Pretargeted α Emitting Radioimmunotherapy Using ^{213}Bi 1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-Tetraacetic Acid-Biotin

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ABSTRACT

Purpose: **The use of an α emitter for radioimmunotherapy has potential advantages compared with β emitters. When administered systemically optimal targeting of intact antibodies requires >24 h, therefore limiting the use of short-lived α emitters. This study investigated the biodistribution of bismuth-labeled biotin in A431 tumor-bearing mice pretargeted with antibody B3-streptavidin (B3-SA) and examined the therapeutic efficacy of the α emitter, ^{213}Bi-labeled biotin.**

Experimental Design: **Biotinidase-resistant 7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA)-biotin was radiolabeled with ^{205,206}Bi or ^{213}Bi. Treatment of tumor-bearing mice began by administration of B3-SA (400 μg) to target the tumor sites for 24 h. Then, an agent containing biotin and galactose groups was used to clear the conjugate from the circulation. Four h later, bismuth-radiolabeled DOTA-biotin was given, and biodistribution or therapy was evaluated. Dose escalation treatment from 3.7–74 MBq was performed, and the effects on tumors of different sizes were investigated. Tumor growth, complete blood cell counts, toxicity, and survival were monitored.**

Results: **Radiolabeled biotin cleared rapidly. Rapid tumor uptake resulted in much higher tumor:nontumor targeting ratios than achieved with the directly labeled monoclonal antibody. Dose escalation revealed that 74 MBq caused acute death of mice, whereas 0.37–37 MBq doses inhibited tumor growth and prolonged survival significantly. Evidence of mild hematological toxicity was noted. At therapeutically effective doses renal toxicity was observed.**

Conclusions: **^{213}Bi-DOTA-biotin, directed by the Pretarget method to tumor-targeted B3-SA, showed a therapeutic effect, although the therapeutic index was low. The source of the toxicity was most likely related to the renal toxicity.**

INTRODUCTION

Clinical studies using β^- particle-emitting radiolabeled monoclonal antibodies (MoAbs) for the radioimmunotherapy of lymphomas have shown promising results (1–4) and have resulted recently in Food and Drug Administration approval of ibritumomab tiuxetan, the first radiolabeled antibody approved for radioimmunotherapy (5, 6). In contrast, numerous studies performed with MoAbs directed against various epithelial tumors have only rarely shown partial or complete remissions (7–10). This lack of antitumor effect is thought to be due to the limited radiation dose delivered together with the lower radiosensitivity of most epithelial tumors when compared with lymphomas. However, in the case of lymphoma, when higher doses were delivered in the setting of myeloablation and bone marrow rescue, a higher incidence of complete remissions and longer duration of remissions are observed (11). Approaches are needed that result in the delivery of higher tumor concentrations of radiolabeled MoAb with relative sparing of normal tissues.

Compared with β^- emitters, α emitters have certain theoretical advantages, higher linear energy transfer with DNA damage that is difficult to repair, reduced nonspecific irradiation to normal tissues around the target cells because of their shorter path lengths (μm), and hypoxia-insensitive cytotoxicity. Bismuth radioisotopes (^{212}Bi and ^{213}Bi) and ^{211}At have been used in preclinical trials, and some clinical applications are currently being explored (12–14). Because of its short half-life (46 min), ^{213}Bi will be limited to systems with rapid delivery and targeting. Rapid delivery can be obtained when targeting circulating cells or cellular targets that are rapidly accessible, such as in the endothelial vasculature, spleen, and bone marrow, or when local delivery can be performed (14–16). In most clinical situations, optimal targeting of both hematological and epithelial malignancies with intact antibodies requires >24 h. Therefore, pretargeting approaches, in which delivery of the large molecular weight antibody and the small molecule radionuclide are uncoupled from each other, appear to be reasonable alternatives for tumor targeting with short-lived radionuclides.

Intact immunoglobulins have limited accessibility into solid tumors because of their size (17). In addition, because of increased pressure gradients in tumors, the kinetics of delivery are slow (18). Furthermore, there is often heterogeneity in the distribution of intact IgG because of binding site barriers (19). Several approaches have been tried to overcome these problems, including utilization of small molecular weight fragments that

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target tumors faster (17) or alternatively using intact antibodies that can be modified for use in pretargeting protocols (20–22). One approach, termed the Pretarget method, has been developed by NeoRx Corporation (Seattle, WA; Refs. 23–25). This approach is based on the streptavidin (SA) biotin system (20). The Pretarget method consists of three steps: (*a*) the antibody-SA conjugate is administered and allowed to target and accumulate in the tumor, thus carrying the SA receptor that can later bind the radiolabeled biotin; (*b*) the nontumor-bound antibody-SA is removed from the circulation by administration of a synthetic clearing agent to prevent it from binding the biotin-radionuclide in the circulation; and (*c*) the biotin-radionuclide is administered i.v. for delivery of radiotherapy. Because of its small size, the radiolabeled biotin extravasates out of the circulation quickly, where it can bind to the SA on the antibody-SA conjugate that has already localized in the tumor. In addition, because of its small size the intravascular radioactivity clears very rapidly through the kidneys. Preclinical studies have characterized the pharmacokinetics of the system and optimized the three reagents used in this approach (25). In addition, preclinical trials with genetically synthesized single-chain Fv-derived tetravalent fusion proteins have also shown favorable kinetics and biodistribution (26). Clinical trials have shown the feasibility of using this Pretarget approach to deliver large radiation doses to target tissue (23, 24). We also demonstrated recently that cures could be achieved in an adult T-cell leukemia scid/nod mice tumor xenograft model using the ^{213}Bi anti-Tac streptavidin conjugate (16).

We have shown previously that B3, an anti-Le^y murine IgG1 monoclonal antibody, can efficiently target A431 tumor xenografts in nude mice (27). This work led to a Phase I therapy trial with ^{111}In and ^{90}Y B3 in patients with a variety of tumor types bearing the target antigen. In those studies, we demonstrated good tumor targeting, but no therapeutic responses were observed, presumably because of the limited dose delivered (28). We have demonstrated recently that the B3 monoclonal antibody conjugated to streptavidin (B3-SA) concentrated significantly in our A431 tumor xenograft model (29). Despite somewhat inhomogeneous tumor concentration, as demonstrated by autoradiography, pretargeted ^{90}Y 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) biotin, with its long-range β^- particle, was able to deliver a sufficient dose to result in tumor cures. In the current study, we investigated the feasibility of the three-step Pretarget approach using radiolabeled bismuth ^{213}Bi-DOTA-biotin and the same B3-SA conjugate.

MATERIALS AND METHODS

Monoclonal Antibody. B3 is a murine IgG1k monoclonal antibody developed by immunizing BALB/c mice with trypsin-treated MCF-7 breast carcinoma cells. B3 reacts with a carbohydrate epitope found on the Le^y and the polyfucosylated-Le^x antigens. The epitope is present on many glycoproteins, and is abundantly and uniformly expressed by most carcinomas, including adenocarcinomas of the stomach, colon, breast, lung, and bladder, and the mucinous cystadenocarcinoma of the ovary (30). The humanized anti-Tac monoclonal antibody (HAT) is a humanized version of murine anti-Tac that recognizes CD25, the cell surface interleukin 2 α receptor, but does not react with the A431 cell line and, thus, was used as a negative control (31, 32). HAT was obtained from Hoffmann LaRoche (Nutley, NJ).

Pretarget Reagents. B3 was conjugated to SA by using succinimidyl 4-(*N*-maleimido-methyl) cyclohexane-1-carboxylate as described previously (25, 29). To determine that the B3-SA conjugate was still functional, ^{111}In-DOTA-biotin was incubated with excess B3-SA. The bound fraction was then determined by paper chromatography developed with saline and was >99% (3MM; Whatman International Ltd., Maidstone, England). The bound fraction was also >99% when measured using avidin gel (Pierce Biotechnology, Rockford, IL). Previous studies showed good localization of B3-SA in our tumor system after administration of 400 μg and optimal tumor localization by 48 h (29). HAT-SA was prepared using chemistry similar to that of B3-SA (16).

Synthetic clearing agent (sCA) was provided by the NeoRx Corp. This reagent consists of a bifunctional moiety with multiple *N*-acetyl-galactosamine residues linked to a single biotin (molecular weight = 8651; Ref. 33). The biotin on the sCA binds rapidly to circulating antibody-SA conjugate. This reagent has been shown to clear antibody-SA conjugates rapidly out of the circulation and into the liver via the Ashwell receptors present in the liver (26). Our previous studies administering 100 μg of sCA to mice receiving 400 μg of radiolabeled B3-SA showed a mean drop of 90% of the circulating antibody-SA conjugate, indicating that the sCA was functional and effective (29). Therefore, the dose of sCA selected for this study was 100 μg. Biotinidase-resistant DOTA-biotin (molecular weight = ~900; NeoRx Corp.) was prepared as described previously (34). B3 was conjugated to CHX-A″ as described previously (35). The CHX-A″ conjugate was then labeled with ^{205,206}Bi for biodistribution studies comparing directly labeled antibody to pretargeted radionuclide-antibody delivery.

Radiolabeling. Although our interest was in radioimmunotherapy with ^{213}Bi, the short half-life and limited availability led us to perform our initial biodistribution studies for proof of concept using the longer lived γ-emitting mixture of ^{205,206}Bi that has a half-life of 15.21 and 6.243 days, respectively. The ^{205,206}Bi was produced in the positron emission tomography department of the NIH by irradiation of a high purity lead target (36) with 25 MeV protons using a CS-30 cyclotron, and purification was performed as described previously (37).

For radioimmunotherapy, ^{213}Bi was used. The ^{213}Bi was eluted from a ^{225}Ac generator (38). The ^{225}Ac (Oak Ridge National Laboratories, Oak Ridge, TN) was dissolved in 0.1 M HNO_3 and loaded onto an MP-50 cation exchange resin preequilibrated with 0.1 M HNO_3. The breakthrough of ^{225}Ac was ~83 parts/million as determined by measuring the ^{213}Bi activity 16 h after elution using a germanium lithium high-resolution detector.

Biotinidase-resistant DOTA-biotin was radiolabeled with ^{205,206}Bi at a specific activity of 1.46 MBq/μg based on the method of Axworthy *et al.* (25). In brief, DOTA-biotin was added to the purified ^{205,206}Bi, and the pH was adjusted to 5–5.5 with 5 M NH_4OAc and then incubated for 12 min at 90°C. To scavenge unreacted Bi, 5 μl of 5 mM DTPA was added to the reaction mixture. The radiochemical yield and bindability of radiolabeled DOTA-biotin was >99% as measured using an

Table 1 Biodistribution of bismuth-labeled DOTA[a]-biotin after pretargeting of B3-SA or directly labeled ^{205,6}Bi-CHX-B3 in nude mice bearing A431 tumor xenografts.

The radioactivity is expressed as %ID/g tissue (mean ± SD).

	^{213}Bi-biotin		^{205,206}Bi-biotin				^{205,6}Bi-CHX-B3
	5 min	15 min	30 min	1 h	2 h	4 h	4 h
Blood	9.29 ± 1.68	5.21 ± 2.83	2.04 ± 0.38	2.14 ± 0.47	2.04 ± 0.37	2.22 ± 0.64	26.42 ± 0.88
Liver	2.62 ± 0.56	1.89 ± 0.53	1.33 ± 0.29	1.16 ± 0.47	1.03 ± 0.19	1.28 ± 0.45	11.45 ± 1.30
Kidney	15.39 ± 2.08	7.86 ± 0.65	2.63 ± 0.30	2.23 ± 0.37	1.93 ± 0.21	2.32 ± 0.39	9.80 ± 0.48
Intestine	1.69 ± 0.51	1.41 ± 1.51	0.49 ± 0.05	0.91 ± 0.53	0.57 ± 0.20	1.25 ± 0.37	2.36 ± 0.27
Stomach	2.43 ± 1.14	0.99 ± 0.33	1.71 ± 0.54	3.51 ± 2.62	2.01 ± 2.29	0.76 ± 0.13	2.16 ± 0.37
Spleen	9.74 ± 11.6	1.07 ± 0.39	0.45 ± 0.08	0.44 ± 0.07	0.42 ± 0.08	0.47 ± 0.13	5.57 ± 0.93
Lung	6.11 ± 0.95	3.06 ± 0.86	1.84 ± 0.27	1.74 ± 0.35	1.40 ± 0.16	1.63 ± 0.33	11.33 ± 2.58
Bone	2.37 ± 0.15	1.57 ± 0.13	0.88 ± 0.25	0.75 ± 0.21	0.57 ± 0.14	0.59 ± 0.18	3.89 ± 0.18
Tumor	5.19 ± 1.60	6.26 ± 1.95	13.06 ± 1.71	10.08 ± 2.90	14.95 ± 2.23	9.25 ± 1.44	6.70 ± 1.90

[a] DOTA, 7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; %ID/g, percentage of the injected dose per gram.

avidin gel. For radioimmunotherapy studies, the DOTA-biotin was labeled with ^{213}Bi as described above at specific activities of 37–88 MBq/μg.

For comparison of biodistribution, B3 CHX-A″antibody conjugate was labeled with ^{205,206}Bi at a specific activity of >37 kBq/μg. Unmodified B3 and B3-SA was also labeled with ^{125}I using *p*-iodobenzoate method (PIB; Perkin-Elmer Life Sciences, Inc., Boston, MA) at specific activity ~37 kBq/μg for immunoreactivity measurements (29).

Cell Line. A431, a human epidermoid carcinoma cell line that expresses the antigen recognized by B3, but not by HAT, was used. This cell line was used for immunoreactivity determination and for the development of tumor xenografts. Cells were grown in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μg/ml) at 37°C in a moist atmosphere with 5% CO_2. Cells were harvested with EDTA-trypsin and resuspended in PBS for immunoreactivity assay and mouse inoculation for tumor xenograft generation.

Immunoreactivity Assay. The immunoreactivity of the B3-SA conjugate used for these experiments was determined previously by labeling with ^{125}I (68–73%) and was in the range of the unmodified ^{125}I-labeled B3 parent MoAb (29). The immunoreactivity of ^{205,206}Bi-labeled B3 CHX-A″ was also determined using the same binding assay. In brief, a constant concentration of ^{205,206}Bi-labeled B3 CHX-A″ (5 ng) was incubated with 2×10^4 to 2×10^6 of A431 cells for 2 h at 4°C. Cell-bound radioactivity was separated by centrifugation and counted in a gamma counter. The cell-bound fraction of ^{205,206}Bi-labeled B3 CHX-A″ was 66%.

Tumor Model. Animal experiments were performed under a protocol approved by NIH Animal Care and Use Committee. Athymic female mice (*nu/nu*) were inoculated s.c. (0.1 ml) with 3×10^6 A431 cells in the right flank. Biodistribution studies were performed ~12 days after inoculation when tumors reached ~0.5 cm in maximal diameter. To evaluate the effect of tumor size on response to radioimmunotherapy with ^{213}Bi-DOTA-biotin, some mice with tumor xenografts were studied at earlier or later times after inoculation when the tumors were smaller or bigger than those generally obtained at 12 days. Seven days before all of the pretargeting experiments, the mice were fed with a biotin-free diet (Biotin Deficient Purina Diet 5836C; Purina Mills, Richmond, IN) to reduce the endogenous biotin level (29). Twenty-four h after injection of radiolabeled DOTA-biotin, their regular diet was resumed. Mice were sacrificed when the tumor size reached >2 cm in the longest diameter, the tumor was ulcerated, or excessive weight loss (>25%) was noted, according to our NIH animal protocol guidelines.

Biodistribution. Tumor-bearing mice were injected via the tail vein with 400 μg/0.2 ml of B3-SA conjugate for pretargeting. After allowing tumor pretargeting of B3-SA for 24 h, 100 μg/0.1 ml of sCA was given to clear the circulating B3-SA conjugate from the blood. Four h later, ^{205,206}Bi-labeled DOTA-biotin (1 μg) typically in 0.2 ml was injected i.v. In all of the studies, 1 μg of DOTA-biotin was used, by adding cold carrier, if necessary. Groups of 5 mice were sacrificed at 0.5, 1, 2, and 4 h after injection of the pretargeted radiolabel. In a separate experiment to determine the reproducibility of pretargeting, the biodistribution measurements were repeated at 1 h (data not shown) and 4 h after pretargeted ^{205,206}Bi-labeled DOTA-biotin was injected. In addition, the biodistribution of ^{205,206}Bi directly labeled to B3-CHX-A″ (5 μg) was determined at 4 h in a separate group of mice and then compared with the pretargeted biodistribution. Additional biodistribution studies were performed at 5 min and 15 min after pretargeted ^{213}Bi-DOTA-biotin was administered. Tumors and all of the major organs were harvested, weighed, and counted in a gamma counter. Counting of the carcasses was also performed to obtain the whole body clearance by accounting for all of the radioactivity in the mice. The percentage of the injected dose per gram (%ID/g) in tissue was calculated for each organ and normalized to a 20-g mouse (Table 1). Tumor:tissue ratios were also determined. To determine whether tumors size had an effect on %ID/g accumulation of pretargeted bismuth-DOTA-biotin we took the largest and the smallest tumor and compared the differences in %ID/g accumulated [(%ID/g large tumor − %ID/g small tumor)/%ID/g small tumor] for each of the biodistribution times (Table 2).

Dosimetry. The mean %ID/g of pretargeted ^{205,206}Bi-DOTA-biotin in tumor (Table 1) at the various time points sampled were extrapolated to ^{213}Bi and corrected for the different half-lives, and the transformed data were used to obtain the area under the time activity curve from 5 min to 4 h using trapezoidal integration (GraphPad Prism, San Diego, CA). After

Table 2 Effect of tumor size on %ID/g[a] of pretargeted bismuth-DOTA-biotin

Time	Small tumor (g)	Large tumor (g)	%difference in uptake[b]
5 min	0.089	0.509	−37
15 min	0.197	0.427	46
30 min	0.151	0.480	−5
1 h	0.247	0.532	29
2 h	0.104	0.234	−14
4 h	0.089	0.575	7
Mean	0.146	0.459	4

[a] %ID/g, percentage of the injected dose per gram; DOTA, 7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid.

[b] %uptake large tumor−%uptake small tumor)/%uptake small tumor.

4 h, the activity was assumed to disappear with the effective terminal half-life of the observed disappearance curve obtained by fitting the biodistribution data to a single exponential (GraphPad Prism). The ^{213}Bi dosimetry was then calculated using the Medical Internal Radiation Dose Committee method (39). The dose delivered was then computed by multiplying the area underneath the curve by the mean energy emitted per nuclear transition of the α particles from ^{213}Bi decay (12).

Therapy. Radioimmunotherapy was performed in A431 tumor-bearing mice using ^{213}Bi DOTA-biotin after pretargeting with B3-SA, and clearance from the blood was performed, as described above in the pretargeting reagent section. Initially, groups of 5 mice each were treated in a dose escalation trial with 3.7, 9.25, 18.5, and 37 MBq of ^{213}Bi-DOTA-biotin and compared with a no-treatment control group (therapy trial 1).

In a second experiment (therapy trial 2), a group of 10 mice was treated (10 days after tumor inoculation) with 37 MBq ^{213}Bi-DOTA-biotin after the same pretargeting approach and compared with a no-treatment control group. These tumors are referred to as of medium size (Table 3). In addition, to evaluate the effect of tumor size on therapeutic response, 2 groups of 10 mice each were treated at 7 days (small tumors) or 13 days (large tumors; Table 3). One group of mice ($n = 5$) bearing medium-size tumors was treated with 74 MBq of ^{213}Bi-DOTA-biotin after pretargeting with B3-SA, and another group ($n = 10$) was treated with 37 MBq of ^{213}Bi-DOTA-biotin after nonspecific pretargeting with HAT-SA, which served as a control.

Response to Therapy and Toxicity Assessment. Tumor growth was monitored by measuring tumor size twice a week for 3 weeks after treatment, and then once a week. A digital caliper was used to measure the tumor in two orthogonal dimensions. The volume was calculated using the formula: (long dimension) × (short dimension)^2/2. In addition, serial total body weight measurements were performed at least weekly.

To determine hematological toxicity, 20 μl of blood was collected from the tail veins using an EDTA-coated capillary tube and diluted to 250 μl with PBS ($n = 5$/group). Complete blood count (CBC), total and differential leukocyte, RBC, and platelet counts were performed before therapy and at weekly intervals thereafter until the time of death to monitor hematological toxicity. CBC from therapy trials 1 and 2 in tumor-bearing mice receiving 37 MBq of ^{213}Bi-DOTA-biotin after pretargeting with B3-SA were combined and analyzed ($n = 10$).

Liver and kidney function parameters were determined in tumor-bearing mice at the time of sacrifice (chemistry required relatively large blood volumes and, thus, they were done at the time of animal euthanasia after therapy trial 1 or therapy trial 2). Because control animals had to be euthanized in <2 weeks, only early CBC and chemistry results were available. In the ^{213}Bi-DOTA-biotin-treated groups after pretargeting, delayed time points were obtained at the time of euthanasia. According to our protocol, animals were sacrificed when tumors were too large, the mice had excessive weight loss, or tumor ulceration occurred. At sacrifice, a blood sample was taken by heart puncture and the serum separated for chemistries. A group of nontumor-bearing mice that received ^{213}Bi-DOTA-biotin without pretargeting had liver and renal function analyses evaluated at 7 days and 21 days after administration of ^{213}Bi-DOTA-biotin.

Nontumor-bearing mice, including nontreatment controls, and groups receiving 1.85 MBq, 9.25 MBq, and 37 MBq ^{213}Bi-DOTA-biotin with and without pretargeting were autopsied at 7 days ($n = 3$/group ^{213}Bi-DOTA-biotin treatment and $n = 4$ control) or 21 days ($n = 3$/group) after treatment with ^{213}Bi-DOTA-biotin and at 6 months ($n = 3$ for control and 1.85 MBq and $n = 4$ receiving 9.25 MBq/group). Tumor-bearing mice receiving treatment with 0, 3.7, 9.25, 18.5, and 37 MBq of ^{213}Bi-DOTA-biotin after pretargeting with B3-SA or with 37 MBq of ^{213}Bi-DOTA-biotin after pretargeting with HAT-SA were autopsied ($n = 2$–3). The time of autopsy of the tumor-bearing mice varied because the time of euthanasia was determined by large tumor size, excessive weight loss, or tumor ulceration. Typically, pathological evaluation of liver, kidney, intestine, lung, bone marrow, and tumor were performed. Tissue samples were harvested and fixed in 10% formalin for pathology studies. The tissues were examined by a veterinary pathologist.

Statistics. Comparison between baseline and follow-up chemistries was done using paired *t* test statistics. Comparison of toxicity after therapy was performed using one-way repeated measure ANOVA when multiple samples were drawn per mouse over time. All of the analyses were performed using Sigmastat software (Jandel, San Rafael, CA). Survival curves

Table 3 Tumor growth (cm^3) after three-step pretargeting

	Days after 37 MBq of ^{213}Bi-DOTA[a]-biotin administration[b]		
	Baseline (−1 day)	7 days	14 days
Control[c]	0.138 ± 0.113	0.870 ± 0.648	1.471 ± 0.843
Medium tumor	0.120 ± 0.051	0.088 ± 0.045	0.024 ± 0.020
Small tumor	0.059 ± 0.024	0.025 ± 0.012	0.009 ± 0.013
Large tumor	0.557 ± 0.280	0.483 ± 0.244	0.257 ± 0.182
HAT-SA[c,d]	0.143 ± 0.080	0.128 ± 0.090	0.128 ± 0.110

[a] DOTA, 7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid.

[b] Control, medium, small, and large tumors were pretargeted with B3-SA.

[c] These mice had tumor size comparable to the medium-size tumors at the time of injection of the pretargeting conjugate.

[d] This group received pretargeting with the nonspecific HAT-SA antibody.

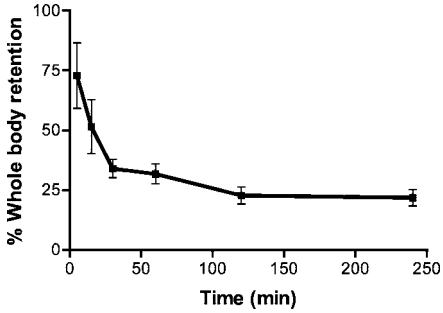

Fig. 1 Whole body retention (mean, $n = 5$) of bismuth-labeled 7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴ -tetraacetic acid-biotin in A431 tumor-bearing mice; *bars*, ± SD. The mice were injected with 400 μg of B3-SA conjugate for pretargeting. After administration of the clearing agent, 1 μg of radiolabeled Bi-7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴ -tetraacetic acid-biotin was administered i.v. The whole body retention of bismuth radioactivity was determined at 5 min to 4 h by summing the activity in all organs and the carcass.

were analyzed using Kaplan-Meier method for survival data (Graph Pad Prism).

RESULTS

Biodistribution Study. The biodistribution of bismuth-labeled DOTA-biotin after pretargeting with B3-SA was evaluated from 5 min to 4 h after administration and compared with that of directly labeled ^{205,206}Bi-CHX-B3″ MoAb. Radioactivity from bismuth-labeled biotin disappeared rapidly from the whole body. Two-thirds of the administered dose was excreted within 30 min after injection (Fig. 1). The %ID/g in the tumor and in all of the major organs is shown in Table 1. The kidney showed high uptake of radioactivity at early time points (15.39%ID/g at 5 min) and exhibited rapid disappearance with only 2.63%ID/g remaining at 30 min. In contrast, tumor uptake of radioactivity was rapid, reaching 13.1%ID/g at 30 min and remaining high up to 4 h after injection (limit of our investigation). These tumor:blood ratios were 4.25 for the pretargeted Bi-DOTA-biotin *versus* 0.25 for the directly radiolabeled ^{205,206}Bi-CHX B3″ antibody at 4 h. Thus, pretargeting showed 17 times higher tumor:blood ratio than directly labeled antibody. To determine the effect of tumor size on %ID/g uptake we compared the %ID/g in the smallest and largest tumor for each biodistribution time point. For the tumors with a mean weight of 0.146 g *versus* those of 0.459 g there was only a mean difference in uptake of 4% ID/g (*t* test, $P = 0.96$; Table 2). When the uptake values were used to determine the area underneath the curve, which is proportional to the radiation dose delivered, the values were similar with 45.3%ID·h/g for the small tumors and 44.3%ID·h/g for the large tumors.

Radiation Dosimetry. ^{213}Bi DOTA-biotin radiation dosimetry was calculated based on the biodistribution data (Table 4) using the MIRD method. The integral of the radioactivity in the major organs and the dosimetry estimates are shown in Table 4. On the basis of our data, the radiation dose to tumor from ^{213}Bi was 21.15 Gy/37MBq and, using a relative biological effectiveness of 5 for α-radiation, we estimated 105.74 Sv/37MBq.

Therapy Studies. A431 tumors in the no-treatment control group of the first therapy trial grew rapidly, from ~0.2 cm^3 in volume immediately before the B3-SA pretargeting to ~2 cm^3 in <2 weeks, and the mean time to quadrupling their tumor size was 4.6 days. These mice were sacrificed when the tumors were >2 cm in length (<10 days). The tumor growth of the no-treatment control tumor-bearing mice in the second therapy study showed a mean time to quadruple the tumor volume of 6.5 days that was somewhat slower than the therapy 1 group and due to a slightly smaller mean baseline volume of 0.138 cm^3 *versus* 0.273 cm^3 for control group of the first therapy. After treatment, inhibition of tumor growth and prolongation of survival were shown with doses of 3.7 MBq of ^{213}Bi or greater ($P < 0.03$, Kaplan-Meier). The median survivals of the groups were 7, 14, 41, 83, and 57 days for no-treatment control, and 3.7, 9.25, 18.5, and 37 MBq of ^{213}Bi-DOTA-biotin pretargeted groups, respectively (Fig. 2). At initiation of the first therapy experiment, the mean tumor sizes were not statistically significantly different between the treatment and the control groups, with the exception of the 18.5 MBq treatment group that by chance had significantly smaller tumors before treatment ($P = 0.04$). The tumor sizes for this group receiving 18.5 MBq ^{213}Bi-DOTA-biotin were approximately 3–5 times smaller than the other groups (mean tumor weights were 0.27, 0.42, 0.22, 0.09, and 0.26 g for the control, and 3.7, 9.25, 18.5, and 37 MBq groups, respectively).

Table 4 Dose to major organs based on 37 MBq administered

	Area under the curve (MBq·h/g)	Dosimetry (Gy)	Dosimetry (Sievert)[a]
Blood	1.258	5.97	29.83
Liver	5.55	2.70	13.49
Kidney	1.628	7.84	39.21
Intestine	0.3710	1.73	8.67
Stomach	0.888	4.22	21.09
Spleen	0.481	2.31	11.57
Lung	0.888	4.26	21.28
Bone	0.407	1.87	9.37
Tumor	4.44	21.15	105.74

[a] Rads were multiplied by a relative biological effectiveness of 5.

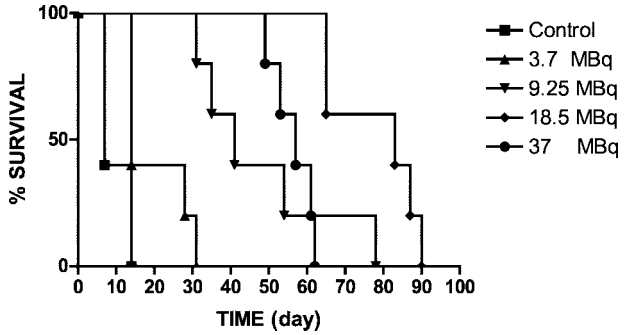

Fig. 2 Kaplan-Meier survival curves of mice bearing A431 tumor xenografts treated with 3.7–37 MBq ^{213}Bi-DOTA-biotin using the three-step pretargeting system with B3-SA and compared with a no-treatment control group. The longest survival was in the 18.5 MBq-DOTA-biotin group that, by chance, had the smallest mean tumor size. ■, control; ▲, 3.7 MBq; ▼, 9.25 MBq; ♦, 18.5 MBq; ●, 37 MBq.

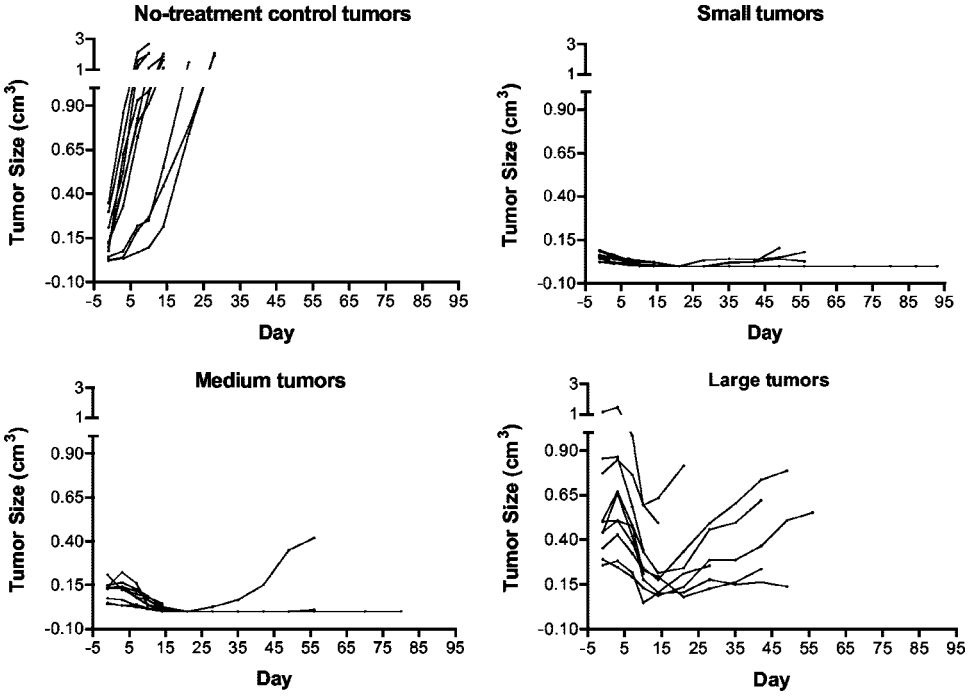

Fig. 3 The effect of tumor size on tumor response was studied. Tumor-bearing mice were treated with 37 MBq ^{213}Bi-7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-biotin using the three-step pretargeting system with B3-SA and were compared with a no-treatment control (medium-size tumor). Mice were treated at various days after A431 tumor cell inoculation, including 7 days (small tumor, mean = 0.059 ± 0.024 g), 10 days (medium tumor, mean = 0.120 ± 0.051 g), and 13 days (large tumor, mean = 0.557 ± 0.280 g) after tumor inoculation. Each *thin line* represents an individual mouse in the group (n = 10), and the *bold line* represents the mean tumor weight. The mean at later times only included tumors of surviving mice.

Results from the second therapy study showed that the 5 mice treated with 74 MBq ^{213}Bi-DOTA-biotin after pretargeted B3-SA died within 5 days after injection. In contrast, groups of mice in the second therapy experiment (Fig. 3) treated with 37 MBq of ^{213}Bi-DOTA-biotin after pretargeting survived much longer. At the beginning of the therapy study, these groups of mice had significantly different tumor sizes that we considered large, medium, and small tumor ($P < 0.05$; Table 3). The median survivals of the mice treated at this dose of 37 MBq were 14 days, 30 days, 40.5 days, 40 days, and 34.5 days, for the control, HAT-SA pretargeted group, and small-, medium-, and large-size tumor groups, respectively, as determined by Kaplan-Meier method. Whereas control mice quadrupled their tumor volume before being sacrificed because of large size, mice receiving 37MBq of ^{213}Bi-DOTA-biotin, including the HAT-SA control, never reached four times their baseline tumor volume (Fig. 3). Mice from the three groups treated with ^{213}Bi-DOTA-biotin after pretargeting with B3-SA showed decreased tumor size (Table 3). Significant differences in median survival were also seen in these three groups when compared with the no-treatment control group (Kaplan-Meier, $P < 0.05$). A trend toward improved survival was also seen in the HAT-SA pretargeted group. More than half of the mice in the medium- and small-size tumor group had no visible tumor at 2–3 weeks post-therapy with B3-SA followed by ^{213}Bi-DOTA (Fig. 3), and the large tumors showed marked inhibition of tumor growth. Mice with small tumors showed a trend toward longer survival than those with large tumors (40.5 days *versus* 34.5 days, respectively; Kaplan-Meier, $P = 0.08$).

In the first therapy trial, 3 of 5 mice with smaller tumors receiving 18.5 MBq of ^{213}Bi-DOTA-biotin had complete disappearance of their tumor, with a recurrence in 2 before their death. In the second therapy trial, some mice treated with 37 MBq of ^{213}Bi-DOTA-biotin also showed complete disappearance of their tumors. In the group of mice with small tumors, 8 of 10 had complete disappearance of their tumor with recurrence in 3, and 5 died of toxicity while visibly tumor free. In the medium-size tumor group, 7 had complete disappearance of their tumor with recurrence in 2, whereas 5 of these mice also died of toxicity while tumor free. In the large tumor group, none of the mice had complete disappearance of their tumors. In summary, the ^{213}Bi-DOTA-biotin pretargeting technique at the 37 MBq dose level showed therapeutic effect in all of the tumor size groups with complete disappearance of tumors in most of the mice with small tumors.

Toxicity. Toxicity evaluation in nontumor-bearing mice was performed. All of the tumor-bearing mice receiving 74 MBq of ^{213}Bi showed acute toxicity with death by 5 days. Shortly after the time of their death, autopsy showed that the kidneys of these mice were visually swollen and pale compared with normal kidneys. Some mice receiving 3.7–37 MBq became less active and died unexpectedly for unknown reasons without large tumor burden. Nontumor-bearing mice receiving ^{213}Bi-DOTA-biotin in doses ranging from 0 to 37 MBq were sacrificed at 7 days, 21 days, and in addition mice receiving 0 (control), 1.85 and 9.26 MBq were also sacrificed at 6 months. Findings of treated groups were compared with control, and the results are shown in Table 5. No pathological findings were seen in the liver and intestines of mice at doses up to 37 MBq compared with controls. The toxicity observed was predominantly in the kidney, marrow, and spleen, and appeared to be dose dependent. No bone marrow hypocellularity was seen at 3 weeks after treatment with up to 37 MBq of ^{213}Bi-DOTA-biotin. At 6 months, pathology of nontumor-bearing mice receiving

Table 5 Pathology mice treated with 0–37 MBq of ^{213}Bi-DOTA[a]-biotin without pretargeting

	Control (0 MBq)			1.85 MBq			9.25 MBq			37 MBq	
Organ	7 days	21 days	6 min	7 days	21 days	6 min	7 days	21 days	6 min	7 days	21 days
Kidney: necrosis	0/7	0/3	0/3	0/3	0/3	0/3	0/3	1/3	1/3	2/3	1/3
Nephropathy	0/7	2/3	0/3	0/3	1/3	0/3	0/3	1/3	2/3	0/3	2/3
Spleen: lymphoid depletion	1/7	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3[b]	2/3	1/3
Bone marrow: hypocellularity	1/7	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3	0/3

[a] DOTA, 7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid.
[b] One mouse developed lymphoma and had involvement of liver, spleen, and lung.

Table 6 Hematologic evaluation following 37 MBq ^{213}Bi-DOTA[a]-biotin after B3-SA and sCA ($n = 10$)

	WBC (K/μl)	Neutrophils (K/μl)	RBC (M/μl)	Lymphocytes (K/μl)	Platelets ($\times 10^6$/μl)
Baseline	11.4 ± 3.1	3.8 ± 2.5	10.6 ± 1.4	5.2 ± 2.4	1.63 ± 0.31
7 days	5.6 ± 3.1	1.9 ± 2.6	9.9 ± 0.6	1.4 ± 1.4	1.08 ± 0.14
14 days	8.8 ± 4.8	4.5 ± 4.7	9.5 ± 1.3	1.8 ± 0.9	1.56 ± 0.43
21 days	9.1 ± 6.1	5.4 ± 6.6	10.2 ± 0.7	2.1 ± 1.1	1.42 ± 0.52
28 days	11.1 ± 4.5	6.2 ± 5.6	10.0 ± 0.8	2.8 ± 2.1	1.20 ± 0.40
35 days	14.1 ± 7.8	6.3 ± 6.6	9.3 ± 1.3	3.9 ± 1.8	1.24 ± 0.59
P RM Anova	$P = 0.002$	$P = 0.178$	$P = 0.086$	$P < 0.001$	$P = 0.002$

[a] DOTA, 7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; sCA, synthetic clearing agent; RM, repeated measure.

1.85 or 9.25 MBq was compared with that of controls. Some evidence of kidney toxicity was seen at 6 months in the histology of the 9.25 MBq-treated mice compared with the control (Table 5). One of the mice in the 9.25 MBq group had histological evidence of lymphoma, but no other significant findings were seen in comparison with the control groups. In addition to pathology, changes in organ weight were evaluated as a possible surrogate of toxicity. There was no significant difference in the weight of the livers or kidneys among these groups. The weight of the spleen of the control group was significantly more than that of the 37 MBq group (mean = 0.107 g *versus* 0.027 g, respectively; $P < 0.05$).

In tumor-bearing mice pretargeted with B3-SA followed by 37 MBq of ^{213}Bi-DOTA-biotin, 2 of 3 mice autopsied at 50–58 days after therapy showed renal papillary necrosis. Evidence of bone marrow hypoplasia was seen in 2 of 3 mice receiving 37 MBq of ^{213}Bi-DOTA-biotin after pretargeting with HAT-SA (mean = 30 days post-treatment) but not in the control or other therapy groups pretargeted with B3-SA. Evidence of liver necrosis was noted in 1 of 3 mice pretargeted with 37 MBq ^{213}Bi-DOTA-biotin after B3-SA or HAT-SA but not at the lower doses.

Tumor-bearing mice receiving 37 MBq of pretargeted ^{213}Bi-DOTA-biotin after B3-SA had their CBCs monitored to evaluate toxicity, and their data are shown in Table 6. A significant drop in lymphocytes was seen by day 7, which recovered by day 35. The platelet count also showed a significant decrease by day 7 that then tended to recover to approximately 75–86% of baseline by 21–35 days. Data for the lower doses is not shown, but a drop in lymphocytes was also seen at the 18.5 MBq and 9.25 MBq ^{213}Bi-DOTA-biotin levels. No platelet, neutrophil, or RBC toxicity was seen at the 9.25 MBq. Platelet toxicity was seen at the 18.5 MBq level, which was in the range of that seen with the 37 MBq dose. No change in neutrophil was seen at 18.5 MBq level.

Mice receiving 37 MBq ^{213}Bi-DOTA-biotin after the nonspecific HAT-SA had a similar CBC toxicity profile compared with those pretargeted with B3-SA, although a significant drop in RBC was also noted (Table 7). Nontumor-bearing mice that received 37 MBq of ^{213}Bi-DOTA-biotin without any pretarget-

Table 7 Hematologic evaluation following 37 MBq ^{213}Bi-DOTA[a]-biotin after HAT-SA and sCA ($n = 5$)

	WBC (K/μl)	Neutrophils (K/μl)	RBC (M/μl)	Lymphocytes (K/μl)	Platelets ($\times 10^6$/μl)
Baseline	11.3 ± 3.0	2.2 ± 2.0	10.7 ± 0.4	6.6 ± 2.6	1.68 ± 0.26
7 days	1.4 ± 1.0	0.8 ± 0.4	7.8 ± 0.4	0.5 ± 0.6	0.66 ± 0.16
14 days	5.9 ± 2.9	0.02 ± 0.01	6.8 ± 1.3	0.2 ± 0.2	0.74 ± 0.30
21 days	12.2 ± 8.1	8.6 ± 9.4	9.8 ± 0.7	1.4 ± 2.0	1.22 ± 0.48
28 days	9.0 ± 1.9	4.0 ± 4.7	8.9 ± 1.2	0.9 ± 0.7	1.08 ± 0.42
35 days	9.4 ± 1.8	0.04 ± 0.02	8.9 ± 1.0	2.9 ± 2.9	0.92 ± 0.15
P RM Anova	$P < 0.001$	$P = 0.086$	$P = 0.001$	$P < 0.001$	$P < 0.001$

[a] DOTA, 7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; sCA, synthetic clearing agent; RM, repeated measure.

ing had similar toxicity that typically recovered by 21 days (data not shown).

Nontumor-bearing mice treated with 37 MBq of ^{213}Bi-DOTA-biotin without pretargeting that were sacrificed at 1 week showed a significant increase in blood urea nitrogen (BUN) compared with the no-treatment controls (26.6 ± 2.9 mg/dl *versus* 17 ± 2.6 mg/dl, respectively). No significant change was seen in the creatinine and liver function tests (bilirubin, alkaline phosphatase, alanine aminotransferase, or aspartate aminotransferase) between controls and the different treatment groups receiving 1.85–37 MBq ^{213}Bi-DOTA-biotin group. Blood chemistry of non-^{213}Bi-DOTA-biotin treated controls (mean sacrifice at 10 days) *versus* 37 MBq of ^{213}Bi-DOTA-biotin after pretargeting with B3-SA (mean sacrifice at 41 days; $n = 4$) or HAT-SA (mean sacrifice at 46 days; $n = 4$) showed an increase in creatinine in the HAT-SA pretargeted group ($P \leq 0.05$) as compared with the controls but no significant increase for the B3-SA pretargeted group (0.1 ± 0.1 mg/dl, 0.2 ± 0.15 mg/dl, *versus* 0.2 + 0.2 mg/dl, respectively). The BUN values were increased significantly in the B3-SA pretargeted group (median 29 mg/dl; $P < 0.05$) compared with the control group (median 19 mg/dl) with a trend toward higher BUN in the HAT-SA pretargeted group (median 21 mg/dl). No significant differences were seen in liver function tests (bilirubin, alkaline phosphatase, alanine aminotransferase, or aspartate aminotransferase) among any of the three groups. In summary, at the doses of ^{213}Bi-DOTA-biotin that showed biologically meaningful efficacy, there was toxicity predominantly affecting the kidney.

DISCUSSION

Similar to our previous studies with ^{111}In or ^{90}Y DOTA-biotin after pretargeting with B3-SA, the current study using ^{213}Bi-DOTA-biotin also demonstrates favorable biodistribution and tumor targeting (29). We limited our biodistribution studies to the 4-h period, because this is the time during which >95% of the ^{213}Bi decays. In terms of biodistribution of radionuclide, the pretargeting approach showed the predicted advantage over the use of directly labeled antibody. The time required to deliver effective doses of the short-lived radionuclide was much shorter, and the tumor:normal tissue ratios were much greater with the pretargeting approach than with directly labeled antibody. In this study we calculated the Gy delivered and also assumed a relative biological effectiveness of 5, which is in the range reported (40). After accounting for the biological effectiveness of ^{213}Bi, the dose estimates to tumors were in the range of our ^{90}Y-DOTA-biotin pretargeting studies and, thus, showed tumor responses, as expected. In this study we showed evidence of a dose-response curve with higher doses generally showing better tumor response than lower doses (Fig. 2). In therapy trial 1, the 18.5 MBq ^{213}Bi group showed better tumor responses than that seen in the 37 MBq group. These differences were felt to be partially attributable to the fact that mice receiving 18.5 MBq initially had smaller tumors (0.09 g), whereas mice receiving 37 MBq had larger tumors (0.26 g).

In contrast to β^- emitters with long-range emissions, the range of α particles is very short and not expected to have a large effect on cells that are not immediately adjacent to the α emitters. Our prior autoradiographic studies have shown that there is an inhomogeneous distribution of antibody within the A431 tumor (29), and thus the short pathlength of ^{213}Bi α-particle is expected to be less successful in irradiating areas of the tumor that have low uptake than the emission of a β^- emitter such as ^{90}Y, which has a mean pathlength of 5 mm. Other studies using α emitters for therapy have also shown poorer tumor responses in larger lesions. Kennel *et al.* (15), using a lung metastasis model, demonstrated that ^{213}Bi targeted with an intact antibody could cure small tumors, but cures were not observed with larger tumors, although there was a tumor growth delay. Similar results in a model system using a ^{213}Bi plasminogen activator inhibitor type 2 construct demonstrated better tumor responses with smaller tumors (41). Similar findings have been reported with an α emitter, ^{212}Bi-labeled antibody (42). Previous studies by our group suggest that, as expected, ^{213}Bi-DOTA-biotin could be quite efficacious in a leukemia model (16, 43) in comparison with the more limited efficacy with tumor nodules. In contrast, the ^{90}Y-DOTA-biotin was more effective in the treatment of nodules than in the treatment of the isolated cells in the leukemia model (16, 29). The latter findings were consistent with our data, where more favorable responses with tumor nodules were seen with ^{90}Y DOTA-biotin after pretargeting (29).

As we escalated the doses of ^{213}Bi-DOTA-biotin there was evidence of improved survival in the various groups studied. Some were complete responders with 18.5 or 37 MBq of ^{213}Bi-DOTA-biotin, but these animals died of radiotoxicity nonetheless. Thus, the therapeutic margin with this α emitter in the treatment of tumor nodules was unfavorable. When larger doses were administered (74 MBq of ^{213}Bi), the animals died rapidly, and whereas no chemistry or histopathology was available, visual examination demonstrated morphological changes in the kidneys suggesting renal toxicity. The control animals had to be sacrificed electively because of large tumor size. Toxicity resulting in death was seen in 2 of 5 of the 3.7 MBq group and in 4 of 5 of the 9.75 MBq group, as well as in 4 of 5 of the 18.5 MBq group and in the entire 37 MBq group. Similarly, in the second therapy trial in which mice received 37 MBq, almost all of the animals receiving ^{213}Bi-DOTA-biotin after pretargeting with B3-SA died of toxicity.

Although plasma chemistry and pathology were available in only a small group of animals, the findings suggest that the main toxicity was renal. Renal toxicity findings were noted in the pathology of both nontumor-bearing animals receiving ^{213}Bi-DOTA-biotin without pretargeting and tumor-bearing mice undergoing ^{213}Bi-DOTA-biotin after pretargeting with B3-SA. Some of these changes were seen as early as 1-week after treatment, whereas in mice responding to treatment, delayed renal toxicity was evident. The renal toxicity was not unexpected given the dosimetry estimates obtained from our biodistribution studies. In general, these studies underestimate the total dose because they average out the dose based on the weight of the entire kidney, whereas most of the activity would be expected to be concentrated in the tubules. Although renal toxicity is usually seen at late time points (month or years) after doses in the range of 20 Gy, when sensitive methods to assess renal effects are used, earlier changes may be observed (44). Behr *et al.* (45) evaluated the effect of high radiation dose to the kidney delivered with an ^{90}Y-Fab. In that studied they showed

that animals receiving >100 Gy to the kidney showed a steep increase in BUN and acute nephritis at 1 week after treatment. Mice receiving an estimated 83 Gy to the kidneys developed renal toxicity and death as early as 5 weeks. In contrast those receiving <66 Gy had no renal toxicity (45). In our pretargeted study doses of ^{90}Y-DOTA-biotin, 72 Gy did not result in renal toxicity (29). The toxicity that we observed in this study occurred early and at estimated doses of ~8 Gy or 40 Sievert if a relative biological effectiveness of 5 is used. These values are much lower than those that resulted in renal toxicity for ^{90}Y Fab or Fab′ (45, 46). In contrast to the finding of Behr *et al.* (46) where the maximal tolerated dose of ^{213}Bi-Fab′ was 54 Gy we observed toxicity at a much lower level of 7.8 Gy. These differences may be related to the microdosimetric distribution of the ^{213}Bi Fab′ *versus* the ^{213}Bi-DOTA-biotin and warrant additional investigation. A less likely possibility as that there was the presence of free actinium, but this is unlikely, because this has not been seen in our delayed assays.

No toxicity was seen in the liver, lung, or gastrointestinal tract based on the autopsy results and plasma chemistries. Bone marrow toxicity was not a major finding at the dose levels used. The main changes in CBC were lymphopenia, which is not surprising because lymphocytes are very radiosensitive. Histological findings of splenic lymphoid depletion and small splenic size were also noted. Some decreases in platelet count were also observed. These tended to occur early and then normalize and were not felt to be responsible for the deaths of any of the mice observed.

The nonspecific radiation delivered with HAT-SA showed a similar toxicity profile in that evidence of an elevated BUN was noted at the 37 MBq ^{213}Bi-DOTA-biotin dose level, and pathological findings were also noted in the kidneys. At this dose, some evidence of a nonspecific tumor response was also noted. Similar nonspecific antitumor effects have also been reported by others (47).

Other studies using a ^{213}Bi-labeled intact antibody in the treatment of tumor masses have also demonstrated toxicity at therapeutic or subtherapeutic doses. Lung toxicity was seen by Kennel *et al.* (48) when targeting lung metastasis with antibodies directed at lung vasculature. Dose-limiting toxicity has been also reported with other α emitters. The use of ^{212}Bi-anti-Tac monoclonal antibody resulted in marrow toxicity (42). Studies with ^{225}Ac showed that a dose of 1 μCi resulted in a wasting syndrome with marrow ablation, splenic atrophy, and some gastrointestinal toxicity (49, 50).

In conclusion, pretargeting of tumor masses with B3-SA results in rapid and high concentration of ^{213}Bi-DOTA-biotin in the tumor that can lead to tumor responses and in some mice can result in complete elimination of tumor xenografts. However, a significant amount of radioactivity is rapidly deposited in the kidneys, resulting in a high dose being delivered to that organ. These results with tumor nodules contrast with those observed in a murine xenograft model of human leukemia, where effective therapy of the isolated malignant cells could be achieved with pretargeting with the α emitter ^{213}Bi with acceptable toxicity. The dose delivered to the mice in the present study involving tumor masses was such that when therapeutic levels were achieved, dose-limiting toxicity resulting in death was observed. Thus, the therapeutic ratio was unfavorable. To improve the therapeutic index, renal radiation protectants may be useful. These results dictate that to progress to clinical trials in humans with pretargeted ^{213}Bi-DOTA-biotin, careful evaluation of the dose delivered to the kidney *in vivo* and a strategy of slow escalation of the dose while carefully evaluating renal function are necessary.

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