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

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ABSTRACT

$^{177}$Lutetium ($^{177}$Lu) is a member of the family of elements known as lanthanides or rare earths. Monoclonal antibody (MAb) CC49, a murine IgGl, 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 $^{177}$Lu-labeled immunoconjugate, $^{177}$Lu-CC49, in an experimental therapy model for human carcinoma. $^{177}$Lu-CC49 was shown to delay the growth of established LS-174T human colon carcinomas in athymic mice at a single dose of 50 μCi. Overt toxicity was observed with the administration of approximately 500 μCi of $^{177}$Lu-CC49 in which 5 of 9 mice died of apparent marrow toxicity. A single administration of 200 or 350 μCi of $^{177}$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 μCi of $^{177}$Lu-CC49 (250 μCi/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 mm³) 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, $^{177}$Lu-MOPC-21. In all dose schedules, a large differential was seen between the therapeutic effects of the $^{177}$Lu-CC49 versus that of the $^{177}$Lu-control MAb. The merits and limitations of the use of $^{177}$Lu-labeled immunoconjugates (in particular, $^{177}$Lu-CC49) are discussed in terms of potential novel therapeutic strategies 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,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 $^{131}$I, $^{90}$Y, $^{212}$Bi, and $^{211}$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 normal 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 (mucosa adjacent to colorectal cancer), and to a much lesser extent, apocrine metaplasia and normal colonic epithelium (15–17).

The first anti-TAG-72 MAb to be used clinically was B72.3 (18). This MAb, coupled to $^{131}$I, $^{111}$In, or $^{252}$I, has been shown to target 70–80% of carcinoma lesions in numerous diagnostic clinical trials (e.g., 19–23).

Preclinical therapy studies with $^{131}$I-B72.3 using a human colon cancer xenograft have shown that a 600-μCi dose will inhibit tumors from growing (6). This dose, however, killed 6 of 10 mice. Dose fractionation protocols using $^{131}$I-B72.3, however, revealed that 2 x 300 or 3 x 200 μCi were much more efficient than one dose of 600 μCi. Moreover, mice could tolerate a dose as high as 900 μCi (300 μCi given 3 times at weekly intervals) which resulted in substantial tumor inhibition. In these $^{131}$I therapy studies, however, a reduced therapeutic component was also observed with control MAb, most likely due to whole body γ-irradiation. The dose-limiting toxicity in these studies was shown to be marrow. Phase I clinical trials with $^{131}$I-B72.3, $^{90}$Y-B72.3, and $^{131}$I-chimeric B72.3(γ4) are currently underway in several institutions.

A series of second-generation anti-TAG-72 MAbs has been developed (24). One of these, CC49, has been shown to have a higher Kᵣ than B72.3 (2.54 versus 16.18 x 10⁶ M⁻¹), react to a higher percentage of tumor cells in a wide range of tumor types, and localize tumors in xenografts more efficiently than B72.3 (15, 24, 25).

We report here the use of a novel radiolabeled MAb conjugate: Lutetium-177 radiolabeled MAb CC49. Lutetium is a member of the family of elements known as lanthanides or rare earths (26). $^{177}$Lu has a half-life of 616 h, β emissions of 497 keV (maximum) and 133 keV (average), and γ emissions of 208 keV (11%) and 113 keV (7%). This latter property allows one to track the radioimmunoconjugate in therapy protocols using external γ scintigraphy. These studies demonstrate the $^{177}$Lu-CC49 immunoconjugate to be efficient in reducing and/or eliminating the growth of human colon carcinoma xenografts with minimal toxicity. Dose fractionation protocols were utilized to reduce or eliminate tumors of larger size. In these studies, moreover, large differences in therapeutic efficacy were seen between $^{177}$Lu-CC49 and a $^{177}$Lu-labeled control MAb.
The potential advantages of $^{177}$Lu-immunoconjugates, and in particular $^{177}$Lu-CC49, for human carcinoma therapy are discussed.

MATERIALS AND METHODS

Monoclonal Antibodies. MAb CC49 (24) is a murine IgG1 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 IgG1 also reactive with TAG-72 and was described previously (18). MOPC-21 (27) was used as an IgG1 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 $^{177}$Lu-CC49 and the $^{177}$LuCC49 Iodo-Gen labeling procedure (28) resulted in >90% precipitable 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% acrylamide. Samples containing 100 μg of protein were separated on a 10% acrylamide gel. 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 d.

$^{177}$Lu Labeling of Mabs. $^{177}$Lu was produced by the neutron irradiation of isotopically enriched $^{176}$LuO$_2$ (44% Lu-176; Oak Ridge National Laboratory, Oak Ridge, TN) at the University of Missouri Research Reactor. Neutron irradiation of $^{176}$Lu to produce $^{177}$Lu also results in the production of $^{178m}$Lu. Under the irradiation conditions used to produce the $^{177}$Lu, there were 4 x 10$^{-4}$ μCi of $^{176m}$Lu/μCi of $^{177}$Lu. Therefore, in the highest $^{177}$Lu dose group (500 μCi) described below, the animals received ~0.02 μCi of $^{176m}$Lu. An extensive GeLi analysis (12-h count time) of an aliquot of the $^{177}$Lu detected no radionuclides (rare earth or otherwise) other than the $^{177}$Lu and the levels of $^{176m}$Lu described above.

A detailed report on the chelation of $^{177}$Lu by the bifunctional chelating agent PA-DOTA, and the subsequent conjugation of the complex to MAb 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 thioisophene and isolated on a reverse-phase cartridge (Alltech, 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-

$^{177}$Lu-PA-DOTA chelate coupled to 18 mg 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 $^{177}$Lu-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 preparation was used as a vehicle control. The solutions were 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 $^{177}$Lu-CC49 Mab to check for percentage of radiolabeled $^{177}$Lu bound to the Mab. The results indicated that >90% of the $^{177}$Lu 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 $^{177}$Lu-CC49 contained 5.46 mCi $^{177}$Lu coupled to 8.6 mg 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 $^{177}$Lu-MOPC-21 contained 5.2 mCi $^{177}$Lu coupled to 5 mg 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 calibration was first performed by preparing a stock solution of $^{177}$Lu 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 $^{177}$Lu-CC49 dose contained 254 μCi and the $^{177}$Lu-MOPC-21 contained 259 μCi. The third $^{177}$Lu-CC49 dose contained 234 μCi in 0.5 ml as measured in a dose calibrator. The $^{177}$Lu-MOPC-21 contained 240 μCi. For ease of presentation, the 3 dose fractions are approximated at 250 μCi each for both $^{177}$Lu-CC49 and $^{177}$Lu-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 in the right flank of 1 x 10$^6$ LS-174T cells in 0.1 ml. For the single-dose titration experiment, 7 days after inoculation, the mice bearing tumors of approximately 30 mm$^3$ were selected and divided into treatment groups. Groups of 8–10 mice each received an i.p. injection of designated amounts of $^{177}$Lu-IgG CC49. For the multiple-dose fractionation experiment, 14 days after tumor transplantation, the mice bearing tumors of approximately 300 mm$^3$ were selected and divided into treatment groups. Groups of 9 or 10 mice received an i.p. injection of approximately 250 μCi of $^{177}$Lu-IgG CC49 or $^{177}$Lu-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
**RESULTS**

**In Vitro Analyses.** $^{177}$Lu-CC49 IgG was first compared with $^{125}$I-CC49 IgG for its ability to bind to the TAG-72-positive LS-174T human colon carcinoma xenograft extract. As seen in Table 1, both radioimmunoconjugates gave similar binding, and neither bound to the TAG-72-negative A375 human melanoma xenograft extract.

Table 1  *In vitro binding of $^{177}$Lu-CC49 IgG*

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

Fig. 1. SDS-PAGE analysis of radiolabeled MAb CC49. $^{177}$Lu-CC49 (lane 1) and $^{125}$I-CC49 (lane 2) were analyzed by SDS-PAGE and subjected to autoradiography. Samples were run under nonreducing (A) and reducing conditions (B) on a 5–20% discontinuous gradient gel.

In Vivo Analyses. A detailed analysis of the pharmacokinetics and tumor versus normal tissue uptake of $^{177}$Lu-CC49 IgG will be described in a forthcoming paper. The Bz-DTPA, Mx-DTPA, and the PA-DOTA chelates, as well as $^{177}$Lu, $^{153}$Sm, and $^{90}$Y 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 $^{177}$Lu-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, the percentage of injected dose/g values of $^{125}$I-labeled CC49 were as follows: tumor, 23.4; liver, 1.3; spleen, 1.2; blood, 1.1; kidneys, 0.4; and lungs, 0.6. The SEM ranged from 0.07 to 0.34 for the $^{177}$Lu-CC49, and for the $^{125}$I-CC49 ranged from 0.18 to 0.56.

Dose Titrations. $^{177}$Lu-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-


**Fig. 3.** Treatment of mice bearing the LS-174T xenograft with $^{177}$Lu-labeled CC49 IgG. Groups of 8–9 mice were treated with $^{177}$Lu-CC49 IgG 7 days after tumor implantation. Tumors were approximately 30 mm$^3$ at time of MAb administration. A, unlabeled CC49. Each line represents the tumor growth in an individual mouse; measurements were taken weekly. †, death of a mouse; •, average tumor sizes of this group. This line is reproduced in B–F for comparisons. B, 50 µCi of $^{177}$Lu-CC49; C, 100 µCi of $^{177}$Lu-CC49; D, 200 µCi of $^{177}$Lu-CC49; E, 350 µCi of $^{177}$Lu-CC49; F, 500 µCi of $^{177}$Lu-CC49.

**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 (µCi)</th>
<th>No. of mice</th>
<th>No. surviving</th>
<th>Tumor reduction*</th>
<th>NETG</th>
<th>No. surviving</th>
<th>NETG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>8 (100)</td>
<td>0</td>
<td>0</td>
<td>0*</td>
<td>NA</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>8 (100)</td>
<td>3 (38)</td>
<td>1 (13)</td>
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<td>3 (38)</td>
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<td>8 (100)</td>
<td>7 (88)</td>
<td>7 (88)</td>
<td>7 (88)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>350</td>
<td>9</td>
<td>9 (100)</td>
<td>7 (78)</td>
<td>7 (78)</td>
<td>7 (78)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>500</td>
<td>9</td>
<td>9 (44)</td>
<td>4 (44)</td>
<td>3 (33)</td>
<td>4 (44)</td>
<td>3 (33)</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.

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

9 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-µCi dose of $^{177}$Lu-CC49 administered to the groups: 50, 100, 200, 350, and 500 µCi, as well as unlabeled CC49. As shown in Fig. 3A, tumors from all mice receiving unlabeled CC49 IgG grew considerably; all mice from this group were sacrificed following day 42 after MAB treatment. Two criteria were used to measure antitumor effects in all 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 criterion was termed NETG. This is defined as a less than doubling in tumor volume post-MAB administration.

In the group of mice receiving 50 µCi $^{177}$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 (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 100 µCi of $^{177}$Lu-CC49 were used (Fig. 3C; 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 µCi 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). Similar results were seen when 350 µCi of $^{177}$Lu-CC49 was used (Fig. 3E; Table 2). When 500 µCi of $^{177}$Lu-CC49 was used, however, only 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 at day 77 (Fig. 3F; Table 2).

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we found that 30-60% of the LS-174T cells express TAG-72. All animals in the
animal 1-1 which was sacrificed on day 70. dose i ¡trai urn study were sacrificed on day 77 post-MAb administration, except
noperoxidase method for tumor cells expressing TAG-72. In previous studies (6)
tumor growth were analyzed histologically by the avidin-biotin-complex immu-
caliper measurements were also analyzed histologically. In 5 of
of an outgrowth of an antigen-negative subpopulation. Original
Table 3, surviving tumors of various sizes were adenocarcino-
tumors that had responded to therapy were biopsied from the
sites in mice that had no evidence of tumor growth by
mas with between 30 and 80% of the cells expressing TAG-72
60% of the cells expressing the TAG-72 antigen. As shown in
77 days after immunotherapy, established tumors and sites of
Histological Studies. Microscopy was performed to analyze
the tumors for histological type and antigenic phenotype. At
177Lu-CC49 resulted in the most favorable antitumor effect for
human colon carcinoma xenograft, with limited toxicity for
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 adenocarcino-
mas with between 30 and 80% of the cells expressing TAG-72
d as detected by MAbs B72.3 and CC49. Thus, those tumors that
mas with between 30 and 80% of the cells expressing TAG-72

Table 3 Gross and histological analyses of tumors in mice treated with

<table>
<thead>
<tr>
<th>Dose (µCi)</th>
<th>Animal no.</th>
<th>Tumor volume (mm³)</th>
<th>% TAG-72-positive tumors</th>
<th>B72.3</th>
<th>CC49</th>
<th>MOPC-21</th>
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<tr>
<td>50</td>
<td>1-1</td>
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<tr>
<td>3-6</td>
<td>0</td>
<td>NTP†</td>
<td>NTP</td>
<td>NTP</td>
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<td>3-7</td>
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<td>NTP</td>
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</tr>
<tr>
<td>350</td>
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<td>5-7</td>
<td>18</td>
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<td>5-9</td>
<td>126</td>
<td>40</td>
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<td>0</td>
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* NTP, no tumor cells present.
† Strong mucin staining.

Groups of 9 or 10 mice bearing established LS-174T human
colon carcinoma xenografts of approximately 300 mm³ were
beared 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

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

<table>
<thead>
<tr>
<th>Dose (µCi)</th>
<th>No lesions</th>
<th>Congestion</th>
<th>Hypocellularity</th>
<th>177Lu-CC49</th>
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<th>Congestion</th>
<th>Hypocellularity</th>
<th>177Lu-MOPC-21</th>
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<td>50 µCi</td>
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<td>2</td>
<td>0</td>
<td>ND</td>
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<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>200 µCi</td>
<td>1</td>
<td>1*</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>350 µCi</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>500 µCi</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>250 µCi × 1 (250 µCi)³</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>250 µCi × 2 (500 µCi)</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>250 µCi × 3 (750 µCi)</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Same animal, two areas.
³ 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 xenografts. 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 × 2 (500 μCi total) $^{177}$Lu-CC49 at days 14 and 21; C, 250 μCi × 3 (750 μCi total) $^{177}$Lu-CC49 at days 14, 21, and 28; D, 250 μCi $^{177}$Lu-MOPC-21 at day 14; E, 250 μCi × 2 (500 μCi total) $^{177}$Lu-MOPC-21 at days 14 and 21; F, 250 μCi × 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

<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>
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<td>CC49</td>
<td>250 μCi × 1</td>
<td>250</td>
<td>10</td>
<td>10 (100)</td>
<td>7 (70)</td>
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<tr>
<td>CC49</td>
<td>250 μCi × 2</td>
<td>500</td>
<td>10</td>
<td>10 (100)</td>
<td>9 (90)</td>
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<tr>
<td>CC49</td>
<td>250 μCi × 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 × 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 × 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 × 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 × 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 immunonjugate 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 immunonjugate. 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 Kᵣ for TAG-72 and reacts to a greater percentage of tumor cells than B72.3 (15). Recently, 123I-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 radioimmunonjugate 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 radioimmunonjugate 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 radioimmunonjugate 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 radioimmunonjugate 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 radioimmunonjugate 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-radioimmunonjugate.

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-immunonjugate 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

6 S. Larson, personal communication.
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. 177Lu-radioimmunoconjugates, and in particular 177Lu-CC49, may thus now be considered as potential candidates for the treatment of a range of human carcinomas.

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REFERENCES


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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