The Journal of NUCLEAR MEDICINE

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J Nucl Med. 1999;40:2014-2020.

The Journal of Nuclear Medicine is published monthly.
SNMMI | Society of Nuclear Medicine and Molecular Imaging
1850 Samuel Morse Drive, Reston, VA 20190.
(Print ISSN: 0161-5505, Online ISSN: 2159-662X)

SNMMI SOCIETY OF NUCLEAR MEDICINE AND MOLECULAR IMAGING

A Clinical Trial of Radioimmunotherapy with ^{67}Cu-2IT-BAT-Lym-1 for Non-Hodgkin's Lymphoma

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Encouraged by the results of ^{131}I-Lym-1 therapy trials for patients with B-cell non-Hodgkin's lymphoma (NHL), this phase I/II clinical trial of ^{67}Cu-2IT-BAT-Lym-1 was conducted in an effort to further improve the therapeutic index of Lym-1-based radioimmunotherapy. Lym-1 is a mouse monoclonal antibody that preferentially targets malignant lymphocytes. ^{67}Cu has beta emissions comparable to those of ^{131}I but has gamma emissions more favorable for imaging. The macrocyclic chelating agent 1,4,7,11-tetraazacyclotetradecane-N,N′,N″,N‴-tetraacetic acid binds ^{67}Cu tightly to form a stable radioimmunoconjugate in vivo. **Methods:** All 12 patients had stage III or IV NHL that had not responded to standard therapy; 11 had intermediate- or high-grade NHL. At 4-wk intervals, patients received up to four doses of ^{67}Cu-2IT-BAT-Lym-1, 0.93 or 1.85–2.22 GBq/m^2 (25 or 50–60 mCi/m^2), with the lower dose used when NHL was detected in the bone marrow. **Results:** ^{67}Cu-2IT-BAT-Lym-1 provided good imaging of NHL and favorable radiation dosimetry. The mean radiation ratios of tumor to body and tumor to marrow were 28:1 and 15:1, respectively. Tumor-to-lung, -kidney and -liver radiation dose ratios were 7.4:1, 5.3:1 and 2.6:1, respectively. This ^{67}Cu-2IT-BAT-Lym-1 trial for patients with chemotherapy-resistant NHL had a response rate of 58% (7/12). No significant nonhematologic toxicity was observed. Hematologic toxicity, especially thrombocytopenia, was dose limiting. **Conclusion:** ^{67}Cu remains an option for future clinical trials. This study established ^{67}Cu-2IT-BAT-Lym-1 as a safe, effective treatment for patients with NHL.

Key Words: antibody; ^{67}Cu; immunotherapy; lymphoma; radiotherapy

J Nucl Med 1999; 40:2014–2020

Despite effective chemotherapy, more than one half of patients with non-Hodgkin's lymphoma (NHL) are not cured, a fact made even more significant given that the incidence of NHL is increasing by 3%–4% per year—50% over the last 15 y (*1*).

Radioimmunotherapy (RIT) is systemic anticancer therapy that uses a monoclonal antibody (MAb), such as Lym-1, to deliver cytotoxic radionuclides specifically to tumors, thus relatively sparing normal tissue. NHL is responsive to external beam radiation therapy, but control of local and regional disease is not necessarily associated with prolonged survival, because NHL is most commonly a systemic disease and progresses outside local radiation fields. RIT has proven effective against NHL (*2–4*), in part because abundant target antigens on malignant lymphocyte cell membranes can be bound by the radiolabeled MAb, even when NHL is disseminated throughout the body.

Lym-1 is a mouse MAb that preferentially targets a discontinuous epitope on the human leukocyte antigen (HLA)-DR10 β subunit expressed on the surface membrane of most B-cell NHLs (*5*). Unmodified Lym-1 had little effectiveness against hematologic malignancies in a phase I clinical trial (*6*); however, ^{131}I-Lym-1 showed promise (*7*). Subsequently, clinical trials were performed to define the safety, toxicity and efficacy of a series of ^{131}I-Lym-1 doses given 2–6 wk apart. In a low-dose trial of ^{131}I-Lym-1, tumor regression occurred in 25 of 30 patients (83%) and 17 (57%) had durable remissions, including 3 complete responses (CRs) (*8*). In a maximum tolerated dose (MTD) trial of ^{131}I-Lym-1, 10 (71%) of 14 entries that received at least two doses of ^{131}I-Lym-1 and 11 (52%) of 21 total entries responded, including 7 CRs (*2*). All three entries in the 3.7-GBq/m^2 cohort had durable CRs. Eighteen (85%) of the 21 patient entries had appreciable tumor regression. Overall, 10 patient entries (19%) had a CR, with a median duration of 8.5 mo. The overall response rate, CRs plus partial responses (PRs), was 56%. A time-dependent proportional hazards model in a multivariate analysis that adjusted for risk factors conclusively showed that response to ^{131}I-Lym-1 therapy was associated with improved survival (*9,10*). Thrombocytopenia was dose limiting and the primary manifestation of radiation-induced myelosuppression (*11*).

After studies with ^{131}I-Lym-1 were complete, methods to further improve RIT were undertaken. The macrocyclic chelator 1,4,7,11-tetraazacyclotetradecane-N,N′,N″,N‴-tetraacetic acid (TETA) was developed to stably bind ^{67}Cu (*12*), thus facilitating its use for RIT (*13,14*). ^{67}Cu emits

Received Sep. 10, 1998; revision accepted May 4, 1999.

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abundant β particles of moderate energy and gamma photons useful for imaging and dosimetry studies but not in too great an abundance to preclude outpatient therapy with relatively high ^{67}Cu doses (*14*). In preclinical studies, ^{67}Cu-labeled MAbs have been shown to deliver higher radiation doses to tumors and higher tumor-to-nontumor dose ratios than their radioiodinated counterparts (*15*). Data from our MTD trial of ^{67}Cu-2IT-6-[p-(bromoacetamido)benzyl]-TETA (BAT)-Lym-1 showed that ^{67}Cu has a long residence time in tumors (a characteristic of radiometals), but ^{67}Cu has no pathway for incorporation into bone, as does ^{90}Y (*14*).

Encouraged by the results of ^{131}I-Lym-1 clinical trials, preclinical studies of macrocycle chelated ^{67}Cu and the MTD trial of ^{67}Cu-2IT-BAT-Lym-1, a phase II therapy trial of ^{67}Cu-2IT-BAT-Lym-1 was conducted. This open label, multiple dose study was intended to determine the efficacy and toxicity of ^{67}Cu-2IT-BAT-Lym-1 when given to B-cell NHL patients who had failed to respond or had relapsed after standard therapy.

MATERIALS AND METHODS

Patient Characteristics

All patients (8 men, 4 women; mean age 51 and 57 y, respectively; combined mean age 53 y) had progressive disease after at least one regimen of chemotherapy (mean 2.6 regimens, range 1–5), and each had received at least one course of an anthracycline-containing regimen before RIT (Table 1). The condition of 3 patients had failed to respond to high-dose chemotherapy with peripheral blood stem cell (PBSC) support. Eleven of 12 patients (92%) had intermediate- or high-grade NHL according to the Working Formulation for Clinical Usage (*16*). Eight patients had stage IV and 4 patients had stage III NHL. Six patients had malignant involvement of the bone marrow. Eight patients had elevated lactate dehydrogenase (LDH) values that were normalized as the percentage greater than the upper limit of each patient's institutional normal value.

Antibody

Lym-1 (Techniclone, Inc., Tustin, CA) is an IgG_{2a} mouse MAb with high affinity against a discontinuous epitope on the HLA-DR10 β subunit antigen on the surface membrane of malignant B lymphocytes (*5*). The antibody-antigen complex is not internalized or released in Raji cell cultures in vitro (*5*); the avidity constant of Lym-1 for its antigen on tumor cell lines is 4.02×10^8/mol. Lym-1 displays antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity against Raji human lymphoma cells in vitro (*17*). Lym-1 was specified as greater than 95% monomeric IgG by polyacrylamide gel electrophoresis and met Food and Drug Administration (FDA) mouse MAb production guidelines for murine virus, mycoplasma, fungus, bacterial contamination, endotoxin, pyrogen, deoxyribonucleic acid content and general safety testing in animals.

Preparation of Agent

The preparation of BAT and its conjugation to Lym-1 through 2-iminothiolane (2IT) (Sigma Chemical Co., St. Louis, MO) has been described previously (*12,18*). The result is the immunoconjugate 2IT-BAT-Lym-1. The mean number of TETA chelating groups conjugated per antibody was measured by metal binding assay to range from 3.7 to 5.8; ratios associated with little or no effect on the function or biodistribution of Lym-1 (*18*). ^{67}Cu (Brookhaven National Laboratory, Upton, NY; and Los Alamos National Laboratory, Los Alamos, NM) was buffered with ammonium citrate and added to 2IT-BAT-Lym-1. The radiolabeling solution was incubated for 30–60 min at room temperature. Na_2 ethylenediaminetetraacetic acid (EDTA) was added to a final concentration of 10

TABLE 1
Patient Characteristics, ^{67}Cu and Radiation Doses and Response

Patient										
No.	Age (y)	Sex	NHL	Bone marrow*	LDH†	GBq/m²/dose	RIT doses (no.)	Total ^{67}Cu (GBq)	Total dose to tumors (Gy) (range)	Response
1	58	F	FSC	+	N	2.22	4	15.73	54.2–70.0	CR
2	51	F	DLC	–	7	2.22	2	5.99	10.1–29.6	PR
3	37	M	FLC	+	N	2.22	1	5.03	6.1–12.7	PR
4	64	F	DSC	–	N	1.85‡	2	5.03	11.4–17.2	PR
5	37	M	DLC	–	44	1.85	1	4.07	5.8–11.3	NR
6	62	M	DSC	–	11	1.85	1	3.89	7.0–16.5	NR
7	51	M	DM	–	21	1.85	1	3.52	9.1–10.7	PR
8	37	M	DLC	–	31	1.85	1	2.78	4.0–4.5	NR
9	58	M	MC	+	N	0.93	3	5.92	3.2–10.3	PR
10	73	M	LCI	+	136	0.93	2	3.77	6.5–7.5	PR
11	55	M	DSC	+	70	0.93	1	1.70	NA	NR
12	54	F	DLC	+	611	0.93	1	1.59	0.6–2.6	NR

*Bone marrow positive or negative as judged on bilateral bone marrow biopsy. Patients 4–12, treated in phase II part of study, were triaged to 1.85 GBq/m² (50 mCi/m²) if no non-Hodgkin's lymphoma (NHL) was detected in bone marrow and 0.93 GBq/m² (25 mCi/m²) if NHL was detected in bone marrow before radioimmunotherapy (RIT).

†Serum lactate dehydrogenase (LDH) value divided by upper limit of institutional normal (N) × 100.

‡First dose was 1.85 GBq/m² and second dose was reduced per protocol because of myelotoxicity.

FSC = follicular small cleaved; CR = complete response; DLC = diffuse large cell; PR = partial response; FLC = follicular large cell; DSC = diffuse small cleaved; NR = no response; DM = diffuse mixed; MC = mantle cell; LCI = large cell immunoblastic.

mmol to complex nonspecifically bound metal ions. ^{67}Cu-2IT-BAT-Lym-1 was purified from ^{67}Cu-EDTA, transferred to saline by G-25 molecular sieving gel chromatography (Sigma, St. Louis, MO) and formulated at 0.037 GBq (1 mCi/mL) in 4% human serum albumin in saline (*19*).

The quality of the radioimmunoconjugate preparations was assessed by cellulose acetate electrophoresis (CAE) and molecular sieving high-performance liquid chromatography (HPLC) as previously described (*20*). Immunoreactivity of ^{67}Cu-2IT-BAT-Lym-1 was assessed by solid-phase radioimmunoassay against partially purified Raji cell homogenates (*20*). For the 15 preparations of ^{67}Cu-2IT-BAT-Lym-1 used for 22 patient doses, the mean ± SD of ^{67}Cu associated with monomeric immunoconjugate by HPLC and CAE was 99% ± 1.5% and 99% ± 1.3%, respectively, and the immunoreactivity was 80% relative to unmodified Lym-1.

Study Design

Adult patients were eligible if their malignant tissue reacted with Lym-1, serum was negative for human anti-mouse antibody (HAMA), liver function tests were less than two times the upper limit of normal, Karnofsky performance score was at least 70% and absolute neutrophil count and platelet count were at least 1500/mm^3 and 100,000/mm^3, respectively. Patients received no other cancer therapy for at least 4 wk, had no evidence of a second neoplasm and had measurable disease at the time of study entry. Patients with greater than 25% of the marrow replaced by NHL were ineligible. Lymphomatous tissue was obtained to determine reactivity with Lym-1. Tissue was placed in media and prepared for flow cytometric analysis; additional fresh tissue was put on ice and underwent immunohistologic evaluation within hours or was immediately frozen for later evaluation. Twenty percent of the cells were required to be Lym-1 reactive, although percentage reactivity did not predict clinical response to radiolabeled Lym-1 (*10*). Before therapy, all patients signed an informed consent for protocols approved by the University of California, Davis, human subjects and radiation use committees under an investigational new drug authorization from the FDA. Patients were treated in an outpatient treatment center. Patients were treated with another dose of ^{67}Cu-2IT-BAT-Lym-1 when toxicities had returned to grade I or better, if NHL was not progressing and serum was negative for HAMA (Table 1). A history was taken and physical examination performed before ^{67}Cu-2IT-BAT-Lym-1 infusions, at about 1 wk after each infusion and monthly for 3 mo after the final therapy. Pharmacokinetics and radiation dosimetry were determined for all patients after each dose of ^{67}Cu-2IT-BAT-Lym-1 (*14*).

Dose and Administration

Three patients in the phase I part of the study received 2.22 GBq/m^2 (60 mCi/m^2) ^{67}Cu-2IT-BAT-Lym-1. The subsequent 9 patients in the phase II part of the study received 1.85 GBq/m^2 (50 mCi/m^2) ^{67}Cu if the pre-RIT bilateral bone marrow biopsy did not detect NHL, or 0.93 GBq/m^2 (25 mCi/m^2) if the bone marrow was found to contain NHL. Patients with grade III or IV hematologic toxicity after a dose were treated with a 50% ^{67}Cu dose reduction after the blood counts returned to grade I or better. Patients were premedicated with 50 mg diphenhydramine and 650 mg acetaminophen. Unconjugated Lym-1 (5 mg) was given before ^{67}Cu-2IT-BAT-Lym-1 to block nonspecific binding sites. Lym-1 and ^{67}Cu-2IT-BAT-Lym-1 were infused at about 0.5 mg/min. Two additional doses of diphenhydramine and acetaminophen were given orally after RIT.

Toxicity Assessment

Vital signs were monitored before at least every 15 min and during and for 2 h after Lym-1 infusion. Subsequently, monitoring continued, but on a less frequent schedule. Each patient underwent renal and liver function tests, a complete blood count and a HAMA assay before and 4–6 wk after each treatment and at intervals of 3–6 mo after the last treatment. Blood counts were also obtained weekly during therapy and afterward until they had recovered. National Cancer Institute Common Toxicity Criteria were used to classify data. Quantitative HAMA assays for reactivity against Lym-1 were performed, as previously described (*21*).

Response Assessment

Tumors were evaluated by physical examination and CT or MRI. Responses were classified as CR, including negative bone marrow examination, or PR, a decrease in the sum of the products of tumor dimensions by at least 50% or tumor volumes by at least 70%. Responses required a durability of 4 wk without evidence of progression. The therapeutic indices were calculated by dividing the highest tumor radiation dose in a patient by that of the patient's organ, whole body or bone marrow radiation dose.

Pharmacokinetics and Radiation Dosimetry

Blood samples were obtained for analysis of radiopharmaceutical content immediately; 10, 30, 60, 120 and 360 min; and daily for approximately 7 d after RIT. Blood radioactivity was counted in a gamma well counter and compared with a standard from the injected dose. The percentage injected dose (%ID) in the blood was calculated for each sample using the body weight to estimate blood volume. HPLC analysis using a molecular sieving column (TSK 3000; Beckman Instruments, Fullerton, CA) with a continuous flow-through radioisotope detector and an ultraviolet (280 nm) monitor was performed on plasma to assess the in vivo stability of ^{67}Cu-2IT-BAT-Lym-1.

Pharmacokinetic data were obtained as previously described (*22*). Briefly, planar images of opposing views were acquired immediately, 4 h and daily for up to 10 d after administration of ^{67}Cu-2IT-BAT-Lym-1 and were coincidence corrected at high counting rates. Cumulated activity in all tissues except the blood was obtained using a monoexponential analysis and converted to a radiation dose using the Medical Internal Radiation Dose (MIRD) formula considering radiation contributed from the target and the remainder of the body (*23*). MIRD S values and reference man masses were used. Tumor sizes were determined at the time of each RIT using caliper or radiographic measurements. A total of 54 tumors were identifiable by imaging of ^{67}Cu-2IT-BAT-Lym-1, but 13 tumors with masses of less than 2 g were excluded to ensure the accuracy of radiation dosimetry.

The bone marrow radiation dose was determined by two methods. The first method addressed contributions from nonpenetrating radiation from blood ^{67}Cu and penetrating radiation from total body ^{67}Cu (*22*). Cumulated ^{67}Cu in blood was obtained by fitting pharmacokinetic data to a biexponential function. The calculation of marrow radiation from the total body assumed a uniform distribution of ^{67}Cu. The second method addressed the marrow-to-marrow, nonpenetrating radiation dose extrapolated from the uptake of ^{67}Cu-2IT-BAT-Lym-1 as imaged in three lumbar vertebrae, as previously described (*14*). It was assumed that the marrow mass in the three lumbar vertebrae was 6.7% of the total red marrow. Cumulated activity in bone marrow and the MIRD S value for nonpenetrating ^{67}Cu radiation were used to determine the radiation dose to marrow from marrow. Total radiation dose to the

bone marrow (Table 2) was calculated by adding the values obtained from the marrow as imaged to the value obtained from the body and blood to marrow.

RESULTS

Dose and Cycles

Patients 1–3 received 2.22 GBq/m^2 ^{67}Cu in the phase I part of the study (Table 2). The next 9 patients participated in the phase II study; 1.85-GBq/m^2 doses of ^{67}Cu-2IT-BAT-Lym-1 were administered if pre-RIT bilateral bone marrow biopsies did not detect NHL, and 0.93-GBq/m^2 doses were given if NHL was detected. Patient 4 received 1.85 GBq/m^2 for the first dose and a reduction to 0.93 GBq/m^2 for the second dose because of hematologic toxicity. Further RIT was prevented by death from NHL (patients 5, 6 and 12), HAMA (patients 3, 7 and 9), noncompliance (patient 11) or disease progression (patients 2, 4, 8 and 10). Patient 1 received all four planned doses of RIT. The maximum administered dose was 15.7 GBq (patient 1), and the minimum was 1.6 GBq (patient 12).

Pharmacokinetics and Radiation Dosimetry

The mean for blood and body radiation dose to bone marrow was 0.08 ± 0.03 Gy/GBq (Table 3). The bone marrow imaging method of estimating the radiation dose to bone marrow gave a mean radiation dose to the bone marrow of 0.19 ± 0.11 Gy/GBq. Total body dose was consistently 0.11–0.14 Gy/GBq. The liver was the organ that had the highest radiation dose from ^{67}Cu-2IT-BAT-Lym-1 and always had a greater radiation dose than did kidneys or lungs.

Fifty-four imaged tumors could be measured by physical or radiologic examination, and 41 met the size criteria for accurate quantification of tumor dose. Individual tumor doses ranged from 0.3 to 5.99 Gy/GBq. The highest

TABLE 3
Radiation Dosimetry for 12 Patients with Non-Hodgkin's Lymphoma

	Mean (Gy/GBq)	SD	Range (Gy/GBq)
Tumor	2.35	0.97	0.30–5.99
Liver	1.24	0.32	0.57–1.97
Lung	0.46	0.19	0.22–0.81
Kidney	0.59	0.22	0.16–0.92
Body	0.11	0.0	0.11–0.14
Blood	0.19	0.08	0.08–0.43
Bone marrow (imaged)*	0.19	0.11	0.05–0.38
Blood and body to marrow†	0.08	0.03	0.05–0.14

*Bone marrow dose determined by imaging of three lumbar vertebrae.

†Bone marrow dose determined by blood-and-body-to-marrow calculation.

TABLE 2
Myelotoxicity

Patient no.	Total dose to bone marrow* (Gy)	Neutrophils			Platelets		
		Maximum toxicity grade	Day of nadir	Grade III/IV toxicity (d)	Maximum toxicity grade	Day of nadir	Grade III/IV toxicity (d)
1	0.92	0	—	0	I	27	0
	0.96	III	16	9	II	9	0
	0.56	0	—	0	0	—	0
	1.37	IV	20	24	IV	11	55
2	0.33	0	—	0	II	26	0
	0.41	IV	13	23	IV	38	63
3	0.58	IV	42	11	IV	44	35
4	1.07	II	25	0	IV	31	17
	0.45	II	44	0	III	43	48
5	0.55	0	—	0	0	—	0
6†	0.86	0	—	0	III	NA	24
7†‡	1.27	III	46	14	IV	46	42
8†‡	0.65	III	60	14	IV	53	84
9	0.85	0	—	0	II	3	0
	0.92	0	—	0	II	3	0
	0.77	0	—	0	II	24	0
10	0.55	0	—	0	0	—	0
	0.35	I	20	0	0	—	0
11	NA	0	—	0	0	—	0
12‡§	0.45	0	—	0	IV	14	7

*Total dose to bone marrow calculated by adding blood-and-body-to-marrow dose to dose calculated from marrow imaging.

†Received one course of external beam radiation therapy before radioimmunotherapy.

‡Received high-dose chemotherapy with peripheral blood stem cell support.

§Received total-body irradiation as part of a conditioning regimen before bone marrow transplantation.

cumulative tumor radiation dose was 70 Gy (Table 1) in patient 1, who received the highest total dose of ^{67}Cu. The therapeutic indices for tumor to lung, tumor to kidney and tumor to liver were 7.4:1, 5.3:1 and 2.6:1, respectively. The therapeutic indices for tumor to bone marrow (body and blood to marrow plus marrow as imaged) (Table 3) and tumor to total body were 15:1 and 28:1, respectively. Targeting of NHL by ^{67}Cu-2IT-BAT-Lym-1 is seen in Figure 1.

Response

This phase I/II clinical trial of ^{67}Cu-2IT-BAT-Lym-1 for patients with NHL had a response rate of 58% (7/12) (Table 1). There was one CR and 6 PRs. The patient who received the highest ^{67}Cu dose (15.7 GBq) and most cycles (four) had a CR. Five patients were able to receive more than one dose of ^{67}Cu-2IT-BAT-Lym-1; all 5 had responded after the first dose. Two of 7 patients who were able to receive only one dose of ^{67}Cu-2IT-BAT-Lym-1 (5 GBq or less) had responses. All 4 patients with normal pre-RIT LDH values responded; 3 of 8 patients (38%) with elevated pre-RIT LDH values responded. One of the 3 patients who previously had high-dose chemotherapy with PBSC support responded. The duration of the CR was 12 mo; the mean duration of the PR was 3 mo (range 1–7 mo).

Five of 12 patients (42%) became HAMA positive an average of 51 d (range 18–79 d) after the initial administration of ^{67}Cu-2IT-BAT-Lym-1. The mean initial positive HAMA titer was 30 μg/mL (range 5.2–78.2 μg/mL), with a positive HAMA defined as greater than 5 μg/mL.

Toxicity

Seven of 8 patients (88%) who received a ^{67}Cu dose of 1.85 GBq/m^2 or more had grade III or IV thrombocytopenia at some time during the RIT protocol, whereas 1 of 4 patients (25%) who received the 0.93-GBq/m^2 ^{67}Cu dose had grade III or IV thrombocytopenia (Table 2). In the 6 patients who had grade III or IV thrombocytopenia and were alive 6 wk after RIT, the mean duration of grade III or IV thrombocytopenia was 49 d and the mean platelet count nadir was day 38. All 3 patients who had previously received high-dose chemotherapy with PBSC support had grade IV thrombocytopenia, including patient 12, who was treated at the 0.93-GBq/m^2 dose level. Despite the dose-limiting thrombocytopenia, significant bleeding did not occur. Five of 8 patients (62%) who received a ^{67}Cu dose of 1.85 GBq/m^2 or more had grade III or IV neutropenia at some time during the RIT protocol (Table 2). None of the patients who received the 0.93-GBq/m^2 (25-mCi/m^2) dose of ^{67}Cu had greater than grade I neutropenia. The mean duration of grade III or IV neutropenia was 16 d, and the mean neutrophil count nadir was day 33. Anemia occurred during the study but was neither dose limiting nor as prominent as the neutropenia and thrombocytopenia. No nonhematologic toxicity greater than grade II was observed.

DISCUSSION

This phase I/II RIT trial used the novel anti-HLA-DR10-directed Lym-1 MAb to deliver the TETA-chelated therapeutic radiometal ^{67}Cu to NHL. ^{67}Cu-2IT-BAT-Lym-1 targeted NHL well in all 12 patients, and despite the fact that 11 of 12 patients had intermediate- or high-grade stage III or IV chemotherapy-resistant NHL, a response rate of 58% was achieved. RIT with ^{131}I-Lym-1 showed considerable potential in our phase I/II clinical trials (*2,8,11*). Other investigators have also achieved encouraging results using RIT for NHL. In a study by Press et al. (*4*), 21 patients, most of whom had low-grade NHL, were treated with ^{131}I-anti-CD20 and 16 patients had CRs. Knox et al. (*24*) treated 18 NHL patients with ^{90}Y-labeled anti-CD20 and achieved a 72% response rate. Kaminski et al. (*3*) used ^{131}I-B1 to treat 28 patients in a study that determined the whole-body MTD dose to be 0.75 Gy (75 rads). Patients with low-grade NHL were particularly responsive, and the overall response rate

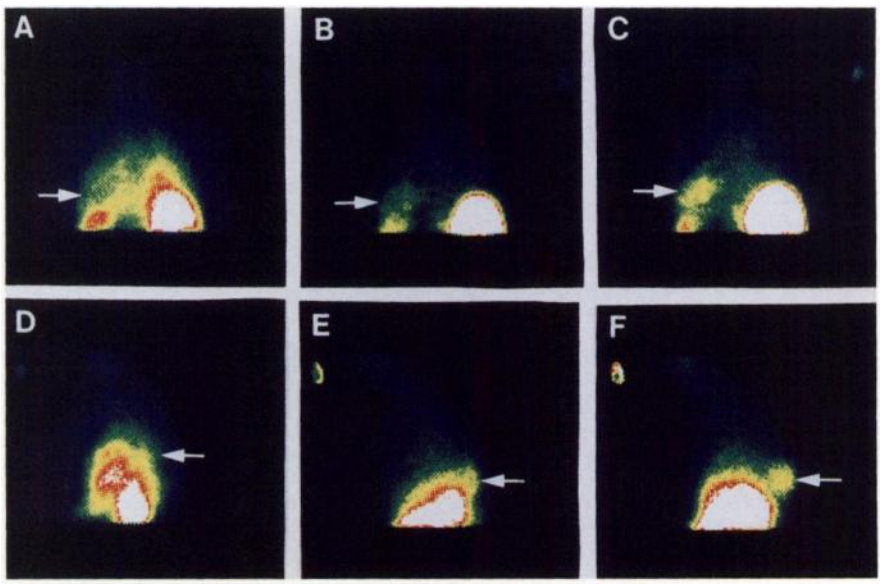

FIGURE 1. Posterior planar images of posterior chest wall lymphomatous mass in NHL patient 10 immediately (A), 3 d (B) and 6 d (C) after infusion of ^{67}Cu-2IT-BAT-Lym-1 and lateral images (with patient facing left) 45 min (D), 3 d (E) and 6 d (F) after infusion. Infused agent is seen in blood pool (A and D), heart is seen anteriorly (D) and liver is seen as large white area (D). Targeting of NHL seen on day 3 (arrows, B and E) and, further, on day 6 (arrows, C and F) shows prolonged residence of ^{67}Cu in NHL.

was 79%. ^{131}I-LL2 (anti-CD22) was used in 8 patients, and cumulative doses of 1.15–3.77 GBq (31–102 mCi) resulted in one CR and two PRs, with minimal toxicity (*25*). Eighteen patients were treated with 3.33–7.40 GBq (90–200 mCi) ^{131}I-OKB7 (anti-CD21); 1 PR and 12 minor responses resulted, and myelotoxicity was the major side effect (*26*). Given the fact that the condition of these patients had failed to respond to standard therapy, the response rates were impressive.

This study used ^{67}Cu and Lym-1 as modalities to enhance the therapeutic index of RIT. The macrocyclic chelating agent TETA was designed specifically to bind copper for conjugation to MAbs (*12*); the result is a radioimmunoconjugate with exceptional stability, high specific activity and complete retention of immunoreactivity (*18,19*).

^{131}I has been the predominant radionuclide for RIT because it is inexpensive, readily available and easily attached to antibodies (*2*). ^{67}Cu also has useful characteristics for RIT, but few trials have been performed with ^{67}Cu (*14,27*), because it is not readily available. The 62-h half-life of ^{67}Cu is similar to the uptake and residence time of antibodies in tumors (*28*). The beta emission (mean energy 141 keV; e_{max} = 577 keV) of ^{67}Cu is comparable to that of ^{131}I, but ^{67}Cu has more favorable gamma emissions (185 keV, 47%, for ^{131}I; 93 keV, 17%, for ^{67}Cu), which are excellent for imaging and expose medical personnel to significantly less radiation than ^{131}I. ^{90}Y has potent beta emissions but no gamma emissions, so ^{111}In is used as its surrogate for imaging. ^{67}Cu is given as outpatient therapy, whereas ^{131}I may require hospitalization because of radiation safety concerns. ^{67}Cu-radiolabeled MAbs have been shown in preclinical studies to deliver higher doses to tumors and higher tumor-to-nontumor dose ratios when compared with their iodinated counterparts (*29,30*), in part because radioiodine is rapidly cleared from tumors (*31,32*). ^{67}Cu is not deposited in bones (as is free ^{90}Y) or bone marrow. Copper is normally stored in the liver, where it can be bound by ceruloplasmin, which is then secreted into the blood (*33*). Some ^{67}Cu can be transferred from radioimmunoconjugates to ceruloplasmin by the liver (*14*). Patients who received ^{67}Cu-2IT-BAT-Lym-1 had a positive or flat slow phase of blood clearance starting 3 d after receiving ^{67}Cu-2IT-BAT-Lym-1 (*14*).

Lym-1 is a useful MAb for RIT, because it binds a variant HLA-DR10 lymphocyte-restricted antigen that is preferentially expressed on malignant lymphocytes (*5*). Relatively small amounts of Lym-1 are required for optimal imaging and effective radionuclide delivery to NHL. Unmodified Lym-1 is ineffective in the treatment of NHL in mice and humans (*6*), thus the data showing promising therapeutic effects are likely caused by the specific delivery of ^{67}Cu to NHL. Results of preliminary clinical trials suggested that ^{67}Cu-2IT-BAT-Lym-1 would be a useful agent for RIT; doses intended for imaging induced tumor regression (*34*). Most investigators use beta-emitting radionuclides, such as ^{131}I, ^{90}Y and ^{67}Cu, to deliver more uniform radiation to the tumor and alleviate the problem of poor tumor penetration by the MAb (*35*). Fractionating RIT into a series of smaller radionuclide and radiation doses, as in this study, is another strategy developed for achieving more uniform tumor irradiation, and fractionation also increases the tolerated radiation dose (*36,37*).

In an MTD study by our group (*2*), 20 patients treated with ^{131}I-Lym-1 had mean lung, kidney, body and blood-and-body-to-marrow dosimetry nearly identical to those reported here for ^{67}Cu-2IT-BAT-Lym-1 (Table 3). The liver dose was higher for ^{67}Cu-2IT-BAT-Lym-1 (1.24 Gy/GBq) than for ^{131}I-Lym-1 (0.4 Gy/GBq) because of transfer of ^{67}Cu to ceruloplasmin in the liver (*14*). No hepatotoxicity was noted in either study. Hematologic toxicity was dose limiting, because neither study used bone marrow or PBSC transplantation. ^{131}I-Lym-1 dosimetry for 42 tumors was 1.1 ± 0.7 Gy/GBq (*2*), whereas the dose to the 54 tumors treated with ^{67}Cu-2IT-BAT-Lym-1 was 2.35 ± 0.97 Gy/GBq (Table 3). In another study, four patients received imaging doses of ^{67}Cu-2IT-BAT-Lym-1 and ^{131}I-Lym-1 less than 26 d apart (*38*). The mean concentration of ^{67}Cu in NHL was 2.8 times that of ^{131}I 48 h after injection, and the mean biologic half-life of ^{67}Cu-2IT-BAT-Lym-1 in tumor was 8.8 d versus 2.3 d for ^{131}I-Lym-1.

In a study using the anticarcinoembryonic antigen MAb35, ^{125}I-MAb35 and ^{67}Cu-MAb were simultaneously injected into six patients before surgery for primary colorectal cancer (*39*). Mean tumor uptake of ^{67}Cu-MAb35 exceeded that of ^{125}I-MAb35 by 40%: 0.0133 versus 0.0095 %ID/g, respectively. The tumor-to-blood ratios were 6.07 and 2.41 for ^{67}Cu-MAb35 and ^{125}I-MAb35, respectively. This study also found a relatively high liver uptake of ^{67}Cu-MAb35.

The MTD of ^{67}Cu-2IT-BAT-Lym-1 was determined to be 2.22 GBq/m^2 (60 mCi/m^2) (*14*). The results of the phase II study suggest that 1.85 GBq/m^2 ^{67}Cu produces hematologic toxicity at the high end of the acceptable range, when given without bone marrow reconstitution. There was minimal toxicity with the 0.93-GBq/m^2 dose. The prolonged nature of thrombocytopenia and, to a lesser extent, neutropenia in the patients who received the 1.85- to 2.22-GBq/m^2 doses reflected not only the radiation effect but also the effect of NHL itself and the extensive chemotherapy to which the bone marrow had been exposed before RIT. Despite myelotoxicity secondary to ^{67}Cu-2IT-BAT-Lym-1, RIT was reasonably well tolerated. There were no instances of bleeding or neutropenic sepsis. On the other hand, the notable absence of nonhematologic toxicity makes it likely that doses could be increased substantially if PBSC support was used.

CONCLUSION

^{67}Cu-2IT-BAT-Lym-1 targeted NHL and provided therapeutic irradiation that resulted in a 58% response rate in patients who had progressive NHL after multiple chemotherapeutic regimens. This was especially impressive given the extensive prior therapy the patients had received and the fact that 11 of 12 had intermediate- or high-grade NHL. Radio-

metals, such as ^{67}Cu, a novel therapeutic radionuclide, although difficult to obtain, remain an option for future clinical trials, because this study established ^{67}Cu-2IT-BAT-Lym-1 as safe, effective treatment for patients with NHL.

ACKNOWLEDGMENTS

This research was supported by grants from the National Cancer Institute (PO1-CA47829) and the U.S. Department of Energy (DOE) (DE-FG03–84ER60233) and by DOE contract DE AC02–76CH00016. The authors acknowledge support by the Veteran's Administration Northern California Healthcare System, Martinez, CA; Owen Lowe, PhD, at the DOE; and Leonard Mausner, PhD, at Brookhaven National Laboratory, Upton, NY.

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