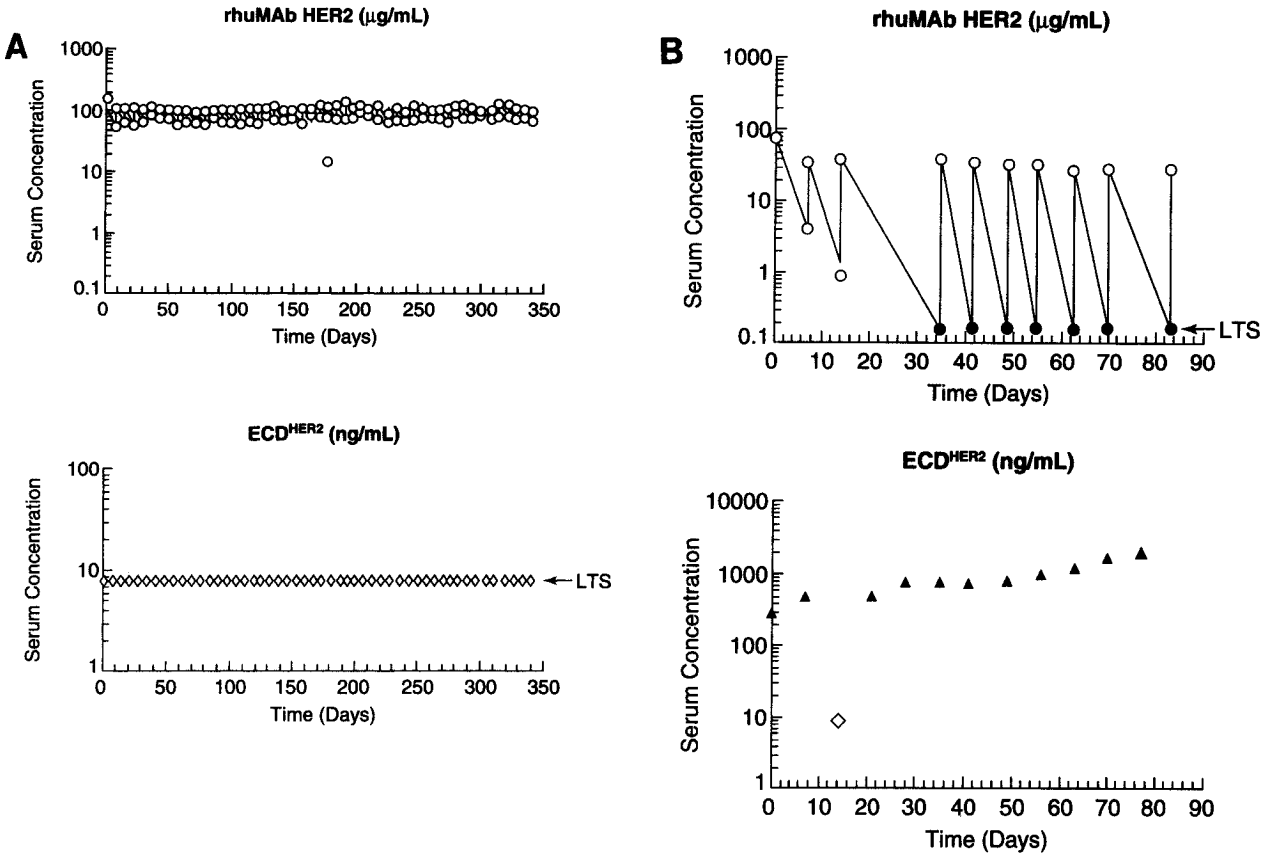

Fig 1. Effect of serum ECD^{HER2} on rhuMAb HER2 pharmacokinetics. Stable serum levels of rhuMAb HER2 in a patient with absence of ECD^{HER2} (A) *v* suboptimal rhuMAb HER2 serum levels in a patient with high ECD^{HER2} (B). Note that log scales on the Y-axis describing the serum ECD^{HER2} differ among charts. LTS, less than lowest assay standard. (○) observed rhuMAb HER2 serum concentration; (●) LTS for rhuMAb HER2 serum concentration; (▲) ECD^{HER2} serum concentration; (◇) LTS for ECD^{HER2} serum concentration.

with rhuMAb HER2. A second declined to continue on the study for personal reasons. The third died of congestive heart failure associated with prior doxorubicin treatment. Among 43 assessable patients, 5 had tumor responses: one patient had a complete remission and four had partial remissions. Therefore, the overall response rate (complete plus partial responses) for assessable patients is 11.6% (95% confidence interval, 4.36 to 25.9). Details of responses are listed in Tables 4 and 5, and examples of the responses are shown in Fig 2.

Table 3. rhuMAb HER2-Related Toxicity

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

NOTE. In number of events of a total of 768 administrations.

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 the patients with either minor or stable disease was 5.1 months. An additional patient had a greater than 50% reduction in the size of the metastatic disease on her mediastinum and chest wall after 2 weeks of treatment. 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 had a response to therapy, but rather 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

Table 4. Response Rate Obtained With rhuMAb HER2 in 43 Assessable Patients

Response	No. of Patients	%
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

consequence, strategies directed at interference 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 MAbs 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, rhuMAb HER2.

Of 43 patients with $p185^{HER2}$-positive tumors assessable for response after treatment with rhuMAb HER2, five experienced a complete or partial remission, for an overall response rate of 11.6%. One additional patient had a greater than 50% shrinking of her cancer that lasted more than 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 the liver and one patient achieved a pathologically-proven complete response of chest wall disease, which has persisted for 24 months. Our patients were selected to have many sites of metastatic involvement, one of the most dire prognostic characteristics regarding response to therapy. This selection was the consequence of the rule that patients with disease involving only bone were ineligible for accrual, because bone is the solitary site of initial metastatic involvement in up to 60% of cases.[27] It is reasonable to hypothesize that the percentage of patients who show objective tumor regression to rhuMAb HER2 will be higher when 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] It would be also of interest to analyze the response rate to rhuMAb HER2 in a patient population with no prior chemotherapy for stage IV disease, since prior experience has shown that untreated patients usually respond better to new anticancer drugs.[29]

Another important point about the probability of response to rhuMAb HER2 concerns the observation that 37% of patients achieved minimal responses or stable disease. In the laboratory, rhuMAb HER2 or the parent antibody 4D5 has been noted to be cytostatic, which causes growth arrest, rather than cytocidal, which causes cell death. In clinical trials of many anticancer drugs, particularly chemotherapy, the achievement of stable disease is not considered a reliable measure of anticancer activity. However, with rhuMAb HER2, stable disease may be an authentic reflection of the biologic action of the drug, which differs markedly from conventional anticancer agents. The unusually long durations of minimal responses and stable disease seen in our trial may relate to this distinction. These data are specially interesting in light of the absence of significant toxicity observed here, for 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 rhuMAb HER2 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 ≥

Table 5. Characteristics of Patients Who Achieved a Response to Treatment

Patient No.	HER2*	Site of Metastatic Disease	Prior Systemic Therapy	Best Response	Duration of Response (months)
1	3+	Chest wall	Doxorubicin	Complete response†	> 24
2	3+	Liver	Doxorubicin, mitoxantrone, paclitaxel	Partial response	6.7
3	2+	Mediastinum	CMFVP, doxorubicin, tamoxifen, paclitaxel	Partial response	7.7
4	3+	Liver + retroperitoneal lymph nodes + bone	CMF, docetaxel	Partial response	1
5	2+	Chest wall	Paclitaxel	Partial response	3.4

Abbreviations: CMFVP, cyclophosphamide, methotrexate, fluorouracil, vincristine, and prednisone; CMF, cyclophosphamide, methotrexate, and fluorouracil.

*By immunohistochemistry: 2+, 25% to 50% of tumor cells with cytoplasmic membrane staining; 3+, > 50% of tumor cells with cytoplasmic membrane staining.

†Patient's complete response was pathologically proven with several biopsies at tumor site. Patient bone scan, head, thoracic, abdominal, and pelvic computed tomographic scans are negative.

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