A Phase I Study of Cantuzumab Mertansine Administered as a Single Intravenous Infusion Once Weekly in Patients with Advanced Solid Tumors

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ABSTRACT

Purpose: **The purpose is to determine the maximum-tolerated dose, assess the toxicities, characterize the pharmacokinetic behavior, and seek preliminary evidence of biological activity of cantuzumab mertansine when administered as a weekly i.v. infusion without interruption.**

Experimental Design: **Patients with incurable solid tumors that expressed the target antigen for cantuzumab mertansine, CanAg, were treated with doses of cantuzumab mertansine ranging from 40 to 138 mg/m^2. The maximum-tolerated dose was defined as the highest dose at which no more than 1 of 6 patients experienced dose-limiting toxicity. Plasma concentrations of cantuzumab mertansine and total humanized antibody were determined, and area under the plasma concentration-time curve (to the last measured concentration) was calculated.**

Results: **Thirty-nine patients received a total of 280 weekly doses of cantuzumab mertansine. Acute, transient elevation of the hepatic transaminases and reversible fatigue were identified as the dose-limiting toxicities at the highest dose level. The maximum-tolerated dose was determined to be 115 mg/m^2/week. Evidence of clinical activity was noted in 3 patients. Pharmacokinetic analyses revealed that the pharmacokinetic variability was moderate, without evidence of dose dependency. Furthermore, the drug had a long terminal half-life (~40 h).**

Conclusions: **This study identified a safe and tolerable dose of the novel immunoconjugate prodrug cantuzumab mertansine. The evidence of antitumor activity suggests that additional clinical development is warranted, with a focus on tumors that express high levels of CanAg and which are known to be sensitive to antimicrotubule agents.**

INTRODUCTION

Cantuzumab mertansine (huC242-DM1) is a tumor-activated prodrug, which is the conjugated product of up to four molecules of the potent maytansinoid antimicrotubule agent DM1 [$N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine $C_{35}H_48ClN_3O_{10}S$] and the humanized monoclonal antibody huC242 (1, 2). HuC242 demonstrates specific binding to the extracellular domain of the tumor-associated carbohydrate antigen known as CanAg (a novel glycoform of MUC1), which is strongly expressed in most pancreatic, biliary, and colorectal cancers (3, 4). It is also expressed in a substantial proportion of gastric cancers (55%), uterine cancers (45%), non-small cell lung cancers (40%), and bladder cancers (40%), whereas normal tissues stain for this antigen only minimally (3) After binding to the external domain of CanAg, the cantuzumab mertansine-CanAg complex is internalized, and the DM1 molecules are released intracellularly by cleavage of the DM1-huC242 disulfide bonds.

DM1 is a derivative of the potent antimicrotubule agent maytansine, (5, 6) synthesized from the maytansinoid fermentation product ansamitocin P-3 (1). The National Cancer Institute originally evaluated maytansine in Phase I and II studies in the 1970s (5–9). It was associated with significant toxicities, namely nausea, vomiting, diarrhea, elevations of liver function tests, lethargy, and peripheral neuropathy. Both complete and partial responses were seen, however, in patients with thymoma, non-Hodgkin's lymphoma, melanoma, acute lymphocytic leukemia, ovarian cancer, and breast cancer (5–9).

Given the early evidence of activity in a variety of human tumors as well as substantial toxicity, maytansine was felt to be appropriate for conjugation to tumor-specific monoclonal antibodies in an effort to improve its therapeutic index. The efforts to link the drug via stable disulfide bonds to human monoclonal antibodies led to the development of DM1, a maytansinoid having a sulfhydryl group. DM1 is 3–10- fold more potent than maytansine with an IC_{50} in the picomolar range and broad cytotoxic activity in a range of human tumors *in vivo* (1). Nonclinical experience demonstrated that the immunoconjugate huC242-DM1 permits the selective delivery of DM1 to CanAg-bearing tumor cells while sparing normal tissue from toxicity (2).

Cantuzumab mertansine has broad activity against a range of CanAg-positive human tumor xenografts (1, 2, 10). At relatively nontoxic doses of 48 mg/m^2 i.v. daily for 5 days, huC242-DM1 resulted in complete regressions and cures of mice bearing human xenografts of Colo 205, HT-29, and LoVo colon cancer

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(2, 10). Complete tumor regressions were also observed using even lower doses of huC242-DM1 (24–27 mg/m^2 daily for 5 days) when mice bearing H441 (lung cancer), SW-1990, BxPC-3, and SU-8686 (pancreatic cancer) were treated (10). In these studies, tumor regressions were not seen in mice treated with maytansine alone, huC242 alone, a mixture of nonconjugated maytansine and huC242, or 5-fluorouracil or irinotecan as single agents at their respective maximally tolerated doses.

The pharmacology and toxicology of huC242-DM1 have been evaluated in both mice that lack the CanAg epitope and in cynomolgus monkeys that have CanAg levels in normal tissue comparable with humans (11). The lethal dose in 10 and 50% (LD_{10} and LD_{50}) of mice was ~228 and 333 mg/m^2, respectively (11). Necropsy revealed the major toxicities to be on the gastrointestinal, hematopoietic, and neuronal tissues. Transient, reversible elevations of the serum hepatic alanine aminotransferase, aspartate aminotransferase (AST), lipase, and amylase were also observed in monkeys. Pathological studies revealed both peak- and cumulative dose-related neurotoxicity in monkeys. The plasma clearance of huC242-DM1 was dose dependent and biphasic, with a terminal half-life ($t_{1/2}$) averaging ~48 h in both mice and monkeys and with a volume of distribution approximately equivalent to the plasma volume (11).

Tolcher *et al.* (11) have published results of a Phase I trial of cantuzumab mertansine given as a single i.v. infusion every 3 weeks. In that study, 37 patients received a total of 110 courses of cantuzumab mertansine at doses ranging from 22 to 295 mg/m^2. On the basis of their experience, the dose recommended for Phase II studies was 235 mg/m^2 given i.v. every 3 weeks. The dose-limiting toxicity (DLT) of cantuzumab mertansine was found to be reversible elevations of hepatic transaminases (11).

We conducted a Phase I trial of cantuzumab mertansine given weekly as an i.v. infusion to patients with advanced cancer. The principal objectives of the study were to determine the maximally tolerated dose of cantuzumab mertansine given weekly as an i.v. infusion, to determine the toxicities of cantuzumab mertansine given on this schedule, and to seek preliminary evidence of anticancer activity in patients with advanced cancer.

PATIENTS AND METHODS

Patient Selection. Patients with histologically confirmed advanced solid tumors that had failed to respond to standard therapy or for which no standard therapy was available were eligible to participate in this study. Although patients whose tumors were expected to express the target antigen CanAg (*e.g.*, colon cancer and pancreatic cancer) were allowed to enroll before immunohistochemical confirmation of the presence of the antigen on their tumor cells, immunohistochemical confirmation of CanAg expression was performed in all patients with available tissue. Other eligibility criteria included bidimensionally measurable or evaluable disease, age at least 18 years, life expectancy at least 12 weeks, an Eastern Cooperative Oncology Group performance status of 0–2, no prior chemotherapy within 4 weeks, no brain metastases, and no coexisting medical problem of sufficient severity to limit compliance with the study. Patients were required to have adequate organ function defined as an absolute neutrophil count of at least 1500/μl, platelet count of at least 100,000/μl, hemoglobin at least 9 g/dl, serum creatinine ≤ 1.5 mg/dl, bilirubin ≤ 1.5 mg/dl; AST, alanine aminotransferase, and alkaline phosphatase ≤ 3 times the institutional upper limit of normal or ≤5 times the institutional upper limit of normal if the elevation was due to hepatic metastases. 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. All patients gave written informed consent according to federal and institutional guidelines. The study was approved by the Institutional Review Board of the University of Chicago before the start of patient enrollment.

Study Design. Cantuzumab mertansine was administered weekly without planned interruption as an i.v. infusion at an initial dose of 40 mg/m^2 (expressed as dose of immunoconjugate protein), which was selected so that the total dose delivered over 21 days (120 mg/m^2) would not exceed the second highest dose level identified as safe in an ongoing Phase I study (11). Cantuzumab mertansine was administered at an infusion rate of 1 mg/min for 15 min and then increased to 3 mg/min if hypersensitivity phenomena were not observed. The study was conducted in two parts, using a modified continual reassessment method for the dose escalation portion of the study. Part 2 of the study was an expanded cohort that was planned to include 30 patients with colorectal, pancreatic, gastric, or non-small cell lung cancer treated at the recommended Phase II dose level.

The National Cancer Institute Common Toxicity Criteria, version 2, was used to grade toxicity. DLT was defined as any grade 4 neutropenia lasting ≥4 days, platelet count < 25,000/μl, or any grade 3 or 4 nonhematological toxicity (excluding nausea and/or vomiting that responded to therapy and alopecia) and was determined during the initial 21-day period of treatment. Cohorts of 3 patients at each dose level were treated. If 1 of 3 patients in a cohort experienced a DLT, the cohort was expanded to 6 patients. The maximum-tolerated dose was defined as the highest dose level at which no more than 1 of 6 patients experienced a DLT during the first 21 days of treatment.

Cantuzumab mertansine was supplied in 20-ml single-use vials by ImmunoGen, Inc. (Cambridge, MA). Each vial contained protein at a concentration of 0.7 mg/ml in a buffered solution (pH 6.5 ± 0.5) comprised of monobasic potassium phosphate (0.57 mg/ml), monobasic sodium phosphate monohydrate (0.20 mg/ml), dibasic sodium phosphate (0.555 mg/ml), and sodium chloride (8.16 mg/ml) in purified water, USP. The drug product was prefiltered twice upon instilling the dose volume into the infusion bag by passing it through a low protein-binding 5-μ filter and was administered to patients through an inline 0.22 μm filter within 8 h of preparation. After infusion, the i.v. line was flushed with fluid to ensure delivery of the full drug dose.

Pretreatment and Follow-Up Studies. Before initiation of therapy, all patients had a history and physical examination, complete neurological examination, including a standardized assessment of extremity sensation, and assessment of performance status. Routine laboratory studies included a complete blood count, differential white blood count, prothrombin and partial thromboplastin times, electrolytes, blood urea nitrogen, serum creatinine, uric acid, glucose, alkaline phosphatase, lac-

tate dehydrogenase, alanine aminotransferase, AST, total bilirubin, calcium, total protein, albumin, cholesterol, triglycerides, amylase and lipase, and urinalysis. Pretreatment studies also included an electrocardiogram, relevant radiological studies for the evaluation of all measurable and evaluable sites of disease, and an assessment of appropriate tumor markers. Radiological studies for assessment of disease status were repeated every 6 weeks. Patients were able to continue treatment if they did not develop progressive disease or experience DLT. A complete response was defined as the disappearance of all measurable and evaluable disease for at least two sequential measurements performed at least 4 weeks apart without worsening of disease-related symptoms or decline in performance status. A partial response was defined as at least a 50% reduction in the sum of the product of the bidimensional measurements of all lesions documented by at least two measurements separated by at least 4 weeks. Increase in the size of any lesion by at least 25% or the appearance of new lesions was considered disease progression. During an initial 21-day determinative period (for assessment of DLTs), patients were observed for 3 h after each treatment. An electrocardiogram was performed within 60 min after completion of dosing. Complete blood counts, chemistries, amylase, and lipase as well as a research nurse's assessment of the clinical status of the patient were performed 24 h after each of the first three doses (days 2, 9, and 16). Laboratory assessments were performed weekly with all subsequent doses.

Immunohistochemical Staining of Tumor Tissues. The distribution of the epitope of CanAg recognized by the murine C242 antibody was determined on sections cut from formalin-fixed, paraffin-embedded tumor biopsies previously obtained from all patients. Staining was done using the avidin-biotin immunoperoxidase technique. A control murine IgG1 antibody was obtained from Coulter Immunology (Hialeah, FL). Staining intensity was scaled from 0 (no staining above background) to 3 (strong staining), whereas the uniformity was scored at homogeneous (>75% of tumor staining positive), heterogeneous (25–75% of tumor staining positive), or focal (≤25% of tumor staining positive).

Table 1 Patient characteristics

Characteristic	No. of patients
No. of patients	39
Sex	
Male	24
Female	15
Age, years	
Median	61
Range	36–81
ECOG[a] performance status	
0	14
1	24
2	1
Prior therapy	
None	2
Chemotherapy only	26
Chemotherapy and radiotherapy	10
Tumor type	
Colon and rectum	24
Pancreas	8
Unknown primary	3
Lung	2
Gastric	1
Peritoneum	1

[a] ECOG, Eastern Cooperative Oncology Group.

Table 2 Doses administered

Dose (mg/m^2/week)	No. of patients	Total no. of doses administered
40	1	6
60	1	6
80	3	16
96	4	96
115	23	134
138	7	22

Bioanalysis and Pharmacokinetics. Pharmacokinetic analyses were performed in a subset of patients in this study. Plasma samples were collected over the first 24 h after the first and third weekly dose. In addition, trough concentrations were obtained just before dosing of the study medication weekly for the first 4 weeks, then every 4 weeks thereafter if subjects remained on study. Plasma cantuzumab mertansine (huC242-DM1 conjugate) and total huC242 antibody levels were determined using methods that have been described in detail elsewhere (11).

Pharmacokinetic Methods. Pharmacokinetic analyses of the individual plasma concentration datasets for cantuzumab mertansine and for the total humanized antibody were performed using standard model independent (noncompartmental) methods (WinNonlin Professional, version 3.2; Pharsight, Inc., Mountain View, CA). The pharmacokinetic parameters included area under the plasma concentration-time curve from the time of dosing to the last observable concentration (AUC_{last}) and maximum observed plasma concentration (Cmax). Where appropriate, the half-life ($t_{1/2}$) was determined using concentration data in the terminal log-linear phase. All computations used the actual sampling times. All reported statistical analyses were conducted using NCSS 2001.[4]

RESULTS

The characteristics of the 39 patients enrolled on the study are listed in Table 1. Patients received a total of 280 weekly infusions of huC242-DM1 at doses ranging from 40 to 138 mg/m^2. The median number of weekly doses administered/patient was 6 (range, 1–62). Four patients required dose reductions for toxicity, 3 at the 138 mg/m^2 dose level, and 1 at the 115 mg/m^2 dose level. Table 2 shows the number of patients treated at each dose level and the number of doses administered.

Toxicity. No toxicity greater than grade 1 was observed at the first three dose levels. As summarized in Table 3, 1 patient experienced an episode of grade 2 fatigue at the 96 mg/m^2 dose level. There were no toxicities greater than grade 1 experienced by the initial cohort of patients treated at the 115 mg/m^2 dose level. The first patient treated at the next dose level, 138 mg/m^2,

[4] Internet address: http://www.ncss.com.

Table 3 Summary of toxicities greater than grade 1 in the 21-day determinative period

Dose level (mg/m^2)	No. of patients	No. of patients with DLT[a]	Elevated AST/ALT		Elevated alkaline phosphatase		Elevated total bilirubin		Elevated amylase/ lipase		Anemia		Fatigue		Nausea		Diarrhea	
			Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3
40	1																	
60	1																	
80	3																	
96	4												1					
115	23	1	7		5	1	1		1	1	2		3		1		1	
138	7	3	1	3[b]			2						2	1[b]				

[a] DLT, dose-limiting toxicity; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[b] This event constituted a DLT.

experienced DLT in the form of grade 3 elevations of alanine aminotransferase and AST and a grade 2 elevation of serum total bilirubin. This patient was known to have bulky liver metastases. For this reason, the planned cohort of 3 patients was expanded to enroll 6 evaluable patients. Two more patients in the expanded cohort experienced DLT in the form of grade 3 elevation of AST. One of these patients also experienced grade 3 fatigue. There were no grade 4 toxicities of any kind noted during the study. Because 3 of 6 patients in the 138 mg/m^2 dose cohort experienced DLT, 115 mg/m^2 was determined to be the maximum-tolerated dose. Therefore, the 115 mg/m^2 dose cohort was expanded. Among the 23 patients treated at the 115 mg/m^2 dose level, the only grade 3 toxicities observed were elevated lipase and elevated alkaline phosphatase, each in 1 patient. The latter event occurred during the first 21 days of treatment and was thus considered a DLT. This was the only DLT at the 115 mg/m^2 dose level. Grade 2 elevations of transaminases (4 patients), alkaline phosphatase (5 patients), amylase (1 patient), and glucose (1 patient) were each observed within the first 42 days of treatment. Two patients experienced grade 2 fatigue, 3 patients experienced grade 2 anemia, and 1 patient each experienced grade 2 nausea or diarrhea. Twenty patients across all dose levels experienced grade 1 peripheral neuropathy consisting of extremity parasthesias and numbness. Three patients experienced grade 2 peripheral neuropathy. There was no convincing evidence of cumulative peripheral neurological toxicity: the patients who were treated the longest (62, 24, and 12 weeks, respectively) reported no more than grade 1 neuropathy. A summary of toxicities experienced in the first 21 days of treatment is presented in Table 3.

Two patients died while enrolled on the study. The first, a 54-year-old man with metastatic rectal cancer and bulky liver metastases and who had received five prior chemotherapy regimens, received two doses of cantuzumab mertansine at 115 mg/m^2. After the second dose, his liver functions were found to be elevated, and he died in hospice 6 days later, probably because of progressive disease. The contribution of cantuzumab mertansine to the patient's death remains indeterminate.

The second patient who died was a 61-year-old woman with lung cancer, previously treated with two chemotherapy regimens, who received three doses of cantuzumab mertansine at 115 mg/m^2. Five days after her last infusion, the patient was admitted to the hospital with fever, nausea, and vomiting and experienced cardiac arrest. The suspected cause of death, based on echocardiographic findings, was massive myocardial infarction. The relationship to the study drug was considered to be unlikely.

Pharmacokinetics. The pharmacokinetic data are presented in Table 4. As the full AUC (extrapolated to infinity) could not be fully characterized, the total plasma clearance cannot be accurately estimated. However, the AUC_{last} provides a lower bound for the extrapolated AUC. On the basis this estimate, the total plasma clearance appears to be <2 ml/min. Interpatient pharmacokinetic variability can be estimated from the dose-normalized AUC_{last} and was moderately variable (CV% of 28–39% at the four highest dose levels). Although there was a trend for dose-normalized AUC to increase with dose level ($r = 0.50$, $P < 0.04$), this is primarily due to the very low AUC in the one patient at the lowest dose level. As the dose-normalized AUC was independent of dose at the four highest dose levels, drug clearance does not appear to be satu-

Table 4 Pharmacokinetic parameters

Dose (mg/m^2)	*n*	AUC_{last} (mg·h/liter)[a]	Cmax (mg/liter)	Vd (liter)	$t_{1/2}$ (h)	AUC_{last}/dose (h/ml)
40	1	216	10.7	5.35	13	3.6
60	1	927	34.0	4.04	22	8.1
80	3	1582 (338)	43.5 (1.2)	5.53 (1.54)	39 (2)	10.2 (2.9)
96	3	1987 (553)	51.2 (5.8)	4.85 (0.77)	37 (9)	10.8 (3.3)
115	5	2650 (999)	73.5 (17.3)	4.06 (1.02)	40 (19)	11.7 (4.2)
138	5	3463 (1131)	80.8 (20.8)	4.58 (0.96)	41 (9)	12.8 (5.0)

[a] AUC_{last}, area under the plasma concentration-time curve from the time of dosing to the last observable concentration; Cmax, maximum observed plasma concentration; Vd, volume of distribution; $t_{1/2}$, terminal half-life.

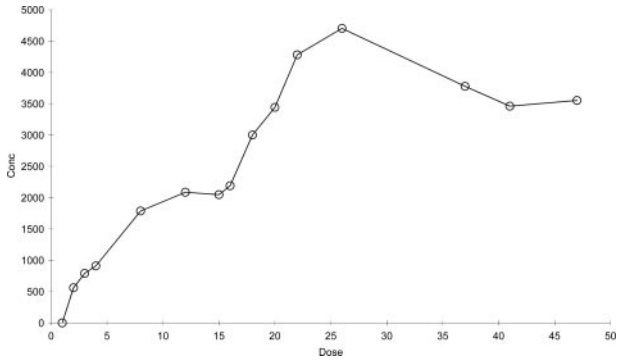

Fig. 1 Trough plasma concentration *versus* number of doses (or weeks of treatment) for a single patient treated at 96 mg/m^2. Drug accumulation is demonstrated by increasing serum trough concentrations of cantuzumab mertansine over time for 1 patient treated for ~1 year.

rable over the dose range studied. Dose-normalized AUC also appears to be independent of body surface area ($r = 0.09$).

Intrapatient variability was assessed by comparing the AUC_{last} on weeks 1 and 3. The AUC_{last} was significantly higher on week 3 than week 1 (2.21 ± 1.11 *versus* 2.81 ± 1.38 mg h/ml, $P < 0.0005$). This appeared to be due both to accumulation of drug (based on detectable trough concentrations), as well as to an increased observed terminal half-life on week 3 (38 ± 13 *versus* 44 ± 14 h, $P < 0.002$). However, given the limited sampling, our analysis may markedly underestimate the true terminal half-life (and AUC), which is likely to be much longer based on both evidence for accumulation (Fig. 1) and the results of another study (11). Drug accumulation (as manifested by increasing trough concentrations over time) was most obvious for the one patient who was on study for the longest duration, as shown in Fig. 1.

Immunohistochemistry for CanAg Expression. All 39 patients enrolled on the study had paraffin-embedded tumor specimens available for immunohistochemical evaluation of CanAg expression. As was permitted by the protocol, one patient with non-small cell lung cancer was enrolled on study and received two doses of cantuzumab mertansine before tumor staining results were available. This patient's tumor tested negative for CanAg, and she was withdrawn from the study after receiving two doses. The tumor immunohistochemistry results for the remaining patients are shown in Table 5.

Antitumor Activity. One patient at 96 mg/m^2 experienced a marked improvement in his clinical status. This patient had not previously received chemotherapy or radiotherapy for his disease. This 57-year-old man with peritoneal carcinomatosis of unknown (probably appendiceal) primary required paracentesis of 5–6 liters of malignant ascites weekly before enrolling on study. He had evaluable but not measurable disease. After six doses of cantuzumab mertansine, the patient's ascites nearly disappeared, and he remained on study for a total of 62 weekly doses, finally experiencing recurrence of his ascites. The malignant cells in his ascitic fluid expressed a 3+ homogeneous pattern of CanAg expression.

Another patient treated at 96 mg/m^2 experienced a marked decline in his CA19-9 associated with stable disease for ~5 months but no objective response. This patient, a 72-year-old man with pancreatic cancer who had previously received chemotherapy and radiation therapy, received a total of 24 doses of cantuzumab mertansine. His CA19-9 at the time of enrollment was 870 units/ml and fell to a nadir of 380 units/ml after 18 doses. It increased again by the 24th dose, and the patient was removed from the study. His tumor expressed a 3+ homogeneous pattern of CanAg expression.

One patient treated at 115 mg/m^2 had a transient reduction of >50% in the sum of cross-dimensional products of two reference lesions in the liver after 4 weekly doses. The patient's diagnosis was adenocarcinoma of unknown primary, probably of gastrointestinal origin, with liver metastases. A follow-up computed tomography scan 7 weeks later revealed that the disease had progressed. Thus, the response could not be confirmed by according to the prospective criteria used in the protocol. Immunohistochemistry of a fine needle biopsy showed a 3+ focal pattern of CanAg expression in the sample of tissue that was stained.

DISCUSSION

Immunoconjugation is a potentially important strategy for delivering effective anticancer therapies that have narrow therapeutic indices. Such is the case with maytansine, which in early studies demonstrated high levels of activity at very low concentrations but also produced severe toxicities and deaths that precluded additional clinical development. Cantuzumab mertansine was selected for clinical development based on its impressive *in vitro* and animal model activity against CanAg-expressing human tumors cells (1, 2). CanAg is widely expressed in human solid tumors (3, 4).

In this study, we confirm the findings of the a previously reported Phase I study that found that cantuzumab mertansine could be administered safely to patients with cancer at doses adequate to achieve antitumor activity (11). On the basis of these clinical results, we believe Phase II development of this agent is warranted, although it is unclear as to whether the weekly or every 3-week schedule is superior.

Given as a weekly i.v. infusion, cantuzumab mertansine was also found to be tolerable and to have evidence of anticancer activity. The recommended Phase II dose based on results of this study is 115 mg/m^2/week, which provides ~30% higher dose density than the every 3-week schedule. Just as in the previous study, the DLT of cantuzumab mertansine given on the weekly schedule was acute, reversible elevation of the hepatic transaminases. In contrast, there was less peripheral neuropathy noted in this study than on the every 3-week schedule. There was no evidence of clinically

Table 5 Tumor CanAg expression

Tumor staining[a]	Homogeneous	Heterogeneous	Homogeneous/ Heterogeneous	Focal
1+				1
2+	3	2		
3+	18	6	2	6

[a] One patient was CanAg negative.

significant cumulative toxicity, including peripheral neuropathy, except mild to moderate fatigue, despite significant drug accumulation. Thus, if one were to choose only one schedule for Phase II development, the weekly schedule may be preferable. A hybrid schedule might also be a consideration, using the previously recommended Phase II dose as a loading dose (~400 mg), followed by 200 mg weekly.

Evidence of antitumor activity was seen in 3 patients. One patient had a fall in his tumor marker and radiographically and clinically stable disease for ~6 months. One patient had clinical benefit with complete disappearance of malignant ascites for a year while receiving treatment with cantuzumab mertansine. A third patient had a transient reduction of >50% in the sum of bidimensional products of two hepatic reference lesions after 4 weekly doses, although this response could not be confirmed on follow-up imaging performed 7 weeks later.

In summary, this Phase I study has identified a safe and tolerable dose of the novel, immunoconjugate prodrug cantuzumab mertansine when given as a weekly i.v. infusion without interruption. A critical issue is identification of the optimal patient population for Phase II studies. Our data and that of the previous Phase I study suggest that antitumor activity is most likely to be observed in Phase II trials in tumors that express high numbers of CanAg molecules on individual cells throughout the tumor. All 3 patients in whom evidence of antitumor activity was seen had 3+ intensity of staining for CanAg on their tumors, 2 patients in a homogeneous pattern and 1 patient in a focal pattern. Tumors that are intrinsically sensitive to antimicrotubule agents would, presumably, also be rational targets (*e.g.*, lung, esophagus, and stomach) for this agent.

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