Phase I Trial of the Trifunctional Anti-HER2 × Anti-CD3 Antibody Ertumaxomab in Metastatic Breast Cancer

Philipp Kiewe,[1] Stephan Hasmüller,[4] Steffen Kahlert,[4] Maja Heinrigs,[5] Brigitte Rack,[5] Alexander Marmé,[3] Agnieszka Korfel,[1] Michael Jäger,[7] Horst Lindhofer,[6] Harald Sommer,[5] Eckhard Thiel,[1] and Michael Untch[2]

Abstract

Purpose: Ertumaxomab is an intact bispecific antibody targeting HER2/neu and CD3 with selective binding to activatory Fcγ type I/III receptors, resulting in the formation of a tri-cell complex between tumor cells, T cells, and accessory cells. Patients with metastatic breast cancer were enrolled into a multicenter phase I dose-escalating trial.

Experimental Design: Three ascending doses of ertumaxomab (10-200 μg) were administered i.v. on day 1, 7 ± 1, and 13 ± 1. Safety and tolerability were the primary objectives. Secondary objectives were antitumor activity and different immunologic variables.

Results: Fifteen out of 17 enrolled patients completed the study. One hundred micrograms was identified as the maximal tolerable single dose. Most drug-related adverse events were mild and transient including fever (94%), rigors (47%), headache (35%), nausea (29%), vomiting (29%). Grades 3 and 4 (Common Toxicity Criteria) were lymphocytopenia (76%) and elevation of liver enzymes (47%). One patient (200 μg dose) developed severe hypotension and respiratory distress syndrome, another patient (150 μg dose) developed a systemic inflammatory response syndrome and acute renal failure. Aggravation of congestive heart failure was seen in one patient with preexisting ventricular dysfunction after administration of the third dose (200 μg). All adverse events were fully reversible. Antitumor response was seen in 5 out of 15 evaluable patients (one with a complete response, two with partial responses, two with stable disease) at dose levels of ≥100 μg. Measurements of cytokines (interleukin-6, interleukin-2, tumor necrosis factor-α, and IFN-γ) suggest a strong T helper cell type 1–associated immune response. The induction of human anti-mouse/anti-rat antibodies was detected in 5 out of 16 (31%) patients.

Discussion: Treatment with triple infusions of ertumaxomab yields a strong immunologic response. Doses up to 100 μg can be safely infused with close monitoring of patients. The observed clinical responses are encouraging and indicate antitumor efficacy.

Metastatic breast cancer is an almost always fatal disease. The median survival time from first manifestation of metastases ranges from 17 to 20 months (1). A number of endocrine, cytotoxic, and biological agents have shown palliative efficacy, but there is no consensual standard of care and treatment often causes substantial adverse effects (2). Epidermal growth factor family member, HER2/neu, is overexpressed in tumor specimens of ~25% to 30% of breast cancer patients (3), and is attributed to more aggressive tumor growth and a worse prognosis (4–6).

The family of epidermal growth factor receptors includes four members, EGFR (ERBB1), HER2/neu (ERBB2), ERBB3, and ERBB4. EGFR and HER2/neu have been intensively pursued as therapeutic targets. Antibodies targeting the extracellular domain of EGFR and HER2/neu as well as small molecular compounds inhibiting intracellular receptor signaling are already in clinical use and have shown clinical efficacy. However, their antitumor effects are often not as strong as predicted from preclinical studies and combination with chemotherapy is preferable (7). Therefore, there is a need for improvement of antitumor activity. Enhancing immunologic effector functions of antibodies reflects one approach improving the efficacy of antibody-based cancer therapy. Successful immune responses against neoplastic cells *in vivo* depend on the cooperation of different classes of immune cells.

Bispecific antibodies are powerful tools for the immunologic treatment of malignant cells. However, the bispecific antibodies

Authors' Affiliations: [1]Department of Haematology, Oncology, and Transfusion Medicine, Charité Campus Benjamin Franklin, Berlin; [2]Department of Gynaecology-Breast Center, Helios Klinikum Berlin-Buch, Berlin; [3]Department of Gynaecology, Universitäts-Frauenklinik, Heidelberg; [4]Department of Gynecology, Klinikum der Ludwig-Maximilians-Universität München-Großhadern; [5]Department of Obstetrics and Gynecology, Klinikum der Ludwig-Maximilians-Universität München-Innenstadt; [6]TRION Pharma, Munich; and [7]TRION Research, Martinsried, Germany

Received 11/9/05; revised 2/13/06; accepted 3/7/06.

Grant support: Fresenius Biotech GmbH, Munich, Germany.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Philipp Kiewe, Department of Haematology, Oncology, and Transfusion Medicine, Charité Campus Benjamin Franklin D-12200, Berlin, Germany. Phone: 49-30-8445-2337; Fax: 49-30-8445-4468; E-mail: philipp.kiewe@charite.de.

doi:10.1158/1078-0432.CCR-05-2436

described thus far normally activate only a single class of effector cells, i.e., either T cells, natural killer cells, FcγRI$^+$, or FcαRI$^+$ cells following binding to an appropriate target molecule of the effector cell (8–11). Here, we report data obtained with a new class of bispecific antibodies consisting of the two potent and evolutionarily related effector subclasses, mouse IgG_{2a} and rat IgG_{2b}.

Ertumaxomab is a trifunctional bispecific monoclonal antibody targeting HER2/neu and CD3. It is produced by a quadroma cell line prepared by the fusion of a specific rat (anti-CD3) and mouse hybridoma cell line (anti-HER2). The heavy chain is composed of murine IgG_{2a} and rat IgG_{2b} subclasses with particularly selective binding to activatory Fcγ type I/III receptors (12, 13). Together with its two antigen-binding sites, ertumaxomab is able to bind HER2/neu-positive tumor cells, T cells, and simultaneously via its Fc portion, Fcγ receptor–positive accessory cells (13, 14). The formation of this postulated tri-cell complex (Fig. 1) results in a physiologic costimulation of the T cell and very efficient tumor cell destruction by various immunologic mechanisms: (*a*) trifunctional antibodies mediate the activation of T cells which are then able to kill tumor cells by, e.g., release of cytokines and lytic enzymes like perforin (14, 15). (*b*) T cells are prevented from anergy by the release of costimulatory cytokines and a crosstalk between costimulatory molecules expressed on T cells and accessory cells. (*c*) Phagocytosis of tumor cells by Fcγ receptor–positive cells, e.g., macrophages and dendritic cells, is another effective mechanism within the tri-cell complex leading to the uptake, processing, and presentation of tumor-associated proteins (13). Finally, as a consequence, a humoral as well as a T cell response against HER2/neu and other potentially unknown tumor-associated peptides could be induced, potentially leading to a long-lasting antitumor immunity facilitated by the involvement of antigen-presenting accessory cells (16).

The ability of ertumaxomab to kill HER2/neu-positive tumor cells was shown in various *in vitro* models using tumor cell lines of different tumor types. Furthermore, ertumaxomab was shown to completely eliminate autologous tumor cells in leukapheresis products of patients with breast carcinoma contaminated with tumor cells (17). Recently, Heiss et al. showed the elimination of autologous tumor cells after i.p. application of ertumaxomab in patients with malignant ascites (18). These data suggest the potential efficacy of this trifunctional antibody for treatment in clinical studies. The fact that ertumaxomab has a different mode of action compared with HER2/neu targeting monoclonal antibody trastuzumab founded the rationale of investigating this trifunctional bispecific monoclonal antibody in a phase I trial including metastatic breast cancer patients with a HER2/neu expression of 1+ to 3+.

A phase I study of ertumaxomab was designed to investigate the safety and tolerability of the systemic administration of ertumaxomab in patients with metastatic breast cancer, and to obtain information on different immunologic surrogates.

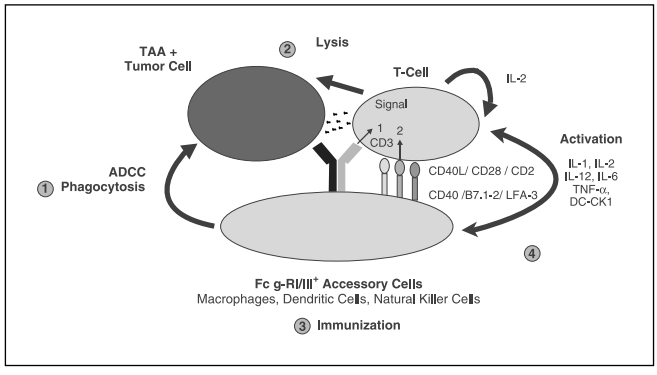

Fig. 1. The postulated tri-cell-complex. The trifunctional antibody is able to accelerate the recognition and destruction of tumor cells by different immunologic mechanisms. *ADCC,* antibody-dependent cellular cytotoxicity; *DC,* dendritic cells; *DC-CK1,* dendritic cell cytokine 1; *LFA,* leukocyte function associated antigen; *TNF-α,* tumor necrosis factor-α; *CD,* cluster of differentiation.

Patients and Methods

Isolation of peripheral blood mononuclear cells from whole blood. Mononuclear cells were purified from the blood of healthy donors using density centrifugation through Ficoll Histopaque (PAN Biotech, Aidenbach, Germany) at 897 × *g*, for 15 minutes at 10°C, acceleration 2, and brake 0. The cells were washed twice with PBS without Mg^{2+} or Ca^{2+} (PAN Biotech) and centrifuged at 458 × *g*, for 10 minutes at 20°C. The supernatant was removed and the pellet was resuspended in 20 mL RPMI 1640, supplemented with 2 mmol/L glutamine, 1 mmol/L sodium pyruvate, and 1 mmol/L nonessential amino acids and 10% FCS (PAN Biotech). Cell number and viability were determined by trypan blue staining (Sigma, Deisenhofen, Germany).

Cell lines and antibodies. SKBR3 cells (breast adenocarcinoma line, ATCC HTB-30), which strongly express the HER2/neu receptor (HER2/neu +3 status in Dako HercepTest) were used as the target cell line. Cytotoxicity experiments were done using the trifunctional antibody ertumaxomab (anti-HER2 × anti-CD3, mouse IgG_{2a} × rat IgG_{2b}; TRION Pharma, Munich, Germany), and the monoclonal antibodies 2502A (anti-HER2, mouse IgG_{2a}; TRION Pharma) and trastuzumab (anti-HER2, human IgG_1; Roche, Penzberg, Germany).

Cytotoxicity assay. Peripheral blood mononuclear cells (PBMC; 2×10^5) and SKBR3 (3×10^4) cells were coincubated for 3 days in a 96-well plate (Greiner Germany) with either trastuzumab (Roche), 2502A (TRION Pharma) or ertumaxomab (TRION Pharma). Each antibody was used at concentrations ranging from 2.5 μg/mL to 5 pg/mL. PBMC coincubated with SKBR3 (allogeneic setting) were used as controls. After 3 days, PBMC were discarded and the proliferation of tumor cells was analyzed using the XTT Cell Proliferation Kit II (Roche). Extinction was measured in a Versamax microplate reader (Molecular Devices) and raw data were analyzed in Excel XP (Microsoft). All experiments were done in duplicate and were repeated with the PBMC of three different healthy donors.

Study design. The study was designed as a German multicenter, uncontrolled, sequential dose-escalating, phase I study. The safety and tolerability of ertumaxomab and the identification of the maximum tolerated dose (MTD) were the primary objectives. MTD was defined as the maximum dose level that at least five out of six patients tolerated without dose-limiting toxicity (DLT). Secondary objectives included antitumor activity, measurement of different immunologic variables [cytokine release, hematological cell populations, and human anti-mouse/anti-rat antibody (HAMA/HARA)].

Decisions regarding the dose levels were made by a Dose Steering Board (DSB) consisting of the investigators of all sites, the physicians responsible for the patients' surveillance from all sites, as well as the study coordinator. The study was conducted according to the principles of the International Conference of Harmonization-Good Clinical Practice and approved by the institutional ethics committees. All relevant authorities were notified according to German drug law.

Patient eligibility. Female patients with histologically confirmed metastatic breast cancer expressing HER2/neu who had no indication

Table 1. Antibody doses applied at each dose level

Dose level (patient cohort)	Dose, μg (day 1)	Dose, μg (day 7 ± 1)	Dose, μg (day 13 ± 1)
1	10	20	20
2	10	50	50
3	10	100	100
3.5	10	150	150
4	10	200	200

NOTE: MTD was determined at dose level 3.

for cytoreductive systemic treatment at the time of enrollment were eligible for the study. HER2/neu expression was defined as weak to strong positivity (1+, 2+, and 3+) by standard immunohistochemistry methods (Dakotest). Patients were required to be 18 to 75 years of age, have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, a life expectancy of at least 6 months, and adequate hematological (hemoglobin, >9 g/dL; platelets, >100/nL; white blood count, >3 and <13/nL), renal (creatinine, <2 mg/dL), and hepatic function (alanine aminotransferase, aspartate aminotransferase, and/or γ-glutamyltransferase <2.5× upper limit of normal, in case of liver metastases, alanine aminotransferase, aspartate aminotransferase, and/or γ-glutamyltransferase <5× upper limit of normal, bilirubin <2× upper limit of normal). Patients with acute or chronic infections, brain metastases, evidence of relevant cardiovascular disease (uncontrolled congestive heart failure, history of myocardial infarction, cardiac arrhythmias, or abnormal echocardiography), severe dyspnea, pulmonary dysfunction, or need for continuous supportive oxygen inhalation, evidence or suspicion of relevant rheumatological disease, and evidence or suspicion of relevant psychiatric impairment were excluded from the study. Any prior anti-HER2 therapy had to be completed 3 months before start of study treatment. All previous chemotherapy, radiation therapy, or investigational therapy had to be discontinued at least 28 days before the start of study treatment and treatment with mitomycin or nitrosurea within 42 days of investigational treatment. Patients were not eligible if they had previous treatment with mouse or rat monoclonal antibody, a positive HAMA/HARA test prior to treatment, or any individual patient condition which, according to the investigator, would present an undue risk during study participation. All patients signed informed consent before receiving the investigational antibody.

Treatment plan and dose escalation. Eligible patients were treated in escalating dose cohorts until MTD was reached. Patients received i.v. ertumaxomab infused during a period of 6 hours once weekly for 3 weeks (day 1, 7 ± 1, and 13 ± 1) as summarized in Table 1. Three patients were to be treated at each dose level without DLT. However, in case of any DLT, three additional patients, up to a maximum of six, could be investigated at that dose level after the DSB had convened to determine how to further proceed. Follow-up visits were scheduled at 1 week (day 19 ± 1), 1 month (day 41 ± 2), and 2 months (day 69 ± 5, end of study) after the last ertumaxomab infusion. Further follow-up visits with open surveillance were done according to the investigator's discretion after additional informed consent.

Drug administration. Ertumaxomab was supplied by TRION Pharma as a sterile, clear, colorless, preservative-free concentrate in prefilled syringes. The concentrate contained 0.1 mg/mL per 100 mmol/L sodium citrate buffer. Each syringe contained 10 μg of antibody to be dissolved in 0.9% sodium chloride solution for i.v. infusion depending on dose level. The antibody was administered as a continuous 6 hour i.v. infusion by a precision infusion pump. Paracetamol (1,000 mg p.o.) and antihistamines (e.g., clemastine, 2 mg i.v.) were given as premedication 30 minutes before the start of infusion. The patients remained under hospital care and surveillance for up to 24 hours after start of the infusion.

Toxicity assessment. Toxic side effects were assessed by clinical examination and laboratory tests and graded according to the National Cancer Institute Common Toxicity Criteria (CTC), version 2 of April 1999. Accordingly, patients were frequently monitored during the 24-hour stay at the hospital and at the scheduled follow-up visits.

Criteria regarded as DLTs were predefined in the study protocol and included any CTC grade ≥3 adverse condition which demanded interruption of the infusion and which could not be relieved within 2 hours by conventional measures, any (delayed) infusion-related CTC grade ≥3 reactions within the first 24 hours after the start of the infusion which could not be relieved readily by conventional measures, any CTC grade ≥3 test abnormality within the first 48 hours after the start of the infusion with regard to serum electrolytes, serum creatinine, or evidence of hemolysis, irreversible CTC grade 4 elevation of liver enzymes within 4 days after antibody infusion, and finally, all National Cancer Institute CTC grade 4 toxicities not mentioned above as well as any CTC grade 3 toxicity considered to be a DLT by the DSB.

Laboratory variables including serum chemistry, hematology, and urinalysis were monitored preinfusion, as well as 6, 12, and 24 hours after the beginning of the infusion. Physical examination including vital signs, 1- and 12-lead electrocardiogram, and echocardiography were also recorded for safety.

Evaluation of antitumor activity. An assessment of tumor status was done at screening and end of study (day 69 ± 1). Further assessments after the end of study were left to the discretion of the investigators. Tumor markers CEA and CA 15-3 were measured at screening, day 41 ± 1, and day 69 ± 1.

Pharmacodynamic variables. The development of HAMA/HARA was evaluated at baseline and at 1 month after the infusion of ertumaxomab. Samples for analysis of cytokines (interleukin-6, tumor necrosis factor-α, and IFN-γ) and hematological markers (lymphocytes, monocytes, granulocytes, ratio of CD3/4 cells, CD3/8 cells, CD19/20 cells, and CD16/56 cells) were obtained before each infusion and at 1, 3, 6, and 24 hours after each infusion, as well as on day 19 ± 1.

Results

Preclinical studies. PBMC from three healthy donors were incubated with +3 HER2/neu-expressing SKBR3 tumor cells at a ratio of ~7:1 and increasing concentrations of the monoclonal anti HER2-antibodies trastuzumab, 2502A or the trifunctional antibody ertumaxomab (anti-HER2 × anti-CD3). After 3 days, the efficacy of the three antibodies to induce tumor cell killing was analyzed.

The monospecific murine monoclonal antibody 2502A revealed only weak antitumor effects even at the highest concentration used. It has been previously shown that other murine IgG_{2a} antibodies are also weak inducers of antitumor activity in comparable cytotoxicity assays (19).

The humanized antibody trastuzumab was able to inhibit tumor cell growth up to ~45% at concentrations from 2.5 μg/mL to 125 ng/mL. At concentrations <125 ng/mL, the antitumor effect is strongly reduced. In contrast, the trifunctional antibody ertumaxomab revealed statistically significant cytotoxicity rates of 97% to 99% against tumor cells at concentrations as low as 50 ng/mL (Fig. 2).

The markedly increased antitumor effect of ertumaxomab compared with the monospecific antibody trastuzumab might be explained by their different proposed modes of action: whereas trastuzumab mainly acts via recruiting immune cells to attack and kill target cells through antibody-dependent cellular cytotoxicity (20), ertumaxomab induces a concerted activation

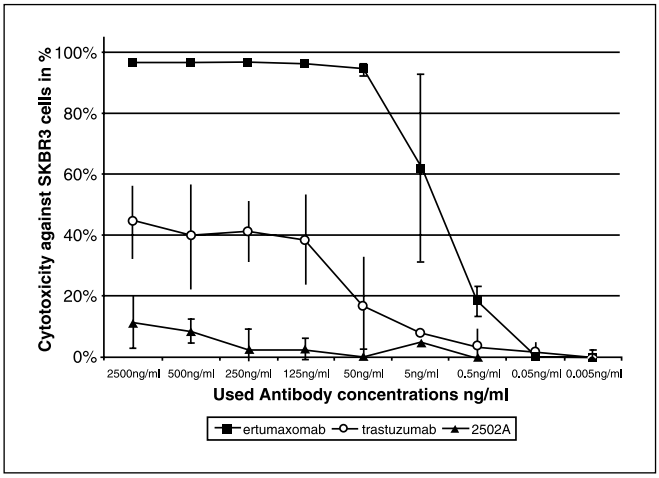

Fig. 2. Cytotoxicity of different HER2/neu antibodies against SKBR3 cells. Cytotoxicity experiments were done in duplicate with the PBMC of three healthy donors. Allogeneic cytotoxicity (median, 2.90%) measured during PBMC and SKBR3 coincubation without the addition of antibodies was subtracted from all samples. Points, median; bars, SE.

of two different immune cell types (T cells and accessory cells) resulting in highly potent tumor cell killing.

Patient characteristics. Fifteen out of 17 patients completed the study, and 14 patients received three complete consecutive infusions. Median age was 60 years, with a range of 41 to 79 years. Fifteen (88%) patients were of Caucasian origin, two (12%) patients were of Oriental origin. Distribution of ECOG performance status: 0 (11 patients, 65%), 1 (5 patients, 29%), 2 (1 patient, 6%). HER2/neu expression according to immunohistochemistry: 1+ (three patients, 18%), 2+ (four patients, 24%), 3+ (nine patients, 53%), and unknown (one patient, 6%).

All but one patient had received prior surgery for breast cancer. A total of 14 patients (82%) had received further antineoplastic treatments like (neo-)adjuvant or palliative chemotherapy, endocrine therapy, anti-HER2 therapy or radiation—not further specified due to incomplete documentation. A list of individual patient characteristics is given in Table 2.

Toxicity. An overview of the most frequent treatment-related toxicities occurring in ≥2 patients is provided in Table 3. Laboratory toxicities CTC grade ≥3 are shown in Table 4. A total of seven patients (41%) experienced 16 serious adverse events. In three patients (18%), the serious adverse events were classified as drug-related.

One patient (no. 15) developed severe hypotension and respiratory distress syndrome after the second dose (200 μg); one patient (no. 13) experienced a systemic inflammatory response syndrome after the second infusion (150 μg), which was accompanied by other severe side effects such as disseminated intravascular coagulation, dyspnea, acute liver failure, and renal failure. These two serious adverse events were fully reversible. Aggravation of congestive heart failure was seen in one patient (no. 14) with preexisting ventricular dysfunction, following the administration of the third dose (200 μg), however, the relation to treatment was uncertain.

Three patients (18%) experienced DLTs, all of which were considered to be certainly related to ertumaxomab and resulted in a reduction of dosing. Among them, were two of the abovementioned serious adverse events, respiratory distress syndrome at 200 μg and systemic inflammatory response syndrome at 150 μg as well as a patient (no. 16) with severe

Table 2. Patient characteristics according to dose group

Group (dose level in μg)	Patient	Age (y)	HER2/neu (IHC/FISH)	ECOG status	HARA (d)	HAMA (d)	Response
10-20-20	1	67	3+	1	500 ng/mL, screen 275 ng/mL, day 19	Negative, screen Negative day 19	
	2	41	3+	1	Negative, day 41	Negative, day 41	
	3	52	2+	0	Negative, day 41	Negative, day 41	
10-50-50	4	62	3+	0	Negative, day 41	Negative, day 41	
	5	60	NA*	0	Negative, day 41	Negative, day 41	
	6	43	1+	0	470 ng/mL, day 41	Negative, day 41	
10-100-100	7	61	3+	1	Negative, day 41	Negative, day 41	CR
	8	61	3+	0	6,600 ng/mL, day 41	27,000 ng/mL, day 41	PR
	9	64	3+	0	Negative, day 41	Negative, day 41	
	10	49	3+	2	Negative, day 41	Negative, day 41	
	11	43	3+	0	3,100 ng/mL, day 41	7,000 ng/mL, day 41	
	12	51	2+	0	1,269 ng/mL, day 41	1,030 ng/mL, day 41	
10-150-150	13†	72	1+	0	Not done	Not done	SD
10-200-200	14	51	1+	1	Negative, day 41	Negative, day 41	
	15†	50	2+, FISH−	1	18,000 ng/mL day 41	100,000 ng/mL, day 41	SD
	16‡	79	2+, FISH+	0	Negative, day 41	Negative, day 41	PR
	17	61	3+	0	Negative, day 41	Negative, day 41	

Abbreviations: IHC, immunohistochemistry; NA, not applicable. CR, complete response; PR, partial response; SD, stable disease.
*1+ or 2+.
† Patient received two infusions.
‡ Patient did not complete third infusion.

Table 3. Treatment-related toxicities occurring in ≥2 patients

Toxicity	All CTCs		CTC grade 3		CTC grade 4	
	No. patients (%)	Adverse events	No. patients (%)	Adverse events	No. patients (%)	Adverse events
All events	17 (100)	139	3 (18)	10	4 (24)	6
Fever	16 (94)	27	—	—	—	—
Rigors	8 (47)	10	—	—	—	—
Headache	6 (35)	7	—	—	—	—
Nausea	5 (29)	14	—	—	—	—
Vomiting	5 (29)	6	—	—	—	—
Pain	4 (24)	6	—	—	—	—
Tachycardia	4 (24)	6	—	—	—	—
Feeling cold	3 (18)	5	—	—	—	—
Hepatic dysfunction	2 (12)	5	1 (6)	1	—	—
Arthralgia	2 (12)	3	—	—	—	—
Herpes simplex	2 (12)	3	—	—	—	—
Systolic hypertension	2 (12)	3	—	—	—	—
Dyspnea	2 (12)	2	2 (12)	2	—	—

rigors and fever at 200 μg which resulted in a premature termination of the third infusion. At the decision of the DSB, three additional patients were then treated within the 10-100-100 μg dose level. No further DLT occurred and 10-100-100 μg was identified as the MTD level. No deaths occurred during the study.

Antitumor activity. Five out of 15 evaluable patients had an antitumor response 2 months after the last ertumaxomab infusion. Complete response of target lesions was seen in one patient (no. 7) with mediastinal lymph node metastases with response duration of at least 6 months without further anticancer treatment. One patient with lung metastases (no. 16) and one with mediastinal and retropectoral lymph node metastases (no. 8) had partial response and two patients (nos. 13 and 15) had stable disease. See Table 2 for individual patient listing. Two patients (nos. 8 and 16) with partial response and two patients (nos. 13 and 15) with stable disease, received concomitant or subsequent endocrine therapy. The distribution of patients with normal or abnormal CEA and CA 15-3 values did not change during the study.

According to the ECOG status, 11 (65%) patients were fully active (ECOG = 0), 5 (29%) patients were restricted in their physical activities (ECOG = 1), and 1 (6%) patient was capable of self-care (ECOG = 2) at screening. At the end of the study, 7 (50%) of the remaining 14 patients were fully active (ECOG = 0), 6 (43%) patients were restricted in their physical activities (ECOG = 1), and 1 (7%) patient was capable of self-care (ECOG = 2). At the end of the study, ECOG status improved in 1 (7%) patient, did not change in 9 (64%) patients, and worsened in 4 (29%) of the 14 patients. No values were available for three patients at the end of the study.

Pharmacodynamic variables. Release of cytokines was observed mainly during the second and third infusions. The changes were most pronounced for interleukin-6. Thirteen patients (77%) had increased interleukin-6 levels 6 hours after the second infusion and 67% of patients 6 hours after the

Table 4. Laboratory toxicities, maximum CTC grades per patient (n = 17)

Laboratory toxicity	Grade 3	Grade 4
	n (%)	*n* (%)
Thrombocytopenia	2 (12)	—
Neutropenia	1 (6)	1 (6)*
Lymphopenia	13 (76)	—
Elevation of γ-glutamyltransferase	7 (41)	1 (6)
Elevation of aspartate aminotransferase	5 (30)	2 (12)
Elevation of alanine aminotransferase	6	1 (6)
Hyperbilirubinemia	2 (12)	—
Elevation of creatinine	—	1 (6)

NOTE: Listed laboratory adverse events were not necessarily drug-related.
*Due to chemotherapy before end of study.

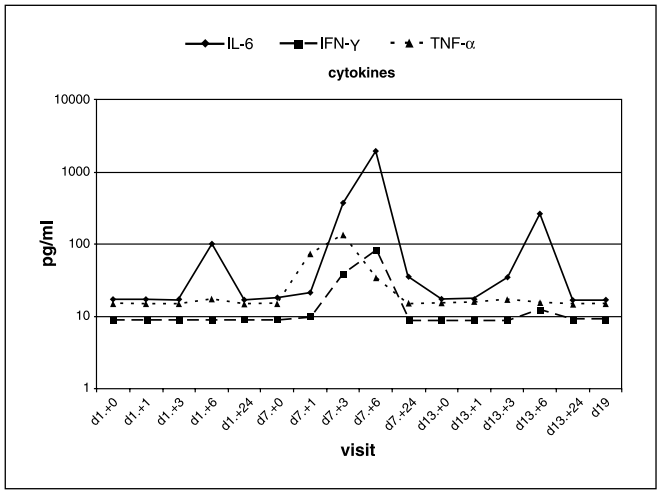

Fig. 3. Mean cytokine release measured in pg/mL for each time point. *X*-axis is discontinuous and describes day and hour after infusion. *Y*-axis in logarithmic scale.

third infusion. Fifty-nine percent had measurable IFN-γ values that followed the same pattern to a lesser extent. Tumor necrosis factor-α release was noted in 71% of patients with maximum levels measured after 3 hours. Most cytokine levels were back at baseline at 24 hours after the start of infusions. Figure 3 plots the mean cytokine release at different points during the trial.

The number of patients with an abnormal CD3/4 ratio was relatively low; abnormal values peaked at 24 hours after the start of the second infusion, in which three patients (18%) had a CD3/4 ratio below lower limit of normal, and two patients (12%) were above the upper limit of normal. Most of the patients with abnormal values during the study also had borderline values or values outside the reference range at pretreatment.

In almost all patients, the CD3/8 ratio decreased at 3 to 6 hours after the start of the second and third infusion and returned to the pretreatment value on day 19. The decrease in CD3/8 ratios was less pronounced in patients in the lower dose groups and patients with low values prior to treatment.

The CD19/20 ratios in dose groups 2 and 3 seemed to increase between 3 and 6 hours after the second and third infusion. No such pattern was observed for the remaining dose groups. No systematic change was observed for CD16/56 or CD14/64 ratios.

HAMA/HARA. HAMA were induced in 4 out of 16 evaluable patients (25%) on day 41. Five out of 16 evaluable patients (31%) had shifted to positive HARA values on day 41. The only patient (no. 6) who converted to a positive HARA value without developing a HAMA response had a very low HARA titer of 470 ng/mL at day 41. Another patient (no. 1) already had a weak positive HARA value (500 ng/mL) at screening, which slightly decreased within 19 days after the first infusion. All other patients who had shifted to positive HAMA values also shifted to a positive HARA value. The number of changes from negative to positive HAMA/HARA values did not show a clear dose dependency to ertumaxomab. It seems, however, that starting with doses >100 μg, the shift to positive HAMA/HARA results is more likely. The patients who shifted from negative to positive HAMA/HARA values did not seem to have a different toxicity profile than the remaining patients.

Discussion

This multicenter, uncontrolled, sequential dose-escalation study was conducted to investigate the safety and tolerability of increasing doses of the trifunctional antibody ertumaxomab (anti-HER2 × anti-CD3). Patients with metastatic breast cancer expressing HER2/neu received up to three i.v. infusions of the study drug. The MTD was established according to the incidence of DLTs by a DSB. Dose escalation was stopped at 200 μg for second and third infusions due to two independent DLTs. An additional dose step of 150 μg was introduced by the DSB because it was felt that the step between 100 and 200 μg had been too large. Only one patient was treated with 10-150-(150 μg) experiencing a DLT after the second infusion. Due to the severity of the event (systemic inflammatory response syndrome), the DSB enrolled three additional patients into dose level 3 with 100 μg for second and third infusions. The DSB considered the doses of 10-100-100 μg of ertumaxomab as high enough to reach a therapeutic effect and a robust stimulation of the immune system.

Treatment with triple infusions of ertumaxomab yields a strong immunologic response. In spite of a pretreatment with paracetamol and antihistamines, almost all patients developed symptoms of cytokine release syndrome with pyrexia and sometimes rigors. Additional toxicities included nausea, vomiting, headache, pain, tachycardia, elevated liver enzymes, arthralgia, systolic hypertension, dyspnea, lymphocytopenia, and herpes simplex reactivation. C-reactive protein values tended to peak at 24 hours after the start of the infusions as well as measurable inflammatory cytokine release during infusions reflecting the inflammatory response. In general, the observed toxicities resemble the side effects seen after the administration of other therapeutic antibodies.

In patients treated with doses up to the MTD, hepatic toxicity with increase in transaminases, γ-glutamyltransferase and bilirubin were mostly of CTC grades 1 or 2. If they were of CTC grade ≥3, they were attributable to other causes, such as treatment with chemotherapy or medical history for all but one patient. In patients treated with doses above the MTD, abnormalities of liver function tests of CTC grades 3 and 4 occurred in the majority of patients and reached the level of DLT in one patient. In all cases, changes of liver function were self-limited and returned to normal after the study drug was discontinued.

Changes in blood pressure and heart rate were observed and could also be attributed to the cytokine release syndrome. In severe cases, they were associated with shock, dyspnea, respiratory distress syndrome, pulmonary edema, and renal failure. These complications contributed most significantly to DLTs. One patient (no. 13) with a dose (150 μg) later found to be above the MTD, experienced a life-threatening event and had to be treated with dialysis to manage acute renal failure. At doses up to the MTD, changes of the cardiovascular and respiratory systems were limited and resolved without sequelae. Possible effects on cardiovascular and respiratory function have to be considered when deciding the treatment for patients with concomitant diseases.

The data seem to indicate a dose relationship regarding the number of CTC grade ≥3 toxicities per patient, the number of serious adverse events and changes in hepatic enzymes. In addition, it seems that more toxicities occurred during the second infusion period than in the remaining study periods. This latter observation might be due to the large dose step between the first and second infusion, and the absence of immunologic tolerance at that time.

The measurements of cytokine release reflect the immunologic response seen particularly in patients experiencing serious adverse events. All dose-limiting events were related to immune reactions and were accompanied by excessive values for interleukin-6, tumor necrosis factor-α, and to a lesser extent, by IFN-γ. The safety data imply that single antibody doses up to 100 μg could be infused safely. The individual patient, however, has to be closely monitored.

The observed immune response with IFN-γ secretion seems to lean towards a pattern of T helper cell type 1 activation, promoting cell-mediated immunity. Therefore, it seems possible that a long-term T cell–mediated immune response may be generated in selected patients comparable to results shown earlier in immunocompetent mouse tumor models (21, 22).

Further cytokine measurements will be necessary to determine the ratio of T helper cell type 1 and 2 immune responses.

Five patients had an objective tumor response 2 months after the first administration of ertumaxomab. One patient (no. 7) with mediastinal lymph node metastases had complete response documented until 6 months after the start of treatment and progressive disease documented after 12 months. Until then, this patient had no further antitumor treatment. One patient (no. 16) with pulmonary metastases achieved partial response 2 months after the beginning of treatment. Further decrease of metastases was seen after 4 months, but trastuzumab had been started in the meantime.

One patient (no. 8) with mediastinal as well as retropectoral lymph node metastases had partial response and another patient (no. 15) with pulmonary as well as pleural metastases had stable disease, however, endocrine therapy in these two patients had been started prior to the study and continued concomitantly.

One patient (no. 13) with pelvic lymph node metastases received only two antibody infusions due to a DLT after the second dose. Nonetheless, stable disease was documented up to 6 months after the start of ertumaxomab, with endocrine therapy starting after the end of the study. Although concomitant application of endocrine therapy is a confounding factor in the response evaluation of one patient with partial response and another patient with stable disease, clinical benefits could be attributed to the effect of the antibody in the remaining three patients.

All patients with partial or complete responses were overexpressing HER2/neu at immunohistochemistry scores of at least 2+ and/or gene amplification detected by fluorescence *in situ* hybridization (FISH). Two occurrences of stable diseases were observed with HER2/neu scores 1+ and 2+, FISH-negative, respectively. However, patients with HER2/neu scores of 1+ and 2+, FISH-negative, were underrepresented at higher dose levels. Clinical benefit seemed to correlate better with a favorable performance status of patients as well as a limited number of previous anticancer therapies and these features were also unevenly distributed among patients with different HER2/neu scores. Larger numbers of patients will be required to determine the dependence of clinical benefit on HER2/neu expression level.

With 25% of patients developing HAMA and 31% of patients developing HARA, the incidence of an immune response against mouse and rat antigens measured on day 41 was rather low. Most clinical trials with i.v. application of other nonhumanized therapeutic antibodies in patients with solid tumors revealed HAMA/HARA rates of 40% to 80% following multiple exposures (23–25). Another trifunctional antibody, catumaxomab (Trion Pharma/Fresenius Biotech, Munich, Germany) induced the development of HAMA/HARA in almost all tested patients (14 of 15) after i.p. application at the end of the study at day 37 (26). The low incidence of HAMA/HARA development in the actual study may indicate a lower immunogenic potential of ertumaxomab after systemic i.v. application compared with the i.p. route. This observation is consistent with earlier investigations demonstrating that the i.p. and s.c. routes are superior to the i.v. route for inducing an immune response (27). However, the relative low incidence of HAMA/HARA responses (31%) provide a rationale for the administration of a second treatment cycle in HAMA-negative patients. The encouraging results obtained in this phase I study warrant further phase II studies in patients with metastatic breast cancer.

References

1. Piccart M. Treatment of advanced breast cancer: current status. Anticancer Drugs 1996;7:5–7.
2. Bernard-Marty C, Cardoso F, Piccart MJ. Facts and controversies in systemic treatment of metastatic breast cancer. Oncologist 2004;9:617–32.
3. Revillion F, Bonneterre J, Peyrat JP. ErbB-2 oncogene in human breast cancer and its clinical significance. Eur J Cancer 1998;34:791–808.
4. Wright C, Angus B, Nicholson S, et al. Expression of c-erbB-2 oncoprotein: a prognostic indicator in human breast cancer. Cancer Res 1989;49:2087–90.
5. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of HER2/neu oncogene. Science 1987;235:177–82.
6. Tandon AK, Clark GM, Chamness GC, Ullrich A, McGuire WL. HER-2/neu oncogene protein and prognosis in breast cancer. J Clin Oncol 1989;7:1120–8.
7. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.
8. Lum LG, Rathore R, Cummings F, et al. Phase I/II study of treatment of stage IV breast cancer with OKT3 × trastuzumab-armed activated T cells. Clin Breast Cancer 2003;4:212–7.
9. Fanger MW, Segal DM, Wunderlich JR. Going both ways: bispecific antibodies and targeted cellular toxicity. FASEB J 1990;4:2846–9.
10. Weiner LM, Clark JI, Ring DB, Alpaugh RK. Clinical development of 2B1, a bispecific murine monoclonal antibody targeting c-erbB-2 and Fc γ RIII. J Hematother 1995;4:453–6.
11. Valerius T, Stockmeyer B, van Spriel AB, et al. FcαRI (CD89) as a novel trigger molecule for bispecific antibody therapy. Blood 1997;90:4485–92.
12. Lindhofer H, Mocikat R, Steipe B, Thierfelder S. Preferential species-restricted heavy/light chain pairing in rat/mouse quadromas. Implications for a single-step purification of bispecific antibodies. J Immunol 1995;155:219–55.
13. Zeidler R, Mysliwietz J, Csanady M, et al. The Fc-region of a new class of intact bispecific antibody mediates activation of accessory cells and NK cells and induces direct phagocytosis of tumor cells. Br J Cancer 2000;83:261.
14. Zeidler R, Reisbach G, Wollenberg B, et al. Simultaneous activation of T-cells and accessory cells by a new class of intact bispecific antibody results in efficient tumor cell killing. J Immunol 1999;163:1246–52.
15. Riesenberg R, Buchner A, Pohla H, Lindhofer H. Lysis of prostate carcinoma cells by trifunctional bispecific antibodies (αEpCAM × αCD3). J Histochem Cytochem 2001;49:911–7.
16. Ruf P, Lindhofer H. Induction of a long-lasting antitumor immunity by a trifunctional bispecific antibody. Blood 2001;98:2526–34.
17. Lindhofer H, Menzel H, Zengerle U, et al. Bispecific antibodies effectively purge cancer cells from peripheral blood stream cell collections without affecting colony forming units. Exp Hematol 1997;25:879.
18. Heiss MM, Strohlein MA, Jager M, et al. Immunotherapy of malignant ascites with trifunctional antibodies. Int J Cancer 2005;117:435–43.
19. Ruf P, Jager M, Ellwart J, Wosch S, Kusterer E, Lindhofer H. Two new trifunctional antibodies for the therapy of human malignant melanoma. Int J Cancer 2004;108:725–32.
20. Gennari R, Menard S, Fagnoni F, et al. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. Clin Cancer Res 2004;10:5650–5.
21. Swain SL, McKenzie DT, Weinberg AD, Hancock W. Characterization of T helper 1 and 2 cell subsets in normal mice. Helper T cells responsible for IL-4 and IL-5 production are present as precursors that require priming before they develop into lymphokine-secreting cells. J Immunol 1988;141:3445–55.
22. Lappin MB, Campbell JD. The Th1-2 classification of cellular immune responses: concepts, current thinking and applications in haematological malignancy. Blood Rev 2000;14:228–39.
23. Mirick GR, Bradt BM, Denardo SJ, Denardo GL. A review of human anti-globulin antibody (HAGA, HAMA, HACA, HAHA) responses to monoclonal antibodies. Not four letter words. Q J Nucl Med Mol Imaging 2004;48:251–7.
24. DeNardo GL, Bradt BM, Mirick GR, DeNardo SJ. Human antiglobulin response to foreign antibodies: therapeutic benefit? Cancer Immunol Immunother 2003;52:309–16.
25. Khazaeli MB, Conry RM, LoBuglio AF. Human immune response to monoclonal antibodies. J Immunother 1994;15:42–52.
26. Jaeger M, Stroehlein MA, Schoberth A, Burges A, Heiss MM, Lindhofer H. Immunotherapy with the trifunctional antibody removal leads to significant elimination of tumor cells from malignant ascites in ovarian cancer: Results of a phase I/II. ASCO Meeting Abstracts 2004: 2504.
27. Stein-Streilein J, Hart DA. Effect of route of immunization on development of antibody-forming cells in hilar lymph nodes. Infect Immun 1980;30:391–6.