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CLINICAL TRIAL REPORT

Cantuzumab mertansine in a three-times a week schedule: a phase I and pharmacokinetic study

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Abstract
Purpose Cantuzumab mertansine (SB-408075; huC242–DM1) is a conjugate of the maytansinoid drug DM1 to the antibody huC242, which targets CanAg antigen. In previous studies, cantuzumab mertansine was considered safe and tolerable, but transaminitis precluded tolerance of higher doses. Based on those studies, it was suggested that treatment at intervals of the half-life of the intact immunoconjugate may allow a higher dose density. This provided the rationale for the three-times weekly treatment explored in this protocol.
Methods Patients with advanced solid tumors and documented CanAg expression were treated with escalating doses of cantuzumab mertansine IV administered three-times a week in a 3 out of 4 weeks schedule. Plasma samples were assayed to determine pharmacokinetic parameters.
Results Twenty patients (pts) with colon (11/20), rectal carcinomas (2/20), or other malignancies (7/20) were treated with doses ranging from 30 to 60 mg/m^2 per day of cantuzumab mertansine IV three-times a week. The maximum tolerated dose (MTD) was 45 mg/m^2, and the dose-limiting toxicity was grade 3 transaminitis. Hepatic, hematologic, and neurosensory effects occurred, but were rarely severe with repetitive treatment at doses of 45 mg/m^2.
Conclusions Treatment with cantuzumab mertansine at 45 mg/m^2 per day three-times weekly × 3-every-4-week schedule proved that a dose-intense treatment with an immunoconjugate can be safely administered. The pharmacokinetic profile of the intact immunoconjugate indicates that the linker is cleaved with a half-life of about 2 days, resulting in faster clearance of the maytansinoid relative to the antibody. Therefore, with the development of second-generation immunoconjugates, there is a need for improvement of the immunoconjugate linker to take full advantage of the slow clearance of full-length antibody molecules.

Keywords Cantuzumab mertansine · SB-408075 · huC242–DM1 · CanAg · Immunoconjugates · Pharmacokinetics · Phase I

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Introduction

After the successful introduction in oncology of monoclonal antibodies such as bevacizumab, cetuximab, or rituximab, many efforts have been attempted to use their high-affinity and antigen specificity for the selective delivery of antineoplasic moieties. Immunoconjugates are monoclonal antibodies covalently linked or chelated to a toxic effector molecule. ^{90}Y-ibritumomab, ^{131}I-tositumomab, and gemtuzumab ozogamicin are examples of approved

immunoconjugates for cancer treatment. Due to their success, many other immunoconjugates are in development (for a review on immunoconjugates, see references [1, 2]).

CanAg is a glycoform of MUC1, a type I transmembrane high molecular weight glycoprotein that provides a protective layer on epithelial surfaces, and is involved in cell–cell interactions, signaling, and metastasis [3]. CanAg is highly expressed in most pancreatic, biliary, and colorectal cancers, as well as in a large proportion of other cancers, but only minimally expressed in normal tissues [4, 5]. huC242 is a humanized monoclonal antibody with high selectivity for an extracellular epitope of CanAg.

The maytansinoid, DM1, is a derivative of maytansine. Maytansine is a natural cytotoxic compound that potently kills cells by preventing the formation of microtubules and depolymerization of extant microtubules. It binds to the same locus as that used by the vinca alkaloids [6, 7].

Based on these, cantuzumab mertansine (also known as huC242–DM1 or SB-4080075) was designed by conjugating several molecules of the potent maytansinoid DM1 to the huC242 antibody. A disulfide linker was used to bind mertansine to the antibody, to permit the selective distribution of the cytotoxic payload to cells bearing the targeted antigen. In preclinical experiments, huC242–DM1 proved to be highly effective for CanAg-positive tumors. It was selective in a dose-dependent fashion, as shown by the fact that the IC50 in CanAg-positive tumor cells in vitro was over 2 logs lower than the IC50 for CanAg-negative cells. In vivo, treatment with huC242–DM1 resulted in complete responses in CanAg-positive gastric, colon, pancreatic, and non-small cell lung cell lines in xenograft tumors [8–10].

Two previous phase I trials have studied the pharmacokinetics and toxicity of cantuzumab mertansine. The first in-human trial [11] examined the treatment of patients with solid tumors with cantuzumab mertansine given once every 3 weeks. The dose-limiting toxicities were elevation in liver enzymes and fatigue at the 295 mg/m^2, establishing a recommended dose of 235 mg/m^2 every 3 weeks for further development. The most common toxicities across cohorts in this trial were acute, transient, and reversible elevations of liver enzymes. The second trial [12] studied escalating weekly treatment in patients with solid tumors. Again, elevations of hepatic transaminases were noted. The pharmacokinetic profile of cantuzumab mertansine in these two trials showed a relatively rapid clearance of the immunoconjugate (t½ about 2 days) and a prolonged half-life of the "naked" antibody (t½ about 9–10 days). This suggested that increasing the frequency of treatment might permit greater exposure to the intact immunoconjugate.

Based on these results, this protocol was initiated to explore the feasibility of a three-times weekly treatment. Our hypothesis is that when the drug is given at intervals that are estimated to be approximately the half-life of the intact immunoconjugate (about 2 days based on 16 patients dosed at 235 mg/m^2), the exposure was analogous to schedules that were highly effective in animal models. The principal objectives of this phase I, and pharmacokinetic study, were to determine: (1) the maximum tolerated dose (MTD) of cantuzumab mertansine administered on a three-times weekly treatment, (2) the toxicities of cantuzumab mertansine, (3) the pharmacokinetic behavior of cantuzumab mertansine in the mentioned schedule.

Materials and methods

Patient selection

Adult patients with histological documentation of advanced or metastatic epithelial solid tumor which were likely to express the CanAg antigen, that were refractory or resistant to standard chemotherapy, or for which no effective standard therapy exists, were selected for this study. Tumors were tested by IHC for CanAg by C242 reactivity. Only patients with tumor biopsies that were scored as having moderate or strong staining (2+ or 3+) were eligible. Tumors were characterized by their staining uniformity as homogeneous (>75% of tumor cells stain), heterogeneous (≤75 to ≥25% of tumor cells stain) or focal (<25% of tumor cells stain).

Eligibility criteria also included the following: age ≥18 years; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; life-expectancy of at least 12 weeks; no chemotherapy, hormonal therapy, immunotherapy, radiotherapy, or surgery within 4 weeks before the first treatment with huC242–DM1 (6 weeks for nitrosureas and mitomycin C) and resolution of toxicities from previous treatments (patients with ≥grade 2 peripheral neuropathy were excluded); measurable or evaluable disease; adequate hematopoietic (absolute neutrophic count ≥1,500/μL, platelet count ≥100,000/μL, hemoglobin ≥9 g/dL, aPTT and PT/INR within 1.5× the upper normal limit), hepatic (albumin ≥2.5 g/dL, total bilirubin ≤1.5× institutional upper normal limit, AST and ALT ≤3× institutional normal upper limit and up to 5× in the case of liver metastases); and renal function (creatinine ≤1.5 g/dL or calculated creatinine clearance ≥60 mL/min according to the method of Cockcroft and Gault); and no concurrent serious infection or coexisting medical problem of sufficient severity to potentially limit full compliance with the protocol. All patients gave written informed consent before entry into the study in accordance with federal and institutional guidelines.

Drug administration

huC242–DM1 was supplied by ImmunoGen Inc., Cambridge, in 20-mL single-use vials at a protein concentration

of 0.7 mg/mL. The protein had an average of 3.5 molecules of DM1 linked to each molecule of huC242 antibody. The drug was formulated for i.v. administration and administered initially at a infusion rate of 1 mg/min and it was increased to 3 mg/min if tolerated.

In a treatment cycle, huC242–DM1 was administered as an IV infusion at an initial dose of 30 mg/m^2, three times per week for three consecutive weeks for a total of nine doses, followed by 1 week of observation. The starting dose and schedule of huC242–DM1 was based on the tolerability noted of this agent in previous studies, not to exceed the second highest dose level identified with the weekly administration. A course of therapy was defined as 28 days from the initiation of treatment, and after the first two cycles, those patients who were both tolerating and benefiting from therapy continued in an extended phase of the protocol.

huC242–DM1 was escalated to a maximum-tolerated dose using a standard Modified Fibonacci scheme. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, Version 2. Cohorts of three patients were entered at each dose level, and dose escalation was permitted only if no dose-limiting toxicities (DLT) were encountered or these were present in less than 2/6 patients. If one patient experienced a DLT at a given dose level, a total of six patients were entered at the dose level. If two of six patients experienced DLT, then dose escalation ceased, and additional patients were entered at the next lower dose to fully characterize the toxicities at the maximum tolerated dose. An analysis of subgroups depending on the extent of liver disease was pre-planned in case that a DLT was observed in patients with extensive metastatic liver disease. Extensive metastatic liver disease was defined as patients with lesions occupying more than a total volume of 10 cm^2 or grade 2 elevation in either bilirubin, ALT, AST, or alkaline phosphatase at baseline and considered to be related with the tumor. The MTD was defined as the highest dose at which less than two of six new patients experienced DLT. DLT was defined as grade 4 thrombocytopenia (<25,000/μL), grade 4 neutropenia (ANC < 500/μL) lasting for at least 4 days, any grade 3 or 4 non-hematological toxicity (excluding nausea or vomiting that responds to therapy, and alopecia), or any toxicity that precludes from receiving a minimum of four of the nine planned doses. A patient who experienced DLT could continue on treatment with one dose-level reduction.

Pretreatment and follow-up studies

Complete medical history, physical examination with complete neurological examination, and routine laboratory studies were performed pretreatment and weekly. Pretreatment studies also included an electrocardiogram, a serum pregnancy test, and relevant radiological studies. Radiological evaluation for disease was repeated after every other course. For patients with measurable disease, Response Evaluation Criteria in Solid Tumors (RECIST) were used.

Special assessments

The level of free CanAg in plasma as well as plasmatic human anti-humanized antibody (HAHA) and human anti-DM1 antibody (HADA) were done at baseline and prior to the second cycle by ELISA methods. For detecting circulating (shedded) CanAg plates were coated with the murine C242 antibody, which was used to capture CanAg from test samples. Bound antigen was detected by its capture of biotinylated murine C242 antibody, and the assay was developed with streptavidin-HRP and TMB reagent. Tumor immunohistochemistry (IHC) for C242 antibody reactivity was performed on tumor biopsy slides or biopsy tissue on all patients. The distribution of the epitope of CanAg recognized by the C242 antibody was determined using the murine C242 monoclonal IgG1 antibody with a murine monoclonal IgG1 antibody control, using the avidin-biotin immunoperoxidase technique [11].

Plasma pharmacokinetic sampling, assay, and analyses

Blood samples for pharmacokinetic analyses were collected pre-dose and immediately post-dose (within 15 min after completion of infusion, for the peak concentrations) at Cycle 1 on days 1, 8, and 19 and Cycle 2 on days 1 and 8. Blood samples were collected into heparinized tubes through an indwelling venous catheter placed in the contralateral arm to the infusion. All blood samples were centrifuged at 3,000*g* for 10 min at 4°C immediately after collection and plasma was stored at −20°C. Plasma concentrations of cantuzumab mertansine conjugate (huC242–DM1) and of the total huC242 antibody (irrespective of the amount of linked DM1) were assayed with anti-huC242 and anti-maytansinoid antibodies by validated ELISA methods. The rate of the cleavage of DM1 from the conjugate was calculated from these measurements. Plasma concentration versus time for each subject was analyzed by standard non-compartmental methods. Maximum observed plasma concentration (C_{max}) and area under the plasma concentration versus time curve (AUC) were calculated from the concentration–time profiles.

Based on the pharmacokinetic profile of the huC242–DM1 immunoconjugate from the first in-human trial [11], a pharmacokinetic model was established. The model was used to simulate new concentration–time relationships

under different dosing regimens. In the present trial, the peak and trough plasma concentrations of intact conjugate and total antibody were measured at the selected time points during the treatment and compared with the simulated curves developed for each dose.

Results

General

Twenty patients, whose pertinent characteristics are listed in Table 1, were recruited between May 2001 and September 2002, and treated with 40 courses of cantuzumab mertansine. They were treated through three planned dose levels, ranging from 30 to 60 mg/m^2 per day. The total numbers of patients treated at each dose level, number of courses, and dose escalation scheme are depicted in Table 2. All patients were fully evaluable for toxicity. The median number of courses administered per patient was 2.0 (range, 1–4). After no or negligible drug-related adverse effects were noted in the first course of the four patients treated at the 30 mg/m^2 dose level, the dose of cantuzumab mertansine was increased to 45 mg/m^2 per day. At 45 mg/m^2 per day, one of the first three patients experienced DLT, which consisted of a grade 3 elevation of liver enzymes; therefore, three additional patients were treated and none of them experienced unacceptable toxicity. In contrast, DLT occurred in the first course of two of the six patients treated at the 60 mg/m^2 per day dose level, which resulted in further patient accrual at the previous lower dose level. There was no apparent trend between extension of the liver involvement and liver toxicity. Therefore, the stratification by liver involvement was not performed. Because none of the other seven patients treated at the 45 mg/m^2 per day dose level experienced DLT, that dose was determined as the maximum tolerated dose (MTD).

Table 1 Patient characteristics

Characteristics	*n*
No. of patients	20
Mean age (range)	55 (32–70)
Sex (M/F)	9/11
Performance status (Southwest Oncology Group)	
0	7 (35%)
1	10 (50%)
2	3 (15%)
Previous therapy	
Chemotherapy	19 (95%)
Radiation therapy	12 (60%)
Biologic or immunotherapy	1 (5 %)
Surgery	16 (80%)
Median (range) no. prior chemotherapy regimens	3 (0–11)
C242 antigen reactivity	
Positive	18 (90%)
Negative	1 (5%)
Not done	1 (5%)
Intensity	
Weak staining	0 (0%)
Moderate staining	6 (33.3%)
Strong staining	12 (66.7%)
Uniformity	
Focal	3 (16.7%)
Heterogeneous	12 (66.7%)
Homogeneous	3 (16.7%)

Table 2 Dose escalation scheme and exposure to study medication

Dose (mg/m^2)	Number of patients	Number of doses administered	Dose limiting toxicities	
			Number	Type
30	4	62	0	N/A
45	10	190	1	Elevated liver enzymes
60	6	71	2	Elevated liver enzymes

Four patients had their dose of medication reduced, all for non-hematological causes. One patient at the 45 mg/m^2 cohort and three patients at the 60 mg/m^2 cohort required dose reduction for liver enzymes abnormalities (grade 3 elevations of AST and/or ALT). None of these patients discontinued the medication due to further toxicity after the dose reduction.

Sixty-five percent of the patients had colorectal cancer (colon cancer, 11/20; rectal cancer, 2/20). Other patients had lung, bile duct, gastric, thymoma, and appendix tumors (other histologies, 7/20). Eighteen patients were positive for C242 staining, one colon cancer patient was negative, and for one patient with thymoma, no sample was tested. The majority of biopsies had strong staining (66.7%). Most biopsies were heterogeneous (66.7%) in uniformity while the remainder had focal (16.7%) or homogeneous (16.7%) staining.

Adverse events

Non-hematologic toxicities

The distributions for most common non-hematological toxicities as a function of dose level are displayed in Table 3. The most frequent reported adverse events across all dose levels were fatigue, nausea/vomiting, increase in ALT or AST, and constipation. At the MTD, the predominant toxicities observed were fatigue, vomiting, and anorexia. The most severe (grade 3 and 4) adverse events were liver

Table 3 Non-hematologic toxicity

	Number of patients experiencing adverse event (including all cycles and all dose levels)		
	Grade 1	Grade 2	Grade 3
Fatigue	7	8	2
Anorexia	9	1	1
Nausea	10	3	0
Vomiting	6	1	0
Constipation	6	4	0
Diarrhea	5	3	0
Abdominal pain	4	2	0
Aspartate aminotransaminase	0	10	3
Alanine aminotransaminase	1	2	3
Bilirubin elevation	0	2	1
Peripheral neuropathy	5	2	0
Myalgia	3	1	0
Allergic reaction	0	2	0
Fever	2	0	0
Flatulence	2	0	0
Hyperglycemia	0	1	1
Hypokaliemia	0	2	0
Hypoproteinemia	0	2	0
Urinary tract infection	5	1	0

enzyme elevations and fatigue. Generally, the hepatic toxicity appeared during the second or third week of treatment and it was reversible, non-cumulative, and manageable, never leading to serious sequelae. At the MTD of 45 mg/m^2, the median onset of significant liver toxicity (grade 3 or 4 elevations of liver enzymes) was 15 days for AST and bilirubin, and 8 days for alkaline phosphatase. The median duration was 6, 2, and 28 days, respectively. There were no delays in treatment due to liver toxicity. Peripheral neuropathy was the most common neurological toxicity, although the events were mild to moderate in intensity. The neuropathy's median time to occurrence was 23 days. Moreover, a worsening of a previously present peripheral neuropathy was seen in six patients.

There were no treatment-related fatal events. Non-fatal serious events during treatment included cholangitis, pyelonephritis, sialadenitis, bacteriemia, bone fracture, hematochezia, dyspnea, dehydration, and allergic reactions. Only the allergic reactions were considered likely to be related with the investigational agent. Two patients had an allergic reaction during infusion of cantuzumab mertansine. Symptoms included fever, rigors, flushing, wheezing, and an asymptomatic decline in systolic blood pressure of about 20 mm/Hg in one case, and lip swelling, feeling chilled, and tachycardia in the other. In both cases, the reaction happened after Cycle 1, the symptoms were successfully treated with dexamethasone, diphenhydramine, and ranitidine, and the patients tolerated well further cantuzumab treatment when premedication was added.

Hematologic toxicities

Hematological toxicities were generally mild (Table 4); there was no grade 3 or 4 toxicity other than lymphocytopenia. Nine patients (45%) had grade 1 leukopenia and five patients had grade 1 or 2 thrombocytopenia (25%). Anemia was the most common hematological side effect noted during treatment, being present in 91% of the patients, and mild (grade 1 or 2 anemia).

Pharmacokinetic results

Pharmacokinetics of intact conjugate and total antibody after administration of huC242–DM1 immunoconjugate

The measured peak and trough concentrations of intact conjugate and total antibody at the selected time points are plotted versus time in Fig. 1a, b, and they were compared with the values simulated by the defined model at the same time points in Table 5. Intact conjugate accumulated modestly with repeated dosing in each cycle (its plasma concentrations were reduced to a negligible level—0.6 μg/mL at

Table 4 Hematologic toxicity

	Number of patients experiencing adverse event per dose level (including all cycles)		
	30 (mg/m^2)	45 (mg/m^2)	60 (mg/m^2)
Leucopenia			
Grade 1–2	2	4	3
Grade 3	0	0	0
Grade 4	0	0	0
Neutropenia			
Grade 1–2	1	0	1
Grade 3	0	0	0
Grade 4	0	0	0
Lymphopenia			
Grade 1–2	4	8	3
Grade 3	1	4	2
Grade 4	0	0	0
Anemia			
Grade 1–2	4	12	5
Grade 3	0	0	0
Grade 4	0	0	0
Thrombocytopenia			
Grade 1–2	2	2	1
Grade 3	0	0	0
Grade 4	0	0	0

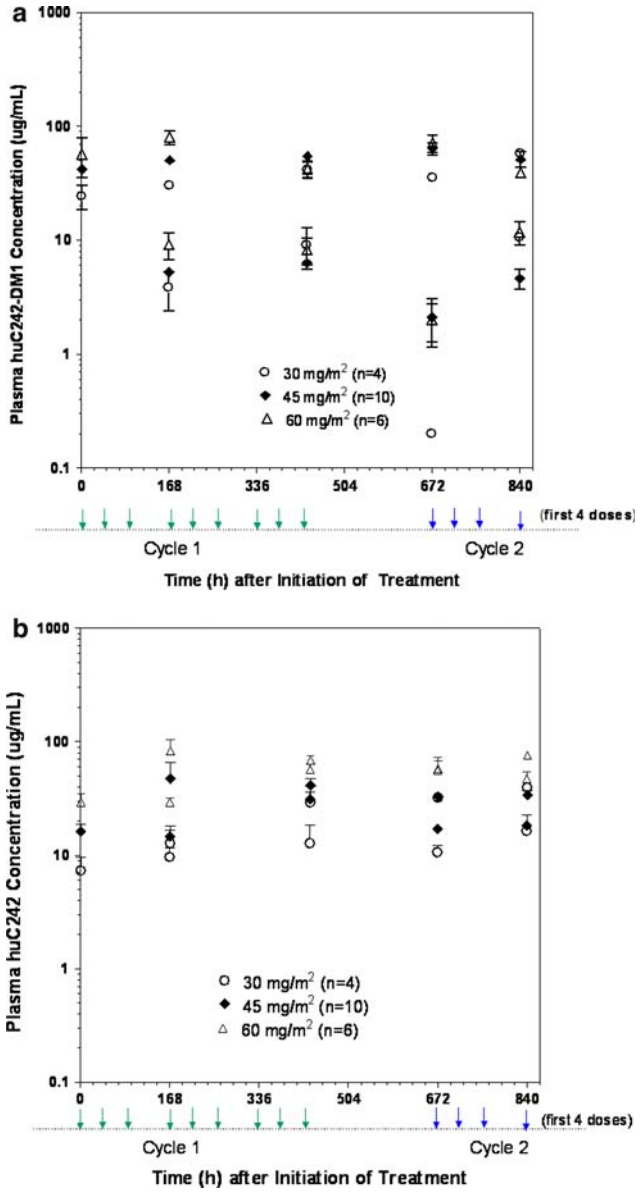

Fig. 1 Peak and trough plasma concentrations of intact conjugate (**a**) and total antibody (**b**) measured at the selected time points after multiple infusions of huC242–DM1 immunoconjugate

the 45 mg/m^2 dose level—prior to the next cycle) while total antibody accumulated as seen in previous trials and as predicted by the model.

The peak and trough concentrations of the conjugate at the nine selected time points over 35 days in this multiple-dose regimen fitted the simulated concentration–time curves well at all three dose levels. The concentrations of total antibody measured at the selected time points did not fit the simulated values as well as the conjugate, especially at the lower dose levels. The measured peak and trough concentrations (mean ± SD) for both conjugate and total antibody were superimposed onto the simulated concentration–time curves for the dose of 45 mg/m^2 in Fig. 2a, b.

Table 5 Comparisons of the measured mean peak and trough concentrations of conjugate (huC242–DM1) and total antibody (huC242) at the selected time points versus the values of conjugate simulated by the defined model at the corresponding time points

Time	30 mg/m^2				45 mg/m^2				60 mg/m^2			
	huC242–DM1		Total C424		huC242–DM1		Total C424		huC242–DM1		Total C424	
h	Simulated (μg/mL)	Measured (μg/mL)	Simulated (μg/mL)	Measured (μg/mL)	Simulated (μg/mL)	Measured (μg/mL)	Simulated (μg/mL)	Measured (μg/mL)	Simulated (μg/mL)	Measured (μg/mL)	Measured (μg/mL)	Measured (μg/mL)
1	25.7	**24.3**	15.3	**7.3**	38.6	**41.8**	22.9	**16.2**	51.4	**58.7**	30.5	**29.3**
168	4.0	**3.8**	19.2	**9.6**	5.9	**5.2**	28.8	**14.6**	7.9	**10.0**	38.4	**29.3**
169	29.6	**30.1**	34.4	**12.7**	44.3	**50.5**	51.6	**47.5**	59.1	**84.0**	68.7	**83.5**
432	7.8	**9.1**	39.7	**12.7**	11.6	**6.3**	59.6	**31.4**	15.5	**8.2**	79.4	**56.7**
433	33.3	**41.6**	54.8	**29**	49.9	**54.6**	82.2	**41.6**	66.5	**42.1**	109.6	**69.4**
672	0.4	**0.2**	26.2	**10.6**	0.6	**2.1**	39.3	**17.2**	0.8	**2.6**	52.4	**55.9**
673	26.1	**35.2**	41.4	**32.1**	39.2	**63.4**	62.1	**32.8**	52.2	**71.1**	82.8	**58.1**
840	4.0	**10.6**	38.4	**16.3**	6.0	**4.6**	57.6	**18.4**	8.0	**11.7**	76.8	**47.2**
841	29.6	**57.8**	53.5	**39.4**	44.5	**51.3**	80.3	**33.8**	59.3	**38.9**	107.1	**76.2**

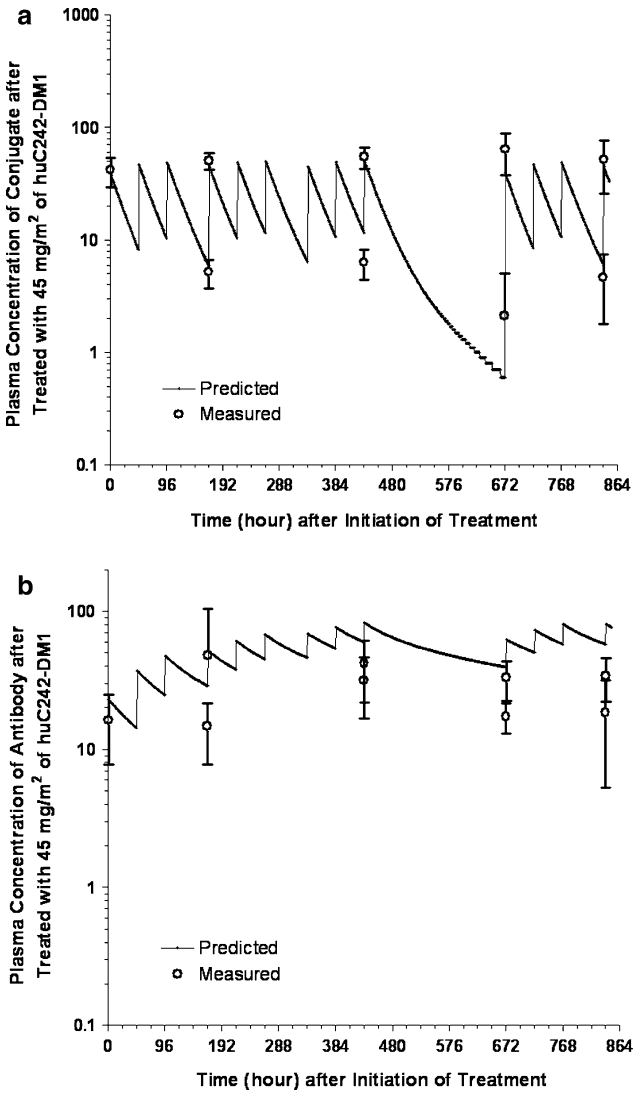

Fig. 2 Fit of the peak and trough conjugate, huC242–DM1, concentrations (*open circle*, mean ± SD) at selected time points after repeat dosing of 45 mg/m^2 of huC242–DM1 (**a**) and total antibody, huC242, (**b**) to the simulated curve derived from the first in-human trial (continuous line, 0–850 h)

Antitumor activity

No objective responses were observed in this study. The best observed response was stable disease seen in two patients with rectal cancer that were treated in the 45 mg/m^2 cohort, and received 27 and 36 doses, respectively. Overall, the average median time to progression in subjects (n = 20) was 7.71 weeks.

Pharmacodynamic results

Circulating antigen: levels of CanAg in plasma

Of the 20 patients on study, seven had pre-dose levels of ≤10 U/mL (undetectable), 12 in the range of 19–191 U/mL, and one patient had a level of 2,242 U/mL prior to treatment with huC242–DM1. Fifteen of the 20 patients were re-assayed for CanAg levels prior to the treatment of the second cycle. CanAg levels remained below the limit of detection in six of seven patients with previously undetectable CanAg (the other patient was not re-assayed). A further seven patients with previous elevations in CanAg recorded undetectable CanAg levels prior to the second cycle. One patient recorded a 2–3 fold fall in CanAg, while only one patient recorded an increase in CanAg (from 19 to 29 U/mL). The changes of CanAg levels in all patients are shown in Fig. 3.

Human anti-humanized C242 antibody and human anti-DM1 antibody

There was no generation of human anti-humanized C242 antibodies (HAHA) or human anti-DM1 antibodies in any patient between baseline and the second cycle evaluation.

Discussion

Maytansinoids are antitubular agents found to be highly effective against tumor cell lines and xenografts, but they were considered unacceptably toxic for clinical development due to severe gastrointestinal symptoms even at low doses [7, 13–16]. It is considered, though, that due to their high cytotoxic potency, they may be good moieties to arm immunoconjugates. Cantuzumab mertansine (also known as huC242–DM1) is an immunoconjugate composed of the humanized monoclonal IgG1 antibody, huC242, and the maytansinoid drug mertansine or DM1 [10].

Initially, when huC242–DM1 was clinically developed, two schedules were tested: once every 3 weeks and once every week [11, 12]. Dose limiting toxicities in these trials were elevation in liver enzymes and fatigue, and not hematotoxicity as might be expected from a cytotoxic drug. It was also observed that compared with the total huC242 antibody administered, the clearance of the immunoconjugate was more rapid and the half-life much shorter, being about 10 days for total antibody (conjugate and unconjugated) versus about 2 days for intact immunoconjugate. This caused an eventual excess of unconjugated antibody and, although it cannot be clinically proven, we hypothesize that this may be detrimental since it could preclude the access of huC242–DM1 to the antigen, decreasing the activity of the immunoconjugate. It was hypothesized that by treating at intervals that are approximately the half-life of the immunoconjugate, higher dose density of the intact immunoconjugate could be achieved, obtaining a safe and active schedule. In the present trial, we tested this hypothesis, treating patients with cantuzumab mertansine on a 3× weekly schedule.

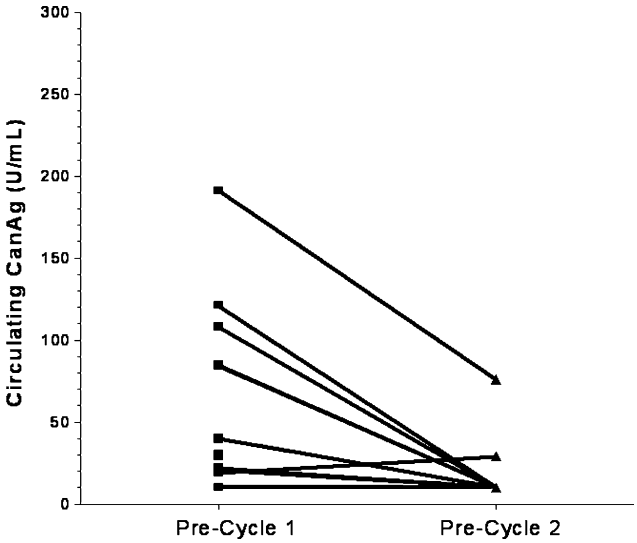

Fig. 3 Changes of circulating CanAg levels pre- versus post-treatment with huC242–DM1

In the current study, dose escalation was limited due to liver enzymes elevation in two patients in the third cohort. Thus, the recommended dose was determined to be 45 mg/m^2 per day. As in previous studies, hematologic toxicity was uncommon and the hepatic toxicity was reversible and manageable. The recommended dose of 45 mg/m^2 per day in the current three-times weekly × 3-every-4-week schedule is equivalent to a dose density of 101 mg/m^2 per week, which is higher than that recommended in the first in-human study (78 mg/m^2 per week, from the recommended 235 mg/m^2 every 3 weeks schedule [11]), but not higher than that recommended in the weekly schedule (115 mg/m^2 every week [12]).

The peak and trough concentrations of intact conjugate and total antibody at the selected time points were compared with the values at the same time points on the concentration–time curves simulated for each dose by a two-compartmental model derived from the data in a prior reported clinical trial. The similarity of pharmacokinetic behavior of the huC242–DM1 conjugate in these two clinical trials was indicated by good correspondence between the measured values in the present trial and the simulated curve. The concentrations of total antibody did not fit the simulated values as well as the conjugate and we hypothesize that there are two main reasons that explain this phenomenon. One possible reason is that the model was based on pharmacokinetic data for the antibody that has a half-life of about 10 days, and the estimated portion of the total $AUC_{0-\infty}$ was about 43.3% ($AUC_{0-504}/AUC_{0-\infty} \times 100\%$). Thus, the pharmacokinetic parameters of that study should be considered as approximate values only, and the simulated curves derived from these data may not accurately reflect the true clearance parameters. The second reason is that the ELISA used in human serum may not perform as well with individual plasma samples. Since the differences narrowed with increasing dose, the predictability of antibody concentrations by the defined model was improved within higher doses.

The clearance of the huC242 antibody component of huC242–DM1 was slower than that of the intact conjugate, and although there was no accumulation of the conjugate (plasma concentrations returned to a negligible level prior to initiation of next cycle), the total antibody had mild accumulation. After dosing, CanAg in plasma was either undetectable or greatly reduced in most tested patients, in testing done immediately prior to the second cycle. The pharmacokinetic data indicate that at this time, there is still a significant amount of antibody present in circulation as determined by an ELISA method utilizing a specific murine anti-huC242 monoclonal antibody for capture of the huC242 antibody from human plasma [11]. Although, since the antigen is multivalent for its carbohydrate epitopes [11], and it is expected that polyvalent antibody–antigen complexes would be rapidly eliminated from circulation, it is nevertheless possible that the lack of detectable antigen in plasma may be due to masking of the antigen by the huC242 antibody already in plasma. If the amount of circulating antigen was comparable to the amount of antibody, it may compromise the ability of the huC242 antibody to bind tumor target cells. However, when plasma was tested with ELISA methods utilizing antigen-capture via immobilized CanAg, similar values for antibody concentration were obtained (data not shown), suggesting that even if circulating CanAg is still present after administration of cantuzumab mertansine, the quantity of antigen epitopes is negligible relative to the number of antibody-binding sites. These findings are consistent with previous observations that circulating CanAg (in the range of values measured in these patients) had little or no effect on the pharmacokinetic properties of cantuzumab mertansine administered as a single dose [11]. There was no generation of human anti-humanized C242 antibodies (HAHA) or human anti-DM1 (HADA) antibodies in any patient at any time-point tested.

Early signs of antitumor activity were observed in this trial, and they were consistent with the previous experience with cantuzumab mertansine, but they may not be as provocative. In the current study, two patients with rectal cancer treated with cantuzumab mertansine at the recommended dose experienced stable disease for 3 and 4 months, respectively. In the previous trials with cantuzumab mertansine, antitumor activity was reported in patients with colorectal carcinoma, pancreatic cancer, and adenocarcinoma of unknown primary that had strong homogeneous staining for CanAg on the tumors [11, 12]. The reported responses in these trials were minor responses, prolonged stable disease, clinical benefit, and

marked decline in tumor markers. In the current trial, only three of the 20 patients had documented homogeneous CanAg expression that was documented as being homogeneous, which may be a factor in the less provocative antitumor activity observed here [18].

In conclusion, huC242–DM1 can be safely administered in a 3× weekly schedule, but a higher dose-density of the intact immunoconjugate cannot be achieved with this schedule when compared with the weekly schedule. Although, there are early signs of activity of huC242–DM1 in tumors with strong intensity of CanAg, dose-related hepatoxicity limits dose escalation. The toxicities observed in the clinical setting may be related with either prematurely released maytansinoid, clearance of the immunoconjugate by the reticuloendothelial system (especially the liver), or a bystander effect on normal liver hepatocytes due to uptake by metastatic tumor in the liver. Demonstration that the immunoconjugate is cleared more rapidly than the antibody component with a concomitant increase in the "naked" antibody provides some support for the first hypothesis.

Based on the results of this third study, we conclude that the premature release of the toxic payload (DM1) may compromise the current formulation of the huC242-conjugate for further development. Therefore, to test the hypothesis that immunoconjugates can selectively deliver chemotherapy to the tumors, there is a need of an improvement in the integrity of the linker. This experience will hopefully lead to further advancement in the tumor-activated prodrug technology in general, and to the development of second-generation immunoconjugates with a better relationship in the antibody–linker–payload combination, such as huC242–DM4 [17].

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