Safety and pharmacokinetics of bivatuzumab mertansine in patients with CD44v6-positive metastatic breast cancer: final results of a phase I study

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The purpose of this study was to investigate the safety, pharmacokinetics and preliminary efficacy of bivatuzumab mertansine in patients with CD44v6-positive metastatic breast cancer. Anthracycline and taxane-pretreated patients with metastatic breast cancer that expressed CD44v6 received one single infusion of bivatuzumab mertansine and were observed for 21 days within one treatment course. Starting dose was 25 mg/m^2, while dose was escalated by increments of 25 mg/m^2. Patients who experienced a disease stabilization were eligible for further courses with bivatuzumab mertansine. Blood serum samples were taken throughout the treatment period for pharmacokinetic analysis. Twenty-four patients were treated at eight different dose levels (25–200 mg/m^2), seven of these patients received more than one course of bivatuzumab mertansine. Two dose-limiting toxicities occurred: one patient treated with 125 mg/m^2 developed transient National Cancer Institute Common Toxicity Criteria grade 4 elevation of liver enzymes; another patient treated at 175 mg/m^2 experienced National Cancer Institute Common Toxicity Criteria grade 3 vomiting. She died from renal failure, which might have been caused by deterioration of pre-existing renal insufficiency. The most common toxicities were transient and mild skin disorders in 75% of patients. As a consequence of one fatal toxic epidermal necrolysis that occurred in a study running in parallel, the clinical trials programme of bivatuzumab mertansine was discontinued. None of the patients developed antibodies against bivatuzumab mertansine. No objective responses were observed. Disease stabilization was achieved in 50% of patients independently of dose level. In conclusion, bivatuzumab mertansine targets CD44v6 and appears to stabilize heavily pretreated metastatic breast cancer that expresses CD44v6. The maximum tolerated dose could not be determined in this trial as the sponsor discontinued the clinical development of bivatuzumab mertansine. *Anti-Cancer Drugs* 18:477–485 © 2007 Lippincott Williams & Wilkins.

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Introduction

More than 200 000 women are diagnosed with breast cancer in the United States per year, which is the most common type of cancer in women [1]. Occult metastases are present in many of those women at the onset and despite systemic treatment, which can eradicate some of those micrometastases, around 40% of women ultimately develop overt metastatic disease [2,3]. Although the use of intensified chemotherapy regimens has failed to demonstrate any survival benefit compared with standard dose therapy for patients with metastatic breast cancer (MBC), the identification of novel therapeutic targets may offer effective treatment alternatives [4,5]. For example, in about 20% of patients tumors overexpress HER2/*neu* with uncontrolled cell growth [6,7]. Therapy of these tumors with trastuzumab, a humanized monoclonal antibody that binds to HER2/*neu*, results in response rates of 15–35% among others via antibody-dependent cellular cytotoxicity [8,9]. A similar approach to improve therapy for patients with MBC is the use of monoclonal antibodies with or without antibody-dependent cellular cytotoxicity, which are labeled with either a radionuclide or a cytotoxic drug to be delivered at the tumor site [10–15].

Bivatuzumab mertansine is a tumor-activated prodrug conjugate. It consists of the cytotoxic maytansinoid mertansine (DM1) covalently linked via disulfide bonds to the humanized monoclonal antibody bivatuzumab.

The cytotoxic macrolide maytansine is a powerful low-molecular-weight inhibitor of tubulin polymerization, thereby disrupting microtubule assembly [16,17]. Although preclinical data suggested that maytansine itself possesses substantial antitumor activity in a variety of solid tumors, further clinical development of the compound was abandoned after phase II testing failed to show significant efficacy [18–23]. DM1 is an analogue of maytansine, a so-called maytansinoid, with a 100–1000-fold higher cytotoxic potency than other clinically used anticancer drugs such as taxanes or anthracyclines [11,24].

Bivatuzumab is a humanized IgG1κ monoclonal antibody with a molecular weight of approximately 143 000 Da. It does not show intrinsic activity such as complement-derived cellular cytotoxicity. Bivatuzumab recognizes the variant domain v6 of the hyaluronate receptor CD44v6, which is preferentially expressed by squamous cell carcinoma of the head and neck, adenocarcinoma of the breast, and nonsmall cell cancer of the lung, which will be the prime targets for bivatuzumab-based immunotherapy [25,26]. Bivatuzumab binds to a subset of epithelial tissues like skin keratinocytes, squamous epithelium of the cervix, epithelium of the cornea and epithelium of the tonsils. Although normal ductal epithelium of the breast is negative for CD44v6, a subset of breast cancers shows expression of this splice variant [27].

Clinical data from three phase I trials with ^{186}Re-radiolabeled bivatuzumab in patients with squamous cell carcinoma of the head and neck, nonsmall cell lung cancer, and adenocarcinoma of the breast showed good tumor localization as well as lack of significant immunogenicity of the radiolabeled antibody [28]. Furthermore, in one phase I trial, [^{186}Re]bivatuzumab demonstrated its ability to target CD44v6-positive tumor cells in breast cancer patients. Bivatuzumab mertansine contains around three DM1 drugs per antibody. On binding to CD44v6-expressing cells, bivatuzumab mertansine is internalized and DM1 is released, resulting in mitotic arrest and cell death.

The present trial was planned to investigate the safety, pharmacokinetics and preliminary efficacy of bivatuzumab mertansine in female patients with MBC that expresses CD44v6 in at least 50% of tumor cells.

Patients and methods

Eligibility requirements included female patients aged 18 years or older with MBC that expresses CD44v6 in at least 50% of tumor cells in primary tumor tissue as assessed by immunohistochemistry, pretreatment with anthracyclines and taxanes, tumor metastases measurable by computed tomography (CT) or magnetic resonance imaging (MRI), life expectancy of at least 6 months, no chemotherapy, radiotherapy or immunotherapy within the last 4 weeks before study entry, adequate organ function, Eastern Cooperative Oncology Group performance score ≤ 2, and signed informed consent.

The trial started following the approval of the institutional ethics committee review board in October 2002. It was conducted following the Declaration of Helsinki (version of 1996) in accordance with the Harmonised Tripartite Guideline for Good Clinical Practice and in accordance with applicable regulatory requirements.

Immunohistochemistry

Paraffin-embedded primary tumor tissue was cut into sections (around 2 μm). Sections were stained using an automated immunohistochemical technique (BioTek TechMate; BioTek Solutions, Newport Beach, California, USA) with strict adherence to the staining protocol. The following primary antibodies were used (clones in parentheses): CD44v6 (VFF18) (Bender Medical Systems, Wien, Austria), estrogen receptor (1D5), progesterone receptor (PR88) and HER2/*neu* (A0485) (all reagents from DakoCytomation, Ely, UK). Hormone receptor positivity was assumed when the semiquantitative score was 3 points or more out of a maximum of 12 points. HER2/*neu* immunoreaction was scored from 0 to 3, only with respect to cell membrane staining. In the case of a HER2/*neu* score 2 + , fluorescent in-situ hybridization was performed.

Pretreatment evaluation and follow-up

Pretreatment evaluation consisted of medical history, physical examination, laboratory examinations (hematology, biochemistry, coagulation parameters, tumor markers, urine analysis, pregnancy test), electrocardiogram, chest radiograph, CT or MRI of measurable metastatic lesions, ophthalmologic examination and immunohistochemistry of primary tumor tissue. During study treatment, patients were assessed for toxicity, pharmacokinetics and efficacy. Target lesions were measured by CT or MRI after 3 weeks following each infusion of bivatuzumab mertansine. Patients who had clinical benefit (i.e. complete or partial remission or stable disease according to the response evaluation criteria in solid tumors) were eligible for further treatment with bivatuzumab mertansine. During follow-up, patients were evaluated every 3 months until progression, lost to follow-up or start of further anticancer treatment.

Administration of bivatuzumab mertansine and dose-escalation plan

Bivatuzumab mertansine was administered on an outpatient basis as one single intravenous infusion over 30 min without premedication with an inline filter followed by a 100-ml wash of the tubing system with 0.9% sodium chloride. Starting dose was 25 mg bivatuzumab mertansine/m^2 body surface and dose was escalated

in 25 mg/m^2 increments up to the maximum tolerated dose (MTD). At least one evaluable patient was entered at each dose level. If the patient did not experience any National Cancer Institute Common Toxicity Criteria version 2 (NCI-CTC) grade 2–4 adverse event during the 3 weeks following infusion, the next patient was treated at the next higher dose level. As soon as one patient experienced a drug-related NCI-CTC grade 2–4 adverse event at a certain dose level, a further two patients had to be treated at that dose level and at least three patients had to be treated at all subsequent dose levels. If a patient experienced dose-limiting toxicity (DLT), the number of patients treated at that dose level had to be increased up to a total of six. DLT was defined as drug-related NCI-CTC grade 4 neutropenia for at least 4 days or complicated by infection, thrombocytopenia of less than 25 000/mm^3, or NCI-CTC grade 3 or 4 nonhematological toxicity except nausea.

In case no more than one patient out of six experienced DLT, the dose was escalated to the next higher level. In case two or more out of six patients experienced DLT, the dose had to be de-escalated to the next lower dose level with a total number of six patients to be treated at that dose level. MTD was defined as the highest dose level at which no more than one patient out of six experienced DLT. The MTD was planned to be defined on the basis of DLT observed during the first 3 weeks. Furthermore, 12 patients were planned to be treated at the MTD. Patients without DLT who had at least disease stabilization from the treatment were eligible for a further treatment course at the same dose level. In addition to disease stabilization or response, the same eligibility criteria had to be fullfilled as at the study entry. No intrapatient dose escalation was allowed.

Evaluation of toxicity and response

Vital signs had to be recorded at screening visit, on day 1 preinfusion, 10, 35, 120, 240 and 480 min after start of infusion of bivatuzumab mertansine, and on days 2, 4, 7, 10, 14 and 21. Safety laboratory parameters were determined at screening visit, on days 1, 2, 7, 14 and 21, and optional on days 4 and 10. The occurrence of adverse events was evaluated at all time points. Response was assessed by tumor measurements and evaluated according to the Response Evaluation Criteria In Solid Tumors [29]. One to 10 target lesions had to be identified at screening by CT or MRI. Re-evaluation had to be done at week 3 after each administration of bivatuzumab mertansine and every 3 months thereafter.

Pharmacokinetic sampling and assays

For measuring the concentration of bivatuzumab mertansine and anti-CD44v6-IgG (which is the sum of intact bivatuzumab mertansine and any IgG antibody recognizing CD44v6), and for detection of antibivatuzumab mertansine antibodies 6 ml whole blood was drawn at screening, on day 1 directly before infusion of bivatuzumab mertansine, 5 min after the end of infusion and a 100-ml wash with NaCl, 120, 240 and 480 min after start of infusion, and on days 2, 4, 7, 10, 14 and 21. Blood was collected in a nonanticoagulant tube. Samples were allowed to clot for not more than 60 min at room temperature, then centrifuged at 4–8°C to prepare serum. Each serum sample (approximately 3 ml) was transferred immediately to a cryotube and was frozen within 30 min after preparation. Serum was stored at –20°C until shipped on dry ice to the sponsor's laboratory for analysis. All samples were analyzed for bivatuzumab mertansine and anti-CD44v6-IgG. For determination of antibivatuzumab mertansine antibodies the samples taken at the screening day, at day 1 before infusion and at day 21 were used. In repeated courses the same sampling scheme was applied without a screening day.

Serum concentrations of bivatuzumab mertansine, anti-CD44v6-IgG and antibivatuzumab mertansine antibodies were determined by fully validated enzyme–linked immunosorbent assays (ELISAs). Bivatuzumab mertansine was quantified using a sandwich-type ELISA with the antigen glutathione S-transferase (GST)-CD44v6 immobilized on the microtiter plates and a selective biotinylated mouse monoclonal anti-DM1 detector antibody. Sandwich complexes formed were detected photometrically after incubation with streptavidin–peroxidase and a chromogenic substrate. The optimized ELISA enables the accurate and precise measurement of the analyte in the range 2–100 ng/ml serum. The lower limit of quantification was about 13pmol/l. All samples were diluted by a factor of at least 1 : 100. Anti-CD44v6-IgG, which is the sum of intact bivatuzumab mertansine and any IgG antibody recognizing CD44v6, was quantified using a sandwich-type ELISA with the antigen GST-CD44v6 immobilized on the microtiter plates and an enzyme-labeled, rabbit polyclonal antihuman detector antibody. Sandwich complexes formed were detected photometrically after incubation with a chromogenic substrate. The ELISA enables the accurate and precise measurement of the analyte in the range 100–4000 ng anti-CD44v6-IgG equivalents/ml serum. The lower limit of quantification was about 0.67 nmol/l. All samples were diluted by a factor of at least 1 : 200. Deconjugated bivatuzumab mertansine concentrations (bivatuzumab without mertansine; however, the linker or parts of the linker might still be present) were calculated by subtracting the measured bivatuzumab mertansine concentrations from the respective measured anti-CD44v6-IgG concentrations. The antibivatuzumab mertansine antibodies were detected using a selective ELISA of the double antigen or bridging type, essentially as described before [28]. Owing to a missing positive human antibivatuzumab mertansine serum, which could have been used as a standard, the validation of the present

assay was carried out using polyclonal rabbit antibivatuzumab mertansine-IgG antibodies that were purified from serum using protein A. This antibody fraction was only available in solutions with a concentration for the whole content of rabbit IgG, which was specific as well as unspecific for bivatuzumab mertansine. It is not possible to quantify the antibody concentrations absolutely in terms of antibody mass per volume. This assay only enabled the relative measurement of antibivatuzumab mertansine concentration in ng reference antibody equivalents/ml. The range of measurement was 10–500 ng equivalents/ml after a 1 : 5 dilution of serum. The first value was also taken as cut-off value, that is all sera $\geq$ 10 ng equivalents/ml indicated an antibivatuzumab mertansine immune response.

The pharmacokinetic parameters of bivatuzumab mertansine and deconjugated bivatuzumab mertansine were calculated according to noncompartmental methods using the WinNonlin software program (Professional, version 4.1; Pharsight, Mountain View, California, USA). Actual sampling times were used for the pharmacokinetic analysis. For predose samples the actual sampling time was set to zero. The pharmacokinetic parameters determined for bivatuzumab mertansine were area under the serum concentration versus time curve (AUC) from time point zero to infinity ($AUC_{0-\infty}$), AUC from time point zero to time point 168 h after drug administration (AUC_{0-168}), maximum serum concentration (C_{max}), time to reach maximum serum concentration (t_{max}), terminal half-life ($t_{1/2}$), total body clearance (CL) and volume of distribution at steady state (V_{ss}); for deconjugated bivatuzumab mertansine $AUC_{0-\infty}$, AUC_{0-168}, C_{max}, t_{max} and $t_{1/2}$, respectively. $AUC_{0-\infty}$, AUC_{0-168} and C_{max} were normalized to 1 mg/m^2 of bivatuzumab mertansine. Individual C_{max} and t_{max} were taken direcly from the serum concentration–time profiles of each participant. The apparent terminal half-life was calculated by dividing ln2 by the terminal rate constant (λ_z), which was estimated from a regression of ln(C) versus time over the terminal log-linear drug disposition portion of the concentration–time profiles. The AUC_{0-168} was calculated using the linear up/log down algorithm. If the drug concentration was smaller than the preceding concentration, the logarithmic method was used. The $AUC_{0-\infty}$ was derived from the formula $AUC_{0-t_z} + C'_{t_z}/\lambda_z$. AUC_{0-t_z} denotes the AUC from time zero to the time of the last quantifiable drug concentration and was calculated by the linear up/log down method as described above. C'_{t_z} is the predicted concentration at the time point t_z, the last time point with a serum concentration above the quantification limit, and λ_z denotes the terminal rate constant. CL was calculated by dividing dose/$AUC_{0-\infty}$ and V_{ss} was calculated as the product of CL and mean residence time (MRT), where MRT was determined according to the following equation: $MRT = [(AUMC_{0-\infty}/AUC_{0-\infty}) - T/2]$, where AUMC is the area under the moment curve and T is the duration of infusion.

Pharmacokinetic results were presented only descriptively. No statistical tests were performed with pharmacokinetic parameters.

Results

From October 2002 to November 2004, 76 patients were screened, resulting in 24 patients eligible for the trial. Those 24 patients with a mean age of 50 years (range 30–68) were treated with bivatuzumab mertansine at the Universities of Heidelberg (23 patients) and Tübingen (one patient), respectively. Eastern Clinical Oncology Group performance score at baseline was 0 in four (17%), 1 in 15 (62%) and 2 in five patients (21%). All patients had received several courses of different chemotherapy protocols (median 7, range 4–12) including anthracyclines and taxanes before entering the study. In addition, 79% had received prior endocrine treatment, 33% prior targeted or immmunotherapy and 92% prior radiotherapy. Patient and tumor characteristics in detail are given in Table 1.

With a starting dose of 25 mg/m^2 the highest dose administered was 200 mg/m^2 (Table 2). Two patients

Table 1 Patient and tumor characteristics

Characteristics	No. of patients (n=24)	Percentage
Age (years)		
Mean	50	
Range	30–68	
ECOG PS		
0	4	17
1	15	62
2	5	21
Hormone receptor status		
ER or PgR-positive	16	67
ER and PgR-negative	8	33
HER2 status		
3+ or 2+/FISH-positive	4	17
0, 1+ or 2+/FISH-negative	19	79
Unknown	1	4
Metastatic site		
Bone	14	58
Soft tissue	10	42
Visceral	19	79
No. of metastatic sites		
1	4	16
2	10	42
>2	10	42
Prior chemotherapy protocols		
Median	7	
Range	4–12	
Anthracycline containing	24	100
Taxane containing	24	100
Prior endocrine therapy	19	79
Prior targeted therapy[a]	6	25
Prior immunotherapies[b]	2	8
Prior radiotherapy	22	92

ECOG PS, Eastern Cooperative Oncology Group performance score; ER, estrogen receptor; FISH, fluorescence in-situ hybridization; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor.
[a]Trastuzumab, four patients; gefitinib, two patients.
[b]MUC1 vaccine, one patient; tumor vaccine therapy, one patient.

experienced DLT. One patient with pre-existing liver and bone metastases treated with 125 mg/m^2 bivatuzumab mertansine developed reversible increases in liver enzymes (observed on days 7–14) NCI-CTC grade 3 (aspartate amino transferase and alanine amino transferase and alkaline phosphatase) and NCI-CTC grade 4 (γ-glutamyl transferase), respectively, which were considered study drug related. A second patient treated with 175 mg/m^2 bivatuzumab mertansine developed drug-related vomiting NCI-CTC grade 3 (day 1 and from day 3 until day 8) with concomitant NCI-CTC grade 1 fever and diarrhea (from day 3 until day 11) requiring hospitalization. The patient died from renal failure. The fatal event might have been caused by deterioration of a pre-existing compensated renal insufficiency, which exacerbated because of vomiting. As a consequence of one fatal toxic epidermal necrolysis (TEN) in a patient with cancer of the esophagus observed within a trial running in parallel to this study the sponsor decided to immediately discontinue the complete clinical trials programme with bivatuzumab mertansine. After consideration of all information concerning the fatal event in that patient and the activity of bivatuzumab mertansine to cause apoptosis of skin keratinocytes with the potential to induce a TEN, the risk-benefit assessment turned negative. Therefore, the MTD within the trial presented here could not be reached.

In this trial no further drug-related adverse events appeared to be of concern with regard to the safety and tolerability of bivatuzumab mertansine. The most frequently reported side effects were transient skin disorders, mostly erythema and rash, which occured in 15 patients (63%) at doses above 75 mg/m^2, but did not exceed NCI-CTC grade 2. In addition, vomiting, nausea, fever, headache and diarrhoea (all NCI-CTC grade ≤ 2 except the one patient with vomiting NCI-CTC grade 3) occured but resolved completely (for details see Table 3).

All 24 patients treated in this single-dose study were assessable for pharmacokinetic analyses. Figures 1 and 2 show the dose-normalized individual and arithmetic mean serum concentration–time profiles of bivatuzumab mertansine and deconjugated bivatuzumab mertansine, respectively. Descriptive statistics of (dose-normalized) pharmacokinetic parameters for bivatuzumab mertansine and deconjugated bivatuzumab mertansine are shown in Table 4. The maximum serum concentrations of on average 0.567 µg/ml/1 mg/m^2 were mainly reached shortly after the end of infusion. Clearance, volume of distribution at steady state and half-life were 1.29 ml/min, 4.54 l and 69.6 h, respectively, after single dosing in the dose range investigated. Individual $AUC_{0-\infty}$ and AUC_{0-168} suggest dose-proportional increase in the exposure to bivatuzumab mertansine and deconjugated bivatuzumab mertansine after a single infusion of 25–200 mg/m^2 of bivatuzumab mertansine. Interindividual variability in all pharmacokinetic parameters of bivatuzumab mertansine and deconjugated bivatuzumab mertansine was relatively low. No significant accumulation of bivatuzumab mertansine and deconjugated bivatuzumab mertansine was observed in the seven patients who received more than one dose of study drug. Figure 3 shows dose-normalized individual serum concentration versus time curves of

Table 2 Dose-escalation scheme and incidence of DLT

	Dose of BIWI 1 (mg/m^2)								
	25	50	75	100	125	150	175	200	Total
No. of patients treated	1	1	1	3	6	3	6	3	24
Mean age	52	56	51	45	47	52	50	55	50
Mean percentage of CD44v6-positive cells	95	60	60	93	86	90	83	80	84
No. of patients with DLT	0	0	0	0	1[a]	0	1[b]	0	2

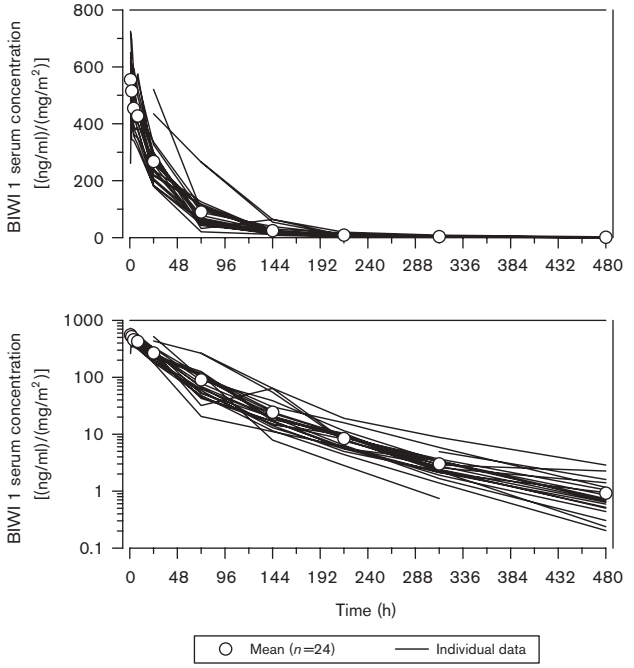
BIWI 1, bivatuzumab mertansine; DLT, dose-limiting toxicity; NCI-CTC, National Cancer Institute Common Toxicity Criteria.
[a]Elevation of γ-glutamyl transferase NCI-CTC grade 4, and of aspartate amino transferase, alanine amino transferase and alkaline phosphatase NCI-CTC grade 3.
[b]Vomiting NCI-CTC grade 3.

Table 3 Observed side effects (all NCI-CTC grade ≤ 2 except one patient who experienced transient vomiting NCI-CTC grade 3); number of patients

	Dose of BIWI 1 (mg/m^2)								
	25	50	75	100	125	150	175	200	Total
Patients treated	1	1	1	3	6	3	6	3	24
Erythema	0	0	0	0	3	0	4	2	9
Exanthema	0	0	0	0	0	3	2	1	6
Vomiting	0	0	0	0	0	0	3	2	5
Nausea	0	0	0	0	0	0	3	1	4
Fever	0	0	0	2	0	0	1	1	4
Headache	0	0	0	1	2	0	0	1	4
Diarrhea	0	0	0	0	0	0	2	1	3

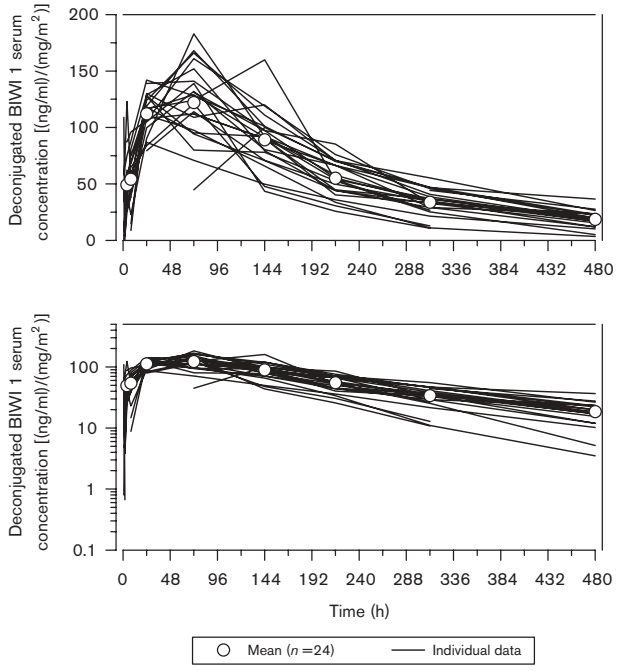
BIWI 1, bivatuzumab mertansine; NCI-CTC, National Cancer Institute Common Toxicity Criteria.

Fig. 1



Dose-normalized individual and arithmetic mean serum concentration versus time curves of bivatuzumab mertansine after a 0.5-h infusion of bivatuzumab mertansine (BIWI 1), course 1 (upper graph: lin scale; lower graph: semi-log scale).

Fig. 2



Dose-normalized individual and arithmetic mean serum concentration versus time curves of deconjugated bivatuzumab mertansine after a 0.5-h infusion of bivatuzumab mertansine (BIWI 1), course 1 (upper graph: lin scale; lower graph: semi-log scale).

bivatuzumab mertansine in the one patient who received four repeated 0.5-h infusions of bivatuzumab mertansine. Bivatuzumab mertansine had no apparent immunogenicity as in none of the patients were antibivatuzumab mertansine antibodies detected.

None of the patients showed an objective response, i.e. either a complete or partial remission to bivatuzumab mertansine, 10 patients had a progressive disease after the first dose of bivatuzumab mertansine (Table 5). As best response, 12 patients (50%) showed stable disease at week 3 after the first dose. Seven of these 12 patients received further treatment with bivatuzumab mertansine. Five patients received one additional course, one patient received two (stable disease after course 1) and another patient three additional courses (stable disease after courses 1 and 2). Ultimately, all patients progressed. Two patients were not evaluable for response owing to missing tumor lesion measurement at the end of trial visit. The characteristics of patients with stable disease after bivatuzumab mertansine are listed in Table 6.

Discussion

Preclinical data with bivatuzumab mertansine, an antibody targeting the cell surface antigen CD44v6 linked to the maytansinoid DM1, suggest antitumor activity in tumors expressing CD44v6 [26,28]. Although not a primary end point in this phase I study, none of the heavily pretreated 24 patients with MBC that expresses CD44v6 showed an objective response following one single dose of 25–200 mg/m^2 of bivatuzumab mertansine. Twelve patients (50%), however, experienced disease stabilization, which indicates activity of single-agent bivatuzumab mertansine in heavily preteated CD44v6-positive MBC. Furthermore, seven of these patients (29%) treated with doses above 100 mg/m^2 received one to three additional treatment courses every 3–4 weeks before progression. No association with clinical or tumorbiologic parameters, e.g. metastatic sites, and no clear dose–response relationship was observed.

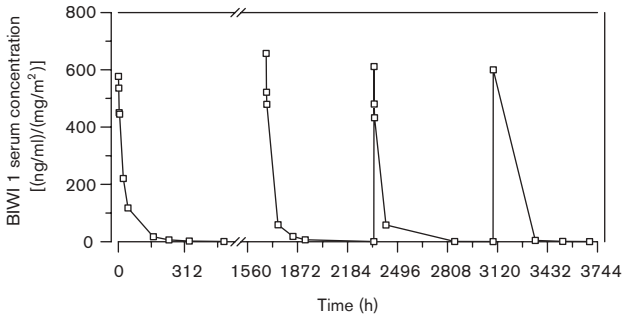
Two patients had DLT. One patient with pre-existing liver and bone metastases treated with 125 mg/m^2 bivatuzumab mertansine developed temporary increases in liver enzymes of NCI-CTC grade 3 and 4, the reason for which is unclear. Rapid decline of liver enzymes within a few days favors a drug-related side effect and argues against progression of liver metastases. Another patient treated with 175 mg/m^2 developed drug-related vomiting of NCI-CTC grade 3. She died from renal failure, which might have been caused by deterioration of a pre-existing renal insufficiency exacerbated owing to dehydration after vomiting. As one fatal TEN occurred in

Table 4 Pharmacokinetic parameters of bivatuzumab mertansine and deconjugated bivatuzumab mertansine in 24 patients: noncompartmental analysis

	Geometric mean (geometric coefficient of variation)						
	$AUC_{0-\infty}$ [(μg/ml*h)/(mg/m^2)]	AUC_{0-168} [(μg/ml*h)/(mg/m^2)]	C_{max} [(μg/ml)/(mg/m^2)]	t_{max}[a] (h)	$t_{1/2}$ (h)	CL (ml/min)	V_{ss} (l)
BIWI 1	22.2 (30.4)	20.5 (29.9)	0.567 (14.2)	0.833, 0.567–4.38	69.6 (25.0)	1.29 (31.0)	4.54 (24.3)
dBIWI 1	31.9 (27.0)	16.3 (21.1)	0.129 (19.4)	70.9, 20.6–169	135 (37.2)	ND	ND

$AUC_{0-\infty}$, area under the serum concentration versus time curve from time point zero to infinity; AUC_{0-168}, area under the serum concentration versus time curve from time point zero to time point 168 h; BIWI 1, bivatuzumab mertansine; dBIWI 1, deconjugated BIWI1; C_{max}, maximum serum concentration; CL, total body clearance; ND, not determined; t_{max}, time to reach maximum serum concentration; $t_{1/2}$, terminal half-life; V_{ss}, volume of distribution at steady state.
[a]Median, range.

Fig. 3



Dose-normalized individual serum concentration versus time curves of bivatuzumab mertansine (BIWI 1) after repeated 0.5-h infusions of bivatuzumab mertansine in the one patient who received four courses of treatment.

a trial running in parallel, the sponsor decided to discontinue immediately the complete clinical trials programme with bivatuzumab mertansine and the MTD in this trial could not be determined. Thus, a conclusive assessment of the efficacy and safety of bivatuzumab mertansine in CD44v6-positive MBC cannot be made.

Pharmacokinetic analyses suggest a dose-proportional increase in the exposure to bivatuzumab mertansine and deconjugated bivatuzumab mertansine after a single infusion of 25–200 mg/m^2 of bivatuzumab mertansine without evidence of accumulation with every-3-weeks dosing. Clearance, volume of distribution at steady state and half-life for bivatuzumab mertansine were 1.29 ml/min, 4.54 l and 69.6 h, respectively. The C_{max} values indicate that bivatuzumab mertansine distributes initially into the plasma water; however, the values for V_{ss} are slightly higher than the volume of the plasma water, suggesting later additional distribution into extravascular spaces. Immunogenicity data provide evidence that bivatuzumab mertansine does not induce human anti-humanized antibodies.

Skin and subcutaneous tissue disorders of NCI-CTC grade 1–2, mostly erythema and exanthema, were the most frequently reported adverse events observed in 63% of patients at doses above 75 mg/m^2. This is in line with the adverse event profile observed in phase I trials running in parallel in patients with squamous cell carcinoma of the head and neck [30,31]. The skin reactions may be explained by the pharmacological properties of bivatuzumab, which binds to and internalizes into cells via the CD44v6 antigen. As skin keratinocytes express CD44v6, they are a potential target tissue for toxicity of bivatuzumab mertansine. Beside those skin reactions none of the observed adverse events reported in this study appears to be of concern with regard to the safety and tolerability of bivatuzumab mertansine providing adeaquate hydration in case of fluid loss.

Although skin reactions were reversible and localized in patients treated in this trial, in a dose-escalation multiple-dose study running in parallel a 67-year-old patient with squamous cell carcinoma of the oesophagus developed fatal TEN after the second weekly dose of 140 mg/m^2 of bivatuzumab mertansine. At that time a total of four phase I dose escalation trials with bivatuzumab mertansine were running in advanced squamous cell carcinoma of the head and neck and oesophagus, and in metastatic or recurrent CD44v6-positive adenocarcinoma of the breast. The highest doses administered were 325 mg/m^2 single dose [30] and 3 weekly doses of 120 mg/m^2 with 1 week rest, respectively, in the trials in advanced squamous cell carcinoma of the head and neck and esophagus. Over 60% of the patients within those studies experienced skin reactions such as rash, pruritus, erythema and localized epidermolysis. These mainly mild to moderate and fully reversible reactions increased in frequency with increasing dose and eventually became dose limiting at 325 mg/m^2 [30]. As there is neither any means to predict which patient will react with reversible skin reactions and which might be at risk to develop a potentially fatal TEN nor is there any known established prophylaxis for TEN, the potential risk for patients on bivatuzumab mertansine outweighed the observed benefit.

In conclusion, the results of all trials indicate that bivatuzumab mertansine targets CD44v6. Thus, it might have been a suitable alternative in the treatment of

Table 5 Best responses to bivatuzumab mertansine and number of courses administered

	Dose of BIWI 1 (mg/m^2)								
	25	50	75	100	125	150	175	200	Total
No. of patients treated	1	1	1	3	6	3	6	3	24
No. of patients with one course	1	1	1	3	3	3	3	2	17
No. of patients with two courses	0	0	0	0	3	0	1	1	5
No. of patients with three or more courses	0	0	0	0	0	0	2	0	2
Complete or partial response	0	0	0	0	0	0	0	0	0
Stable disease	1	1	0	0	3	1	4	2	12
Progressive disease	0	0	1	3	3	1	1	1	10
Not evaluable	0	0	0	0	0	1	1	0	2

BIWI 1, bivatuzumab mertansine.

Table 6 Characteristics of patients with stable disease after bivatuzumab mertansine

No.	Dose of BIWI 1 (mg/m^2)	Age	ER	PgR	HER2	MS	No. of MS	No. of prior chemotherapy protocols
1	25	52	p	p	p	Bone visceral soft tissue	5	12
2	50	56	p	p	n	Bone visceral	4	4
3	125	33	n	n	n	Bone	2	3
4	125	54	n	n	n	Soft tissue	2	6
5	125	64	p	p	n	Bone	1	7
6	150	59	p	p	n	Visceral	1	4
7	175	49	n	n	n	Visceral soft tissue	1	5
8	175	38	p	p	p	Bone soft tissue	2	4
9	175	50	n	n	n	Visceral	1	7
10	175	42	p	p	n	Soft tissue visceral	3	8
11	200	49	p	p	n	Soft tissue visceral	3	10
12	200	63	n	n	n	Soft tissue	1	5

BIWI 1, bivatuzumab mertansine; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; MS, metastatic site; n, negative; p, positive; PgR, progesterone receptor.

malignant tumors expressing CD44v6 such as MBC. It, however, became evident that the main toxicity of bivatuzumab mertansine, i.e., skin reactions explained by the strong expression of CD44v6 on skin keratinocytes, whereas mostly mild to moderate and reversible, could become serious and ultimately fatal. The inability to predict such a fatal event turned the risk-benefit assessment negative and prompted the sponsor to discontinue the clinical development with bivatuzumab mertansine.

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