Phase I Trial of a Novel Anti-GD2 Monoclonal Antibody, Hu14.18K322A, Designed to Decrease Toxicity in Children With Refractory or Recurrent Neuroblastoma

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ABSTRACT

Purpose

The addition of immunotherapy, including a combination of anti-GD2 monoclonal antibody (mAb), ch14.18, and cytokines, improves outcome for patients with high-risk neuroblastoma. However, this therapy is limited by ch14.18-related toxicities that may be partially mediated by complement activation. We report the results of a phase I trial to determine the maximum-tolerated dose (MTD), safety profile, and pharmacokinetics of hu14.18K322A, a humanized anti-GD2 mAb with a single point mutation (K322A) that reduces complement-dependent lysis.

Patients and Methods

Eligible patients with refractory or recurrent neuroblastoma received escalating doses of hu14.18K322A ranging from 2 to 70 mg/m² per day for 4 consecutive days every 28 days (one course).

Results

Thirty-eight patients (23 males; median age, 7.2 years) received a median of two courses (range, one to 15). Dose-limiting grade 3 or 4 toxicities occurred in four of 36 evaluable patients and were characterized by cough, asthenia, sensory neuropathy, anorexia, serum sickness, and hypertensive encephalopathy. The most common non-dose-limiting grade 3 or 4 toxicities during course one were pain (68%) and fever (21%). Six of 31 patients evaluable for response by iodine-123 metaiodobenzylguanidine score had objective responses (four complete responses; two partial responses). The first-course pharmacokinetics of hu14.18K322A were best described by a two-compartment linear model. Median hu14.18K322A α (initial phase) and β (terminal phase) half-lives were 1.74 and 21.1 days, respectively.

Conclusion

The MTD, and recommended phase II dose, of hu14.18K322A is 60 mg/m² per day for 4 days. Adverse effects, predominately pain, were manageable and improved with subsequent courses.

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INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor in childhood. Addition of monoclonal antibodies (mAbs) targeting disialoganglioside GD2, a sialic acid-containing glycosphingolipid uniformly expressed on the surface of most neuroblastoma cells, to standard therapy has improved survival rates in patients with high-risk disease.^{1,2} The anti-GD2 antibodies currently being tested for clinical use include human-mouse chimeric 14.18 (ch14.18), humanized 14.18 fused to interleukin-2 (hu14.18-IL2), and murine 3F8.³ In a recent Children's Oncology Group study, patients with high-risk neuroblastoma who had at least a partial

response to standard induction chemotherapy and were randomly assigned to receive ch14.18 combined with cytokines and isotretinoin in the maintenance phase had longer event-free and overall survival compared with those who received isotretinoin alone.² Similarly, in an update of the Cooperative German Neuroblastoma Trials, patients who received ch14.18 had better long-term outcome.^{1,4} Despite this improved outcome, the use of anti-GD2 antibodies is limited by their toxicity, primarily pain, which may be partially mediated by complement activation,⁵ and by their immunogenicity.

Hu14.18K322A is a humanized mAb that contains fully human amino acid sequences for immunoglobulin G1 heavy and kappa light chains, and the

complementarity-determining regions correspond to the antigen binding sequences of the murine 14.18 antibody. The resulting hu14.18 antibody is approximately 98% derived from human genes, theoretically making it less immunogenic. In addition, hu14.18K322A has a single point mutation (K322A) designed to prevent activation of the complement cascade.^{5,6} Hu14.18K322A is produced in a YB2/0 rat myeloma cell line that has decreased fucosylation activity, potentially resulting in increased antibody-dependent cellular cytotoxicity (ADCC).^{7,8} In vitro analyses have shown that hu14.18K322A retains the binding specificity and ADCC capabilities of ch14.18, with virtually no complement-dependent lysis.⁵ Furthermore, in vivo analyses in rats documented less dysesthesia with hu14.18K322A than with ch14.18.⁵ Thus, hu14.18K322A has the potential to cause less complement-mediated pain and fewer hypersensitivity reactions than ch14.18.

We conducted a phase I trial of hu14.18K322A in children with refractory or recurrent neuroblastoma to define the toxicity profile, dose-limiting toxicities (DLTs), maximum-tolerated dose (MTD), pharmacokinetics, and immunogenicity of this novel anti-GD2 antibody.

PATIENTS AND METHODS

Patient Population

Eligibility criteria included: neuroblastoma recurrent/refractory to standard therapy and age $\leq$ 21 years. Other organ-specific and prior therapy inclusion/exclusion criteria are provided in the Appendix (online only).

Written informed consent was obtained from parents or legal guardians. Patients were treated at St Jude Children's Research Hospital on a protocol approved by the institutional review board.

Drug Supply and Administration

YB2/0 rat myeloma cells transfected with the expression plasmid pdHL7s-hu14.18 (K322A; hu14.18 antibody with lysine-322 in the CH2 domain replaced by alanine) were provided to St Jude and Children's GMP (Memphis, TN) by Merck Serono (Darmstadt, Germany), and hu14.18K322A was manufactured for clinical use by Children's GMP. Hu14.18K322A was administered on an inpatient basis intravenously over 4 hours daily for 4 consecutive days every 28 days (one course). Patients were premedicated with diphenhydramine or hydroxyzine and acetaminophen before each infusion. At the discretion of the treating physician, premedication with morphine sulfate or an alternative was provided for analgesia.

Study Design

This was a first-in-humans study; nine dose levels were evaluated, from 2 to 70 mg/m² per day. The study used a two-stage modified Storer's phase I design.^{9,10} Once a single patient experienced grade $\geq$ 2 toxicity, the study converted to a standard 3 + 3 phase I dose-escalation design, where three to six patients would be treated at each level. The highest dose at which fewer than two of six patients experienced DLT during course one (definition provided in Appendix, online only) was considered the MTD.

Toxicities were graded according to the Common Terminology Criteria for Adverse Events (version 3.0). Pain was graded based on the reported pain level per the age-appropriate pain scale (eg, Numeric or Faces Pain Scale; pain level of 1 to 4 was considered grade 1; 5 to 7, grade 2; and 8 to 10, grade 3). Pretreatment and on-study evaluations to monitor toxicity and criteria for subsequent courses and dose modification and definitions for duration of response are provided in the Appendix (online only). Tumor response was reported using RECIST (version 1.0)¹¹ and iodine-123 metaiodobenzylguanidine (MIBG) score (Curie scale).^{12,13}

Pharmacokinetic and Human Antihuman Antibody Studies

Serial blood samples (3 mL) for pharmacokinetic studies were collected on days 1 and 4 of courses one and two (preinfusion and 1, 2, 4, 8 [day 1 only],

12, and 20 hours after infusion) and on days 8, 11, 15, 21, and 28. Patients were also monitored for the development of human antihuman antibody (HABA). With each course of treatment, blood samples (3 mL) were collected before the first infusion (day 1) and on days 8, 15, and 28. Serum concentration of hu14.18K322A and HABA were measured by enzyme-linked immunosorbent assay, similarly to those used previously.¹⁴⁻¹⁶ Brief details are provided in the Appendix (online only).

Pharmacokinetic parameters during course one were determined using the NONMEM 7.2 population pharmacokinetic program.¹⁷ Details of the pharmacokinetic model are provided in the Appendix (online only).

Statistical Analyses

Pharmacokinetic parameters for individual patients were summarized using standard descriptive statistical methods. Linear regression analysis or the Jonckheere-Terpstra trend test was used to examine the association between pharmacokinetic parameters versus dose and HABA. Dose linearity for maximum plasma concentration (C_{max}) and area under the concentration-time curve from time zero to infinity (AUC_{0-}) were examined using linear regression by log-transformed values (power model).¹⁸

RESULTS

Patient Characteristics

Thirty-nine patients were enrolled, and 132 courses of therapy (median, two per patient; range, one to 15) were administered. Five patients received more than the four courses of planned therapy because of clinical benefit. Patient characteristics are summarized in Table 1.

Toxicity and MTD

The number of patients enrolled and evaluable at each dose level and DLTs are summarized in Table 2. The first patient treated at dose-level one experienced grade 2 cough. Thus, the traditional 3 + 3 design was used afterward. No DLTs were observed at dose levels one or two. At dose level three (6 mg/m²), one patient had grade 3 cough. No DLTs were observed at levels four through seven. At level eight (60 mg/m²), one patient experienced DLT of grade 3 asthenia. However, at level nine (70 mg/m²), the first two patients experienced DLTs, one grade 3 anorexia and sensory neuropathy and one grade 3 serum sickness and grade 4 hypertensive encephalopathy. On the basis of these DLTs, the MTD was established as 60 mg/m².

All treatment-related toxicities observed in $>$ 20% of patients are summarized in Table 3. Toxicities were primarily observed during the 4 days of infusion, except ocular or visual changes. The infusion rate was modified from 4 to 8 hours in three patients to ameliorate hypotension, hypoxia, and cough in one patient each who received 20, 20, and 60 mg/m² per day of hu14.18K322A, respectively. Four patients were taken off study because of unacceptable toxicity, including one each during course one with cough, serum sickness, and prolonged neutropenia (likely postinfectious) and one during course nine with hypersensitivity reaction (wheezing). All toxicities were reversible (including those observed in patients receiving $>$ four courses), and no patient died as a consequence of therapy.

The most common grade 3 or 4 toxicity was pain, followed by fever and hyponatremia. One patient had a febrile seizure. No sequelae were observed from hyponatremia. Pain was predominantly localized to the abdomen or extremity. Pain was worst on day 1 of course one, improved with each subsequent course, and in almost all cases resolved 24 hours after the last infusion on day 4. Twenty-six (68%) of 38 patients who received course one had at

Table 1. Patient Demographic and Clinical Characteristics (N = 39)

Characteristic	No. of Patients
Age, years	
Median	7.2
Range	2.6-16.2
Sex	
Male	24
Female	15
Weight, kg	
Median	22.1
Range	12.2-84.9
Body surface area, m ²	
Median	0.87
Range	0.56-2.02
Race	
White	26
Black	4
Other	9
Prior therapy	
No. of chemotherapy regimens per patient	
Median	4
Range	1-11
Radiotherapy	38
Autologous stem-cell transplantation	36
Syngeneic stem-cell transplantation	1
Anti-GD2 antibody therapy	3*
Disease burden at study entry†‡	
1	4
2	11
3	3
4	20

Abbreviations: CT, computed tomography; HVA, homovanillic acid; MIBG, metaiodobenzylguanidine; VMA, vanillylmandelic acid.
 *Iodine-131 3F8, n = 2; hu14.18-IL2, n = 1.
 †Semiautomatically scored (1 to 4) based on following criteria: score 1, elevated urine catecholamines (VMA/HVA) and/or < 10% bone marrow involvement only; score 2, > 10% bone marrow involvement, one to two MIBG-avid sites, and/or CT lesion measuring < 1 to 3 cm in longest diameter; score 3, > 50% bone marrow involvement, three to five MIBG-avid sites, and/or CT lesion measuring > 3 to 5 cm in longest diameter; and score 4, CT lesion measuring > 5 cm in longest diameter and/or > five MIBG-avid sites.
 ‡Excludes patient who withdrew consent before receiving study drug.

Table 2. Summary of Patients Treated and DLTs by Dose Level

Dose Level	Dose (mg/m ² per day)	No. of Patients Enrolled	No. of Patients Evaluable for DLT*	DLT
1	2	5	3	—
2	4	3	3	—
3	6	6	6	Grade 3 cough (n = 1)
4	10	3	3	—
5	20	3	3	—
6	40	3	3	—
7	50	3	3	—
8	60	11†	10	Grade 3 asthenia (n = 1)
9	70	2	2	Grade 3 anorexia, sensory neuropathy (n = 1); grade 3 serum sickness and grade 4 hypertensive encephalopathy (n = 1)

Abbreviation: DLT, dose-limiting toxicity.
 *Three patients not evaluable for DLT: withdrawal of consent before receiving antibody (n = 1), progressive disease before completion of first course (n = 1), and replaced because of grade 4 infection unrelated to study drug (n = 1).
 †Additional patients were enrolled at maximum-tolerated dose until six patients had completed four courses of therapy.

Pharmacokinetics

Pharmacokinetic studies were performed in 37 patients who received 4 consecutive days of antibody during course one. Of those, 34 had serial sampling on days 1 and 4. Using population pharmacokinetic methods, a two-compartment model provided the optimal fit. Pharmacokinetic parameters are summarized in Table 4. Median hu14.18K322A α half-life ($T_{1/2\alpha}$) and beta half-life ($T_{1/2\beta}$) were 1.74 days (range, 0.95 to 2.21 days) and 21.1 days (range, 4.89 to 271.0 days), respectively. Clearance and volume distribution were not dose related. A slightly longer $T_{1/2\alpha}$ was observed with increased doses of hu14.18K322A ($P = .008$). Similar trends were seen in $T_{1/2\beta}$ but were not statistically significant ($P = .069$). $C_{\max}$ and AUC_{0-} (Fig 1) increased with dose ($P < .001$ for each), and each showed linear dose-proportional increases (95% CI of slope of $\ln [\text{dose}]$, 0.92 to 1.13 for $C_{\max}$ and 0.88 to 1.06 for AUC_{0-}). Figure 2 shows the predicted serum concentrations for patients receiving 60 mg/m² of hu14.18K322A during course one based on the observed serum concentrations on days 1 and 4.

HAHA Response

Of the 37 patients evaluated, 15 (40%) had an HAHA response, as determined by showing a reproducible increase of > 0.7 OD units¹⁹ from their baseline value in the HAHA assay. There was no clear association between patients who had an HAHA response and any allergic or autoimmune toxicity. One patient had a hypersensitivity reaction only after course nine at 60 mg/m². This patient was HAHA negative for courses one to four but did develop a strong HAHA response in course five, which was not different before course nine than before courses six to eight. A second HAHA-positive patient had serum sickness in course one at 70 mg/m². In addition, the association between the dose of hu14.18K322A and the development of an HAHA response was not statistically significant ($P = .106$). HAHA responses were not typically detected until day ≥ 10 during course one (only three patients had HAHA response before day 10). The association

least one report of grade 3 pain during course one, whereas only eight (33%) of 24 patients who received course two reported grade 3 pain. Details of narcotic use are summarized in the Appendix (online only). Although the highest grade of pain reported with the first two dose levels was lower than those reported with subsequent levels, there was no significant increase in narcotic use with increased hu14.18K322A dose.

During the study, 20 patients (51%) had ocular or visual abnormalities best characterized as Adie's pupil (mydriasis, photophobia, and abnormal accommodation). This usually developed in the first course (16 patients) and was more common at higher dose levels; it was identified in more than three quarters of the patients treated at ≥ 40 mg/m². Nine of these patients were prescribed reading glasses. These ocular findings did not worsen with subsequent courses. Of the 20 patients with ocular findings, 16 had documented resolution at a median of 80 days (range, 1 to 365 days) from onset. Four patients had ongoing, but improving, ocular symptoms at the off-study date for progressive disease.

Table 3. Treatment-Related Toxicity (> 20% of patients)

Toxicity	Course One (n = 38)*				Courses Two to Four (n = 24)			
	Grade 1 or 2		Grade 3 or 4		Grade 1 or 2		Grade 3 or 4	
	No.	%	No.	%	No.	%	No.	%
Nonhematologic								
Pain	11	29	26	68	10	42	12	50
Sinus tachycardia	27	71			16	67		
Fever without neutropenia	25	66	8	21	14	58		
Weight loss	22	58			8	33		
Hypoalbuminemia	20	53			9	38		
Anorexia	19	50	1	3	8	33		
Alanine aminotransferase	20	53			15	63	1	4
Aspartate aminotransferase	19	50			12	50	2	8
Nausea	19	50			15	63		
Hyponatremia	17	45	4	11	12	50		
Hypertension	16	42	1	3	12	50		
Vomiting	16	42			9	38		
Ocular/visual	16	42			13†	54		
Fatigue (asthenia, lethargy, malaise)	15	39	1	3	8	33		
Cough	15	39	1	3	13	54		
Hyperglycemia	11	29			11	46	1	4
Pruritis/itching	9	24			5	21		
Hypokalemia	7	18	4	11	10	42		
Hypophosphatemia	7	18			5	21		
Rash/desquamation	5	13			6	25		
Hypotension					4	17		
Elevated alkaline phosphatase	5	13			5	21		
Hematologic								
Hemoglobin	11	29	3	8	9	38		
Leukopenia	7	18	1	3	8	33	1	4
Platelets	6	16	1	3	6	25	2	8
Lymphopenia	5	13	5	13	6	25	2	8
Neutrophils	5	13			6	25	3	13

NOTE. Grades according to National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). Each adverse event was counted once (any course, highest grade) for each patient during course one and also once for each patient during courses two to four.

*Thirty-nine patients enrolled; one patient excluded because of withdrawal of consent before administration of any study drug.

†No. includes nine patients who had visual/ocular abnormalities in course one that persisted during courses two to four.

between an increase of HAHA and pharmacokinetic parameters such as clearance, volume distribution, $T_{1/2\alpha}$, C_{max} , and AUC_{0-} was not statistically significant. However, a longer $T_{1/2\beta}$ was noted, with a larger increase in HAHA in course one ($P = .022$), suggesting that an HAHA response could influence the terminal elimination phase in course one or pharmacokinetics in subsequent courses. For patients receiving at least two courses of hu14.18K322A, the magnitude of the HAHA response in course one (increase in OD value) was associated with lower C_{max} values in course two than in course one (data not shown; $P < .001$), again suggesting that HAHA response may influence pharmacokinetic parameters.

Tumor Response

Tumor responses are shown in Figure 3. No objective responses were observed using RECIST. However, two patients had partial re-

sponses (best response after two courses of treatment at 6 and 60 mg/m²), and four patients had complete responses by MIBG score (best response one each after two, four, six, and 12 courses; two after treatment at 20 mg/m², and two at 60 mg/m²). Median duration of response was 3.4 months (range, 1.1 months to 2.3 years). Nine patients had stable disease for at least two courses by RECIST or MIBG score, with a median duration of 7.0 months (range, 1.8 to 33.7 months). Twelve patients experienced disease progression after the first course. Median time to progression for the remaining patients was 3.6 months (range, 1.8 to 12.9 months).

DISCUSSION

We found the MTD of hu14.18K322A administered daily for 4 consecutive days to be 60 mg/m². DLTs were cough, asthenia, anorexia, sensory neuropathy, serum sickness, and hypertensive encephalopathy. The spectrum of adverse effects, the most common of which were pain, fever, and tachycardia, was similar to that previously described with the parent antibody, ch14.18, in children,^{4,20,21} except that none of our patients had potentially life-threatening capillary leak syndrome. These adverse effects were mostly restricted to the 4 days of infusion and could be managed acceptably with standard premedications and analgesia. Our study was designed as a dose-finding safety study. It is difficult to make a direct comparison between hu14.18K322A and other anti-GD2 antibodies, given the different products and manufacturing techniques, varying schedules of administration, and different criteria for ascertainment of toxicity. For these reasons, it is not possible to say with certainty whether the MTD of hu14.18K322A is higher than that of ch14.18 or whether the degree and duration of adverse effects, particularly pain, are less. The comparison would require a large randomized trial. However, in treating distinct patients with hu14.18K322A at the MTD of 60 mg/m² or with ch14.18 (combined with cytokines) at 25 mg/m², our clinical impression is that the duration and severity of pain in patients receiving hu14.18K322A are less, as suggested from the preclinical data.⁶

An interesting adverse effect of anti-GD2 antibodies is the ocular abnormality Adie's pupil. Kremens et al²² reported symptoms of mydriasis and accommodation deficits in 10 (11%) of 85 patients treated with ch14.18 (20 mg/m² daily for 4 days) and hypothesized that the abnormalities resulted from GD2 antibody binding to neural structures in the ciliary or sphincter muscles of the eye. Ozkaynak et al²³ noted mydriasis with or without blurred or double vision in six of 79 courses of ch14.18 in combination with granulocyte-macrophage colony-stimulating factor. In contrast, we observed a significantly higher incidence of ocular abnormalities in our study (up to 50%). Symptoms were detected much earlier, and most occurred during the first course. Possible explanations are the frequency of monitoring (ophthalmology assessments before each course), the older patient population able to verbalize vision changes, and the longer exposure time (because of longer half-life) and higher dose of hu14.18K322A. Fortunately, the abnormalities were correctable with prescription glasses and reversible or improving after completion of therapy in all patients at the time of last follow-up.

Objective responses and prolonged stabilization of disease were observed in our cohort of heavily pretreated patients. Six of 34 evaluable patients showed objective responses by protocol-defined criteria. As expected from preclinical^{24,25} and clinical data,²⁶ these responses seemed

Table 4. Hu14.18K322A Pharmacokinetic Parameters for Course One Based on Two-Compartment Model

Dose (mg/m ² per day)	No. Of Patients	CL (L/day)		C _{max} (mg/L)		AUC _{0-∞} (mg × day/L)		T _{1/2α} (day)		T _{1/2β} (day)		V _c (L)	
		Median	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
2	4	0.87	0.34-1.39	1.37	0.42-2.46	9.37	4.14-16.24	1.41	1.02-1.58	15.11	5.67-23.60	3.52	1.37-6.67
4	3	0.50	0.38-0.57	4.39	2.94-7.46	23.77	17.34-35.98	1.74	1.71-1.75	30.60	29.70-36.00	1.38	1.19-1.99
6	5	0.83	0.57-1.48	5.42	4.90-7.54	34.71	30.81-50.80	1.45	1.10-1.96	11.50	4.89-17.40	3.00	2.17-5.57
10	3	0.75	0.61-0.99	8.94	6.42-9.21	53.77	43.32-57.82	1.67	0.95-2.16	13.50	13.30-68.30	2.36	2.01-4.00
20	3	0.82	0.49-0.95	20.09	14.01-20.49	97.17	95.38-129.98	1.70	1.65-1.96	9.52	6.01-128.00	2.34	2.22-3.64
40	3	1.23	0.81-2.20	25.28	23.57-27.11	146.91	127.48-150.31	1.91	1.54-2.15	46.70	36.90-112.00	3.66	2.83-7.83
50	3	0.70	0.60-0.80	46.53	33.45-53.52	231.92	190.61-268.01	1.85	1.33-2.13	62.60	34.50-79.60	2.35	1.77-2.57
60	11	0.72	0.45-1.06	59.15	25.03-74.44	302.10	168.84-428.03	1.85	1.33-2.13	13.50	6.92-201.00	2.08	1.45-4.19
70	2	0.74	0.70-0.77	67.63	61.56-73.70	341.00	326.00-356.00	1.75	1.58-1.92	146.05	21.10-271.00	2.18	1.87-2.49
All	37	0.75	0.34-2.20	23.57	0.42-74.44	129.98	4.14-428.03	1.74	0.95-2.21	21.10	4.89-271.00	2.36	1.19-7.83

Abbreviations: AUC_{0-∞}, area under the concentration-time curve from time zero to infinity; C_{max}, maximum plasma concentration; CL, clearance; T_{1/2α}, half-life alpha; T_{1/2β}, half-life beta; V_c, volume of distribution.

more likely in patients who did not have bulky disease (ie, measurable by RECIST).

After four consecutive daily doses, median T_{1/2α} and T_{1/2β} of hu14.18K322A from the two-compartment pharmacokinetic model were 1.7 and 21.1 days, respectively, with wide variability in T_{1/2β}. The variability may have resulted in part from the HAHA response in a subset of patients. Nonetheless, the T_{1/2β} was similar to those reported for other humanized mAbs (eg, bevacizumab, 19.9 days; rituximab, 19.7 days; and trastuzumab, 28.5 days).²⁷⁻²⁹ In contrast, Uttenreuther-Fischer et al³⁰ described an average T_{1/2β} of 4.8 days for ch14.18, which is shorter than that found in our study. The shorter elimination half-life reported for ch14.18 may: one, be the result of the expected greater immunogenicity of the chimeric form of the antibody, possibly promoting faster clearance; two, reflect differences in the assays used in these studies to measure serum levels of the mAbs for pharmacokinetic calculations;

or three, reflect differences in clearance resulting from the K322A mutation or decreased fucosylation of hu14.18K322A. For largely historical reasons, the administration of anti-GD2 mAbs to patients with neuroblastoma has primarily followed a daily schedule for three or four sequential doses. However, because the T_{1/2α} and T_{1/2β} for this agent are comparable to those for other tumor-reactive mAbs that are normally administered weekly or every 2 weeks, and because this type of dosing should maintain longer exposure of tumor tissue to biologically meaningful levels of mAb, we are moving forward with analyses of toxicity, antitumor effect, and pharmacokinetics of weekly hu14.18K322A.

Development of HAHA and its significance is always a concern with genetically engineered mAbs. Forty percent of our patients had an HAHA response during the first course of therapy. Development of HAHA did not seem to correlate with dose or toxicity; however, it did

Fig 1. Course one (A) maximum plasma concentration (C_{max}) and (B) area under the concentration-time curve (AUC) plotted by dose.

Fig 2. Predicted (blue line) and average predicted (gold line) hu14.18K322A serum levels for 11 patients treated at 60 mg/m² (maximum-tolerated dose) based on two-compartment model. Blue dots represent observed hu14.18K322A serum levels for representative patient who had median body-surface area among patients treated at 60 mg/m². Peak serum level on day 4 (immediately after four daily infusions) was greater than that after first infusion on day 1. Elimination of agent after peak on day 1 showed similar pattern to that seen after peak on day 4.

seem to influence the $T_{1/2\beta}$ and the levels of hu14.18K322A in course two. The immunologic mechanisms for these observations require further study.

Because hu14.18K322A was designed to reduce complement activation, complement levels were monitored during course one (Appendix, online only). In contrast to reports of a decrease in complement levels with other anti-GD2 antibodies,^{21,31-33} we noted a significant increase in serum complement levels between days 1 and 5, suggesting a lack of consumption of these proteins as a result of complement fixation. We were unable to demonstrate a dose-dependent rise in serum complement because of the small sample size per dose level (data not shown).

Preclinical data suggest that enhanced antitumor effects should be obtained by ADCC when tumor-reactive mAbs are combined with agents that augment the ability of effector cells to mediate ADCC.^{24,34-37} Subsequent testing of hu14.18K322A may incorporate these concepts and determine whether the MTD, in combination with ADCC inducers, remains greater than that of ch14.18 in such combinations.³⁴ Preclinical data also suggest that certain chemotherapies potentiate the *in vivo* ADCC of certain mAbs.³⁸⁻⁴³ Augmented access to tumors damaged by chemotherapy might outweigh the potential inhibition of ADCC resulting from chemotherapy-induced transient immune suppression. In this regard, infusions of effector cells able to mediate ADCC have potentiated *in vivo* ADCC effects in preclinical models.⁴⁴ We recently opened a pilot clinical trial of this concept, combining hu14.18K322A with conventional chemotherapy and with infusions of allogeneic natural killer cells for patients with relapsed/refractory neuroblastoma (Clinicaltrials.gov NCT01576692).

Finally, because the clinical benefit of anti-GD2 mAb has clearly been seen in neuroblastoma, much of the ongoing testing of anti-GD2 strategies has been focused on this rare disease. However, more common malignancies are characterized by GD2 expression, including melanoma, small-cell lung cancer, and sarcomas.⁴⁵⁻⁵¹ Novel strategies using hu14.18K322A may be developed and tested in these diseases.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Fariba Navid, Paul M. Sondel, Raymond Barfield, Jianrong Wu, Wayne Furman, Lisa McGregor, Stephen Gillies, Rupert Handgretinger, Victor M. Santana

Fig 3. Tumor response in patients treated with hu14.18K322A. Tumor response was assessed by two methods: metaiodobenzylguanidine (MIBG) score and RECIST. No patient met criteria for objective response by RECIST. Majority of patients (n = 31) had sites of disease apparent by MIBG. Using MIBG score, tumor response (complete response [CR]/partial response [PR]) was observed in six patients. Five of these patients had disease that was only apparent by MIBG. One patient had an MIBG-avid lesion that resolved (CR by MIBG score; persistent abnormalities measurable by RECIST have remained stable for 3 months off therapy). BM, bone marrow; HVA, homovanillic acid; PD, progressive disease; SD, stable disease; VMA, vanillylmandelic acid. (*) Two patients not evaluable for response (both withdrew consent; one before receiving any study drug, and one after second dose of drug during course one). (†) MIBG-avid lesion resolved; lesions measurable by RECIST stable. (‡) One patient was removed from therapy after course one because of toxicity; four patients were removed from therapy at end of course two because of PD. (§) Removed from therapy after course one because of toxicity.

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Appendix

Eligibility Criteria

Patients had to have fully recovered from the acute toxic effects of all prior therapy; have life expectancy ≥ 8 weeks and Karnofsky/Lansky performance score ≥ 50 ; have no neurologic deficits or peripheral neuropathy grade ≥ 2 ; have received no biologic therapy within 7 days, no irradiation within 2 weeks, no myelosuppressive, investigational, immunosuppressive, immunostimulatory, or immunomodulatory therapy within 2 weeks, and no hematopoietic growth factors within 1 week before study entry; have negative pregnancy test if female; not be breastfeeding if female; and agree to use an effective contraceptive method if male or female of reproductive potential. Patients with prior CNS disease were eligible provided CNS disease had been treated and been clinically stable for 4 weeks before study entry. Patients with known sensitivity to other recombinant human antibodies and uncontrolled infection were not eligible. Patients with prior exposure to other anti-GD2 monoclonal antibodies were eligible if serologic testing showed absence of detectable antibody to humanized 14.18 (hu14.18). Laboratory criteria for enrollment included an absolute neutrophil count $\geq 750/\text{m}^3$, total bilirubin $\leq 2 \times$ upper limit of normal (ULN) for age, ALT $\leq 2.5 \times$ ULN for age, glomerular filtration rate $\geq 70 \text{ mL/min/1.73 m}^2$ or normal serum creatinine for age, shortening fraction $\geq 27\%$, and corrected QT interval ≤ 450 .

Definition of Dose-Limiting Toxicity

Dose-limiting toxicity was defined as any toxicity $\geq$ Common Terminology Criteria for Adverse Events grade 3 or hypotension (symptomatic or systolic or diastolic blood pressure 15% below baseline unresponsive to fluid resuscitation and 50% reduction in antibody infusion rate) that occurred during the first course of therapy, with the following exceptions: medically managed grade 3 nausea and vomiting; grade ≥ 3 pain requiring intravenous narcotics no more than 48 hours after completing antibody infusion; grade ≥ 3 fever lasting < 6 hours and controllable with antipyretics; grade 3 skin toxicity that improved with treatment (eg, intravenous antihistamines) within 48 hours after completing antibody infusion; grade ≥ 3 metabolic or other related laboratory value that improved with or without treatment within 48 hours after completing antibody infusion; grade ≥ 3 elevations in ALT or bilirubin that returned to $\leq 2.5 \times$ ULN for age and $\leq 1.5 \times$ ULN for age, respectively, within 1 week of completing antibody infusion; grade 3 infection; and grade ≥ 3 hematologic toxicity that improved to at least grade 2 or to pretherapy baseline values within 1 week after antibody infusion. Patients who required platelet or RBC transfusions to begin therapy and patients with disease in the bone marrow were not evaluable for hematologic dose-limiting toxicity.

Pretreatment and On-Study Evaluations and Definition of Duration of Response

Pretreatment evaluations included medical history, physical examination, performance status assessment, echocardiogram and ECG, complete blood count with differential, serum electrolytes, renal and liver function studies, urinalysis, eye examination, bone marrow biopsy/aspirates, metaiodobenzylguanidine (MIBG), brain magnetic resonance imaging or computed tomography (CT) to exclude CNS disease, and chest, abdomen, and pelvis CT. During each course, weekly physical examinations, complete blood count with differential, serum electrolytes, and renal and liver function studies were performed. At the end of courses one and two, and then after every other course, all pretreatment evaluations (including disease evaluations) were repeated.

Duration of response was defined as the interval from the date of objective response (complete or partial response) to the date of disease progression or date of going off study or of last contact. Duration of stable disease was defined as the interval from the date of treatment initiation to the date of disease progression or date of going off study or of last contact. Time to progression for all patients was defined as the interval from the date of treatment initiation to the date of disease progression; patients without progression were censored at the date at which they went off study or date of last contact.

Subsequent Courses and Dose Modification

A course could be repeated if the patient had at least stable disease and had recovered from the prior course of therapy such that symptoms resolved to grade ≤ 1 or baseline. Patients were taken off protocol therapy if toxicity persisted ≥ 2 weeks, QT/QT_c interval was > 60 milliseconds over baseline, they developed hypotension unresponsive to fluid resuscitation and had reduced antibody infusion rate, or they had grade 3 or 4 hypersensitivity reaction. Patients who required systemic corticosteroids, except as replacement for adrenal insufficiency and premedication for prevention of transfusion or imaging contrast agent–related allergic reaction, were not permitted to continue study therapy. Otherwise, patients could continue study treatment for four courses if there was no disease progression or unacceptable toxicity. Additional courses, beyond the planned four courses, were considered for patients who had clinical benefit.

Pharmacokinetic Studies

Serum concentration of hu14.18K322A was measured using an enzyme-linked immunosorbent assay (ELISA) method. Plates were coated with 1A7 anti-idiotypic antibody (from K. Foon, MD, and M. Chatterjee, PhD, and Titan Pharmaceuticals, Scottsdale, AZ)⁴⁹ and

blocked. Patient serum samples in appropriate dilutions were applied into the wells (in duplicate). Plates were incubated at 4°C overnight; the next day, they were washed and incubated with the secondary antibody (sheep antihuman IgG1-HRP) for 3 hours at room temperature. After washing, enzyme substrate was added, and after 15 minutes of incubation, the reaction was stopped, and absorbency of plate wells was measured using an ELISA reader (Tecan, Männedorf, Switzerland). The standard curve (range, 0 to 50 ng/mL) was constructed using a purified preparation of hu14.18K322A obtained from Children's GMP.

The NONMEM 7.2 population pharmacokinetic program¹⁷ was used to perform a compartmental analysis of the course-one hu14.18K322A concentration data. Pharmacokinetic parameters for individual patients such as clearance (CL) and volume distribution (V) were estimated from an empiric Bayesian method post hoc in a two-compartment model (ADVAN3 and TRANS4), including body-mass index (BMI) as a proportional covariate model with first-order conditional estimation. Maximum plasma concentration was estimated 1 hour after the end of the infusion on day 4 from the two-compartment model. Interindividual variability in pharmacokinetics was described by an exponential error model, and the residual error was modeled as an additive error. Initial ($T_{1/2\alpha} = \ln^2/\lambda_1$) and terminal ($T_{1/2\beta} = \ln^2/\lambda_2$) half-lives were derived parameters, where λ_1 and λ_2 are rate constants for the initial and terminal phases derived from CL, Q (intercompartmental clearance), V_c (central volume), and V_p (peripheral volume); area under the concentration-time curve from time zero to infinity was calculated as the total dose divided by CL.

Pharmacokinetic data were based on body-surface area (mg/m^2) and showed that maximum serum concentrations and drug exposure increased with increasing dose based on body-surface area. When the data were evaluated by unit of weight (mg/kg) or by lean body mass (mg/kg ; calculated lean body mass), a similar dose-dependent correlation was seen (data not shown). These comparisons indicated that dosing of hu14.18K322A based on body-surface area seems justified for this patient population. Only two of the 39 patients were overweight ($\text{BMI} \geq 25 \text{ kg}/\text{m}^2$), and none were obese ($\text{BMI} \geq 30 \text{ kg}/\text{m}^2$); hence, conclusions on the pharmacokinetics of hu14.18K322A in obese patients cannot be made from this study.

Because tumor volume might influence pharmacokinetic values, all enrolled patients were semiquantitatively scored (1 to 4) for tumor burden on study entry based on the following criteria: score 1, elevated vanillylmandelic acid/homovanillic acid and/or < 10% bone marrow involvement only; score 2, > 10% bone marrow involvement, one to two MIBG-avid sites, and/or CT lesion measuring < 1 to 3 cm in longest diameter; score 3, > 50% bone marrow involvement, three to five MIBG-avid sites, and/or CT lesion measuring > 3 to 5 cm in longest diameter; and score 4, CT lesion measuring > 5 cm in longest diameter and/or > five MIBG-avid sites. There was no significant association between tumor burden and any pharmacokinetic parameter (data not shown). In addition, WBC count, absolute neutrophil count, and absolute lymphocyte count before treatment showed no significant association with any pharmacokinetic parameter (data not shown).

Human Antihuman Antibody Studies

Serum samples were assayed for human antihuman antibody (HAHA) using an ELISA bridging assay. Plates were coated with hu14.18 K322A antibody and blocked. Patient serum samples diluted at a ratio of one to five were applied to the wells in duplicate, incubated overnight at 4°C, and then washed. Biotinylated hu14.18K322A was added to the wells for 3 hours at room temperature and then washed. ExtrAvidin—alkaline phosphatase (Sigma-Aldrich, St Louis, MO) was added for 1 hour at room temperature. After this incubation and washing, *p*-nitrophenyl phosphate substrate was added to the wells, and the reaction was carried out until control wells showed a 2.5-OD readout at 405/492 nm. Optical density units obtained for each pair of duplicate wells reflected anti-hu14.18K322A reactivity in this bridging assay system.

Complement Studies

Blood samples for serum complement levels (C3, C4, and CH50) were collected before hu14.18K322A infusion on day 1, on day 5, and before infusion day 1, course two. Samples were analyzed in the clinical laboratory. Changes in serum complement levels were examined using the exact Wilcoxon signed rank test.

Thirty-five patients had results available for analysis of all three complement levels (C3, C4, and CH50) for days 1 and 5 of course one, and 23 patients had results available for day 1 of course two. Significant increases in C3, C4, and CH50 were observed between days 1 and 5 of course one (all $P < .001$). However, there was no evidence of any difference in complement level between day 1 of course one and day 1 of course two, indicating that the levels had returned to near baseline ($P > .43$) at the start of course two.

Narcotic Requirements During Hu14.18K322A Infusion

Eleven patients (29%) had patient-controlled analgesia initiated during the first course, and two patients (8%) had it initiated during the second course. All other patients were managed with bolus doses of narcotics as needed. In these patients, the total, average, and median numbers of narcotic doses administered during course one, day 1, decreased from 101, 3.9, and four (range, zero to seven) to 45, 1.7, and one (range, zero to seven) on day 4, respectively.