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Antimelanoma Monoclonal Antibody–Ricin A Chain Immunoconjugate (XMMME-001-RTA) Plus Cyclophosphamide in the Treatment of Metastatic Malignant Melanoma: Results of a Phase II Trial

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Summary: Prior studies with the XMMME-001-RTA immunoconjugate composed of an antimelanoma monoclonal antibody and ricin A chain demonstrated some antitumor activity. However, almost all patients studied developed human antimurine antibodies and antiricin antibodies. In an effort to abrogate these host anti-immunotoxin immune responses and thus enhance antitumor activity, we treated 20 patients with the immunoconjugate plus a single dose of intravenous cyclophosphamide. An overall response rate of 20% was observed—predominantly in pulmonary and soft tissue nodules. There was no diminution in antibody responses against either the murine antibody or the ricin moiety. Further studies to elucidate the role of cyclophosphamide in monoclonal antibody therapy are planned. **Key Words:** Immunoconjugate—Antimelanoma monoclonal antibody—Ricin A chain—Human antimurine antibodies—Cyclophosphamide.

We have tested an antimelanoma monoclonal antibody conjugated to ricin A chain in patients with metastatic malignant melanoma. XMMME-001-RTA, an IgG_2 murine monoclonal antibody, recognizes two high molecular weight antigens of 220 kDa and greater than 500 kDa, and is conjugated to purified ricin A chain by SPDP reaction. Prior animal and phase I studies have demonstrated the safety of this agent (1,2). Phase II studies suggested potential clinical usefulness of this immunotoxin after a single course, with a small number of patients achieving durable partial remissions (3).

In these previous studies, all patients tested mounted a host antibody response against both murine immunoglobulin and ricin A chain moieties of the immunotoxin. Cyclophosphamide given with or shortly after sensitization to a new antigen has been shown in animal (4,5) and human studies (6) to blunt humoral responses to new antigens. In an animal model designed to test the effect of various immunosuppressive drugs on antibody responses, Santos et al. immunized rats with sheep red blood cells (SRBCs) and at various times in relation to immunization-administered cyclophosphamide, methotrexate, or 6-mercaptopurine (6). The animals were then bled periodically and peak anti-SRBC antibody titers were measured. Cyclophosphamide was a powerful inhibitor of the humoral response particularly when administered one or several days prior to immunization. In a comparison of the immunosuppressive capacity of drugs, cyclophospha-

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mide and methotrexate were far superior to 6-mercaptopurine. In an early clinical study (7), patients were immunized with either the V1 antigen (a purified polysaccaride) or a *Pasturella tularensis* vaccine. Immunosuppressive drugs including cyclophosphamide and 6-mercaptopurine were administered either prior to or following immunization. All patients who received cyclophosphamide (7 mg/kg i.v. daily for 7 days prior to antigen challenge) showed no rise in antibody titer while under observation.

More recent animal studies (8) used a Balb/c mouse model in which animals were sensitized with alloantigens comprised of spleen, thymus, and lymph node cells from C3H mice. Two dosages (20% LD_{50} and 60% LD_{50}) of a number of different immunosuppressive agents were administered at various times in relation to antigen challenge. Cyclophosphamide in doses of 102 and 306 mg/kg both showed strong suppression of antibody responses when given with or shortly after the immunizing antigen.

In an effort to enhance antitumor responses and abrogate the host anti-immunotoxin antibody response, cyclophosphamide was added to this antimelanoma immunotoxic protocol. A single large dose of cyclophosphamide (1,000 mg/m^2 i.v.) given immediately following immunotoxin infusion was selected based on preclinical and animal studies, so that the immunosuppressive agent was given essentially along with the potential antigen.

MATERIALS AND METHODS

Patients

Patients over age 18 years with histologically documented malignant melanoma with measurable metastatic disease were eligible for study. Other eligibility criteria included Karnofsky performance status ≥80% and life expectancy of at least 12 weeks. Patients were required to have adequate bone marrow, renal, and liver function. Prior systemic therapy for metastatic melanoma was allowed. Patients with resected, irradiated brain metastases and stable head computed tomography (CT) scan were eligible. This study was approved by the New York University Medical Center IRB and all patients signed written informed consent prior to treatment. Patients were ineligible if they had previously been treated with murine monoclonal antibodies or ricin A chain containing toxins.

Immunotoxin and Cyclophosphamide

The immunotoxin XMMME-001-RTA was provided by XOMA Corporation (Berkeley, CA, U.S.A.) (9). The immunotoxin is a murine monoclonal antibody of the IgG_{2a} subclass (MW 150,000±) to which ricin toxin A chain (RTA) (MW 30,000) is covalently coupled. The conjugation technique has been previously described (1,9). The ricin A chain is purified by affinity chromatography using an anti-ricin B chain column. Briefly, the antibody is activated with *N*-succinimidyl-3-(2-pyridyldithio)proprionate followed by addition of affinity-purified ricin A chain that has been reduced with dithiothreitol. The immunotoxin is then purified by gel chromatography. It is provided in a sterile, pyrogen-free formulation at a concentration of 1.0 mg/ml in 0.9% phosphate-buffered saline solution, pH 7.0. The binding specificity of the antibody as determined by enzyme-linked immunosorbent assay, radioimmunoassay, flow cytometry, and immunoperoxidase techniques demonstrates binding with all melanomas tested by frozen section and the majority of melanoma cell lines. There is no binding with other tumors or normal tissues with the exception of pigmented nevi and some cytoplasmic binding of vascular endothelium. Specificity of the ricin-conjugated immunotoxin is identical to that of the unlabeled antibody and binding activity is only minimally reduced by conjugation. Lots of 30 mg were shipped in 10 mg vials. Each patient was treated with a single lot of immunotoxin.

Commercially prepared cyclophosphamide was used.

Treatment Plan

Prior to each treatment, each patient had both a skin test and, if negative, an intravenous (i.v.) test dose. Skin tests were performed by subcutaneously injecting 0.1 ml of saline containing 0.01 mg of immunotoxin. Skin tests were considered to be negative if erythema and induration at the site were less than 5 mm in greatest diameter at 30 min. After a negative skin test, patients received an intravenous challenge with 1 ml containing 0.2 mg of immunotoxin. Patients were monitored closely and vital signs were recorded for 30 min following the i.v. test dose. If no adverse reaction developed, patients were then treated with an intravenous infusion of immunotoxin at a dose of 0.4 mg/kg in 150 cc of normal saline over 30–60 min. Thirty minutes

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after the completion of immunotoxin infusion, patients received intravenous cyclophosphamide at a dose of 1,000 mg/m^2.

Physical examination, with measurement of indicator lesions, and laboratory tests were performed at baseline and weekly for 1 month on an outpatient basis and less frequently thereafter. Patients were followed for tumor response for a minimum of 8 weeks after the initiation of treatment. Laboratory tests included blood samples analyzed for complete blood count with white blood cell differential, platelet count, and serum chemistry tests including electrolytes, urea nitrogen, creatinine, and liver enzymes as well as albumin and total serum protein. Serum samples for quantitative determinations of human antimurine and antiricin immunoglobulins were obtained before treatment and at weekly intervals thereafter.

Assay for Human Antimurine and Antiricin Antibodies

The antibody response to immunotoxin components was measured in all patients by a previously described enzyme immunoassay method (1). Patients' sera were obtained prior to treatment and at weekly intervals thereafter. Appropriate serial dilutions (1:10 + 1:10^5) were prepared and added to microtiter plates that contained either the adsorbed murine antimelanoma monoclonal antibody or adsorbed ricin A chain. The plates were washed and incubated for 1 h at room temperature with goat anti-human IgG antibody or anti-human IgM conjugated to alkaline phosphatase (Zymed Laboratories, South San Francisco, CA, U.S.A.). Another wash was followed by addition of *p*-nitrophenyl phosphate (Sigma Laboratories, St. Louis, MO, U.S.A.). This reaction produced a color titration measured spectrophotometrically at 405 nm. Titration curves were generated for each serum sample and immune responses were expressed as a response ratio: the ratio of the end-point dilution of the serum sample showing maximum response to the end-point dilution of the pretreatment serum sample.

Evaluation of Tumor Responses

Patients were examined weekly for 4 weeks and then every 2 weeks for 4 weeks following treatment in order to evaluate tumor response. Palpable disease was assessed by weekly examination and direct measurement of the perpendicular diameters of all measurable nodules. CT scans and chest radiographs were used to evaluate visceral disease and were obtained every 4 weeks. Complete response was defined as the disappearance of all measurable tumor. Partial response was defined as a reduction of all measurable tumors by at least 50% of the sum of the product of the two greatest diameters present, in the absence of any new lesions or any tumor enlargement. Mixed response was a reduction in size of some measurable tumors by at least 50%, but either no change or progressive disease in other tumors. Minimal response was a reduction in size of less than 50% in some tumors. Stable disease was no objective change in all measurable tumors. Progressive disease was an increase in size of measurable tumors by at least 25% or the appearance of new lesions. The duration of response was defined from the date of therapy until the date of progressive disease, most recent follow-up, or death. All responses were required to persist for at least 30 days.

RESULTS

Patients

Twenty patients were entered. Their characteristics are detailed in Table 1. The median age was 58.5 years (range of 38–73 years). Twelve were male and 8 were female. Nine patients (45%) had received no prior treatment for metastatic melanoma, whereas 11 patients (55%) had been previously

TABLE 1. *Patient characteristics*

	No. (%)
Age	
Median (years)	58.5
Range	38–73
Sex	
Male	12 (60)
Female	8 (40)
No prior treatment	9 (45)
Prior treatment	11 (55)
Chemotherapy	7 (35)
Radiotherapy	5 (25)
Immunotherapy	6 (30)
Sites of metastatic disease	
Soft tissue/subcutaneous/lymph nodes	9 (45)
Lung	7 (35)
Liver	4 (20)
Spleen	5 (25)
Brain	2 (10)
Adrenal	1 (5)

No. of patients = 20.

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treated with chemotherapy, radiation therapy, immunotherapy, or a combination of these. Most patients had more than one site of metastatic disease. The predominant areas of involvement included skin and soft tissue, lung, and liver. Two patients with resected and irradiated brain metastases were treated on this protocol.

Toxicity

All patients were evaluable for toxicity. Overall, the combination treatment was well tolerated and toxicity was manageable. Patterns of toxicity are outlined in Table 2. No patient had a positive reaction to the skin test dose. No patient developed hypotension, tachycardia, rash, hives, or wheezing during the intravenous test dose of immunotoxin. One patient had an episode of sneezing during the i.v. test dose with no other symptoms. After receiving approximately 30 cc of the intravenous infusion dose, he developed facial flushing, increased lacrimation, and swelling of the lower lip. He had no dyspnea, wheezing, stridor, rash, hypotension, tachycardia, or fever. The infusion was discontinued and the patient was given 50 mg of diphenhydramine by i.v. bolus. His symptoms resolved, and the immunotoxin infusion was resumed. The treatment was completed at a slower infusion rate and was well tolerated.

Other immediate toxicities from this regimen included nausea and vomiting in 18/20 (90%) patients in the first 24–48 h (mild in 13 patients, moderate in 5 patients), which was felt to be due to cyclophosphamide. Low-grade fevers were seen in 4/20 (20%) patients during the first 72 h after treatment. Most patients complained of constitutional symptoms consisting of fatigue, malaise, myalgias, and arthralgias during the first several days after treatment. This was reflected in a general decline in performance status by at least 10–20%, and resolved with return to baseline performance status by the middle of the second week after treatment.

Within the first 2 weeks following treatment, hypoalbuminemia was noted in 15/20 (75%) patients, manifested by decreases in serum albumin by less than 0.2 g/dl in 5 patients, ≥0.2–0.5 g/dl in 3 patients, and greater than 0.5 g/dl in 7 patients. Only five of these patients developed clinically evident peripheral edema: three patients had mild ankle swelling, one patient had lower extremity edema that was treated with oral furosemide, and one patient developed significant swelling of the left arm that had been the site of his primary melanoma and left axillary lymphadenectomy. The upper extremity edema was managed with oral diuretics, arm elevation, and an elastic arm stocking and resolved within 1 week. Three patients reported mild to moderate dyspnea—but pulmonary edema was not documented on chest radiographs and on no occasion were rales or wheezing appreciated on auscultation.

One patient became acutely ill within 24 h of treatment. She had a history of resected and irradiated brain metastases. A CT scan of the brain done less than 4 weeks prior to treatment showed no evidence of involvement, and her pretreatment neurologic exam was unremarkable. Nonetheless, she developed grand mal seizures on the evening following treatment. A repeat CT scan the next day revealed the presence of multiple new brain metastases. Furthermore, this patient remained hospitalized and developed grade 4 neutropenia, grade 3 anemia, fever, and sepsis. She had been heavily

TABLE 2. *Toxicity results*

Grade	None (0)	Mild (1)	Moderate (2)	Severe (3)	Life-threatening (4)
Fever	16	3	1	0	0
Fatigue	4	5	7	4	0
Malaise	11	9	0	0	0
Myalgias	10	8	2	0	0
Arthralgias	18	1	0	1	0
Dyspnea	17	2	1	0	0
Edema	15	3	1	1	0
Decreased albumin	5	5	3	7	0
Nausea/vomiting	2	13	5	0	0
Neutropenia	7	6	5	1	1
Anemia	17	1	1	1	0
Decrease in performance status	2	6	8	3	1
Seizures[a]	19	0	1	0	0

[a] See the discussion in the Toxicity section.

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pretreated with multiple cytotoxic and myelosuppressive regimens. Despite a measureable partial response in her non-central nervous system tumor, the patient expired 43 days following treatment, related to nadir sepsis and brain metastases. There were no other deaths during the study period.

Clinical Responses

Clinical outcomes of responding patients are detailed in Table 3. There were no complete responses to treatment. Four (20%) partial responses were seen. One patient achieved a partial response as measured by disappearance of several pulmonary nodules and reduction of all others by at least 50%. Early response was noted on the chest radiograph within 1 month after treatment, and improvement continued over 2–3 months. This patient received a second immunotoxin treatment 4 months after his initial treatment and the lesions then stabilized for a total response duration of 1 year. During this time, the patient was clinically well, and worked regularly. A second patient had a partial response of skin, soft tissue, and lymph node disease including multiple tumors involving the gastrointestinal tract. He reported tenderness of the responding subcutaneous and soft tissue nodules during the first week after treatment. These became erythematous and warm, and over the next 2–3 weeks gradually became softer and smaller. Some nodules disappeared completely. The duration of response was 10 weeks. Two other patients had partial responses in subcutaneous and soft tissue nodules of 6 and 15 weeks duration. Of interest, responding nodules in general did not grow at the time of disease progression. Progressive disease was usually characterized by growth of nonresponding nodules or appearance of new metastases. One patient (5%) had a minor response characterized by a 30% reduction in a soft tissue pelvic mass of greater than 56 weeks duration. She has required no further treatment of her melanoma. Two patients (10%) had mixed responses in which there was a 50% decrease in the size of soft tissue metastases but progressive disease in visceral lesions. Three patients (15%) had stable disease throughout the study period and 10 patients (50%) had progressive disease.

Immune Responses

In 13 patients, pre- and posttreatment titers of human antimurine antibodies and antiricin antibodies were determined separately, and in seven patients antibody titers against the complete immunotoxin were measured. In all instances but one, antibody titers against the mouse immunoglobulin, ricin A chain, and whole immunotoxin rose after treatment. Patient #122 did not mount an antibody response against the ricin moiety of the immunoconjugate but did produce a response ratio of 7.5 in the human antimurine antibody (HAMA) response. Baseline, maximum end-point titers, and day 28 titers are shown in Tables 4–6. The maximum response ratios are displayed graphically in Fig. 1. The median HAMA response ratio was 1.25 (range of 3–100). The median response ratio to the ricin A chain component was 32 (range of 1–250). In the seven patients so tested, the median response ratio to the whole immunotoxin was 13.3 (range of 4.5–62.5).

The single dose of 1,000 mg/m^2 of cyclophosphamide used in this study neither abrogated the pro-

TABLE 3. *Clinical features of responding patients*

Patient no.	Age (years)/sex	Sites of disease	Prior therapy	Response	Duration (weeks)
121	73/M	Lung	None	PR	53
124	45/M	Soft tissue, skin, lymph nodes, GI tract	Chemotherapy	PR	10
130	59/F	Soft tissue, skin, lymph nodes, brain	Chemotherapy/RT	PR	6
138	73/F	Soft tissue, lymph nodes	None	PR	15
125	58/F	Lymph nodes	None	Minor	56+
133	46/M	Lymph nodes	None	Mixed	6 (progression in spleen, continued response in lymph nodes)
135	52/M	Soft tissue, skin, GI tract	None	Mixed	15 (progression in GI tract but continued response in skin and soft tissue)

PR, partial response.

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