Therapeutic Advantage of Pretargeted Radioimmuno therapy 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 μCi of 90Y-IgG, yet mild in the pretargeted animals given 500 or 700 μCi of the 90Y-peptide. Evidence of end-stage renal disease was found at 900 μCi of the pretargeted 90Y-peptide whereas animals given 700 μCi 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-μCi dose of the pretargeted peptide over a 96-hour period was increased 2.5-fold (48 μCi/g) compared with 150 μCi of 90Y-IgG (18.9 μCi/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 6-C12 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
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 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 hapten at enhance and stabilize the hapten binding to the bsMAb in the tumor (20). HSG peptides coupled with compounds capable of binding $^{90}$Y to 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 $\sim 80$ kDa, fully humanized bsMAb that can divalent bind carcinoembryonic antigen (CEA) and monovalently bind HSG, have previously been described (21). $^{125}$I-hBS14 was prepared to a specific activity of $\sim 16$ mCi/mg using 0.1 mL of 1.3 mmol/L lysine 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 $^{111}$InCl$_3$ (IsoTex, Friendsville, TX) or $^{99m}$Tc$_3$ (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 $^{99m}$Tc, as previously described (17), to a specific activity of $\sim 590$ 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, $\sim 18.5 \pm 1.4$ g) were given 100 to 200 $\mu$Ci of $^{90}$Y-hMN-14 IgG i.v. with each dose containing 50 $\mu$g of DOTA-hMN-14 IgG and 500 or 700 $\mu$Ci of the pretargeted $^{90}$Y-peptide. Pretargeted groups were given i.v. 244 or 342 $\mu$g 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, $^{131}I$-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 $\mu$L) was added to 50 $\mu$L 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 $\mu$L) was read on a fluorescence-activated cell sorting scan (FACS Calibur, 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/NCr/Taconic, Taconic Farms). At $\sim 6$ 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 $\sim 0.34 \pm 0.03$ 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 $50 \mu$g 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 $\geq 2.5$ 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 mCi of $^{90}$Y-hMN-14 IgG or 500 mCi of $^{90}$Y peptide pretargeted 24 hours earlier using 244 mCi 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 (Bremsstrahlung 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 μCi/g effective data were used to derive area under the curve (AUC) and predicted average μCi/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 90Y-IgG, using the physical half-life of 90Y. The average μCi/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 111In-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 a 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 111In-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 90Y-peptide and 90Y-hMN-14 immunoglobulin G. Figure 1 illustrates the biological clearance data for the pretargeted 90Y-peptide and the 90Y-anti-CEA IgG, as well as effective curves that are adjusted to reflect the administered activities for each treatment that subsequent data suggest are safely tolerated. Table 2 provides the calculated AUC and average μCi/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 90Y-peptide alone (not shown). At all times, tumor/nontumor ratios were highly favorable for the pretargeted 90Y-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 90Y-IgG occurred after several days, but as indicated in the effective curve, the μCi/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 90Y-IgG (Table 2). Based on the AUC ratios, pretargeting has an appreciable tumor/blood ratio advantage over 90Y-IgG whereas 90Y-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 90Y-IgG (48.8 versus 18.9 μCi/g). These differences are similar to those reported previously using the 111In-labeled peptide (16), indicating that the 111In-peptide is a useful surrogate for monitoring 90Y-peptide biodistribution.

Evaluation of hematologic toxicity. All animals given 90Y-hMN-14 IgG experienced severe hematologic toxicity 1 week after treatment. The decrease in leukocytes ranged from an average of 83.5 ± 4.7%, compared with baseline counts, in animals given 100 μCi of 90Y-IgG to 92.8 ± 2.8% in animals given 200 μCi of 90Y-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 ≥90% decrease in leukocytes), which

<table>
<thead>
<tr>
<th>Table 1. Assessing optimal conditions for pretargeting</th>
<th>%ID/g</th>
<th>Tumor/nontumor ratio</th>
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<tbody>
<tr>
<td></td>
<td>125I-hBS14</td>
<td>111In-peptide</td>
</tr>
<tr>
<td>10:1, 24 h</td>
<td></td>
<td></td>
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<tr>
<td>Tumor</td>
<td>6.7 ± 1.7</td>
<td>20.6 ± 8.5</td>
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<tr>
<td>(0.25 ± 0.04 g), n = 4</td>
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<tr>
<td>Liver</td>
<td>0.35 ± 0.03</td>
<td>0.64 ± 0.13</td>
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<tr>
<td>Kidney</td>
<td>0.69 ± 0.09</td>
<td>4.02 ± 0.88</td>
</tr>
<tr>
<td>Blood</td>
<td>0.52 ± 0.07</td>
<td>0.85 ± 0.21</td>
</tr>
<tr>
<td>50:1, 48 h</td>
<td></td>
<td></td>
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<tr>
<td>Tumor</td>
<td>3.2 ± 0.4</td>
<td>20.0 ± 10.9</td>
</tr>
<tr>
<td>(0.26 ± 0.05 g), n = 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.16 ± 0.02</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.40 ± 0.03</td>
<td>2.62 ± 0.32</td>
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<tr>
<td>Blood</td>
<td>0.15 ± 0.03</td>
<td>0.63 ± 0.17</td>
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</table>

NOTE: Animals received 40 μg (0.5 nmol) or 200 μg (2.5 nmol) of hBS14 (each containing 7 μCi of 125I-hBS14). After 24 or 48 hours, 0.05 nmol of 111In-peptide (29.3 μCi) was given. Animals were necropsied 3 hours later. Values represent means ± SD. Weights of tumors and number of animals are shown 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 ± 32.5% and 25.9 ± 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 cm³ (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 cm³ 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 cm³ 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 cm³ 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 cm³ 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,

<table>
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<tr>
<th>Pretarget 90Y-peptide (700 μCi)</th>
<th>90Y-hMN-14 IgG (150 μCi)</th>
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<tbody>
<tr>
<td><strong>AUC</strong>&lt;sup&gt;+&lt;/sup&gt; μCi/g (96 h)</td>
<td><strong>AUC</strong>&lt;sup&gt;+&lt;/sup&gt; μCi/g (96 h)</td>
</tr>
<tr>
<td>Tumor</td>
<td>5,346</td>
</tr>
<tr>
<td>Liver</td>
<td>437 (12)&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1,175 (5)</td>
</tr>
<tr>
<td>Blood</td>
<td>217 (25)</td>
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</table>

<sup>+</sup>μCi/g h, effective.

<sup>+</sup>Tumor AUC/nontumor AUC ratio in parentheses.
multifocal glomerulonephritis similar to that seen in animals given 700 μCi of the pretargeted ⁹⁰Y-peptide.

Survival analysis (i.e., time to reach ≥2.5 cm³) 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 ⁹⁰Y-hMN-14 IgG–treated control animals. Thus, the quantitative assessment of ⁹⁰Y 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 F(ab′)₂ (15, 16, 19, 26–30). Many of the streptavidin-based studies have used ⁹⁰Y as the therapeutic radionuclide but this is the first report using a bsMAB pretargeting system where ⁹⁰Y 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 ⁹⁰Y-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 ⁹⁰Y-biotin or ⁹⁰Y-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 ⁹⁰Y-biotin indicated that gastrointestinal toxicity was dose-limiting due to the uptake of ⁹⁰Y-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 ⁹⁰Y-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 ⁹⁰Y-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 ⁹⁰Y-labeled somatostatin receptor peptide that will likely be similar for pretargeting procedures (37). There is also a new multicompartment 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. 3. Whole-body clearance of ⁹⁰Y 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.

Fig. 4. Survival analysis based on time to progression to 2.5 cm³. Nude mice bearing GW-39 tumors averaging 0.34 cm³ (n = 12) were injected i.v. with 244 μg (3.05 nmol) of the bsMABs, followed 24 hours later with 700 μCi (0.305 nmol). Other animals were given 150 μCi (n = 12) or 190 μCi (n = 12) of ⁹⁰Y-DOTA-hMN-14 IgG (50 μg). Controls include untreated animals (n = 11) or animals given 1.0 mCi (0.61 nmol) of the ⁹⁰Y-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 ⁹⁰Y-IgG doses.
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 90Y-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 μCi/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 90Y-IgG. Importantly, too, the tumor/kidney AUC ratio was ~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.

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/90Y-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 gemicitabine (46) and paclitaxel (47) in animals bearing a colorectal and a medullary thyroid xenograft, respectively, has been reported. Fractionated or multiple cycles of pretreated 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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Fig. 5. Histology of kidney taken from an animal given 900 μCi of the pretargeted 90Y-peptide at ~400 magnification. Amorphous protein cast filling the lumen of an expanded renal tubule is evidence of changes that are typical of end-stage kidney disease.

References

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