Efficacy and Toxicity of $^{67}$Cu-2IT-BAT-Lym-1 Radioimmunoconjugate in Mice Implanted with Human Burkitt’s Lymphoma (Raji)

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ABSTRACT

Radioimmunotherapy has shown promising results for treatment of radiosensitive malignancies such as lymphoma. Positive responses have been reported in patients with non-Hodgkin’s lymphoma treated with $^{131}$I-radiolabeled Lym-1, a mouse anti-lymphoma monoclonal antibody. In this study, the efficacy of $^{67}$Cu-radiolabeled Lym-1 was examined. Nude mice bearing human Burkitt’s lymphoma (Raji) tumors (20–524 mm$^3$) were treated with 12.4, 14.8, 18.5, and 23.3 MBq of $^{67}$Cu-2IT-BAT-Lym-1. Tumor size was measured to assess efficacy, and mouse weight, blood counts, and mortality were monitored to assess toxicity. In mice treated with 12.4, 14.8, and 18.5 MBq of $^{67}$Cu-2IT-BAT-Lym-1, 50% (9 of 18), 42% (5 of 12), and 50% (3 of 6) of tumors achieved remission or cure; 33% of tumors were cured overall; and significant regrowth delay was observed. The 23.3 MBq dose group did not yield meaningful efficacy data because of high mortality. In control groups receiving 14.8 and 18.5 MBq of the isotype-matched nonspecific monoclonal antibody radioimmunoconjugate, $^{67}$Cu-2IT-BAT-L6, 0% (0 of 15) and 17% (2 of 12) of tumors achieved a response; hence, targeted delivery of radiation was the dominant antitumor mechanism of $^{67}$Cu-2IT-BAT-Lym-1. LD$_{50/30}$ for mice treated with $^{67}$Cu-2IT-BAT-Lym-1 and -L6 were 21.6 and 20.6 MBq, respectively. In conclusion, $^{67}$Cu-2IT-BAT-Lym-1 provided a therapeutic and frequently curative dose of radiation to tumors with modest toxicity.

INTRODUCTION

Despite continuing advances in conventional therapy for NHL, $^1$ 40 to 70% of patients with intermediate- and high-grade NHL fail to achieve long term disease-free survival, and no curative treatment has been established for patients with low-grade NHL (1–4). RIT, using MABs against tumor-associated antigens to deliver cytotoxic radionuclides to tumors, has been particularly promising in the treatment of radiosensitive malignancies such as lymphoma (4–13).

$^{131}$I has been the predominant radionuclide for RIT because it is inexpensive, widely available, and readily incorporated into proteins (4–9, 14–16). However, $^{131}$I has other characteristics that limit its utility for RIT. The abundant high energy gamma radiations of $^{131}$I increase the radiation exposure to medical personnel compared to other candidate radionuclides (15–17). Additionally, rapid clearance of radioiodine from tumors can reduce the radiation dose to tumor by iodinated MABs (18–23). For these reasons, there is strong interest in other radionuclides for RIT, particularly $^6$Cu, which has excellent physical and biochemical properties for radioimmunotherapy. Its half life of 62 h is well matched to the uptake and residence time of antibodies in the tumor (17, 24).

$^{67}$Cu has a beta emission (mean energy, 141 keV; $e_{\text{max}}$, 577 keV) comparable to that of $^{131}$I but more favorable gamma emissions (185 keV, 47%; 93 keV, 17%) that are excellent for imaging but contribute significantly less radiation exposure to medical personnel. Radiation safety concerns may affect the amount of time a patient must remain isolated in the hospital following treatment with $^{131}$I immunoconjugate; in contrast, it is anticipated that patients receiving relatively high doses of $^{67}$Cu immunoconjugate could be treated as outpatients.

A potential advantage of $^{67}$Cu for RIT is its enhancement of the therapeutic index of radioimmunotherapeutic agents. In theoretical modeling of radiation absorbed doses, $^{67}$Cu was predicted to give almost a 25% better tumor:non-tumor dose ratio than $^{131}$I if the biological data remained constant (25). In fact, the biological data favor $^{67}$Cu even further. $^{67}$Cu-radiolabeled MABs have been shown in preclinical studies to deliver higher doses to tumor and higher tumor:non-tumor dose ratios when compared to their iodinated counterparts (26, 27). These results...
have been substantiated by preliminary data from our clinical trials of $^{67}$Cu-2IT-BAT-Lym-1, in which an exceptionally long residence time of $^{67}$Cu on tumor was observed (28, 29).

In pioneering studies to develop the potential of $^{67}$Cu for RIT, the macrocytic chelating agent TETA was designed specifically to bind copper for conjugation to MAbs (30). In our group, the bifunctional TETA derivative BAT is conjugated to mouse anti-lymphoma IgG$_2$ MAb Lym-1 via 2IT to prepare the immunoconjugate 2IT-BAT-Lym-1 (31–33). Under well-characterized radiolabeling conditions, 2IT-BAT-Lym-1 has been shown to bind $^{67}$Cu rapidly and selectively to prepare radioimmunoconjugates of exceptional stability, high specific activity, and with complete retention of immunoreactivity (32, 34, 35).

Tumor responses and modest toxicity have been reported previously in clinical trials of RIT of malignant lymphoma using $^{131}$I-radioiodinated Lym-1 (2, 9). In this study, to evaluate the potential of $^{67}$Cu-radioiodinated Lym-1 for RIT, we investigated the efficacy and toxicity of the radioimmunoconjugate $^{67}$Cu-2IT-BAT-Lym-1 in mice bearing Raji tumor xenografts. To investigate the antitumor effect of antibody-mediated targeted delivery of radiation versus nonspecifically delivered radiation, we performed similar studies with an isotype-matched, nonspecific radioimmunoconjugate, $^{67}$Cu-2IT-BAT-L6.

**MATERIALS AND METHODS**

**Antibodies.** Lym-1 (Techniclone, Inc., Tustin, CA), an IgG2a MAb that was produced in mice immunized against naked nuclei of human Burkitt’s lymphoma cells, reacts with greater than 80% of B-cell lymphomas and 40% of B-cell chronic lymphocytic leukemias (2, 36). Lym-1 recognizes a Mr 31,000–35,000 antigen that is present on the surface of both normal and malignant B-cells, although the antibody has greater avidity for malignant cells (36). The antibody-antigen complex is not internalized or released in Raji cell cultures in vitro. L6 (Oncogen, Seattle, WA), a mouse anti-adenocarcinoma IgG2a MAb generated using non-small cell lung carcinoma pleural effusion as an immunogen, reacts with a membrane antigen found on human adenocarcinoma cells of the lung, colon, ovary, and breast (37, 38).

**Radiation Counting.** An ionization chamber dose calibrator (Capintec CRC-12; Capintec, Inc., Pittsburgh, PA) was used to measure radioimmunoconjugate doses. Total body activities of mice were measured with a dual sodium iodide crystal probe system (Picker Nuclear, North Haven, CT). All other samples were counted in a well counter (Pharmacia LKB 1282; Pharmacia Biotech, Inc., Piscataway, NJ).

**Preparation of $^{67}$Cu-2IT-BAT-Lym-1 and $^{67}$Cu-2IT-BAT-L6.** BAT was prepared as described previously (30, 33) and conjugated to Lym-1 and L6 via 2IT by standard methods as follows (32, 33). Conjugations were conducted in 0.1 M tetraethylammonium phosphate buffer, pH 8 to 9, for 30 min at 37°C. The concentrations of MABs, 2IT, and BAT were 7 to 27 mg/ml, 0.5 to 3.0 mm, and 1.0 to 6.0 mm, respectively; in every reaction, the concentration of 2IT was one-half the concentration of BAT. The immunoconjugates were purified and transferred to 0.1 M ammonium citrate buffer, pH 5.5, by centrifuged column chromatography (39, 40) or open column gel chromatography (Sephadex G50; Sigma Chemical Co., St. Louis, MO).

The mean number of TETA chelating groups conjugated per antibody was measured by metal binding assay (32, 40). The 2IT-BAT-Lym-1 conjugates prepared had 1.3 to 5.8 TETA groups per antibody values of 1.3–5.8, a range shown previously to be consistent with high retention of immunoreactivity and high tumor uptake (32). The single 2IT-BAT-L6 conjugate had 4.1 TETA groups per antibody.

The radioimmunoconjugates 2IT-BAT-Lym-1 and -L6 were radioiodinated with $^{131}$I by methods described previously (34) as follows. $^{67}$Cu, produced by proton spallation on zinc oxide targets (BNL, Upton, NY, or LANL, Los Alamos, NM) in 0.1 to 0.5 m HCl, was buffered with 2 m ammonium citrate (pH 7.7) to a final pH of 5.5. Buffered $^{67}$Cu was added to 2IT-BAT-Lym-1 or -L6, and the radiolabeling solution was incubated for 30–60 min at room temperature. Na$_2$EDTA was added to a final concentration of 10 mM to complex any nonspecifically bound metal ions. The solution was incubated for 15–30 min at room temperature. Radiolabeled conjugate was purified from $^{67}$Cu-EDTA and transferred to saline by centrifuged column chromatography or open column gel chromatography. In two radiolabelings of $^{67}$Cu-2IT-BAT-Lym-1, radioactive yields of 77 and 78% were achieved, and products with specific activities of 54 and 108 MBq $^{67}$Cu per mg Lym-1 were obtained. One radiolabeling of $^{67}$Cu-2IT-BAT-L6 was performed. The radioactive yield was 88%, and the specific activity of product was 116 MBq $^{67}$Cu per mg L6.

The quality of the radioimmunoconjugate preparations was assessed by CAE, HPLC, and RIA. CAE (Gelman Sciences, Inc., Ann Arbor, MI) was performed using 0.05 m sodium barbital buffer, pH 8.6. A current of 5 mA per strip was applied. Samples were electrophoreses for 11 and 45 min. At 11 min, free chelates were resolved from immunoconjugates. At 45 min, monomeric immunoconjugates were resolved from aggregated species. HPLC (Beckman 332; Beckman, San Ramon, CA) was performed using a molecular sieving column (Beckman SEC-3000) eluted in 0.1 m sodium phosphate, 0.1 m potassium sulfate, and 0.025% (v/v) sodium azide (pH 7.1). The flow rate was 1.0 ml/min. Radiolabeled MAbs and conjugates were detected by UV absorbance at 280 nm (Beckman 160 detector) and radioactivity (Beckman 170 detector). Immunoreactivity of $^{67}$Cu-2IT-BAT-Lym-1 was assessed by solid-phase RIA against partially purified Raji cell homogenates, as described previously (41). Immunoreactivity of $^{67}$Cu-2IT-BAT-L6 was assessed by HBT 3477 cell binding RIA as described previously (42, 43).

In every preparation of $^{67}$Cu-2IT-BAT-Lym-1 and -L6, greater than 95% of $^{67}$Cu was associated with monomeric immunocomplex by HPLC and CAE, and the relative immunoreactivity was assayed to be greater than 90% versus $^{125}$I-Lym-1 and -L6 reference standards, respectively.

**Implantation of Tumors.** Female athymic nude/nude mice (Harlan Sprague Dawley, Frederick, MD) were maintained according to University of California Animal Care guidelines on normal diet ad libitum and under pathogen-free conditions. Five mice were housed per cage. To minimize ambient radiation dose, bedding was changed daily for 1 week following treatment with $^{67}$Cu-2IT-BAT-Lym-1 or -L6 and twice weekly thereafter. The mice received body exposures of 400 rads by external beam irradiation to suppress immune response to the Raji xenografts (44, 45). Two to 5 days later, the mice were given s.c. injections.
of 2–5 × 10⁶ Raji cells in the lower abdomen; each mouse received two injections to establish up to two tumors per mouse. Among 190 injections in 95 mice, 136 tumors developed in 86 mice. Although the tumors were somewhat variable in size and growth rate, 97 tumors were well established, i.e., 20 mm³ or larger, at the time of treatment with radiolabeled immunonjugate. All mice were used for the toxicity studies (mortality, mouse weight, and blood counts); only mice bearing tumors at the time of treatment were used to evaluate efficacy.

Efficacy and Toxicity Studies. The mice were divided into dose level groups of 8–19 mice with comparable distributions of tumor sizes among groups. Therapy groups received i.v. injections of 12.4 MBq (335 μCi)/231 μg, 14.8 MBq (400 μCi)/276 μg, 18.5 MBq (500 μCi)/345 μg, 20.9 MBq (565 μCi)/194 μg, or 23.3 MBq (630 μCi)/434 μg of ⁶⁷Cu-²¹T-BAT-Lym-1. Control groups received 14.8 MBq/128 μg, 18.5 MBq/159 μg, or 23.3 MBq/201 μg of ⁶⁷Cu-²¹T-BAT-Lym-6. To promote uniform blood clearance, unmodified L6 was added to doses of ⁶⁷Cu-²¹T-BAT-Lym-1 and -L6 as needed to increase total IgG₅ immunoglobulin to 300 μg (9, 46). (No unmodified ChL6 was added to the 18.5 and 23.3 MBq ⁶⁷Cu-²¹T-BAT-Lym-1 doses.) A control group of 12 mice with 18 tumors was left untreated. RBC, platelet, and WBC counts were normal at the time of treatment. Survival was monitored daily; mouse weight and tumor size were measured 2–3 times per week for 12 weeks postinjection or until death occurred. Tumors were measured in three orthogonal diameters with a caliper, and tumor volume, \( V \), was calculated by the formula for hemiellipsoids:

\[
V = \frac{4}{3} \pi \left( \frac{d_1}{2} \times \frac{d_2}{2} \times \frac{d_3}{2} \right)
\]

RBC, platelet, and WBC counts were measured 2–3 times per week for 12 weeks postinjection as follows. Blood samples were collected from tail veins using 2-μl microcapillary pipettes, and samples from all mice within a dose group were pooled. The pooled samples were diluted 1:200 in PBS [10 mm NaPi/0.9% (w/v) NaCl (aq), pH 7.4] for RBC counts, 1:100 in 1% (w/v) ammonium oxalate for platelet counts, and 1:20 in 3% (w/v) acetic acid for WBC counts. The diluted blood cells were counted using a hemocytometer and light microscopy at a magnification of ×100 (WBCs) or ×450 (RBCs and platelets).

Total body clearance was evaluated by taking activity measurements immediately after injection and 1, 24, 48, 72, 96, and 120 h postinjection. Blood clearance was evaluated by collecting 10-μl blood samples from the tail vein immediately after injection and 1, 4, 24, 48, 72, 96, and 120 h postinjection. The 20.9 MBq ⁶⁷Cu-²¹T-BAT-Lym-1 dose group consisted entirely of untumored mice and was not used to evaluate efficacy. Tumor volumes, mouse weights, total body activities, and blood activities in all mice alive on the day of measurement were used to calculate the mean values for a dose group on that day.

\[4 \text{ K. L. Turner, Harlan Sprague Dawley, personal written communication.}\]

Analysis of Tumoricidal Effect. Initial tumor volumes were measured one day before treatment with ⁶⁷Cu-²¹T-BAT-Lym-1 or -L6. In the calculation of mean tumor size, tumors that had completely regressed, including those which never subsequently regrew, were considered tumors with a volume of 0.

TRD is commonly used to evaluate the effect of therapy on tumor size over an extended period of time (47–49). TRD for each dose group was defined as the mean number of days required for treated tumors to grow to two times their initial volume minus the mean number of days for untreated tumors to grow to two times their initial volume. TRD data were examined by the Jonckheere-Terpstra Exact test, which is designed for multiple group comparisons (50).

Mortality Calculations. Cumulative mortality curves were constructed and logistic regression analysis was used to calculate estimates of LD₅₀/₅₀ dose (SAS System software; SAS Institute, Inc., Cary, NC). Confidence intervals were calculated by Fieller's method (51). To corroborate these results, estimates of LD₅₀/₅₀ dose were also calculated by the Reed-Muench method (52, 53).

Radiation Dosimetry. Cumulated activity for the total body was obtained by integration of monoexponential analysis of body clearance data. Cumulated activity for the blood was obtained by integration of biexponential analysis of blood clearance data. Cumulated activities for the liver and tumor were estimated by using a trapezoidal fit of the activity/time curve, based on the results of previous studies of the biodistribution of ⁶⁷Cu- and ⁶⁸Cu-²¹T-BAT-Lym-1 in Raji-tumored mice (32, 45). To calculate total dose to liver, tumor, and marrow, the penetrating radiation from the body of the mice was added to the nonpenetrating radiation from the targeting tissue itself (54). A simplifying assumption of uniformly distributed activity in the total body was used to calculate the contribution from penetrating radiation. The absorbed fraction of photons in a 20-g mouse was obtained from MIRD data (55). A mean energy per nuclear transition of 8.9 (cGy/g)/(MBq·h) was used to calculate the nonpenetrating radiation of ⁶⁷Cu in the targeting tissue (56). A specific activity for blood in marrow of 0.25 was used to calculate the nonpenetrating dose to marrow from blood (57).

RESULTS

Tumoricidal Effect. Tumor responses were categorized as follows: cure, tumor disappeared and did not regrow during the 84 day study; CR, tumor disappeared but later regrew; and PR, tumor decreased by 50% or more and regrew. Only tumors that began to shrink within 3 weeks of treatment were classified as PR, CR, or cure, because earlier studies of the natural history of Raji tumors and their response to RIT indicated that RIT-induced tumor response occurred within this period (44).

The following observations about tumoricidal effect are restricted to doses of 18.5 MBq or less of ⁶⁷Cu-²¹T-BAT-Lym-1 or -L6, because 23.3 MBq was too toxic to yield meaningful efficacy data, and to tumors whose initial volume was 20 mm³ or greater at the time of treatment, unless stated otherwise. Responses (PR, CR, or cure) were achieved in a high percentage of tumors in mice treated with ⁶⁷Cu-²¹T-BAT-Lym-1, although over the range of dose examined, no correlation was observed between dose and percentage responding
Efficacy and Toxicity of $^{67}$Cu-2IT-BAT-Lym-1

Table 1  Tumor responses to varying doses of $^{67}$Cu-2IT-BAT-Lym-1 and -L6 in well-established tumors with initial volume 20 mm³ or greater

<table>
<thead>
<tr>
<th>Dose, MBq</th>
<th>n, tumors/ mice</th>
<th>Initial tumor volume, mean (range), mm³</th>
<th>PR</th>
<th>CR</th>
<th>Cures</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>18/12</td>
<td>78 (25–188)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$^{67}$Cu-2IT-BAT-Lym-1</td>
<td>12.4</td>
<td>18/15</td>
<td>115 (21–524)</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>14.8</td>
<td>12/9</td>
<td>103 (31–333)</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td>18.5</td>
<td>6/5</td>
<td>196 (51–421)</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>$^{67}$Cu-2IT-BAT-L6</td>
<td>14.8</td>
<td>15/9</td>
<td>128 (46–252)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18.5</td>
<td>12/8</td>
<td>179 (62–429)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>17</td>
</tr>
</tbody>
</table>

*Response = cure + CR + PR.

Table 2  Tumoricidal effect of $^{67}$Cu-2IT-BAT-Lym-1 on Raji tumor xenografts of varying initial volume

<table>
<thead>
<tr>
<th>Initial tumor volume, range, mm³</th>
<th>n, tumors</th>
<th>Mean dose, MBq</th>
<th>Cures</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2, palpable, not measurable</td>
<td>12</td>
<td>15.6</td>
<td>12 (100)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>2–19</td>
<td>11</td>
<td>12.8</td>
<td>7 (64)</td>
<td>7 (64)</td>
</tr>
<tr>
<td>20–200</td>
<td>29</td>
<td>14.1</td>
<td>12 (41)</td>
<td>17 (59)</td>
</tr>
<tr>
<td>201–524</td>
<td>7</td>
<td>14.8</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Response = cure + CR + PR.

(Table 1). Overall, among tumors of initial volume 20–524 mm³ treated with 12.4–18.5 MBq of $^{67}$Cu-2IT-BAT-Lym-1, 47% (17 of 36) achieved a tumor response, and 33% (12 of 36) were cured. The mean (±1 SD) time to disappearance of cured tumors were 19 (+8) days. A correlation between the initial volume of the tumor and the percentage responding was observed (Table 2). Among tumors in mice treated with 12.4–18.5 MBq of $^{67}$Cu-2IT-BAT-Lym-1, no tumors with an initial volume greater than 200 mm³ achieved a response, whereas 64% (7 of 11) tumors of initial size 2–19 mm³ were cured; furthermore, 100% (12 of 12) tumors that were palpable but too small to measure at the time of treatment were cured.

Tumors not cured by treatment with $^{67}$Cu-2IT-BAT-Lym-1 exhibited significant TRD. Treatment with $^{67}$Cu-2IT-BAT-Lym-1 significantly increased the time required for tumors to grow to two times their initial size versus untreated control tumors (Jonekheere-Terpstra Exact test, $P = 0.008$). The mean time required for untreated tumors to grow to two times their initial volume was 4 (range, 2–12) days; comparable values for tumors in mice treated with 12.4, 14.8, and 18.5 MBq of $^{67}$Cu-2IT-BAT-Lym-1 were 22 (1–59), 32 (5–58), and 43 (28–49) days, respectively. Hence, TRD for tumors in mice treated with 12.4, 14.8, and 18.5 MBq of $^{67}$Cu-2IT-BAT-Lym-1 were 18, 28, and 39 days, respectively.

Among 27 tumors treated with the nonspecific radioimmunoconjugate, $^{67}$Cu-2IT-BAT-L6, 2 tumors in one mouse were cured (Table 1). Treatment with $^{67}$Cu-2IT-BAT-L6 significantly increased the time required for tumors to grow to two times their initial size versus untreated control tumors (Jonekheere-Terpstra

Fig. 1  Mean volume of Raji tumors (initial tumor volume range, 20–200 mm³) in groups of mice after treatment with varying doses of $^{67}$Cu-2IT-BAT-Lym-1 and -L6. Anti-lymphoma $^{67}$Cu-2IT-BAT-Lym-1 inhibited tumor growth to a greater extent than nonspecific $^{67}$Cu-2IT-BAT-L6.

Fig. 2  Tumor growth versus time in tumor groups receiving $^{67}$Cu-2IT-BAT-Lym-1 and -L6. Anti-lymphoma $^{67}$Cu-2IT-BAT-Lym-1 inhibited tumor growth to a greater extent than nonspecific $^{67}$Cu-2IT-BAT-L6.

Estimates of LD<sub>50/30</sub> doses by logistic regression (95% confidence interval) were calculated to be 21.6 (19.3–27.8) MBq and 20.6 (17.3–25.8) MBq for $^{67}$Cu-2IT-BAT-Lym-1 and -L6, respectively (Fig. 3). These results were corroborated by Reed-Muench analysis of the mortality data, from which LD<sub>50/30</sub> doses were calculated to be 20.4 and 21.3 MBq for $^{67}$Cu-2IT-BAT-Lym-1 and -L6, respectively.
The survival of mice after injection of $^{67}$Cu-2IT-BAT-Lym-1 (A) or $^{67}$Cu-2IT-BAT-L6 (B). Similar mortality patterns were observed with similar doses of the two radioimmunoconjugates.

Fig. 4 Mean weights of groups of mice that received $^{67}$Cu-2IT-BAT-Lym-1 (A) or $^{67}$Cu-2IT-BAT-L6 (B). Among mice receiving 18.5 MBq or less of either radioimmunoconjugate, loss of weight, if any, was mild and transient. Among groups receiving higher doses, mice receiving 23.3 MBq of $^{67}$Cu-2IT-BAT-Lym-1 exhibited an especially severe and prolonged loss of weight, significantly greater than that of other dose groups. Data points are mean weights; bars, SD. For clarity, error bars are shown only on the day at which the 23.3 MBq dose groups reached their weight nadir.

Fig. 3 Mortality rate 30 days after treatment with $^{67}$Cu-2IT-BAT-Lym-1 or -L6. Predicted probability of mortality rate was calculated using logistic regression analysis. Data points represent 8–19 mice; bars, 95% confidence interval.

The group of untumored mice receiving 20.9 MBq of $^{67}$Cu-2IT-BAT-Lym-1 were treated to verify a preliminary estimate of the LD$_{50/30}$. Untumored mice had been used for this group to assure that deaths were not related to tumor. The deaths of 6 of 13 mice (Fig. 2) corroborated the earlier results.

Weight Loss. Mice receiving 23.3 MBq of $^{67}$Cu-2IT-BAT-Lym-1 exhibited severe and prolonged weight loss (Fig. 4), reaching a nadir at day 24 with the loss of 28% of initial body weight. Mice receiving 23.3 MBq of $^{67}$Cu-2IT-BAT-L6 reached a weight nadir at day 18 with the loss of 13% of initial body weight. Among mice receiving 18.5 MBq or less of either radioimmunoconjugate, weight loss, if any, was mild and transient.

Myelotoxicity. There was a similar hematopoietic pattern among mice treated with $^{67}$Cu-2IT-BAT-Lym-1 and -L6 (Fig. 5). WBC and platelet counts declined 4–7 days postinjection, and RBCs, due to their longer lifetimes, fell later and to a
Efficacy and Toxicity of $^{67}$Cu-$^{11}$T-BAT-Lym-1

In this study, $^{67}$Cu-$^{11}$T-BAT-Lym-1 had a substantial therapeutic effect with modest toxicity in mice bearing Raji tumors. Although human tumors in immunocompromised mice are not ideal to predict antitumor activity in humans, providing a curative dose of radiation to tumor mice without inducing lethal bone marrow failure is a singular result. Furthermore, this experimental model used the high-grade, $bcl-2$-positive Raji cell line, which is less radiosensitive than most other lymphoma cell lines (60, 61).

Therapy with $^{131}$I-radiolabeled Lym-1 showed considerable potential in our Phase I-II trials in patients (2, 9); therefore, it is currently in an industry-sponsored Phase III multicenter trial. Comparatively, the therapeutic efficacy of $^{67}$Cu-$^{11}$T-BAT-Lym-1 was shown in the current study to be superior to that of $^{131}$I-Lym-1, as evaluated previously in the Raji-tumored athymic mouse model. The doses administered and the tumor sizes at the time of treatment in the $^{131}$I study and the current $^{67}$Cu study were similar. Mice treated with 12.4 MBq of $^{67}$Cu-$^{11}$T-BAT-Lym-1 achieved twice the cure rate and three times the

**DISCUSSION**

In this study, $^{67}$Cu-$^{11}$T-BAT-Lym-1 achieved twice the cure rate and three times the

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**Pharmacokinetics**

In the case of both radioimmunoconjugates, total body clearance of $^{67}$Cu was monoexponential, and the blood clearance was biexponential. Based on pooled data from all dose groups, differences in clearance rates among different dose groups were not statistically significant. The biological $T_{1/2}$ of $^{67}$Cu in the blood was 2.0 (±0.3) h and 194 (±10) h for the fast and slow clearance phases, respectively.

In mice treated with $^{67}$Cu-$^{11}$T-BAT-Lym-1, differences in clearance rates among different dose groups were not statistically significant. Based on pooled data from all dose groups, the biological $T_{1/2}$ of $^{67}$Cu in the body was 157 (±3) h, and the biological $T_{1/2}$ of $^{67}$Cu in the blood was 2.0 (±0.2) h and 122 (±9) h for the fast and slow clearance phases, respectively.

**Dosimetry**

Despite the modest differences in pharmacokinetics between the two radioimmunoconjugates, mice given $^{67}$Cu-$^{11}$T-BAT-Lym-1 and -L6 received similar radiation doses to marrow and total body per MBq administered. In mice treated with $^{67}$Cu-$^{11}$T-BAT-Lym-1, the radiation dose to total body was 32.4 cGy/MBq. Penetrating radiation from the body contributed 2% or less of the total radiation dose to marrow, liver, and tumor; the total radiation doses to marrow, liver, and tumor were estimated to be 40.8, 41.1, and 144 cGy/MBq (1.51, 1.52, and 5.33 cGy/μCi), respectively. Hence, the LD$_{50/30}$ dose of 12.4 MBq of $^{67}$Cu-$^{11}$T-BAT-Lym-1, at which 50% of established tumors achieved a response, corresponded to radiation doses to total body, marrow, liver, and tumor of 400, 510, 510, and 1790 cGy, respectively. In mice treated with $^{67}$Cu-$^{11}$T-BAT-Lym-L6, the radiation dose to total body and marrow were 29.2 and 32.7 cGy/MBq, respectively.

The LD$_{50/30}$ dose of $^{67}$Cu-$^{11}$T-BAT-Lym-1, 21.6 MBq, corresponded to a total body radiation dose of 700 cGy. The LD$_{50/30}$ dose of $^{67}$Cu-$^{11}$T-BAT-L6, 20.6 MBq, corresponded to a total body radiation dose of 600 cGy. Comparatively, the LD$_{50/30}$ for an acute total body radiation dose to BALB/c mice from external beam radiation has been reported as 550–750 cGy (58, 59).

**REFERENCES**

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Supplementary material available online at [canceres.com](http://canceres.com) (10).
A similar rate of regression was observed with 67Cu-2IT-BAT-L6. A similar rate of regression was observed with 67Cu-2IT-BAT-L6, in contrast to the absence of therapeutic effect of unmodified Ly-1 or nonspecific 67Cu-2IT-BAT-L6, indicates that targeted delivery of radiation by 67Cu-2IT-BAT-Lym-1 was the dominant antitumor mechanism of the radioimmunoconjugate.

Greater resistance to RIT with increasing tumor size in xenografted animal models, previously reported elsewhere (64, 65), was also observed in this study. Furthermore, there was a surprising absence of dose-dependent therapeutic effect over the range 12.4–18.5 MBq, corresponding to a range of radiation dose to tumor of 1790–2660 cGy. Both observations demonstrate the potential for improvement of the treatment modality, e.g., by dose fractionation or addition of synergistic or debulking agents.

Results of clinical trials, although preliminary, have tended to confirm the promise of 67Cu-2IT-BAT-Lym-1 as an agent for RIT; in one patient, a dose intended for imaging induced tumor regression (28, 29). Most of the challenges involved in developing 67Cu as a practical agent for RIT have been met. The radiometal itself is produced to high standards of chemical and radiochemical purity (66) by BNL and LANL. Recently, BNL has improved the specific activity of its 67Cu product by a factor of five (67). The macrocyclic chelating agent, TETA, was developed to bind copper "tightly," i.e., to form a kinetically stable copper chelate in vivo. TETA has been shown to bind copper selectively, quickly, and completely, forming copper chelates of exceptional kinetic stability (33, 35, 68). TETA-MAb immunoconjugates such as 2IT-BAT-Lym-1 are readily prepared with full retention of immunoreactivity by well-documented methods (32, 33). In short, the methodology for the dependable preparation of 67Cu-radiolabeled immunoconjugates in therapeutic amounts is in place.

Although the implementation of 67Cu protocols has been impeded by the limited availability of the radionuclide, this problem can be readily solved. The capacity for year-round production of 67Cu in quantities to support therapy protocols exists at BNL and LANL. In addition, an alternate method of production that would extend the capability to produce 67Cu to lower energy proton accelerators is currently under investigation (69).

In conclusion, this study has shown the efficacy of 67Cu-2IT-BAT-Lym-1 for the treatment of human lymphoma xenografts in mice. The results suggest that 67Cu radioimmunoconjugates can make an important contribution to RIT and support further clinical evaluation of 67Cu-2IT-BAT-Lym-1.

**ACKNOWLEDGMENTS**

We gratefully acknowledge Dr. Justin Moran (University of California at Davis, Davis, CA) for the preparation of BAT; Cheng-Yi Xiong (University of California Davis Medical Center, Davis, CA) for assistance with the mouse studies; Dr. Kathleen R. Lamborn (University of California, San Francisco, CA) for biostatistical advice; and Dr. Robert T. O'Donnell (University of California Davis Medical Center) for helpful discussions.

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**Fig. 6** Total body (---) and blood (----) clearance of 67Cu from mice treated with 67Cu-2IT-BAT-Lym-1 (A) or 67Cu-2IT-BAT-L6 (B). Total body clearances were monoeponential, and blood clearances were biexponential. There were no statistically significant differences in clearance rates among dose groups receiving the same radioimmunoconjugate; dose group data were pooled to calculate clearance rates of the radioimmunoconjugates (see "Results"). Data points are the mean %ID, per dose group; bars, SD, pooled data.
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