Biodistribution, Pharmacokinetic, and Imaging Studies with $^{186}$Re-labeled NR-LU-10 Whole Antibody in LS174T Colonic Tumor-bearing Mice

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ABSTRACT

Biodistribution, pharmacokinetic, and radioimaging studies were performed with $^{186}$Re-labeled NR-LU-10 whole antibody in athymic nude mice bearing the LS174T tumor growing either s.c. or in an experimental hepatic metastasis model. NR-LU-10 is an IgG2b murine monoclonal antibody (MAb) that reacts with virtually all human tumors of epithelial origin. NR-BC-1, an IgG2b murine MAb that reacts with normal human B-cell and B malignancies, was used as an isotype-matched control. These MAbs were radiolabeled with $^{182}$Re (3.7-day physical half-life; 1.07-MeV $\beta$ particle and 137-keV $\gamma$, 9% abundance) by a preformed chelate approach using the triamide thiolate ligand system. $^{186}$Re-labeled NR-LU-10 (50 $\mu$Ci) was injected into nude mice bearing LS174T tumors growing s.c. Biodistribution studies revealed that the LS174T tumor retained the highest concentration of $^{186}$Re-labeled NR-LU-10 (5.3% injected dose/g) at day 6. The tumor/blood ratio ranged from 0.1:1 to 10:1 by day 6, the last day of analysis. In contrast the tumor/blood ratio of $^{186}$Re-labeled NR-BC-1, the isotype-matched MAb control, was 1:1 on day 6. Pharmacokinetic analysis indicated that the $t_{1/2}$ of NR-LU-10 for blood and other tissues ranged from 21 to 25 h, while the $t_{1/2}$ for the LS174T tumor averaged 52 h. The area under the curve for tumor compared to blood was 2.6- to 5.7-fold higher than the area under the curve for all other tissues and organs. The mean residence time for NR-LU-10 in blood and all other organs ranged from 23 to 26 h, while the mean residence time for NR-LU-10 in the LS174T tumor was 72 h. Scintigraphic images revealed selective uptake of the $^{186}$Re-labeled NR-LU-10, but not of the $^{186}$Re-labeled NR-BC-1, at the LS174T tumor site. Studies in an experimental model of hepatic metastasis revealed a selective pattern of $^{186}$Re-labeled NR-LU-10 accumulation. Scintigraphic images of the LS174T tumor growing within the athymic nude mouse liver were obtained. The biodistribution, pharmacokinetic, and scintigraphic image results suggest that $^{186}$Re-labeled NR-LU-10 shows promise as a therapeutic agent for gastrointestinal cancer.

INTRODUCTION

With the advent of monoclonal antibodies there has been a renewed interest in their use for targeted delivery (1). Drugs, toxins, and radionuclides have been coupled to MAbs\(^2\) to form immunonoconjugates with potential for more selective destruction of disseminated cancer. The impetus for treating malignant disease with antibody-targeted radionuclides is 3-fold. First, the deposition of $\beta$ emission from $^{186}$Re spans multiple cell diameters, 90% deposition within 2 mm. Consequently, tumor cells devoid of antigen can be destroyed by the radiation cross-fire from adjacent antibody-bearing cells. Second, since the initial imaging studies (2, 3) with MAbs using $^{131}$I-labeled ant carcinoembryonic antigen, there have been improvements in radio-labeling applications, using $\gamma$ and positron tumor imaging. These results indicate that several radionuclides can be stably labeled and can selectively concentrate in tumors. Third, clinical responses with antibody-targeted radionuclides by using $^{131}$I-labeled MAbs have been observed (4-7). These trials have demonstrated only modest therapeutic success and several factors have limited the therapeutic efficacy of $^{131}$I-labeled radioimmunoconjugates. Initial iodination radiolabeling used typical oxidative methods that can cause MAbs to lose immunoreactivity. In vivo, dehalogenation also can occur and results in non-selective uptake of radioiodine in both the thyroid and the stomach and reduces tumor retention. With the development of methods to attach metal chelating groups to proteins a wide range of $\beta$-emitting isotopes, including $^{67}$Cu (8), $^{90}$Y (9, 10), $^{153}$Sm (11), and radioisotopes of rhenium ($^{186}$Re and $^{188}$Re) can be attached to MAbs.

Wessels and Rogus (12) have suggested that $^{186}$Re or $^{188}$Re an optimal radionuclide are a half-life of 3.7 days, which is long enough for tumor localization but short enough to minimize toxicity to whole body; a low abundance of 137 keV photons (9%) that allow imaging with a minimal nonspecific radiation dose; an intermediate energy $\beta$ emission comparable to $^{131}$I, and decay to stable daughters so that additional toxicity is of no concern. Rhenium has a structural chemistry similar to technetium and can be stably coupled to MAbs by using the diamide-dimercaptide preformed chelate approach (13). MAbs are labeled by conjugation of a $^{186}$Re mercaptoacetylglycyl-glycyl-$\gamma$-aminobutyryl active ester agent. The stability of this radioimmunoconjugate is high with less than 2% loss over a 24-h period (14).

In this study $^{186}$Re was chelated to NR-LU-10, a murine IgG2b MAb that recognizes a 40-kilodalton glycoprotein expressed on most carcinomas of epithelial origin (15). To evaluate the potential of this RIC for cancer therapy, biodistribution and imaging studies were performed in nude mice bearing LS174T tumors growing s.c. as well as in experimental hepatic metastases in athymic nu/nu mice.

MATERIALS AND METHODS

Monoclonal Antibodies. NR-LU-10 is an IgG2b murine MAb reactive with the M, 40,000 glycoprotein which is expressed on most carcinomas of epithelial origin (15). NR-BC-1 is an IgG2b murine MAb reactive with the M, 40,000 glycoprotein which is expressed on most carcinomas of epithelial origin (15). NR-BC-1 is an IgG2b murine MAb reactive with the M, 40,000 glycoprotein which is expressed on most carcinomas of epithelial origin (15).

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The abbreviations used are: MAb, monoclonal antibody; ID/g, injected dose/ g; MRT, mean residence time; AUC, area under the curve; RIC, radioimmunoconjugate; HPLC, high pressure liquid chromatography; FBS, fetal bovine serum; PBS, phosphate-buffered saline; MFI, mean fluorescence intensity; ITLC, instant thin layer chromatography.

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Cell Lines. LS174T and HT29 are human colon adenocarcinoma cell lines and ASPC1 and SU86 are human pancreatic adenocarcinoma cell lines. These cell lines of epithelial origin are maintained as a monolayer in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% FBS (Gibco) and a penicillin-streptomycin-neomycin antibiotic mixture (x100, Gibco) at 37°C under 5% CO2. Cells were removed from flasks by brief exposure to trypsin-EDTA solution consisting of 0.5 g/liter of trypsin (1:250) and 0.2 g/liter of EDTA in Hanks’ balanced salt solution without calcium or magnesium. Cells were washed and subcultured at a dilution of 1:5.

Raji, Daudi, and EB3 are malignant B-lymphoblastoid cell lines derived from patients with Burkitt’s lymphoma. These cell lines were grown in suspension culture and maintained at 37°C in a humidified atmosphere under 5% CO2. Cells were passaged every 2–3 days in RPMI 1640 supplemented with 10% FBS and penicillin-streptomycin-neomycin antibiotics. All cell lines were obtained from the American Type Culture Collection.

Indirect Immunofluorescence. Reactivity of NR-LU-10 and NR-BC-1 to the above cell lines was determined by flow cytometry analysis. Cells were harvested and adjusted to 1 x 10^6/ml in RPMI 1640 containing 2.5% FBS; 100 μl of cell suspension were added to a 12- x 75-mm tube. Saturating amounts of antibody (1 μg/ml) were added to cells and incubated at 4°C for 30 min. Cells were washed once with PBS solution, resuspended in 100 μl of 1:4 dilution of rabbit antimouse conjugated with fluorescein isothiocyanate (DAKO P161, Denmark) and incubated at 4°C for 30 min. After washing, cells were analyzed for immunofluorescence with a FACSCAN cell sorter (Becton-Dickinson, San Jose, CA). Cells were gated using forward and 90-degree light scatter to exclude dead cells and debris and were analyzed with a 488 nm argon laser.

Immunoreactive Fraction. Immunoreactivity, at infinite antigen excess, was measured by the method of Lindmo et al. (19). A constant amount of radiolabeled MAb (0.5-1 ng) was added to increasing concentrations of live cells (2-10 x 10^6/ml). The suspension was rotated for 120 min at room temperature, centrifuged, and washed 3 times with PBS. After final centrifugation, the cells were counted for radioactivity using a gamma scintillation counter (Beckman Model 5500B). Nonspecific binding was measured by adding an excess of unlabeled MAb to live cells. After a 120-min incubation, the suspension was centrifuged and washed 3 times with PBS. For the final wash, radiolabeled MAb was added and cell binding was determined as described above. For each specific concentration, four replicate samples were analyzed and a statistical analysis was performed. The data were graphically expressed with inverse cell binding on the ordinate and inverse cell concentration on the abscissa. The immunoreactive fraction, at infinite antigen excess, was determined by linear extrapolation to the ordinate.

Nude Mice. Athymic nude female ICR/Swiss mice, 4–5 weeks old, were obtained from Taconic Farms, Inc. (Germantown, NY). Mice were kept under sterile conditions in a laminar flow rack (Lab Products, Inc., Rochelle Park, NY) in cages with filter bonnets. They were fed a sterilized mouse diet and acidified tap water ad libitum.

LS174T Xenografts. Xenografts of LS174T tumor cells were established by injecting 5 x 10^6 cells s.c. in the left or right flank when mice were 6–8 weeks old. For experimental hepatic metastasis, nude mice 6–8 weeks old were anesthetized by i.p. injection of 55 mg pentobarbital/kg. A midline incision was made and the ileocolic vein was exposed. LS174T tumor cells, 1 x 10^6 in 0.1 ml, were injected into the vein with a 30-gauge needle and 1–ml tuberculin syringe. A cotton-tipped applicator was placed over the injection site for 1 min to prevent excessive bleeding. After the ecum was returned to its proper position in the abdomen, the peritoneal membrane was closed with the use of 5-0 chromic suture (Ethibon), and the skin was closed with stainless steel clips (20).

Biodistribution and Pharmacokinetic Analysis. In vivo tissue distribution and pharmacokinetic analysis were conducted in nu/nu mice bearing LS174T tumors. Groups of 3-4 animals were sacrificed and dissected at 4, 24, 72, 120, and 144 h after i.v. injection. Tissues and organs were immediately removed, rinsed with saline, blotted dry, weighed, and placed in counting tubes. The samples of blood, tumor, spleen, liver, heart, lung, kidney, muscle, skin, small intestine, stomach, and femur were counted in a well-type gamma counter (Beckman Instruments, Model 5500B). Results of labeled MAb biodistribution were expressed as a percentage of injected dose per g tissue and as tissue:blood ratios of the concentration (cpm/g) in the tissues relative to the level of radioactivity in plasma.

Table 1 Cell surface phenotype of established cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Histotype</th>
<th>NR-LU-10^a (%)</th>
<th>Total MFI*</th>
<th>NR-BC-1^a (%)</th>
<th>Total MFI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPC-1</td>
<td>Pancreatic adenocarcinoma</td>
<td>78.6</td>
<td>125.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SU-86</td>
<td>Pancreatic adenocarcinoma</td>
<td>60.1</td>
<td>111.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LS174T</td>
<td>Colon adenocarcinoma</td>
<td>86.1</td>
<td>131.3</td>
<td>2.7</td>
<td>36</td>
</tr>
<tr>
<td>HT-39</td>
<td>Colon adenocarcinoma</td>
<td>45.0</td>
<td>120.7</td>
<td>0.6</td>
<td>36</td>
</tr>
<tr>
<td>RAJJ-I</td>
<td>B-cell lymphoma</td>
<td>0.4</td>
<td>—</td>
<td>90.2</td>
<td>412</td>
</tr>
<tr>
<td>EB3</td>
<td>B-cell lymphoma</td>
<td>1.5</td>
<td>—</td>
<td>77.3</td>
<td>109</td>
</tr>
<tr>
<td>Daudi-I</td>
<td>B-cell lymphoma</td>
<td>4.7</td>
<td>44</td>
<td>93.9</td>
<td>403</td>
</tr>
</tbody>
</table>

* NR-LU-10, an IgG2b murine MAb that recognizes a PAN carcinoma antigen.
* MFI, mean fluorescence intensity.
* NR-BC-1, an IgG2b murine MAb that recognizes a Class II HLA-DR variant on B-cell and B-cell lymphoproliferative disease.
* —, none.
Table 2 Biodistribution of $^{186}$Re-labeled NR-LU-10 administered i.v. in athymic mice bearing LS174T colon carcinoma xenografts

Biodistribution of 50 μCi $^{186}$Re-labeled NR-LU-10 in athymic nude mice bearing s.c. LS174T colon carcinoma. At 4, 24, 72, 120, and 144 h following i.v. injections, tissues were dissected and weighed. The radioactivity (cpm/g) was counted and corrected for physical decay. The percentage of the injected dose per g of tissue was calculated from these data. The results are mean ± SD of 3-4 mice. The tumor weight is expressed in g.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>4 (N = 4)</th>
<th>24 (N = 4)</th>
<th>72 (N = 4)</th>
<th>120 (N = 4)</th>
<th>144 (N = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>18.62 ± 2.80</td>
<td>8.23 ± 1.17</td>
<td>2.26 ± 0.34</td>
<td>1.05 ± 0.98</td>
<td>0.49 ± 0.18</td>
</tr>
<tr>
<td>Skin</td>
<td>3.32 ± 1.00</td>
<td>1.83 ± 0.50</td>
<td>0.48 ± 0.28</td>
<td>0.39 ± 0.45</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>Heart</td>
<td>3.59 ± 0.29</td>
<td>1.73 ± 0.33</td>
<td>0.23 ± 0.19</td>
<td>0.19 ± 0.38</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.91 ± 1.79</td>
<td>1.61 ± 0.68</td>
<td>0.16 ± 0.12</td>
<td>0.21 ± 0.02</td>
<td>0.24 ± 0.07</td>
</tr>
<tr>
<td>Liver</td>
<td>4.70 ± 3.32</td>
<td>2.29 ± 0.72</td>
<td>0.32 ± 0.13</td>
<td>0.35 ± 0.11</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.63 ± 0.17</td>
<td>0.35 ± 0.18</td>
<td>0.10 ± 0.17</td>
<td>0.07 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Intestine</td>
<td>1.31 ± 0.56</td>
<td>0.73 ± 0.43</td>
<td>0.07 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.58 ± 0.42</td>
<td>0.67 ± 0.14</td>
<td>0.09 ± 0.05</td>
<td>0.11 ± 0.01</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.69 ± 1.13</td>
<td>1.75 ± 0.27</td>
<td>0.18 ± 0.06</td>
<td>0.20 ± 0.03</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.89 ± 1.54</td>
<td>1.29 ± 0.58</td>
<td>0.17 ± 0.11</td>
<td>0.26 ± 0.11</td>
<td>0.24 ± 0.11</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.25 ± 0.25</td>
<td>0.40 ± 0.12</td>
<td>0.03 ± 0.01</td>
<td>0.06 ± 0.02</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Bone</td>
<td>1.78 ± 0.20</td>
<td>2.70 ± 2.67</td>
<td>0.16 ± 0.03</td>
<td>0.17 ± 0.01</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Tail</td>
<td>1.28 ± 0.31</td>
<td>0.87 ± 0.50</td>
<td>0.32 ± 0.01</td>
<td>0.21 ± 0.05</td>
<td>0.12 ± 0.00</td>
</tr>
<tr>
<td>Tumor</td>
<td>1.95 ± 1.07</td>
<td>9.82 ± 1.27</td>
<td>6.71 ± 0.75</td>
<td>5.97 ± 2.12</td>
<td>5.31 ± 2.24</td>
</tr>
<tr>
<td>Tumor wt</td>
<td>1.2 ± 0.28</td>
<td>1.2 ± 0.42</td>
<td>1.9 ± 0.18</td>
<td>1.6 ± 0.09</td>
<td>1.6 ± 0.08</td>
</tr>
</tbody>
</table>

Table 3 Pharmacokinetic analysis of $^{186}$Re-labeled NR-LU-10 in LS174T tumor-bearing mice

Table 4 Tumor:normal tissue ratios for LS174T tumor

**RESULTS**

Radiolabeling of Monoclonal Antibodies and Characterization of Products. When 5-mg aliquots of NR-LU-10 and NR-BC-1 were labeled with 30 mCi of $^{186}$Re, the efficiency of incorporation of $^{186}$Re ranged from 30 to 40% with specific activities ranging from 0.5 to 2.5 mCi/mg. The radiochemical yield determined by ITLC was greater than 95%. By HPLC analysis, 97% of $^{186}$Re-labeled NR-LU-10 resolved as a single peak with an apparent molecular weight of 150,000. The immunoreactive to the blood at selected time points. For pharmacokinetic analysis, tissue concentrations (cpm/g) of the groups of mice at each time point were averaged. The mean concentration versus time (C × t) data were analysis by least squares nonlinear regression to calculate the coefficients and rate constants after fitting these data to the appropriate 1- or 2-compartment open model. Concentration (C) data were weighted as 1/C². Pharmacokinetic parameters (AUC, MRT, blood clearance, tissue, or organ half-life, etc.) were generated by using the LAGRAN program.

Imaging Studies. Mice with tumors were given i.v. injections of 50 μCi of $^{186}$Re-labeled MAb. Scintigraphic imaging was performed 6 days after MAb administration. Mice were anesthetized with sodium pentobarbital (55 mg/kg), and imaging was performed with a General Electric STARCAM gamma camera equipped with a 4-mm pinhole collimator. Analogue and digital images were acquired from the dorsal view with the collimator positioned approximately 7 cm from the animal. Images were acquired from approximately 30,000 accumulated counts resulting in imaging times ranging from 5 to 15 min. Digital images were normalized with a General Electric STARCAM computer to produce visually similar levels of activity in the central torso. Tumor and whole body regions of interest were identified on each mouse image.

Table 2 Biodistribution of $^{186}$Re-labeled NR-LU-10 WHOLE ANTIBODY IN TUMOR-BEARING MICE

Table 3 Pharmacokinetic analysis of $^{186}$Re-labeled NR-LU-10 in LS174T colon carcinoma xenografts

Table 4 Tumor:normal tissue ratios for LS174T tumor

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* Terminal elimination half-life.
* Correlation coefficient of regression line.
Whole Body Scintigraphic Image

**LS174T SC Tumor**

**Positive**

**Negative**

Fig. 4. Whole body scintigraphic image of athymic nude mice bearing s.c. LS174T tumors given injections i.v. of 50 μCi of ¹⁸⁶Re-labeled NR-LU-10 or 50 μCi of ¹⁸⁶Re-labeled NR-BC-1. Scanning was performed on day 6. Background subtraction was not utilized.

Fig. 5. Biodistribution of 50 μCi ¹⁸⁶Re-labeled NR-LU-10 in athymic nude mice bearing s.c. LS174T tumors and LS174T tumors growing as an experimental hepatic metastasis. At 144 h following i.v. injection the mice were sacrificed and the tissues were dissected and weighed. The radioactivity (cpm/g) was counted and corrected for physical decay. The percentage of the injected dose per g of tissue was calculated from these data. The results are mean ± SD of 3–4 mice. BL, blood; SK, skin; HE, heart; LU, lung; LI, liver; PA, pancreas; IN, intestine; ST, stomach; SP, spleen; KI, kidney; MU, muscle; BO, bone; TA, tail; TU, tumor.

Fraction at infinite antigen excess was 0.57.

Flow Cytometric Analysis of NR-LU-10 and NR-BC-1 with Human Malignant Cell Lines. Reactivity of NR-LU-10 and NR-BC-1 on human malignant cell lines was determined by indirect immunofluorescence analysis. The results in Table 1 indicate that the epitope recognized by NR-LU-10 was highly expressed on gastrointestinal cell lines (LS174T, ASPC-1, SU-86, HT-29) but not on B-cell lines (Raji, EB-3, Daudi). Conversely, the epitope recognized by NR-BC-1 was highly expressed on B-cell lines but not on tumors of gastrointestinal origin.

Biodistribution and Pharmacokinetics of Radiolabeled NR-LU-10. The tissue biodistribution of ¹⁸⁶Re-labeled NR-LU-10 was determined in athymic nude mice bearing s.c. antigen-positive LS174T tumors ranging in size from 1.2–1.9 g. The nude mice received 50 μCi i.v. of ¹⁸⁶Re-labeled NR-LU-10 and were sacrificed at intervals up to 6 days. The results of the biodistribution study are shown in Table 2. By 24 h, the concentration of ¹⁸⁶Re-labeled NR-LU-10 present in the tumor was 9.8% ID/g and in blood was 8.2% ID/g. All other organs had markedly lower concentrations of ¹⁸⁶Re-labeled NR-LU-10 (<3.0% ID/g). The percentage of ID/g for blood declined from 8.2 to 0.49%, whereas the percentage of ID/g for the tumor declined from 9.8 to 5.3% on day 1 and day 6, respectively, resulting in a maximal tumor: blood ratio of 10.8 by day 6 (Fig. 2). All of the normal organs had retained less than 0.5% ID/g by 72 h. The tumor:tissue ratios for blood, lung, liver, kidney, and spleen are shown in Fig. 3. With the exception of blood, peak tumor:tissue ratios were obtained 3 days after i.v. injection of ¹⁸⁶Re-labeled NR-LU-10. They ranged from 20:1 to 40:1. Peak tumor: blood ratios were obtained on day 6 (10.8).

Pharmacokinetic parameters and noncompartmental calculations derived by regression analysis from the concentration versus time data are summarized in Table 3. Peak tissue concentrations of NR-LU-10 in normal tissues and organs occurred 4 h after i.v. injection, whereas peak levels in LS174T tumors occurred 24 h after injection. Terminal elimination (t½/2) half-lives of NR-LU-10 from blood and all normal organs ranged from 21 to 25 h while the t½/2 β from the LS174T tumor was 52.5 h. The total AUC for LS174T tumor was 3.8–5.7 times the AUC of all other normal organs. The MRT of ¹⁸⁶Re-labeled NR-LU-10 was 77 h in the LS174T tumor. The MRT in blood and other normal organs ranged from 23 to 26 h.

Selectivity of ¹⁸⁶Re-labeled NR-LU-10 Localization. The relative tissue biodistribution of ¹⁸⁶Re-labeled NR-LU-10 was compared to ¹⁸⁶Re-labeled NR-BC-1 in mice bearing LS174T s.c. tumors. ¹⁸⁶Re-labeled NR-BC-1 gave a different pattern of biodistribution than ¹⁸⁶Re-labeled NR-LU-10 in nude mice bearing LS174T tumors. The tumor:tissue ratios of these two MAb's are presented in Table 4. The tumor:tissue ratios for ¹⁸⁶Re-labeled NR-LU-10 ranged from 11:1 to 104:1. In contrast, the tumor:tissue ratios for ¹⁸⁶Re-labeled NR-BC-1 ranged from 4:1 to 10:1.
from 1:1 to 8:1, suggesting no preferential uptake of \(^{186}\text{Re}\)-labeled NR-BC-1 in the LS174T tumor system. These results were confirmed by whole body scintigraphic studies.

**Imaging of Tumor-bearing Mice.** The mice bearing LS174T s.c. tumors were given injections i.v. of 50 \(\mu\)Ci of either \(^{186}\text{Re}\)-labeled NR-LU-10 or \(^{186}\text{Re}\)-labeled NR-BC-1 and whole body scintigraphic images were obtained 6 days later. The results in Fig. 4 indicate preferential uptake of NR-LU-10 at the s.c. tumor site with marginal uptake in the blood pool and normal tissues. As for the \(^{186}\text{Re}\)-labeled control (NR-BC-1), no difference in uptake was observed between the tumor site, the blood pool, and normal tissues.

**Biodistribution Studies and Imaging Studies in Nude Mice Bearing LS174T Experimental Hepatic Metastasis.** The relative biodistribution of \(^{186}\text{Re}\)-labeled NR-LU-10 was compared in mice bearing LS174T tumors growing either s.c. or as an experimental hepatic metastasis. The results in Fig. 5 indicate at day 6 a similar distribution pattern of \(^{186}\text{Re}\)-labeled NR-LU-10 in both xenograft models.

Scintigraphic images were also obtained with mice bearing LS174T hepatic metastases. The scintigraphic image in Fig. 6 reveals preferential uptake of \(^{186}\text{Re}\)-labeled NR-LU-10 at the tumor site.

**DISCUSSION**

The physical properties of \(^{186}\text{Re}\) make it an excellent candidate radionuclide for radioimmunotherapy when it is covalently attached to MAbs (13). A stable \(^{186}\text{Re}\) radiopharmaceutical has been prepared by conjugating a preformed \(^{186}\text{Re}\) active ester complex to NR-LU-10, an IgG2b murine MAB (14). A preliminary evaluation of \(^{186}\text{Re}\)-labeled NR-LU-10 therapeutic potential has been performed in nude mice bearing s.c. xenografts of SHT-1, a human small cell lung carcinoma cell line (22). Significant antitumor responses were observed with efficacious tumor growth control. While NR-LU-10 was generated against a human lung cancer cell line, it recognizes a variety of tumors of epithelial origin. Thus, the therapeutic potential of \(^{186}\text{Re}\)-labeled NR-LU-10 as a radiopharmaceutical can be evaluated in a variety of different tumor systems.

In this study we evaluated the therapeutic potential of \(^{186}\text{Re}\)-labeled NR-LU-10 for gastrointestinal cancer. These studies were performed with the LS174T tumor cell line growing both as a s.c. xenograft and as an experimental hepatic metastasis in athymic nu/nu mice. The nude mouse xenograft model can evaluate the potential of a radiopharmaceutical for both imaging and therapy of tumors, since it indicates the relative amount of radiopharmaceutical retained and its residence time in the tumor and normal tissues. The target:normal tissue relationship required for radioimmunotherapy is no greater than that for radioimmunoimaging if there is prolonged residence time of the radiopharmaceutical on the tumor cell (8).

Selective tumor accumulation of \(^{186}\text{Re}\)-labeled NR-LU-10 was observed, as the relative uptake in the LS174T tumor far exceeded highly vascularized tissues and organ concentrations. All normal tissues and organs with the exception of blood showed minimal uptake and more rapid clearance when compared to the LS174T tumor. While the AUC for the LS174T tumor and blood were comparable, both were 3.8–5.7 times higher than all other normal tissues and organs. However, the terminal elimination half-lives for the LS174T tumor and blood were different. The elimination half-life for blood was 2.4 times more rapid than for the LS174T tumor. Peak tumor: blood ratios appeared 5 days after i.v. injection, resulting in a tumor: blood ratio greater than 11:1. In contrast, the tumor: blood ratio for NR-BC-1 (the isotype-matched control) was only 1:1, indicating lack of selective concentration at the tumor site. The MRT (77 h) of the radiopharmaceutical was 3 times greater in the LS174T tumor than in all other normal organs and tissues. This extended MRT favorably compares to other radiopharmaceuticals that demonstrated control of tumor growth (8).

The radioimaging data also provided evidence of preferential...
accumulation of the radiopharmaceutical at the tumor site. 18Re-labeled NR-LU-10 accumulated at the LS174T tumor site. In contrast, no significant accumulation was observed with the control MAb NR-BC-1 at the LS174T tumor site. The long accumulation and retention of the radiopharmaceutical is a desirable attribute for radioimmunotherapy, since prolonged contact with the radiopharmaceutical within the tumor cells dictates its effectiveness. However, the prolonged distribution of the radiopharmaceutical in the blood pool can result in a significant radiation dose delivered to all vascular organs. Potential toxic effects may be minimized if fractionated doses are delivered instead of a single activity dose.

Parallel studies were performed in athymic nude mice bearing LS174T experimental hepatic metastases. LS174T experimental hepatic metastases were established by implantation of LS174T tumor cells into the portal system through the ileocolic vein. This procedure has been successfully used to establish hepatic metastasis with MCA-38 colonic tumor cells in C57BL/6J mice (20). This model simulates the natural history of the development of metastases from colon cancer and provides a model that can more realistically assess the therapeutic potential of RIC.

The biodistribution pattern of 18Re-labeled NR-LU-10 in mice bearing LS174T experimental hepatic metastases was similar to the biodistribution pattern observed with mice bearing LS174T s.c. tumors of comparable size. Furthermore, LS174T tumors growing within the parenchyma of the nude mouse liver could be successfully imaged. The ability to image the uptake of a radiopharmaceutical in a tumor growing within a major internal organ is important for several reasons. Images can document uptake and distribution of a therapeutic dose of radionuclide. Furthermore, scanning can be used to document the effects of therapy. The availability of this model will enable us to pursue more realistic therapeutic evaluations of radiotherapeutics. These studies are currently under way.

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Biodistribution, Pharmacokinetic, and Imaging Studies with $^{186}$ Re-labeled NR-LU-10 Whole Antibody in LS174T Colonic Tumor-bearing Mice

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