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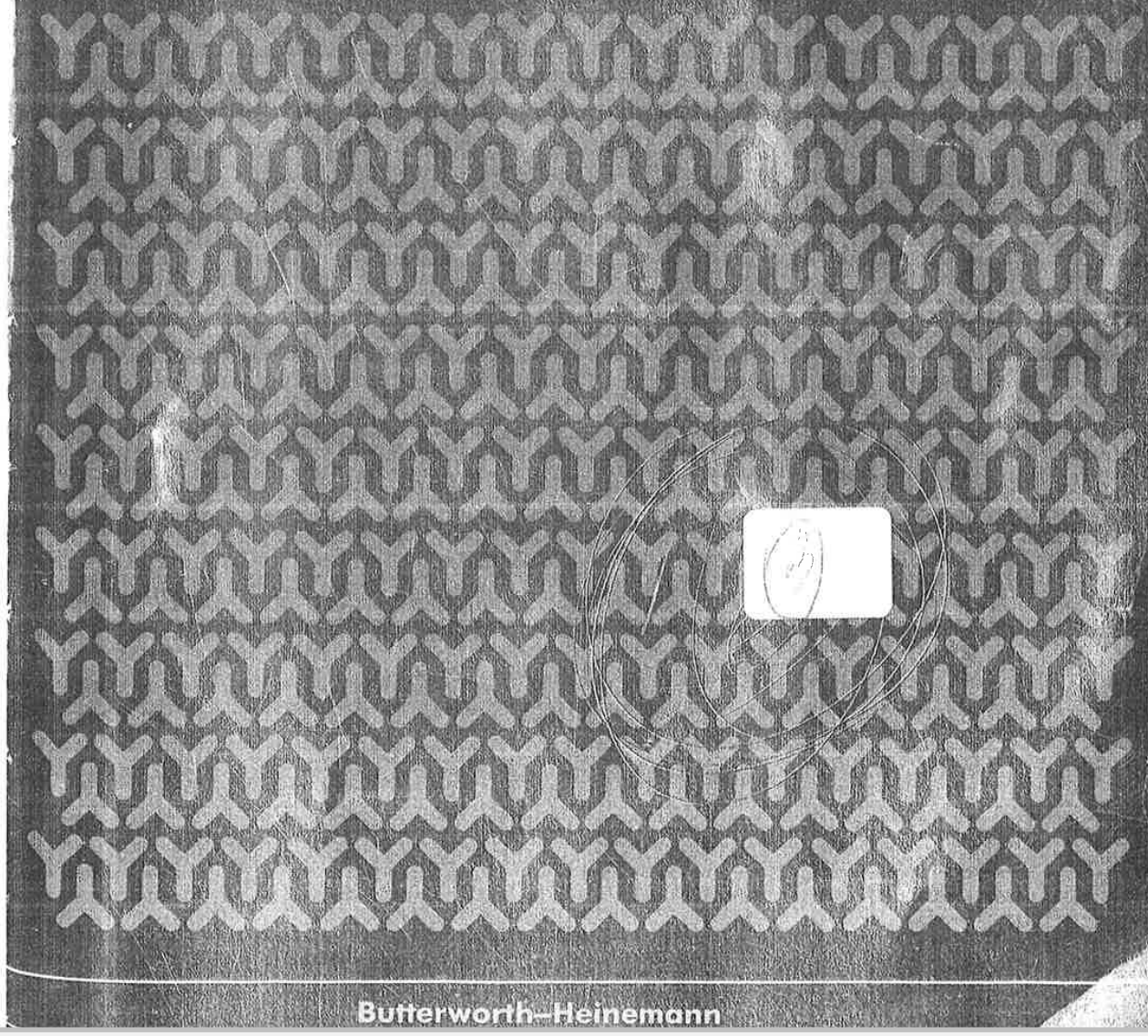

Butterworth–Heinemann

Single-dose murine monoclonal antibody ricin A chain immunotoxin in the treatment of metastatic melanoma: a phase I trial

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To determine the maximally tolerated dose of a ricin A chain-conjugated antimelanoma antibody (XomaZyme-Mel), 20 patients with metastatic melanoma were treated with escalating doses of the murine immunotoxin given as single intravenous infusion over 30 minutes. The starting dose was 0.6 mg/kg and was escalated in five groups to a maximum of 1.6 mg/kg. The maximally tolerated dose was 1.25 mg/kg as three of six patients treated at 1.6 mg/kg developed unacceptable toxicity. The dose-limiting toxicity consisted of profound fatigue, myalgias, and arthralgias. These occurred within 4 days and resolved in 7 to 10 days. Other non–dose-limiting toxicities encountered consisted of hypoalbuminemia, weight gain, peripheral edema, mild hypotension, and flu-like syndrome; the severity of these was also dose related. In addition, two allergic reactions occurred, one severe. There was one durable complete response of 12+ months' duration and one brief mixed response lasting 3 months. We conclude that the maximum tolerated single dose of XomaZyme-Mel is 1.25 mg/kg. Phase I studies evaluating 1.25 mg/kg given in multiple doses at 2- to 4-week intervals and phase II studies to determine the response rate of a single 1.25 mg/kg dose are warranted.

Keywords: Melanoma; monoclonal antibody; immunotoxin

Introduction

The incidence of malignant melanoma is increasing steadily. Among the white population in California and Australia, there are at least 15 cases per 100,000 individuals, with double that rate in Hawaii.[1] Approximately 28,000 new cases were expected in the United States during 1990. At the present rate of increase it is estimated that by the year 2000, one of every 100 white individuals in the United States will develop malignant melanoma.[2] Surgery has been successful in the early treatment of the primary tumor, but in its disseminated form melanoma has been poorly responsive to most conventional types of therapy. With this in mind, there is an obvious need for newer modalities of treatment to be used separately or in combination with current strategies.

The development of hybridoma methodology has greatly increased our ability to detect and characterize tumor-associated antigens. Monoclonal antibodies (MoAbs) have been used as therapeutic agents in a variety of malignancies,[3–12] including melanoma.[13–18] A number of melanoma-associated antigens have been described. The most common are high molecular weight cytoplasmic and membrane-bound antigens,[19–22] p97,[22,23] and the melanoma-associated gangliosides GD2, GM2, 9-0-acetylated GD3, and GD3.[13,14,24,25]

The MoAb L72, directed to the ganglioside GD2, has been used for intralesional therapy of cutaneous nodules resulting in regression of a significant number of treated nodules.[13] Systemically, murine MoAbs recognizing high molecular weight antigens, the p97 melanoma-associated antigen, and the ganglioside GD3 have been administered intravenously in escalating doses with no response.[15–18] The antiganglioside GD3 MoAb R24, an IgG3, has been associated with three partial and two mixed responses in 14 patients treated in this manner,[16] possibly because of more efficient

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mediation of complement and cell-mediated lysis of antibody-coated tumor cells. This highlights a possible limitation to the effectiveness of MoAbs: the finite capacity of the immune effector system to eliminate antibody-coated tumor cells resultant from immunosuppression due to prior treatments or inherent in the disease process itself.

Because meaningful responses are unlikely to be achieved with naked MoAbs, conjugates with radioisotopes or toxins have been developed. With regard to melanoma radioimmunotherapy, Larsen *et al*[26] administered therapeutic doses of ^{131}I-labeled Fab' (50 to 150 mCi) fragments of anti-p97 MoAb, with stabilization of disease in two patients and a partial response in a third. In the area of immunotoxins, Spitler *et al.*[27] conducted a phase I trial with murine MoAbs coupled to the ricin A chain. Doses ranged from 0.01 mg/kg/d for 5 days to 1 mg/kg/d for 4 days. Encouraging clinical results were observed and toxicity was acceptable. Based on these data we implemented a phase I trial of XomaZyme-Mel in metastatic melanoma with the goal of determining the maximum tolerated dose (MTD) and of gaining additional information regarding efficacy.

Methods

Monoclonal antibody

For the generation of the hybridoma, the hybridization, cloning, and recloning were performed according to the conventional procedure described by Kohler and Milstein[28] with minor modifications.[29] BALB/c mice were immunized with cultured human melanoma cells. The spleen cells were harvested and fused with the 8-azaguanine-ristant murine myeloma line P3-X63-Ag8 in the presence of polyethylene glycol. The cells were cultured overnight, and resuspended in medium containing hypoxanthine, aminopterin, and thymidine. They were then cloned. Hybridomas secreting antibodies with the appropriate specificity were subcloned twice by limiting dilution using BALB/c splenocytes as feeder cells.

The monoclonal antibody used in the preparation of the immunotoxin was produced from murine ascites and was purified by XOMA Corporation (Berkeley, CA, USA) using a staphylococcal protein A column with elution at pH 3.5. It is an IgG2a antibody and reacts with melanoma-associated antigens having molecular weights of 220,000 and over 500,000. The hybridoma and the monoclonal antibody have been fully characterized in accordance with guidelines proposed by the Food and Drug Administration in "*Points to Consider in the Production of Monoclonal Antibody Products for Human Use.*" On frozen sections, the antibody shows minimal reactivity with normal tissues except for vascular endothelium, in which the reactivity appears to be cytoplasmic. The antibody also cross-reacts with nevus cells. The purified antibody contains neither virus nor parental hybridoma DNA or RNA.[30] Both the hybridoma and the purified antibody are free of other murine viral contaminants as determined by the mouse antibody production test.

Ricin toxin A chain

The ricin toxin A chain (RTA) was purified from castor beans by a series of column-based separations, including immunoaffinity chromatography.[31] The RTA was greater than 95% pure, as judged by SDS-PAGE, and contained no detectable ricin or ricin toxin B chain by any assay including immunoprecipitation. The IC_{50} level of the purified RTA as measured by a reticulocyte lysate assay[31] was less than 10 pM. This assay measures inhibition of protein synthesis in a cell-free system. In a mouse toxicity assay, RTA injected into BALB/c mice at 10 mg/kg produced no deaths.

XomaZyme-Mel immunotoxin

The immunotoxin, consisting of the murine monoclonal antimelanoma antibody conjugated to ricin A chain, is produced by XOMA Corporation. The conjugation technique has been described in detail elsewhere.[32] The immunotoxin 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. Preclinical testing demonstrated binding specificity of the immunotoxin similar to that of the unmodified antibody and cytotoxic antimelanoma activity both *in vitro* and *in vivo*.

Patient population and treatment plan

Patients entered on this study met the following criteria: age greater than 18 years, histologically documented melanoma, measurable metastatic lesions beyond regional lymph nodes, life expectancy of at least 3 months, performance status greater than 80% (Karnofsky), leukocyte count higher than 4,000/dl, platelet count higher than 150,000/dl, serum albumin higher than 3.5 g/dl, hematocrit higher than 30% with no history of blood transfusions for 3 weeks prior to treatment, creatinine less than two times normal, bilirubin less than two times normal, and written informed consent approved by the institutional review board. Patients were excluded for the following reasons: brain metastases, chemotherapy or biologic agents within 4 weeks of study entry, nitrosoureas within 6 weeks prior to study, anticoagulation therapy, pregnancy or lactation, history of prior therapy with murine antigens or known allergy to murine antigens, significant heart disease defined as uncontrolled arrhythmias, congestive heart failure, and uncontrolled angina or myocardial infarction within 6 months of study entry.

The treatment plan called for a single intravenous infusion of the immunotoxin over 30 minutes. The starting dose was 0.6 mg/kg and was escalated in stepwise fashion by 25% until the MTD was determined. Prior to administration of the drug, a skin test was performed during which 0.1 ml containing 10 μg of immunotoxin (diluted with normal saline) was injected

intradermally and the patient was observed for 30 minutes. The skin test was considered negative if the resultant wheal was less than 5 mm; an intravenous challenge dose of 0.2 mg to 0.4 mg was then administered. If no reaction occurred after 30 minutes, the full dose diluted in normal saline was administered as a slow intravenous infusion over 30 minutes under close medical supervision. The patients were observed for 24 hours and were then assessed for toxicity using a standard toxicity scale based on World Health Organization and criteria at days 4 to 5 and 7 to 8.

The MTD was defined as that dose level which did not produce grade IV toxicity (excluding allergy). Tumor response was assessed at days 14, 28, and 60.

A complete response was defined as the disappearance of all detectable disease for at least 28 days, partial response as the average reduction of the product of the largest diameter and its perpendicular of each tumor mass by at least 50% for at least 28 days as measured on clinical examination or radiographic studies, no response as average decrease of measurable disease by less than 50% for at least 28 days, and progressive disease as any measurable increase in tumor size or the appearance of new lesions. Duration of response was measured from the date of first documentation of response to the date of disease progression, death, or last patient contact. Survival was calculated from the date of starting the first cycle of therapy until the date of death or last patient contact. If patients responded and subsequently relapsed they were eligible to receive a second course of treatment as long as they met the entry criteria.

Results

Twenty eligible patients were registered prior to treatment and received a total of 22 courses of treatment on this study. Patient characteristics are listed in *Table 1*. There were 13 men and seven women. The median age was 46 years (range, 24 to 69 years). All but three patients were previously treated, usually with immunotherapy, chemotherapy, or combined modalities.

All 20 patients are evaluable for toxicity. Acute side effects are detailed in *Table 2*. The frequency and severity was generally dose related. Six patients were treated at the 1.6 mg/kg dose level, and three developed dose-limiting toxicity manifested as grade IV fatigue (reduction of patients activity to <25%). Also noted at this dose level were two episodes of grade III toxicity: fatigue (reduction of activity to 25% to 50% of

Table 1. Patient characteristics

Patient No.	Sex	Age (yr)	Sites of metastases	Performance status	Dose level (mg/kg)	Prior therapy	Maximum weight increase (% total body weight)	Albumin nadir (% change)	Maximum temperature (°C)
1	F	41	Lung	100	0.6	Imuvert	11.6	4.2	36.8
2	M	34	SQ	100	0.6	IFN, IL-2/LAK	13.9	2.8	36.4
3	F	30	LN	100	0.8	IFN	33.3	6.7	38.1
4	M	58	SQ, LN, lung	100	0.8	RT	25.6	ND	37.4
5	F	41	SQ	100	0.8	BCG, ARA-C, CDDP, Imuvert	18.4	5.8	38.2
5b	F	41	SQ	100	0.8	BCG, ARA-C, CDDP, Imuvert	15.9	ND	38.7
6	F	24	SQ	100	1.0	IFN	14.2	6.1	37.3
7	M	69	LN, lung	100	1.0	None	26.6	3.9	37.6
8	F	45	SQ	100	1.0	IFN, BCG	24.3	8.9	37.4
9	M	36	Brain, SQ, Bowel, lung	80	1.0	RT, Vinblastine/CDDP, IFN	30.9	ND	37.5
10	M	58	SQ, LN	100	1.25	None	29.4	3.4	37.4
11	F	46	SQ, lung	90	1.25	Piritrexim, Imuvert	23.4	3.6	39.1
11b	F	46	SQ	90	1.25	Piritrexim, Imuvert	—	—	—
12	M	57	SQ, LN	100	1.25	None	35.2	6.6	37.8
13	M	49	SQ, lung	100	1.25	DTIC, IL-2, IFN, RT	14.2	2.2	37.5
14	M	37	SQ, LN, lung	100	1.25	DTIC	15.3	4.9	38.5
15	M	50	Lung	100	1.6	IFN, Imuvert, combination chemo	34.2	6.7	38.0
16	F	36	SQ, LN	100	1.6	Imuvert, Piritrexim, combination chemo, BCG	21.4	10.9	37.5
17	M	35	SQ, LN, lung	100	1.6	IFN, combination chemo	23.5	7.7	38.1
18	M	69	Lung, LN	100	1.6	IL-2/IFN/ACT-D, TNF, CDDP	24.4	4.1	37.7
19	M	60	SQ, lung, kidney	100	1.6	IL-2/LAK, carboplatin, RT	34.9	5.7	37.5
20	M	54	Lung	100	1.6	BCG, IL-2/IFN	29.4	1.7	38.4

Abbreviations: SQ, squamous; LN, lymph node; IFN, interferon; IL-2/LAK, interleukin-2/lymphocyte-activated killer cells; RT, radiation therapy; BCG, bacille Calmette-Guérin; ARA-C, cytosine arabinoside; CDDP, cisplatin; DTIC, dacarbazine; combination chemo, dacarbazine, cisplatin, BCNU, and tamoxifen; ACT-D, actinomycin D; TNF, tumor necrosis factor.

Table 2. Toxicity of XomaZyme-Mel at various dose levels

	Dose level (mg/kg)																				
	0.6				0.8				1.0				1.25				1.6				Total	
Toxicity grade	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	N	%
Fatigue	1	—	—	—	1	—	1	—	1	2	—	—	—	1	2	—	—	1	1	3	14	70
Arthralgias/myalgias	2	—	—	—	1	1	—	—	1	1	—	—	—	2	—	—	—	3	1	—	12	60
Weight gain	—	—	—	—	1	—	—	—	2	—	—	—	—	1	—	—	3	—	—	—	7	35
Hypoalbuminemia	—	—	—	—	3	—	—	—	3	—	—	—	1	1	—	—	4	1	—	—	13	65
Proteinuria	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	4	1	—	—	6	30
Fever	—	—	—	—	2	—	—	—	—	—	—	—	1	1	1	—	4	—	—	—	9	45
Allergy	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	2	10
Total	3	—	—	—	8	1	1	1	8	3	—	—	4	5	3	—	15	6	2	3		

normal) and arthralgias/myalgias (requiring narcotics). The mean serum albumin fell from 4.10 ± 0.42 g/dl before treatment to a nadir of 3.11 ± 0.46 g/dl. This was associated with a median weight gain of 5.3% ± 1.9% of total body weight, usually with peripheral edema and signs of mild hypovolemia such as tachycardia and a modest decrease in blood pressure. Serum creatinine did not change significantly from 0.89 ± 0.19 mg/dl pretreatment to 1.05 ± 0.39 mg/dl posttreatment. Clinically relevant proteinuria was not observed. These effects usually occurred within 4 days of drug administration and gradually resolved over the course of the next 7 to 10 days.

Three patients also experienced fever (>38.5°C) to a maximum of 39.1°C and other constitutional symptoms such as anorexia but these were usually mild and self-limited. There was no evidence of hematologic toxicity or coagulopathy. Likewise, liver enzymes, urinalysis, cardiac enzymes, and serial electrocardiograms remained stable.

Two patients suffered allergic reactions. At the completion of the infusion, one patient (no. 7, *Table 1*) developed a rash, watery eyes, and swollen tongue, which responded to diphenhydramine. Another patient (no. 4, *Table 1*) had a 9-mm wheal on skin testing and a negative intravenous challenge. After beginning the dose of immunotoxin, the patient became flushed, nauseated, and light-headed. Blood pressure dropped to 54/0. This quickly responded to interruption of the infusion, epinephrine, hydrocortisone, and diphenhydramine.

All 20 patients were evaluable for response. Fifteen progressed within 60 days and were removed from study. The remaining five patients had a response or stable disease lasting at least 60 days. Two of these (nos. 5 and 11, *Table 1*) had objective responses. Patient no. 5 had a mixed response at day 60 and was retreated at the same dose level at day 95. By day 35 of the second course, this patient had progressed and was removed from study. Patient no. 11 developed complete disappearance of lung and subcutaneous metastases gradually over the course of 1 year without further treatment. It was then felt that this patient had relapsed at a solitary subcutaneous site and was retreated at the same dose level. However, the patient continued to complain of pain; a fine needle aspirate of the suspected relapse was performed and resulted positive for melanoma. This was resected 4 months after retreatment. There was no evidence of melanoma in the surgical specimen and the patient remains well and free of disease 24 months after initial treatment. The remaining three responding patients progressed at 3, 4, and 8 months.

Discussion

We have studied the administration of a single dose of murine monoclonal antibody conjugated with RTA in patients with metastatic melanoma. Patients were treated at five dose levels and data suggest that 1.25 mg/kg represents the MTD. Higher doses cause severe fatigue and myalgias/arthralgias in the majority of patients, both of which are reversible. The spectrum of toxicities encountered also includes flu-like syndrome and hypoalbuminemia with weight gain, tachycardia, and mild hypotension suggesting vascular leak. No bone marrow, neurologic, cardiac, hepatic, or renal toxicity was encountered. All of the side effects appeared to be dose related. Two additional patients developed allergic reactions, one severe.

Although the MTD of 1.25 mg/kg is somewhat subjective, we believe this is appropriate for the outpatient setting. Higher doses could be administered with the intent of admitting patients to intensive care units. Further studies to evaluate the effect of 1.25 mg/kg of XomaZyme-Mel in multiple doses every 2 weeks are warranted.

Most patients in the present study were heavily pretreated, and only a single dose of immunotoxin was used. Despite this there was an objective response, a mixed response, and three additional patients with stable disease for 60 days or longer. The proposed mechanism of action of this immunotoxin is the delivery of RTA into the ribosome with interruption of protein synthesis and cell death. Improvement of the antimelanoma activity might be possible by increasing deliv-

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