High-Dose Therapy with $^{90}$Yttrium-labeled Monoclonal Antibody CC49: A Phase I Trial

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ABSTRACT
A Phase I trial of increasing administered activities of $^{90}$yttrium ($^{90}$Y)-labeled monoclonal antibody (MAb) CC49 was conducted to determine whether extrahematopoietic toxicity occurred with this radioimmunoconjugate. Twelve patients with various gastrointestinal tract cancers were administered a tracer dose of $^{111}$In-labeled MAb CC49 for biodistribution and pharmacokinetic studies. Patients then underwent a single treatment with increasing administered activities of $^{90}$Y-labeled MAb CC49 (0.3, 0.4, and 0.5 mCi/kg). Biodistribution studies, using $^{111}$In-labeled MAb CC49 as a surrogate, were determined using planar and single photon emission computed tomography imaging. Pharmacokinetic studies were performed by measuring radioactivity in blood samples taken at intervals after radioimmunoconjugate infusions. Tissue biopsies of tumor metastases and related normal tissues (liver and bone marrow) were obtained for radioactivity measurements. Radiation dosimetry estimates were calculated using these data. Toxicity was evaluated using the National Cancer Institute Common Toxicity Criteria. No dose limiting extrahematopoietic toxicity was identified in the range of administered activities used in this study. Radioimmunolocalization based on planar and single photon emission computed tomography images $^{111}$In-labeled MAb CC49 showed heterogeneous (nonspecific) liver and splenic uptake. Liver metastases were usually hypofocalic, and extrahepatic metastases showed faint to moderate uptake. The $\alpha$ and $\beta$ half-lives of $^{111}$In-labeled MAb CC49 and $^{90}$Y-labeled MAb CC49 in the blood were similar. Absorbed radiation dose estimates in metastatic tumor sites ranged from 180 to 3000 cGy. The percentage of injected dose/kg of tumor ranged from 1.12 to 18.14; however, tumor:normal liver ratios were consistently <1. No objective responses were observed. Doses of up to 0.5 mCi/kg could be administered with reversible grade IV myelotoxicity. Absorbed radiation dose in tumor was suboptimal, even at the highest administered activity level. Deposition of $^{90}$Y in liver was high, and estimates of absorbed dose in liver equaled or exceeded that which could be achieved in metastatic tumor sites. Strategies to enhance access of radioimmunoconjugates in tumor and diminish deposition in the liver need to be developed for effective treatment using MAb CC49 with chelated radiometals.

INTRODUCTION
Although the 5-year survival rates for many gastrointestinal adenocarcinomas appear to be improving, treatment of recurrent or metastatic disease remains problematic. Systemic chemotherapy is often used in an attempt to achieve tumor control in patients with metastatic gastrointestinal tract cancer. Objective response rates range from <10% (e.g., gemcitabine therapy in advanced pancreatic cancer; Ref. 1) to ~40% (e.g., 5-FU-based) regimens in metastatic gastric cancer; Ref. 2). External beam radiotherapy is commonly used for either local control or palliative intent in patients with unresectable or metastatic gastrointestinal tract cancers. Because of the local nature of external beam radiotherapy, this therapy does not often affect the natural history of the disease or overall survival in patients with metastatic gastrointestinal tract cancers. However, the use of radiation, particularly in combination with chemotherapy, has proved to be of major importance for local control and possibly survival in esophageal (3), pancreatic (4), and rectal cancer (5). Obviously, a major disadvantage of radiation therapy lies in the necessary limitation of treatment volume or dose delivery in an effort to spare normal tissues.

Systemic radioimmunotherapy is an experimental approach that allows for selective targeting of radioactivity to tumor tissue while hopefully sparing normal organs. MAbs that target selective tumor-associated antigens have been developed for the purposes of cancer imaging and therapy. In solid tumors, the use of radioimmunoconjugates for radioimmunodetection has proved to be successful; clinical studies have documented that it is possible to identify occult disease in patients suspected of...
Therapy with 90 Y-labeled CC49

MATERIALS AND METHODS

Therapy with 90 Y-labeled CC49 activity selection, definition of maximum tolerated dose, and evaluable patients. Administered activities selected for study were 0.3, 0.4, and 0.5 mCi/kg. The specific activity of the radiolabeled MAb was ~9.6 mCi/mg (range, 8.1–11.9). Each dose was checked for the lack of pyrogens and the radiochemical purity of the MAb prior to administration. Each administered dose was adjusted to give a total protein dose of 5 mg and diluted with 0.9% NaCl injection images were acquired on the second and fifth day (2 and 5). Both planar and SPECT image acquisition were performed using a dual-headed gamma camera system. For regional SPECT acquisitions, the number of counts ranged from approximately 50,000–100,000 counts/frame. In planar views, a minimum of 1 million counts was acquired from the diagnostic and postradioimmunodetection studies because of its higher affinity and more rapid plasma clearance compared with MAb B72.3. MAb CC49 recognizes an epitope distinct from MAb B72.3 and exhibits higher reactivity to gastric, colonic, and pancreatic adenocarcinomas (12). Preclinical studies in athymic mice bearing human tumor xenografts showed improved targeting and a 3-5-fold greater therapeutic efficacy for MAb CC49 labeled with either 111In (13) or 90Y (13) than similarly labeled B72.3.

The following report details a Phase I trial of dose escalation of 90Y-labeled MAb CC49 in a setting of hematopoietic stem cell support in patients with metastatic gastrointestinal adenocarcinomas. Because 90Y has a pure beta energy emission, 111In-labeled MAb CC49 was used as a surrogate for radio-metal-labeled MAb uptake for biodistribution and dosimetric calculations.

MATERIALS AND METHODS

Patient Eligibility. Patients were required to have a histological diagnosis of metastatic or unresectable adenocarcinoma of the colorectum, stomach, pancreas, or esophagus. Immunoreactivity with MAb CC49 using standard immunohistochemical and immunocytochemical techniques, is preferentially expressed in adenocarcinomas (8). The antigen is uncommonly expressed in normal tissues, with the exception of secretory endometrium (9). In an effort to improve on the parental MAb B72.3, Muraro et al. (10) and Colcher et al. (11) developed and described a series of second generation MABs that also react with TAG-72. Murine MAb CC49 is a member of the initial library of antibodies produced and was selected for further clinical studies because of its higher affinity and more rapid plasma clearance compared with MAb B72.3. MAb CC49 recognizes an epitope distinct from MAb B72.3 and exhibits higher reactivity to gastric, colonic, and pancreatic adenocarcinomas (12). Preclinical studies in athymic mice bearing human tumor xenografts showed improved targeting and a 3-5-fold greater therapeutic efficacy for MAb CC49 labeled with either 111In (13) or 90Y (13) than similarly labeled B72.3.

The following report details a Phase I trial of dose escalation of 90Y-labeled MAb CC49 in a setting of hematopoietic stem cell support in patients with metastatic gastrointestinal adenocarcinomas. Because 90Y has a pure beta energy emission, 111In-labeled MAb CC49 was used as a surrogate for radio-metal-labeled MAb uptake for biodistribution and dosimetric calculations.

MATERIALS AND METHODS

Patient Eligibility. Patients were required to have a histological diagnosis of metastatic or unresectable adenocarcinoma of the colorectum, stomach, pancreas, or esophagus. Immunoreactivity with MAb CC49 using standard immunohistochemical analysis was documented in either a metastatic or primary lesion. Only patients with 30% or greater tissue positivity (based on a visual estimate) were eligible. Patients were required to be between 19 and 65 years of age and to have an Eastern Cooperative Oncology Group performance status of 0 or 1. Adequate hematological (white blood count, >3,500/mm³; platelet count, >150,000/mm³; internormal ratio, <2.0; partial thromboplastin time, <37.0 s) and organ function (bilirubin and serum creatinine <2.0 mg/dl, AST and alkaline phosphatase less than five times the upper limits of normal) was also required. Prior therapy was permitted and could include single- or multiple-agent chemotherapy, either alone or in combination with radiation therapy. Other exclusion criteria included patients with serious active medical illnesses (infection, heart disease, diabetes, hypertension, and others) requiring active medical management. Patients who had received prior immunotherapy with murine antibody were excluded. Patients who previously participated in radioimmunodetection studies with a murine MAb-based reagent were required to demonstrate a negative HAMA titer. All patients gave informed consent.

Treatment Schedule. Initial evaluation included a physical examination, histology review, immunohistochemistry, baseline blood work, chest X-ray examination, and CT scan of the abdomen and/or thorax. Subsequently, prior to treatment all patients underwent collection and cryopreservation of hematopoietic stem cells adequate for one autologous transplant. On day −3, an imaging dose of 111In-labeled MAb CC49 (~5 mCi; 5 mg of IgG) was given i.v. Whole-body exposure rate measurements were obtained using triplicate readings with standard geometry at distances of 1 meter from the mediastinum using a calibrated dose rate meter. These readings were continued daily until 90Y-labeled MAb CC49 was administered. In addition, blood samples (serum and whole blood) were obtained immediately after administration and at 30 min and 1, 2, and 3–4 h and daily for quantification of circulating 111In-labeled MAb CC49 activity. Daily total urine samples were obtained for 3 days after the infusion for pharmacokinetic measurement. Daily planar image acquisitions were performed for 5 days. SPECT images were acquired on the second and fifth day (~2 and +1). Both planar and SPECT image acquisition were performed using a dual-headed gamma camera system. For regional SPECT acquisitions, the number of counts ranged from approximately 50,000–100,000 counts/frame. In planar views, a minimum of 1 million counts was acquired from the diagnostic and postradioimmunodetection studies.

On test day 0, the selected therapy activity of 90Y-labeled MAb CC49 was administered through one port of a double lumen, indwelling central line. Blood samples were obtained for pharmacokinetic studies for 7 days after the therapy dose, and 24-h urine samples were collected for 3 days. Bone marrow aspirates and biopsies of accessible liver metastases and uninvolved liver were obtained 5–7 days after treatment.

Posttreatment Follow-Up. Patients were evaluated weekly for toxicity by clinical examination, complete blood count, and chemistry profile. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria and the Supplementary Toxicity Criteria for Bone Marrow Transplantation. Patients who achieved grade IV neutropenia received supportive care with prophylactic ciprofloxacin and granulocyte-colony stimulating factor. In addition, routine support for myelosuppression was provided including administration of appropriate blood products. Patients were admitted to the hospital only for fever that could not be controlled with outpatient antibiotic therapy. The protocol called for infusion of cryopreserved hematopoietic stem cells if patients did not recover to grade III neutropenia within 5 days of the initiation of granulocyte-colony stimulating factor. CT scans of the abdomen and/or thorax were repeated at 8-week intervals to determine response to treatment.
to 15–25 ml and infused i.v. over 20–30 min through an indwelling central line. Patients were prehydrated with 1–2 liters of 5% dextrose in 0.45% NaCl for injection.

If no grade III or IV extrahematopoietic toxicity occurred over a 4-week observation period after the initial three patients placed on a given activity level, the amount of $^{90}Y$ activity was escalated for the successive group of three patients. If any instance of grade III or IV extrahematopoietic toxicity had been observed, three additional patients would have been treated at the existing dose level, and the dose would have been escalated only if no further instances of grade III or IV toxicity was observed. If at any time two instances of grade III or IV extrahematopoietic toxicity and/or grade IV or V bone marrow supplementary toxicity had been observed at a given dosage level, entry onto that level would be terminated. The maximum tolerated dose was defined as the highest dose in which no more than one instance (maximum sample size, 6) of grade III or IV extrahematopoietic toxicity or grade IV or V bone marrow supplementary toxicity was observed.

All patients were evaluable for toxicity. The protocol also demanded that a minimum of three patients would be evaluable for dosimetry estimations at each dose level. Because of the anticipated problem of $^{111}$In deposition in liver, a minimum of at least three patients with extrahematopoietic metastases evaluable for dosimetry estimations in tumor were entered onto each dose level.

**Response Criteria.** Tumor measurements were recorded in centimeters using the longest diameter and perpendicular diameter at the widest portion of the tumor. A complete response was defined as total resolution of all measurable sites of disease for a minimum of 8 weeks. A partial response was defined as a 50% or greater decrease in the sum of the products of the perpendicular dimensions of all measurable lesions for a minimum of 4 weeks without the appearance of new lesions. Stable disease was defined as no change in measurable lesions or the appearance of new lesions. Stable disease was defined as a 50% or greater decrease in the sum of the products of the perpendicular dimensions of all measurable lesions for a minimum of 8 weeks without the appearance of new lesions. Stable disease was defined as a 50% or greater decrease in the sum of the products of the perpendicular dimensions of all measurable lesions for a minimum of 8 weeks without the appearance of new lesions. Duration of response was measured from the time of achievement of response to progression.

**Preparation and Administration of the Radioimmunoconjugate.** MAb CC49 modified with an average of two $2\text{-}p\text{-aminophenyl-1,4,7,10-tetraazacyclododecane-N,N',N''',N'''\text{-tetraacetic acid residues}}$ per molecule antibody (CC49-PA-DOTA) was stored at $-70^\circ\text{C}$ in sterile 2-ml plastic vials. All procedures were performed in a biological safety cabinet using aseptic techniques. Prior to radiolabeling, the antibody solution was brought to room temperature, and the vial was swabbed with alcohol.

To 1 mg of CC49-PA-DOTA in 0.2 ml of 0.05 M ammonium acetate (pH 6), $^{111}$InCl$_3$ (~10 nCi) in 0.05 M HCl was added. An aliquot of indium[III] to produce a metal:PA-DOTA molar ratio of 1:1 was added to improve the incorporation of the radiometal into the MAb chelate complex. The mixture was incubated at 37°C for 30 min. The reaction progress was monitored at 20 and 30 min on ITLC strips (developed with 0.9% saline). The reaction was terminated by the addition of 0.01 M DTPA in water. The $^{111}$In incorporation yield was verified on ITLC strips. The purification of $^{111}$In-labeled CC49-PA-DOTA was done on a sterile Sephadex G-50 column (1.5 × 30 cm) equilibrated with 0.05 M PBS (pH 7.4). The radioimmunoconjugate eluting in the void volume was collected into sterile vials, and the radioactivity was assayed in a Capintec dose calibrator.

The radiolabeling of CC49-PA-DOTA with $^{90}$Y was accomplished in an identical manner as described for $^{111}$In with the following minor modifications; the amount of CC49-PA-DOTA was increased to 4 mg and the reaction time was increased to 45 min.

Radiolabeled antibody preparations had to contain <5% free radionuclide (ITLC) and <175 endotoxin units/dose. The samples were also tested for immunoreactivity (solid-phase RIA) using the RhoChek assay (RhoMed, Albuquerque, NM), purity integrity [SDS-PAGE (5–20% gradient gel, SDS-PAGE) and high-performance liquid chromatography], and sterility; this set of tests was completed after the administration of the radiolabeled antibody.

The specified amount of radiolabeled MAb CC49 to be administered to the patient was diluted to 25 ml of saline containing 1% human serum albumin and then given over 20–30 min, followed by a 25-ml flush of normal saline infused over 15 min. Although the amount of MAb CC49 was different for each of the $^{111}$In-labeled MAb CC49 and $^{90}$Y-labeled MAb CC49 administrations, the total amount of administered protein was kept constant at 5 mg for each administration by the addition of unlabeled MAb. We have shown previously (14) that there was no apparent difference in the pharmacokinetics of MAb CC49 administered at 1–1.6 mg/patient for the diagnostic studies and MAb doses of up to >70 mg given as the therapy dose. The $T_{1/2}$ was 39.7 ± 10.4 versus 46.1 ± 10.6 h. Because 5 mg was sufficient to conjugate the desired amount of $^{90}$Y activity, it was decided to standardize the administered amount of protein to the highest amount necessary for the studies. The amount of radiolabeled MAb given to the patient was determined by measuring the amount of the $^{111}$In or $^{90}$Y in the syringe prior to and after patient administration; the difference of these numbers was used as the patient dose.

**Image Interpretation.** $^{111}$In-labeled MAb CC49 planar and SPECT images were interpreted with full knowledge of known disease sites and with direct comparison to CT images. Localization of activity in tumor sites were classified as minimal, moderate, or marked. A region of interest was drawn around the area of uptake and another in a representative background area. The mean counts/pixel in each region of interest was used to determine uptake ratios. Minimal localization was defined as faintly seen above background activity. Moderate localization was defined as easily identified, but activity was no more than approximately two to four times greater than background. Marked localization was defined as an intense activity more than four times greater than background.

**Pharmacokinetic Studies.** To determine the pharmacokinetics of radiolabeled MAb CC49, blood samples were drawn immediately after administration and then at 0.5, 1, 2, and 3–4 h and at 1, 2, 3, 4, 5, and 7 days. The concentration of $^{111}$In-labeled and $^{90}$Y-labeled MAb CC49 in these samples was measured in a well-type NaI (TI) gamma scintillation counter cali-
brated with $^{90}$Y standard from the National Institute for Science and Technology. $^{111}$In was counted using a window of 150–510 keV. When measuring the activity of $^{90}$Y-labeled MAb CC49 in blood samples, a 511–2000 keV window was used on a gamma scintillation counter to measure Bremsstrahlung radiation resulting from the $^{90}$Y decay. At these high-energy settings, there was virtually no crossover from the $^{111}$In-labeled MAb CC49 that was also present in these samples.

**Measurement of HAMA.** Serial blood samples were screened for the presence of HAMA using a sandwich solid-phase RIA as described previously (15). This assay uses an isotype-matched MAb [B6.2 (IgG1)] not related to the TAG-72 antigen. Serum from a cynomolgus monkey positive for anti-mouse immunoglobulin antibodies is used as a reference standard to determine the amount of immunoglobulins bound by the serum samples (expressed as ng of immunoglobulin bound per ml of serum).

**Analysis of Bone Marrow Aspirates and Tissue Biopsies.** When possible, sternal bone marrow aspirates and needle biopsies of liver and liver metastases of $\sim 10$ mg in mass were obtained at 3–7 days after the administration of $^{90}$Y-labeled MAb CC49 (approximately 8–10 days after $^{111}$In-labeled MAb CC49 administration). The Bremsstrahlung radiation resulting from the $^{90}$Y decay in these samples was measured using a gamma scintillation counter (511–2000 keV) to eliminate any possible crossover from the residual $^{111}$In activity. All of the samples were adjusted to 1 ml and counted to obtain a consistent sample size to obtain a reproducible counting efficiency for the $^{90}$Y. There were virtually no indium counts detected in the biopsies after the passage of three to four physical half-lives of the radionuclide. The needle biopsies were placed in 1 ml of formalin prior to counting.

**Radiation Dosimetry for Tumors and Normal Tissues.** A complete description of the methodology for determining radiation absorbed dose estimates for tumors and normal tissues in this study has been presented elsewhere (16). Quantitative gamma camera imaging of $^{111}$In-labeled MAb CC49 and direct measurements of the activity of $^{90}$Y-labeled MAb CC49 in blood, tumors, and normal liver were used in absorbed dose calculations. Whole-body imaging commenced in $<2$ h after administration of $^{111}$In-labeled MAb CC49 and was repeated at 24, 48, and 72 h. SPECT acquisitions were carried out at 24 and 72 h. Administered activities of $^{111}$In-labeled MAb CC49 ranged from 3 to 5 mCi, and all images were acquired on a dual-headed gamma camera system. Energy windows of 15% were used in all planar and SPECT acquisitions and were centered on the two photopeaks of $^{111}$In. The information about the activity of $^{111}$In-labeled MAb CC49 in SPECT slices, and whole-body images was used to generate time-activity curves for $^{90}$Y-labeled MAb CC49, in conjunction with measurements in tissue samples. The tissue activities calculated from the SPECT images of the $^{111}$In-labeled MAb CC49 correlated with absolute measurements made of liver and tumor biopsies (17).

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age</th>
<th>Primary disease site</th>
<th>Metastatic disease site(s)</th>
<th>Prior therapy</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>F</td>
<td>45</td>
<td>Pancreas</td>
<td>Liver, nodes, peritoneum</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>02</td>
<td>F</td>
<td>63</td>
<td>Pancreas</td>
<td>Locally advanced</td>
<td>None</td>
<td>Moderate</td>
</tr>
<tr>
<td>03</td>
<td>F</td>
<td>45</td>
<td>Pancreas</td>
<td>Liver</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>04</td>
<td>M</td>
<td>54</td>
<td>Colon</td>
<td>Nodes</td>
<td>5-FU/leucovorin</td>
<td>Minimal</td>
</tr>
<tr>
<td>05</td>
<td>M</td>
<td>52</td>
<td>Appendix</td>
<td>Liver</td>
<td>Heated mitomycin C, protracted infusion 5-FU/leucovorin</td>
<td>Minimal: pleura, None: liver</td>
</tr>
<tr>
<td>06</td>
<td>M</td>
<td>63</td>
<td>Cholangiocarcinoma</td>
<td>Peritoneal implants</td>
<td>None</td>
<td>Moderate</td>
</tr>
<tr>
<td>07</td>
<td>M</td>
<td>65</td>
<td>Pancreas</td>
<td>Liver</td>
<td>5-FU/mitomycin C/persantin, etoposide/carboplatin</td>
<td>None (photopenic region)</td>
</tr>
<tr>
<td>08</td>
<td>M</td>
<td>57</td>
<td>Esophagus</td>
<td>Liver</td>
<td>5-FU, protracted infusion 5-FU/leucovorin, cisplatin/etoposide, carboplatin/etoposide, radiation</td>
<td>None (photopenic region): liver, Minimal: periaortic nodes</td>
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<tr>
<td>09</td>
<td>M</td>
<td>53</td>
<td>Colon</td>
<td>Peritoneal implants</td>
<td>Adjuvant 5-FU/levamisole, Theratope, protracted infusion 5-FU/leucovorin</td>
<td>Moderate</td>
</tr>
<tr>
<td>010</td>
<td>M</td>
<td>62</td>
<td>Colon</td>
<td>Liver</td>
<td>5-FU/leucovorin</td>
<td>None</td>
</tr>
<tr>
<td>011</td>
<td>F</td>
<td>50</td>
<td>Rectal</td>
<td>Liver, lung</td>
<td>DTCi/cisplatin, 5-FU, 5-FU/leucovorin, Theratope, radiation</td>
<td>Minimal: lung, photopenic regions with rim of moderate activity peripherally; liver</td>
</tr>
<tr>
<td>012</td>
<td>F</td>
<td>47</td>
<td>Colon</td>
<td>Liver</td>
<td>FUDR, $^*$ 5-FU/leucovorin</td>
<td>Photopenic regions with small foci of moderate activity peripherally</td>
</tr>
</tbody>
</table>

*FUDR, fluorouracil.*
activity measurements in whole blood. It has been suggested that a reduction factor of 0.2–0.4 be used for the activity in blood to account for the difference in activity concentrations in blood and red marrow (15). In this study, a reduction factor of 0.3 was used to scale from blood activity to marrow activity in absorbed dose calculations.

RESULTS

Patient Demographics. Twelve patients were enrolled in this study. There were 6 men and 6 women. The median age was 52 years (range, 45 to 65 years). Five patients had colorectal cancer, four had pancreatic carcinoma, and one patient each had esophageus carcinoma, cholangiocarcinoma, and carcinoma of the appendix. Sites of metastases are shown in Table 1. One patient with extensive locoregional involvement with pancreatic adenocarcinoma did not have metastatic disease. Eight patients had prior treatment. This included two patients who received 5-FU alone or in combination with levamisole, leucovorin, or both. Six patients received multiple drug therapies. Two patients received prior radiation: sites included esophageus (patient 08) and rectum (patient 11). All patients underwent collection of hematopoietic stem cells.

Radioimmunoconjugate Preparation. Both radioisotopes produced stable radioimmunoconjugates of CC49-PA-DOTA. The average yield of 111In incorporation was slightly higher than 90Y, 58.7% versus 56.4%. The radiochemical purity for all radioimmunoconjugate preparation ranged from 99.7 to 99.9%. Specific activities of 111In-labeled antibodies were ~6.2 mCi/mg (range, 3.1 to 8.2) of CC49-PA-DOTA, which corresponds to approximately 1 in 50 molecules of antibody carrying 111In. The 90Y-labeled conjugates were prepared with a specific activity of 9.6 mCi/mg (range, 8.1 to 11.9), which corresponds to about 1 in 30 molecules of antibody having one 90Y.

The immunoreactivity of the radiolabeled MAbs were evaluated in a solid-phase immunoassay. The binding assay results indicated a slightly greater retention of immunoreactivity for 111In-labeled CC49-PA-DOTA compared with the 90Y-labeled CC49-PA-DOTA, 87.9 ± 4% versus 79.1 ± 4.4%, respectively.

Imaging Results. Table 1 also profiles the extent of disease in 12 patients enrolled in the study along with the imaging information obtained from images acquired after the diagnostic dose. Three patients had no detectable localization of labeled antibody, and an additional three patients had no more than minimal localization in any known lesion. Six patients had at least one focus of moderate activity at a known disease site. Only one patient had marked localization at any site, this being in the primary tumor.

In general, liver lesions were difficult to identify because of the high nonspecific activity seen in normal liver with 111In. Lesions were more likely to be seen as photopenic regions rather than enhanced activity, although patients 11 and 12 did have some small areas of increased activity peripheral to photopenic liver lesions. Pancreatic lesions were often difficult to differentiate from immediately adjacent normal liver activity. Fig. 1 is a typical planar image from a patient with peritoneal carcinomatosis.

Pharmacokinetics. The levels of radioactivity in the blood were measured to evaluate the clearance rates. Pharmacokinetic analysis of these samples obtained after the infusion of the 111In-labeled MAb CC49 (for imaging) and the 90Y-labeled MAb CC49 (for therapy) showed very similar clearance rates. The effective $T_{1/2}$ and $T_{90}$ for 111In-labeled MAb CC49 were 5.44 h (range, 1.91–9.35) and 59.83 h (range, 33.21–89.93), respectively. For the 90Y-labeled MAb CC49, the $T_{1/2}$ and $T_{90}$ were 3.89 h (range, 1.48–7.93) and 47.38 h (range, 28.14–65.69), respectively.

Biopsy Data. Sternal aspirates were obtained on all patients. Biopsies from liver metastases and of normal liver were obtained on 8 and 10 patients, respectively. Although the percentage of injected dose/kg (range, 1.12–18.14) was somewhat higher than we had observed with 131I, the tumor:normal liver ratio was consistently <1 because of the retention of the 111In and 90Y in the normal liver. Table 2 shows the percentage of injected dose/kg for the tumor, normal liver, and the tumor: normal liver ratios, tumor: blood, and bone marrow: blood. Although there may be heterogeneity in the biopsies, the measured activities in the tissues correlated well with the SPECT image analyses. The tumor: blood ratio ranged from 1 to >36:1.

Radiation Absorbed Dose Estimates. A summary of the administered activities of 90Y-labeled MAb CC49 and mean values of the absorbed dose in tumors and normal tissues are given in Table 3. Within the range of administered activities of 90Y-labeled MAb CC49, the absorbed dose estimates observed were 200–3000 cGy, averaging 35 cGy/mCi administered (range, 7–81).

Toxicity. Table 4 summarizes the toxicities seen on this trial. The predominant toxicity, as expected, is hematopoietic. None of the patients reached the criteria for hematopoietic stem cell rescue. However, cryopreserved bone marrow was returned to patient 2 for prolonged thrombocytopenia after therapy and...
Therapy with 90 Y-labeled CC49

Therapy with 90 Y-labeled CC49 was administered as a palliative measure for control of extension into the duodenum, and external beam radiation therapy was recommended for Phase II testing. Unfortunately, Phase II trials using this activity failed to show clinical efficacy in patients with metastatic colon and prostate cancer (19, 20). We have conducted previously a trial using very high administered activities, the maximum absorbed dose of 131 I-labeled MAb CC49 (up to 300 mCi/m 2 ). Even with very high administered activities, the maximum absorbed dose estimate in tumor sites was 3300 cGy (14).

DISCUSSION

131 I-labeled MAb CC49 has undergone extensive clinical evaluation. Consistent with therapeutic studies using other radioimmunoconjugates, a Phase I trial of 131 I-labeled MAb CC49 conducted at Memorial Sloan Kettering Cancer Center demonstrated myelosuppression due to bystander radiotoxicity from the circulating radioimmunoconjugate (18). Because this toxicity was dose limiting, an administered activity of 75 mCi/m2 was recommended for Phase II testing. Unfortunately, Phase II trials using this activity failed to show clinical efficacy in patients with metastatic colon and prostate cancer (19, 20). We have conducted previously a trial using very high administered activities of 131 I-labeled MAb CC49 (up to 300 mCi/m2). Even with very high administered activities, the maximum absorbed dose estimate in tumor sites was 3300 cGy (14).

In addition to suboptimal absorbed dose estimates, another concern arose from studying autoradiographs of 131 I-labeled MAb CC49 in tumor biopsies after treatment. Although these biopsies showed good penetration of radiolabel throughout the core biopsy fragments, areas of low or no activity resulting from heterogeneous antibody uptake were evident. Although it is possible that the heterogeneity seen in tissues may have been a result of a low administered antibody dose, it is also possible that other factors, such as high osmotic pressure, prevented antibody access to these sites. Thus, this finding prompted us to study 90 Y in this subsequent Phase I trial. 90 Y has a higher response for up to 30 days after administration of the diagnostic dose of 111 In-labeled MAb CC49 (followed by therapeutic 90 Y-labeled MAb CC49) in six of eight patients evaluated. The median HAMA quantitation was 521 mg/ml (range, 1–22,374 ng/ml) at 2 months after the study; 2 patients remained negative, 2 patients had modest levels of HAMA (<1000 ng/ml), 3 patients had between 1000 and 10,000 ng/ml HAMA, and one patient had 10,000 ng/ml HAMA.

Response to Treatment. There were no objective responses observed in this trial. Two patients (nos. 10 and 11) demonstrated stable disease durable for 4 and 2 months after treatment, respectively.

patient 9 in an attempt to shorten aplasia in the face of rapidly progressing disease.

Although isolated grade 3 and 4 nonhematological events were recorded during the conduct of this trial, all of these events were felt to be attributable to either progressing disease or comorbid conditions. One patient on the second dose level developed gastrointestinal bleeding because of direct tumor extension into the duodenum, and external beam radiation therapy was administered as a palliative measure for control of gastrointestinal bleeding. This was felt to be warranted because grade 3 or 4 thrombocytopenia was likely to be encountered in these patients with very high administered activities, the maximum absorbed dose estimate in tumor sites was 3300 cGy (14).
average energy β emission compared with 131I (937 keV versus 183 keV). This property can potentially permit more bystander radiotoxicity to overcome heterogeneous targeting to antigen. Furthermore, this radiometal can be stably chelated to the antibody using a macrocyclic structure. This stable linkage provides a theoretical advantage over directly labeled 131I, which can undergo some dehalogenation in vivo.

Although there can be variations between the normal biodistribution of 111In- and 90Y-conjugated antibodies, preclinical studies support the use of 111In as a surrogate for 90Y deposition in tumor sites. Using patient specific dosimetry of 111In/90Y-conjugated MAb CC49, we have calculated absorbed dose estimates in metastatic tumor sites of up to 3000 cGy for the highest administered activity levels. This dose delivery is comparable with absorbed dose estimates with very high administered activities of 131I-labeled MAb CC49 (20). However, the administered activities of 131I-labeled MAb CC49 required to achieve this dose deposition caused severe myelosuppression requiring administration of hematopoietic stem cell support. Reversible myelosuppression was observed with the administered activities of 90Y-labeled MAb CC49 used in this trial. Thus, within the range of activities studied, it would appear that higher tumor dose deposition can be achieved with 90Y using administered activities that are less toxic than observed with 131I.

However, we also calculated a disappointingly high uptake of 90Y-labeled MAb CC49 in normal liver. There are very few other published studies of treatment using systemic administration of 90Y-conjugated antibody in Phase I and II trials in patients with solid tumors. Wong et al. (21) reported on three patients treated with 90Y-conjugated chimeric T84.66 and ant carcinoembryonic antigen antibody. Using administered activities of 5 mCi/kg, no serious hematopoietic toxicity was observed. However, even with this low administered activity, the estimated total radiation dose to liver ranged from 234 to 432 cGy. Absorbed dose estimates for tumor were not reported.

Although the original goal of our trial was to determine whether extrahematopoietic toxicity occurred with 90Y-labeled MAb CC49, a decision was made to cease dose escalation at 0.5 mCi/kg because of the observation of high absorbed dose estimates in normal liver tissue. Thus, this study provides clinical confirmation for earlier observations by Wang et al. (22) in beagle dogs. In their studies, animals treated with high activities of 90Y-labeled MAb B72.3 antibody experienced liver toxicity including liver failure. Fortunately, this finding may not present a critical hurdle for therapy of hematological malignancies that are more radiosensitive. Experience with an 90Y-conjugated anti-CD-20 antibody in the treatment of lymphoma suggests that therapeutic activity is seen with administered activities in the range used for our study (23).

Nonetheless, the high deposition of 90Y-labeled MAb CC49 in normal liver is problematic. There are no antigentic targets for MAb CC49 in normal liver, and free 90Y is known to accumulate in bone and/or bone marrow. This finding could have resulted from infusion of antibody damaged during labeling. However, we saw no evidence of such damage in our postlabeling quality assurance assays (high-performance liquid chromatography, ITLC, and immunoreactivity assays). Thus, we assume that the accumulation of 90Y in the liver is a result of normal liver clearance of either the antibody or antigen-antibody complexes carrying the stably chelated 90Y. Paik et al. (24) has suggested that alteration in chelation and linker chemistry may reduce liver uptake of antibodies; thus, it is possible that this liver deposition could be modified using novel chelate-linker conjugates. Other approaches could involve confusion of cold antibody or pretargeting. This latter strategy is being pursued in the development of MAb NR-LU-10 for therapy (25). Streptavidin-conjugated antibody is first administered to bind to the tumor target. Biotin-bound albumin is used to “washout” circulating MAb, which is then followed by 90Y conjugated to biotin. This strategy avoids nonspecific uptake of 90Y and can conceivably reduce both myelosuppression and liver uptake. In fact, the dose-limiting toxicity with MAb-NR-LU-10 and 90Y-labeled biotin (120 mCi/M2) using this strategy is diarrhea.

The HAMA response seen in this trial was predicted by

### Table 4 90Y-labeled CC49 toxicity summary

<table>
<thead>
<tr>
<th>90Y administered activity level</th>
<th>0.3 mCi/kg (n = 5)</th>
<th>0.4 mCi/kg (n = 4)</th>
<th>0.5 mCi/kg (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity grade:</td>
<td>1–2</td>
<td>3</td>
<td>1–2</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anemia</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Anorexia</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Change in taste</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fever without infection</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspnea</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* One patient in this group had rapidly progressing disease and was placed on hospice, refusing further toxicity follow-up after week 5.

* One patient in this group developed gastrointestinal bleeding because of direct tumor extension. Thus, toxicity for this patient reflects the addition of the radiation therapy.

* Hemorrhage was limited to epistaxis with no sequela.

* Gastrointestinal bleeding was secondary to direct tumor extension into the duodenum and was not believed to be related to therapy.
earlier studies. Higher dose deposition in tumors may also require multiple administrations of $^{131}$I- or $^{90}$Y-conjugated MAb CC49. MAb CC49 has been humanized and further engineered to delete the C4b2 portion of the Fc fragment (26). This antibody is predicted to be less immunogenic and will be the focus of our future studies of radioimmunoconjugate therapy.

To our knowledge, this is the first clinical trial using systemic administration of $^{90}$Y-conjugated antibody in which tissue biopsies of tumor targets and organs at risk have been obtained in an effort to validate dosimetry estimates. The extremely low tumor:normal tissue ratios seen in this study raises concern about the future of $^{90}$Y-conjugated antibodies for radioimmunoconjugate therapy in solid tumors. Future studies with radiometals will need to focus on strategies to minimize liver uptake or dramatically improve tumor targeting and absolute accumulation of radioactivity in tumor sites. However, high-dose deposition in tumor with a single and well-tolerated administration of $^{90}$Y-labeled MAb CC49 raises hope that successful radioimmunotherapy can be achieved using sequential treatments with a less immunogenic engineered antibody.

ACKNOWLEDGMENTS

We acknowledge the important contributions of the following individuals: Dave Jacobson and Frank Ruta (Radiation Safety); Kay Devish (laboratory support); Mary Capadano and Bev Schmidt (nurse coordinators); nurses in the University Treatment Center and Clinic (nursing care); and Lisa Stillahn (manuscript preparation).

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11. Colcher, D., Minelli, M. F., Roselli, M., Muraro, R., Simpson-Milenic, D., and Schlom, J. Radioimmunoconjugate therapy in solid tumors. Future studies with radiometals will need to focus on strategies to minimize liver uptake or dramatically improve tumor targeting and absolute accumulation of radioactivity in tumor sites. However, high-dose deposition in tumor with a single and well-tolerated administration of $^{90}$Y-labeled MAb CC49 raises hope that successful radioimmunotherapy can be achieved using sequential treatments with a less immunogenic engineered antibody.

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Margaret Tempero, Peter Leichner, Janina Baranowska-Kortylewicz, et al.


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