

## A Phase I Dose Escalation Study with Anti-CD44v6 Bivatuzumab Mertansine in Patients with Incurable Squamous Cell Carcinoma of the Head and Neck or Esophagus

Bernard M. Tjink,<sup>1</sup> Jan Buter,<sup>2</sup> Remco de Bree,<sup>1</sup> Giuseppe Giaccone,<sup>2</sup> Margreet S. Lang,<sup>3</sup> Alexander Staab,<sup>4</sup> C. René Leemans,<sup>1</sup> and Guus A.M.S. van Dongen<sup>1</sup>

**Abstract** **Purpose:** To assess safety, pharmacokinetics, maximum tolerated dose, and preliminary efficacy of bivatuzumab mertansine. Bivatuzumab is a humanized monoclonal antibody directed against CD44v6, which previously seemed to be safe in phase I radioimmunotherapy trials, whereas the conjugated mertansine is a potent maytansine derivative.

**Experimental Design:** Patients with incurable squamous cell carcinoma of the head and neck or esophagus were eligible. Bivatuzumab was given weekly for 3 consecutive weeks by i.v. infusion. One patient was planned to be treated at each dose tier as long as toxicity did not reach grade 2; otherwise, three patients had to be treated until dose-limiting toxicity occurred. Starting dose was 20 mg/m<sup>2</sup> and dose was subsequently escalated in steps of 20 mg/m<sup>2</sup>. Patients without disease progression and not experiencing dose-limiting toxicity were eligible for repeated courses. Blood serum samples were taken throughout the treatment period to determine the pharmacokinetic properties of bivatuzumab mertansine and to assess the human anti – bivatuzumab mertansine antibody response.

**Results:** Seven patients received a total of 23 weekly doses of bivatuzumab mertansine. One patient at the 100 mg/m<sup>2</sup> and one at the 120 mg/m<sup>2</sup> level experienced stable disease during treatment phase but also developed grade 1 skin toxicity (desquamation). One of them received a second treatment course. At the highest dose level achieved in this study (140 mg/m<sup>2</sup>), one patient developed toxic epidermal necrolysis after two infusions and died. Massive apoptosis of skin keratinocytes had occurred, whereas only symptomatic therapy for skin toxicity was available. The risk-benefit assessment of all patients treated in the total phase I program (4 clinical trials, 70 patients) turned out to be negative after consideration of this case of a toxic epidermal necrolysis and the skin-related adverse events observed in the other trials. Therefore, development of the conjugate was discontinued. Interindividual variability in pharmacokinetic variables was low and exposure to BIW1 increased proportionally with dose. No anti – bivatuzumab mertansine reactions were observed.

**Conclusion:** The main toxicity of bivatuzumab mertansine was directed against the skin, most probably due to CD44v6 expression in this tissue. The majority of skin reactions was reversible; however, one fatal drug-related adverse event had occurred. Clinical development was discontinued before reaching maximum tolerated dose.

Squamous cell carcinoma, the predominant histologic type among tumors of the head and neck, accounts for ~5% of all malignant tumors in Europe and the United States. In 2000, an estimated 551,100 new cases of head and neck squamous cell

carcinoma of the oral cavity, pharynx, or larynx were diagnosed worldwide (1). Early stages (stage I/II) of head and neck squamous cell carcinoma generally have a good prognosis after surgery or radiotherapy. Unfortunately, prognosis is much worse for advanced stage disease (stage III/IV), which is diagnosed in about two thirds of all head and neck squamous cell carcinoma patients. Despite improvements in locoregional treatment modalities, the rate of locoregional recurrence is still close to 40%, whereas ~25% of these patients develop distant metastases. Autopsy studies have shown incidences of distant metastases in up to 57% (2–5). It is plausible that many advanced stage head and neck squamous cell carcinoma patients harbor residual tumor cells after surgery and radiotherapy. The role of adjuvant chemotherapy for this group of patients is limited, and therefore the development of an effective adjuvant systemic treatment is a major challenge.

The incidence of esophageal cancer amounts to 13,000 new cases in the United States alone with >12,000 cancer-related deaths per year (6). The majority of patients is diagnosed with

**Authors' Affiliations:** Departments of <sup>1</sup>Otolaryngology/Head and Neck Surgery and <sup>2</sup>Medical Oncology, VU University Medical Center, Amsterdam, the Netherlands; <sup>3</sup>Boehringer Ingelheim BV, Alkmaar, the Netherlands; and <sup>4</sup>Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany

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**Requests for reprints:** Guus A.M.S. van Dongen, Department of Otolaryngology/Head and Neck Surgery, VU University Medical Center, De Boelelaan 1117, P.O. Box 7057, 1007 MB Amsterdam, the Netherlands. Phone: 31-20-444-0953; Fax: 31-20-444-3688; E-mail: gams.vandongen@vumc.nl.

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locally advanced disease with median survival rates of 12 to 14 months, or with metastatic disease with survival rates of <12 months. Systemic therapy in this disease has modest activity, and new treatment modalities are urgently needed in this disease as well.

Selective targeting of head and neck squamous cell carcinoma by use of monoclonal antibodies (mAb) might contribute to a more effective treatment of advanced stage disease. Previous efforts of our group focused on the development of radioimmunoconjugates for radioimmunotherapy because head and neck squamous cell carcinoma is relatively radiosensitive. In our selection of a suitable target antigen for head and neck squamous cell carcinoma, the E48 antigen (7, 8) and CD44 splice variants containing the v6 domain (CD44v6) seemed to be the most promising thus far.

In the past decade, encouraging progress was obtained with the development of CD44v6-directed radioimmunotherapy in head and neck squamous cell carcinoma. For this purpose, the chimeric mAb U36 (cmAb U36) and the humanized mAb BIWA 4 (bivatuzumab), both directed against CD44v6, have been evaluated (9, 10). Although not a primary objective, phase I dose escalation radioimmunotherapy studies with  $^{186}\text{Re}$ -labeled cmAb U36 and  $^{186}\text{Re}$ -labeled bivatuzumab showed promising antitumor effects with consistent stable disease at higher radioactivity dose levels (11–13). The only toxicity observed in the radioimmunotherapy studies was myelotoxicity caused by circulating radioimmunoconjugate and, in some patients, mild oral mucositis. In these studies, bivatuzumab seemed to have low immunogenicity. Binding of these mAbs to the target antigen resulted in internalization of the antibody (14).

Based on aforementioned promising clinical results, we hypothesized that coupling of a nonradioactive cytotoxic drug to bivatuzumab instead of radionuclides might provide us with a second promising option for adjuvant therapy in head and neck cancer. From such conjugate no bone marrow toxicity has to be expected, and therefore perspectives might arise for combined treatment with conventional chemotherapeutics. Besides, omission of radioactivity will solve several issues related to logistics and radiation safety. Therefore, we decided to start the development and clinical evaluation of bivatuzumab mertansine.

Bivatuzumab mertansine is a tumor-activated prodrug conjugate, which consists of the cytotoxic mertansine covalently linked to bivatuzumab. After binding to CD44v6, the complex can be internalized, and the mertansine molecules will be released intracellularly by cleavage of the antibody-mertansine disulfide bonds. The origin of mertansine and the clinical evaluation of a first mertansine-antibody conjugate have recently been described by Tolcher et al. (15) and Helft et al. (16). Mertansine [ $N^2$ -deacetyl- $N^2$ -(3-mercapto-1-oxopropyl)-maytansine  $\text{C}_{35}\text{H}_{48}\text{ClN}_3\text{O}_{10}\text{S}$ ] is a derivative of the antimicrotubule agent maytansine and has a 100- to 1,000-fold higher cytotoxic potency than other clinically used anticancer drugs such as taxanes or anthracyclines (17, 18). On intracellular delivery and release, mertansine inhibits tubulin polymerization, thereby disrupting microtubule assembly (19, 20); as a result, mitotic arrest and tumor cell death occur. A phase I trial with cantuzumab mertansine (huC242-DM1), 235 mg/m<sup>2</sup> given i.v. once every 3 weeks in patients with solid malignancy, showed minor responses in chemotherapy-refractory patients without severe toxic effects (15).

In the phase I trial presented herein, bivatuzumab mertansine was given weekly for 3 weeks as an i.v. infusion to patients with incurable carcinoma of the head and neck or esophagus. The principal objectives of the study were to assess the safety, maximum tolerated dose (MTD), and preliminary efficacy and to determine the pharmacokinetics and immunogenicity of bivatuzumab mertansine. Patients without disease progression and not experiencing dose limiting toxicity (DLT) were eligible for repeated courses.

## Patients and Methods

### Patient selection

This was a phase I study with an accelerated dose titration. The study was approved by the Institutional Review Board of the VU University Medical Center (Amsterdam, the Netherlands) and written informed consent was required from all patients.

Patients with histologically confirmed squamous cell carcinoma of the head and neck or esophagus with local and/or regional recurrent disease or distant metastases and who were refractory to or not amenable to established treatments were eligible for participation in this study. Other eligibility criteria included clinically or radiologically (by computed tomography, magnetic resonance imaging, or ultrasound) evaluable tumor deposits, age of at least 18 years, life expectancy of at least 3 months, an Eastern Cooperative Oncology Group performance score  $\leq 2$ , and without prior chemotherapy, radiotherapy, or immunotherapy within the past 4 weeks, brain metastases requiring therapy, known secondary malignancy requiring therapy, active infectious disease, and neuropathy grade  $\geq 2$  or concomitant non-oncological diseases that could interfere with the evaluation of the safety of the trial drug. Expression of CD44v6 was assessed by immunohistochemistry on archival tumor samples, but this was not done before patient entry in the study, as >95% of the squamous carcinomas of the head and neck and esophagus are known to show homogeneous expression of the antigen (10).

Patients were required to have adequate organ function defined as an absolute neutrophil count of at least 1,500/mm<sup>3</sup>, platelet count of at least 100,000/mm<sup>3</sup>, bilirubin  $\leq 26$   $\mu\text{mol/L}$ , serum creatinine  $\leq 132$   $\mu\text{mol/L}$ , and aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase  $\leq 3$  times the upper limit of normal. Pregnant and lactating women were excluded from participation in the study, and all patients with reproductive potential were required to use an effective contraceptive method if they were sexually active.

### Bivatuzumab, bivatuzumab mertansine, and CD44v6

Bivatuzumab (BIWA 4), an immunoglobulin G1 (IgG)-1 mAb against CD44v6, is the humanized version of BIWA 1 and was generated, produced, and characterized by Boehringer Ingelheim (Germany) as previously described (13).

The CD44 protein family consists of isoforms, encoded by standard exons and up to nine alternatively spliced variant exons (v2-v10), which are expressed in a tissue-specific way and involved in physiologic functions like signal transduction, growth factor binding, cell adhesion, and cell migration. Expression of v6-containing CD44 variants has been related to aggressive behavior of various tumor types and was shown to be particularly high and homogeneous on the outer cell surface of squamous cell carcinoma of the head and neck, esophagus, lung, cervix, and vulva (21, 22). Expression was also found in some normal tissues such as skin keratinocytes, squamous epithelium of the cervix, epithelium of the cornea, and epithelium of the tonsils. No evidence was found for variability of CD44v6 expression between skin biopsies of different individuals. Immunohistochemical screening of a representative profile of normal human and cynomolgus monkey tissues revealed that CD44v6 expression was almost identical in man and monkey (23). Bivatuzumab does not cross-react with murine CD44v6.

On the basis of aforementioned data, before starting the present clinical study with bivatuzumab mertansine, toxicity studies had been done with the same conjugate in cynomolgus monkeys.<sup>5</sup> In one of the studies, animals ( $n = 3$  per sex per group) had been treated by once weekly i.v. administration for 6 weeks followed by a 4-week recovery period at doses of 0.5, 1.5, and 4 mg/kg. No mortality had occurred and none of the animals had to be sacrificed prematurely. Exfoliation of the skin was noted for males and females in all dose groups from day 8 of study. In the mid dose, exfoliation was predominantly located at the femoral region. In the high dose, exfoliation progressed to scabs and was reported in the femoral regions, legs, arms, and chest. Pigmentation was seen at the injection site of all treated animals. Microscopically epidermal necrosis and epidermal hyperplasia, as well as prominent melanin in the stratum basale, were noted. Body weight, food consumption, electrocardiogram, and blood pressure were unremarkable. Whereas no differences in hematology were noted between treated animals and controls, a lower percentage of neutrophils was detected in the bone marrow. The changes had returned to pretreatment values after end of recovery. Increases of aspartate aminotransferase and alanine aminotransferase by a factor of 2 in the mid and high doses were not considered to be of biological significance. Ophthalmologic examination revealed increased pigmentation from week 3 onwards; however, no microscopic changes were observed in the eye. At the end of the recovery period, however, the intensity of pigmentation had decreased. Based on these findings and the safety assessment of the eye findings, the highest nonsevere toxic dose of bivatuzumab mertansine following once weekly administration to cynomolgus for 6 weeks was 4 mg/kg. This reflects an  $AUC_{0-\infty}$  of 2,410  $\mu\text{g} \cdot \text{h/mL}$  and a  $C_{\text{max}}$  of 105  $\mu\text{g/mL}$  (at first dose).

Treatment of mice bearing established A431 or FaDu human SCC xenografts with a single cycle of five daily i.v. doses of bivatuzumab mertansine resulted in dose-dependent antitumor efficacy with complete, long-lasting tumor regressions observed at well-tolerated doses (2.1-21 mg/kg/d). The unconjugated antibody given at the highest dose level showed no effect on tumor growth. In the FaDu model, an alternative treatment schedule (once weekly for 4 weeks) was tested in addition and resulted in comparable antitumor efficacy (24).

Starting clinical studies was considered to be justified because toxicities were expected to be completely reversible, and the potential benefit of immunotherapy with bivatuzumab mertansine was anticipated to outweigh these risks.

### Study design

Bivatuzumab mertansine was administered i.v. in 30 minutes as a weekly infusion for 3 weeks at 7-day interval. The dose of bivatuzumab mertansine was fixed within one course of three infusions, starting at 20  $\text{mg/m}^2$  body surface area (expressed as dose of immunoconjugate protein). Body surface area was calculated according to Du Bois and Du Bois (25). Doses were escalated in increments of 20  $\text{mg/m}^2$ . Patients were observed for 4 weeks after first infusion to evaluate experiences of toxicities. The National Cancer Institute Common Toxicity Criteria, version 2, was used to grade toxicity. DLT was defined as drug-related grade 3 or 4 nonhematologic toxicity (excluding nausea and/or vomiting) or drug-related grade 4 neutropenia for  $\geq 4$  days and/or complicated by infection or drug-related thrombocytopenia  $< 25,000/\text{mm}^3$ . One patient was planned to be treated at each dose level as long as any drug-related toxicity did not exceed grade 1 during first course; otherwise, three patients had to be treated until DLT occurred. When at the ongoing dose level one patient experienced DLT, the number of patients treated at that dose level had to be increased to a total of six. When no more patient experienced DLT, the dose was to be escalated to the next level. When two (or more) of six patients experienced DLT, three additional patients would be treated at one dose tier below unless six patients had already been treated at that dose tier. MTD was defined

as the highest dose at which no more than one of six patients experienced DLT. Another 12 patients would be included at the MTD dose level after MTD had been established until 18 patients had been treated at MTD. Patients without disease progression and not experiencing DLT during the 4 weeks after start of treatment were eligible for a second course. This course had to be started at least 3 weeks after the last infusion of the previous course.

During the course of this study, another three dose escalation studies were being conducted with bivatuzumab mertansine in six other centers. One trial was conducted in advanced head and neck carcinoma with single infusion and two trials in metastatic or recurrent CD44v6-positive adenocarcinoma of the breast with either single infusion or three infusions once weekly.

Bivatuzumab mertansine was supplied in 20-mL single-use vials by Boehringer Ingelheim (Biberach, Germany). Each vial contained protein at a concentration of 2.0 mg/mL in PBS (pH 6.5) containing 0.02% Tween 20. The manufacturing process was designed to give a yield of 3.5 to 4 molecules of mertansine coupled per molecule of bivatuzumab on average. Free mertansine was detected at a concentration of 300 ng/mg (0.03%). The drug product was not prefiltered on instilling the dose volume into the infusion bag and was administered to patients through an inline 1.2- $\mu\text{m}$  filter (B. Braun AG, Melsungen, Germany) within 6 hours of preparation. After 30-minute infusion, the i.v. line was flushed with 100 mL of 0.9% NaCl fluid to ensure delivery of the full drug dose.

### Pretreatment and follow-up studies

Before initiation of therapy, all patients had a history and general physical examination including ENT examination, vital signs, electrocardiogram, and chest X-ray. Preclinical toxicity studies in cynomolgus monkeys had revealed a dose- and time-dependent increase in exfoliation of the skin. In addition, pigmentation of the skin of the eyelids and pigmentation of sclerae and peripheral cornea were detected. Therefore, in this trial, besides a general physical examination, an ophthalmologic examination was done, which included slit lamp examination of sclera and cornea in mydriasis, assessment of the eyelids for reddening or dermal exfoliation of the skin, conjunctival mucosa ulcerations, blepharitis of the lid margins, madarosis of the lashes, visual acuity, intraocular pressure, inspection of the crystalline lens, and funduscopy in mydriasis. In addition, pigmentation of sclera and cornea was assessed. Several registered drugs cause pigmentation of the cornea without any effect on vision. Routine laboratory studies included full blood cell counts, reticulocyte count, chemistries, and International Normalized Ratio/prothrombin time and partial thromboplastin time, and urine analysis.

Appropriate radiological studies for the evaluation of all measurable and evaluable sites of disease were done. Radiological studies for assessment of disease status were repeated after 4 weeks. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors after each course (26). During the 4-week period of treatment, patients were observed for 24 hours after the first infusion and for 8 hours after the second and third infusion. Blood samples for complete blood counts, chemistries, and coagulation variables were obtained just before the infusion (on days 1, 8, and 15), 48 hours after the infusion (on days 3, 10, and 17), and 1 week after the last infusion (day 22). On days 1, 3, 6, 8, 10, 13, 15, 17, 20, 22, and 24, an assessment of the clinical status of the patient was done. On day 29, at the end-of-trial visit, all pretreatment studies were repeated, including tumor staging and assessment.

### Immunohistochemical staining of tumor tissues

Expression of CD44v6 in tumor samples was retrospectively assessed by immunohistochemistry. To this end, archival tumor biopsies of the patients, obtained at earlier presentation of the patient at the clinic, were stained for CD44v6 expression. Staining was done using the streptavidin-biotin-immunoperoxidase technique with the murine anti-CD44v6 mAb BIWA 1 (also designated VFF18, Bender Med Systems,

<sup>5</sup> Unpublished data.

Vienna, Austria) as primary antibody. Staining intensity for CD44v6 was scored positive [with the three gradings, 1 (+), 2 (++), 3 (+++)] or negative or not evaluable; in case of positivity, the percentage of positive cells was estimated.

### Serum pharmacokinetic sampling and assays

Serum samples for the determination of bivatuzumab mertansine and anti-CD44v6-IgG (which is the sum of intact bivatuzumab mertansine plus any IgG antibody recognizing CD44v6) were collected at the following time points: just before the start of each of the three infusions, at 35 minutes, 2 hours, 8 hours, 3 days, and 6 days after start of each of the three infusions. Additional samples were collected after the third infusion within a course at days 22, 24, and 29. In repeated courses, a reduced sampling scheme was applied.

Evaluation of the immunogenicity of the bivatuzumab mertansine conjugate was conducted by determination of human anti-bivatuzumab mertansine antibodies in serum samples taken before administration of bivatuzumab mertansine (screening visit) at all 3 infusion days before start of infusion and at end-of-trial visit (2 weeks after last administration).

At each sampling time point, 6 mL of blood were drawn to obtain ~3 mL of serum for the determination of bivatuzumab mertansine, anti-CD44v6-IgG, and human anti-bivatuzumab mertansine antibodies. Serum concentrations of bivatuzumab mertansine, anti-CD44v6-IgG, and anti-bivatuzumab mertansine antibodies were determined with fully validated ELISAs.

**Bivatuzumab mertansine ELISA.** Intact bivatuzumab mertansine was quantified with a sandwich-type ELISA with the antigen glutathione S-transferase-CD44v6 immobilized on the microtiter plates and a selective, biotinylated, mouse monoclonal anti-mertansine detector antibody. Sandwich complexes formed were detected photometrically after incubation with streptavidin-peroxidase and a chromogenic substrate. The optimized ELISA enabled the accurate and precise measurement of the analyte in the range of 2 to 100 ng/mL serum. The lower limit of quantification was ~13 pmol/L. All samples were diluted by a factor of at least 1:100.

**Anti-CD44v6-IgG ELISA.** The analyte, which is the sum of intact bivatuzumab mertansine plus any IgG antibody recognizing CD44v6, was quantified with a sandwich-type ELISA with the antigen glutathione S-transferase-CD44v6 immobilized on the microtiter plates and an enzyme-labeled rabbit polyclonal anti-human IgG as detector antibody. Sandwich complexes formed were detected photometrically after incubation with a chromogenic substrate. The optimized ELISA enabled the accurate and precise measurement of the analyte in the range of 100 to 4,000 ng anti-CD44v6-IgG equivalents/mL serum. The lower limit of quantification was ~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.

**Anti-bivatuzumab mertansine antibody ELISA.** The anti-bivatuzumab mertansine antibodies were detected with a selective ELISA of the double antigen or bridging type, essentially as described before (13). Due to a missing positive human anti-bivatuzumab mertansine serum, which could have been used as standard, the validation of the present assay was done with polyclonal rabbit anti-bivatuzumab 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 anti-bivatuzumab mertansine concentration in nanograms of reference antibody equivalents per milliliter. The range of measurement was 10 to 500 ng equivalents/mL after a 1:5 dilution of serum. The first

value was also taken as cutoff value (i.e., all sera  $\geq 10$  ng equivalents/mL indicated an anti-bivatuzumab mertansine immune response).

### Pharmacokinetic analysis

The pharmacokinetic analysis of bivatuzumab mertansine and deconjugated bivatuzumab mertansine was carried out by noncompartmental analysis of the serum concentration-time data using the WinNonlin software program (Professional, version 4.1, Pharsight Corp., Mountain View, CA). Actual sampling times were used for the pharmacokinetic analysis. The following variables were determined for each of the three administrations for each of the two substances: area under the serum concentration-time curve from 0 to 168 hours ( $AUC_{0-168}$ ), the maximum serum concentration ( $C_{max}$ ), the time to reach the maximum concentration ( $t_{max}$ ), and the terminal half-life ( $t_{1/2}$ ). As pharmacokinetics of bivatuzumab mertansine were at steady state after the third administration, the variables are assigned a (ss) denoting steady-state. The variables  $AUC_{0-168/\tau,ss}$  and  $C_{max(ss)}$  were dose normalized;  $\tau$  stands for the 168-hour dosing interval. Clearance ( $CL_{ss}$ ) and volume of distribution ( $V_{ss}$ ) at steady state were in addition calculated for bivatuzumab mertansine. And for both substances, the accumulation index for AUC ( $R_{A,AUC}$ ) and  $C_{max}$  ( $R_{A,Cmax}$ ) was determined. Individual  $C_{max(ss)}$  and  $t_{max(ss)}$  values were determined directly from the serum concentration-time profiles of each subject. The apparent terminal half-life was calculated by dividing  $\ln 2$  by the terminal rate constant [ $\lambda_{z(ss)}$ ], which was estimated from a regression of  $\ln(C)$  versus time over the terminal log-linear drug disposition portion of the concentration-time profiles.  $AUC_{0-168/\tau,ss}$  was calculated using the linear up/log down algorithm.  $CL_{ss}$  was calculated by dividing the dose by  $AUC_{\tau,ss}$ .  $V_{ss}$  was calculated by the product of  $CL_{ss}$  and mean residence time at steady state ( $MRT_{ss}$ ), where  $MRT_{ss}$  was calculated according to the following equation:  $MRT_{ss} = (AUMC_{ss} / AUC_{\tau,ss}) - (T \times 0.5)$ , where  $AUMC_{ss}$  is the area under the first moment curve at steady state and  $T$  is the duration of infusion. The accumulation indices were calculated by dividing the  $C_{max}$  and  $AUC_{0-168}$  values after the third administration by the respective values after the first administration.

## Results

All patients were included between October 2003 and November 2004. The characteristics of the seven patients enrolled in the study are listed in Table 1, whereas the treatment data are shown by Table 2. Patients received a total of 23 weekly infusions of bivatuzumab mertansine at doses ranging from 20 to 140 mg/m<sup>2</sup>. The median number of weekly doses administered per patient was 3 (range, 2-6).

**Toxicity.** No drug-related toxicity greater than grade 1 was observed during the first course of the first six dose levels. One patient in the 60 mg/m<sup>2</sup> dose level group experienced a non-drug-related grade 2 skin infection due to tumor growth through the skin. Two patients experienced a grade 1 desquamation of the skin at the 100 and 120 mg/m<sup>2</sup> dose level groups, respectively. In the first patient, desquamation started 6 days after first infusion and ended 34 days after third infusion, whereas in the second patient, symptoms started 6 days after second infusion and ended 16 days after third infusion. Both patients with desquamation showed stable disease during treatment phase. After disappearance of skin toxicity, another course of three infusions was given to the patient in the 100 mg/m<sup>2</sup> dose level starting 37 days after third infusion in the first course. Again, a grade 1 toxicity (desquamation of the skin) was seen during this treatment phase starting 4 days after the first infusion of the second course. After 1 week (4 days after second infusion), this toxicity

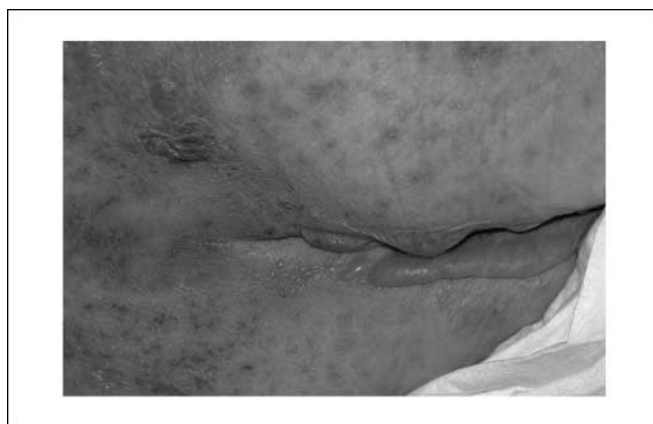
**Table 1.** Patient and tumor characteristics

Characteristics	No. patients
No. patients	7
Sex	
Male	6
Female	1
Age (y)	
Median	67
Range	50-73
ECOG performance status	
0	1
1	3
2	3
Prior therapy	
Radiotherapy	1
Surgery and radiotherapy	4
Chemoradiation	1
Surgery and chemoradiation	1
Tumor type	
Oral cavity	1
Larynx	1
Hypopharynx	2
Esophagus	2
Nose	1

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

was classified as grade 2 (desquamation of the skin). Toxicity had totally disappeared without scars 18 days after the third infusion in the second course. After the second course, progressive disease occurred. The patient treated in the 120 mg/m<sup>2</sup> dose level did not receive another course due to treatment of radionecrosis of the mandible.

The patient treated at the next dose level, 140 mg/m<sup>2</sup>, experienced a grade 1 skin toxicity (desquamation of the skin) 5 days after the first infusion. Three days after the second infusion, this patient experienced a DLT: a grade 4 skin toxicity resulting in death (Fig. 1). At autopsy of this patient, gross inspection showed loss of epidermis on large parts of the body (back from shoulders to buttocks, groins, back of the right arm, and several small loci) and bullae on both malleoli (Fig. 2). Moreover, the skin of the whole body came off on touch (Nikolsky sign). Microscopy of both affected (next to the bullae on the ankles) and unaffected skin showed separation of the epidermis from the dermis with extensive apoptosis of keratinocytes in the basal and suprabasal layers. The skin toxicity observed was a toxic



**Fig. 1.** Desquamation of skin between buttocks. Photo was taken at start of symptoms 2 days after second injection of bivatuzumab mertansine 140 mg/m<sup>2</sup> (patient no. 7).

epidermal necrosis, consisting of a full lysis of epidermis. The patient died within 2 days after start of symptoms. No other drugs had been administered that could have caused the toxic epidermal necrosis.

With respect to peripheral neuropathy and transaminases, no toxicities of biological significance as noted in the anti-MUC1-mertansine clinical studies were seen (15, 16). No other toxicities were seen during the study. No critical ophthalmologic changes were detected.

**Pharmacokinetics.** Figures 3 and 4 show the dose-normalized individual and geometric mean serum concentration-time profiles of bivatuzumab mertansine and deconjugated bivatuzumab mertansine, respectively. Bivatuzumab mertansine serum concentration-time profiles are almost superimposable after each of the three infusions, whereas the serum concentration-time profiles of deconjugated bivatuzumab mertansine after multiple dosing show accumulation.

Descriptive statistics of (dose-normalized) pharmacokinetic variables for bivatuzumab mertansine and deconjugated bivatuzumab mertansine are shown in Tables 3 and 4. The maximum serum concentrations were mainly reached at the end of infusion. 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. Clearance, volume of distribution at steady-state, and half-life were 1.51 mL/min, 5.25 L, and 69.1 hours,

**Table 2.** Dose levels of bivatuzumab mertansine administered

Dose (mg/m <sup>2</sup> /wk)	No. patients	BSA (m <sup>2</sup> )*	Total no. doses administered	Cumulative dose (mg)
20	1	1.9	3	114
40	1	1.9	3	228
60	1	1.8	3	324
80	1	1.8	3	432
100	1	2.1/2.0 <sup>†</sup>	3 + 3	630 + 600
120	1	1.6	3	576
140	1	1.7	2	476

\*BSA, body surface area according to Du Bois and Du Bois (25).

<sup>†</sup>Weight was decreased at start second course; body surface area was recalculated.

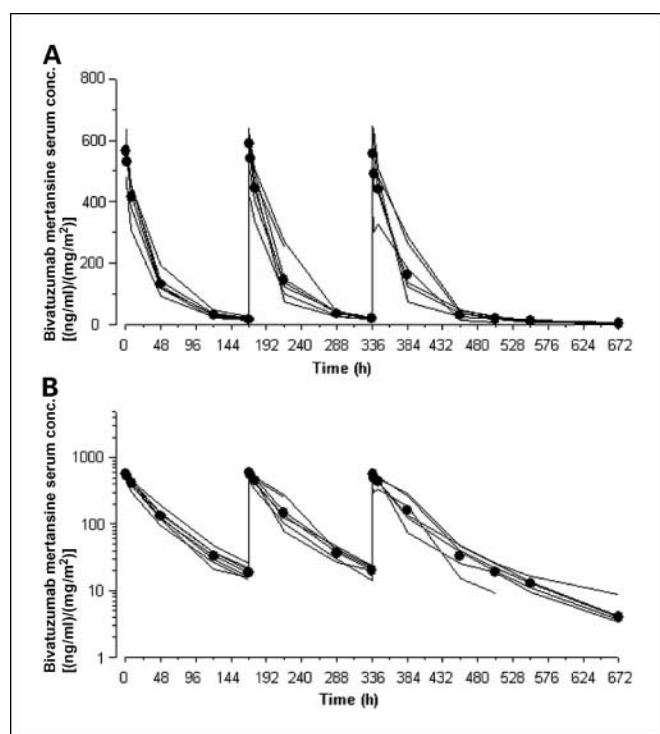


**Fig. 2.** Bulla on the ankle. Photo taken during autopsy 1 week after second injection of bivatuzumab mertansine 140 mg/m<sup>2</sup> (patient no. 7). Apoptosis of cells was observed both in affected and nonaffected skin.

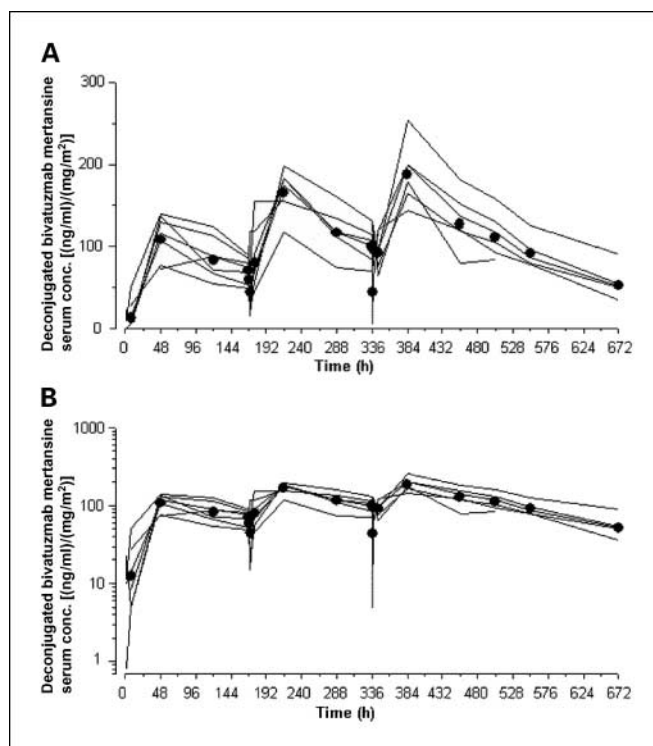
respectively. Individual dose-normalized AUC<sub>0-168</sub> values show no dependency on dose.

No significant accumulation of bivatuzumab mertansine took place during the first and third administration as shown by the accumulation indices for C<sub>max</sub> and AUC of 0.99 and 1.07. Due to the long half-life of deconjugated bivatuzumab mertansine (152-183 hours), accumulation was observed. Accumulation factors of 1.75 (C<sub>max</sub>) and 1.9 (AUC) were determined between the first and third injection.

Interindividual variability in all pharmacokinetic variables of bivatuzumab mertansine and deconjugated bivatuzumab mertansine was relatively small. The pharmacokinetic variables/profiles of conjugated and deconjugated bivatuzumab mertansine



**Fig. 3.** Individual (—) and geometric mean (●) serum concentration-time profiles of bivatuzumab mertansine during a 3-week once weekly infusion regimen, normalized to 1 mg/m<sup>2</sup>. A, linear scale. B, semi-log scale.



**Fig. 4.** Individual (—) and geometric mean (●) serum concentration-time profiles of deconjugated bivatuzumab mertansine during a 3-week once weekly infusion regimen, normalized to 1 mg/m<sup>2</sup>. A, linear scale. B, semi-log scale.

sine of the patient in dose group 140 mg/m<sup>2</sup> were similar to the pharmacokinetic variables/profiles observed in the other patients.

**CD44v6 expression, human anti-bivatuzumab mertansine response, and antitumor activity.** From five of seven treated patients, archival tumor biopsies were available for immunohistochemical assessment of CD44v6 expression. From two patients, no biopsies were available because chemoradiation therapy had been done instead of surgery. All tested specimens, and 100% of the cells within the specimen, were strongly positive [+++] for CD44v6 expression. In none of the seven patients a human anti-bivatuzumab mertansine response was detected.

Two patients at 100 and 120 mg/m<sup>2</sup>, respectively, experienced stable disease. The 100 mg/m<sup>2</sup> dose group patient was treated because of a supraclavicular lymph node metastasis, which was refractory to radiotherapy. Patient had previously received surgery and radiotherapy because of a squamous cell carcinoma of the nose (ala nasi). After a first course of three infusions, there was stable disease. After another treatment of three infusions, tumor size increased. During this period, the patient developed grade 2 skin toxicity. The second patient with stable disease was treated because of a lung metastasis, which did not enlarge during treatment. Because of radionecrosis of the mandible, the patient did not receive an additional course of three infusions. During surgery of the mandible a few weeks later, a locoregional recurrence was detected at the primary tumor site.

## Discussion

The objective of the study was to determine the MTD of three weekly doses of bivatuzumab mertansine in patients with

**Table 3.** Descriptive statistics of noncompartmental pharmacokinetic variables of bivatuzumab mertansine after a 3-week once weekly infusion regimen ( $n = 7$ , weeks 1 and 2;  $n = 6$ , week 3)

Week		AUC <sub>0-168</sub> * [( $\mu\text{g} \cdot \text{h/mL}$ )/( $\text{mg/m}^2$ )]	C <sub>max</sub> * [( $\mu\text{g/mL}$ )/( $\text{mg/m}^2$ )]	t <sub>max</sub> [h] <sup>†</sup>	t <sub>1/2</sub> [h]	CL <sub>ss</sub> [mL/min]	V <sub>ss</sub> [L]
1	gMean	19.9	0.568	0.75	42.1		
	gCV (%)	16.3	13.2	0.75-2.08	4.45		
2	gMean	20.2	0.564	0.783	40.2		
	gCV (%)	14.5	16.4	0.667-2.17	26.5		
3 <sup>‡</sup>	gMean	20.4	0.559	0.875	69.1	1.51	5.25
	gCV (%)	18.9	17.8	0.75-2.75	14.7	21.5	15.0

Abbreviations: gMean, geometric mean; gCV, geometric coefficient of variation.

\*Dose normalized.

<sup>†</sup>Median and range.

<sup>‡</sup>Variables at steady-state.

histologically confirmed squamous cell carcinoma of the head and neck or esophagus. A total of seven patients were treated at doses ranging from 20 to 140 mg/m<sup>2</sup>. The MTD could not be properly assessed due to the premature termination of this trial.

The reason for the premature termination was a fatal case of toxic epidermal necrolysis in a patient with squamous cell carcinoma of the esophagus. In view of similar, albeit less severe, unpredictable skin toxicities seen in parallel studies, a decision was taken to halt the development of this agent and all studies were terminated. In the patient who died of toxicity, there were no signs of spongiotic dermatitis. Immunofluorescence microscopy of the skin revealed no specific pattern. Immunofluorescence at the epidermal-dermal junction was negative (IgG, IgA, IgM, C1q, C3,  $\kappa$ ,  $\lambda$ ), ruling out paraneoplastic bullous pemphigoid.

During preclinical toxicity studies in cynomolgus monkeys and during the course of this and parallel clinical trials, it became evident that the main toxicity of bivatuzumab mertansine was directed against the skin. This can be explained by the expression of CD44v6 on skin keratinocytes. Accumulation of administered anti-CD44v6 mAbs in the basal layers of the skin had been observed in previous studies (10). The majority of skin reactions in the present trial were mild to moderate and fully reversible. In the present study, two patients had received a higher total dose of bivatuzumab mertansine during their first course than the patient with fatal toxic epidermal necrolysis (Table 2), whereas skin toxicity for these patients did not exceed grade 1.

In addition, in a parallel study with single infusion of bivatuzumab mertansine, head and neck cancer patients had received doses as high as 325 mg/m<sup>2</sup> (27). Although two patients treated at this dose level experienced DLT, with some signs of epidermolysis, toxicity had been totally reversible. The previous dose level of 300 mg/m<sup>2</sup> had well been tolerated. It is therefore difficult to predict when severe skin toxicity would occur and it does not seem to be strictly dose dependent.

In preclinical toxicity studies in cynomolgus monkeys, nonsevere dose-related reversible skin toxicity of bivatuzumab mertansine had been observed following weekly administration of 0.5, 1.5, and 4 mg/kg for 6 weeks. The 4 mg/kg dose resulted in a mean AUC<sub>0-∞</sub> of 2,410  $\mu\text{g} \cdot \text{h/mL}$  and a mean C<sub>max</sub> of 105  $\mu\text{g/mL}$  after the first dose. In the patients treated with 20 to 140 mg/m<sup>2</sup>, who received three instead of six administrations, the AUC at steady state (AUC<sub>τ,ss</sub>) ranged from 464 to 2,600  $\mu\text{g} \cdot \text{h/mL}$  and the C<sub>max</sub> at steady state (C<sub>max,ss</sub>) ranged from 12.8 to 69.5  $\mu\text{g/mL}$ . According to these data, the starting dose of 20 mg/m<sup>2</sup> and dose escalation steps of 20 mg seem to be cautious also in retrospect.

According to most recent insights, toxic epidermal necrolysis is considered to be a rare disorder characterized by extensive epidermal death, caused by an idiosyncratic drug reaction. The estimated annual incidence of toxic epidermal necrolysis is 1 to 2 per million population and the mortality rate is high (28). CTLs are involved in this reaction. Management of toxic epidermal necrolysis requires intensive care unit admission

**Table 4.** Descriptive statistics of pharmacokinetic variables of deconjugated bivatuzumab mertansine after a 3-week once weekly infusion regimen ( $n = 7$ , weeks 1 and 2;  $n = 6$ , week 3)

Week		AUC <sub>0-168</sub> * [( $\mu\text{g} \cdot \text{h/mL}$ )/( $\text{mg/m}^2$ )]	C <sub>max</sub> * [( $\mu\text{g/mL}$ )/( $\text{mg/m}^2$ )]	t <sub>max</sub> [h] <sup>†</sup>	t <sub>1/2</sub> [h]
1	gMean	13.3	0.111	48.6	183
	gCV (%)	24.0	23.1	47.3-120	38.5
2	gMean	20.8	0.166	47.5	166
	gCV (%)	21.2	19.1	6.03-73.3	32.1
3 <sup>‡</sup>	gMean	23.8	0.187	36	152
	gCV (%)	20.1	19.7	21.7-73.3	10.4

\*Dose normalized.

<sup>†</sup>Median and range.

<sup>‡</sup>Variables at steady-state (ss).

for extensive supportive care and protection of the exposed dermis and mucosal surfaces, respiratory care, and appropriate nutritional support with maintenance of strict normoglycemia. All possible triggering drugs should be stopped. No specific treatment regimen has been described. Treatment with immunosuppressive drugs can be considered, although the risk of developing secondary infection will increase. Use of corticosteroids is not recommended. Plasmapheresis may have benefit, but the working mechanism is still unclear. Conflicting results with antiapoptotic measures have been reported. Whereas, in general, the pathogenesis of toxic epidermal necrolysis is not understood (28), one may assume that, in the present study, toxic epidermal necrolysis was caused by direct eradication of keratinocytes by mAb-targeted mertansine. A complicating factor in bivatuzumab mertansine-induced toxic epidermal necrolysis is the long circulating time of the conjugate, which made immediate stopping of the trigger impossible.

In the present study, no indications were found for toxicity related to free mertansine as a result of deconjugation. Neural and gastrointestinal toxicities are the side effects of the unconjugated drug, as reported in the phase I and II studies done in the 1980s with maytansine in unconjugated form (29–32). Nevertheless, data presented in Fig. 4 suggest a slow but substantial deconjugation of bivatuzumab mertansine in the circulation. Substantial deconjugation was also previously observed with the cantuzumab mertansine conjugate, for which the same conjugation chemistry had been applied (15). In two patients of the latter study, also free mertansine plasma concentrations were measured with an ELISA of a protein-free extract of blood. Mertansine was detected in the plasma up to 48 hours following treatment, and at all time points the free mertansine represented <1% of the total circulating mertansine (antibody-conjugated mertansine plus free mertansine). These data indicate rapid elimination of free mertansine from the circulation on deconjugation.

Expression of CD44v6 is probably not selective enough for tumor cells to allow safe antibody-based cancer therapy. This

conclusion is probably reasonable for approaches in which supertoxic drugs like mertansine are used, but may not necessarily be the case for other approaches. Phase I dose escalation radioimmunotherapy studies with the anti-CD44v6 conjugates  $^{186}\text{Re}$ -cmAb U36 and  $^{186}\text{Re}$ -bivatuzumab showed promising antitumor effects with consistent stable disease at the higher radioactivity dose levels (11–13). In one of these studies, transplantation of autologous blood progenitor cells was applied (11). This procedure enabled the doubling of the MTD of  $^{186}\text{Re}$ -cmAb U36 and resulted in stabilization of disease in the majority of patients and in all patients treated at the MTD level. No skin toxicity was observed in these radioimmunotherapy studies, whereas just a small proportion of patients experienced mucositis. In general, mucositis was less severe than experienced by these patients during a previous course of external beam irradiation. Among others, two aspects make radioimmunotherapy fundamentally different from therapy with mAb-mertansine conjugates. First, radiation deposition with  $^{186}\text{Re}$  is relatively slow due to the long half-life of 89 hours of this  $\beta$ -emitting radionuclide. Second, whereas the energy and path length (5 mm) of the  $\beta$ -emission of  $^{186}\text{Re}$  are ideal for irradiation of small-sized to medium-sized tumors (0.5–1 cm; ref. 33), toxicity in the skin is just moderate due to the fact that a large part of the disintegration energy dissipates outside the distribution volume of this tissue (especially the basal cell layer). This explains diminished skin toxicity in case of radioimmunotherapy. As a corollary, radiation absorption will also be relatively small in small tumor cell clusters, and therefore radioimmunotherapy with  $^{186}\text{Re}$ -labeled anti-CD44v6 mAbs will most probably not be effective as single-modality adjuvant.

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