Targeting Human Cancer Xenografts with Monoclonal Antibodies Labeled Using Radioiodinated, Diethylenetriaminepentaaetatic Acid-appended Peptides

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Abstract
A new nonmetabolizable peptide approach to the production of residualizing radioiodine was evaluated in nude mice bearing xenografts of human lung adenocarcinoma (Calu-3) and B-cell lymphoma (Ramos). Monoclonal antibodies (MAbs) RS7 (anti-epithelial glycoprotein-1) and LL2 (anti-CD22) were radioiodinated using the thiol-reactive diethylenetriaminepentaaetic acid-o-peptide adducts IMP-R1 and IMP-R2. 125I-IMP-R1- and 125I-IMP-R2-labeled MAbs were compared to the MAbs iodinated by the conventional chloramine-T approach, 111In, and 131I-dilactitolyamine (DLT). In vivo biodistribution studies demonstrated a significant improvement in the tumor accumulation of radiolabel using the 125I-IMP-R1 labeled MAbs compared with the conventionally iodinated antibodies. For example, at day 7, the percentage of injected dose per gram of tissue in Calu-3 was 7.9 ± 4.1% and 18.1 ± 7.9% (P < 0.05) for the conventional 131I- and 125I-IMP-R1-RS7, respectively, and tumor:nontumor ratios were 2.6–4.5-fold higher with the 125I-IMP-R1-RS7. It is estimated that 125I-IMP-R1-RS7 would deliver a dose to tumor (at the estimated maximum tolerated dose) 3.9 times greater than conventional 131I-labeled RS7, 1.4 times greater than 99mTc-labeled RS7, and 0.7 times that of 123I-DLT-labeled RS7. Tumor accumulation of 125I-IMP-R2-RS7 was also improved compared with conventionally iodinated antibody. However, this label also caused a large increase in kidney accretion. Similar improvements in tumor accumulation and tumor:nontumor ratios were observed when 125I-IMP-R1-LL2 was used in the Ramos model. IMP-R1 offers a practical and useful residualizing radiiodine label because labeling efficiency is at least 10 times greater than that of the residualizing label DLT, without MAb aggregation. Structural modifications can be envisioned for further improvements in radioiodine incorporation, specific activity, and tumor dosimetry, and efforts along these lines are under way.

Introduction
The catabolism of iodinated proteins results in the generation of iodotyrosine within lysosomes and its release from the cell (1, 2). This contrasts with the fate of radiometal chelate-labeled proteins, where the radiometal remains trapped inside the lysosomes after catabolism (3–5). To prevent the rapid loss of radioiodine from target cells, and thus increase the radiation dose delivered to tumors following radioimmunotherapy with 131I-labeled MAbs, novel methodologies for the incorporation of iodine to protein have been developed. In these approaches, the iodine residue is conjugated to antibody via adducts that become lysosomally trapped. These radioiodine constructs have been called “residualizing” labels. The relative size and hydrophilicity of the adduct restricts its diffusion out of the lysosomal compartment.
Recent work with residualizing adducts has focused on nonmetabolizable carbohydrate-tyramine adducts, including DLT (6, 7) and tyramine-cellobiose (8). A substantial advantage in tumor retention, both in vitro and in vivo, has been observed when residualizing iodine is used. For example, when MAb RS7 was used in nude mice bearing human lung tumor xenografts, the %ID/g in tumor at day 7 increased 7-fold when the 131I-DLT-labeled antibody was used (7). In addition, 131I-DLT-labeled antibody was shown to be therapeutically more effective in the Calu-3 xenograft model than conventionally radioiodinated antibody (9). However, the low overall conjugation yields (overall radioiodine incorporation of 3–6%), and resulting low-specific activity product (0.5–1.0 mCi/mg) obtained in the preparation of 131I-DLT-labeled antibody makes the development of this agent for clinical use impractical.
We recently described the syntheses of two residualizing substrates that can be used to radioiodinate MAbs at greatly improved conjugation yields and specific activities (10). These substrates are thiol-reactive DTPA-peptide adducts that are capable of being iodinated. The peptides are assembled using α-amino acids. These substrates are referred to as IMP-R1, maleimide-X-Gly-d-Tyr-d-Lys(TC-Bz-DTPA), and IMP-R2, [maleimide-X-d-Ala-d-Tyr-d-Tyr-d-Lys2](DTPA), where X is 4-(N-maleimidomethyl)cyclohexane-l-carboxyl. Radiodination of the peptides followed by conjugation to disulfide-re-
duced MAbs resulted in 40–90% overall yields, with specific activities of 2–11 mCi/mg and less than 3% aggregation. Two internalizing MAbs, RS7 (anti-epithelial glycoprotein-1) and LL2 (anti-CD22), labeled by this procedure exhibited a 2–3-fold better retention in Calu-3 and Ramos cell lines, respectively, than with the residualizing label DLT, without MAb aggregation.

In vivo studies. Tumors were propagated in female nu/nu mice (Taconic Farms, Germantown, NY) at 6–8 weeks of age by s.c. injection of Calu-3 or Ramos cells, which had been propagated in tissue culture. Radioiodinated antibodies were injected i.v., via the lateral tail vein, into the tumor-bearing animals. Details on the quantities of radioisotope injected for each experiment are indicated in Table 1. Typically, 10–μCi doses of 125I-labeled MAbs were injected into each nude mouse. Because of the shorter half-life of 131I, 131I-MAbs were used at 20–25 μCi/nude mouse, except in the paired label study of 125I-IMP-R1-L2 and 131I-LL2(CT), where 10 μCi/nude mouse of each label was used. The animals were sacrificed at the times indicated, and the radioactivity in the tumor, liver, spleen, kidneys, lungs, small and large intestines, muscle, bone (whole femur), and blood was determined in a gamma-scintillation counter. Results were

Materials and Methods

MAbs and Cell Lines. The production and characterization of MAbs RS7 and LL2 have been described previously (12–15). The antibodies were purified from ascites fluid by passage through a protein A-immunoadsorbent column.

Calu-3, a human adenocarcinoma of the lung cell line, and Ramos, a human Burkitt’s lymphoma cell line, were purchased from American Type Culture Collection (Rockville, MD). The cells were grown in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM).

Radioiodinations. Synthesis and radioiodination of IMP-R1 and IMP-R2, and conjugation to disulfide-reduced MAbs have been described (10). Briefly, the peptides were synthesized on the solid phase by the standard fluorenylmethyloxy carbonyl strategy (16), using differential amine protections for the side chain amino group of D-lysine and the amino group at the peptide’s NH₂ terminus. Deprotections of these amino groups under different conditions enabled the introduction of DTPA to the D-lysine and the maleimide group at the NH₂ terminus. The peptides were radioiodinated by the conventional CT method, excess unreacted iodine was quenched with 4-hydroxyphenylacetic acid, and the radioiodinated peptides were conjugated to dithiothreitol-reduced MAbs.

For comparison, MAbs were also directly radioiodinated with 131I and 125I (New England Nuclear, North Billerica, MA) by the CT method (17) and with DLT (7). As expected from previous studies (7), overall incorporation of 125I was low, 4%, when the DLT labeling methodology was used. 111In-labeled MAbs were prepared as described previously (4), using indium purchased from New England Nuclear. The bifunctional chelate used in the preparation of the 111In radioimmunoconjugates was the p-isothiocyanatobenzyl derivative of DTPA. Specific activities and overall incorporation yields of the radiolabeled MAbs preparations used in the biodistribution studies are listed in Table 1.

Assessment of immunoreactivity after radiolabeling was performed with a direct cell binding assay (14). The percentages of specific binding for the 125I-labeled MAbs and 131I-labeled MAbs are shown in Table 1. Values ranged from 52% to 75% for all preparations.

In Vivo Studies. Tumors were propagated in female nu/nu mice (Taconic Farms, Germantown, NY) at 6–8 weeks of age by s.c. injection of Calu-3 or Ramos cells, which had been propagated in tissue culture. Radioiodinated antibodies were injected i.v., via the lateral tail vein, into the tumor-bearing animals. Details on the quantities of radioisotope injected for each experiment are indicated in Table 1. Typically, 10–μCi doses of 125I-labeled MAbs were injected into each nude mouse. Because of the shorter half-life of 131I, 131I-MAbs were used at 20–25 μCi/nude mouse, except in the paired label study of 125I-IMP-R1-L2 and 131I-LL2(CT), where 10 μCi/nude mouse of each label was used. The animals were sacrificed at the times indicated, and the radioactivity in the tumor, liver, spleen, kidneys, lungs, small and large intestines, muscle, bone (whole femur), and blood was determined in a gamma-scintillation counter. Results were

### Table 1: Details of labeled MAbs preparations

<table>
<thead>
<tr>
<th>Label</th>
<th>MAb</th>
<th>Overall incorporation (%)</th>
<th>Specific activity (mCi/mg)</th>
<th>Biodistribution data shown in Fig.</th>
<th>T/NT data shown in Fig.</th>
<th>μCi/animal</th>
<th>Specific binding (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125I-IMP-R1</td>
<td>RS7</td>
<td>40</td>
<td>1.8</td>
<td>1A</td>
<td>2A</td>
<td>10</td>
<td>58.5</td>
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<tr>
<td>131I(DLT)</td>
<td>RS7</td>
<td>84</td>
<td>15.3</td>
<td>1B</td>
<td>2A</td>
<td>25</td>
<td>52.7</td>
</tr>
<tr>
<td>131I-IMP-R1</td>
<td>RS7</td>
<td>4</td>
<td>0.9</td>
<td>1C</td>
<td>2B</td>
<td>10</td>
<td>72.3</td>
</tr>
<tr>
<td>131I-IMP-R1</td>
<td>RS7</td>
<td>31</td>
<td>0.8</td>
<td>Not shown</td>
<td>2B</td>
<td>20</td>
<td>57.6</td>
</tr>
<tr>
<td>131I-IMP-R2</td>
<td>RS7</td>
<td>75</td>
<td>6.0</td>
<td>Not shown</td>
<td>2D</td>
<td>10</td>
<td>73.1</td>
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<tr>
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<td>LL2</td>
<td>34</td>
<td>1.0</td>
<td>4A</td>
<td>5A</td>
<td>10</td>
<td>ND</td>
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<tr>
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<td>LL2</td>
<td>81</td>
<td>12.9</td>
<td>4B</td>
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<td>10</td>
<td>ND</td>
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<tr>
<td>131I-IMP-R2</td>
<td>LL2</td>
<td>86</td>
<td>5.4</td>
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<tr>
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<td>LL2</td>
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<td>12.3</td>
<td>Not shown</td>
<td>5B</td>
<td>20</td>
<td>ND</td>
</tr>
</tbody>
</table>

* T/NT, tumor/nontumor; ND, not determined.

Note: The table includes details of the labeled MAbs preparations used in the biodistribution studies. The MAbs were radioiodinated using different methods and are compared in terms of specific activity and biodistribution in tumor and nontumor tissues.
Fig 1  Biodistribution of radiolabeled RS7 in nude mice bearing Calu-3 tumors. Mice with tumors received injections i.v. on day 0 with the radiolabels in each panel and sacrificed successively. Results of four biodistribution studies are shown: $^{125}$I-IMP-R1-RS7 (A) versus $^{131}$I-RS7(CT) (B); $^{125}$I-DLT-RS7 (C) versus $^{131}$I-IMP-R1-RS7 (data not shown); $^{111}$In-labeled RS7 (D) and $^{125}$I-IMP-R2-RS7 (E) versus $^{131}$I-RS7(CT) (data not shown).

corrected for physical decay and are given as the mean ± SD of 3–5 animals per group. Statistical differences were calculated using the Student t test.

Radiation dose estimates were determined from the biodistribution data, as described (7). Calculations were performed by first integrating the trapezoidal regions (for tumors) or exponential regions (for normal tissues) defined by the time-activity data (corrected for physical decay). To generate conservative dose estimates, which avoid overestimation of the tumor cumulative dose, a zero-time value of zero was assumed for the trapezoidal fit of the tumor. For the other tissues, the zero time point was extrapolated according to the exponential curve. The resulting integral for each organ is converted to cGy/mCi using S-values appropriate for isotope and organ weight. These S-values were calculated for each isotope by assuming uniformly distributed activity in small unit-density spheres (18).
Results

Two new residualizing iodine labels, IMP-R1 and IMP-R2, were evaluated for their ability to deliver radioiodine to tumor when conjugated to MAbs that recognize tumor-associated antigens. Targeting of MAb RS7, a murine IgG1, was studied using nude mice bearing xenografts of Calu-3, a human lung adenocarcinoma cell line. Radioiodine accretion in three double-label biodistribution studies [125I-IMP-R1-RS7 versus 131I-RS7(CT), 131I-IMP-R1-RS7 versus 125I-DLT-RS7; and 125I-IMP-R2-RS7 versus 131I-RS7(CT)] was compared to the accretion of 111In-labeled RS7 in this model. 111In was used in the biodistribution experiments as a surrogate for 90Y, which would be used therapeutically. This substitution was made because the γ emissions of 111In allow counting of the tissues in the gamma counter and these two radiometals have been shown to yield similar distribution results. Fig. 1 summarizes the results of these biodistribution studies. In the paired label study of 125I-IMP-R1-RS7 versus 131I-RS7(CT) (Fig. 1, A and B), 125I-IMP-R1-RS7 demonstrated a markedly significant increase in tumor accretion of radiolabel, 18.1 ± 7.9% compared with 7.9 ± 4.1% (P < 0.05) at day 7. The accretion of the two labeled MAbs in liver and spleen was not significantly different. However, blood clearance was significantly faster when 125I-IMP-R1-RS7 was used [%ID/g at day 7 was 4.5 ± 1.0% compared with 9.4 ± 1.8% with 131I-RS7(CT); P < 0.01], as was clearance from the lungs (P < 0.02). Elimination rate constants (K_e) were 0.120 d^{-1} and 0.072 d^{-1} for 125I-IMP-R1-RS7 and 131I-RS7(CT), respectively, in blood, corresponding to half-lives of 5.78 and 9.59 days. In addition, higher levels of 125I-IMP-R1-RS7 were observed in the kidney, with %ID/g values at day 7 of 4.6 ± 0.7% compared with 3.1 ± 0.6% (P < 0.01) for the 131I-RS7(CT). Because we previously had demonstrated that the residualizing label 125I-DLT yields a large increase in tumor accretion of radioiodine compared with the conventional iodine-labeled antibody (7), 131I-IMP-R1-RS7 was also compared directly with 125I-DLT-RS7 in a paired label study (Fig. 1C; %ID/g 131I-IMP-R1-RS7 data are not shown because the data are similar to 125I-IMP-R1-RS7 in Fig. 1A). 131I-IMP-R1-RS7 accretion in tumor was approximately one-third the level obtained with the 125I-DLT. For example, in this study, at day 7, the %ID/g in Calu-3 was 48.6 ± 6.3% for 125I-DLT-RS7 and 15.7 ± 2.1% for 131I-IMP-R1-RS7 (P < 0.001). However, the more rapid clearance of 131I-IMP-R1-RS7 from the blood and normal organs, with the exception of kidney, led to similar tumor:nontumor ratios for 131I-IMP-R1-RS7 and 125I-DLT-RS7, as described further below. As seen in earlier studies with 88Y-RS7 (7), the biodistribution of 111In-labeled RS7 (Fig. 1D) was similar to that of 125I-DLT-RS7 (Fig. 1C).

Fig. 1E shows the %ID/g of the second residualizing substrate, 125I-IMP-R2-RS7, established in a paired-label study of 125I-IMP-R2-RS7 versus 131I-RS7(CT) [paired 131I-RS7(CT) data not shown]. Consistent with the result observed with other residualizing labels, tumor accretion of 125I-IMP-R2-RS7 was significantly greater than that of 131I-RS7(CT) [%ID/g at day 7 was 8.9 ± 2.2% compared with 4.0 ± 2.4% for 131I-RS7(CT); P < 0.001]. However, when this label was used radioiodine accretion in the kidney was higher than in the tumor (%ID/g at day 7 of 14.7 ± 3.4% in kidney). Liver and spleen uptake were also increased compared with that of the conventionally iodinated antibody. The increase in blood clearance of 125I-IMP-
R2-RS7 was even greater than that observed with $^{125}$I-IMP-R1-RS7, with a %ID/g at day 7 of 1.2 ± 0.4% in blood compared with 5.8 ± 2.0% for the $^{131}$I-RS7(CT) ($P < 0.01$). Elimination rate constants in this study were 0.286 d$^{-1}$ and 0.140 d$^{-1}$ for $^{125}$I-IMP-R2-RS7 and $^{131}$I-RS7(CT), respectively, in blood, corresponding to half-lives of 2.42 and 4.94 days.

The day-7 tumor:nontumor ratios for these studies are shown in Fig. 2. In the comparison of $^{125}$I-IMP-R1-RS7 with $^{131}$I-RS7(CT) (Fig. 2A), a 4.9-fold increase in the tumor: blood ratio was observed when the residualizing label was used. Increases in tumor: nontumor ratios for liver, spleen, lungs, small and large intestines, muscle, and bone ranged from 2.6- to 4.5-fold. The lowest increase in the tumor: nontumor ratio was observed in the kidney. However, even in kidney a 60% increase was seen in this parameter. The comparison of $^{131}$I-IMP-R1-RS7 with $^{125}$I-DLT-RS7 (Fig. 2B) showed that $^{131}$I-IMP-R1-RS7 yielded somewhat lower tumor:nontumor ratios than $^{125}$I-DLT-RS7, 13-29% lower for the liver, spleen, and lungs. The tumor: blood ratio was 4.2 ± 0.8 with $^{131}$I-IMP-R1-RS7, 35% lower than the value observed when $^{125}$I-DLT-RS7 was used. The largest difference between $^{131}$I-IMP-R1-RS7 and $^{125}$I-DLT-RS7 was observed in the tumor:kidney ratio, where the ratio with $^{131}$I-IMP-R1-RS7 was only 35% of that with $^{125}$I-DLT-RS7. Thus, the new residualizing label IMP-R1 yields marked increases in tumor:nontumor ratios when compared with the conventional CT iodination, yet it is not as effective as the DLT.

Fig. 3 Mean cumulative absorbed dose in tissues following injection of radiolabeled RS7 in nude mice bearing Calu-3 tumors. Results calculated from data in Fig. 1 and normalized to 1500 cGy cumulative absorbed dose to blood.
Fig. 4 Biodistribution of radiolabeled LL2 in nude mice bearing Ramos tumors. Mice with tumors received injections i.v. on day 0 with the radiolabels listed in each panel and sacrificed successively. Results of two biodistribution studies are shown: $^{125}$I-IMP-R1-LL2 (A) versus $^{131}$I-LL2(CT) (B); and $^{125}$I-IMP-R2-LL2 (C) versus $^{131}$I-LL2(CT) (data not shown).

tumor:nontumor ratios obtained with $^{111}$In-RS7 (Fig. 2C) were similar to the $^{125}$I-DLT values. Fig. 2D shows a comparison of tumor:nontumor ratios using RS7 labeled with the second residualizing adduct $^{125}$I-IMP-R2 to $^{131}$I-RS7(CT). Although the tumor:lungs and tumor:blood ratios were increased 7- and 11.5-fold, respectively, compared with the conventional CT label, there was no significant change in the tumor: liver: and tumor:spleen ratios. Moreover, the tumor: kidney ratio with $^{125}$I-IMP-R2-RS7 was only 33% that of the $^{131}$I-RS7(CT).

Cumulative absorbed radiation doses were calculated from the biodistribution data shown in Fig. 1. Cumulative absorbed doses for $^{131}$I-IMP-R1-RS7, $^{131}$I-DLT-RS7, and $^{131}$I-IMP-R2-RS7 were calculated using the $^{125}$I-labeled MAb biodistribution data. Similarly, cumulative absorbed doses for $^{90}$Y-labeled RS7 were calculated using the $^{111}$In-labeled RS7 biodistribution data shown in Fig. 1D. Doses were normalized to blood and are presented in Fig. 3 as dose to tissue that would be delivered at the estimated MTD. MTD values were estimated as the administered dose that would yield an absorbed dose to blood of 1500 cGy (7). As shown in Fig. 3A, it was estimated that $^{131}$I-IMP-R1-RS7 would deliver a dose to tumor (at the estimated MTD) 3.9-fold greater than conventional $^{131}$I-RS7(CT), 1.4-fold greater than $^{90}$Y-labeled RS7, and 0.7-fold that of the $^{125}$I-DLT-labeled RS7. At this dose of $^{131}$I-IMP-R1-RS7, the absorbed doses to all normal tissues, including kidney, were calculated to be below toxic levels. Toxic levels for these organs are taken to be 2000 cGy to lung and kidney and 3000 cGy to liver (19). In contrast to the results obtained using $^{125}$I-IMP-R1-RS7, the results shown in Fig. 3B indicate that $^{131}$I-IMP-R2-RS7 would not yield as great an improvement on the therapeutic efficacy of $^{131}$I-RS7(CT) because of the large doses delivered to kidney, liver, and spleen by $^{131}$I-IMP-R2-RS7. These data indicate that in a dose escalation study of $^{131}$I-IMP-R2-RS7, the kidneys would receive a dose-limiting toxicity before bone marrow, thus the dose that delivers 2000 cGy to kidney would be the estimated MTD. The dose to tumor at the MTD was estimated to be 1536 cGy, which although still higher than the tumor dose obtained at the MTD of $^{131}$I-RS7(CT), was only 35% of the dose to tumor obtained at the MTD of $^{131}$I-IMP-R1-RS7.

To study whether the results obtained with radiiodinated IMP-R1- and IMP-R2-labeled RS7 in the Calu-3 model can be generalized to other MAbs and tumor types, the murine IgG2a MAb LL2 was also evaluated using these new adducts. The targeting of $^{131}$I-IMP-R1-LL2 and $^{125}$I-IMP-R2-LL2 was compared with that of $^{131}$I-LL2(CT) in a series of double-label experiments in nude mice bearing xenografts of Ramos, a hu-
man B-cell lymphoma. Fig. 4 summarizes the results of these studies. As with MAb RS7, the use of the residualizing label $^{125}$I-IMP-R1-LL2 (Fig. 4A) caused a greater than 2-fold increase in tumor accretion of radiolabel, 8.9 ± 3.0% compared with 3.6 ± 0.7% ($P = 0.06$) at day 5. Again, there was not a significant difference in the accretion of the two labeled MAbs in liver and spleen, and the blood clearance was significantly faster when the $^{125}$I-IMP-R1-labeled MAb was used (3.4 ± 0.7% compared with 6.3 ± 1.3% with $^{131}$I-LL2(CT) at day 5; $P < 0.02$) leading to more rapid clearance from the lungs and other normal organs. $K_e$ was 0.245 d⁻¹ for $^{125}$I-IMP-R1-LL2 in the blood versus 0.168 d⁻¹ for $^{131}$I-LL2(CT). Increased uptake by the kidney was also observed using $^{125}$I-IMP-R2-LL2, with %ID/g values at day 5 of 7.2 ± 0.6% compared with 2.5 ± 0.5% for the $^{131}$I-LL2(CT) ($P < 0.001$). These levels of accretion led to increases in tumor:nontumor ratios of 2.5–4.5-fold in the liver, spleen, lungs, and blood, and a small decrease in tumor:kidney ratio (Fig. 5). From dosimetry calculations based on these data (Fig. 6), it was estimated that at a dose to blood of 1500 cGy, $^{131}$I-IMP-R2-LL2 would deliver a dose to tumor of 4830 cGy compared with the 997 cGy that would be delivered by conventional $^{131}$I(CT)-labeled LL2. If the administered dose of $^{131}$I-IMP-R2-LL2 was adjusted to deliver a cumulative absorbed dose to kidney of 2000 cGy, the dose delivered to tumor would be 2823 cGy. This value represents a 2.8-fold increase compared with the dose to tumor at the MTD of $^{131}$I(CT)-labeled LL2, and the liver, spleen, and lungs would all receive doses less than or equal to those delivered at the MTD of $^{131}$I(CT)-labeled LL2.

The results of the biodistribution study comparing $^{125}$I-IMP-R2-LL2 (Fig. 4C) versus $^{131}$I-LL2(CT) [paired $^{131}$I-LL2(CT) data not shown] also corroborate the properties of IMP-R2-labeled MAb accretion seen with RS7. $^{125}$I-IMP-R2-LL2 accretion in tumor was significantly greater than that of $^{131}$I-LL2(CT) (%ID/g at day 5 was 8.3 ± 2.6% compared with 1.7 ± 0.4%, respectively; $P < 0.001$), the accretion in the kidney was markedly elevated (%ID/g at day 5 of 22.4 ± 3.2% for $^{125}$I-IMP-R2-LL2 compared with 1.7 ± 0.4 for the $^{131}$I-LL2(CT); $P < 0.001$), and there was rapid blood clearance of $^{125}$I-IMP-R2-LL2 (%ID/g at day 5 of 2.8 ± 0.8% compared with 4.6 ± 1.1% for the $^{131}$I-LL2(CT); $P < 0.02$). $K_e$ was 0.287 d⁻¹ for $^{125}$I-IMP-R2-LL2 in the blood versus 0.202 d⁻¹ for $^{131}$I-LL2(CT). Tumor:nontumor ratios and dosimetry data shown in Figs. 5B and 6B show that the kidneys would be the dose-limiting organ if $^{131}$I-IMP-R2-labeled LL2 was used for therapy. For the cumulative dose to kidney to be below 2000 cGy, the estimated dose to tumor would be lowered to a dose approximately equal to that delivered by the MTD (myelotoxic dose) of $^{131}$I-LL2(CT).

**Discussion**

Although residualizing iodine radiolabels have been shown to accumulate within tumor cells to a greater extent than conventional iodine labels (5, 7–9, 20–23), these labels have not become widely used. This can be attributed to the complex
labeling procedures, low level of radioiodine incorporation, low specific activity, and problems with MAb aggregation. We report here that radioiodinated, DTPA-appended peptides are residualizing iodine labels that overcome many of the limitations that have impeded the development of residualizing iodine for clinical use. When the radioiodinated, DTPA-appended peptide IMP-R1 is used, residualizing $^{131}$I-labeled MAbs can be prepared at sufficient yields and specific activities and without aggregation or loss of immunoreactivity; these $^{131}$I-labeled MAbs are able to deliver a greatly elevated radiation dose to tumors.

In this report, we demonstrated that in two model systems the use of radioiodine-IMP-R1-labeled MAbs increases the $^{131}$I/g accreting in target tumors by more than 2-fold compared with MAbs labeled using conventional iodination. This factor combined with an increased rate of clearance from the blood led to tumor:nontumor ratios that ranged from 2.5 to 4.6-fold greater than those obtained using conventional iodination by the CT method. Estimated cumulative absorbed doses to tumor at MTD are 2.8- and 3.9-fold larger in the B-cell lymphoma and adenocarcinoma of the lung models, respectively.

IMP-R2 was synthesized to further improve MAb radioiodination yields. The increased tyrosine and maleimide content of IMP-R2, which contains two tetrapeptides, each with two tyrosines and a maleimide group attached to DTPA dianhydride, resulted in substantially higher yields and specific activities of radioiodinated MAB than IMP-R1 and also residualized (10). However, the elevated doses delivered to normal organs, specifically the kidneys, preclude the use of this substrate for radioimmunotherapy.

The reason for the faster blood clearance and increased accretion in the kidney of radioiodinated IMP-R2-labeled MAbs, and to a lesser extent IMP-R1-labeled MAbs, remains to be established. However, the difference in kidney accretion/retention between IMP-R1- and IMP-R2-labeled MAbs may be due to differences in lipophilicity and charge of the two adducts. When comparing the structures of IMP-R1 and IMP-R2, it can be seen that the latter contains a total of five carboxylic acid groups (three from DTPA and one each from L-lysines) and two tetrapeptide strands. This contrasts with six carboxylic acid groups and a tripeptide in the IMP-R1 structure. The high nontarget uptake may be hypothesized as arising due to the lipophilicity, as well as lower negative charge of a putative metabolite of the labeled MAbs. In explaining the differential normal organ uptakes of a series of $^{64}$Cu- and $^{67}$Cu-labeled MAb conjugates in in vivo animal biodistribution experiments, Rogers et al., (24) concluded that the charge and lipophilicity of the chelates played a major role in kidney uptake. It is possible that the labeled IgG molecules are first catabolized elsewhere, such as in the liver or spleen, and the catabolite is either excreted renally or retained in kidney, depending on whether the catabolite is hydrophilic or lipophilic. Other approaches to make the IMP-R2 substrate more usable for in vivo applications by altering the hydrophilicity profile of the molecule are planned.

In summary, IMP-R1 offers a practical and useful residualizing radioiodine label because labeling efficiency is at least 10-fold greater than that of the residualizing label DLT, without MAb aggregation or loss of immunoreactivity. Significant improvements in the tumor accretion of radiolabel were seen when the $^{131}$I-IMP-R1-labeled-MAbs were compared with the conventional $^{131}$I-labeled antibodies in two model systems differing in both tumor type and MAb isotype. These results demonstrate that the DTPA-peptide approach for the design of residualizing radioiodine yields an enhanced therapeutic index in vivo, thus extending our previous in vitro observations of enhanced tumor cell retention. $^{131}$I-IMP-R1-labeled MAbs are currently under evaluation for therapeutic efficacy in these models. It is important to note, however, that the DTPA-peptide approach has a great deal of flexibility and can be adapted to synthesize bifunctional substrates differing in the tyrosine content, maleimide content, number of amino acids, and the mode of attachment to DTPA. Modifications along these lines, which may lead to further improvements in radioiodine incorporation, specific activity, and biodistribution, are in progress.

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References


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