Phase II Study of Receptor-Enhanced Chemosensitivity Using Recombinant Humanized Anti-p185$^{HER2/neu}$ Monoclonal Antibody Plus Cisplatin in Patients With HER2/*neu*-Overexpressing Metastatic Breast Cancer Refractory to Chemotherapy Treatment

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Purpose: To determine the toxicity, pharmacokinetics, response rate, and response duration of intravenous (IV) administration of recombinant, humanized anti-p185^{HER2} monoclonal antibody (rhuMAb HER2) plus cisplatin (CDDP) in a phase II, open-label, multicenter clinical trial for patients with HER2/*neu*-overexpressing metastatic breast cancer.

Patients and Methods: The study population consisted of extensively pretreated advanced breast cancer patients with HER2/*neu* overexpression and disease progression during standard chemotherapy. Patients received a loading dose of rhuMAb HER2 (250 mg IV) on day 0, followed by weekly doses of 100 mg IV for 9 weeks. Patients received CDDP (75 mg/m^2) on days 1, 29, and 57.

Results: Of 37 patients assessable for response, nine (24.3%) achieved a PR, nine (24.3%) had a minor response or stable disease, and disease progression occurred in 19 (51.3%). The median response duration was 5.3 months (range, 1.6-18). Grade III or IV toxicity was observed in 22 of 39 patients (56%). The toxicity profile reflected that expected from CDDP alone with the most common toxicities being cytopenias (n = 10), nausea/vomiting (n = 9), and asthenia (n = 5). Mean pharmacokinetic parameters of rhuMAb HER2 were unaltered by coadministration of CDDP.

Conclusion: The use of rhuMAb HER2 in combination with CDDP in patients with HER2/*neu*-overexpressing metastatic breast cancer results in objective clinical response rates higher than those reported previously for CDDP alone, or rhuMAb HER2 alone. In addition, the combination results in no apparent increase in toxicity. Finally, the pharmacology of rhuMAb HER2 was unaffected by coadministration with CDDP.

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THE HER2/*neu* GENE encodes a 185-kd transmembrane protein that is a member of the type I family of growth factor receptors. Amplification of this gene is found in approximately 25% of human breast cancers and results in overexpression of the 185-kd encoded receptor tyrosine kinase, which is homologous to the epidermal growth factor receptor (EGFR). Overexpression of p185$^{HER2/neu}$ is an independent predictor of both relapse-free and overall survival in patients with breast cancer.[1-4] In addition, overexpression of this gene has prognostic significance in patients with ovarian,[2] gastric,[5] endometrial,[6] and salivary gland malignancies.[7] In breast cancer, overexpression of HER2/*neu* is also associated with a number of other adverse prognostic factors that include advanced pathologic stage,[4] number of metastatic axillary lymph nodes,[2] absence of estrogen and progesterone receptors,[8] increased S-phase fraction,[9] DNA ploidy,[10] and high nuclear grade.[11] A role for the HER2/*neu* alteration in metastasis has also been suggested given the increased occurrence of visceral metastasis[12] and micrometastatic bone marrow disease in patients with HER2/*neu* overexpression.[13] Like many other cell-surface receptors, a soluble form of the extracellular domain (ECD) of p185$^{HER2/neu}$ can be shed from the surface of tumor cells and is detectable in the sera of experimental animals that bear HER2/*neu*-overexpressing xenografts, as well as in the sera of approximately 20% to 25% of patients with locally advanced or metastatic breast cancer.[14-16] Patients with elevated serum levels of shed HER2/*neu* ECD have a decreased response to hormonal therapy and shortened overall survival compared with patients without shed HER2/*neu* ECD.[14,16]

A murine monoclonal anti-HER2 antibody, 4D5, known to have antiproliferative activity against HER2/*neu*-overex-

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pressing human breast carcinoma cells in vitro and against breast cancer xenografts with HER2/*neu* overexpression in vivo, was humanized, which resulted in a human immunoglobulin (IgG1) molecule with retained murine sequences only in the complementarity determining regions. The resultant molecule has improved binding affinity to the extracellular domain of HER2/*neu* (kd = 0.1 nM *v* 0.3 nM for murine 4D5) and similar growth inhibitory activity against HER2/*neu*-overexpressing cell lines and xenografts.[17]

Previous work has shown that treatment with monoclonal antibodies directed against EGFR in combination with the cytotoxic drug cisplatin (CDDP) resulted in a marked reduction in both size and number of human epidermoid carcinoma xenografts that overexpressed EGFR.[18] Using a similar experimental approach, we have shown a synergistic, cytocidal effect against cell lines and xenografts with HER2/*neu* overexpression by using monoclonal anti-HER2/*neu* antibodies plus CDDP.[19] The mechanism of this effect appears to involve a decreased capacity of HER2/*neu*-overexpressing cells to repair CDDP-induced DNA adducts after pretreatment with anti-HER2/*neu* antibodies.[19-21] This activity, which we have termed receptor-enhanced chemosensitivity (REC), has potential clinical application based on the fact that (1) the dose-effect relationship of the anti-HER2/*neu* antibody plus CDDP is synergistic, (2) this synergistic effect is specific for cells that overexpress the HER2/*neu* receptor, (3) the combination of CDDP plus anti-HER2/*neu* antibody results in a two-log increase in cell killing, and (4) the combination yields pathologic complete remissions against HER2/*neu*-overexpressing human breast carcinoma xenografts in athymic mice.[19]

Pursuant to these preclinical observations, a series of phase I clinical trials were initiated and conducted at the University of California at Los Angeles to determine the safety and pharmacology of the murine monoclonal antibody 4D5, as well as the recombinant, humanized anti-p185^{HER2} antibody monoclonal (rhuMAb HER2), both alone and in combination with CDDP. These studies showed that the pharmacokinetics of rhuMAb HER2 were predictable, and that the doses delivered achieved a target trough serum concentration of 10 to 20 μg/mL, which is associated with antitumor activity in preclinical models. In addition, administration of this anti-HER2/*neu* antibody was safe; the only toxicity was low-grade fever that occurred with the first infusion and/or pain at the site of known tumor deposits in a minority of patients. Moreover, these studies showed that rhuMAb HER2 was not immunogenic in contrast to murine monoclonal antibody 4D5. Finally, the phase I studies showed that the combination of rhuMAb HER2 and CDDP showed significant antitumor efficacy, with four of 15 patients who achieved objective responses, which included three partial responses and one sustained complete remission that lasted in excess of 5.5 years without subsequent treatment. Based on these findings, we designed the current phase II trial with the following objectives: (1) to determine the overall response rate and response duration of intravenous (IV) rhuMAb HER2 plus CDDP in an open-label, multicenter clinical trial for patients with HER2/*neu*-overexpressing metastatic breast cancer who have shown disease progression while undergoing standard chemotherapy treatment; (2) to document the tolerance and toxicity of rhuMAb HER2 plus CDDP; and (3) to determine the pharmacokinetics of rhuMAb HER2 when administered in combination with CDDP.

PATIENTS AND METHODS

Eligibility Criteria

Women aged from 18 to 75 years with a primary histologic diagnosis of invasive breast cancer, with radiographically or visually measurable and assessable metastatic disease documented by physical examination or radiographic findings, were considered for enrollment. Patients were required to have evidence of overexpression (2 + to 3 +) of the HER2/*neu* proto-oncogene in their malignant cells as determined by immunohistochemical analysis (Roche Biomedical Laboratories, Research Triangle Park, NC), and were required to have documentation of objective tumor progression while receiving active chemotherapy for breast cancer. No therapy of any kind (cytotoxic, cytokine, or hormonal) was allowed within the 3 weeks before study entry. In addition, no therapy with mitomycin or nitrosoureas was allowed within 6 weeks of study entry. A Karnofsky performance status (KPS) greater than 60%; life expectancy of 3 months or greater; normal serum calcium level ($\leq$ 10.5 mg/dL); and preserved cardiac, renal (serum creatinine level $\leq$ 1.5 mg/dL, creatinine clearance $\geq$ 60 mL/min, $\leq$ 2 + proteinuria), hepatic (bilirubin level $\leq$ 1.5 mg/dL), pulmonary (forced expiratory volume in 1 second $\geq$ 70% of predicted value), hematologic (WBC count $\geq$ 3,000/μL, granulocyte count $\geq$ 1,500/μL, platelet count $\geq$ 125,000/μL), and coagulation (prothrombin time < 14 seconds, partial thromoplastin time < 35 seconds) function were all required. All patients signed a written, internal review board–approved, informed consent document. Patients were excluded for active infection, pregnancy or lactation, significant cardiac disease (New York Heart Association class III or IV), known hemorrhagic diathesis, hepatic metastases that involved greater than 50% of the liver parenchyma, lymphangitic pulmonary metastasis, CNS metastasis, bone-only metastasis, prior treatment with CDDP or other cisplatin analogues, previous therapy with a monoclonal or polyclonal antibody, or concomitant use of any investigational agent.

Study Design

Eligible patients received a 250-mg loading dose of rhuMAb HER2 IV day 0, followed by 100 mg IV weekly for a total of eight doses. Patients also received CDDP 75 mg/m^2 day 1 of treatment, with repeat doses on days 29 and 57. Clinical response was assessed on day 70. Responsive patients or patients with stable disease were eligible for entry onto a maintenance phase program after day 70. The maintenance phase protocol consisted of rhuMAb HER2 100 mg IV weekly plus CDDP 75 mg/m^2 IV every 4 weeks until disease progression or prohibitive toxicity ensued.

Treatment Plan

A baseline pretreatment evaluation that included a complete history and physical examination, 12-lead ECG, chest radiograph, serum pregnancy test, complete blood count, urinalysis, creatinine clearance, serum chemistries (which included hepatic function tests), coagulation studies, hepatitis serologies, audiologic testing, pulmonary function tests, and baseline tumor measurements was performed within 2 weeks before study entry. Study patients were monitored weekly by physical examination, complete blood counts, serum chemistries, and coagulation studies. All rhuMAb HER2 doses were administered in 250 mL of 0.9% sodium chloride solution infused IV over 90 minutes. Vital signs were recorded before each dose, at the end of the infusion, and 1 hour postinfusion. Serum samples were collected just before and 1 hour after each rhuMAb HER2 dose for pharmacokinetic analysis of rhuMAb HER2, presence of shed p185^{HER2} ECD, and detection of anti–rhuMAb HER2 antibodies. All CDDP doses were administered 1 day after scheduled rhuMAb HER2 doses, consisted of CDDP 75 mg/m^2 diluted in 500 mL of 0.9% sodium chloride solution, and were administered IV over 60 minutes after hydration with a minimum of 500 mL of 5% dextrose/0.9% sodium chloride solution. After CDDP administration, patients received an additional 500 mL of 5% dextrose/0.9% sodium chloride solution. Additional hydration, mannitol, furosemide, and electrolyte solutions were administered as medically indicated. Antiemetic therapy consisted of dexamethasone 20 mg IV before CDDP administration and ondansetron 0.15 mg/kg IV before CDDP administration and 1.5 and 3.5 hours after the CDDP infusion. A graded toxicity scale based on the modified National Cancer Institute criteria was used to assess toxicity. Dose modification of CDDP to 50% of the original dose was performed for grades I to II nephrotoxicity or grades III to IV gastrointestinal toxicity. For any other grades III to IV toxicity, treatment was reinstituted at doses of CDDP of 50 mg/m^2 and rhuMAb HER2 of 50 mg IV after resolution of toxicity. Response criteria were defined as follows: complete response, disappearance of all radiographically and/or visually apparent tumor; partial response, reduction of at least 50% in the sum of the products of the perpendicular diameters of all measurable lesions with no new lesions detected; minor response, a reduction of 25% to 49% in the sum of the products of the perpendicular diameters of all measurable lesions with no new lesions detected; stable disease, not meeting the criteria for response or progression; and progressive disease, objective evidence of an increase of 25% in any measurable lesion or the appearance of any new lesion. All objective responses were assessed by an independent response evaluation committee comprised of a medical oncologist and radiologist who were otherwise not involved in the conduct of this study.

*Detection of HER2/*neu *Oncogene Overexpression in Clinical Tissue Specimens*

Patterns of HER2/*neu* expression were evaluated by a modification of published immunohistochemical techniques that used a murine monoclonal antibody (4D5) directed against HER2/*neu*.[2,22] Four-micron sections from formalin-fixed, paraffin-embedded tissues were cut and mounted on positively charged slides. Tissue sections were deparaffinized and endogenous peroxidase activity was quenched with 1% hydrogen peroxide in methanol. Sections were digested with 1 mg/mL of protease in phosphate buffered saline (PBS) and allowed to incubate with horse serum to block nonspecific antibody binding. Primary antibody (4D5; Genentech, Inc, South San Francisco, CA) was applied (10 μg/mL) and sections were allowed to incubate at 4°C for 18 hours. Sections were washed with PBS and treated with a biotinylated antimouse secondary antibody (Vector Laboratories, Inc, Burlingame, CA). After rinsing with PBS, sections were incubated with avidin-biotinylated enzyme complex (Vector Laboratories, Inc). Sections were then rinsed in PBS, and antibody binding was detected by staining with a diaminobenzidine/hydrogen peroxide chromogen solution. Sections were rinsed in deionized water, counterstained in Harris hematoxylin, dehydrated through graded alcohols, cleared in xylene, and coverslipped. The scoring system for interpretation of HER2/*neu* immunostaining is as follows: 0, 10% or less of tumor cells show any level of positive staining; 1 +, barely perceptible light membranous rimming that may not totally encircle the cell membrane; 2 +, light to moderate membranous rimming that totally encircles the membrane; and 3 +, moderate to strong membrane rimming that totally encircles the membrane.

Pharmacokinetics of rhuMAb HER2

The concentration of rhuMAb HER2 in serum was measured by means of an enzyme-linked immunosorbent assay (ELISA) with the ECD of p185^{HER2} as the coat antigen. In this ELISA format, 100 μL of p185^{HER2} (Genentech, Inc) was added to MaxiSorp 96-well microtiter plates (Nunc, Roskilde, Denmark) at 1 mg/mL in 0.05 mol/L of sodium carbonate, pH 9.6. After overnight incubation at 2° to 8°C, the plates were washed three times with ELISA wash buffer (PBS that contained 0.05% Tween-20) using a Biotek EL304 platewasher (Bio-tek Instruments, Inc, Winooski, VT). The plates were then blocked with 200 μL per well of ELISA diluent (PBS that contained 0.5% bovine serum albumin [BSA]; 0.05% Tween-20; and 0.05% Proclin300, pH 7.2) for 1 to 2 hours at ambient temperature with agitation. After blocking, plates were washed again three times with ELISA wash buffer. Subsequently, 100 μL of standards, samples, or controls were added to duplicate wells and allowed to incubate for 1 hour at ambient temperature. The standard curve range for the assay is 1.56 to 100 μg/mL. After the sample/standard incubation, the plates were washed six times with ELISA wash buffer and 100 μl of goat antihuman IgG Fc-horseradish peroxidase (HRP), freshly diluted to its optimal concentration in ELISA diluent, was added to the plates. After a 1-hour incubation, the plates were washed six times in ELISA wash buffer and 100 μL of PBS, pH 7.2, that contained 2.2 mmol/L of orthophenylene diamine (Sigma Chemical Co, St Louis, MO) and 0.012% (vol/vol) hydrogen peroxide (Sigma Chemical Co) were added to each well. When color had fully developed, the reaction was stopped with 100 μL per well of 4.5 mol/L of sulfuric acid. The absorbencies of the well contents were read at 492 nm minus 405 nm reference absorbance using an automatic plate reader (Molecular Devices, Palo Alto, CA). A four-parameter curve fit program was used to generate the standard curve, from which sample and control concentrations were interpolated.

Detection of Anti-rhuMAb HER2 Antibodies in Serum

An antibody titer ELISA was developed to measure the presence and titer of antibodies against rhuMAb HER2 in human sera. The positive control in the ELISA was an antiserum prepared against rhuMAb HER2 in cynomologous monkeys. The negative control was a human serum pool prepared from a panel of healthy donors. Briefly, 100 μL of rhuMAb HER2 was added to 96-well microtiter plates at 1 μg/mL in 0.05 mol/L of sodium carbonate buffer, pH 9.6. After overnight incubation at 4°C, plates were washed with ELISA wash buffer (PBS that contained 0.05% Tween-20 and 0.01% thimerosal) and blocked for 1 hour with ELISA diluent (PBS that contained 0.05% Tween-20, 0.5% BSA, and 0.01% thimerosal). Subsequently, 50 μL of sample, positive control, or negative control and 50 μL of biotin-rhuMAb HER2 were added to appropriate wells and allowed to incubate for 1 hour at room temperature (RT). The titer of the positive control was determined by an initial 1:100 dilution of the sample followed by serial 1:2 dilutions. The plates were washed in ELISA wash buffer, then 100 μL of PBS that contained 2.2 mmol/L of orthophenylene diamine (OPD) and 0.012% hydrogen peroxide was added to each well. The colorimetric reaction was quenched with 100 μL of 4.5 mol/L of sulfuric acid and absorbance was measured at 492-nm wavelength in an automated plate reader. Intra-assay and interassay variability averaged 2.6% and 12.1%, respectively.

Detection of p185$^{HER2/neu}$ ECD in Serum

The method for detection of shed HER2 ECD levels in serum is an ELISA-based assay and has been described in detail elsewhere.[23] Briefly, the ELISA uses pairs of anti-HER2 monoclonal antibodies (Genentech, Inc) that recognize mutually exclusive determinants of the ECD of p185$^{HER2/neu}$. Wells were coated overnight at 4°C with MAb 7F3, which does not compete with rhuMAb HER2 for shed HER2 ECD binding. Assay standards (recombinant, p185$^{HER2/neu}$ ECD) and patient samples were added to appropriate wells and allowed to incubate for 2 hours. After a wash step, secondary antibody was added (MAb 4D5 to detect free shed HER2 ECD and MAb 2C4 to detect total shed HER2 ECD) for 2 hours. The bound conjugate was detected with OPD substrate and the resulting absorbance was measured at a 490-nm wavelength. The range of the assay is 2.75 to 1,800 ng/mL in serum or plasma.

RESULTS

Patient Characteristics

Thirty-nine patients were enrolled onto the study, and their characteristics are listed in Table 1. Patients ranged in age from 29 to 75 years. Eighty-six percent of the patients had a KPS of 90% or greater. High levels of HER2/*neu* overexpression (3+) were observed in 82% of the patients. Twenty-four of 37 patients (65%) who had measurements of serum shed HER2/*neu* ECD performed before treatment had levels greater than 2.75 ng/mL (the lower limit of detection in the ELISA assay). Only one third of the patients for whom hormone receptor data were available were either estrogen receptor– or progesterone receptor–positive, consistent with previous studies that showed an inverse correlation between HER2/*neu* overexpression and hormone receptor expression.[24] Twenty-seven of the 39 patients (69%) were postmenopausal at diagnosis, and a majority of the patients had a high disease burden, with 18 of 39 patients (46%) who had three or more sites of metastatic disease. This patient population had been heavily pretreated before study entry, with 35 of 39 patients (90%) in whom two or more prior chemotherapeutic regimens had failed for metastatic disease. In addition, all patients had to show resistance to standard chemotherapy as evidenced by tumor progression while receiving chemotherapy treatment to be eligible for this trial.

Table 1. Patient Characteristics

Characteristic	No.	%
Age, years		
Mean	50	
Range	29-75	
Karnofsky performance status, % (n = 37)		
100	22	59
90	10	27
80	4	11
70	1	3
Level of HER2/*neu* overexpression		
2 +	7	18
3 +	32	82
Detection of shed HER2 ECD (n = 37)		
Receptor status		
Estrogen receptor–positive (n = 37)	13	35
Progesterone receptor–positive (n = 36)		
Menopausal status		
Premenopausal	10	26
Postmenopausal	27	69
Perimenopausal	2	5
No. of metastatic sites		
1	7	18
2	14	36
≥ 3	18	46
Sites of metastasis		
Lung	19	49
Lymph node	19	49
Bone	18	46
Chest wall/skin	17	44
Liver	14	36
Breast	4	10
Ovary	1	2.5
Eye	1	2.5
No. of prior chemotherapy regimens for metastatic disease		
1	4	10
2	18	46
≥ 3	17	44
Prior hormonal therapy	21	54
Prior radiotherapy	27	69

NOTE. N = 39.

Toxicity

Toxicity data are listed in Table 2. During the main study, 105 cycles of CDDP were administered in combination with 314 weekly IV doses of rhuMAb HER2. During the maintenance phase, an additional 81 cycles of CDDP and 434 doses of rhuMAb HER2 were administered. Thirty-seven patients had KPS assessed at baseline and at least once during the course of the study. The KPS remained unchanged in 19 patients (the majority of whom had

Table 2. Grade 3 or 4 Clinical Adverse Events, Irrespective of Causality

	Grade 3		Grade 4	
Event	No.	%	No.	%
Main study (n = 39)				
Accidental injury	1	3	0	0
Back pain	1	3	0	0
Infection	2	5	0	0
Dyspnea	2	5	0	0
Anorexia	1	3	0	0
Nausea and/or vomiting	7	18	0	0
Increased AST	1	3	0	0
Increased alkaline phosphatase	1	3	0	0
Hyperbilirubinemia	3	8	1	3
Nephrotoxicity	0	0	1	3
Asthenia	5	13	0	0
Hypertonia	1	3	0	0
Leukopenia	2	5	0	0
Anemia	3	8	0	0
Thrombocytopenia	4	10	0	0
Maintenance phase (n = 19)				
Hyperglycemia	1	5	0	0
Sepsis	0	0	1	5
Cardiomyopathy	1	5	0	0
Nausea and/or vomiting	2	10	1	5
Hyperbilirubinemia	1	5	0	0
Peripheral neuropathy	2	10	0	0
Leukopenia	1	5	0	0
Anemia	1	5	0	0
Thrombocytopenia	4	21	0	0

KPS > 80%), improved in two patients, and decreased in 16 patients. Four patients experienced weight loss in excess of 10% of their baseline body weight during the study. Four patients experienced fever greater than 38°C during rhuMAb HER2 infusion or at the postinfusion measurement. There was no significant difference in blood pressure between pretreatment and posttreatment measurements across all treatment days. During the main study, 22 of 39 patients (56%) experienced at least one episode of grade III or IV toxicity. The most frequent grade III toxicities observed were nausea and/or vomiting in seven patients (18%), asthenia in five patients (13%), thrombocytopenia in four patients (10%), anemia in three patients (8%), and leukopenia in two patients (5%). One episode of reversible grade IV nephrotoxicity was registered during the main study, and this patient's renal function recovered after 9 days. One patient developed grade IV hyperbilirubinemia during the main study. This event was believed to be disease-related rather than treatment-related. During the maintenance phase, 10 of 19 patients (53%) experienced grade III or IV toxicity. The most frequent grade III toxicities were thrombocytopenia, four patients (21%); nausea and/or vomiting, two patients (10%); and peripheral neuropathy, two patients (19%). Two patients experienced grade IV toxicity during the maintenance phase, one patient with sepsis and another with gastrointestinal toxicity. Grade III/IV toxicity reported as possibly related to rhuMAb HER2 was infrequent and was reported in six of 39 patients (15%). These events consisted of grade III cytopenia in three patients, grade III nausea or anorexia in two patients, grade III asthenia in one patient, and grade III hyperbilirubinemia in one patient. In most of these cases, we were not able to dissociate toxicity possibly related to rhuMAb HER2 from toxicity likely caused by CDDP or the patient's underlying disease. There was no report of grade IV toxicity attributable to rhuMAb HER2 administration. We observed no evidence of increased toxicity in those tissues that are known to express $p185^{HER2}$, ie, lung, gastrointestinal tract, CNS, or skin, nor was there any toxicity at the IV injection site. Three patients discontinued the main study treatment because of toxicity or intercurrent medical illness (two with nephrotoxicity and one with hepatic failure), and one patient discontinued the maintenance phase as a result of cardiomyopathy. The latter patient had a cumulative anthracycline dose of 420 mg/m^2 and had also received prior chest-wall irradiation. This patient also had a history of concurrent hypertension and diabetes. Four patients died before day 70; however, in each case, patients had been removed from the study because of disease progression before death. In summary, the toxicities observed were consistent with those previously reported for CDDP alone in a heavily pretreated population of breast cancer patients (Table 3).

Response and Response Duration

Tumor response was evaluated on day 70 during the main study and every 10 weeks during the maintenance phase protocol. Patients with symptoms or suspected progressive disease could have tumor assessment at any time during the course of the study. Thirty-seven of the 39 patients enrolled (95%) were assessable for tumor response (Table 4). Assessable patients were defined as those who met all eligibility criteria, received at least one dose of therapy, and underwent response evaluation other than at baseline. Patient deaths before tumor evaluation were considered assessable (progressive disease). Two patients were not assessable for tumor response because of adverse events before tumor assessment. During the main study, the median number of doses of rhuMAb HER2 per patient was nine (range, one to nine doses). The median number of doses of CDDP per patient was three, and the average administered dose was 74 mg/m^2. Three patients missed one dose of CDDP or received dose reduction as dictated by the study guidelines. Nineteen patients (49%) entered the maintenance phase protocol, in

Table 3. Studies of Single-Agent Cisplatin in Patients With Previously Treated Metastatic Breast Cancer

Investigator	Dosage	No.	CR	PR	Response Duration	Nephrotoxicity* (%)	Neurotoxicity†	Gastrointestinal Toxicity	Leukopenia‡ (%)	Thrombocytopenia
Bull et al[38] (1978)	70 mg/m² every 3 weeks	16	0	2	21-85 days	8	36% Hearing loss	97% Severe nausea	44	47% < 50,000/μL × 7 days
Samal et al[39] (1978)	15 mg/m²/d × 5 every 4 weeks or 120 mg/m² every 4 weeks	23	0	0	NA	0	9% Tinnitus 4% Ataxia/lethargy	70% Nausea and vomiting	29-50	50%-57% < 100,000/μL
Yap et al[35] (1978)	100 mg/m² every 3-4 weeks or 20 mg/m² × 5 every 4 weeks	26	0	0	NA	21-33	8% Tinnitus 4% Hearing loss	17%-57% Moderate to severe nausea and vomiting	NR	NR
Ostrow et al[36] (1980)	100 mg/m² every 3-4 weeks	17	1	0	3 months	35	29% Hearing loss 6% Optic neuritis 6% Peripheral neuropathy	100% Nausea and vomiting, frequently severe	33	6% < 20,000/μL
Forastiere et al[37] (1982)§	60 mg/m² every 3 weeks or 120 mg/m² every 3-4 weeks	37	0	5	1-6 months	13.5	66% Tinnitus 33% Hearing loss (5% tinnitus/hearing loss with 60 mg/m²)	100% Nausea nd vomiting	3-7	1%-2% < 50,000/μL
Total		119	1	7	21 days-6 months	0-35	5%-66% Tinnitus/hearing loss 0%-6% Peripheral neuropathy	17%-97% Nausea and vomiting frequently severe	3-50	1%-47% < 50,000/μL
Overall response rate	7% (95% CI, 2 to 11)									

Abbreviations: CR, complete response; PR, partial response; NA, not available; NR, not reported.
*Creatinine level > 2 mg/dL.
†High-frequency hearing loss > 20 dB.
‡WBC count < 3,000/μL.
§All 5 responses in this study occurred in the 120-mg/m² arm.

which the median number of rhuMAb HER2 doses per patient was 17 (range, three to 75 doses), and the median number of CDDP doses per patient was four (range, one to 10 doses), with an average CDDP dose of 65 mg/m². Ten patients required dose reductions of CDDP during the maintenance phase. The maximum number of rhuMAb HER2 doses received by any patient was 84.

Of the 37 patients assessable for tumor response, eight patients had a partial response documented during the main study, and one additional patient had a partial response that occurred during the maintenance phase. There were no complete responses in this study. Three patients met the criteria for minor response and six patients had stable disease during the main study. Disease progression was observed in 19 patients. The overall objective response rate among assessable patients (main study plus maintenance phase) was 24% (nine of 37 patients), all of whom had partial responses (95% confidence interval [CI], 12.4 to 41.6). The overall response rate for all patients (intent-to-treat population) was 23% (nine of 39 patients; 95% CI, 11.7 to 39.7). The sites of response were lung (n = 5), lymph node (n = 2), chest wall/skin (n = 6), and liver (n = 1). The median response duration was 5.3 months (range, 1.6 to 18 months). The characteristics of the responders are listed in Table 5. Pretreatment clinical variables were evaluated to determine if any variable was predictive of clinical response. Neither age, KPS, body weight, degree of HER2/*neu* overexpression, prior treatment, number or sites of metastases, hormone receptor status, or pretreatment shed HER2/*neu* ECD serum concentration correlated with clinical response. Furthermore, we found no association between fever during the rhuMAb HER2 loading dose and clinical response, nor did febrile reaction to rhuMAb HER2 corre-

Table 4. Response Data for Main Study Plus Maintenance Phase Among 37 Assessable Patients

Response	No. of Patients	%
Complete response	0	0
Partial response	9	24.3
Overall response	9	24.3
Minor response	3	8
Stable disease	6	16
Disease progression	19	51.3

Table 5. Characteristics of Patients Who Responded to CDDP Plus rhuMAb HER2

Patient Age (years)	No. of Prior Chemotherapeutic Regimens for Metastatic Disease	HER2 Expression	Site(s) of Objective Response	Maximum Shed HER2/*neu* ECD (μg/mL)	Response Duration (months)	Time to Tumor Progression (months)
39	2	3+	Chest wall, supraclavicular	0.201	7.7	10.0
63	1	3+	Chest wall	0.056	5.3	7.5
45	2	2+	Lung	0.019	4.0	6.7
39	5	3+	Liver	2.21	1.6	4.4
68	2	3+	Lung	0.048	9.4	11.5
41	1	2+	Lung, supraclavicular	0.004	2.7	8.4
37	1	3+	Chest wall, lung	0.004	6.7	9.1
50	1	3+	Chest wall	None detected	18	20.5
43	0	3+	Chest wall, lung	0.062	4.7	7.0

NOTE. All nine patients had objective partial responses.

late with pretreatment shed HER2/*neu* ECD concentration in serum.

rhuMAb HER2 Pharmacokinetics

To determine the effect of concomitant CDDP administration on rhuMAb HER2 pharmacokinetics, we compared the mean pharmacokinetic parameters measured in the current study with those in patients from a previously reported phase II clinical trial of rhuMAb HER2 alone in patients with HER2/*neu*-overexpressing advanced breast cancer (Table 6).[25] These data show that there is no significant difference in rhuMAb HER2 pharmacokinetics with the coadministration of CDDP when compared with treatment with antibody alone.

In addition, there was an inverse relationship between rhuMAb HER2 serum half-life and serum shed HER2 ECD of 0.5 μg/mL or greater. Indeed, patients with any measurable shed HER2/*neu* ECD serum level, compared with patients without measurable circulating ECD, had lower mean trough rhuMAb HER2 concentrations (18.7 *v* 43.6 μg/mL; $P = .0001$) across all time points (n = 443 observations; Fig 1). Among the subset of patients with measurable shed HER2/*neu* ECD levels, an inverse log-linear relationship between rhuMAb HER2 trough and shed HER2/*neu* ECD serum levels ($R = 0.79$; $P = .0001$) was seen (Fig 2). Although shed HER2/*neu* ECD may interfere with the quantitation of rhuMAb HER2, significant loss of quantitation of trough rhuMAb HER2 concentration was not observed unless the ratio of rhuMAb HER2 to shed HER2/*neu* ECD concentration was less than 10:1. Therefore, the observed relationship between rhuMAb HER2 serum concentration and serum shed HER2/*neu* ECD cannot be explained solely on the basis of assay interference.

*Correlation of Shed HER2/*neu *ECD Levels and Clinical Response*

The relationship between the maximum serum shed HER2/*neu* ECD concentration and level of HER2/*neu* expression (2 + *v* 3 +), as well as disease burden (number of metastatic sites), is shown in Fig 3. These data suggest a relationship between the maximum observed shed HER2/*neu* serum concentration with both degree of HER2/*neu* overexpression and disease burden, although these correlations did not reach statistical significance, perhaps because of the small numbers of patients in each of these subgroups. Clinical pretreatment variables, which included level of HER2/*neu* overexpression, number of metastatic sites, and serum shed HER2/*neu* ECD, were not predictive of clinical outcome; however, the difference in serum shed HER2/*neu* ECD concentration (day 70 posttreatment *v* day 0 pretreatment) was significantly associated with clinical outcome (disease progression *v* stable or responsive disease; $P = .008$, Fisher's exact test). Patients with disease progression showed a significant increase in serum shed HER2/*neu* ECD over

Table 6. Mean Pharmacokinetic Parameters

Study	Half-Life (days)	Shed HER-2 ECD (μg/mL)	Max Cpeak (μg/mL)	Max Ctrough (μg/mL)	Mean Css (4 μg/mL)	No. of Patients Max Ctrough < 10 μg/mL*
rhuMAb HER2 alone	9.2 ± 5.3 (n = 39)	< 0.5	113 ± 35 (n = 46)	54 ± 32 (n = 45)	52.3 ± 23.6 (n = 45)	7/40
	2.9 ± 3.2 (n = 6)	≥ 0.5				
rhuMAb HER2 + CDDP	11.0 ± 4.4 (n = 30)	< 0.5	121 ± 84 (n = 37)	85 ± 18.1 (n = 37)	50.1 ± 21.6 (n = 37)	12/37
	4.0 ± 2.6 (n = 7)	≥ 0.5				

Abbreviations: Max Cpeak, maximum concentration peak; Max Ctrough, maximum concentration trough; Css, mean concentration steady state.
*Number of patients with at least one minimum rhuMAb HER2 through concentration less than 10 μg/mL.

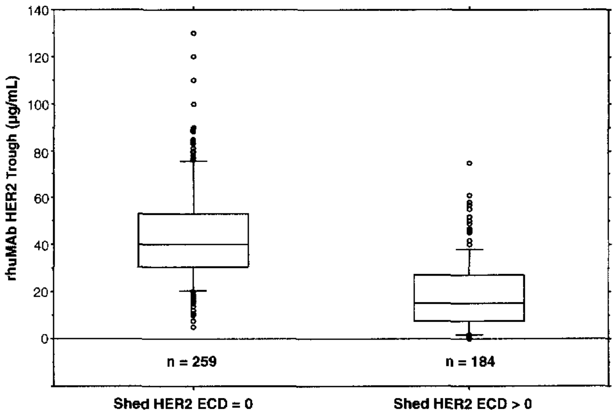

Fig 1. Comparison of serum (rhuMAb HER2)$_{trough}$ for all patients at all time points. Patients with detectable levels of shed HER2/*neu* ECD had lower mean (rhuMAb HER2)$_{trough}$ (18.7 µg/mL *v* 43.6 µg/mL, *P* = .0001) compared with patients without detectable serum shed HER2/*neu* ECD.

time (paired two-tail *t* test, *P* = .0006; Fig 4A). Patients with stable or responsive disease had a significant decrease in serum HER2/*neu* ECD after CDDP plus rhuMAb HER2 therapy (*P* = .004; Fig 4B); however, in these patients, a decrease in serum shed HER2/*neu* ECD concentration was not sufficient to discriminate between patients with stable disease and those with objective clinical responses, because five of six patients with stable disease had a decrease in shed HER2/*neu* ECD that was not associated with a measurable objective clinical response.

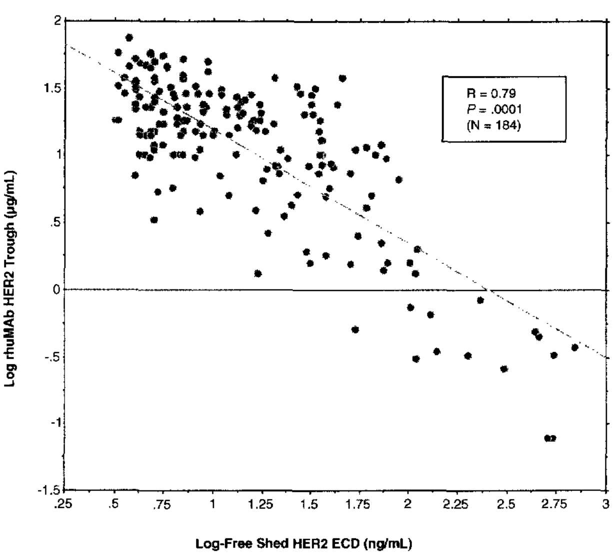

Fig 2. Measurement of serum trough rhuMAb HER2 concentration among the subset of patients with detectable levels of shed HER2/*neu* ECD. These data demonstrate an inverse relationship between serum shed HER2/*neu* ECD and rhuMAb HER2 trough concentration (*P* = .0001).

Human Antihumanized Antibody Responses

Seven hundred forty-eight doses of rhuMAb HER2 were administered in this study. There have been no cases in which measurable antibodies to rhuMAb HER2 were detected despite repeated weekly IV exposure, in some cases for many months. In addition, there were no reports of severe allergic reactions, such as bronchospasm, hypotension, urticaria, or eosinophilia after rhuMAb HER2 administration.

DISCUSSION

Therapeutic antitumor antibodies have long been proposed as an attractive approach to cancer therapy. However, despite many years of research, this approach to cancer therapy has not gained wide use in clinical practice for many reasons, which include (1) the lack of antitumor efficacy of antibodies in human clinical trials, (2) the lack of antibody specificity for tumor targets, (3) the induction of immune responses against nonhuman antibodies that preclude the use of multiple doses, and (4) the technical limitations to large-scale production and purification of monoclonal antibodies. Amplification and overexpression of the HER2/*neu* proto-oncogene occurs in 25% to 30% of breast cancers that provide a tumor-selective target for antibodies directed against p185$^{HER2/neu}$. Previous studies showed that a murine monoclonal antibody, 4D5, directed against the ECD of the HER2/*neu* protein, has growth inhibitory effects on malignant cells that overexpress this receptor.[26] This anti-HER2 antibody has been humanized so that it is less capable of eliciting either human antimurine or antihumanized antibody responses. Humanized antibodies may also be capable of affecting antitumor immune responses. We have previously shown the clinical efficacy of rhuMAb HER2 alone in a phase II clinical study,[25] and the combination of rhuMAb HER2 with CDDP had significant antitumor efficacy against HER2/*neu*-overexpressing breast cancers in a phase I clinical trial.

The mechanism of action of CDDP involves the formation of an equated cisplatin species that can react bifunctionally with DNA, which results in inter- and intrastrand cisplatin-DNA adducts that lead to inhibition of DNA synthesis.[27-30] One mechanism of resistance to CDDP involves removal of cisplatin-DNA adducts through DNA excision-repair processes.[31,32] Experimental data suggest that this DNA repair activity can be significantly decreased by the binding of ligands (EGF) to or antibodies against cell-surface type I receptor tyrosine kinases, such as the EGFR or p185$^{HER2/neu}$.[19,33,34] This phenomenon translates into a two-log increase in CDDP killing of cells that is specific to those cells that overexpress the HER2 receptor.[19] In preclinical in

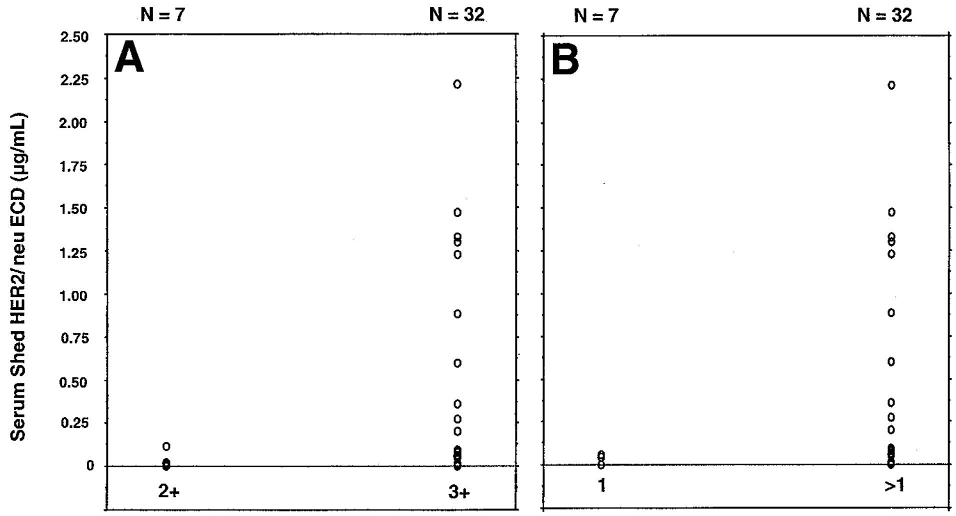

Fig 3. Maximum total serum shed HER2/*neu* ECD levels according to level of HER2/*neu* overexpression (A) and the number of metastatic sites (B). These data suggest a possible association between shed HER2/*neu* ECD, both with degree of HER2/*neu* overexpression and with number of metastatic sites.

vivo breast xenograft models, this resulted in the cure of some animals.

To assess the response of previously treated patients with metastatic breast cancer to CDDP alone, we have reviewed the literature and compared that experience to the results in this study. To date, there have been five reports on the use of CDDP as a single agent in previously treated patients with advanced breast cancer.[35-39] These studies are listed in Table 3. Of 119 patients treated with a variety of CDDP administration schedules, only one complete response and seven partial responses were seen for an overall objective response rate of 7% (95% CI, 2 to 11). The duration of responses ranged from 21 days to 6 months, and no responses were seen in patients treated with doses of less than 70 mg/m^2.

In the current trial, we administered rhuMAb HER2 in combination with CDDP to 39 patients with advanced breast cancer who had disease progression while receiving chemotherapy before study entry. We observed nine objective partial responses (eight during the main study and one during the maintenance program) among 37 patients who were assessable for response for an overall objective response rate of 24% (95% CI, 12.4 to 41.6). The sites of response were diverse (lung, liver, lymph node, and chest wall/skin), and the response duration ranged from 1.6 to 18 months, with a median response duration of 5.3 months. Clinical response data in a trial run in parallel to this study, which used weekly IV administration of rhuMAb HER2 as a single agent in patients with metastatic breast cancer with

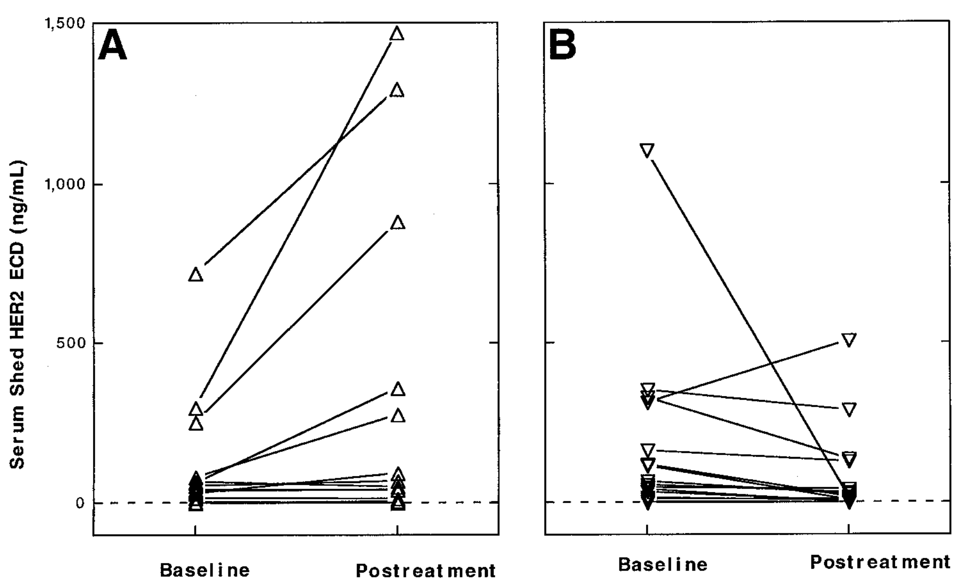

Fig 4. (A) Shed HER2/*neu* ECD at baseline (pretreatment) and day 70 (posttreatment) for patients with disease progression demonstrating a significant increase in shed HER2/*neu* ECD (P = .006). (B) For patients with stable or responsive disease, there was a significant decrease in shed HER2/*neu* ECD (P = .004).

the same dose and dosing schedule used in the current study, showed an objective response rate of 12% (95% CI, 4 to 26), with mild to moderate fever and chills reported as the predominate toxicity.[25]

There are at least four potential explanations for the observed objective clinical responses seen in the current trial. First, some of the patients may have responded to CDDP alone; however, the response rate observed in the current study is substantially higher than those reported previously for single-agent CDDP, especially considering the relatively modest CDDP dose administered and the requirement for demonstration of resistance to ongoing treatment with cytotoxic drugs in patients enrolled onto this study. Furthermore, overexpression of HER2/*neu* has been associated with CDDP resistance in previous studies.[40-44] It is, therefore, less likely that the observed objective response rate resulted from a unique sensitivity to CDDP among patients with HER2/*neu*-overexpressing breast cancer. Second, it is also possible that some of the clinical responses observed were caused by the action of rhuMAb HER2 alone. However, in the previously published parallel trial, the objective response rate to rhuMAb HER2 alone was approximately one half (12%) that seen in this study. On binding to $p185^{HER2/neu}$, rhuMAb HER2 causes downregulation of $p185^{HER2/neu}$ expression and disrupts the formation of HER2/HER3 and HER2/HER4 heterodimers. These events are accompanied by a decrease in cell proliferation.[45,46] Because this effect is not cytotoxic, administration of rhuMAb HER2 alone may not be expected to result in objective clinical responses; however, the rhuMAb HER2 antibody used in this study has been engineered to elicit antibody-dependent cellular cytotoxicity by host natural-killer cells, macrophages, and neutrophils, and this may have resulted in some of the observed objective clinical responses.[47] A third mechanism to account for clinical responses with CDDP plus rhuMAb HER2 might simply be the additive effects from each agent alone. Finally, it is possible that downregulation and/or inactivation of HER2/*neu* receptor activity by rhuMAb HER2 may result in increased response to CDDP by decreasing cellular repair of cisplatin-induced DNA adducts, thus effecting the clinical responses seen with this treatment. This latter hypothesis is supported by data that indicate such a mechanism is operative in HER2/*neu*-overexpressing breast cancer cells when treated with anti-HER2/*neu* antibodies.[19]

The toxicity observed in this study essentially parallels that reported previously for single-agent CDDP in a similar patient population in which treatment was frequently accompanied by gastrointestinal, renal, neurologic, and hematologic toxicity (Table 3). There was no evidence that rhuMAb HER2 enhanced the toxicity of CDDP. Moreover, the toxicity data from the current clinical trial are consistent with preclinical data that indicate that the enhanced cytotoxicity of CDDP mediated by rhuMAb HER2 is restricted to cells that contain HER2/*neu* amplification/overexpression.[19] This clinical observation validates the specificity proposed in the REC model and shows that the increased CDDP killing effects are unique to cells that contain the HER2/*neu* alteration. The only observed toxicity unique to rhuMAb HER2 infusion was mild to moderate fever and chills in a minority of patients, usually during or just after the rhuMAb HER2 loading dose. This phenomenon was also noted in earlier phase I and II clinical trials with this drug.[25] We found no correlation between febrile reaction to rhuMAb HER2 and serum HER2/*neu* ECD, nor was there any correlation between fever and clinical response.

Coadministration of CDDP with rhuMAb HER2 had no measurable effect on the mean pharmacokinetic parameters of IV administered rhuMAb HER2 compared with values obtained with weekly IV administration of rhuMAb HER2 as a single agent (Table 6). Furthermore, in light of the distinct mechanisms of elimination for these two agents (clearance of IgG through the reticuloendothelial system and predominantly renal elimination of CDDP), as well as the absence of any increase in toxicity attributable to CDDP, we believe it is unlikely that rhuMAb HER2 impacts significantly on CDDP pharmacokinetics, although the pharmacology of CDDP was not directly evaluated in this study.

Twenty-four of 37 patients (65%) who had measurements of serum shed HER2/*neu* ECD performed before treatment had detectable serum levels in a sensitive ELISA assay. As noted in the previous study of single-agent rhuMAb HER2, serum HER2/*neu* ECD was associated with rhuMAb HER2 pharmacokinetics (Table 6; Figs 1 and 2). There are three potential mechanisms that may account for this observation. First, rhuMAb HER2 may bind to HER2/*neu* ECD, which results in an antigen/antibody complex formation that results in a more rapid clearance of rhuMAb HER2 from serum. Second, the serum shed HER2/*neu* ECD may merely be a marker of high disease burden that reflects an increased rhuMAb HER2 binding by tumor cells that results in shortened rhuMAb HER2 serum half-life. This hypothesis is supported by the observation of a significant inverse correlation between serum rhuMAb HER2 concentration and xenograft volume, which is independent of serum shed HER2/*neu* ECD in athymic mice that bear HER2/*neu*-overexpressing breast carcinoma xenografts.[48] The third possibility is that there may have been interference in the assay used to measure serum rhuMAb HER2 caused by the presence of serum HER2/*neu* ECD, which resulted in artifactually low rhuMAb HER2 measurements in the patients with high levels of shed HER2/*neu* ECD. Significant loss of quantitation of rhuMAb HER2, however, is not observed unless the ratio of serum rhuMAb HER2 to shed HER2/*neu* ECD is less than 10:1. This occurred in only a small number of samples in this study. The extent to which any or all of these mechanisms account for the observed pharmacokinetic relationships between shed HER2/*neu* ECD and rhuMAb HER2 concentrations cannot be determined from the current data set and requires further study. Pretreatment serum HER2/*neu* ECD levels did not correlate with clinical response to CDDP plus rhuMAb HER2 therapy. This apparent lack of correlation between pretreatment shed HER2/*neu* ECD and clinical response may be caused by lack of statistical power to detect such a difference, because there were only 24 patients with measurable levels of shed HER2/*neu* ECD before treatment. An important aspect of the current data is the demonstration that measurable serum HER2/*neu* ECD does not preclude clinical response to CDDP plus rhuMAb HER2 because eight of the nine responders had measurable shed HER2/*neu* ECD during the course of this study, which included one patient with a very high level (2.21 μg/mL).

We were unable to identify any clinical pretreatment variables that correlated with clinical outcome, which included age, KPS, body weight, degree of HER2/*neu* overexpression, prior treatment, number or sites of metastases, hormone-receptor status, or pretreatment shed HER2/*neu* ECD serum concentration. Although pretreatment serum HER2/*neu* ECD did not correlate with response to rhuMAb HER2 plus CDDP, we found that trends in shed HER2/*neu* ECD over time did correlate with overall clinical outcome, specifically with disease progression. Nine of 13 patients with progressive disease showed an increase in shed HER2/*neu* ECD during treatment, and six of six patients with objective partial response and measurable serum levels showed a decrease in shed HER2/*neu* ECD after treatment with the combination. A significant decrease in shed HER2/*neu* ECD, however, was also evident in five of six patients with stable disease, as well as in one patient with disease progression, which indicated a decrease in HER2/*neu* ECD concentration alone was not sufficient to discriminate between stable disease and objective clinical response in this small data set. These data further suggest that serial measurement of serum HER2/*neu* ECD may have limited use as a predictive factor for objective clinical response, but it may be a useful marker of treatment failure in patients who continue to show increases in serum HER2/*neu* ECD during treatment.

This is the first report of a therapeutic strategy that uses a combination of chemotherapy plus anti-HER2/*neu* antibody. The REC approach shows activity in patients with breast cancer with prior clinical resistance to chemotherapy, and the objective response rate of approximately 24% after treatment with CDDP plus rhuMAb HER2 holds promise that the REC strategy that used rhuMAb HER2 with cytotoxic drugs may be a viable therapeutic approach that warrants further study. We have tested the combination of rhuMAb HER2 with other chemotherapeutic agents known to be active in breast cancer in preclinical models both in vitro and in vivo. These studies showed evidence for an additive or synergistic efficacy of rhuMAb HER2 in combination with alkylating agents, epipodophyllotoxins, taxanes, vinca alkaloids, anthracyclines, and some antimetabolites[49,50] (R.J. Pietras et al, unpublished data, May 1997). These preclinical studies formed the basis for an ongoing phase III clinical trial that evaluates combinations of rhuMAb HER2 with doxorubicin plus cyclophosphamide or paclitaxel in previously untreated patients with HER2/*neu*-overexpressing metastatic breast cancer. The results from this trial will shed further light on the clinical utility of the combined HER2/*neu* antibody/chemotherapy approach.

REFERENCES

1. Slamon DJ, Clark GM, Wong SG, et al: Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. Science 235:177-182, 1987

2. Slamon DJ, Godolphin W, Jones LA, et al: Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. Science 244:707-712, 1989

3. Press MF, Pike MC, Chazin VR, et al: Her-2/*neu* expression in node-negative breast cancer: Direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. Cancer Res 53:4960-4970, 1993

4. Seshadri R, Firgaira FA, Horsfall DJ, et al: Clinical significance of HER-2/*neu* oncogene amplification in primary breast cancer. The South Australian Breast Cancer Study Group. J Clin Oncol 11:1936-1942, 1993

5. Yonemura Y, Ninomiya I, Yamaguchi A, et al: Evaluation of immunoreactivity for *erb*B-2 protein as a marker of poor short-term prognosis in gastric cancer. Cancer Res 51:1034-1038, 1991

6. Berchuck A, Rodriguez G, Kinney RB, et al: Overexpression of HER-2/*neu* in endometrial cancer is associated with advanced stage disease. Am J Obstet Gynecol 164:15-21, 1991

7. Press MF, Pike MC, Hung G, et al: Amplification and overexpression of HER-2/*neu* in carcinomas of the salivary gland: Correlation with poor prognosis. Cancer Res 54:5675-5682, 1994

8. Quenel N, Wafflart J, Bonichon F, et al: The prognostic value of c-*erb*B2 in primary breast carcinomas: A study of 942 cases. Breast Cancer Res Treat 35:283-291, 1995

9. Borg A, Baldetorp B, Ferno M, et al: *ERB*B2 amplification in breast cancer with a high rate of proliferation. Oncogene 6:137-143, 1991

10. Stal O, Sullivan S, Sun XF, et al: Simultaneous analysis of c-*erb*B-2 expression and DNA content in breast cancer using flow cytometry. Cytometry 16:160-168, 1994

11. Berger MS, Locher GW, Saurer S, et al: Correlation of c-*erb*B-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. Cancer Res 48:1238-1243, 1988

12. Kallioniemi OP, Holli K, Visakorpi T, et al: Association of c-*erb*B-2 protein overexpression with high rate of cell proliferation, increased risk of visceral metastasis and poor long-term survival in breast cancer. Int J Cancer 49:650-655, 1991

13. Pantel K, Schlimok G, Braun S, et al: Differential expression of proliferation-associated molecules in individual micrometastatic carcinoma cells. J Natl Cancer Inst 85:1419-1424, 1993

14. Yamauchi H, O'Neill A, Gelman R, et al: Prediction of response to antiestrogen therapy in advanced breast cancer patients by pretreatment circulating levels of extracellular domain of the HER-2/c-*neu* protein. J Clin Oncol 15:2518-2525, 1997

15. Volas GH, Leitzel K, Teramoto Y, et al: Serial serum c-*erb*B-2 levels in patients with breast carcinoma. Cancer 78:267-272, 1996

16. Leitzel K, Teramoto Y, Konrad K, et al: Elevated serum c-*erb*B-2 antigen levels and decreased response to hormone therapy of breast cancer. J Clin Oncol 13:1129-1135, 1995

17. Carter P, Presta L, Gorman CM, et al: Humanization of an anti-p185HER2 antibody for human cancer therapy. Proc Natl Acad Sci U S A 89:4285-4289, 1992

18. Aboud-Pirak E, Hurwitz E, Pirak ME, et al: Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma in vitro and in nude mice. J Natl Cancer Inst 80:1605-1611, 1988

19. Pietras RJ, Fendly BM, Chazin VR, et al: Antibody to HER-2/*neu* receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. Oncogene 9:1829-1838, 1994

20. Arteaga CL, Winnier AR, Poirier MC, et al: p185c-*erb*B-2 signal enhances cisplatin-induced cytotoxicity in human breast carcinoma cells: Association between an oncogenic receptor tyrosine kinase and drug-induced DNA repair. Cancer Res 54:3758-3765, 1994

21. Hancock MC, Langton BC, Chan T, et al: A monoclonal antibody against the c-*erb*B-2 protein enhances the cytotoxicity of cis-diamminedichloroplatinum against human breast and ovarian tumor cell lines. Cancer Res 51:4575-4580, 1991

22. Fendly BM, Winget M, Hudziak RM, et al: Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/*neu* gene product. Cancer Res 50:1550-1558, 1990

23. Sias PE, Kotts CE, Vetterlein D, et al: ELISA for quantitation of the extracellular domain of p185HER2 in biological fluids. J Immunol Methods 132:73-80, 1990

24. Marsigliante S, Muscella A, Ciardo V, et al: Enzyme-linked immunosorbent assay of HER-2/*neu* gene product (p185) in breast cancer: Its correlation with sex steroid receptors, cathepsin D and histologic grades. Cancer Lett 75:195-206, 1993

25. Baselga J, Tripathy D, Mendelsohn J, et al: Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/*neu*-overexpressing metastatic breast cancer. J Clin Oncol 14:737-744, 1996

26. Chazin VR, Kaleko M, Miller AD, et al: Transformation mediated by the human HER-2 gene independent of the epidermal growth factor receptor. Oncogene 7:1859-1866, 1992

27. Roberts JJ, Pascoe JM: Cross-linking of complementary strands of DNA in mammalian cells by antitumour platinum compounds. Nature 235:282-284, 1972

28. Rosenberg B: Possible mechanisms for the antitumor activity of platinum coordination complexes. Cancer Chemother Rep 59:589-598, 1975

29. Rice JA, Crothers DM, Pinto AL, et al: The major adduct of the antitumor drug cis-diamminedichloroplatinum(II) with DNA bends the duplex by approximately equal to 40 degrees toward the major groove. Proc Natl Acad Sci U S A 85:4158-4161, 1988

30. Takahara PM, Rosenzweig AC, Frederick CA, et al: Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. Nature 377:649-652, 1995

31. Zamble DB, Mu D, Reardon JT, et al: Repair of cisplatin-DNA adducts by the mammalian excision nuclease. Biochem 35:10004-10013, 1996

32. Marshall JL, Andrews PA: Preclinical and clinical experience with cisplatin resistance. Hematol Oncol Clin North Am 9:415-429, 1995

33. Christen RD, Hom DK, Porter DC, et al: Epidermal growth factor regulates the in vitro sensitivity of human ovarian carcinoma cells to cisplatin. J Clin Invest 86:1632-1640, 1990

34. Isonishi S, Jekunen AP, Hom DK, et al: Modulation of cisplatin sensitivity and growth rate of an ovarian carcinoma cell line by bombesin and tumor necrosis factor-alpha. J Clin Invest 90:1436-1442, 1992

35. Yap HY, Salem P, Hortobagyi GN, et al: Phase II study of cis-dichlorodiammineplatinum(II) in advanced breast cancer. Cancer Treat Rep 62:405-408, 1978

36. Ostrow S, Egorin M, Aisner J, et al: High-dose cis-diamminedichloro-platinum therapy in patients with advanced breast cancer: Pharmacokinetics, toxicity, and therapeutic efficacy. Cancer Clin Trials 3:23-27, 1980

37. Forastiere AA, Hakes TB, Wittes JT, et al: Cisplatin in the treatment of metastatic breast carcinoma: A prospective randomized trial of two dosage schedules. Am J Clin Oncol 5:243-247, 1982

38. Bull T, Anderson ME, Lippman JG, et al: A phase II trial of cis-dichlorodiammine platinum II (CIS-DDP) in breast and ovarian carcinomas. Proc Am Assoc Cancer Res 19:87, 1978 (abstr 345)

39. Samal B, Vaithevicius V, Singhakowenta A: Cis-diamminedichloroplatinum (CDDP) in advanced breast and colorectal carcinomas. Proc Am Assoc Clin Oncol 19:347, 1978 (abstr 164)

40. Tsai CM, Chang KT, Perng RP, et al: Correlation of intrinsic chemoresistance of non–small-cell lung cancer cell lines with HER-2/*neu* gene expression but not with *ras* gene mutations. J Natl Cancer Inst 85:897-901, 1993

41. Tsai CM, Yu D, Chang KT, et al: Enhanced chemoresistance by elevation of p185*neu* levels in HER-2/*neu*–transfected human lung cancer cells. J Natl Cancer Inst 87:682-684, 1995

42. Benz CC, Scott GK, Sarup JC, et al: Estrogen-dependent, tamoxifen-resistant tumorigenic growth of MCF-7 cells transfected with HER2/*neu*. Breast Cancer Res Treat 24:85-95, 1993

43. Pegram MD, Finn RS, Arzoo K, et al: The effect of HER-2/*neu* overexpression on chemotherapeutic drug sensitivity in human breast and ovarian cancer cells. Oncogene 15:537-547, 1997

44. Felip E, Del Campo JM, Rubio D, et al: Overexpression of c-*erb*B-2 in epithelial ovarian cancer. Prognostic value and relationship with response to chemotherapy. Cancer 75:2147-2152, 1995

45. Reese D, Arboleda J, Twaddell J, et al: Effects of the 4D5 antibody on HER-2/*neu* heterodimerization with other class I receptors in human breast cancer cells. Proc Am Assoc Cancer Res 37:51, 1996 (abstr 353)

46. Klapper LN, Vaisman N, Hurwitz E, et al: A subclass of

tumor-inhibitory monoclonal antibodies to *Erb*B-2/HER2 blocks crosstalk with growth factor receptors. Oncogene 14:2099-2109, 1997

47. Pegram MD, Baly D, Wirth C, et al: Antibody dependent cell-mediated cytotoxicity in breast cancer patients in phase III clinical trials of a humanized anti-HER2 antibody. Proc Am Assoc Cancer Res 38:602, 1997 (abstr 4044)

48. Hsu S, Pegram M, Pietras R, et al: Therapeutic advantage of chemotherapy drugs in combination with recombinant, humanized anti–HER-2/*neu* monoclonal antibody (rhuMAb HER-2) against human breast cancer cells and xenografts with HER-2/*neu* overexpression. Proc Basic Clin Aspects Breast Cancer: A special conference of the Am Assoc Cancer Res, Keystone, CO, March 7-12, 1997 (abstr A-39)

49. Pegram MD, Pietras RJ, Slamon DJ: Monoclonal antibody to HER-2/*neu* gene product potentiates cytotoxicity of carboplatin and doxorubicin in human breast tumor cells. Proc Am Assoc Cancer Res 33:442, 1992 (abstr 2639)

50. Lopez AM, Pegram MD, Landaw EM, et al: Models to assess combination therapy interactions: Optimal timing of anti–HER-2 antibody and doxorubicin in breast cancer. Proc Am Assoc Cancer Res 38:605, 1997 (abstr 4061)

51. Pietras RJ, Pegram MD, Finn RS, et al: Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. (submitted, 1997).