Am J Clin Oncol (CCT) 18(4): 307–312, 1995.

Phase I Study of Monoclonal Antibody-Ricin A Chain Immunoconjugate Xomazyme-791 in Patients with Metastatic Colon Cancer

P. M. LoRusso, D.O., P. L. Lomen, M.D., B. G. Redman, D.O., E. Poplin, M.D., J. J. Bander, M.D., and M. Valdivieso, M.D.

The immunoconjugate XMMCO-791/RTA consists of ricin A chain bound to a murine monoclonal antibody MoAb 791T. This monoclonal antibody (MoAb) binds to a glycoprotein of 72 kD, which is expressed on human colorectal carcinoma, ovarian carcinoma, and osteogenic sarcoma. XMMCO-791/RTA was tested in a Phase I trial with proposed dose escalation steps of 0.02, 0.04, 0.15, and 0.2 mg/kg per day. Twelve patients with metastatic colorectal carcinoma were treated at 0.02, 0.03, and 0.04 mg/kg per day dose levels administered over 1 hour on days 1–5. Study-related toxicities were hypotension (6 patients); greater than 10% weight gain (6 patients); peripheral edema (9 patients); fever (4 patients); confusion (3 patients); diarrhea (3 patients); proteinuria, as identified by dipstick (3 patients), greater than 0.6 mg/dl decrease in serum albumin (11 patients); greater than 25% decrease in oncotic pressure (10 patients), and a decrease in ionized calcium (8 patients). Six patients received a second course of treatment. HAMA levels developed in 9 patients and titers increased with number of courses administered. Decreased overall toxicity, in comparison to the first course, was noted, but one patient had an allergic-type response (hypotension, crushing chest pain, diaphoresis) after the test dose of the second course (HAMA level > 10,000 IgG). Life-threatening toxicity in the form of fluid shift, resulting in noncardiac pulmonary edema and third-spacing occurred after course 1 in 1 of 3 patients at the 0.04 mg/kg per day level. No further dose escalation was attempted and no antitumor activity was seen.

Key Words: Xomazyme 791—Monoclonal antibody—Immunoconjugate—Colorectal carcinoma.

The technology permitting the development of continuous cultures of fused cells secreting antibody of predefined specificity was described by Kohler and colleagues in 1975 (1). Since that time, hybridoma technology has provided monoclonal antibodies for a variety of purposes, including the diagnosis and therapy of malignancy in humans.

The concept of drug targeting through the use of monoclonal antibodies (MoAb) directed against human tumor-associated antigens is actively being investigated against a wide variety of human malignancies (2–5). In theory, coupling of a cytotoxic agent to a monoclonal antibody (MoAb) will allow specific delivery of the agent to the cancer cell with decreased toxicity to the normal cell lacking the antigen. One such moiety is the monoclonal antibody 791T/36 (MoAb 791T/36) coupled to ricin toxin A chain (Xomazyme-791, Xoma Corporation, Berkeley, California).

MoAb 791T/36 recognizes the gp72-kD antigen that is expressed on the tumor cells derived from ovarian, colorectal, and osteogenic sarcoma tissues (6–10). In diagnostic imaging studies, this MoAb bound to over 80% of colorectal and ovarian tumor cells (7–9). The ricin toxin A chain (RTA) is a ribosomal-inactivating protein, which exerts its cytotoxic action by enzymatically modifying the 28S ribosomal subunit, thereby inhibiting protein synthesis (11). RTA does not bind to cells, is not internalized efficiently, and is functionally inactive as a tumoricidal agent. However, by coupling RTA to MoAb 791T/36, RTA has the potential of being targeted to the tumor cells with resultant internalization and cytotoxicity (12). Preclinical studies confirmed cytotoxic activity against human colorectal carcinoma of the conjugate RTA/MoAb791T/36 (Xomazyme-791) (13), and a Phase I trial in patients with metastatic colorectal carcinoma was conducted

From the Divisions of Hematology/Oncology and Critical Care Medicine, Department of Internal Medicine, Wayne State University School of Medicine, Harper Hospital, Detroit, Michigan, and Xoma Corporation, Berkeley, California, U.S.A.

This work was supported in part by Xoma Corporation and NIH/NCI PO-1 #CA46560.

Address correspondence and reprint requests to Dr. Patricia Mucci LoRusso, Harper Hospital, Division of Hematology and Oncology, P.O. Box 02143, Detroit, MI 48201, U.S.A.

using immunoconjugate in which the ricin compound consisted of RTA_{30} and RTA_{33} (2).

The immunoconjugate used in this study contained only the less glycosylated isoenzyme of ricin toxic A chain, RTA_{30}. This immunoconjugate was developed in an effort to improve the pharmacokinetic properties of the compound, primarily to prolong serum residence time to improve tumor penetration.

MATERIALS AND METHODS

Immunoconjugate

The immunoconjugate Xomazyme-791 was prepared by coupling ricin A chain (RTA) to a monoclonal antibody XMMCO-791 that recognizes a 72-kD glycoprotein present on the surface of human sarcoma and ovarian and colorectal tumors. XMMCO-791 MoAb is of the IgG2b immunoglobulin subclass with kappa light chains. It was produced in the ascites of specific pathogen-free BALB/c mice and purified using standard procedures (data on file, Xoma Corporation). RTA (2 isoenzymes-RTA_{30} and RTA_{33}) is a polypeptide derived from castor beans that is known to inhibit ribosomal function. When coupled to a monoclonal antibody that binds to a cell surface antigen, it is internalized into the cell with resulting cell death. It is estimated that one molecule is sufficient to cause cell death.

Several preclinical studies of XOMAZYME-791 in mice xenografts showed an inhibition of growth of tumor cell lines (C170, HT29) with increase in survival time of animals. It was also found that the immunoconjugate consisting of XMMCO-791 antibody and isozyme RTA_{30} immunoconjugate in the tumor was threefold more XMMCO 791-RTA_{33} immunoconjugate. The ratio is similar to the ratio of serum residence times, thus increased residence time likely contributed to the increase in the amount of immunoconjugate delivered to the tumor (reference on file, Xoma Corporation).

Xomazyme-791 was supplied as a solution containing 1 mg/ml of the Xomazyme-791 immunoconjugate. The molecular weight of RTA is 30 and the molecular weight of the antibody is approximately 150. Thus, about 17% of the total molecular weight is contributed by RTA_{30}. The product was diluted 1:25 with normal saline before administration and filtered through a 0.22-μm low-protein binding filter (Millex-6v). The final dilution was administered from a Travenol intravenous pack.

Patient Profile and Methods

Adult patients with metastatic colorectal adenocarcinoma were eligible for this study. Pretreatment requirements included a Karnofsky performance status of $\geq$70%, a total bilirubin $<$ 2.0 mg/dl, a serum albumin of $\geq$3.0 g/dl, normal prothrombin and partial thromboplastin time, serum creatinine $<$ 1.5 mg/dl, granulocytes $>$ 1,500/mm^3, normal brain computed tomogram, presence of measurable disease, and no significant cardiac or pulmonary disease. No chemotherapy and/or radiation therapy could have been administered within 4 weeks of Xomazyme-791 administration. All patients were required to give informed consent prior to initiation of treatment.

Xomazyme-791 was administered as a 1-hour intravenous infusion daily for 5 consecutive days with a 16-day rest period (one 21-day cycle). On day 1 of each cycle, patients received an 80-μg IV bolus test dose. If no acute toxicities were noted with the test dose, the remaining portion of the calculated day 1 dose of XMMCO-791/RTA_{30} was infused. Based on animal studies, a starting dose of 0.02 mg/kg per day immunoconjugate for 5 days, with subsequent dose escalations to 0.04, 0.08, 0.15, and 0.2 mg/kg per day immunoconjugate were proposed. Three patients were to be enrolled at each escalating step until a maximum tolerated dose was reached. A total of 6 patients were scheduled for treatment at the maximum tolerated dose level. No intragroup, intrapatient, or intercycle dose escalation was permitted. To maximize potential patient benefits from tumor response, a two-cycle regimen was devised. A second cycle was initiated (toxicity permitting) on day 21 of cycle one. Daily weight, physical examinations, and laboratory evaluations were done, including complete blood count with differential, serum chemistry profile, direct and ionized calcium, urinalysis, and arterial oncotic pressures. Days 1 and 5 evaluation included protein electrophoresis to help identify components of protein loss during therapy, urinary electrolytes, and plasma hemoglobin to detect intravascular hemolysis. Serum samples for human anti-mouse antibody levels (HAMA) were drawn on days 0, 6, 15, and 21. Carcinoembryonic antigen (CEA) samples were drawn on days 1, 21, and 42. A baseline chest radiograph and electrocardiogram were done pretreatment.

The starting dose of Xomazyme-791 was 0.02 mg/kg per day. The second scheduled dose level was 0.04 mg/kg per day. After encountering unacceptable toxicity at the 0.04-mg/kg per day dose level, an intermediate dose of 0.03 mg/kg per day was selected as a potential maximum tolerated dose.

Standard tumor response criteria were used. Disappearance of all known lesions with development of no new lesions for a minimum of 4 weeks was judged a complete response. Partial response was defined as a reduction of at least 50% of the sum of the perpendic-

ular diameters of all indicator lesions lasting 4 weeks minimum without appearance of new lesions or progression of disease at other sites. Progression was defined as a ≥50% increase of the sum of the perpendicular diameters of all measurable lesions or the appearance of new lesions. Stable disease was defined as a decrease in tumor size from 0 to 50% or a steady state not qualifying for increasing disease of at least 8 weeks duration. To be evaluable for response, patients must have received one complete course of treatment defined as a 21-day cycle. Serum levels of CEA were monitored but not used in the definition of response.

RESULTS

Twelve patients with advanced, measurable, histologically proven metastatic colorectal adenocarcinoma were treated with Xomazyme-791. Ages ranged from 46 to 69 (median: 60). Median Karnofsky performance status was 80% (range: 70–100%). Previously, 11 patients were treated with 5-fluorouracil. Four patients had previously received radiation therapy in an adjuvant setting. No patient received prior immunotherapy. All patients had elevated serum carcinomembryonic antigen levels (range: 5.2–1978; median: 57 IU; normal: 0–2.5 for nonsmokers, ≤5.0 for smokers). All 12 patients were evaluable for response and toxicity.

In this study, 9 patients (75%) had liver metastasis, 5 patients (42%) had pelvic and retroperitoneal lymph node involvement, 6 patients (50%) had lung involvement, 4 patients (33%) had lung and liver involvement, and 4 patients (33%) had abdominal and pelvic disease (excluding lymph nodes).

Study-related toxicities were observed at all dose levels and appeared to be dose-dependent (Table 1). A decrease in blood pressure was gradual in onset, usually beginning on days 3 to 4. At 0.02 mg/kg per day maximum decrease in systolic blood pressure was 20–29 mmHg, occurring in 1 patient during the first course and 2 patients during the second course. Neither fluid challenge nor pressors were administered. At 0.04 mg/kg per day (the second step in dose escalation), 3 of 3 patients experienced a minimum of 20 mmHg decrease in systolic blood pressure, while 2 of 3 patients responded to intravenous fluids. The third patient developed profound, sustained, symptomatic hypotension requiring therapy with multiple pressor agents, albumin, and fluids. At 0.03 mg/kg per day, hypotension was noted in 3 of 6 patients. Fluid supplementation was given to these patients. Fluid administration was begun when systolic blood pressure consistently decreased more than 20 mmHg below the baseline reading. No patient, however, developed clinical symptoms related to their hypotension. Fluid administration was maintained until near normalization of systolic pressure, often requiring an additional day of hospitalization.

Dose-dependent changes were noted in serum albumin and total serum protein (Table 1). All patients had >3.0 mg/dl pretreatment serum albumin levels. At the 0.02-mg/kg per day dose, there was no significant decrease in serum albumin or protein. In the first cycle of 0.03 mg/kg per day, grade I to grade III hypoalbuminemia was noted in 4 of 6 patients, and 6 of 6 patients had a decrease of total serum protein. At the dose of 0.04 mg/kg per day, grades II-IV decrease of serum albumin and grades I-III decrease of total protein was noted in 3 of 3 patients. In patients who received a second course of therapy (0.02 and 0.03 mg/kg per day dose), there was neither hypoalbuminemia nor hypoproteinemia observed. Protein electrophoresis was done on patients treated at the 0.03 mg/kg/day dose level and did not demonstrate any specific subset contributing to the protein depletion. Dipstick of urine for protein ranged from 0 to 2+.

Coinciding with the hypoalbuminemia was an observed weight gain. Again, this was seen predominately with the first course of therapy (Table 1). At a dose of

TABLE 1. *Toxicities: number of patients (grade)*

Dose (mg/kg/day)[a]	N[b]	↓BP[c]	↓Albumin	↓Protein	↑Weight	Peripheral edema	Diarrhea	Neuromood	Drug fever	↓Calcium	
										Total	Ionized
0.02 (cycle 1)	3	1 (I)	0	0	3 (2–I; 1–II)	3 (1–II; 2–III)	0	0	0	1	0
(cycle 2)	3	2 (I)	0	0	0	0	0	0	0	0	0
0.03 (cycle 1)	6	3 (2–I; 1–IV)	4 (3–I; 1–III)	6 (4–I; 2–II)	5 (2–I; 1–II; 1–III; 1–IV)	6 (1–II; 2–III; 3–IV)	2 (II)	4 (I)	6 (1–I; 5–II)	4	4
(cycle 2)	3	1 (I)	0	0	1 (1–I)	3 (2–I; 1–II)	2 (II)	4 (I)	6 (1–I; 5–II)	2	0
0.04	3	3 (II, III, IV)	3 (II, III, IV)	3 (I, II, III)	3 (II, III, IV)	3 (IV)	1 (III)	2 (1–II; 1–III)	3 (2–II; 1–IV)	Not drawn	Not drawn

[a] Based on dose of immunotoxin.
[b] Number of patients treated.
[c] Blood pressure decrement.

0.02 mg/kg per day, grades I to II weight gain was seen in all three patients during the first cycle. At 0.03 mg/kg per day, grade IV toxicity was noted in the first cycle. At the 0.04 mg/kg per day dose level, grades II to IV weight gain was noted. In those patients receiving a second cycle of therapy, weight gain was less marked, again seemingly corresponding to serum albumin levels.

Significant peripheral edema was also noted and appeared to be dose-dependent (Table 1). Furosemide administration did not minimize the weight gain and, in fact, led to additional decrease of blood pressure. Gentle administration of diuretics in conjunction with albumin was of minimal benefit. Other dose-dependent toxicities included diarrhea, mood changes and fever (Table 1).

All patients had normal levels of ionized calcium at the onset of treatment. In those patients in whom ionized calcium levels decreased to borderline or below normal levels (Table 1), a decrease in systolic and diastolic blood pressure was also noted. The rest of the serum electrolytes remained stable and urinary electrolytes were noncontributory.

In several patients treated at the 0.03 mg/kg per day dose, serial arterial oncotic pressures were determined to identify a possible correlation between serum albumin levels and blood pressure alterations. Where measurements were available, we found a consistent downward trend in arterial oncotic pressure. A decrease in blood pressure paralleled a decrease in arterial oncotic pressure.

Blood urea nitrogen and creatinine were monitored closely and were unremarkable, except for one patient who developed severe hypotension at the 0.04 mg/kg per day dose level. This patient developed a clinical picture consistent with prerenal azotemia.

Human anti-murine antibody (HAMA) titers were available in all 12 patients pre- and posttherapy for course 1, and in 3 of 6 patients for course 2 (Table 2). In 3 of 12 patients, the pretreatment HAMA levels were slightly increased (Patients 8, 10, and 12). In Patient 8, it was not until the second course of therapy that a significant increase in HAMA level was noted, with an IgG HAMA rising to 2,000. IgM HAMA in this patient remained at baseline levels. HAMA levels measured after treatment in Patient 10 showed an increase of IgM-800 and IgG-1800. This patient was treated at the 0.04-mg/kg per day dose and demonstrated the least overall clinical toxicities of three patients treated at that level. In the third patient with high pretreatment HAMA levels (Patient 12), the titers did not increase after course 1. This patient had an intermediate level of clinical toxicities encountered by patients at the 0.04-mg/kg per day dose level. Overall, after course 1, HAMA levels rose in 8 of 12 patients tested. Three patients in whom HAMA were measured after course 2, demonstrated incremental increases in HAMA levels.

Patient 6 had a HAMA level of >10,000 after completion of course 1. Upon administration of the test dose for cycle 2, the patient had an acute episode of crushing chest pain, diaphoresis, hypotension, and tachycardia. Symptoms abated approximately 10 minutes after onset. Protocol therapy was discontinued in this patient.

No tumor response was observed. The dose-limiting toxicity appeared to be related to fluid shifts.

DISCUSSION

This Phase I study of Xomazyme-791 immunoconjugate in patients with metastatic colorectal cancer has evaluated clinical toxicities in patients treated at 0.02, 0.03, and 0.04 mg/kg per day for 5 days, with a repeat course at 21 days. Several study-related toxicities occurred in a dose-dependent fashion. As assessed by

TABLE 2. *HAMA levels*

Patient	Dose (mg/kg/day)	Pretherapy		Postcourse 1		Postcourse 2	
		1 gM	1 gG	1 gM	1 gG	1 gM	1 gG
1	0.02	<1/10	<1/10	0	2,200	0	3,800
2	0.02	<1/10	<1/10	0	710		
3	0.02	0	0	0	0		
4	0.03	0	0	10	500		
5	0.03	0	0	100	100		
6	0.03	0	0	130	>10,000	200	>10,000
7	0.03	0	0	0	0		
8	0.03	10	20	0	15	10	2,000
9	0.03	0	0	100	100		
10	0.04	130	0	800	1,800		
11	0.04	0	0	0	500		
12	0.04	0	100	0	40		

computed tomography scans of measurable disease, no signs of objective clinical response were seen in the 12 patients treated.

Fluid shifts resulting in noncardiac pulmonary edema and third-spacing was the principal dose-limiting treatment related toxicity. This toxicity may have a pathogenetic component of increased vascular permeability. The fluid shift and fluid retention most likely contributed to the hypotension and increased weight observed in several patients. It appears the pathophysiology of fluid shifting was similar to what is seen in a "capillary leak syndrome." The end result of "capillary leak syndrome" is third spacing of fluid and, if severe enough, progressive decline in systemic vascular resistance, followed by a decrease in mean arterial pressure, despite increases in cardiac output (changes similar to those seen in septic shock). Calcium supplementation therapy (calcium chloride) resulted in an increase of blood pressure with increasing levels of ionized calcium. We have also observed a similar phenomenon in association with high-dose IL-2 therapy (15). It is of interest to note that DeNardo and coworkers found a beneficial effect of increased vascular permeability in their preclinical models (Reference on file: XOMA Corporation). In their analysis, such an increase was credited with improved delivery of tumoricidal agent to the tumor.

Some clinical toxicity might be related to cross-reactivity of the XMMCO-791 antibody with normal human tissue. During pre-Phase I trials, XMMCO-791 showed a low level of reactivity with human tissues. It bound weakly and nonspecifically to human red blood cells and to macrophages. There was weak binding to granulocytes and to a proportion of phytohemagglutinin-stimulated lymphoblasts, which have been shown to express the gp72 antigen. There was no binding to progenitor cells or to normal lymphocytes. Immunoperoxidase staining demonstrated that XMMCO-791 bound to predominately noncellular stromal elements. The vascular endothelium of some capillaries and venules in varying tissues were weakly positive, but most of the venules were negative. XMMCO-791 showed positive reactivity with about 10% of the alveolar epithelium and macrophages in lung tissue in 2 of 4 autopsy specimens. There was very faint reactivity with less than 10% of the glomeruli in 1 of 4 kidneys tested. Other tissue that showed some reactivity were epithelium of the lumen of skin ducts and glandular epithelium of colon. In the fetal tissue that was tested, there was some reactivity in the alveolar and bronchial epithelium of the lung and tubule epithelium of the kidney (Reference on file: XOMA Corporation). Antigen shedding by cells binding immunoconjugate was not detected (Personal communication, Dr. Vera Byers).

In several patients who completed a second course of therapy, the degree of study-related toxicity was not as pronounced as in course 1. Weight loss, hypoalbuminemia, hypocalcemia, and hypotension were all attenuated (Table 1). The reasons for this observation are unclear, although we can postulate that HAMA response causes a more rapid clearance through formation of antibody/immunoconjugate complexes decreasing the likelihood of immunoconjugate-induced clinical toxicities (16).

An earlier Phase I dose escalation study was carried out in which 17 patients with metastatic colorectal cancer were treated with doses of immunotoxin Xomazyme-791 ranging from 0.02 to 0.2 mg/kg per day in a 1-hour intravenous infusion for a 5-day course (2). In this study, the ricin A immunotoxin contained two isoenzymes (molecular weight: 30 and 33; RTA_{30} and RTA_{33}). There was no documented response, although there was some suggestion of tumor regression (drop in CEA levels, decreased size of lesions on CT scans, chest radiograph, and physical examination) in 5 of the 17 patients treated (2). Side effects in the previous study included a composite of signs and symptoms thought to be generic to RTA immunotoxin, including decreased serum albumin, weight gain with secondary peripheral edema, elevated temperatures, asymptomatic proteinuria, mild fatigue, and reversible mental status changes (2). There was no life-threatening toxicity noted.

Pharmacokinetics done in a preclinical (mice) study have determined the half-life alpha phase of the RTA_{30} immunoconjugate to be 0.7 hours and the beta phase half-life to be 20.4 hours. In the previously published Phase I study of Xomazyme-791, attempts were made to measure serum concentrations of 791/RTA (2). The assay available was not sensitive enough to detect serum levels. Since the study reported here used one-tenth of the dose used in the first Phase I trial, no attempt was made to measure serum levels. Hence no definitive correlation between serum levels in the responding animals versus nonresponding patients can be made.

It is of interest to note that 3 of the 12 patients had elevated pretreatment IgG or IgM HAMA levels. There was no obvious reason for this phenomenon. Several patients developed elevation of HAMA titers postinfusion, but levels did not correlate with the type or severity of adverse effects observed during the study.

In this Phase I study using Xomazyme-791 immunoconjugate in patients with metastatic colorectal carcinoma, it was found that at a dose of 0.03 mg/kg per day $\times$ 5 days, the compound has clinically acceptable side effects. Even though we saw no objective tumor response, Phase II studies should be considered to define therapeutic utility of this immunoconjugate in

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.