Monoclonal Antibody-based Therapy of a Human Tumor Xenograft with a \textsuperscript{177}Lutetium-labeled Immunoconjugate

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ABSTRACT

\textsuperscript{177}Lutetium (\textsuperscript{177}Lu) is a member of the family of elements known as lanthanides or rare earths. Monoclonal antibody (MAb) CC49, a murine IgG1, which is reactive with the tumor-associated antigen, TAG-72, has been shown previously to react with a wide range of human carcinomas; CC49 reacts to a different epitope on the TAG-72 molecule than MAb B72.3 and has a higher binding affinity. We report here the first use of a \textsuperscript{177}Lu-labeled immunoconjugate, \textsuperscript{177}Lu-CC49, in an experimental therapy model for human carcinoma. \textsuperscript{177}Lu-CC49 was shown to delay the growth of established LS-174T human colon carcinomas in athymic mice at a single dose of 50 \textmu Ci. Overt toxicity was observed with the administration of \textsuperscript{177}Lu-CC49 in which 5 of 9 mice died of apparent marrow toxicity. A single administration of 200 or 350 \textmu Ci of \textsuperscript{177}Lu-CC49, however, was shown to eliminate established tumors through the 77-day observation period after MAb administration. Dose fractionation experiments revealed that at least 750 \textmu Ci of \textsuperscript{177}Lu-CC49 (250 \textmu Ci/week for 3 consecutive weeks) was well tolerated in that 9 of 10 mice survived. Moreover, this dose schedule was able to eliminate the growth of relatively large (300 mm\textsuperscript{3}) human colon tumor xenografts in 90\% of the animals treated. Single-dose and dose fractionation studies were also carried out with an isotype-matched control MAb, \textsuperscript{177}Lu-MOPC-21. In all dose schedules, a large differential was seen between the therapeutic effects of the \textsuperscript{177}Lu-CC49 versus that of the \textsuperscript{177}Lu-control MAb. The merits and limitations of the use of \textsuperscript{177}Lu-labeled immunoconjugates (in particular, \textsuperscript{177}Lu-CC49) are discussed in terms of potential novel therapeutics for human carcinoma.

INTRODUCTION

Radioimmunotherapy of human tumors is now in a process of evolution. There are several major parameters that are currently being evaluated: (a) the choice of MAb,\textsuperscript{4} (b) the choice of radionuclide, (c) the choice of chelate used to couple a radionuclide to MAb, and (d) several other parameters such as the route of MAb administration, the size of the target tumor, and the heterogeneity, if any, of the target antigen. These have been discussed in detail in review articles (1, 2).

Several radionuclides have been used in experimental protocols using xenograft models. These include \textsuperscript{131}I, \textsuperscript{90}Y, \textsuperscript{212}Bi, and \textsuperscript{201}Tl. At among others (3–10). Each has its own advantages and disadvantages as to availability, ease of handling for the medical workers, convenience (i.e., need for hospitalization of patients after treatment), and toxicity for normal tissues. Differences in half-lives and maximum and average energies have implications for tumors that have antigenic heterogeneity, toxicity to non-target tissues, route of administration, MAb forms (whole IgG, fragments, recombinant forms, and modified constructs), and size of tumor masses. The chelate of choice is also important in keeping the radionuclide bound to the MAb molecule and, perhaps, for radionuclide metabolism.

The choice of MAb is of obvious importance for selective or differential reactivity to tumor. Our laboratory has developed a series of MAbs to a pancarcinoma tumor-associated antigen which has been termed "tumor-associated glycoprotein (TAG-72)" (11–13). TAG-72 is expressed on adenocarcinomas of the gastrointestinal tract, ovarian and endometrial carcinomas, non-small cell lung adenocarcinomas, pancreatic carcinomas, and mammary carcinomas (14, 15). Normal tissues that express TAG-72 are secretory endometrium, transitional mucosa (mucosal surface of the gastrointestinal tract), and carcinomas of the endometrium (16). A large differential was seen between the therapeutic effects of the \textsuperscript{177}Lu-CC49 versus that of the \textsuperscript{177}Lu-control MAb. The merits and limitations of the use of \textsuperscript{177}Lu-labeled immunoconjugates (in particular, \textsuperscript{177}Lu-CC49) are discussed in terms of potential novel therapeutics for human carcinoma.

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4 The abbreviations used are: MAb, monoclonal antibody; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; NETG, no evidence of tumor growth; HPLC, high-performance liquid chromatography.
MATERIALS AND METHODS

Monoclonal Antibodies. MAB CC49 (24) is a murine IgGl which was obtained by immunizing mice with purified TAG-72. Its properties and range of reactivities were described previously (15, 25). MAB B72.3 is a murine IgGl also reactive with TAG-72 and was described previously (18). MOPC-21 (27) was used as an IgGl isotype-matched control murine MAB. This MAB has been shown to be negative for binding to the LS-174T xenograft and gives <2:1 tumor to normal tissue ratios in vivo (28). MOPC-21 was purchased from Sigma Chemical Company (St. Louis, MO).

Solid-Phase Radioimmunoassays. The immunoreactivity of the radiolabeled MAB CC49 was assessed by solid-phase radioimmunoassay, as previously described (28), using TAG-72-positive extract of a colon carcinoma xenograft (LS-174T). An extract of a melanoma xenograft (A375) was used as a TAG-72-negative control for nonspecific binding. The proportion of protein-bound radiolabel versus free radiolabel was determined by precipitation with cold 10% trichloroacetic acid. Both the 177Lu-CC49 and the 177Lu-CC49 Iodo-Gen labeling procedure (28) resulted in >90% radioactive counts.

High-Performance Liquid Chromatography. The integrity of the radiolabeled antibody was examined using HPLC. The analyses were performed using Spherogel-TSK 2000 SW (0.75 x 30 cm; Beckman Instruments, Inc., Berkeley, CA) and Spherogel-TSK 3000 SW (0.75 x 30 cm) columns in tandem, equilibrated in 67 mM sodium phosphate containing 100 mM KCl, pH 6.8. Radiolabeled MAB CC49 (200,000 cpm/50 µl) was applied and the columns were run at a flow rate of 0.5 ml/min. The protein was detected by absorbance at 280 nm; the radioactivity was measured using a Beckman radioisotope detector (model 170).

SDS-PAGE. Radiolabeled MAB CC49 was also analyzed using discontinuous SDS-PAGE using the method of Laemmli (29). Samples were analyzed under nonreducing conditions and reducing conditions (0.5% β-mercaptoethanol, 3 min at 100°C) using a gradient gel of 5–20% (w/v) acrylamide (14 x 12.5 x 0.15 cm). A stacking gel of 3% acrylamide was used. The radiolabeled antibody was visualized by autoradiography using Kodak (Rochester, NY) XAR X-ray film and DuPont (Wilmington, DE) Lightning-Plus intensifying screens. Films were exposed at ~70°C for 1–2 h.

177Lu Labeling of MABs. 177Lu was produced by the neutron irradiation of isotopically enriched 176LuO3 (44% Lu-176; Oak Ridge National Laboratory, Oak Ridge, TN) at the University of Missouri Research Reactor. Neutron irradiation of 176Lu to produce 177Lu also results in the production of 176mLu. Under the irradiation conditions used to produce the 177Lu, there were 4 x 10⁻⁴ µCi of 177mLu/µCi of 177Lu. Therefore, in the highest 177Lu dose group (500 µCi) described below, the animals received ~0.02 µCi of 177mLu. An extensive GeLi analysis (12-h count time) of an aliquot of the 177Lu detected no radionuclides (rare earth or otherwise) other than the 177Lu and the levels of 177mLu described above.

A detailed description of the chelation of 177Lu by the bifunctional chelating agent PA-DOTA, and the subsequent conjugation of the complex to MABs is described elsewhere. Briefly, following irradiation, the target is dissolved in dilute HCl and then added to a buffered solution containing the PA-DOTA. The resulting complex is activated with thiohephosgene and isolated on a reverse-phase cartridge (Altech, Deerfield, IL), eluted with 30% acetonitrile and added to a buffered solution (20 mM carbonate, pH 9.5) containing the MAB. Conjugation of the complex to the antibody occurs via reaction of the aryl isothiocyanate group of the complex with lysine amino groups of the MAB. Following conjugation the labeled protein is separated from any non-conjugated complex via size exclusion chromatography.

A solution containing 18.44 µCi of 177Lu-PA-DOTA chelate coupled to 18 µg of CC49 IgG was prepared in 4 ml for MAB administration. For each mouse group (50, 100, 200, 350, and 500 µCi), an aliquot containing the appropriate µCi amount of 177Lu-CC49 was diluted to a final volume of 7 ml with PBS. Each 0.5-ml dose (per mouse) contained 54, 121, 244, 362, and 524 µCi, respectively, as measured in a dose calibrator (Capintec, Montvale, NJ). These values, for ease of presentation, are presented as 50, 100, 200, 350, and 500 µCi. For the control group, each mouse received 388 µg of unlabeled CC49 in 0.5 ml. This proportion concentration was approximated to the same amount administered to the animals receiving the 350-µCi dose. An instant thin-layer chromatography ITLC (type-SG/85% methanol solvent) quality assessment procedure was conducted on a small sample of the 177Lu-CC49 MAB to check for percentage of radiolabeled 177Lu bound to the MAB. The results indicated that >90% of the 177Lu was protein bound on the day of injection.

The following procedures were carried out for the dose fractionation study. For the first dose, the radionuclide conjugate of 177Lu-CC49 contained 5.46 mCi 177Lu coupled to 8.6 µg of CC49 IgG. This was diluted in phosphate-buffered saline to obtain a 273 µCi dose in 0.5 ml as measured in a dose calibrator. The 177Lu-MOPC-21 contained 5.2 mCi 177Lu coupled to 5 µg of MOPC-21 IgG. This was diluted in phosphate-buffered saline to obtain a 276-µCi dose in 0.5 ml as measured in a dose calibrator. The dose calibrator was first prepared a stock solution of 177Lu and determining the activity of the solution by counting an aliquot in a known geometry on a GeLi detector which was calibrated using National Bureau of Standards traceable standards. The standard solution was then used to calibrate (i.e., determine the proper setting) for a variety of geometries and containers in the dose calibrator.

The second and third doses were produced as described above, using fresh preparations. The second 0.5-ml 177Lu-CC49 dose contained 254 µCi and the 177Lu-MOPC-21 contained 259 µCi. The third 177Lu-CC49 dose contained 234 µCi in 0.5 ml as measured in a dose calibrator. The 177Lu-MOPC-21 contained 240 µCi. For ease of presentation, the 3 dose fractions are approximated at 250 µCi each for both 177Lu-CC49 and 177Lu-MOPC-21.

Tumor Inhibition Studies. The LS-174T cell line (CL188) (30) was obtained from the American Type Culture Collection (Rockville, MD). This cell line was established from a human mucinous adenocarcinoma of the colon. The cells were grown in Eagle's minimum essential medium supplemented with 1% nonessential amino acids (100 mM), including 1% glutamine (200 mM), 10% heat-inactivated fetal calf serum, and gentamicin (50 µg/ml). Cells were passaged weekly at a 1:10 dilution. They grew as a monolayer and were harvested with the use of 0.1% trypsin in 0.5 mM EDTA. The cells were washed twice in serum-free minimum essential medium before being injected into athymic mice.

Female athymic mice (NCr-nu) were obtained from the Frederick Cancer Research Facility (Frederick, MD), at 4–6 weeks of age. The mice were given a s.c. injection of the right flank of 1 x 10⁶ LS-174T cells in 0.1 ml. For the single-dose titration experiment, 7 days after inoculation, the mice bearing tumors of approximately 30 mm³ were selected and divided into treatment groups. Groups of 8–10 mice each received an i.p. injection of designated amounts of 177Lu-IgG CC49. For the multiple-dose fractionation experiment, 14 days after tumor transplantation, the mice bearing tumors of approximately 300 mm³ were selected and divided into treatment groups. Groups of 9 or 10 mice received an i.p. injection of approximately 250 µCi of 177Lu-IgG CC49 or 177Lu-IgG MOPC-21 at weekly intervals for 1, 2, or 3 doses. The interval of 7 days between doses was chosen because we have shown (31) that, at day 7, >90% of i.p. administered MABs are cleared from the blood pool, thus reducing the potential for marrow toxicity and enabling the marrow to regenerate. The tumor growth was measured in 2 diameters weekly with a precision caliper, until the mice were sacrificed. Results are expressed as tumor volume as described previously (6).

Histological Studies. The tumor or site of tumor inoculation, spleen, bone marrow, lung, and small intestine were obtained from selected mice.
**Table 1 In vitro binding of 

<table>
<thead>
<tr>
<th>Immunoconjugate</th>
<th>Input (cpm)</th>
<th>LS-174T</th>
<th>A375</th>
</tr>
</thead>
<tbody>
<tr>
<td>177Lu-CC49</td>
<td>41,203</td>
<td>41.0</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>11,834</td>
<td>55.0</td>
<td>1.7</td>
</tr>
<tr>
<td>125I-CC49</td>
<td>40,785</td>
<td>37.4</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>11,460</td>
<td>36.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**RESULTS**

**In Vitro Analyses.** 

177Lu-CC49 IgG was first compared with 

125I-CC49 IgG for its ability to bind to the TAG-72-positive LS-174T human colon carcinoma xenograft using a solid-phase radioimmunoassay. As seen in Table 1, both radioimmunoconjugates gave similar binding, and neither bound to the TAG-72-negative A375 human melanoma.

The 177Lu-CC49 and 125I-CC49 IgGs were then analyzed in SDS-PAGE with and without reduction with β-mercaptoethanol. As shown in Fig. 1, the 177Lu-CC49 IgG migrated as a homogeneous band at approximately 150 kDa and separated into the homogeneous bands characteristic of heavy and light chains with reduction. The 177Lu- and 125I-labeled CC49 IgGs were also analyzed via HPLC and homogeneity with no evidence of aggregation was observed (Fig. 2). One of the characteristics of the 177Lu-PA-DOTA complex is its very high thermodynamic and kinetic stability. Since 177Lu is a γ emitter, it was easy to determine whether radioactivity was being retained on columns and filters due either to colloid formation or binding to the resins. Studies have been carried out which demonstrate that the small amount of nonprotein-bound 177Lu is present as the PA-DOTA complex and not as uncomplexed metal. In the 177Lu-labeled materials used for the animal studies described below, the amount of nonprotein-bound radioactivity was <1% in all cases.

**In Vivo Binding.** A detailed analysis of the pharmacokinetics and tumor versus normal tissue uptake of 177Lu-CC49 IgG will be described in a forthcoming paper. The Bz-DTPA, Mx-DTPA, and the PA-DOTA chelates, as well as 177Lu, 153Sm, and 90Y using the PA-DOTA chelate will be compared. The following percentage of injected dose/g values at 168 h after MAb administration using the LS-174T xenograft model (4–5 animals/group) described here were obtained with 177Lu-PA-DOTA-CC49: tumor, 81.1; liver, 10.0; spleen, 5.3; blood, 2.6; kidneys, 2.9; lungs, 1.5; and bone (femur), 1.0. This corresponds to the following tumor to normal tissue ratios: liver, 8.9; spleen, 15.1; blood, 37.3; kidneys, 26.3; lungs, 58.3; and femur, 77.2. As reported previously (25), the percentage of injected dose/g values of 125I-labeled CC49 were as follows: tumor, 23.4; liver, 1.3; spleen, 1.2; bone, 1.1; kidneys, 0.4; and lungs, 0.6. The SEM ranged from 0.07 to 0.34 for the 177Lu-CC49, and for the 125I-CC49 ranged from 0.18 to 0.56.

**Dose Titrations.** 177Lu-CC49 was administered to groups of 10 mice bearing the LS-174T human colon carcinoma xenograft which had been transplanted 7 days previously and was approx-
**Table 2. Dose titration of **\(^{177}\)Lu-CC49 IgG for the ability to inhibit the growth of the LS-174T human colon carcinoma xenograft.**

<table>
<thead>
<tr>
<th>Dose ((\mu)Ci)</th>
<th>No. of mice</th>
<th>Day 42 post-MAb</th>
<th>Day 77 post-MAb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. surviving</td>
<td>Tumor reduction*</td>
<td>NETG</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>8 (100)</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>8 (100)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>100</td>
<td>8</td>
<td>8 (100)</td>
<td>5 (63)</td>
</tr>
<tr>
<td>200</td>
<td>8</td>
<td>8 (100)</td>
<td>7 (88)</td>
</tr>
<tr>
<td>350</td>
<td>9</td>
<td>9 (100)</td>
<td>7 (78)</td>
</tr>
<tr>
<td>500</td>
<td>9</td>
<td>4 (44)</td>
<td>4 (44)</td>
</tr>
</tbody>
</table>

* Tumor reduction, a <10-fold increase in tumor volume post-MAb administration. The average increase in the control group was 107-fold, with a minimum value of a 26-fold tumor volume increase; NETG, less than doubling in tumor volume post-MAb administration. Numbers in parentheses, percentages from total number of mice injected. Mice were treated with MAb 7 days after tumor transplantation. Tumors were approximately 30 mm\(^3\) in volume.

* All mice were sacrificed after day 42 of observation because of large size and/or rapid growth of tumors.

* All mice were sacrificed after day 70-post-MAb treatment because of large size of most tumors.

It thus appears from these studies that the 200-\(\mu\)Ci dose of groups. The first is termed “tumor reduction.” This is arbitrarily defined as a <10-fold increase in tumor volume post-MAb administration. The average tumor volume increase in the control group receiving unlabeled CC49 was 107-fold, with a minimum value of a 26-fold tumor volume increase. The second criteria was termed NETG. This is defined as a less than doubling in tumor volume post-MAb administration.

In the group of mice receiving 50 \(\mu\)Ci \(^{177}\)Lu-CC49, an antitumor effect was seen in some of the mice (Fig. 3B). At day 42 post-MAb administration, 3 mice showed tumor reduction by the above criteria and one of these showed no evidence of tumor growth in the group of mice receiving 100 \(\mu\)Ci \(^{177}\)Lu-CC49 (Fig. 3B; Table 2). By day 70 post-MAb administration, however, all of the mice in this group were sacrificed because of rapid growth of most tumors.

More pronounced antitumor effects were seen when 200 \(\mu\)Ci of \(^{177}\)Lu-CC49 were used (Fig. 3B; Table 2). At day 42 post-MAb administration, one half of the mice showed marked reduction in tumor growth and 3 of these showed no evidence of tumor growth. By day 77 post-MAb administration, however, all mice did show some evidence of tumor growth.

A substantial and long-lasting antitumor effect was seen when 200 \(\mu\)Ci of \(^{177}\)Lu-CC49 was used. At day 77, one of 8 mice had died of tumor, one had a very small tumor, and 6 showed no evidence of tumor growth (Fig. 3D). Very similar results were seen when 350 \(\mu\)Ci of \(^{177}\)Lu-CC49 was used (Fig. 3E; Table 2). When 500 \(\mu\)Ci of \(^{177}\)Lu-CC49 was used, however, 5 of 9 mice died of toxicity by the day 42 observation point. The remaining 4 mice showed no evidence of tumor growth or minimal tumor growth by day 77 (Fig. 3F; Table 2).

It thus appears from these studies that the 200-\(\mu\)Ci dose of
we found that 30-60% of the LS-174T cells express TAG-72. All animals in the dose titration study were sacrificed on day 77 post-MAb administration, except one animal which was sacrificed on day 70.

In previous studies (6), tumor growth were analyzed histologically by the avidin-biotin-complex immunoperoxidase method for tumor cells expressing TAG-72. Original outgrowth of an antigen-negative subpopulation. Original escaped the radioimmunotherapy were probably not the result of an outgrowth of an antigen-negative subpopulation. Original tumor sites in mice that had no evidence of tumor growth by microscopy were biopsied from the host.

Histological Studies. Microscopy was performed to analyze the tumors for histological type and antigenic phenotype. At 77 days after immunotherapy, established tumors and sites of tumors that had responded to therapy were biopsied from the various dose groups. We previously showed (6) that the LS-174T xenograft is an adenocarcinoma with approximately 30-60% of the cells expressing the TAG-72 antigen. As shown in Table 3, surviving tumors of various sizes were adenocarcinomas with between 30 and 80% of the cells expressing TAG-72. 60% of the cells expressing the TAG-72 antigen. As shown in Table 3, surviving tumors of various sizes were adenocarcinomas with between 30 and 80% of the cells expressing TAG-72.

Table 3 Gross and histological analyses of tumors in mice treated with 177Lu-CC49

<table>
<thead>
<tr>
<th>Dose (µCi)</th>
<th>Animal no.</th>
<th>Tumor volume (mm³)</th>
<th>% TAG-72-positive tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1-1</td>
<td>3790</td>
<td>30 30 30 0</td>
</tr>
<tr>
<td>100</td>
<td>2-6</td>
<td>2137</td>
<td>60 60 60 0</td>
</tr>
<tr>
<td>200</td>
<td>3-3</td>
<td>320</td>
<td>30 50 0</td>
</tr>
<tr>
<td>350</td>
<td>4-2</td>
<td>9.5</td>
<td>5 30 0</td>
</tr>
<tr>
<td>500</td>
<td>5-3</td>
<td>14</td>
<td>NTP NTP NTP</td>
</tr>
<tr>
<td>750</td>
<td>5-7</td>
<td>18</td>
<td>NTP NTP NTP</td>
</tr>
<tr>
<td>750</td>
<td>5-9</td>
<td>126</td>
<td>40 40 0</td>
</tr>
</tbody>
</table>

* NTP, no tumor cells present.  
* Strong mucin staining.

177Lu-CC49 resulted in the most favorable antitumor effect for the human colon carcinoma xenograft, with limited toxicity for the host.

Histological Studies. Microscopy was performed to analyze the tumors for histological type and antigenic phenotype. At 77 days after immunotherapy, established tumors and sites of tumors that had responded to therapy were biopsied from the various dose groups. We previously showed (6) that the LS-174T xenograft is an adenocarcinoma with approximately 30-60% of the cells expressing the TAG-72 antigen. As shown in Table 3, surviving tumors of various sizes were adenocarcinomas with between 30 and 80% of the cells expressing TAG-72 as detected by MAbs B72.3 and CC49. Thus, those tumors that were examined. Animals receiving moderate levels of radiolaabeled CC49 showed sinusoidal congestion, but animals receiving the higher amounts of radiation showed marked hypocellularity of a normally 95–100% cellular marrow (Table 4). This is consistent with previously described effects of radiation on the bone marrow (33). Of significance, 14 of 17 mice in the 200- and 350-µCi groups survived the 77-day observation period; thus, the atrophy and congestion observed in the marrows of these groups were reversible. In fact, marrows in animals receiving 200, 350, and 500 µCi and surviving 77 days showed no pathological changes.

Other tissues were examined for radiation toxicity. No pathological changes were seen in any femoral bone specimens. Spleen, small intestine, and lung from 2 mice in each of the 200-, 350-, and 500-µCi groups showed no pathological changes at day 77.

All mice from each of the 6 groups were also monitored for body weights. Averages at the end of the observation periods were 22.0, 21.9, 24.3, 25.9, 26.8, and 21.3 g for the 50-, 100-, 200-, 350-, 500-, and 0-µCi groups, respectively. As can be seen, if anything, a slight increase in body weight was observed in the mice receiving the higher doses. In a second experiment, no differences in weights were observed for mice receiving unlabeled CC49, and 177Lu-CC49 at 50, 100, and 200 µCi.

Dose Fractionation Studies. Additional studies were undertaken with three goals. The first was to determine whether dose fractionation of 177Lu-CC49 could reduce toxicity and consequently increase therapeutic efficacy. The second goal was to determine the differential (if one existed) between specific and nonspecific radiotherapeutic effects by comparing single or multiple doses of 177Lu-CC49 with that of an isotype-matched control MAb, 177Lu-MOPC-21. The third goal was to determine whether 177Lu-CC49 could reduce the growth of relatively large human colon cancer xenografts (approximately 300-mm³ volume versus approximately 30-mm³ volume in the previous studies).

Groups of 9 or 10 mice bearing established LS-174T human colorectal carcinoma xenografts of approximately 300 mm³ were treated with either 1, 2, or 3 doses of 250 µCi of 177Lu-CC49 at weekly intervals; parallel groups were treated with control 177Lu-MOPC-21 MAb at the same doses and specific activities. The actual number of animals surviving or with no evidence of tumor growth is given in Table 5. As shown in Fig. 4A and Table 5, one dose of 250 µCi 177Lu-CC49 was sufficient to reduce the growth of the large LS-174T xenografts. Fig. 4, B and C, shows an even greater reduction with two and three doses of 250 µCi of MAb (resulting in total doses of 500 and 750 µCi, respectively). In both of these dose groups 90% (9 of 10) of the mice showed no evidence of tumor growth through the observation period; thus, the atrophy and congestion observed in the marrows of these groups were reversible. In fact, marrows in animals receiving 200, 350, and 500 µCi and surviving 77 days showed no pathological changes.

Table 4 Histological analysis of marrows in mice receiving 177Lu-CC49 or 177Lu-MOPC-21

<table>
<thead>
<tr>
<th>Dose</th>
<th>No lesions</th>
<th>Congestion</th>
<th>Hypocellularity</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µCi</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>100 µCi</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>200 µCi</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>350 µCi</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>500 µCi</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>250 µCi x 1 (250 µCi)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>250 µCi x 2 (500 µCi)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>250 µCi x 3 (750 µCi)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* NTP, no tumor cells present.  
* Strong mucin staining.  
* Same animal, two areas.  
* Numbers in parentheses, total dose.  
* ND, not done.
Fig. 4. Dose fractionation of $^{177}$Lu-CC49 and $^{177}$Lu-MOPC-21 versus growth of the LS-174T human colon carcinoma xenograft. Groups of 9–10 mice bearing tumors of approximately 300 mm$^3$ were treated with $^{177}$Lu-CC49 or $^{177}$Lu-MOPC-21 at 14, 21, 28 days after tumor implantation (arrows). Each line, growth of tumor in an individual mouse. – – – – , growth of tumors in mice treated with unlabeled MAb CC49, as indicated in Fig. 3; †, death of a mouse. A, 250 µCi $^{177}$Lu-CC49 at day 14; B, 250 µCi $\times$ 2 (500 µCi total) $^{177}$Lu-CC49 at days 14 and 21; C, 250 µCi $\times$ 3 (750 µCi total) $^{177}$Lu-CC49 at days 14 and 21, and 28; D, 250 µCi $^{177}$Lu-MOPC-21 at day 14; E, 250 µCi $\times$ 2 (500 µCi total) $^{177}$Lu-MOPC-21 at days 14 and 21; F, 250 µCi $\times$ 3 (750 µCi total) $^{177}$Lu-MOPC-21 at days 14, 21, and 28.

Table 5. Dose fractionation of $^{177}$Lu-CC49 and $^{177}$Lu control MAb MOPC-21 to inhibit the growth of the LS-174T human colon carcinoma xenografts

Mice were given one to three injections of 250 µCi $^{177}$Lu-CC49 or $^{177}$Lu-MOPC-21 beginning day 14 after tumor implantation. Injections for multiple-dose schedules were administered 1 week apart. The average tumor volume at day 14 for all animals was approximately 300 mm$^3$. Numbers in parentheses, percentages. Tumor sizes were measured weekly. Values given are at day 56.

<table>
<thead>
<tr>
<th>MAb</th>
<th>Dose schedule</th>
<th>Total dose (µCi)</th>
<th>No. of animals</th>
<th>Surviving mice</th>
<th>NETG</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC49</td>
<td>250 µCi $\times$ 1</td>
<td>250</td>
<td>10</td>
<td>10 (100)</td>
<td>7 (70)</td>
</tr>
<tr>
<td>CC49</td>
<td>250 µCi $\times$ 2</td>
<td>500</td>
<td>10</td>
<td>10 (100)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>CC49</td>
<td>250 µCi $\times$ 3</td>
<td>750</td>
<td>10</td>
<td>9 (90)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>MOPC-21</td>
<td>250 µCi $\times$ 1</td>
<td>250</td>
<td>9</td>
<td>9 (100)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>MOPC-21</td>
<td>250 µCi $\times$ 2</td>
<td>500</td>
<td>9</td>
<td>9 (100)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>MOPC-21</td>
<td>250 µCi $\times$ 3</td>
<td>750</td>
<td>9</td>
<td>9 (100)</td>
<td>4 (44)</td>
</tr>
</tbody>
</table>

the 56-day observation period (Table 5). It should be pointed out that when 500 µCi of $^{177}$Lu-CC49 was given in one administration 5 of 9 (56%) mice died of toxicity (Table 2). On the other hand, 9 of 10 animals survived the 750-µCi dose when it was fractionated ($3 \times 250$ µCi), thus reinforcing the advantage of dose fractionation of radioconjugated MABs.

Fig. 4, D–F, also demonstrates the great differential between nonspecific (MOPC-21) and specific (CC-49) effects of a $^{177}$Lu-immunoconjugate. This differential effect was even more striking than the effects seen previously (6) between $^{131}$I-B72.3 and $^{131}$I-control MAB in the same xenograft model system. This is probably due to the greater nonspecific effect of an $^{131}$I-labeled MAB due to the greater path length of the $^{131}$I-$\gamma$ emission and the higher tumor uptake of CC49 as compared to B72.3.

Histological Studies. Microscopy was performed to analyze the tumors for histological type and antigenic phenotype at day 56. Established tumors and sites of tumors that had responded to therapy were biopsied from the various dose groups. Surviving tumors of various sizes were adenocarcinomas with between 30 and 80% of the cells expressing TAG-72 as detected by MAb CC49. Again, those that escaped the radioimmunotherapy were probably not the result of an outgrowth of an antigen-negative subpopulation. Original tumor sites in mice that had no evidence of tumor growth by caliper measurement ($n = 12$) were also analyzed histologically. In one case, no histological evidence of tumor cells was found, and in two, rare neoplastic cells could not be excluded. Most of the remaining cases revealed only microscopic clusters of potentially viable neoplastic cells.

Although selection of antigen-negative tumors was not found in treatment-resistant groups, a histological dimorphism was observed. The LS-174T xenograft is a human colon tumor cell line. It is a moderately differentiated adenocarcinoma showing gland formation with mucin secretion. The nuclear to cytoplasmic ratio is high and the cytoplasm is basophilic. Mitoses are frequent. This morphology was observed in untreated and treatment-resistant animals.

Histologically intact neoplastic cells in animals showing response to radiolabeled MABs showed a poorly differentiated adenocarcinoma with little gland formation but increased extracellular mucin often appeared in pools. The cells showed a decreased nuclear to cytoplasmic ratio with nuclear pleomorphism and multinucleation, cytoplasmic eosinophilia, and absence of mitotic activity. The histopathological changes observed in the radioimmunotreated group are consistent with previously described changes in tissues exposed to high-dose radiation and termed "reproductive death" (34).
Histological studies were also performed to analyze various tissue sites for evidence of radiation toxicity. Two mice from each of the three groups (250, 500, and 750 μCi) were sacrificed 14 days after MAB administration, and the femoral and proximal tibial marrows were examined. Marrows from 5 of 6 animals receiving radiolabeled CC49 showed congestion (Table 4). Of significance, four of these animals received equal or more radiation than those in the dose titration study; however, no marrow atrophy was found.

Other tissues were examined for radiation toxicity. Spleens showed increased polymorphonuclear leukocytes and congestion of the red pulp at 14 days after MAB treatment. No pathological changes were seen in any femoral bone specimens. Livers, spleens, small intestine, and lung from 2 mice in each of the 3 groups showed no pathological changes at day 56.

These studies thus demonstrate (a) the difference between specific and nonspecific effects of a 177Lu-labeled MAB, (b) the advantage of dose fractionation, and (c) the ability of the 177Lu-CC49 immunoconjugate to eliminate a relatively large (300 mm³) human colon carcinoma xenograft.

**DISCUSSION**

The studies reported here are the first to utilize 177Lu in a radiotherapeutic immunoconjugate. The MAB used, CC49, is reactive with the same antigen as B72.3, i.e., TAG-72, but the two MAbS have been shown to react with different epitopes (24). Moreover, MAB CC49 has a higher Ks for TAG-72 and reacts to a greater percentage of tumor cells than B72.2 (15).

Recently, 131I-labeled CC49 was used in diagnostic clinical trials with an intraoperative hand-held γ-detecting probe. In a limited number of primary and metastatic colorectal cancer patients, CC49 was shown to detect tumors in 80% (8 of 10) of cases (35). In an ongoing phase I trial with 131I-CC49, metastatic tumor was also visualized by γ-scanning in the majority of cases.6

It is important to point out that virtually every radionuclide that can potentially be used therapeutically in a radioimmunoconjugate has its own advantages and disadvantages. One must thus consider each radionuclide in terms of its use with a given MAb or modified MAB construct, the type of tumor being treated, the route of MAB administration, and the size of the tumor mass(es) in question. Indeed, some of the answers to the pros and cons of various radionuclides and MABs may well have to be developed via empirical analyses of clinical trial data.

For example, 177Lu has several advantages and some disadvantages when compared to 90Y or 131I for use as a radioimmunoconjugate for the treatment of solid tumors. 177Lu has a half-life of 161 h (6.7 days) as compared to a half-life of 2.7 days for 90Y and 8 days for 131I. The longer half-life of 177Lu when compared to 90Y may be considered an advantage in terms of marrow toxicity arising from radioimmunoconjugate in the circulation in that a smaller fraction of the total radioactive decays occur, whereas circulating levels of the MAB are high. Similarly, a greater fraction of the total decays occur on the tumor. These advantages are possible with this system because of the fact that anti-TAG-72 MABs have been shown to have a relatively long residence time on tumors (up to 21 days) (28); moreover, it may also take several days for the radioimmunoconjugate to penetrate larger tumor masses. The advantage of reduced marrow dose as a result of the longer half-life of 177Lu arises because the longer half-life allows for clearance of the antibody from circulation. As a result, a proportionately larger fraction of the total decays occur after blood levels are reduced and the marrow exposure is therefore lower. The longer half-life of 177Lu (relative to 90Y) is a disadvantage in that it requires more stringent chelation properties of the bifunctional chelating agent used to attach the radionuclide to the MAB. Since the in vivo radioactivity persists for a longer time, the rate of loss of the radionuclide from the MAB while in circulation must be very low in order to avoid uptake of 177Lu in normal tissues such as bone.

Comparisons of the β and γ emissions as well as other properties of these three radionuclides are also of interest. A problem with 131I is the γ-rays which are emitted in high abundance at a high energy (364 keV, 84%). These long range emissions may contribute to unwanted marrow toxicity, present a problem for handling of 131I by health care workers, and may also affect the amount of time a patient must stay relatively isolated in the hospital after MAB treatment. Some chemical forms of iodine are also volatile. 90Y, on the other hand, is a pure β emitter and therefore cannot be efficiently detected via scintillation imaging to define tumor localization. The γ-rays emitted by 177Lu are moderate in energy and of relatively low abundance (208 keV, 11%; 113 keV, 7%) which enables one to define the radiolocalization of the radioimmunoconjugate via scintillation imaging when it is being used in therapeutic trials. It is also anticipated that patients receiving relatively high doses of 177Lu could potentially be released as outpatients following treatment with a 177Lu-radioimmunoconjugate.

90Y emits β particles having a maximum energy of 2280 keV and an average energy of 935 keV. Assuming a cell diameter of 20 μm, this means that, when deposited on a cell surface, 90Y can potentially kill at an average of 150–200 cell diameters with some potential killing at distances of 500–600 cell diameters. 177Lu emits β particles with a maximum energy of 497 keV and an average energy of 133 keV. This translates into its ability to potentially kill cells at approximately 12 cell diameters from a cell on whose surface it is deposited, with a maximum cell-killing potential of approximately 50 cell diameters. While the extended range of the 90Y β particles is a great advantage in terms of tumor cell antigenic heterogeneity, it also means that for smaller tumors (diameter, <1 cm) a significant fraction of the radiation dose arising from radiolabeled MABs localized to tumor cells is deposited outside of the tumor (36). The shorter range 177Lu β-rays, while not as good as 90Y in terms of dealing with tumor cell antigenic heterogeneity, may be sufficient for many solid tumors. In the LS-174T xenograft model in which approximately 30–80% of cells express TAG-72, the 177Lu-immunoconjugate apparently has the ability to efficiently eliminate tumor masses as described in this report.

The shortened path length of 177Lu cell killing as compared to 90Y has significant potential, however, in terms of reduced marrow toxicity. It was previously demonstrated (37) that, when deposited in cortical bone, high energy β emitters such as 90Y have the ability to irradiate all areas of the marrow, while with lower energy emitters, such as 177Lu, the reduced path length will spare much of the marrow. This difference between 90Y and 177Lu may not necessarily be seen in murine xenograft studies because of the small size of the bones (and thus diameter of the marrow) in the mouse as compared to humans. Finally, the relatively low γ and shorter path length β emissions of 177Lu reduce the problems of handling by health care personnel.

The studies reported here demonstrate that 177Lu-labeled...
CC49 can clearly elicit markedly reduced tumor growth, if not cure, in a model using a relatively large established human colon carcinoma xenograft, with very limited toxicity. 

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Monoclonal Antibody-based Therapy of a Human Tumor Xenograft with a \(^{177}\)Lutetium-labeled Immunoconjugate

Jeffrey Schlom, Kathleen Siler, Diane E. Milenic, et al.


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