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Phase I Trial of the Murine Monoclonal Anti-G_{D2} Antibody 14G2a in Metastatic Melanoma¹

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ABSTRACT

In a phase I trial, 12 patients with G_{D2} antigen-positive metastatic melanoma received the murine anti-G_{D2} monoclonal antibody 14G2a. The monoclonal antibody was administered in four doses over an 8-day period with total dose ranging from 10 to 120 mg. All patients receiving >10 mg of 14G2a experienced transient abdominal/pelvic pain during the antibody infusion. Five patients had a delayed extremity pain syndrome following the third and fourth antibody infusion. Four of the five patients developed neurological toxicity, including two patients with significant although reversible motor neuropathy. Two of the patients developed hyponatremia secondary to a syndrome of inappropriate antidiuretic hormone. All 12 patients developed high levels of human anti-14G2a antibody. The plasma half-life of 14G2a was 42 ± 6 (SD) h. One patient each had a partial response, mixed response, and stable disease, respectively. The very modest antitumor activity accompanied by dose-limiting neurological toxicity at total doses >80 mg may restrict the clinical utility of murine 14G2a.

INTRODUCTION

The therapeutic use of biological reagents in the treatment of malignant melanoma continues to be an area of ongoing investigation (1-3). A number of antigens that are highly expressed on melanoma cells have been identified (4-7). Several MoAbs³ directed against these antigens have been developed and have demonstrated potent antitumor activity *in vitro* (8-11). The disialoganglioside G_{D2} is preferentially expressed on tumors of neuroectodermal origin, including malignant melanoma, small cell carcinoma of the lung, and neuroblastoma (4, 10, 12). The G_{D2} antigen is expressed on microprocesses emanating from the tumor cell surface and is thought to play an important role in the attachment of tumor cells (to extracellular substratum) in tissue culture as well as *in vivo* (4, 13). In tissue culture, anti-G_{D2} antibodies cause detachment and aggregation of antigen-expressing tumor cells (4). The murine monoclonal anti-G_{D2} antibody 14.18 (IgG3) and its IgG2a switch variant 14G2a were developed by Mujoo *et al.* (10, 14) and mediate antibody-dependent cellular cytotoxicity as well as complement-dependent lysis of neuroblastoma and melanoma cell lines *in vitro*. Preliminary studies with this MoAb have reported antitumor activity in children with recurrent, therapy-resistant neuroblastoma (15). Cheung *et al.* (16) have reported objective antitumor responses in adult patients with neuroblastoma and metastatic melanoma using 3F8, an unrelated anti-G_{D2} MoAb. The disialoganglioside antigen G_{D3}, a precursor of G_{D2} (17), has also

been the target of therapeutic MoAbs. Investigators using the anti-G_{D3} MoAb R24 demonstrated objective responses in patients with malignant melanoma (18, 19). We report a phase I clinical trial of the murine anti-G_{D2} MoAb 14G2a in the treatment of patients with metastatic melanoma.

MATERIALS AND METHODS

Patient Selection. Selection criteria permitted the inclusion of only those patients with G_{D2} antigen-positive metastatic melanoma. The presence of the target antigen was determined by immunoperoxidase technique on frozen sections of fresh biopsy specimen (10, 20). Patients had to have an Eastern Cooperative Oncology Group performance status of 0-2 and not have received more than one prior trial of chemotherapy. Patients with symptomatic central nervous system metastases were excluded. Therapy was administered at least 4 weeks after prior chemotherapy or radiotherapy and patients had to have recovered from any side effects of previous treatment. Laboratory selection criteria included liver function tests no greater than 4-fold above normal, < 2 mg/dl serum creatinine, and < 30 mg/dl blood urea nitrogen. Patients had to have small or moderate tumor volume, defined as follows: small tumor burden, all lesions <5 cm in diameter; and moderate tumor burden at least one lesion >5 cm and none >10 cm.

Treatment Regimen and Antibody Administration. The MoAb 14G2a was produced by Abbott Laboratories and provided by the Biologic Response Modifier Program of the National Cancer Institute (IND BB3159). The protocol was approved by the National Cancer Institute and by the Institutional Review Board of the University of Alabama at Birmingham. All patients were required to sign an informed consent at the time of enrollment.

The strategy for MoAb administration was to establish a regimen which would produce continuous circulating levels of antibody for approximately 10 days. We thus administered antibody on days 1, 3, 5, and 8, similar to our previous schedule with murine MoAb 17-1A (21). Patients in group 1 received a total dose of 10 mg subdivided into 1-, 1-, and 4-, and 4-mg doses. Patients in group 2 received a total dose of 100 mg subdivided into 10, 10, 40, and 40 mg. Because of toxicity in group 2, patients in group 3 were given a reduced total dose of 80 mg subdivided into four 20-mg doses. Following completion of this dose level, group 4 patients received a total dose of 120 mg subdivided into four 30-mg doses (Table 1).

The antibody was provided in 10-ml vials containing 50 mg of antibody (5 mg/ml) and was stored at 4°C. At the time of treatment, the antibody was diluted in 200 ml of normal saline and infused over 60 min. Patients experiencing infusion-related toxicity with the 60-min infusion had the following dose infused over 4 h. Since this did not appear to influence the toxicity, all subsequent infusions were administered over 60 min regardless of antibody dose. All patients received a 0.5-mg i.v. test dose prior to each infusion followed by 30 min of careful monitoring for evidence of allergic reaction. If no evidence of an adverse reaction was observed, the therapeutic dose was infused and vital signs were determined at four 15-min intervals and then at four 30-min intervals or more frequently if required. To allow the study of MoAb localizing to tumor *in vivo*, patients 2-12 received 0.5 mg of ¹³¹I-labeled 14G2a (5-10 mCi) together with the first therapeutic dose (day 1). The MoAb was radiolabeled using standard iodogen methodology followed by separation of free iodine and quality control testing as previously described (22). The immunoreactivity of the radiolabeled

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³ The abbreviations used are: MoAb, monoclonal antibody; HAMA, human anti-mouse antibody; SIADH, syndrome of inappropriate antidiuretic hormone.

antibody was consistently >60%. Patient 1 did not receive the imaging dose due to a technical problem during radiolabeling. The patients also received 10 drops of saturated solution of potassium iodide 1 day prior to the radiolabeled dose and for 14 days thereafter for thyroid protection.

Patient Follow-up and Response Criteria. Patients were hospitalized in the General Clinical Research Center for the duration of therapy. Complete blood count, serum chemistry, and serum complement levels were drawn prior to each infusion, 24 h following the end of therapy, and weekly thereafter for a total of 4 weeks. Serum samples for pharmacokinetics were obtained at zero time and 1, 2, 4, 12, 24, 36, and 48 h following the day 1 dose and at zero time, and 4, 8, and 24 h following each subsequent dose. Samples for human anti-mouse antibody response were drawn prior to therapy and weekly for 4 weeks and monthly thereafter. All patients were reevaluated for evidence of objective response 4 weeks from the start of therapy. The criteria for response were (all tumor measurements consisted of the longest perpendicular diameters): (a) complete response, disappearance of all clinical evidence of active tumor (objectively measurable and "evaluable" tumor sites for a minimum of 4 weeks. The patient must be free of any symptoms related to cancer; (b) partial response, 50% or greater decrease in the sum of the products of the perpendicular diameters of all objectively measurable lesions; significant improvement in all evaluable (nonmeasurable) tumor sites. No simultaneous increase in the size of any lesion or the appearance of new lesions may occur. The response must be maintained for at least 4 weeks. (c) stable disease, steady state or response less than partial remission or progression for a minimum of 6 weeks. There may be no appearance of new lesions and no worsening of the symptoms; (d) mixed response, complete regression of some index lesions combined with <50% increase in size of others; and (e) progression, an increase of at least 50% in the size of any measured lesions or the appearance of new lesion(s).

Radioimmune Imaging. Patients 2–12 underwent imaging with a large-field-of-view scintillation gamma camera equipped with a high-energy parallel-hole collimator on days 3 and 5. Whole-body as well as regions of interest were imaged on day 3. Areas of antibody uptake as well as sites of known disease were again imaged on day 5.

Pharmacokinetics of MoAb 14G2a. The pharmacokinetics of the murine MoAb 14G2a was determined in patients 2–12 based upon the disappearance of the radiolabeled tracer dose administered on day 1. The radioactivity of 1-ml serum samples collected at different time intervals was measured using an LKB 1282 Compugamma CS counter. The cpm were converted to dpm and back-corrected to zero time for radiation decay. The radioactivity counts were then utilized to calculate the pharmacokinetic parameters as previously described (23). Results were comparable to those obtained using an assay for circulating mouse antibody (data not shown).

Human Antibody to Murine 14G2a. Human antibody to murine MoAb 14G2a was assayed using the "double antigen assay" previously described (23). Briefly, 100 μ l of patient serum in triplicate were incubated with a 14G2a-coated polystyrene bead (Precision Plastic Ball, Chicago, IL) for 1 h on a laboratory oscillator at room temperature. Following incubation, the beads were washed with phosphate-buffered saline, pH 7.2, and incubated with 200 ng of ¹²⁵I-labeled MoAb 14G2a (approximately 200,000 cpm) for 1 h at room temperature. The beads were again washed in phosphate-buffered saline and the bead-associated radioactivity was determined on a Micromedic automatic gamma counter. The mean cpm bound per 100 μ l of serum were converted to ng of ¹²⁵I-labeled MoAb 14G2a and results are expressed as ng 14G2a bound/ml of patient serum. Serum from normal individuals bound 13 \pm 5 ng/ml of 14G2a (n = 20). Prior to therapy, the patient's sera bound 13 \pm 6 ng/ml (n = 11). This minimal binding represents nonspecific binding since it is not competitively inhibited by excess antigen (23).

Values >2 times the pretreatment level were considered positive. The linear range for the antibody assay is between 10 and 200 ng/ml bound. Serum samples yielding anti-14G2a values higher than 200 ng/ml underwent 10-fold serial dilution with normal human serum until values were in the linear range of the assay.

Data Analysis and Statistical Methods. The pharmacokinetic parameters for circulating antibody were estimated by fitting to a one- and two-compartment model. SIPHAR program (SIMED, Creteil, France) and SAS NLIN procedure (24) were used for nonlinear estimation by least squares method. Analyses of variance and covariance were used to compare the significance of differences in pharmacokinetic parameters among individual patients and dose groups (21, 24).

RESULTS

Patient Characteristics. The clinical aspects of the trial are summarized in Table 1. Twelve patients with metastatic melanoma received MoAb 14G2a at total doses ranging from 10 to 120 mg. All patients had previously undergone resection of their primary lesion. Three patients had received prior chemotherapy and one patient had received prior cranial irradiation. Two of the patients (patients 4 and 5) had undergone multiple surgical procedures for resection of regional recurrences and had regional disease at the time of entry. All other patients had disseminated disease at enrollment (see Table 3 for sites of metastatic disease). Six of the 12 patients had small tumor burden and the remaining 6 patients had moderate tumor burden (for criteria see "Patient Selection").

Toxicity. The two patients who received 10 mg total dose experienced no side effects. For the remaining patients, pain was the most frequent toxicity associated with 14G2a administration (Table 2). Two separate pain syndromes were observed in this trial. Nine of 12 patients experienced "pain during infusion". The pain occurred midway into the antibody infusion, lasted for the duration of the antibody administration, and generally subsided within 1–2 h following the end of infusion. The pain was described as mild to moderate (Grade 2) by four patients and severe (Grade 3) by five patients and was localized primarily to the abdomen and pelvis. Female patients described the pain as menstrual or labor-like. Mild to moderate pain generally responded to oral analgesics such as acetaminophen/oxycodon while severe pain required i.v. morphine for control (total dose, 5–40 mg). Prolonging the infusion time from 1 to 4 h did not alter the pain characteristics.

In addition, a "delayed extremity pain" syndrome was observed in five patients (patients 4, 5, 8, 11, and 12). This pain was readily distinguishable from the pain during infusion and occurred in all cases approximately 24 h following the third and

Table 1 Clinical characteristics

Patient	Total dose (mg)	Fractions (mg)	Prior therapy	Performance status ^a	Tumor burden	Response
Group 1						
1	10	1, 1, 4, 4	S, ^b R	0	Sm	P
2	10	1, 1, 4, 4	S, C	1	Sm	P
Group 2						
3	100	10, 10, 40, 40	S	2	Mod	P
4	100	10, 10, 40, 40	S, C	1	Sm	PR
5	60	10, 10, 40	S, C	1	Sm	MR
Group 3						
6	80	20, 20, 20, 20	S	0	Mod	P
7	80	20, 20, 20, 20	S	2	Mod	P
8	80	20, 20, 20, 20	S	0	Mod	P
9	80	20, 20, 20, 20	S	1	Mod	P
10	80	20, 20, 20, 20	S	0	Mod	SD
Group 4						
11	120	30, 30, 30, 30	S	0	Sm	P
12	120	30, 30, 30, 30	S	0	Sm	P

^a Performance status on Eastern Cooperative Oncology Group scale (0–4).

^b S, surgery; R, radiotherapy; C, chemotherapy; Sm, small; Mod, moderate; PR, partial response; MR, mixed response; SD, stable disease; P, progressive disease.

Table 2 Toxicity associated with 14G2a therapy^a

Patient	Total dose (mg)	Pain during infusion	Delayed extremity pain	Neurotoxicity	Hypонатremia	Fever
Group 1						
1	10	0	0	0	0	
2	10	0	0	0	0	
Group 2						
3	100	2	0	0	0	
4	100	3	3	2 (S) ^b 4 (M)	0	3
5	60	3	3	3 (C)	2	
Group 3						
6	80	0	0	0	0	
7	80	2	0	0	0	
8	80	3	3	0	0	
9	80	2	0	0	0	
10	80	2	0	0	0	
Group 4						
11	120	3	2	2 (S)	0	
12	120	3	3	4 (M) 4 (C)	0	

^a Toxicity reported by National Cancer Institute Consensus grading system (0–4).

^b S, neurosensory; M, neuromuscular; C, neurocortical.

fourth infusions. The pain was described as severe and burning and affected both hands and both legs below the knee. The pain required i.v. morphine for control in all patients except patient 11 who responded to self-administered anti-inflammatory agents and local heat application to the extremities. The pain generally was resolved completely within 24–48 h except in patient 4 who had recurrence of the extremity pain following discharge from the hospital.

Four of the five patients who experienced the delayed extremity pain also developed neurological toxicity (patients 4, 5, 11, and 12). Patient 4 noted recurrence of the severe distal leg and hand pain approximately 5 days following the last antibody dose. Two days later he was admitted to his local hospital with intractable leg pain accompanied by paresthesia and leg weakness. He was unable to walk without assistance because of the leg weakness and required a continuous infusion of morphine for pain control. He underwent physical therapy and was discharged 10 days later with improvement in the weakness and decrease in pain. Two weeks following his discharge, neurological examination revealed persistent decreased strength in the proximal thigh muscles, which was more pronounced on the right. Deep tendon reflexes were decreased in both lower extremities. Sensory examination revealed paresthesia in both lower extremities with a stocking distribution. The patient also complained of a cold sensation in both his hands and feet. Electromyography studies conducted at our institution revealed evidence of denervation in the proximal and distal muscles of both lower extremities, which was more pronounced on the right. Biopsy of the right sural nerve revealed demyelination and a mononuclear infiltrate in the endoneural and perivascular space. Immunohistological studies demonstrated G_{D2} on the patient's nerve fibers. MoAb 14G2a was not detected on the sural nerve tissue (the biopsy was performed approximately 4 weeks after completion of MoAb therapy). The patient's clinical picture was compatible with the diagnosis of mononeuritis multiplex/polyradiculopathy. The patient refused steroid therapy. His neurological function gradually improved and returned to base line over the ensuing 4 to 6 weeks. At last follow-up (20 weeks from the end of therapy), the patient had regained full

sensory as well as motor function but continued to complain of dysthermia in the hands and feet.

Patient 5 developed mental confusion and disorientation 24 h following the third dose of 14G2a. The altered mental status coincided with onset of the delayed extremity pain. The patient was incidentally noted to have a serum sodium of 124 mg/dl (with a normal electrolyte profile 3 days prior). Serum and urine osmolality studies were consistent with SIADH. The patient's hyponatremia corrected promptly with fluid restriction. She continued, however, to be confused and disoriented for approximately 1 week. Evaluation including computed tomography scan of the head revealed no evidence of a specific abnormality. The patient did not receive the fourth dose of 14G2a.

Patient 11 complained of severe extremity pain and loss of sensation in both lower extremities on the day following the fourth antibody infusion. The patient was at home and resorted to taking antiinflammatory agents and applying local heat to the lower extremities with apparent relief of symptoms. He was seen by his local physician the next day and was noted to have a completely normal neurological examination by that time. There were no further symptoms or signs of neurological damage.

Patient 12 received the same dose of antibody as patient 11. Six h following the last antibody infusion she complained of inability to void. This was followed by progressive lower extremity weakness over the next 24 h to the point where she was unable to raise her legs or stand without assistance. Neurological examination and nerve conduction studies were compatible with a neurogenic bladder and motor radiculopathy involving the L2–4 and S1 nerve roots. Cerebral and spinal magnetic resonance imaging scans revealed no evidence of intracranial metastasis or cord compression. The patient also had a serum sodium of 113 mg/dl (with a normal electrolyte profile 2 days prior). Serum and urine osmolality studies were compatible with SIADH. While hyponatremic, the patient had a grand mal seizure. Treatment was started with 80 mg of prednisone daily for presumptive inflammatory myelopathy. Three weeks later, she had regained full bladder control and within 6 weeks was able to ambulate with a walker. At last follow-up (16 weeks from the end of therapy), she was able to ambulate without assistance but had not attained full motor strength.

Six of the patients (patients 3, 4, 5, 8, 9, and 12) demonstrated a > 25% decrease in the total serum hemolytic complement between days 1 and 9. No other laboratory alteration was observed in the patient population. One patient (patient 4) developed transient fever up to 104°C following both the third and fourth antibody infusions. None of the patients developed hypertension during antibody therapy.

Clinical Response. One patient each had a partial response, a mixed response, and stable disease at 4-week follow-up (Table 1). Patient 4 had pathologically documented complete resolution of two of four s.c. index lesions, each measuring 1 x 1 cm (partial response). Patient 5 had approximately 50 small (5–7-mm) cutaneous melanotic lesions on the inner aspect of the left ankle as well as an inguinal lymph node measuring 2 x 1 cm. She had erythema and pruritus at the site of the small cutaneous deposits during infusion therapy. Blanching of lesions was observed 1 week following therapy. Three lesions biopsied 1 week following therapy revealed necrotic tissue without residual melanoma. At 4-week follow-up, some of the lesions had regressed while others showed <50% increase in size. The index inguinal lymph node measured 1 x 1 cm (mixed response). Patient 10 had a pelvic mass as well as inguinal adenopathy originating

from melanoma of the vulva. Both sites showed no change in size at 4- and 8-week follow-up (stable disease). All other patients had disease progression.

Radioimmune imaging. Eight of 11 patients (73%) had MoAb localization to at least 1 tumor site by radioimmune imaging (Table 3). Of a total of 34 lesions documented by either physical examination or conventional diagnostic studies, 13 (38%) were visualized upon imaging. With the exception of one single lesion that was observed only on day 5, all other lesions were visualized equally well on days 3 and 5.

Pharmacokinetics. The plasma disappearance curves of radiolabeled 14G2a best fit a one-compartment model. The plasma half-life ranged from 30 to 49 h with an overall mean $T_{1/2}$ of 42 ± 6 h (Table 4). High-performance liquid chromatography analysis of randomly selected serum samples revealed the radioactivity to be associated with the IgG fraction with no evidence of free iodine. The plasma half-life, volume of distribution, and clearance rate did not vary significantly among patients receiving different doses of unlabeled MoAb on day 1.

HAMA. Table 5 lists the antibody response to murine 14G2a over a 4-week period. Prior to therapy, all patients except patient 3 had baseline levels of 14G2a binding similar to those of healthy control donors. All 12 patients developed antibody to murine 14G2a. Patients frequently required a 1:100- to 1:1000-fold dilution of their serum to accurately quantitate the magnitude of the antibody response. Antibody response was detectable by day 10 in all except three patients (patients 2, 11,

Table 3 Radioimmune imaging

Patient	Total dose (mg)	Metastatic site	Size (cm)	Imaging ^a
Group 1				
2	10	Cutaneous Liver Pelvic mass	Nm ^b <2 Nm	- - +
Group 2				
3	100	Liver Pelvic mass	5 × 2 4.5	+ +
4	100	Cutaneous	1	-
5	60	Cutaneous (multiple) Inguinal LN	<1 2 × 1	+ +
Group 3				
6	80	Liver Adrenal	3 × 3	- +
7	80	Oral mass Liver Axillary LN Lung	4 × 4 5 × 5 3 × 5 1.5	- - - -
8	80	Pelvic mass Spleen Chest wall	5 × 10 4.5 × 4.5 1 × 1	+ - -
9	80	Thigh (s.c.) Submandible LN Inguinal LN Periaortic LN Lung	Nm Nm 2 × 3 3 × 2.5 2 × 2	+ + + - -
10	80	Right inguinal LN Left inguinal LN Pelvic mass	1.5 × 2 2 × 2 Nm	- - +
Group 4				
11	120	Axillary LN Mediastinal LN Adrenal Right lung Left lung	2 × 2 5 × 3 1.5 × 2 1 2 × 2	- - - - -
12	120	Thigh (s.c.) Lung	6 1.5 × 1	+ -

^a Radioimmune imaging.
^b Nm, nonmeasurable; LN, lymph node; -, not visualized; +, unequivocal uptake of labeled MoAb.

Table 4 Pharmacokinetics of MoAb 14G2a

Patient	Dose (mg) ^a	T _{1/2} (h)	V _{d_{ss}} ^b (ml/kg)	MRT ^c (h)	CL ^d (ml/h/kg)	R ²
2	1	49	50	71	0.71	99.0
3	10	30	58	43	1.33	97.2
4	10	49	41	71	0.58	98.4
5	10	48	58	69	0.84	99.4
6	20	40	52	58	0.90	99.1
7	20	33	36	47	0.76	99.1
8	20	36	36	52	0.70	98.9
9	20	40	59	58	1.01	98.5
10	20	47	44	67	0.65	99.0
11	30	44	61	63	0.96	98.9
12	30	48	48	69	0.69	97.6
Mean ± SD		42 ± 6	50 ± 9	61 ± 9	0.83 ± 0.21	98.5 ± 0.8

^a Dose of unlabeled 14G2a administered with the 0.5-mg radiolabeled dose on day 1.

^b V_{d_{ss}}, volume of distribution at steady state; MRT, mean residence time; CL, clearance.

Table 5 Human antibody response to murine 14G2a

Patient	Pre-14G2a	Days following first dose				
		10	11	15	22	29
Group 1						
1	15 ^a	45	232	14,080	9,960	6,660
2	15	18	800	111,800	123,700	106,600
Group 2						
3	115	1,527	NT ^b	86,000	NT	NT
4	24	57	2190	25,600	NT	17,980
5	12	13,080	NT	15,910	15,570	NT
Group 3						
6	16	1,609	NT	11,580	6,350	5,040
7	10	755	NT	5,010	2,390	91
8	4	264	NT	113	51	27
9	17	72	956	4,880	8,330	622
10	7	1,356	NT	29,030	6,750	NT
Group 4						
11	4	4	48	1,795	953	747
12	19	7	159	9,850	6,760	2,091

^a ng of ¹²⁵I-labeled 14G2a bound/ml of serum.

^b NT, not tested.

and 12). Peak levels of human anti-14G2a antibody were evident by day 15 in ten patients and by day 22 in the remaining two patients (patients 2 and 9). Antibody to 14G2a persisted in all patients and could be detected as far out as day 122 (data not shown). The antibody response was not dose dependent in that the highest antibody response was noted in patient 2 (10-mg dose) and the lowest level of immune response occurred in patient 8 (80-mg dose).

DISCUSSION

Administration of the anti-GD2 murine MoAb 14G2a in this trial was associated with a spectrum of unusual and unanticipated side effects not observed with other antiganglioside MoAbs. Two distinct pain syndromes were observed in our trial. Nine of ten patients receiving >10 mg 14G2a developed a syndrome of severe abdominal/pelvic pain during each antibody infusion. Five patients also experienced a delayed extremity pain syndrome following the third and fourth infusion. Neurotoxicity was noted in four of these five patients. Two patients developed hyponatremia secondary to SIADH. The maximum tolerated total dose was determined to be 80 mg since dose-limiting neurotoxicity occurred at 100–120 mg. The antitumor response was modest and one patient each had a partial response, mixed response, and stable disease.

The abdominal pain associated with 14G2a infusion is similar to that reported with the unrelated anti-G_{D2} MoAb 3F8 (16). This side effect appears to be characteristic of anti-G_{D2} antibodies since no comparable toxicity has been observed in patients receiving the anti-G_{D3} MoAb R24 (18, 19). Chest tightness has been reported with R24 administered alone (19) or when combined with interleukin 2 (25). The G_{D2} (but not G_{D3}) antigen has been found on peripheral nerve tissue (20) and some investigators have speculated that the infusion-related pain syndrome may be due to binding of antibody to peripheral pain fibers (16). The location of the pain (abdominal as opposed to peripheral extremity) and the rather rapid cessation of pain following completion of antibody infusion (despite high levels of circulating antibody), however, cannot be accounted for by such a scenario. We have demonstrated that 14G2a binds to smooth muscle of the gastrointestinal tract, as well as the surrounding mesenteric blood vessels.⁴ Whether binding of antibody to these sites may cause smooth muscle and vascular spasm and thereby contribute to the abdominal pain is unclear. Toxicity related to rate of antibody infusion has been observed with other murine monoclonal antibodies (18, 26, 27). These symptoms have been ascribed to binding of antibody to nontumor sites (26) or due to immune complex and/or aggregate formation (27). These symptoms frequently respond to slowing of the infusion rate (26, 27). Prolonging the infusion time of 14G2a from 1 to 4 h in this trial did not alter the pain. Administration of 14G2a as a continuous infusion over 24 h has also been associated with a similar pain syndrome (15, 28). It thus appears that the pain during infusion is not simply dependent on the infusion rate and etiology of this toxicity still remains to be determined. Given the transient nature of this toxicity and adequate control obtained with i.v. morphine, pain during infusion was not dose limiting in this study. Hypertension was not observed in this trial, although both 3F8 and high-dose R24 therapy have been associated with blood pressure elevation (16, 29).

The delayed extremity pain syndrome observed with 14G2a occurred approximately 24 h following the third and fourth infusion. None of the three patients receiving the 10-mg dose, two of six patients receiving 60–80-mg doses, and three of four patients receiving >100 mg 14G2a experienced this side effect. Except for the higher dose of antibody administered, no specific clinical characteristic identified those patients predisposed to this side effect. It is noteworthy that four of the five patients experiencing the extremity pain syndrome also developed neurological toxicity following their last infusion.

Neurological toxicity associated with 14G2a therapy consisted primarily of sensory and motor neuropathy. Altered mental status was noted in two patients, both of whom had concomitant hyponatremia secondary to SIADH. Patients 4 and 12 developed an acute demyelinating process with severe motor neuropathy that reversed over the ensuing months. Both patients received >80 mg antibody. This finding was unanticipated since no such side effect was observed in our trial using comparable doses of the related human/mouse chimeric anti-G_{D2} MoAb ch 14.18 (30, 31). The precise etiology of the neurotoxicity associated with 14G2a and absence thereof with ch 14.18 remains unclear. All patients exposed to 14G2a developed high levels of HAMA while ch 14.18 therapy has been associated with a weak immune response directed at the variable region (32). Patients 4 and 12 both had a much more rapid

clearance of the day 8 dose as compared to the day 1 infusion. No circulating 14G2a was detectable after the 48-h time point (day 10) (data not shown). Circulating HAMA was detected by day 11 (Table 5). Whether the demyelinating neuropathy could have been caused by binding of 14G2a/anti-14G2a immune complexes to the peripheral nerve with resulting immune-mediated cytotoxicity remains speculative. The role of the day 8 dose and HAMA on the subsequent development of acute neurotoxicity remains unclear and cannot be conclusively ascertained from our data. Neither of our patients had nerve biopsies at the time of acute neurotoxicity looking for bound antibody and/or complement deposition. Interestingly, four of the five patients who developed neurological side effects had a >25% reduction in total hemolytic complement during therapy.

The pharmacokinetics of 14G2a followed a one-compartment model similar to what has been observed with other murine reagents (21, 33). The immune response to 14G2a, however, exceeds that previously described for m-17-1A (21) or m-B72.3 (33) by 2 to 3 logs using similar assay techniques. Our results demonstrated no correlation between the degree of immune response and the dose of antibody administered.

No major responses were observed at the doses of 14G2a used in this trial and the maximum tolerated dose was determined to be 80 mg (total dose). In previous trials of 3F8 and R24 higher doses could be administered and favorable clinical responses could be achieved (16, 18, 19). Ongoing studies with the chimeric MoAb ch 14.18 have not been associated with any dose-limiting toxicity thus far (31) and we are proceeding with dose escalation using this reagent.

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