Radioimmunotherapy with a $^{64}$Cu-labeled monoclonal antibody: A comparison with $^{67}$Cu

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ABSTRACT $^{67}$Cu ($\lambda_1 = 62$ h) has demonstrated potential as a radionuclide for radioimmunotherapy, but limited availability severely restricts its widespread use. $^{64}$Cu ($\lambda_1 = 12.8$ h) has been shown to have comparable effectiveness in vitro and in vivo. The present study was undertaken to examine the therapeutic potential of $^{64}$Cu- and $^{67}$Cu-bromoacetamidobenzyl-1,4,8,11-tetraazacyclotetradecane-$N,N',N'',N'''$-tetraacetic acid (BAT)-2-iminothiolane (2IT)-1A3 (1A3 is a mouse anti-human colorectal cancer mAb) for treatment of GW39 human colon carcinoma carried in hamster thigs. Hamsters were injected with $^{64}$Cu- or $^{67}$Cu-BAT-2IT-1A3 or Cu-labeled nonspecific IgG (MOPC) or saline. Hamsters were killed 6-7 months after therapy or when tumors were ≥10 g. Of the hamsters with small tumors (mean weight 0.43 ± 0.25 g), 87.5% were disease-free 7 months after treatment with 2 mCi (1 Ci = 37 GBq) of $^{64}$Cu-BAT-2IT-1A3 or 0.4 mCi of $^{67}$Cu-BAT-2IT-1A3. The mean tumor doses at these activities of $^{64}$Cu- and $^{67}$Cu-BAT-2IT-1A3 were 586 and 1269 rad (1 rad = 0.01 Gy), respectively. In contrast, 76% of hamsters treated with 2 mCi of $^{64}$Cu-BAT-2IT-MOPC or 0.4 mCi of $^{67}$Cu-BAT-2IT-MOPC had to be killed before 6 months because of tumor growth. When hamsters with larger tumors (mean weight 0.66 ± 0.11 g) were treated with $^{64}$Cu- or $^{67}$Cu-BAT-2IT-1A3, survival was extended compared with controls, but only one animal remained tumor-free to 6 months. These results demonstrate that $^{64}$Cu- and $^{67}$Cu-BAT-2IT-1A3 given in a single administered dose can eradicate small tumors without significant host toxicity, but additional strategies to deliver higher tumor doses will be needed for larger tumors.

Tumor-selective mAbs labeled with cytotoxic radionuclides offer potential for treating many types of cancer (1, 2). The most widely used radionuclides for radioimmunotherapy (RIT) include $\beta$ emitters ($^{131}$I, $^{90}$Y, $^{67}$Cu, and $^{188}$Re), $\alpha$ emitters ($^{211}$At and $^{212}$Bi), and auger electron emitters ($^{125}$I). Preclinical RIT studies with these radionuclides have shown variable results, depending upon the isotope, dosage, and animal model system. Most studies with $^{131}$I-labeled intact mAb have demonstrated inhibition, but not long-term remission, of tumor growth (3–7) or partial remission in that some animals in the successfully treated group had tumor recurrences (3, 8–10). In studies reporting partial remissions, only small, young tumors were affected (3, 8–10). In studies with larger, older tumors, remission was not observed, and $^{131}$I doses at or near maximum tolerance were necessary for tumor inhibition, resulting in increased host toxicity with lengthy recovery intervals (8, 11). $^{90}$Y-labeled mAbs have shown therapeutic potential; however, bone marrow toxicity has limited the administered dose (12, 13). In RIT studies using xenografts of small cell lung carcinoma in nude mice, $^{186}$Re-labeled mAb produced tumor growth delays, but most tumors recurred (14). RIT with $^{212}$Bi-labeled mAb proved effective in eradicating most lymphoma and leukemic cells (15, 16), but the 60-min half-life of this isotope may limit its routine applicability with solid tumors. $^{211}$At-labeled mAb $^{51}$Cr, an mAb reactive with gliomas, prolonged survival in a meningial tumor model (17), but the short range of $\alpha$ particles (50–100 μm) limits the utility of $^{211}$At for RIT of solid tumors.

In vivo studies comparing the therapeutic potential of $^{64}$Cu with that of $^{67}$Cu have shown both isotopes to be equally effective on a per-decay basis in inhibiting cell growth and DNA synthesis (18). Recently, we have confirmed these results for free $^{64}$Cu and $^{67}$Cu and also have shown that $^{64}$Cu and $^{67}$Cu linked to bromoacetamidobenzyl-1,4,8,11-tetraazacyclotetradecane-$N,N',N'',N'''$-tetraacetic acid (BAT)-2-iminothiolane (2IT)-1A3 (1A3 is a mouse anti-human colorectal cancer mAb developed in our laboratory) exhibited similar per-decay tumor growth inhibition in vivo (19). These similar therapeutic effects occurred despite the different decay schemes of $^{64}$Cu and $^{67}$Cu; $^{64}$Cu decays by electron capture (41%), $\beta^-$ decay [0.573 MeV (1 eV = 1.602 × 10$^{-19}$ J), 40%], and $\beta^+$ decay [0.656 MeV, 19%], accompanied by emission of annihilation radiation (0.511 MeV, 38%) and $\gamma$ photons (1.34 MeV, 0.5%). This decay scheme permits pretherapy dosimetry estimation by quantitative positron emission tomography (PET). $^{67}$Cu releases abundant $\beta^-$ particles (0.59 MeV, 100%) as well as $\gamma$ emissions (0.092 MeV, 23%; 0.184 MeV, 30%) suitable for diagnostic imaging. $^{67}$Cu is accelerator-produced sporadically throughout the year, whereas $^{64}$Cu is currently produced on a weekly basis at Missouri University Research Reactor. Both the high cost and limited availability of $^{67}$Cu have restricted its applicability. In contrast, $^{64}$Cu is relatively inexpensive and consistently available at high specific activity.

mAb 1A3 is of the IgG1, $\kappa$ isotype (20) and binds to antigen(s) extracted in the methanol phase of a Folch extract. The recognized 1A3 antigen is expressed strongly in human colorectal adenocarcinomas and weakly, or not at all, in normal tissues, including normal colon (20). $^{64}$Cu-BAT-2IT-1A3 is a stable compound with high immunoreactivity and excellent tumor localization in the GW39 human colon cancer-hamster model (21). $^{64}$Cu-BAT-2IT-1A3 is currently in clinical immunoscintigraphy trials using PET detection methods and has proved to be a safe, effective imaging agent (22).

In this report we summarize our RIT results with both $^{64}$Cu- and $^{67}$Cu-BAT-2IT-1A3 in the GW39 human colon cancer-hamster model. This report of in vivo therapy with a $^{64}$Cu-labeled mAb shows the effectiveness of this newly described conjugate in inhibiting tumor growth and, under select conditions, causing complete tumor remission. In addition, absorbed doses to tumors and normal human organs are presented.

Abbreviations: RIT, radioimmunotherapy; BAT, bromoacetamidobenzyl-1,4,8,11-tetraazacyclotetradecane-$N,N',N'',N'''$-tetraacetic acid; 2IT, 2-iminothiolane; PET, positron emission tomography.

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MATERIALS AND METHODS

mAbs. mAb 1A3 was purified from serum-free media by Invitron (St. Louis, MO) with proprietary methods. Cells producing control mouse plasmacytoma MOPC 21 (IgG 
\kappa) were grown in vitro by Endotronics (Minneapolis, MN) with a hollow fiber system. MOPC was purified from this medium with E-Z-Sep (Middlesex Sciences Inc., Foxborough, MA) and Q Sepharose (Sigma). Radio-TLC was accomplished with a BIOSCAN system 200 Imaging Scanner (Bioscan, Washington, DC). Radioactivity was measured with a Beckman gamma counter. Fast protein liquid chromatography was performed as described (21).

Radioisotopes. 64Cu (t1/2 = 12.8 h) was produced and purified at the University of Missouri, Columbia Research Reactor, via the fast-neutron reaction on a natural zinc target as described (23). The specific activity of 64Cu ranged from 19,000 to 58,000 Ci/mmol (1 Ci = 37 GBq) at the time of receipt. 67Cu was produced at Brookhaven (Upton, NY) and Los Alamos National Laboratories (Los Alamos, NM) with a specific activity of 128–640 Ci/mmol.

Conjugation and Labeling. The synthesis of BAT was accomplished as described (24) with minor alterations. BAT was conjugated to 1A3 and MOPC with the linking agent 2IT and labeled with 64Cu or 67Cu as described (21).

Quality Control. Fast protein liquid chromatography was performed on a sample of each radiolabeled preparation to assess the purity as described (21). The immunoreactivity of each radiolabeled antibody preparation was routinely determined by use of suspensions of GW39 human colon cancer cells expressing 1A3 antigen as targets under conditions of antigen excess (25).

Animal Model. The GW39 human colon cancer xenografts were propagated in 7- to 9-week-old male Golden Syrian hamsters (26). Tumor cell suspensions with ≥90% viability [25% (vol/vol), 0.5 ml, ~8.2 × 10^6 cells] were injected in the right thigh of hamsters. The tumor grows exponentially in this model for 10–14 days, after which some necrosis becomes apparent. All mAbs or control buffers were given by intracardiac injection. All animal experiments were performed in compliance with guidelines specified by Washington University and Jewish Hospital Animal Studies Committees.

RIT Studies. RIT studies were performed on hamsters carrying small, 2-day-old or large, 7-day-old tumors and injected with 1–3 mCi (400 μg) of 64Cu-BAT-2IT-1A3 or 64Cu-BAT-2IT-MOPC, or 0.2–0.6 mCi (400 μg) of 67Cu-BAT-2IT-1A3 or 67Cu-BAT-2IT-MOPC. Since both the equilibrium dose constants for nonpenetrating radiations are similar for 64Cu and 67Cu (0.267 and 0.303, respectively) and since similar lethality was noted in cell kill experiments (unpublished data), these administered activities were selected to deliver similar total numbers of radioactive decays of the radionuclides based on their half-lives. Control animals received saline only. Hamsters were killed when tumors were ≥10 g (~3 cm in diameter) or after surviving tumor-free for 6–7 months. The 10-g end point was selected for humane reasons. During the first month of therapy, hamsters were weighed two times a week, and 5–10 hamsters were used for each condition; data from repeat experiments were combined. At the time of death, tumors were weighed and normal hamster tissues were examined.

Dosimetry Studies. Dosimetry was estimated from biodistribution data obtained in the hamster model for both 64Cu- and 67Cu-BAT-2IT-1A3. Tumors were implanted either 2 or 7 days before injection of radiolabeled mAb (30–50 μg mAb; 2.4–3.5 μCi/μg). Groups of four to five hamsters were killed 1–120 h after mAb injection. Tumors and tissues were weighed and then counted in an NaI(Tl) auto gamma well counter. S values for tumors (mean dose per unit cumulative activity) were calculated, assuming activity to be uniformly distributed within a tumor sphere with a mean weight of 0.4 g (small tumors) or 0.66 g (large tumors), with the Medical Internal Radiation Dose (MIRD) schema (27, 28). The S value for 64Cu is 0.573 rad/μCi-h (1 rad = 0.01 Gy) for small tumors and 0.386 rad/μCi-h for large tumors. For 67Cu, the S value is 0.668 rad/μCi-h for small tumors and 0.456 rad/μCi-h for large tumors (28, 29).

For normal human organs, cumulative activity (μCi-h) per mCi per organ was determined by assuming the same %ID per organ in the human as the hamster, then integrating the area under time-activity curves with the computer program KALEIDA GRAPH. The S values for 64Cu and 67Cu were obtained from MIRDose3 (28). Bone activity was assumed to be located half in trabecular bone and half in the cortical bone. The cumulative activity in the marrow was determined as described (29).

Statistical Methods. To compare survival among different treatment groups, the SAS Lifetests procedure was used to generate Kaplan–Meier probability density plots, and these data were then analyzed using log-rank, Wilcoxon, and -2log(LR) statistics and the P values were reported.

RESULTS

Radiolabeling of mAbs. Specific activities of 10 μCi/μg (64Cu-BAT-2IT-1A3) or 2 μCi/μg (64Cu-BAT-2IT-1A3) were achieved with corresponding immunoreactivity values of 88% and 83%. Specific activities of 64Cu (64Cu-BAT-2IT-MOPC) or 1.5 μCi/μg (64Cu-BAT-2IT-MOPC) were achieved with 3–26% binding in the immunoreactivity assay. Fast protein liquid chromatography indicated radiochemical purity of >95% for 64Cu- and 67Cu-BAT-2IT-1A3 and >85% for 64Cu- and 67Cu-BAT-2IT-MOPC.

RIT Studies. These studies showed therapeutic effects of 64Cu- and 67Cu-BAT-2IT-1A3 in the GW39 hamster model. The results with 2-day-old tumors (Fig. 1A) showed significantly (P < 0.0007) improved survival rates for hamsters treated with high doses of 64Cu- and 67Cu-BAT-2IT-1A3 compared with both 64Cu- and 67Cu-BAT-2IT-MOPC- and saline-treated control hamsters. Seven months after treatment (duration of experiment), 82% of hamsters (14 of 17) treated with 2 mCi of 64Cu-BAT-2IT-1A3 and 93% of hamsters (14 of 15) survival.
15) treated with 0.4 mCi of $^{64}$Cu-BAT-2IT-1A3 were alive and tumor-free. In contrast, 98% of hamsters (46 of 47) treated with saline had to be killed by 7 weeks because their tumors had grown to ≥10 g. Hamsters treated with high doses of $^{64}$Cu- or $^{65}$Cu-BAT-2IT-MOPC (2 or 0.4 mCi, respectively) or with low doses of $^{64}$Cu- or $^{65}$Cu-BAT-2IT-1A3 (1 or 0.2 mCi, respectively) had intermediate survival. These groups showed prolonged survival compared with saline-treated controls (P < 0.01), but the majority of tumors regrew by the end of the experiment. A survival of 11% was seen with both 1 mCi of $^{64}$Cu-BAT-2IT-1A3 (1 of 9) and 2 mCi of $^{64}$Cu-BAT-2IT-MOPC (2 of 18). Treatment with 0.2 mCi of $^{64}$Cu-BAT-2IT-1A3 resulted in 25% survival (2 of 8), whereas 0.4 mCi of $^{67}$Cu-BAT-2IT-MOPC gave 37.5% survival (6 of 16).

In all RIT treatment groups, hamsters appeared healthy. They gained weight at the same rate as untreated controls and had no gross visceral abnormalities or histologic abnormalities of the liver or kidneys.

Several tumors that regrew after RIT with mAb 1A3 were examined by immunoperoxidase techniques to determine whether 1A3 antigen was still being expressed by surviving GW39 cells. In all six tumors examined, 1A3 antigen staining was similar in both pattern and intensity to control tumors of the same size.

The effectiveness of each treatment protocol was also evaluated by determining the mean lifespan of each hamster group. Most hamsters (25 of 32) treated with high dose $^{64}$Cu- or $^{67}$Cu-BAT-2IT-1A3 were alive and tumor-free at the 7-month (245-day) experimental end point. In contrast, saline-treated hamsters had to be killed because of the large tumor size by 35 ± 21 days. Nonspecific Cu-labeled MOPC and low-dose Cu-labeled 1A3 showed intermediate results with lifespan prolonged two to four times that of saline-treated hamsters.

Despite the use of larger doses in the studies of animals with large, 7-day-old tumors, only one complete response was observed (0.6 mCi of $^{67}$Cu-labeled 1A3). All other tumors reached the 10-g end point before 6 months. The mean lifespan of hamsters receiving 3 mCi of $^{64}$Cu-BAT-2IT-1A3 (48.9 ± 14.8 days) was only slightly extended compared with that of hamsters treated with 3 mCi of $^{64}$Cu-BAT-2IT-MOPC (33.7 ± 4.4 days) or saline (33.0 ± 16.7 days). With 0.6 mCi of $^{67}$Cu-BAT-2IT-1A3, the mean lifespan (107.5 ± 69.1 days) was extended more than 2-fold compared with that of hamsters treated with 0.6 mCi of $^{67}$Cu-BAT-2IT-MOPC (45.4 ± 6.4 days) and saline-treated control hamsters (41.7 ± 11.13 days).

**Dosimetry.** The tumor doses in hamsters with small, 2-day-old tumors injected with 2 mCi of $^{64}$Cu-BAT-2IT-1A3 or 0.4 mCi of $^{67}$Cu-BAT-2IT-1A3 were 586 rad (293 rad/mCi) and 1269 rad (3174 rad/mCi), respectively. The corresponding tumor doses in hamsters bearing large, 7-day-old tumors injected with 3 mCi of $^{64}$Cu-BAT-2IT-1A3 or 0.6 mCi of $^{67}$Cu-BAT-2IT-1A3 were 330 rad (110 rad/mCi) and 706 rad (1176 rad/mCi), respectively. Normal organ absorbed doses for $^{64}$Cu- and $^{67}$Cu-labeled 1A3 (Table 1) show the critical organs for $^{64}$Cu- and $^{67}$Cu-BAT-2IT-1A3 were the upper large intestine (0.51 rad/mCi) and bone surface (4.77 rad/mCi), respectively.

**DISCUSSION**

Our results show that $^{64}$Cu linked to an mAb is capable of inhibiting tumor cell growth in an animal model. The anti-colorectal-cancer mAb (1A3) labeled with $^{64}$Cu or $^{67}$Cu caused complete remission of small tumors in the GW39—hamster model. $^{64}$Cu- or $^{67}$Cu-labeled nonspecific control mAb (MOPC), at the highest administered activity, was also able to temporarily suppress tumor growth, but most of these MOPC-treated tumors recurred in the 6–7 months of this study. Further, no signs of toxicity were observed in hamsters treated with either $^{64}$Cu- or $^{67}$Cu-BAT-2IT-1A3 at administered activities that caused total tumor suppression.

The RIT studies have established that $^{64}$Cu can inhibit tumor growth when linked to a tumor-selective mAb. These studies have also demonstrated that despite differing decay schemes, $^{64}$Cu and $^{67}$Cu have similar therapeutic effects in this model. Tumor remission occurred with a 586-rad tumor dose for $^{64}$Cu-BAT-2IT-1A3 and a 1269-rad tumor dose for $^{67}$Cu-BAT-2IT-1A3. With both copper isotopes, the radiation absorbed dose was delivered at a similar, low mean dose rate (~8 rad/h for $^{64}$Cu and ~6 rad/h for $^{67}$Cu, determined using the time interval required to deliver 90% of the dose). In these experiments, to account for the different half-lives of $^{64}$Cu and $^{67}$Cu, five times more $^{64}$Cu-BAT-2IT-1A3 than the $^{67}$Cu agent was administered. The absorbed doses to the tumor that caused remission were about two times greater for $^{67}$Cu than for $^{64}$Cu. This result is partly due to the fact that maximal tumor uptake of intact mAb 1A3 did not occur until 24 h after injection, when the $^{64}$Cu had decayed through about two half-lives. Increased tumor delivery of $^{64}$Cu may be achieved with the use of agents, such as mAb fragments, that have more rapid tumor uptake (4, 30) or with pretargeting approaches (31).

Table 2 compares our results with those in other RIT studies that similarly used intact mAb in colon cancer models. The results indicate that tumor size at time of treatment is critical and that, to cause remission of bigger (~0.3 cm³) tumors, larger doses are required than were administered in most studies, including our own. In the study that had comparable tumor remission frequencies for both small and large tumors (3, 11), the tumor dose to large tumors was 3-fold (7200 rad) that received by small tumors (2400 rad). In none of the other reported studies with comparable sized, or larger tumors, did tumor dose approach this level (4, 5, 32–34). A study using a combination of four $^{131}$I anti-carcinoembryonic antigen F(ab')2 fragments also was successful at causing regression of large T380 colon tumors (0.5–1.5 g) at an absorbed dose of 9000 rad (35). Tumor regression has also been seen in dose fractionation studies (7, 11, 36). The range of absorbed tumor doses shown to cause tumor cell growth inhibition and regression in a variety of preclinical models has been reviewed (2). Tumor doses ranging from 19 to 18,700 rad were reported (2). This variation may in part reflect tumor size at initiation of therapy (8, 11), inherent differences of tumor radiosensitivity, and different assumptions made for dosimetry calculations with different models (37).

**Table 1. Human absorbed dose estimates**

<table>
<thead>
<tr>
<th>Organ</th>
<th>$^{64}$Cu-TETA-1A3, rad/mCi (mGy/MBq)</th>
<th>$^{67}$Cu-TETA-1A3, rad/mCi (mGy/MBq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenals</td>
<td>0.114 (0.032)</td>
<td>0.346 (0.094)</td>
</tr>
<tr>
<td>Bladder wall</td>
<td>0.069 (0.019)</td>
<td>0.235 (0.064)</td>
</tr>
<tr>
<td>Bone surfaces</td>
<td>0.216 (0.058)</td>
<td>4.770 (1.289)</td>
</tr>
<tr>
<td>Stomach wall</td>
<td>0.230 (0.062)</td>
<td>0.989 (0.267)</td>
</tr>
<tr>
<td>SI</td>
<td>0.179 (0.048)</td>
<td>0.695 (0.188)</td>
</tr>
<tr>
<td>ULI</td>
<td>0.508 (0.137)</td>
<td>2.252 (0.609)</td>
</tr>
<tr>
<td>LLI</td>
<td>0.475 (0.128)</td>
<td>2.347 (0.634)</td>
</tr>
<tr>
<td>Heart wall</td>
<td>0.119 (0.032)</td>
<td>0.417 (0.113)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.329 (0.089)</td>
<td>1.703 (0.460)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.465 (0.126)</td>
<td>1.881 (0.508)</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.131 (0.035)</td>
<td>0.453 (0.122)</td>
</tr>
<tr>
<td>Red marrow</td>
<td>0.249 (0.067)</td>
<td>0.996 (0.269)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.082 (0.022)</td>
<td>0.289 (0.078)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.136 (0.037)</td>
<td>0.625 (0.169)</td>
</tr>
</tbody>
</table>

Tumor dosimetry was estimated from biodistribution data obtained in the hamster model for both $^{64}$Cu- and $^{65}$Cu-BAT-2IT-1A3, as detailed in Materials and Methods. SI, small intestine; ULI, upper large intestine; LLI, lower large intestine.
Survival studies with hamsters carrying large (7-day-old) tumors at initiation of therapy showed considerable variation in responsiveness to both $^{64}$Cu- and $^{64}$Cu-BAT-21T-1A3 with negligible tumor remission. The absorbed dose for large tumors in animals injected with 3 mCi of $^{64}$Cu-BAT-21T-1A3 was 303 rad compared with an absorbed dose of 586 rad for small tumors, and for hamsters injected with 0.6 mCi of $^{67}$Cu-BAT-21T-1A3, the dose to the large tumors was 706 rad compared with an absorbed dose of 1269 rad for small tumors. Tumor dosimetry in the smaller tumors suggests that administered activities of 7 mCi of $^{64}$Cu-BAT-21T-1A3 and 0.8 mCi of $^{67}$Cu-BAT-21T-1A3 would be more effective at inhibiting growth of these larger tumors. To minimize bone marrow and liver toxicity at these doses, dose fractionation may be required. Several studies have documented that both reduced toxicity and dose escalation can be achieved with dose fractionation (7, 16, 35–37).

Other difficulties in targeting large tumors include the observation that small tumors have a greater fractional uptake of the administered dose of mAb per unit mass of tumor than do large tumors (3, 35). Additionally, larger quantities of mAbs may be needed to effect tumor saturation in large tumors than in small ones (20), and small tumors, in general, are more radiosensitive than large tumors. Also, with large tumors, penetration of intact mAb into the tumor is slow; this problem is frequently ameliorated by use of mAb fragments (4, 30). Ideally, the design of experiments to maximize mAb tumor uptake in combination with dose fractionation approaches may produce higher success rates with large tumors, as others have shown (7, 14, 36, 37).

Currently, dose fractionation studies with $^{64}$Cu- and $^{67}$Cu-labeled mAb are difficult because of the limited availability of both radionuclides. In this study, 90% of the total dose to the tumor was delivered within 66 h of injection of the $^{64}$Cu-labeled mAb. Theoretically, by 66 h, any cell(s) with clonogenic potential would be capable of initiating tumor regrowth. Thus, additional doses of $^{64}$Cu-BAT-21T-1A3 should ideally be given within 2–5 days of the initial dose to affect the greatest number of tumor cells. This protocol should optimize any potential therapeutic enhancement of dose fractionation, while minimizing increases of human anti-mouse antibody (HAMA) in a clinical setting, since the primary HAMA response takes more than 1 week to occur (38). When $^{64}$Cu becomes available on a daily basis, future studies using $^{64}$Cu-BAT-21T-1A3 will include dose fractionation with short time intervals to assess therapeutic effects on larger tumors as well as to examine effects on normal tissue.

Our immunoperoxidase staining results in those tumors that regrew suggest that antigen modulation was not responsible for tumor regrowth, as has been suggested to be the case with other tumor models (13).

In biodistribution studies, mean tumor/muscle ratios of 11.5 and 60.0 were seen with $^{64}$Cu-labeled MOPC and 1A3, respectively, indicating some tumor binding by MOPC (ref. 21; unpublished data). The observation that high doses of $^{64}$Cu-BAT-21T-MOPC were less effective at inhibiting tumor growth (11% survival) than $^{67}$Cu-BAT-21T-MOPC (37.5% survival) may reflect effects of the longer half-life of $^{67}$Cu, the variability inherent in the model system, or both.

In a study using $^{67}$Cu-BAT-21T-Lym-1 (Lym-1 is an antilymphoma mAb), no toxicity at doses of 155–165 μCi was seen in nude mice carrying Raji lymphoma tumors (39); further, this radiopharmaceutical has been safely administered in clinical RIT studies with minimal toxic effects (40). No in vivo therapy studies using $^{64}$Cu-labeled mAb have been reported previously. In this study, the lack of toxicity observed in hamsters with doses of $^{64}$Cu-BAT-21T-1A3 that caused tumor regression suggests that the therapeutic potential of this radionuclide should be studied further with mAbs as well as with other tumor-targeting agents.

In the United States, $^{67}$Cu can be obtained only from high-energy accelerator facilities. Currently, only Brookhaven and Los Alamos National Laboratories produce $^{67}$Cu. Moreover, published studies with $^{67}$Cu-BAT-21T-Lym-1 report results with only three to five patients (40), primarily because of the limited availability of the radionuclide. At present, the availability of $^{64}$Cu is also limited, because it can be produced only at a small number of nuclear reactor facilities. However, clinical Phase 1/II PET imaging studies with $^{64}$Cu-BAT-21T-1A3 have included over 36 patients during a 12-month period (22). For the therapy studies described here, $^{64}$Cu was obtained two to three times per month from Missouri University Research Reactor. The production of $^{64}$Cu on a biomedical cyclotron from a $^{64}$Ni target has been described (41, 42). At Washington University, we are currently developing the technology to produce large amounts of $^{64}$Cu on a biomedical cyclotron on a daily basis; in preliminary studies 600 mCi have been produced following a 3-h cyclotron irradiation (43).

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Table 2. Colon cancer preclinical RIT results*

<table>
<thead>
<tr>
<th>Model system</th>
<th>mAb</th>
<th>Radionuclide</th>
<th>Tumor size†</th>
<th>Activity administered, mCi</th>
<th>Mean tumor dose, rad</th>
<th>Tumor response‡</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GW39†</td>
<td>1A3</td>
<td>$^{64}$Cu</td>
<td>0.2 g</td>
<td>2.0</td>
<td>586</td>
<td>82%</td>
<td>**</td>
</tr>
<tr>
<td>GW39†</td>
<td>1A3</td>
<td>$^{64}$Cu</td>
<td>0.6 g</td>
<td>3.0</td>
<td>330</td>
<td>GI</td>
<td>**</td>
</tr>
<tr>
<td>GW39†</td>
<td>1A3</td>
<td>$^{67}$Cu</td>
<td>0.2 g</td>
<td>0.4</td>
<td>1269</td>
<td>93%</td>
<td>**</td>
</tr>
<tr>
<td>GW39†</td>
<td>1A3</td>
<td>$^{67}$Cu</td>
<td>0.6 g</td>
<td>0.6</td>
<td>706</td>
<td>GI</td>
<td>**</td>
</tr>
<tr>
<td>GW39‡</td>
<td>NP4</td>
<td>$^{131}$I</td>
<td>&lt;0.2 cm³</td>
<td>1.0</td>
<td>2400</td>
<td>55%</td>
<td>3</td>
</tr>
<tr>
<td>GW39‡</td>
<td>NP4</td>
<td>$^{131}$I</td>
<td>0.3–0.45 cm³</td>
<td>3.0</td>
<td>7200</td>
<td>55%</td>
<td>11</td>
</tr>
<tr>
<td>GW39‡</td>
<td>NP4</td>
<td>$^{90}$Y</td>
<td>0.3 cm³</td>
<td>0.05</td>
<td>1603</td>
<td>GI</td>
<td>32</td>
</tr>
<tr>
<td>COLO 205§</td>
<td></td>
<td>$^{17}$-1A</td>
<td>0.27 cm³</td>
<td>1.0</td>
<td>700</td>
<td>GI</td>
<td>3</td>
</tr>
<tr>
<td>T808§</td>
<td></td>
<td>$^{35}$B,B17</td>
<td>$^{131}$I</td>
<td>0.5 cm³</td>
<td>5642</td>
<td>GI</td>
<td>4</td>
</tr>
<tr>
<td>LS1747§</td>
<td>17-1A</td>
<td>$^{131}$I</td>
<td>0.52 cm³</td>
<td>0.15</td>
<td>953</td>
<td>GI</td>
<td>33</td>
</tr>
<tr>
<td>LS1747§</td>
<td>17-1A</td>
<td>$^{90}$Y</td>
<td>0.52 cm³</td>
<td>0.25</td>
<td>1790</td>
<td>GI</td>
<td>33</td>
</tr>
<tr>
<td>LS1747§</td>
<td>ZCE</td>
<td>0.025</td>
<td>0.5 cm³</td>
<td>0.12</td>
<td>3400</td>
<td>GI</td>
<td>34</td>
</tr>
</tbody>
</table>

*Included are RIT studies that have positive results in colorectal cancer models and that used intact mAbs and presented tumor dosimetry. Indicated tumor carried in 1 hamster thigh, 2 hamster cheek pouch, or 3 nude mice.
†Tumor size at the time of RIT treatment in g or volume.
‡Tumor remission or tumor growth inhibition (GI). Studies with GI had no permanent remission.
**Present study.

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