

Therapeutic Advantage of Pretargeted Radioimmunotherapy Using a Recombinant Bispecific Antibody in a Human Colon Cancer Xenograft

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Abstract Purpose: To assess if pretargeting, using a combination of a recombinant bispecific antibody (bsMAb) that binds divalently to carcinoembryonic antigen (CEA) and monovalently to the hapten histamine-succinyl-glycine and a ⁹⁰Y-peptide, improves therapeutic efficacy in a human colon cancer-nude mouse xenograft compared with control animals given ⁹⁰Y-humanized anti-CEA immunoglobulin G (IgG).
Experimental Design: Clearance and biodistribution were monitored by whole-body readings and necropsy. Animals were monitored for 34 weeks with a determination of residual disease and renal pathology in survivors. Hematologic toxicity was assessed separately in non-tumor-bearing NIH Swiss mice.
Results: Hematologic toxicity was severe at doses of 100 to 200 μ Ci of ⁹⁰Y-IgG, yet mild in the pretargeted animals given 500 or 700 μ Ci of the ⁹⁰Y-peptide. Evidence of end-stage renal disease was found at 900 μ Ci of the pretargeted ⁹⁰Y-peptide whereas animals given 700 μ Ci showed only mild renal pathology, similar to that seen in control animals given ⁹⁰Y-IgG. Biodistribution data indicated that the average amount of tumor radioactivity by a 700- μ Ci dose of the pretargeted peptide over a 96-hour period was increased 2.5-fold (48 μ Ci/g) compared with 150 μ Ci of ⁹⁰Y-IgG (18.9 μ Ci/g). At these doses, survival (i.e., time to progression to 2.5 cm³) was significantly improved ($P < 0.04$) compared with ⁹⁰Y-IgG, with ablation of about one third of the tumors, whereas viable tumor was present in all of the ⁹⁰Y-IgG – treated animals.
Conclusion: Pretargeting increases the amount of radioactivity delivered to colorectal tumors sufficiently to improve the therapeutic index and responses as compared with conventional radioimmunotherapy.

Although radioconjugates have been approved for imaging several types of cancer and are approved for the treatment of certain types of non-Hodgkin's lymphoma (1, 2), there continues to be a need to improve these agents, especially for the treatment of the more radioresistant solid cancers. Radionuclide targeting may be improved through the use of a variety of molecular-engineered antibodies (3–5). Radiolabeled scFv (~25 kDa) and diabodies (~50 kDa) have

extremely rapid blood clearance, yielding excellent tumor/nontumor ratios for better imaging but with significantly lower tumor uptake compared with the intact immunoglobulin G (IgG; refs. 3–5). However, when directly coupled to molecules ≤ 60 kDa, many radionuclides of therapeutic interest, except radioiodine, become trapped in the kidneys, resulting in highly unfavorable tumor/kidney ratios (6–9). Striving to meet the challenge of balancing good tumor uptake with rapid blood clearance, investigators have turned to molecules with somewhat higher molecular weights, including δ -C_H2 minibodies, tetravalent scFv, and divalent scFv-Fc constructs with a mutated Fc-receptor, all of which have faster blood clearance than an IgG molecule. These larger molecules have higher tumor uptake than the smaller, faster-clearing antibody fragments or constructs (10–13).

An alternative to administration of directly radiolabeled antibodies is pretargeting (14). All pretargeting procedures separate the targeting of the antibody from the delivery of the radionuclide. After the tumor is targeted with an unlabeled molecule, the radioactivity is administered conjugated to a small molecule, such as biotin or a peptide, that rapidly clears from the blood, thus achieving excellent tumor/blood ratios. Despite the exceptionally fast clearance of the radioactivity from the circulation, under optimal conditions tumor uptake can rival that of a directly radiolabeled IgG, with peak accretion

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occurring within an hour (15, 16). Importantly, too, the radiolabeled compounds used for pretargeting have minimal retention in the kidneys and, therefore, even tumor/kidney ratios with a pretargeted ^{111}In -labeled compound greatly exceed that of directly radiolabeled Fab' (16, 17). There are several reports of improved therapeutic responses with different pretargeting strategies (15, 16, 18, 19). We are developing a bispecific antibody (bsMAB) pretargeting method with an antihapten antibody that recognizes a novel hapten [histamine-succinyl-glycine (HSG); ref. 17]. Unlike most other bsMAB systems where the antihapten arm specifically binds to a radiolabeled chelate, the HSG hapten is not involved directly in the binding of the radionuclide, and therefore peptides could conceivably be synthesized with a wide variety of compounds to bind radionuclides or, for that matter, other agents (e.g., paramagnetic, fluorescent, etc.) for disease detection or treatment. The core peptide structure (e.g., four to five amino acids) contains two HSG haptens to enhance and stabilize the hapten binding to the bsMAB in the tumor (20). HSG peptides coupled with compounds capable of binding $^{99\text{m}}\text{Tc}$ for imaging and 7,10-tetra-azacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA) for use with ^{111}In , ^{177}Lu , or ^{90}Y have been described (16–18). In this report, in animal models, the toxicity associated with ^{90}Y -IgG and the pretargeted ^{90}Y -peptide was examined and therapeutic responses were compared.

Materials and Methods

Antibody and peptide reagents. The bsMAB, designated hBS14, was provided by IBC Pharmaceuticals, Inc. (Morris Plains, NJ). The binding properties and preparation of hBS14, a ~80 kDa, fully humanized bsMAB that can divalently bind carcinoembryonic antigen (CEA) and monovalently bind HSG, have previously been described (21). ^{125}I -hBS14 was prepared to a specific activity of ~16 mCi/mg using 0.1 mL of 1.3 mmol/L tyrosine to terminate the reaction (21). The radiolabeled product was >95% pure by size-exclusion high-performance liquid chromatography and instant TLC, and when mixed with a 10-fold mole excess of CEA (Scripps Laboratories, San Diego, CA), >95% of the product shifted to a higher molecular weight by high-performance liquid chromatography analysis.

Immunomedics, Inc. (Morris Plains, NJ) provided the DOTA-conjugated humanized anti-CEA IgG, hMN-14 (22), and the di-HSG-DOTA-peptide, IMP-241 (17). The DOTA-conjugated hMN-14 was prepared and radiolabeled as previously described (23) using $^{111}\text{InCl}_3$ (IsoTex, Friendsville, TX) or $^{90}\text{YCl}_3$ (Perkin-Elmer, Boston, MA). The final radiolabeled product had a specific activity of 5 mCi/mg. By size-exclusion high-performance liquid chromatography, the product migrated as a well-defined single peak, and when excess CEA was added, >95% of the radiolabeled IgG migrated to a higher molecular weight. The HSG peptide was radiolabeled with ^{111}In or ^{90}Y , as previously described (17), to a specific activity of ~590 and 1,640 Ci/mmol, respectively. Instant TLC indicated >97% of the radioactivity was bound, and when mixed with 10-fold mole excess of hBS14, >97% of the radioactivity shifted to the molecular size of the bsMAB; following the addition of CEA to this complex, >95% of the radiolabeled peptide and bsMAB shifted further to a higher molecular weight signifying the binding of the radiolabeled peptide to the bsMAB and the bsMAB to CEA.

Animal studies. All animal studies were done in accordance with the Institutional Animal Care and Use Committee–approved protocol. Hematologic toxicity was assessed in non-tumor-bearing, female, NIH Swiss mice (Taconic Farms, Inc., Germantown, NY). Groups of eight animals (5 weeks old; average body weight, 18.5 ± 1.4 g) were given 100 to 200 μCi of ^{90}Y -hMN-14 IgG i.v. with each dose containing 50 μg of DOTA-hMN-14 IgG and 500 or 700 μCi of the pretargeted

^{90}Y -peptide. Pretargeted groups were given i.v. 244 or 342 μg of hBS14 (3.05 and 4.27 nmol, respectively) 24 hours before the i.v. injection of the ^{90}Y -peptide (0.305 and 0.427 nmol, respectively). Biodistribution studies done in advance of this testing with the ^{111}In -hMN-14 IgG, ^{125}I -hBS14 bsMAB, and ^{111}In -peptide using these same pretargeting conditions showed similar clearance and organ uptake in the NIH Swiss mice as in nude mice (not shown), and therefore similar toxicities would be expected. Prior to treatment, all mice were bled retroorbitally under local anesthesia, followed by weekly bleeding until recovery to baseline. A separate group of untreated animals also was monitored weekly. Blood drawn in a calibrated capillary tube (70 μL) was added to 50 μL of heparin followed by a 10-minute incubation in 1.0 mL of an ammonium chloride lysing buffer. The pellet was washed twice in PBS followed by resuspension in 1.0 mL of buffered formalin. The sample (30 μL) was read on a fluorescence-activated cell sorting scan (FACSCalibur, Becton Dickinson, San Jose, CA) that was previously calibrated using antibody standards (Becton Dickinson Pharmingen, San Jose, CA) against mouse leukocyte markers. Total leukocyte counts derived for each treated animal were compared with the average leukocyte counts derived from all animals ($n = 54$) at the onset of study and expressed as the average percent change \pm SD for each treatment group.

Biodistribution and therapy studies were done in female athymic nude mice (NCr nu:NCI/Taconic, Taconic Farms). At ~6 weeks of age, mice were implanted s.c. with a suspension of a CEA-producing, human colonic carcinoma cell line, GW-39 (24). Two weeks later, baseline body weight and three-dimensional tumor size measurements were made and treatment was initiated. Groups of 12 mice (averaging 22–24 g) bearing 0.34 ± 0.03 cm^3 tumors were given an i.v. injection of the bsMAB followed 1 day later with an i.v. injection of ^{90}Y -peptide. Other groups of animals received an i.v. injection of the ^{90}Y -hMN-14 IgG ($n = 12$) or the ^{90}Y -peptide alone (not pretargeted; $n = 11$) whereas other animals were not treated ($n = 11$). All ^{90}Y -IgG doses were supplemented with unlabeled DOTA-hMN-14 IgG so that a fixed protein dose of 50 μg was given to all animals at each dose level. ^{90}Y activity was transferred to a 3-mL glass vial and read in a Capintec Model CRC-15R dose calibrator (Ramsey, NJ) that was previously calibrated against a National Institute of Standards and Technology standard using appropriate vessel/geometry controls. The volume required to deliver the prescribed dose was determined, drawn in syringes, and rechecked in the dose calibrator before injection.

Individual animals were marked, with five animals housed per cage. Cage bedding was changed several times the first week to remove the excreted radioactivity from the environment. Tumors and body weight were measured weekly and more frequently if required (e.g., rapid tumor progression or $\geq 10\%$ loss in body weight). Animals with tumors exceeding 2.5 cm^3 or with evidence of morbidity ($\geq 20\%$ loss in body weight) were euthanized, with tumors and kidneys placed in formalin for later histologic analysis. Monitoring continued for 34 weeks, at which time all surviving animals were necropsied. Any residual tumor masses and kidneys were placed in formalin for histologic analysis. All tissue samples were encoded to indicate their treatment group and embedded in paraffin. Five- to eight-micron sections were cut at different depths throughout the tumor and for the kidneys, sections were taken from the longitudinal midsection. Coded H&E-stained sections were read independently by a veterinary pathologist. Survival analysis was based on the time required for tumor to reach ≥ 2.5 cm^3 . Animals otherwise removed were censored from the survival analysis. Survival analysis was done using the GraphPad Prism 4.0 software (GraphPad Software, San Diego, CA) and the log-rank test.

Whole-body clearance was determined by placing each of the treated animals in a dose calibrator, starting at 3 hours after the ^{90}Y injection and then 24 and 48 hours later. A separate group of tumor-bearing animals, receiving either 190 μCi of ^{90}Y -hMN-14 IgG or 500 μCi of ^{90}Y peptide pretargeted 24 hours earlier using 244 μg of hBS14, were necropsied 24, 48, 96, and 120 hours after the IgG, and 3, 24, 48, and 96 hours after the pretargeted peptide. These animals were anesthetized

and bled by cardiac puncture. Tumors and tissues were weighed, solubilized at 37°C in Solvable (Perkin-Elmer), and then counted in a gamma counter (Bremstrahlung radiation) along with a standard prepared from the injected materials, thereby allowing for determination of decay-corrected (biological) % of injected dose/g and tumor/nontumor ratios. The $\mu\text{Ci/g}$ effective data were used to derive area under the curve (AUC) and predicted average $\mu\text{Ci/g}$ activity in the tissues (i.e., dose rate). A trapezoidal model was used to define the clearance of radioactivity from the tumors and all normal tissues. The AUC includes an estimate of the residual activity either using the calculated effective half-life from the last data collection point or, as in the case with ^{90}Y -IgG, using the physical half-life of ^{90}Y . The average $\mu\text{Ci/g}$ was truncated at 96 hours for both treatments.

Results

Selecting optimized pretargeting conditions. Based on our prior experience in optimizing bsMAb-pretargeting procedures (25), a comparison was made for the pretargeting of the ^{111}In -peptide using a bsMAb/peptide ratio of 10:1 with a 24-hour interval or 50:1 with a 48-hour interval. As shown in Table 1, the % of injected dose/g of the hBS14 bsMAb in blood and tumor was reduced when the longer interval was used but because five times more bsMAb had been given at the 50:1 ratio, there was ~2.4-fold more moles of bsMAb in the tumor compared with the 10:1 ratio when given at 24 hours. Nevertheless, the higher bsMAb dose did not increase tumor uptake of ^{111}In -peptide or have an appreciable effect on tumor/nontumor ratio. Thus, all future studies were done using a 10:1 hBS14/peptide mole ratio with a 24-hour interval.

Biodistribution of the pretargeted ^{90}Y -peptide and ^{90}Y -hMN-14 immunoglobulin G. Figure 1 illustrates the biological clearance data for the pretargeted ^{90}Y -peptide and the ^{90}Y -anti-CEA IgG, as well as effective curves that are adjusted to reflect the administered activities for each treatment that subsequent data

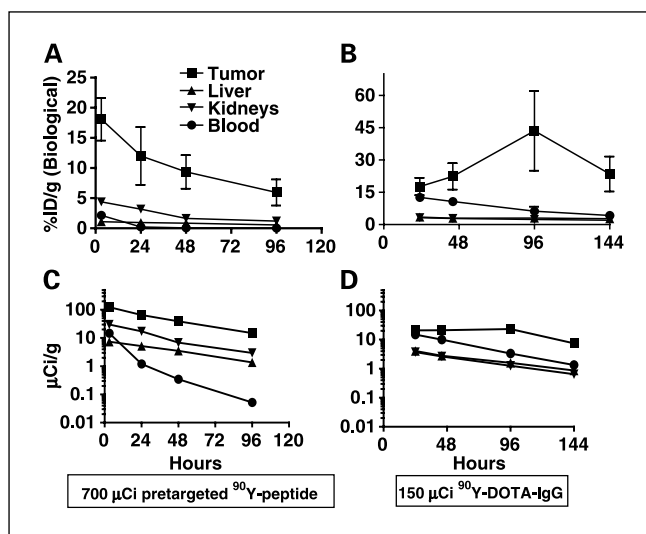


Fig. 1. Biodistribution of pretargeted ^{90}Y -peptide or ^{90}Y -IgG in nude mice bearing GW-39 xenografts. For pretargeting, animals were given i.v. 244 μg of the bsMAb followed 24 hours later with 0.305 nmol of the ^{90}Y -peptide. For ^{90}Y -IgG, animals were given i.v. 50 μg of the ^{90}Y -DOTA-IgG. A and B, biological data, respectively; C and D, corresponding effective data at the indicated administered activity. Tumors averaged 0.14 to 0.25 g ($n = 5$).

suggest are safely tolerated. Table 2 provides the calculated AUC and average $\mu\text{Ci/g}$ found in these tissues. The pretargeted peptide had a rapid uptake in the tumor, followed by a steady decline. Tumor uptake of the pretargeted peptide was more than 200 times higher than that observed with the ^{90}Y -peptide alone (not shown). At all times, tumor/nontumor ratios were highly favorable for the pretargeted ^{90}Y -peptide (e.g., at 3 hours, tumor/liver, kidney, and blood ratios were already 17.5 ± 6.1 , 4.1 ± 0.7 , and 9.1 ± 4.2 , respectively). In contrast, maximum tumor accretion of ^{90}Y -IgG occurred after several days, but as indicated in the effective curve, the $\mu\text{Ci/g}$ content in the tumors was relatively constant. At 24 hours, tumor/nontumor ratios were 5.4 ± 1.8 , 5.7 ± 1.8 , and 1.4 ± 0.2 for the liver, kidneys and blood, respectively. An assessment of the AUC data generated from the effective clearance curves that were adjusted to safely tolerated activities for each treatment suggests that pretargeting can deliver ~1.7 times more radioactivity than ^{90}Y -IgG (Table 2). Based on the AUC ratios, pretargeting has an appreciable tumor/blood ratio advantage over ^{90}Y -IgG whereas ^{90}Y -IgG has a better tumor/kidney ratio. Pretargeting also maintains an average of nearly 2.5-fold more radioactivity in the tumor over a 96-hour period compared with ^{90}Y -IgG (48.8 versus 18.9 $\mu\text{Ci/g}$). These differences are similar to those reported previously using the ^{111}In -labeled peptide (16), indicating that the ^{111}In -peptide is a useful surrogate for monitoring ^{90}Y -peptide biodistribution.

Evaluation of hematologic toxicity. All animals given ^{90}Y -hMN-14 IgG experienced severe hematologic toxicity 1 week after treatment. The decrease in leukocytes ranged from an average of $83.5 \pm 4.7\%$, compared with baseline counts, in animals given 100 μCi of ^{90}Y -IgG to $92.8 \pm 2.8\%$ in animals given 200 μCi of ^{90}Y -IgG (Fig. 2). Six of the eight animals in the 200 μCi group and seven of the eight animals in the 175 μCi group experienced excessive loss in body weight within 2 weeks of treatment (with $\geq 90\%$ decrease in leukocytes), which

Table 1. Assessing optimal conditions for pretargeting

	%ID/g		Tumor/ nontumor ratio
	^{125}I -hBS14	^{111}In -peptide	^{111}In -peptide
10:1, 24 h			
Tumor	6.7 \pm 1.7	20.6 \pm 8.5	—
(0.25 \pm 0.04 g), <i>n</i> = 4			
Liver	0.35 \pm 0.03	0.64 \pm 0.13	31.7 \pm 9.5
Kidney	0.69 \pm 0.09	4.02 \pm 0.88	5.1 \pm 1.8
Blood	0.52 \pm 0.07	0.85 \pm 0.21	24.1 \pm 6.2
50:1, 48 h			
Tumor	3.2 \pm 0.4	20.0 \pm 10.9	—
(0.26 \pm 0.05 g), <i>n</i> = 5			
Liver	0.16 \pm 0.02	0.25 \pm 0.05	79.9 \pm 40.6
Kidney	0.40 \pm 0.03	2.62 \pm 0.32	7.6 \pm 4.0
Blood	0.15 \pm 0.03	0.63 \pm 0.17	33.0 \pm 19.9

NOTE: Animals received 40 μg (0.5 nmol) or 200 μg (2.5 nmol) of hBS14 (each containing 7 μCi of ^{125}I -hBS14). After 24 or 48 hours, 0.05 nmol of ^{111}In -peptide (29.3 μCi) was given. Animals were necropsied 3 hours later. Values represent means \pm SD. Weights of tumors and number of animals are shown in parentheses.

Table 2. Effective uptake data

	Pretargeted ⁹⁰ Y-peptide (700 μCi)		⁹⁰ Y-hMN-14 IgG (150 μCi)	
	AUC*	μCi/g (96 h)	AUC*	μCi/g (96 h)
Tumor	5,346	48.8	3,231	18.9
Liver	437 (12) [†]	3.8	359 (9)	2.4
Kidneys	1,175 (5)	11.0	299 (11)	2.2
Blood	217 (25)	2.2	960 (3)	8.1

*μCi h/g, effective.
[†]Tumor AUC/nontumor AUC ratio in parentheses.

resulted in their removal from the study. Thus, animals of this starting size (i.e., body weights averaging ~18.5 g) were unable to tolerate ≥ 175 μCi of ⁹⁰Y-IgG. In the remaining animals given 100 or 145 μCi of ⁹⁰Y-IgG, the leukocytes required 6 weeks for recovery. In contrast, animals given 500 and 700 μCi of the pretargeted ⁹⁰Y-peptide experienced only minor decreases in their leukocyte count, with nadirs occurring at 1 to 2 weeks posttreatment ($18.4 \pm 32.5\%$ and $25.9 \pm 22.9\%$, respectively), and recovered fully by week 3. Hence, hematologic toxicity with just 100 μCi of ⁹⁰Y-IgG was even more severe than 700 μCi of the pretargeted ⁹⁰Y-peptide.

Evaluation of therapeutic response. Based on the hematologic toxicity data, we elected to test doses of 150 ± 4 and 189 ± 3 μCi. The ~190 μCi dose was included because the body weights of the nude mice were ~20% higher than the NIH Swiss mice, and therefore this higher dose may be tolerated. Because hematologic toxicity was not dose-limiting to the mice given ~700 μCi of the pretargeted ⁹⁰Y-peptide and because earlier studies suggested as much as 1.0 mCi would be tolerated (16), doses of 708 ± 7 and 902 ± 6 μCi were tested.

Figure 3 shows the whole-body clearance of the ⁹⁰Y-radioactivity from the animals given the pretargeted peptide or the IgG. In the pretargeting groups, nearly 80% of the total injected activity was eliminated within 3 hours, decreasing to a level similar to that found in the animals given ⁹⁰Y-IgG. After this initial loss of activity, the majority of the remaining activity cleared from the blood could be accounted for by ⁹⁰Y physical decay. However, animals given the pretargeted ⁹⁰Y-peptide had nearly 2-fold less activity in the body by 48 hours than that measured in the animals given ⁹⁰Y-IgG (e.g., 90.2 ± 1.8 and 72.2 ± 2.1 μCi for ~190 and ~150 μCi ⁹⁰Y-IgG injected activity compared with 54.4 ± 10.5 and 48.1 ± 11.7 μCi for the ~900 and ~700 μCi pretargeted ⁹⁰Y-peptide injected activity). This is likely a reflection of a gradual dissociation of a portion ⁹⁰Y-peptide bound to the bsMAb, with the peptide eliminated subsequently, but we cannot rule out localized peptide degradation.

All untreated animals and animals given 1.0 mCi of the ⁹⁰Y-peptide alone were removed from the study within 2 to 4 weeks because tumors exceeded 2.5 cm^3 (Fig. 4). Most of the tumors in all the specific treatment groups also increased in size within the first 1 to 2 weeks, but by only ~1.5- to 2-fold, and then tumor growth was arrested in nearly all animals. Only in the pretargeting groups was there evidence of substantial shrinkage of several of the tumor masses (three at 700 μCi dose and four

at the 900 μCi dose), which essentially disappeared within 9 to 14 weeks.

At the end of the 34-week monitoring period, a total of six animals remained in the group of animals given 700 μCi of the pretargeted ⁹⁰Y-peptide. Two had no evidence of tumor whereas a residual mass of 0.1 cm^3 that was removed from one other animal showed no evidence of viable tumor, and therefore this treatment was credited for the tumor ablation in 3 animals. The other three animals had tumors of 1.0 to 2.3 cm^3 that had discrete areas of viable tumor, as well as pockets of previously killed cells. Another six animals were removed during the 34-week monitoring period due to tumor progression or excessive weight loss, and two were found dead at weeks 17 and 24. One animal removed from study at 19 weeks only had a residual mass of 0.043 cm^3 that showed no histologic evidence of viable tumor, and one found dead also had a residual mass too small to measure, but we were unable to obtain a specimen for histologic assessment. In sum, histologic examination indicated that 700 μCi of the pretargeted ⁹⁰Y-peptide resulted in the ablation of 4 of 12 tumors with an additional unconfirmed eradication of tumor. Kidneys were examined in 10 of 12 animals and revealed 3 to be normal, 6 with evidence of mild, multifocal glomerulonephritis, and 1 with mild to moderate multifocal perivascular lymphocytic infiltrates in the corticomedullary junction.

At the 900 μCi dose, five animals remained after 34 weeks. Whereas only one of the kidneys from the animals at 34 weeks had evidence of moderate changes, two animals removed earlier had evidence of end-stage renal disease (Fig. 5). Thus, 900 μCi of the pretargeted ⁹⁰Y-peptide was considered an excessive dose and, therefore, although there were substantial antitumor responses in this group, including evidence of five cures, a formal assessment of the antitumor response in this group was considered inappropriate.

At 34 weeks, four animals remained in each of the ⁹⁰Y-hMN-14 IgG groups. Three tumors (one at the 150 μCi dose and two in the 190 μCi dose) had been stable at 0.330 , 0.669 , and 0.897 cm^3 for several months whereas the others had been progressing slowly toward the end of the study. Histology, however, revealed pockets of viable tumor in each of these tumors, and therefore no evidence of cures was found in any of the tumors of animals given the ⁹⁰Y-hMN-14 IgG. Six of the animals given 150 μCi of ⁹⁰Y-IgG and all five of the animals examined at 34 weeks after receiving 190 μCi of ⁹⁰Y-IgG had minimal,

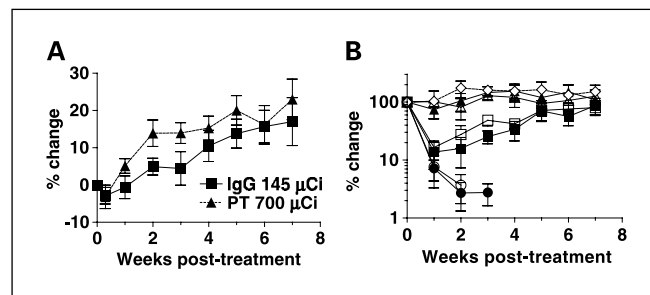


Fig. 2. Toxicity of pretargeting and direct targeting in NIH Swiss mice. *A*, body weight (doses indicated). *B*, leukocyte count; ●, ○, ■, and □, 200, 175, 145, and 100 μCi ⁹⁰Y-anti-CEA IgG, respectively; ▲, △, and ◇, 700 and 500 μCi of the pretargeted ⁹⁰Y-peptide and animals given buffer alone, respectively. PT, pretargeting.

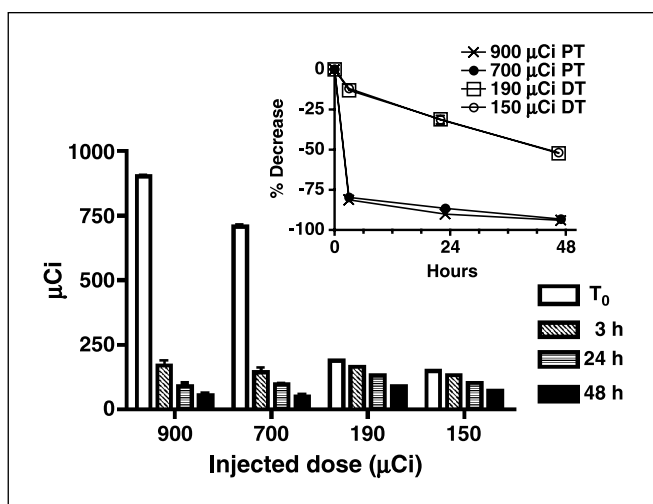


Fig. 3. Whole-body clearance of ^{90}Y in treated animals. Individual animals in each group were placed in a dose calibrator at the times indicated. Inset, % decrease compared with the initial reading (PT, pretargeting; DT, direct targeting). $n = 12$ per group.

multifocal glomerulonephritis similar to that seen in animals given 700 μCi of the pretargeted ^{90}Y -peptide.

Survival analysis (i.e., time to reach $\geq 2.5 \text{ cm}^3$) indicated that the pretargeting procedure significantly improved survival ($P < 0.04$). In addition, the 700 μCi pretargeting dose resulted in the complete ablation of 4 of 12 and possibly 5 tumors whereas no cures were found in any of the ^{90}Y -hMN-14 IgG-treated control animals. Thus, the quantitative assessment of ^{90}Y uptake and retention in tumors, as well as evidence for significant improvements in therapeutic response, favors the use of pretargeting as a means of delivering radionuclides for cancer therapy.

Discussion

Several reports have found that pretargeted radionuclides, using either an avidin/biotin-based or a bsMAB-based approach, improve therapeutic response when compared with the same radionuclide delivered by a directly radiolabeled IgG or $\text{F}(\text{ab}')_2$ (15, 16, 19, 26–30). Many of the streptavidin-based studies have used ^{90}Y as the therapeutic radionuclide but this is the first report using a bsMAB pretargeting system where ^{90}Y has been studied in a solid tumor model and shown to have a therapeutic advantage, leading to tumor ablation in one third of the animals after a single dose of the radiolabeled peptide.

The most significant challenge in this study was how to objectively compare the efficacy of a directly targeted versus that of a pretargeted radionuclide when the dose-limiting toxicity for each of these treatments involves a different organ system. Hematologic toxicity was relatively mild in the pretargeted animals receiving 700 μCi of the ^{90}Y -peptide, suggesting that substantially higher doses could have been tolerated. However, when a large portion of the radionuclide is filtered through the kidneys, it is important to carefully assess the potential for renal toxicity. Severe renal toxicity associated with directly radiolabeled Fab' (31) and diabodies (32) has been reported in mice but there is a considerably higher retention of these agents in the kidneys than that seen with ^{90}Y -

biotin or ^{90}Y -peptide. There has been an extensive examination of renal toxicity associated with the use of radiolabeled somatostatin receptor peptides in rats and humans (33, 34), which suggested that renal toxicity requires several months to be detected. Clinical studies using the NR-LU-10 IgG-streptavidin conjugate and ^{90}Y -biotin indicated that gastrointestinal toxicity was dose-limiting due to the uptake of ^{90}Y -biotin in the intestine but this was related to the specificity of the NR-LU-10 antibody for the gastrointestinal system (35, 36). However, several patients also developed elevated serum creatinine ≥ 6 months after treatment. Thus, our preclinical study was designed to monitor animals for at least 6 months using direct histologic examination of the kidneys to assess toxicity. Renal toxicity was observed in 2 of 10 assessable animals given 900 μCi of the pretargeted ^{90}Y -peptide whereas other animals given this dose only had mild to moderate histologic changes, indicating that lower doses should be used. Although evidence for some degree of renal toxicity has previously been observed (26, 29), other studies have not reported renal damage with doses as high as 1.0 mCi of pretargeted ^{90}Y -biotin, but these investigations may have been terminated prematurely or evaluated in only a small number of animals. Because the distribution of radionuclides can be monitored easily in patients by external scintigraphy, the risk for renal toxicity can be reduced by the use of dosimetry and with an understanding of radiation dose-response relationships. There is already considerable clinical experience in determining dose-response relationships for a ^{90}Y -labeled somatostatin receptor peptide that will likely be similar for pretargeting procedures (37). There is also a new multicompartiment model for renal dosimetry that has been developed (38) and may improve the ability to relate renal toxicity with renal uptake of the injected radionuclides. Preclinical and clinical studies also suggest that radiation-induced renal toxicity can be reduced with the use of captopril, an angiotensin-converting enzyme inhibitor or an angiotensin II receptor blocker (39, 40). Thus, whereas careful assessment of renal accretion will be required for pretargeting procedures, it is clear from the preclinical evidence that at safely

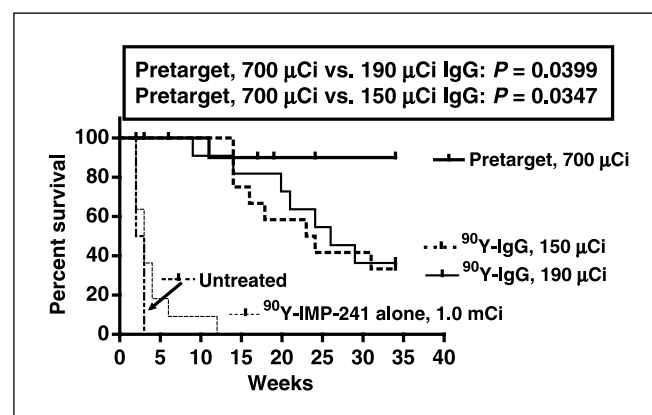


Fig. 4. Survival analysis based on time to progression to 2.5 cm^3 . Nude mice bearing GW-39 tumors averaging 0.34 cm^3 ($n = 12$) were injected i.v. with 244 μg (3.05 nmol) of the bsMAB, followed 24 hours later with 700 μCi (0.305 nmol). Other animals were given 150 μCi ($n = 12$) or 190 μCi ($n = 12$) of ^{90}Y -DOTA-hMN-14 IgG (50 μg). Controls include untreated animals ($n = 11$) or animals given 1.0 mCi (0.61 nmol) of the ^{90}Y -peptide alone ($n = 11$). Tumor size was monitored weekly. A significant survival advantage was found for the 700 μCi pretargeting dose compared with either of the ^{90}Y -IgG doses.

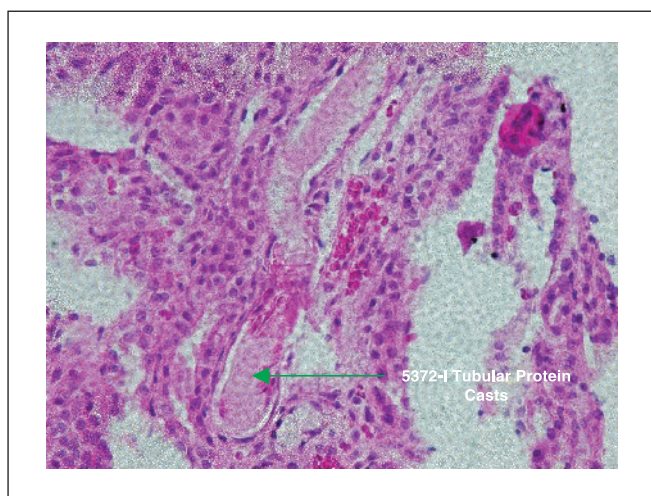


Fig. 5. Histology of kidney taken from an animal given 900 μCi of the pretargeted ^{90}Y -peptide at $\times 400$ magnification. Amorphous protein cast filling the lumen of an expanded renal tubule is evidence of changes that are typical of end-state kidney disease.

tolerated doses, pretargeting can improve therapeutic responses at doses that do not result in renal damage and without the severe hematologic toxicity associated with directly radiolabeled IgG.

A dose of 700 μCi of the pretargeted ^{90}Y -peptide was a more effective treatment procedure than the directly radiolabeled antibody based on the survival data, the number of tumors cured by the treatment, and the targeting results showing a ~ 1.7 -fold higher AUC delivered to the tumor with a 2.5-fold higher average $\mu\text{Ci/g}$ at doses that we now consider to be equitoxic. Furthermore, the 700 μCi dose resulted only in a mild, multifocal glomerulonephritis that was similar to the histologic findings in animals given either 150 or 190 μCi of ^{90}Y -IgG. Importantly, too, the tumor/kidney AUC ratio was $\sim 5:1$ for pretargeting. Therefore, these data collectively suggest that objective clinical responses might be achieved with this pretargeting procedure, assuming that these ratios can be attained.

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This study and the work of others are highly supportive of continuing clinical studies with pretargeted radionuclides. Whereas the initial clinical experience in advanced metastatic colorectal cancer using the NR-LU-10-streptavidin/ ^{90}Y -biotin pretargeting procedure was disappointing (36), clinical data continue to confirm the major finding of preclinical studies: pretargeting improves the tumor/nontumor radiation-absorbed dose ratios whereas the radiation-absorbed dose to tumors compares favorably to that reported for a directly radiolabeled antibody (41, 42). However, it is likely that additional measures will need to be undertaken to achieve significant therapeutic effects in solid tumors. For example, there is evidence that when used to treat cancer locally or as minimal disease, directly radiolabeled antibodies are effective (1, 43–45); thus, pretargeting perhaps could further improve responses in these settings. Pretargeting with bispecific humanized antibodies is also ideally suited for a variety of treatment options, including fractionated or multiple cycles of treatment, or even combinations with chemotherapy agents that themselves are myelosuppressive. Improved efficacy of combining pretargeting with gemcitabine (46) and paclitaxel (47) in animals bearing a colorectal and a medullary thyroid xenograft, respectively, has been reported. Fractionated or multiple cycles of pretargeted treatments will require the procedure to be less immunogenic than the streptavidin conjugates or fusion proteins tested to date (41, 42). Here, a bsMAb pretargeting strategy using a humanized construct would have a distinct advantage. However, a thorough assessment of renal tolerance to fractionated or multiple cycles of treatment will be required.

In conclusion, this bsMAb pretargeting system has been shown to deliver higher doses of radioactivity to tumors in the model studied than directly radiolabeled IgG, thus supporting clinical testing to assess its prospects for improving the therapy of solid tumors.

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