Phase I Radioimmunotherapy Trial with Iodine-131-CC49 in Metastatic Colon Carcinoma

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CC49 is a murine monoclonal antibody (MAb) that reacts against the TAG-72 antigen. We carried out a Phase I study with escalating doses of ^{131}I-CC49 in patients with advanced colorectal cancer expressing the TAG-72 antigen to determine the dose-limiting toxicity and therapeutic efficacy, if any, of the radioimmunoconjugate. **Methods:** Twenty-four patients with TAG-72- expressing colorectal cancer were treated with escalating doses of ^{131}I-CC49 starting at 15 mCi/m^2 and going up to 90 mCi/m^2 of ^{131}I labeled to 20 mg MAb CC49. Patients were selected if TAG-72 was expressed in ≥50% of cells in previously resected tumor and at least one metastasis was demonstrable on standard imaging such as CT. All patients had failed conventional chemotherapy and had not received prior radiotherapy or murine MAb. Patients were under radiation isolation precautions until whole-body radioactivity decreased to ≤5 mR/hr at 1 m. Whole-body scintigrams were obtained prior to discharge and 1 and 2 wk after infusion in all patients. SPECT imaging was carried out at least once in all patients. **Results:** All patients had excellent targeting of radioactivity to known tumor sites. There was no nonhematologic toxicity. Hematologic toxicity was more pronounced in those patients who had received extensive prior chemotherapy. There were no major responses. All patients developed an immune response (HAMA) within 4 wk of therapy. **Conclusion:** Radioimmunotherapy with ^{131}I-CC49 is safe and there is significant therapeutic efficacy in this Phase I trial at the doses studied. There is excellent targeting of radioactivity to antigen-positive tumors. Dose-limiting toxicity is hematopoietic, with the maximum tolerated dose in this group of heavily pretreated patients being 75 mCi/m^2.

Key Words: radioimmunotherapy; iodine-131-CC49; hematopoietic toxicity; colorectal carcinoma

J Nucl Med 1995; 36:586–592

TAG-72 is a tumor-associated mucin that is widely expressed in adenocarcinomas, particularly colorectal, ovary, breast, prostate and lung cancers (*1*). Radiolabeled antibodies which target TAG-72 have been extensively studied for possible improved tumor diagnosis and treatment (*2,3*). The "first generation" anti-TAG-72 MAb, B72.3, has been used in radio-immunodiagnosis and radioimmunotherapy (RIT) trials in over 1000 patients (*3*).

Monoclonal antibody (MAb) CC49 is a murine IgG_1 that reacts against the TAG-72 antigen, found in most differentiated adenocarcinomas (*4*). CC49 is a "second generation" MAb developed against the TAG-72 antigen and has been shown to localize in antigen-expressing tumors. As its antigen affinity is high (about 2×10^{10} M^{-1} (*4*)), it is being studied for its potential usefulness in the diagnosis and treatment of adenocarcinomas. In our hands, MAb CC49 has been somewhat better than B72.3 for localizing to colorectal carcinoma. In 10 patients who received both MAbs simultaneously prior to surgery, there was a 60% improvement in tumor-to-serum ratios with CC49 a week after infusion, with absolute tumor concentration of CC49 being greater, albeit not statistically significant in this small group of patients, than that of B72.3 (*5*). We also found that absolute tumor uptake of CC49 was better at the 20-mg dose than at the 1-mg dose and decided that the optimum mass amount of MAb CC49 for clinical use would be 20 mg.

Antigen heterogeneity is a limitation to optimum targeting of MAb to tumor. The TAG-72 antigen is expressed heterogeneously in most adenocarcinomas (*2*). An important consideration in the design of a RIT trial would therefore be "adequate" antigen expression in tumor tissue. Recent studies in colorectal cancer have shown that TAG-72 antigen expression does not vary significantly between primary and metastatic tumors; analysis of antigen expression and distribution in the primary tumor can therefore aid in the selection of an appropriate MAb for RIT. While many antigens can only be detected in fresh-frozen tissue specimens, the TAG-72 antigen is preserved in paraffin fixed tissue, permitting retrospective review of antigen expression and distribution if necessary.

Given the excellent targeting of CC49 to colonic adenocarcinomas in pre-surgical clinical studies carried out at this center (*5*) and elsewhere (*6*), we decided to undertake, under a contract (NO1-CA-97609) with the National Cancer Institute (NCI), a Phase I RIT trial with escalating doses of ^{131}I-CC49 in patients with advanced colorectal

Received Feb. 22, 1994; revision accepted Aug. 22, 1994.

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cancer. The availability of immunohistochemical screening of TAG-72 in paraffin-fixed tissue made it possible to study the primary tumor. We therefore decided to treat only those patients whose tumors had adequate antigen expression, assuming that TAG-72 expression in the primary and metastatic lesion would be comparable. While homogeneous antigen expression, i.e., 100% of cells reactive in a representative high-power field, in all tumor sites, is the desired goal, we feel that tumor is "adequately" antigen-positive if half or more of tumor cells in an average high-power field in an available tumor specimen express antigen. Of 32 patients evaluated, 24 patients with advanced colon cancer had a TAG-72-expressing tumor with more than 50% of cells in a high-power field being positive for the antigen. We conducted a Phase I RIT trial with ^{131}I-CC49 in these 24 patients.

MATERIALS AND METHODS

Patients

Twenty-four patients with measurable metastatic colorectal carcinoma were studied. Requirements included no prior radiotherapy or mouse MAb, tumor tissue reactivity (≥50% reactivity with CC49 in an average high power field), and adequate renal, hepatic and hematopoietic function. The Phase I RIT study was approved by the Institution Review Board at this center. Informed consent was obtained from all patients. All patients underwent standard imaging modalities including CT within 2 wk prior to and 4 wk after therapy. Patients received 20 mg of CC49 labeled with escalating amounts of ^{131}I as follows: the first group of three patients received 15 mCi/m^2 and subsequent escalation, in groups of three patients, was in 15-mCi/m^2 increments; at the 75- and 90-mCi/m^2 doses, six patients each were studied. Patient characteristics are summarized in Table 1. All patients had failed 5-fluoro-uracil (5-FU) and leucovorin therapy prior to MAb infusion. None had received any therapy for at least 4 wk prior to entry into this protocol. The median time elapsed between prior therapy and entry into this trial was 8 wk. All patients received saturated solution of potassium iodide (SSKI, 10 drops orally three times daily) for 2 wk starting the day of radiolabeled MAb administration. Patients who were retreated received the same amount of radiolabeled MAb as in their first infusion.

All pre- and postradioimmunotherapy CT scans were evaluated by a radiologist (S.H.) familiar with the clinical history. Responses were graded as follows:

1. Progression: Increase in size and/or number of lesions.
2. Stable disease: No change in lesion size or number.
3. Major response: ≥50% reduction in sum of greatest diameters of measurable lesions, with no increase in size of any lesion.
4. Minor response: <50% reduction in sum of greatest diameters of measurable lesions, with no increase in size of any lesion.

All retreated patients had stable disease on CT scan evaluation 4 wk after administration of the first dose of antibody. The retreatment was carried out 6–8 wk after the first therapy (Table 2).

TABLE 1
Patient Characteristics

Patient no.	Antibody dose (mCi/m^2)	Age (yr)	Sex	Serum CEA (ng/ml serum)	Disease extent
1	15	70	F	29	Liver
2	15	70	F	33	Liver, lungs
3	15	75	F	173	Liver
4	30	76	F	160	Liver, abdomen
5	30	39	F	55	Liver
6	30	40	M	552	Liver, lungs
7	45	72	M	80	Liver, abdomen
8	45	57	F	312	Liver, abdomen
9	45	31	F	16	Liver, abdomen
10	60	65	M	950	Liver
11	60	72	M	78	Liver, abdomen, lungs
12	60	56	M	3810	Liver, abdomen
13	75	60	F	178	Liver, abdomen
14	75	61	F	9260	Liver, abdomen
15	75	75	M	255	Liver, abdomen
16	75	58	M	162	Liver
17	75	50	F	634	Liver, abdomen
18	75	82	M	316	Liver, lungs
19	90	29	F	61	Liver, abdomen
20	90	54	M	19	Liver
21	90	39	M	976	Liver, abdomen
22	90	70	M	268	Liver
23	90	29	M	110	Abdomen, pelvis
24	90	51	F	575	Liver, abdomen, lungs

Monoclonal Antibody CC49

Clinic-grade CC49 was provided by the Division of Cancer Therapy, National Cancer Institute. CC49 was labeled with ^{131}I using the iodogen (Pierce, Inc., Rockford, IL) method. Briefly, the requisite amount of MAb and radioiodine were mixed in sterile 10-ml glass vials precoated with iodogen and incubated at room temperature for 15 min. The radiolabeled MAb was separated from the mixture by passage through a sterile BioGel P6 (BioRad Inc., Melville, NY) size-exclusion column. Thin-layer chromatography of an aliquot was carried out and the preparation utilized only if the percent of protein-bound radioactivity was ≥95%.

Radioimmunoreactivity

In every patient, radiolabeled MAb was assayed for radioimmunoreactivity using the method described by Lindmo et al. (7). Briefly, appropriate dilutions of each MAb were added in triplicate to wells of microtiter plates precoated with antigen-positive (LS174T) and antigen-negative (A435) cell extract and incubated at 4°C overnight. The percent of bound radioactivity was plotted against dilution to obtain the percent binding at conditions of antigen excess.

Administration of Radiolabeled MAb

All MAb preparations were used after passage through a 0.2-μ filter. Unlabeled CC49 (0.5 mg) was initially administered as an intravenous slow bolus, and patients were monitored for symptoms with vital signs being measured every 15 min. If there was no adverse reaction noticed within half an hour of this test dose, the radiolabeled MAb, diluted in 100 ml of 5% HSA in normal saline was administered as an intravenous infusion over 1 hr. Radiolabeled MAb was injected into the patient prior to determination of radioimmunoreactivity. The 1-hr infusion was felt to be slow enough to monitor for possible anaphylactoid and other, un-

TABLE 2
Individual Patient Doses of ^{131}I

Patient no.	Antibody dose (mCi/m^2)	^{131}I dose (mCi)	Whole-body $T_{1/2}$ (hr)	Serum $T_{1/2}$ (hr)	Thrombocytopenia
4	(30) First infusion	45.0	37.4	38.2	None
4	(30) Second infusion	45.6	45.5	39.4	None
5	(30) First infusion	58.4	52.6	48.7	None
5	(30) Second infusion	50.5	14.75	11.8	None
6	(30)	58.5	78.00	43.2	None
7	(45)	70.3	54.5	51.6	None
8	(45)	88.1	60.5	58.4	None
9	(45)	64.0	30.0	23.7	None
10	(60) First infusion	114.0	74.0	68.2	Grade II
10	(60) Second infusion	100.0	21.2	22.3	None
11	(60)	125.0	38.8	36.9	None
12	(60)	147.0	51.5	48.9	None
13	(75)	154.0	53.0	49.3	Grade II
14	(75)	115.0	51.3	52.4	Grade II
15	(75)	140.0	66.2	63.5	Grade IV
16	(75)	130.0	33.7	34.1	None
17	(75) First infusion	180.0	46.3	47.1	None
17	(75) Second infusion	186.0	11.7	6.4	None
18	(75) First infusion	124.0	47.4	46.8	Grade III
	(75) Second infusion	117.0	15.2	14.9	None
19	(90) First infusion	140.0	28.0	36.7	Grade IV lymphopenia*
	(90) Second infusion	135.0	11.4	13.2	None
20	(90)	200.0	49.6	54.1	Grade II
21	(90)	178.0	37.9	28.3	Grade IV
22	(90)	228.0	42.9	46.1	Grade III
23	(90)	209.0	40.1	41.3	Grade I
24	(90)	175.0	37.0	35.4	Grade IV

*This patient had Grade IV lymphopenia with Grade II thrombocytopenia. No other hematopoietic toxicity was seen.

known, acute adverse events. Patients' vital signs were monitored for at least 4 hr after completion of antibody infusion. Whole-body measurements of radiation were obtained using an ionization chamber calibrated to measure radiation dose (in mR/hr) both at the body surface and 1 m from the patient. These measurements were obtained at least daily until discharge; the patient was discharged from hospital only if the radiation dose at 1 m from the patient was ≤5 mR/hr. Subsequent measurement at 1 wk was also obtained in all patients. Patients remained in radiation isolation until their whole-body radiation had reached acceptable (≤5 mR/hr at 1 m) limits.

Pharmacokinetics, Whole-Body Counting and Radioimmunoscintigraphy

Blood was obtained for determination of radiolabeled MAb clearance immediately following antibody infusion, daily subsequently while the patients were in the hospital and weekly thereafter up to 4 wk after infusion. Anterior and posterior whole-body ^{131}I images were obtained prior to discharge from the hospital and 1 and 2 wk after infusion. SPECT of the relevant areas was obtained at 1 and, where possible, 2 wk after infusion.

In Vitro Studies

Samples of serum (0.5 ml each) were counted in a gamma counter along with appropriate dilutions of the standards and biopsy specimens. All serum and standard counts were obtained at the same time. Samples were counted in the ^{131}I window in a gamma well scintillation counter (LKB Wallac, Piscataway, NJ). Samples were counted to less than 1% relative error, according to the criteria of Loevinger and Berman (*8*).

Samples of pre-infusion, 2-wk and 4-wk sera were incubated with ^{125}I-CC49 and analyzed by HPLC to determine the presence of human antimouse antibody. These assays were carried out at least 4 wk after infusion, to minimize the contribution of ^{131}I-CC49. The test was considered positive for HAMA if the proportion of radioactivity seen at 300 KD was ≥15%.

RESULTS

Twenty-four patients with measurable metastatic colorectal carcinoma were studied (Table 1). Of the 32 patients evaluated for the study during this period, 24 had positive immunostaining of tumor with CC49. The labeling method was simple and efficient. The procedure reproducibly resulted in ≥90% incorporation of radioiodine to antibody as determined by thin-layer chromatography. Immunoreactivity of ^{131}I-CC49 was always ≥55%. There was no correlation between immunoreactivity and specific activity, which ranged from 10 to 30 mCi of ^{131}I per milligram of MAb CC49.

None of the 24 patients had side effects during infusion of the test dose. One of 24 patients treated initially with the radiolabeled MAb had urticaria 30 min after completion of therapy; this resolved promptly following intravenous

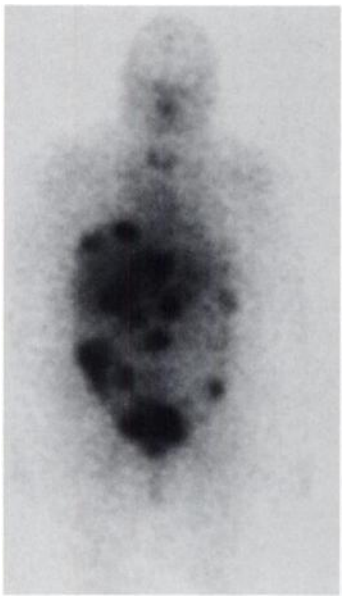

FIGURE 1. Anterior whole-body image obtained a week after intravenous infusion of 155 mCi/20 mg ^{131}I-CC49 (Patient 13). Extent of abnormal uptake in omentum and peritoneum was far greater than anticipated from a concurrent contrast-enhanced CT scan.

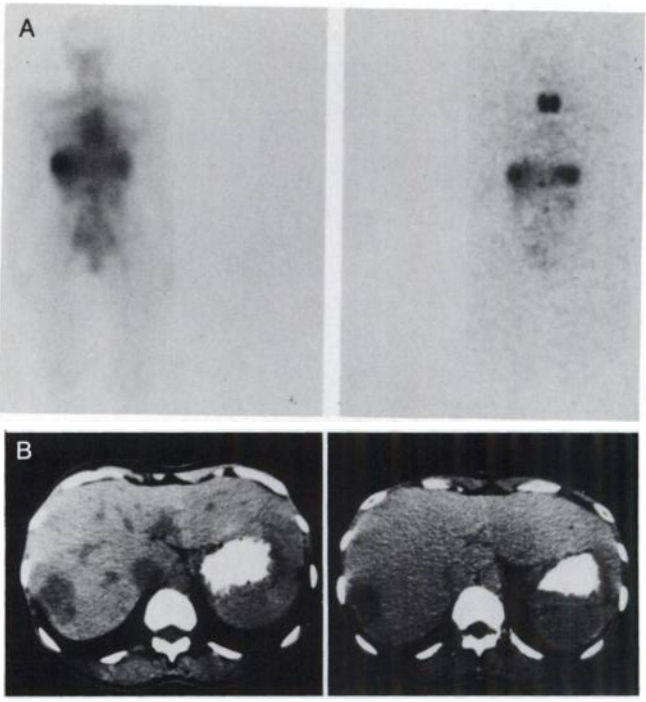

FIGURE 2. (A) Anterior whole-body images of a Patient 4 with hepatic metastatic disease a week after the first infusion (left) and after the second infusion (right) of ^{131}I-CC49 (30 mCi/m^2). (B) Noncontrast enhanced CT slice through the liver of the same patient prior to (left) and 8 wk after (right) radioimmunotherapy.

diphenhydramine. Of the six patients retreated, two had minor symptoms during the second infusion consisting primarily of itching in the palms and flushing of the face which resolved promptly after intravenous administration of 50 mg of diphenhydramine. All patients received the entire second dose. At doses ≥75 mCi/m^2, patients experienced considerable fatigue during the week after treatment; this resolved over the following week. In two patients at the 90-mCi/m^2 dose, there was significant knee and calf pain starting approximately 10 days after MAb infusion; this was well controlled with oral nonsteroidal anti-inflammatory drugs. In these patients, there were no changes in serum complement levels or immune complex formation (data not shown).

Imaging

More than 95% of all CT-detected lesions were visualized by radioimmunoscintigraphic scans (MAb scans). Radionuclide scans were interpreted as positive if there were focal, persistent areas of increased tracer concentration in the liver. Nodal areas of increased radiotracer concentration were interpreted as positive only if the uptake was very intense and persistent, or if nondraining mesenteric nodes were involved. SPECT delineated lesion extent better than did the planar images. In general, extrahepatic abdominal disease was better visualized by MAb scans than by CT. These data have been published elsewhere (*9*). Lesion visualization was best at a week after MAb infusion. Splenic uptake of ^{131}I-CC49 was greater than could be accounted for by vascular distribution. Co-registration of CT and SPECT images enabled identification of sites of increased radioactive uptake.

Figure 1 shows an anterior whole-body image obtained a week after administration of 75 mCi/m^2 ^{131}I-CC49 (total dose: 155 mCi). This 62-yr-old female (Patient 13) had hepatic and intra-abdominal disease visualized on CT. However, the extent of peritoneal and para-aortic disease was far better visualized on the antibody image than on CT.

A 30-yr-old female with cecal carcinoma had had a resection of the bowel lesion followed by adjuvant 5-FU and leucovorin therapy (Patient 5). She subsequently had hepatic metastases and received intra-arterial FUDR. When progression of hepatic disease was seen, the patient was treated with 30 mCi/m^2 of ^{131}I-CC49 (total dose 58 mCi). In Figure 2A, the left panel shows an anterior whole-body image obtained 1 wk after therapy. A CT scan obtained 4 wk after therapy showed stable disease. The patient was accordingly retreated, despite being positive for HAMA, with the same dose of ^{131}I-CC49. An anterior whole-body image obtained 1 wk after the second administration of radiolabeled antibody is shown in Figure 2A, right panel. Dehalogenation of MAb resulted in increased thyroid uptake and faster clearance of radioactivity; increased uptake in the liver and spleen is probably a result of immune complex deposition. This patient had disease limited to the liver, and a minor response in liver lesions was seen following the two courses of radioimmunotherapy. The pretherapy CT scan detailing the liver lesions is shown in Figure 2B, left, while a similar slice obtained 4 wk after the second RIT shows resolution of some liver disease (Fig. 2B, right). This patient had 42% reduction in the size of her liver lesions, and thus was classified as a minor response. No other responses were seen in this Phase I study.

Six patients were re-treated between 6 and 8 wk after the

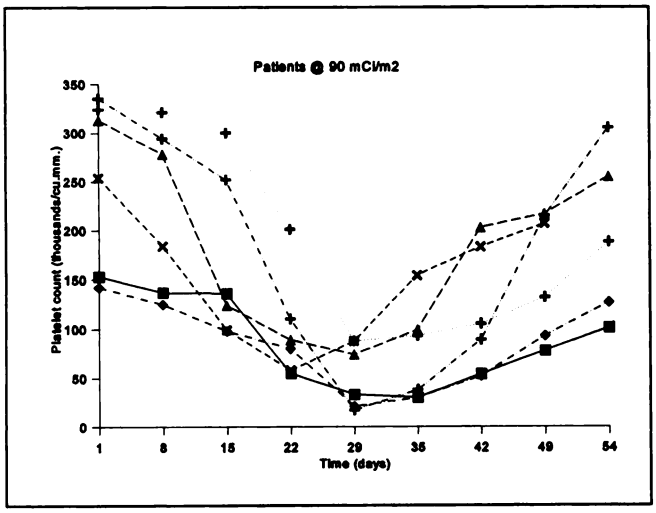

FIGURE 3. Platelet counts versus time in six patients treated with 90 mCi/m^2 ^{131}I-CC49.

first dose of radioimmunotherapy. Although the patient in Figure 2 showed tumor uptake following the second infusion, there was no tumor visualization in the other five patients. In all patients there was evidence of increased dehalogenation manifest by increased thyroid uptake (despite comparable amounts of SSKI) and faster whole body and serum clearance.

Pharmacokinetics

MAb CC49 showed a monoexponential clearance of blood radioactivity. Table 2 shows the effective half-life of serum and whole-body radioactivity in 21 patients who received between 30 and 90 mCi/m^2. The actual dose of radioiodine administered is also detailed. There was close correlation between serum and whole-body clearance of radioactivity. In several patients, aliquots of sera were tested by the trichloroacetic acid method to determine serum protein-bound radioactivity; in all samples obtained from patients after the first infusion, ≥95% of radioactivity in serum was protein-bound. A subset of these sera were assayed by size-exclusion HPLC: more than 95% of radioactivity was seen to be antibody-associated. In contrast, there was variable, up to 25%, free radioiodine noted in sera of patients following retreatment.

Repeat RIT resulted in faster whole-body and serum clearance in five of six patients. Table 2 details the effective whole-body half-life in these six patients for each infusion.

Human Antimouse Antibody (HAMA)

HAMA levels were positive in all patients 4 wk after infusion.

Toxicity

There was no nonhematologic toxicity. There was no hematologic toxicity noted in any patient up to the 60-mCi/m^2 dose. At the 60-mCi/m^2 dose, one of three patients had thrombocytopenia that reached a Grade II (50–75 thousand platelets/μl) nadir approximately 3 wk after infusion. None of the other patients at this dose level had > Grade II thrombocytopenia. One of six patients at the 75-mCi/m^2 dose, and two of six at the 90-mCi/m^2 dose, had Grade IV thrombocytopenia, and one other patient at the 90-mCi/m^2 dose had Grade IV lymphopenia. Thrombocytopenia was seen to nadir at around 4 wk and return to baseline levels by 8 wk post-treatment. Figure 3 is a graph showing the temporal course of thrombocytopenia depression in patients treated at the 90-mCi/m^2 dose. Leukocyte depression tended to follow thrombocyte depression with

TABLE 3
Details of Prior Chemotherapy in Addition to 5-Fluorouracil and Leucovorin and Hematologic Toxicity in Patients Receiving ≥60 mCi/m^2 ^{131}I-CC49

Patient no.	Antibody dose (mCi/m^2)	Prior chemotherapy*	Hematologic toxicity†
10	60	BOF/Strep	Grade II (108 → 62)
11	60	None	None
12	60	None	None
13	75	None	Grade II (338 → 52)
14	75	None	Grade II (632 → 57)
15	75	BOF/Strep., Mitomycin	Grade IV (331 → 17)
16	75	None	None
17	75	None	None
18	75	BOF/Strep	Grade III (248 → 40)
19	90	None	Grade IV lymph. (1.2 → 0.4)‡
20	90	None	Grade II (254 → 58)
21	90	BOF/Strep, Mitomycin	Grade IV (335 → 17)
22	90	BOF/Strep	Grade III (153 → 27)
23	90	None	Grade I (324 → 88)
24	90	Mitomycin	Grade IV (249 → 21)

*Refers to chemotherapy in addition to 5-fluorouracil and leucovorin. BOF/Strep: BCNU, Vincristine, 5FU and streptozotocin.
†Platelet counts, in thousands/μl, in parentheses.
‡Only Patient 19 had lymphopenia expressed also in thousands/μl.

recovery also occurring after recovery of the platelet count. Except in the one patient at the 60-mCi/m^2 dose, there was no hematologic toxicity following the second infusion of ^{131}I-CC49 (Table 3).

Table 3 also lists chemotherapy received in addition to 5FU and leucovorin. Toxicity was related to prior chemotherapy and was greater in those patients who had received mitomycin-C and/or nitrosoureas.

Therapeutic Response

No major responses were seen in this Phase I study. Six patients had stable disease 4 wk after administration of the first dose of ^{131}I-CC49. These patients received a second dose. One of these patients (Patient 5, Fig. 2) had a minor response following the second dose of therapy, seen 4 wk after the second dose lasting for an additional 4 wk. The others showed progression of disease on followup CT scans.

DISCUSSION

This study confirms the excellent localization characteristics and relative lack of toxicity of ^{131}I-labeled MAb CC49. Patients were preselected for antigen expression, i.e., ≥50% of tumor cells staining strongly with the antibody with strong reactivity of surrounding mucin if any. This preselection enabled us to target >95% of known tumor sites. Moreover, there was excellent concordance between extent of disease as measured by standard imaging modalities such as CT scans, and as measured by scintigraphy following administration of radiolabeled MAb. The excellent targeting to >95% of known lesions in these patients also suggests that antigen heterogeneity may not be very variable between the primary and metastatic colorectal cancer tumors.

We administered doses of up to 90 mCi/m^2 of ^{131}I-CC49 without any significant nonhematologic toxicity. Mild toxicity was limited to fevers of up to 38.5°C in a few patients, usually occurring the day after therapy; and extremity myalgia and arthralgia in two patients at the 90-mCi/m^2 dose. This latter occurrence, at about 2 wk after MAb administration, had the clinical hallmarks of serum sickness. However, there were no confirming laboratory correlates such as changes in complement levels or the presence of serum immune complexes, and symptoms were easily controlled with nonsteroidal anti-inflammatory drugs. Apart from these self-limiting minor toxicities, the treatment was very well-tolerated.

Hematologic toxicity was dose-limiting: thrombocytopenia occurred in 10 of 12 patients who received ≥75 mCi/m^2 of ^{131}I. Platelet depression was more marked in patients who had received additional chemotherapy other than 5FU and leucovorin. While the number of patients is small, we feel that in general, hematopoietic toxicity is directly related to the number of chemotherapeutic regimens received. This trial in addition to a recently completed Phase I RIT trial using another anti-colorectal MAb, A33, labeled with ^{131}I, also suggested that the extent and duration of toxicity was greatest in patients who had received mitomycin (*10*). The duration of the nadir was generally not more than 4 wk.

Iodine-131-CC49 does not localize in marrow. Myelosuppression arises from the passive irradiation of marrow cells by the long-range beta-minus radiations of ^{131}I circulating in the blood. It should therefore be comparable for all ^{131}I-labeled murine IgGs that have similar clearance following intravenous administration and no specific marrow targeting. Several groups have sought to define nonmyeloid toxicity by carrying out studies with high dose ^{131}I-MAb using bone marrow transplant or stem cell rescue (*11,12*).

While the treatment was well tolerated, there were no major responses. It seems likely that lethal amounts of radiation to tumor will require multiple injections of radiolabeled MAb (*13*). Animal studies have shown a greater effectiveness with multiple divided doses than with single doses of radiation (*14,15*). However, in all 24 patients studied, there was development of HAMA within 4 wk of MAb administration. Six patients were re-treated between 4 and 8 wk after the first dose; in all but one of these patients, retreatment was accompanied by rapid clearance of radioactivity from the body, accompanied by signs of immune complexing (noted on HPLC, as well as demonstrated by unusually high uptake in the liver and spleen) and dehalogenation (evidenced by increased thyroid uptake). This underscores the importance of developing nonimmunogenic forms of antibodies that can be used repeatedly. Alternatively, an immune modulator such as cyclosporin can be used to blunt the HAMA response and permit multiple infusions of radiolabeled MAb (*16*). We are currently studying another immune modulator, deoxyspergualine, in a clinical trial to test this hypothesis.

Several other groups have studied radiolabeled MAbs in solid tumors (*10–17*). With the exception of neuroblastoma treatment, none have reported any major responses to date. However, RIT holds great promise in hematologic neoplasms including B-cell lymphoma and myelogenous leukemia (*18–20*). In both, the neoplasm is easily accessible to the radioimmunoconjugate. Moreover, the bulky nature of adenocarcinoma and the change in tumor vasculature make it difficult for the MAb to rapidly penetrate into tumor.

Various approaches are currently being studied to circumvent these problems. Reduce the size of the molecule; this will increase clearance and reduce immunogenicity. Single-chain antigen-binding proteins or sFv are being studied in this regard (*21*). An important consideration, however, would be reduced absolute tumor uptake, as well as possible reduced tumor retention time of these smaller molecules (*22*). Humanization of the constant regions of the murine MAb (resulting in a "chimeric" antibody (*23*)) or of all but the antigen-binding portions of the MAb (*24*) (resulting in a "humanized" MAb) should result in decreased immunogenicity permitting repeat administration. Clinical trials with chimeric MAbs have shown that some are immunogenic while others are not; the immunogenicity

depends on the characteristics of the idiotype (*25*). Humanized antibodies against solid tumors have been far fewer, and early data suggest that they may not be immunogenic and may therefore permit repeat administration. An important consideration is the potential prolongation in serum circulation time. This has been shown in several clinical trials to be widely varying: chimeric 17-1A had comparable circulation times to its murine counterpart (*26*), while the clearance of chimeric IgG_4 B72.3 was much longer than that of murine IgG_1 B72.3 (*27*). In a trial in myelogenous leukemia carried out at this center, the clearance of humanized IgG_1 M195 was comparable to that of murine IgG_{2a} M195 (*28*). The benefit of repeat administration will need to be weighed against the disadvantage of increased circulation time (with resultant increased bone marrow radiation dose) in each individual system.

CONCLUSIONS

Iodine-131-CC49 was safe at doses of up to 75 mCi/m^2 in heavily pretreated patients with TAG-72 expressing colon cancer, targeting to >95% of multiple tumor sites. Dose-limiting toxicity was hematopoietic, with thrombocytopenia being predominant. One patient had a minor response. Further studies, including a modified Phase I study to evaluate the MTD in patients who have not received chemotherapy other than 5-FU/leucovorin, a Phase II study with ^{131}I-CC49 at the 75-mCi/m^2 dose level (or higher, depending on the results of the previously mentioned study), and nonimmunogenic forms of this MAb, are warranted in patients whose tumors express ≥50% of the antigen.

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