Clinical Experience with Rhenium-186-Labeled Monoclonal Antibodies for Radioimmunotherapy: Results of Phase I Trials

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Rhenium is a radionuclide with physical and chemical properties suitable for radioimmunotherapy. Two Phase I trials were carried out using ^{186}Re-labeled murine monoclonal antibodies. Patients with refractory metastatic epithelial carcinoma received single doses of either ^{186}Re-labeled intact NR-LU-10, a pancarcinoma antibody, 25-120 mCi/m^2 (n = 15) or ^{186}Re-labeled F(ab')$_2$ fragment of NR-CO-02, an anti-CEA variant antibody, 25-200 mCi/m^2 (n = 31). Prior to radioimmunotherapy, tumor localization of antibody was confirmed by ^{99m}Tc-labeled NR-LU-10 Fab or ^{99m}Tc-labeled NR-CO-02 F(ab')$_2$ imaging. Dose-limiting myelosuppression was observed at 120 mCi/m^2 following ^{186}Re-NR-LU-10 intact antibody and at 150 mCi/m^2 following NR-CO-02 F(ab')$_2$ fragment in heavily pretreated patients. In patients with minimal prior therapy, a maximum tolerated dose for NR-CO-02 F(ab')$_2$ was not reached by 200 mCi/m^2. Non-marrow toxicity was minimal. Human anti-mouse antibody developed in all patients receiving intact NR-LU-10, and in 86% patients receiving F(ab')$_2$ NR-CO-02. One patient treated with ^{186}Re NR-CO-02 achieved a partial response. We conclude that ^{186}Re-labeled antibody can be safely administered with significant toxicity limited to marrow.

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Following reports of tumor regressions in experimental animal systems using radiolabeled monoclonal antibodies (Mabs) (*1–4*), numerous investigators have performed radioimmunotherapy trials in man. Encouraging preliminary results have been reported using ^{131}I-labeled intact monoclonal antibodies to treat patients with cutaneous T-cell lymphoma (*5*), non-Hodgkin's lymphoma (*6,7*), or neuroblastoma (*8*) and using an ^{131}I-labeled Fab fragment (*9*) to treat a melanoma patient. Clinical trials with ^{90}Y-labeled polyclonal and monoclonal antibodies have been carried out in patients with hepatoma or lymphoma (*10*) and in patients with ovarian cancer following intraperitoneal administration (*11*). The dose-limiting toxicity for both these radionuclides has been myelosuppression with maximum tolerated doses (MTD) of approximately 150 mCi for ^{131}I and 15–30 mCi for ^{90}Y-labeled monoclonal antibodies.

Rhenium-186 is an attractive radionuclide for radioimmunotherapy (*12*). The 3.7 day half-life is compatible with the pharmacokinetics of tumor localization and clearance of murine Mabs. Rhenium-186 has a medium-energy beta particle (91% abundance) that is suitable for radioimmunotherapy; its maximum energy is 1.07 MeV and 90% of the energy from a point source is delivered within 2 mm of the source (X_{90}) (*13*). The 137 keV gamma photon from ^{186}Re is ideal for gamma camera imaging even at high doses. Its low energy and low abundance (9%) and the very small fraction (0.05%) of higher energy gamma photons (>600 keV) result in minimal radiation exposure to medical personnel compared with ^{131}I.

Rhenium-186 has been used as a therapeutic agent in patients: ^{186}Re colloid for the treatment of rheumatoid arthritis (*14*) and ^{186}Re-(Sn)HEDP as palliative treatment for painful bone metastases in prostate cancer (*15*). Phase I trials in patients with metastatic cancer was carried out using two ^{186}Re-labeled murine monoclonal antibodies, NR-LU-10, a pancarcinoma antibody, and NR-CO-02, an anti-CEA variant antibody. The primary goal was to determine the toxicity and MTD of these ^{186}Re-labeled antibodies.

Rhenium and technetium belong to Group VIIa of the periodic table and have similar structural and chelation chemistries. Both can be stably linked to antibodies using a preformed amide thiolate chelate method (*16,17*). Technetium-99m and ^{186}Re conjugated antibodies show similar biodistribution in the nude mouse/human xenograft system (*16,17*). Patients were selected for these ^{186}Re immunoconjugate therapy trials if they demonstrated satisfactory localization of the ^{99m}Tc immunoconjugate to tumor.

In this paper, we describe the pharmacokinetics, toxicity

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and MTD of ^{186}Re-labeled intact NR-LU-10, and ^{186}Re-labeled F(ab')$_2$ fragment of NR-CO-02. These immunoconjugates have different specificities and immunoreactivities and cannot be compared with respect to imaging and tumor uptake. All patients were evaluated for tumor response and for the development of human antimouse antibody (HAMA). Radiation dose to normal organs and tumor will be summarized in this report and described in detail separately. A preliminary report of the initial 10 patients treated with NR-CO-02 F(ab')$_2$ has been published (*18*).

METHODS

Patients

Patients with histologically confirmed carcinoma of the lung, colon, rectum, breast, ovary or kidney were eligible for these studies if they had evaluable tumor refractory to standard treatment. In the NR-CO-02 study, all patients had serum elevated CEA, greater than 5 ng/ml. Patients were required to be at least 4 wk from their most recent chemotherapy or radiation therapy and to have a Karnofsky performance status of >60%, no other serious concurrent illness, creatinine less than 1.8 mg/dl, bilirubin less than 2 mg/dl, platelet count greater than 150,000/μl and white blood count greater than 3,500/μl.

The eligibility criteria in the ^{186}Re-NR-CO-02 F(ab')$_2$ study were modified after the initial 25 patients to exclude heavily pretreated patients (i.e., patients who had received prior radiotherapy to the pelvis or axial skeleton, or prior chemotherapy with alkylating agents or antibiotic class chemotherapy drugs). Only patients with prior anti-metabolite chemotherapy (e.g., 5-FU alone or 5-FU and leucovorin or methotrexate) were eligible.

Tumor extent and volume were determined by CT scan prior to therapy. Baseline HAMA levels were measured (*19*), and patients were excluded if the antibody titer was greater than two standard deviations above the geometric mean of a control population. The HAMA level was not required prior to ^{186}Re infusion if this was within 15 days of the imaging study because our data indicated that a HAMA response would not develop in this interval. Patient demographics are shown in Table 1. Each patient entered into these trials was given a patient study number. In the NR-CO-02 study, the first two digits indicated whether the ^{186}Re immunoconjugate was administered intravenously (#40.) or intra-arterially (#30.). The studies were conducted under Investigational New Drug Applications with the Office of Biologics, Research and Review, Food and Drug Administration, and were approved by the Institutional Review Board of the Virginia Mason Medical Center. Patients reviewed and signed informed consent after thorough explanation of the studies.

Antibodies

NR-LU-10 is a murine IgG$_{2b}$ Mab that recognizes a 40 kD glycoprotein antigen expressed by several epithelial tumors including carcinoma of the lung, colon, ovary, breast and other adenocarcinomas (*20,21*). The target antigen for NR-LU-10 has not been fully characterized.

NR-CO-02 is a murine IgG$_1$ Mab that was elicited using immuno-absorbants of lectins combined with peripheral protein extracts of xenografted colon adenocarcinomas (*22*). The antibody recognizes an antigen expressed on an uncharacterized subspecies of carcinoembryonic antigen (CEA). Tumor reactivity was demonstrated in vitro to colorectal, breast, lung, ovarian, gastric, prostate and cervical cancer using standard immunohistological techniques.

TABLE 1
Patient Demographics

Characteristics	Patients receiving ^{186}Re-antibody	
	NR-LU-10	NR-CO-02
Patients		
Number	15	31
M/F	9/6	19/12
Age		
Range	42–72	32–81
Diagnosis (no. of pts):		
Colorectal	10	27
Lung	2	2
Ovarian	2	—
Gastric	—	2
Renal	1	—
Prior Therapy (no. of pts):		
None or antimetabolites only	6	17
Radiation ± antimetabolites	3	6
Intensive chemo ± radiation	6	8

Antibody Production

Antibodies were produced by in vitro fermentation, purified, and tested for purity, lack of pyrogenicity, sterility and absence of contamination by mycoplasma, viruses or polynucleotides. Since the short half-life of ^{99m}Tc is not compatible with imaging using a whole antibody, the intact NR-LU-10 antibody was papain digested to yield the Fab fragment for labeling with ^{99m}Tc for imaging. The intact NR-LU-10 antibody labeled with ^{186}Re was then used for therapy. NR-CO-02 was pepsin cleaved to the F(ab')$_2$ fragment with an intermediate half-life, which was labeled with ^{99m}Tc for imaging and with ^{186}Re for therapy using the same ligand system. The fragments were purified by column chromatography and re-tested as outlined above. Bulk solutions of the antibody and the fragments were aseptically vialed and stored at pH 7 in phosphate-buffered saline.

Labeling

The ^{99m}Tc labeling of antibody Fab fragments has been previously described (*23*). The labeling procedures for NR-CO-02 F(ab')$_2$ with ^{99m}Tc and ^{186}Re and intact NR-LU-10 with ^{186}Re were similar. Following reduction of [^{186}Re]perrhenate (100–600 mCi, 0.5 ml) by stannous ion, the reduced ^{186}Re or ^{99m}Tc was then exchanged into the tetrafluorophenyl activated ester of mercaptoacetylglycylglycyl-gamma-aminobutyrate (MAG$_2$-GABA, 0.4–0.9 mg) by incubation at 95°C for 30 min (Fig. 1). Following conjugation to the antibody, the crude conjugates were purified by either gel permeation chromatography (30 cc acrylamide) or by ion-exchange chromatography (5 QAE cc cartridges in series) and eluted with a phosphate/ascorbate saline buffer at pH 7 in human serum albumin to yield the ^{186}Re-labeled immunoconjugate. The preparation was finally diluted to 30 ml with 0.9% saline for administration to the patient.

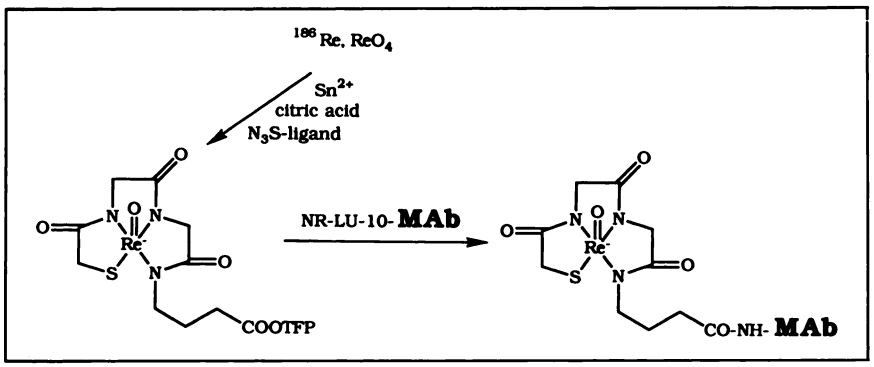

FIGURE 1. Preformed chelate antibody labeling schema for ^{186}Re-immunoconjugates.

Quality Control

Prior to patient injection, all ^{186}Re antibody preparations were assessed for radiochemical purity by ITLC (12% trichloroacetic acid; release criteria: ≥90%), percent monomeric antibody by size exclusion chromatography (HPLC; Zorbax diol; 0.2 *M* phosphate buffer, pH 6.8; release criteria: ≥85%), endotoxin by a LAL/ELISA technique (release criteria: 5 EU/mL or less) and cell-binding immunoreactivity (CBIR) by a solid-phase assay.

In the 15 NR-LU-10 preparations administered, the mean ITLC value was 95% ± 2%. HPLC of the preparations indicated that 98% ± 1% of the radiolabeled protein was monomeric with the major ^{186}Re impurity being Re-MAG_2-COOH, the free acid form of the MAG_2-GABA complex. Levels of endotoxin averaged 0.23 ± 0.01 EU/ml. CBIR was 74% ± 13%, equivalent to >95% of control ^{125}I-labeled antibody.

The mean radiochemical purity for the ^{186}Re-NR-CO-02 preparation was 95% ± 2%. HPLC was 97% ± 3%; perrhenate was the major impurity when gel permeation chromatography was used, while ^{186}Re-MAG_2-COOH was the major impurity when the ion exchange procedure was used. Mean endotoxin level was 0.26 ± 0.37 EU/ml. Cell-binding immunoreactivity of NR-CO-02 was 43% ± 17%, equivalent to >95% of control ^{125}I-labeled antibody.

Study Design

Patients were selected for ^{186}Re therapy on the basis of positive tumor targeting by ^{99m}Tc-labeled immunoconjugate. This was based on a subjective impression of localization of activity at the site of known tumor. The 15 patients treated with ^{186}Re NR-LU-10 received the ^{186}Re immunoconjugate therapy 5 to 165 (median 8) days after the imaging study. NR-LU-10 antibody, 40 ± 7 mg labeled with escalating doses of ^{186}Re (45–260 mCi), was infused intravenously over 5–7 minutes.

Thirty-one patients received ^{186}Re-labeled NR-CO-02 F(ab')$_2$, generally within 15 days of the ^{99m}Tc-labeled immunoconjugate administration. Patients received 10 mg of unlabeled antibody fragment followed by 36 ± 5 mg of the ^{186}Re-labeled antibody fragment (25–336 mCi), administered over approximately 5 min. Five patients with disease predominantly in the liver received ^{186}Re-NR-CO-02 F(ab')$_2$ intra-arterially by hepatic artery catheter. Intra-arterial administration was by infusion pump over 1 hr: one patient at 60 mCi/m^2, two at 90 mCi/m^2 and two at the 125 mCi/m^2 dose levels.

Gamma camera images were acquired immediately and 3, 20, 44, 68, and 140 hr following injection. Most patients were imaged with SPECT at 70 hr, using 64 projections and registered in a 64 × 64 matrix. The study duration was determined by the ability of the patient to tolerate SPECT acquisition times, which ranged from 30 to 40 sec per angle. A medium-energy collimator was used to acquire counts from the 137 keV photon of ^{186}Re (*24*). A GE 400AT Starcam II digital system was used to acquire and process data. Quantitative planar imaging determined the activity in the source organs at each timepoint using the conjugate-view method (*25*). The radiation absorbed dose was determined by the Medical Internal Radiation Dose Committee (MIRD) method (*26–30*). Serial blood, urine and stool specimens were obtained for 6 days in order to determine radiolabel clearance. Urinary metabolites were assessed by reverse-phase HPLC. Patients were hospitalized in private rooms for medical care or until the radiation exposure was below the limit set by the hospital radioactive materials license for ^{131}I.

At least three patients were studied at each dose level prior to dose escalation. This occurred only after at least one patient had been observed for 6 wk and no unacceptable toxicity was noted. The dose levels studied were 25, 60, 90 and 120 mCi/m^2 for NR-LU-10 and 25 mCi, then 25, 60, 90, 125, 150, 175 and 200 mCi/m^2 for NR-CO-02. Unacceptable toxicity was defined as three patients with grade III or two patients with Grade IV toxicity in the same organ system using criteria adapted from the World Health Organization (*31*) and the Eastern Cooperative Oncology Group (*32*). The MTD was defined as the dose level immediately below that which resulted in unacceptable toxicity.

Patient Follow-up

Interval history, physical examination and routine laboratory studies to assess toxicity were repeated 1, 2 and 4 days following administration of the ^{186}Re-labeled antibody and then weekly for 8 wk. At 4–6 wk following ^{186}Re administration, patients were assessed for tumor response by standard oncologic response criteria (*32*).

HAMA Methods

Serum was obtained at intervals to evaluate HAMA formation (*19*). The criterion for a positive response was a HAMA titer twice the patient's baseline titer and above the geometric mean plus two standard deviations of a normal population (i.e., 4.6 normal serum (NS) units for NR-LU-10 or 10.7 NS units for NR-CO-02).

RESULTS

Imaging

Images of tumor uptake with ^{99m}Tc-NR-LU-10 Fab and ^{186}Re-NR-LU-10 intact antibody were similar (Fig. 2), as

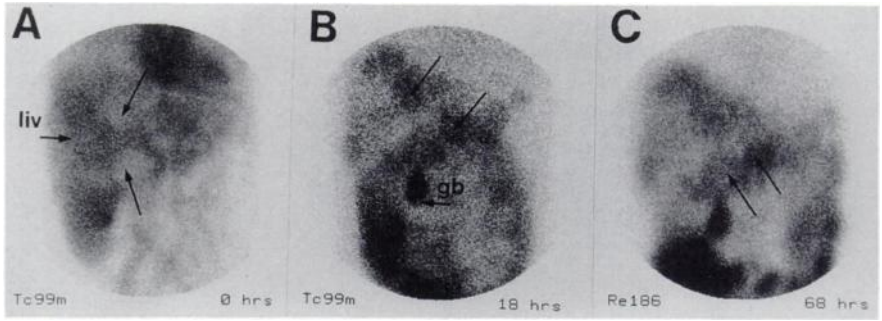

FIGURE 2. Anterior abdominal images in a patient with multiple hepatic metastases from colon cancer injected with ^{99m}Tc-NR-LU-10 Fab (A,B) and ^{186}Re-NR-LU-10 whole antibody (C). (A) Immediately following ^{99m}Tc Fab injection. Note multiple photopenic hepatic metastases, (B) 18 hr following ^{99m}Tc-Fab injection and (C) 68 hr following ^{186}Re-antibody. Long arrows indicate sites of metastases, and demonstrate localization of immunoconjugate. Symbols in figures: Liv = normal liver and gb = gallbladder.

were the images with ^{99m}Tc- and ^{186}Re-NR-CO-02 F(ab')$_2$. With the exception of one patient, all tumors visualized on the ^{99m}Tc studies were again visualized following administration of ^{186}Re immunoconjugate. The single patient (#36) in whom known liver metastases were not visualized with ^{186}Re-NR-LU-10 experienced an acute hypersensitivity reaction following infusion. The 3.7-day physical half-life of ^{186}Re allowed improved tumor visualization after 24 hr, and tumors became more prominent as background serum activity decreased over the 6-day duration of the ^{186}Re study. Activity was seen only in the periphery of many large hepatic tumors. Pulmonary metastases were often difficult to identify on planar images. SPECT imaging was helpful in assessing uptake, particularly in patients with multiple small pulmonary nodules in whom discrete lesions could not be identified on planar images. Metastases to lymph nodes were only visualized in superficial nodes. Occult lesions were detected following both ^{186}Re immunoconjugates in liver, lung, bone, brain, lymph nodes and muscle.

Pharmacokinetics

Mean serum, urine and fecal clearance curves from patients who received NR-LU-10 are shown in Figure 3A. Serum clearance in 12 patients demonstrated biexponential clearance with a mean alpha $t_{½}$ (distribution phase) of 4.7 ± 2.6 (s.d.) hr and a mean beta $t_{½}$ (elimination phase) of 26.3 ± 4.9 hr. Serum clearance from three patients best fit a one-compartment model with mean monoexponential $t_{½}$ of 25.6 ± 4.5 hr.

Kidneys were the primary route of radiolabel excretion. Mean cumulative excretion in the urine by 6 days was 65% ± 12%. The radiolabeled material excreted in the urine consisted of low molecular weight catabolites of the antibody fragment (*34*). The lysine adduct of the Re-MAG$_2$-GABA complex appeared in the urine by 1–2 hr and was the major catabolite at all times. In addition, small amounts (≤10%) of the N-acetylated lysine adduct, free acid and perrhenate were found. The mean cumulative activity in feces in 11 patients was 15% ± 6% over 6 days.

The mean serum clearance and urinary excretion of the ^{99m}Tc- and ^{186}Re-labeled NR-CO-02 F(ab')$_2$ are shown in Figure 3B. Serum clearance best fit a one-compartment model in most patients with mean ± s.d. monoexponential $t_{½}$ of 11 ± 5 hr and 14 ± 7 hr, respectively, for ^{99m}Tc and ^{186}Re. With the limitation that data for the ^{99m}Tc immunoconjugate are available only for 24 hr, these curves are similar to each other, i.e., in 20 patients who received ^{99m}Tc and ^{186}Re F(ab')$_2$ intravenously and had adequate data for serum clearance determinations, the paired mo-

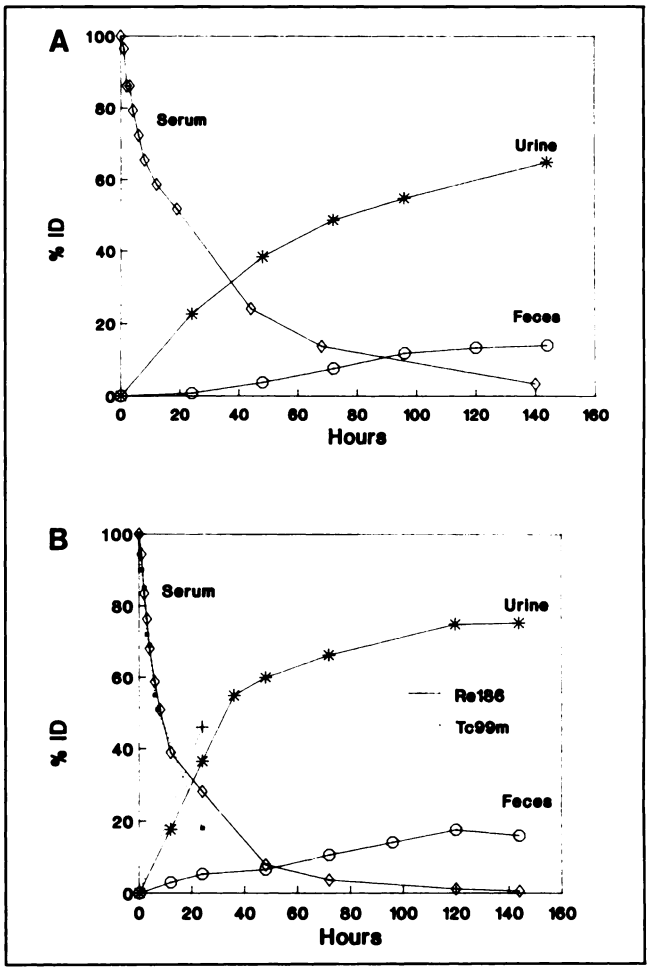

FIGURE 3. Pharmacokinetics of immunoconjugates. Urine and fecal radioactivity are expressed as cumulative percent of the injected dose excreted. Serum radioactivity is expressed as percent of serum radioactivity immediately after injection. (A) ^{186}Re-NR-LU-10 and (B) ^{99m}Tc- and ^{186}Re-F(ab')$_2$ NR-CO-02.

noexponential half-times were closely correlated by t-test ($p < 0.001$) and coefficient of determination ($r^2 = 0.77$).

The mean percent of the injected dose of NR-CO-02 F(ab')$_2$ excreted in the urine by 24 hr postinfusion was 43% ± 16% for the ^{99m}Tc patients and 36% ± 17% for the ^{186}Re-NR-CO-02 patients (not different statistically). By 6 days postinjection, 75% ± 16% (n = 29) of the injected ^{186}Re-NR-CO-02 had been excreted in the urine. Again, urinary metabolites were predominantly the lysine and N-acetyl lysine adducts of the ^{186}Re-MAG$_2$-GABA chelate with small amounts (<10%) of ^{186}Re-MAG$_2$ acid complex and perrhenate, indicating that loss of free ^{186}Re from the intact immunoconjugate was negligible. The mean cumulative fecal excretion of ^{186}Re-NR-CO-02 F(ab')$_2$ in 20 patients was 16% ± 7% of the injected dose.

Intra-arterial ^{186}Re-labeled immunoconjugates had serum clearance curves virtually identical to those observed after intravenous administration (data not shown). Gamma camera clearance half-times from the liver and from hepatic tumors were also similar after intravenous and intra-arterial administrations. With the limited number of intra-arterial patients, we were unable to determine any appreciable difference in biodistribution between intravenous and intra-arterial administration.

Dosimetry

Average absorbed dose to normal organs for the two trials is summarized in Table 2. The kidneys received the highest dose for both immunoconjugates followed by the liver, while whole-body and marrow doses were considerably lower. The absorbed dose to tumor was estimated at the predominant site of disease. For NR-LU-10, the absorbed dose estimates for 20 tumors in 15 patients ranged from 0.35 to 17.7 rad/mCi, mean 6.3 ± 4.8 rad/mCi. The tumor-to-whole-body dose ratio ranged from 0.38 to 21. For the NR-CO-02 F(ab')$_2$, mean tumor dose for 41 sites in 23 patients was 4.0 ± 3.8 rad/mCi and ranged from 0.4 to 18.6 rad/mCi. The tumor-to-whole body dose ratio ranged from 0.9 to 54.6. The heterogeneity of tumors created variable uptake that is well illustrated in the multiple hepatic metastases in Figure 2. In this patient, dose to tumor following ^{186}Re-NR-LU-10 varied from 8.25 to 17.7 rad/mCi.

Tumor biopsies were obtained from four lymph nodes and two subcutaneous nodules. The percent injected dose per gram of tumor tissue (%ID/g) averaged 0.004% ± 0.002% (range from 0.001% to 0.007%) in four NR-LU-10 samples at 26 to 168 hr, and were 0.002% and 0.004% ID/g in two NR-CO-02 samples at 68 hours.

TABLE 2
Rhenium-186 Normal Organ Dosimetry Absorbed Dose (rad/mCi)

	Intact NR-LU-10		F(ab')$_2$ NR-CO-02	
	Mean	s.d.	Mean	s.d.
Whole body	0.6	0.2	0.4	0.1
Marrow	0.6	0.2	0.4	0.1
Liver	2.9	0.5	1.7	0.3
Lung	1.4	0.0	0.9	0.3
Kidney	5.7	3.0	3.5	1.5
Small intestine	0.2	0.0	0.1	0.0
Upper large intestine	0.5	0.2	0.5	0.2
Lower large intestine	1.4	0.5	1.2	0.4
Testes	1.4	1.4	0.5	0.4
Ovary	0.6	0.2	0.3	0.1
Thyroid	2.5	1.7	not visualized	

Clinical Observations

Non-marrow toxicity following administration of ^{186}Re-NR-LU-10 antibody was mild and is summarized in Table 3. Low-grade fever (<103°F) in six patients (40%) and mild nausea in eight patients (53%; with vomiting in one patient) lasted 1–2 days following treatment. Liver function test (LFT) abnormalities in 11 patients (67%) consisted of clinically asymptomatic, mild elevations of SGPT, SGOT, bilirubin or LDH, generally less than 2.5 times normal, beginning 1–4 days following injection and resolving within 3 wk. One patient (#18) developed a symptom complex of transient hypotension, irregular pulse, fever, nausea, vomiting, diarrhea and minimal elevation of serum creatinine, SGOT and bilirubin within 12 hr of immunoconjugate administration. Whether these symptoms were related to the antibody infusion or to an intercurrent illness could not be determined. Another patient (#36) developed facial edema without other allergic manifestations 30 min following administration of ^{186}Re-NR-LU-10. He had a history of atopy and of prior exposure to murine Mab, but his baseline HAMA level was normal. A third patient (#39) developed fever, myalgias and increased LFTs lasting 7 days.

Mild hematologic toxicity was seen up to the 90 mCi/m^2 dose level, with platelet and granulocyte depression recovering spontaneously after 4 wk. The time course of white blood cell and platelet suppression following administration of ^{186}Re-NR-LU-10 for all patients at each dose level is shown in Figure 4. A single patient (#49) demonstrated bone marrow uptake on his images, perhaps related to a previous myeloproliferative disorder. He developed severe marrow toxicity requiring platelet support at a relatively low administered dose (79 mCi/m^2). As indicated in Table 3, three of the four patients who received 120 mCi/m^2 developed platelet count suppression below 25,000/μl or white cell count suppression below 2,000/μl. Hence, 120 mCi/m^2 was determined to be the toxic dose and 90 mCi/m^2 the MTD.

Despite estimates of a substantial dose to the kidneys (Table 2) from excretion of ^{186}Re-labeled antibody fragments and cross-reactivity of NR-LU-10 with renal tubule epithelial cells, and to the thyroid from cross-reactivity of NR-LU-10 with thyroid epithelium (*20*), no clinical toxicity in these organs has been encountered to date.

TABLE 3
Rhenium-186-NR-LU-10 Toxicity

Patient no.	Dose level (mCi/m^2)	^{186}Re dose (mCi)	Whole-Body dose (rad)	Nadir counts $\times 10^{-3}/\mu l$‡ WBC	Platelet	Other adverse reactions†
18*	25	58	24	2.4	259	Fever, LFT, N/V, diarrhea, proteinuria, creat
36*	25	50	20	3.3	161	Allergic
37	25	45	25	6.9	226	Fever
39	60	97	72	2.6	198	Fever, diarrhea, myalgia, LFT(3+)
40	60	94	63	7.6	333	Fever, LFT, diarrhea: N/V(2+)
28*	60	78	75	3.3	124	Fever, LFT, N/V, proteinuria
42*	60	111	52	3.2	105	N/V
43*	60	124	59	2.9	58	LFT
48*	90	146	69	3.4	171	LFT
46	90	179	93	3.3	124	LFT
49	90	146	112	0.9(4+)	22(4+)	LFT, N/V
50*	120	215	128	0.9(4+)	10(4+)	LFT, N/V, diarrhea, proteinuria, rash
51*	120	237	135	1.6(3+)	50	Fever, N/V
54*	120	180	166	2.5	16(4+)	LFT, N/V
55	120	259	86	2.7	86	LFT, N/V

* Patients with prior radiation or intensive chemotherapy.

† LFT = liver function test abnormalities; N/V = nausea and/or vomiting; creat = creatinine elevation; RUQ = right upper quadrant. Severity of reactions was as follows: Fever = 100–102.9°F, fever(2+) = 103–105°F for <6 hr; LFT (2+) = 2.5–4.9 × normal, LFT = <2.5 × normal, LFT(3+) = ≤10 × normal, and LFT(4+) = >10 × normal; Lipase(3+) = ≤10 × normal; RUQ pain = little or no therapy required, RUQ pain(2+) = treated as an outpatient; N/V = nausea, N/V(2+) = transient vomiting, N/V(3+) = vomiting requiring intravenous fluids; vertigo, epistaxis and hypotension-transient, requiring no therapy; and creatine(3+) = 3–5 × normal.

‡ Severity of hematological toxicity: Grade 3 = WBC nadir of 1.0–1.9 × $10^{-3}/\mu l$ or platelet nadir of 25–49 × $10^{-3}/\mu l$; Grade 4 = WBC nadir < 1.0 × $10^{-3}/\mu l$ or platelet nadir of < 25 × $10^{-3}/\mu l$ indicated as (3+) or (4+).

Toxicity in the patients who received ^{186}Re-NR-CO-02 F(ab')$_2$ was also mild and is presented in Table 4. (One patient, #40.01, died of progressive tumor 11 days after receiving 25 mCi of the immunoconjugate and was excluded from this toxicity analysis). Fever <103°F was seen in 15 patients (48%), nausea or vomiting in 8 patients (26%), transient increase in LFT in 14 patients (45%), pain in the right upper quadrant in patients with known liver metastases in 3 patients and increase in lipase, vertigo, epistaxis and hypotension in one patient each. One patient sustained a hepatic infarction after intra-arterial immunoconjugate administration which was attributed to mechanical vascular occlusion from the catheter placement.

Table 5 examines the hematologic toxicity of patients treated with ^{186}Re-NR-CO-02 F(ab')$_2$ in relation to prior therapy. At the 150 mCi/m^2 dose level among patients treated previously with either radiation or chemotherapy more intensive than antimetabolites, two of five patients had nadir platelet counts of less than 50,000/μl and two of five patients had nadir WBCs of less than 2,000/μl. Therefore, 150 mCi/m^2 was determined to be the toxic dose and 125 mCi/m^2 the MTD in patients with intensive prior therapy. In contrast, among patients previously untreated or treated with antimetabolites only, the MTD was not reached by 200 mCi/m^2.

No symptoms of gastrointestinal radiation toxicity have been observed in spite of the prominent activity in the intestinal tract observed on the images. This activity is largely due to the excretion of ^{186}Re-antibody metabolites in the stools rather than uptake in the bowel mucosa as determined by movement of activity on the images during the study period consistent with peristalsis, and by direct measurement of the radioactivity in the stools and the bile in one patient (data not shown). The range of the ^{186}Re beta particle is such that radioactivity in the bowel contents would not penetrate the bowel mucosa, and therefore the absorbed dose to the intestinal mucosa has not been clinically significant.

Tumor Response

In the ^{186}Re-NR-LU-10 antibody trial, no tumor responses were observed. No tumor markers were available in these patients to indicate any biological effect on tumors.

One patient (#30.04) treated with an intra-arterial dose of 142 mCi (83 mCi/m^2) of NR-CO-02 F(ab')$_2$ achieved a

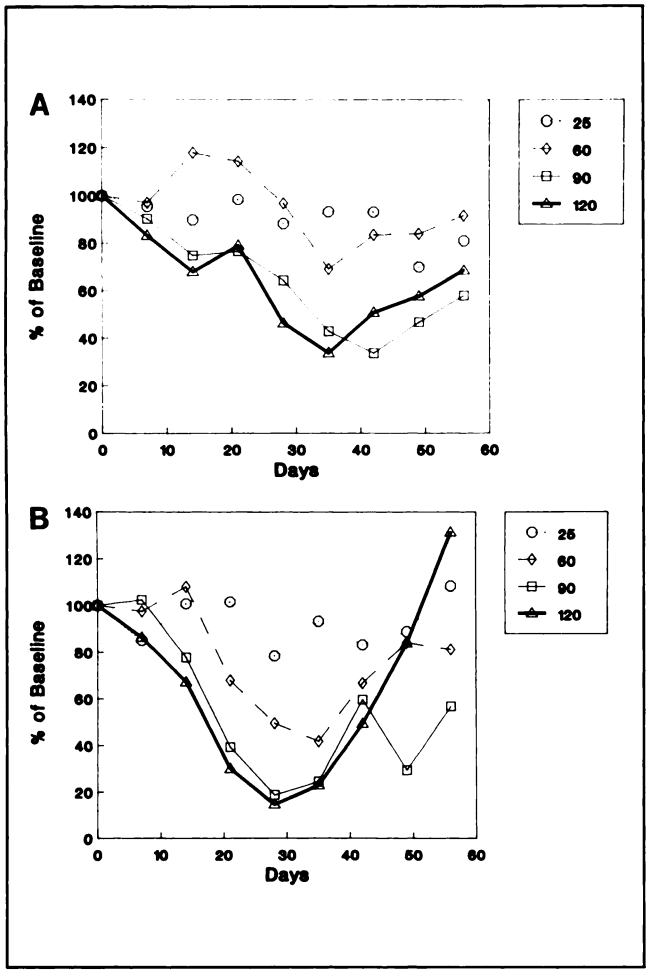

FIGURE 4. Mean blood counts for all patients at each dose level following ^{186}Re-NR-LU-10, expressed as percent of baseline count. (A) White cell count and (B) platelet count.

partial response. This 50-yr-old man with refractory colon cancer metastatic to the liver demonstrated improvement in Karnofsky performance status, LFT (SGOT 248 to 81 IU) and CEA (148 to 14.6 ng/ml) and reduction in liver size by 13 days after infusion. On Day 30, an abdominal CT scan showed a 53% reduction in bidimensional tumor measurements (Figure 5). Because of the objective response, absence of toxicity and lack of HAMA formation, the patient was retreated with 89 mCi/m^2 of ^{186}Re-immunoconjugate intra-arterially 66 days after the initial infusion. By Day 27 after the second dose, the patient developed mild myelosuppression (WBC = 2300/μl, platelets = 50,000/μl). He also demonstrated a further 57% decrease in tumor area (Fig. 5) and again further improvement in liver function tests. The patient remained clinically well for 7 mo after which his tumor progressed.

Using standard response criteria, 11 other patients showed stable disease 4–6 wk following administration of ^{186}Re-NR-CO-02 F(ab')$_2$, while 19 patients showed progressive disease. Ten patients demonstrated a fall of more than 30% in their CEA, and of these 10 patients, 1 had a partial response, 5 had stable disease and 4 had progressive disease.

HAMA Response

The mean post-treatment HAMA responses are shown in Figure 6. All patients had a positive response following intact NR-LU-10 and 80% developed positive HAMA titers by Week 2. The mean response peaked at Week 8 at approximately 1000 NS units. All patients evaluated at Weeks 12 and 16 had persistently elevated HAMA titers.

Eighteen patients who received only a single antibody administration for the NR-CO-02 F(ab')$_2$ imaging study were evaluated. Elevated HAMA titers were seen in 11 of these patients. The mean peak titer was approximately 10 NS units at 6 wk. Twenty-four of 28 (86%) patients who received a second F(ab')$_2$ administration for ^{186}Re therapy developed elevated HAMA titers. The earliest positive response was observed at Week 1. The mean response peaked at Week 4 at approximately 100 NS units. All patients who developed elevated HAMA levels did so by Week 6.

DISCUSSION

We have demonstrated that ^{186}Re can be stably conjugated to intact Mab or antibody fragments and administered in doses up to 349 mCi (200 mCi/m^2). The MAG$_2$-GABA preformed chelate method for conjugation of ^{186}Re to murine antibody has been predictable and reproducible throughout the dose range studied. It allowed the use of low-specific activity ^{186}Re at clinically relevant levels with no evidence of aggregation, loss of immunoreactivity or transchelation of ^{186}Re to other proteins. The thyroid was not visualized after either ^{99m}Tc- or ^{186}Re-F(ab')$_2$ NR-CO-02. This was achieved without any attempt to block the thyroid, thus demonstrating by sensitive bioassay that the labeling procedures produce stable chelates since free pertechnetate (and presumably perrhenate) would localize to the thyroid. Thyroid visualization after NR-LU-10 was due to cross-reactivity with normal thyroid tissue (*20*).

The predominant and significant toxicity observed was hematologic and occurred at a somewhat lower dose level with the intact antibody than the F(ab')$_2$ fragment, presumably because of the longer circulating half-life of intact antibody. We did not consistently encounter this toxicity until doses in excess of 90–125 mCi/m^2 ^{186}Re were administered. We determined the MTD for ^{186}Re-NR-LU-10 to be 90 mCi ^{186}Re/m^2. At 120 mCi/m^2, significant platelet and granulocyte suppression were observed. However, the one patient at this dose level who had received no prior therapy (#55) experienced only mild toxicity. In addition, mild non-hematological toxicity was observed in these patients as described above and in Table 3. These toxicities did not correlate with radiation dose, and a constant antibody dose was administered. The frequent observation of mild liver function abnormality in this study and the absence of similar toxicity in studies with other murine

TABLE 4
Rhenium-186-NR-CO-02 F(ab′)$_2$ Toxicity

Patient no.	Dose level (mCi/m^2)	^{186}Re dose administered (mCi)	Absorbed dose: Whole-Body dose (rads)	Nadir counts × $10^{-3}/\mu l$‡: WBC	Nadir counts × $10^{-3}/\mu l$‡: Platelets	Other adverse reactions†
40.04*	25	51	20	3.3	131	
40.06*	25	47	27	6.2	475	
40.03	25	52	23	4.6	311	
30.02	60	100	20	8.2	249	Fever, LFT(2+)
40.10	60	100	30	8.0	416	Fever, LFT(4+)
40.07	60	105	45	6.1	122	
40.12*	60	149	49	5.4	171	Lipase(3+)
30.03*	90	147	62	3.0	79	Fever, LFT(3+), RUQ pain
30.04*	90	142	54	3.8	168	Fever
40.16*	90	132	56	6.4	197	
40.14*	90	184	72	3.8	234	Fever
40.20	90	175	34	10.8	133	Fever, LFT
40.13	90	173	89	2.3	183	Fever, N/V, vertigo
30.07	125	202	106	4.8	104	Fever, LFT creat(3+)
30.14	125	254	76	7.1	177	
40.19*	125	244	75	3.8	189	
40.17	125	217	72	5.4	153	NV(2+), LFT
40.34*	125	296	91	1.4(3+)	38(3+)	LFT
40.21	125	248	71	10.6	481	LFT
40.33*	150	255	124	0.5(4+)	52	RUQ pain(2+)
40.31*	150	281	77	2.9	124	Fever(2+), N/V(2+), LFT
40.23*	150	236	107	2.0	48(3+)	Fever, N/V, LFT
40.18*	150	199	113	7.6	422	Fever
40.28*	150	262	86	1.6(3+)	14(4+)	Fever, N/V, LFT
40.37	150	301	111	3.4	339	N/V(3+), LFT(4+)
40.35	150	349	119	2.5	60	Fever, N/V
40.38	175	274	117	4.8	104	Fever, LFT
40.39	175	336	115	6.0	8(4+)	Epistaxis
40.25	175	290	165	3.5	66	Fever, N/V, LFT(2+), RUQ pain, hypotension
40.40	200	336	161	3.9	57	LFT

See Table 3 for footnote definitions.

TABLE 5
Hematological Toxicity After ^{186}Re-F(ab′)$_2$ NR-CO-02 in Relation to Prior Therapy

Dose level (mCi/m^2)	Cell line	Prior Therapy: None or antimetabolites only				Radiation or intensive chemotherapy				
125	WBC	4.8*	5.4	7.1	10.6	1.4 (3+)	3.8			
	Platelet	104	153	177	481	38 (3+)	189			
150	WBC	2.5	3.4			0.5 (4+)	1.6 (3+)	2.0	2.9	7.6
	Platelet	60	339	339	52	52	14 (4+)	48 (3+)	124	422
175	WBC	3.5	4.8	6.0						
	Platelet	66	104	8 (4+)						
200	WBC	3.9								
	Platelet	57								

* WBC and platelet nadir counts (in thousands/μl) are given vertically for individual patients.

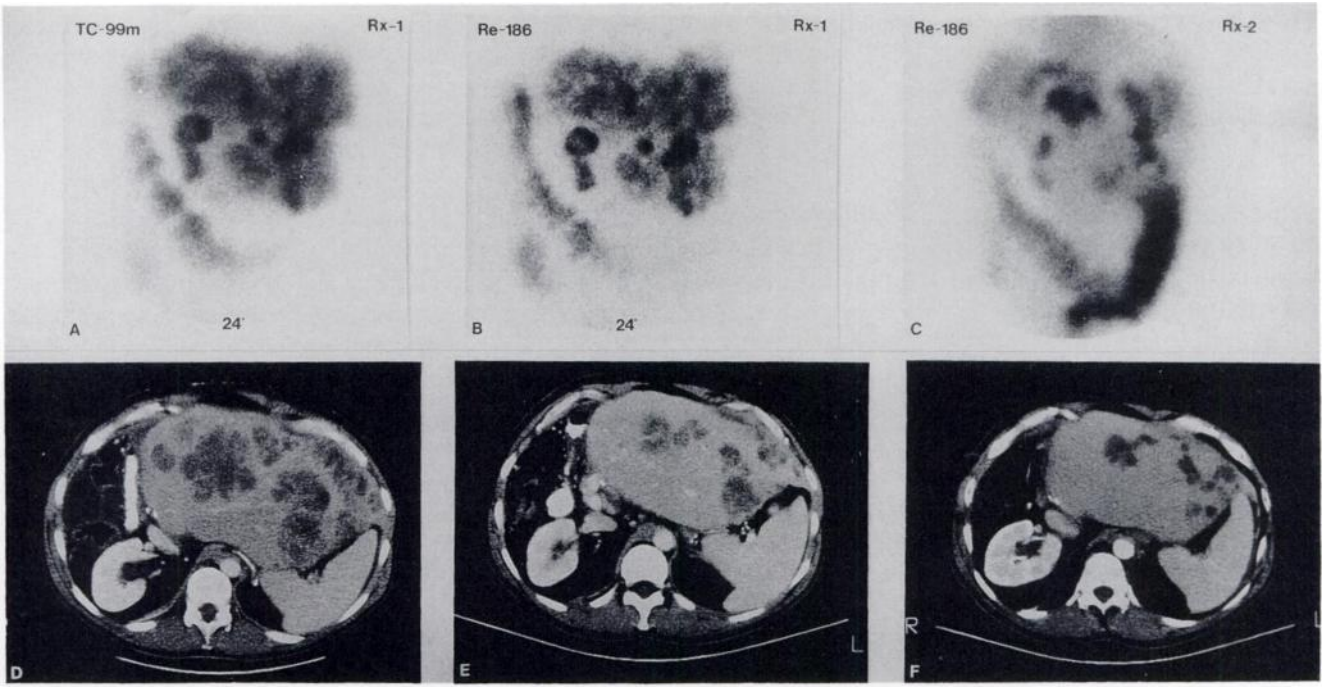

FIGURE 5. Patient 30.04 who responded twice to ^{186}Re-NR-CO-02 F(ab')$_2$. Anterior abdominal gamma camera images showing radionuclide accumulation in multiple hepatic metastases: (A) ^{99m}Tc-NR-CO-02 images prior to therapy; (B) at 20 hr following 142 mCi ^{186}Re-NR-CO-02; and (C) at 72 hr following 146 mCi ^{186}Re-NR-CO-02 (second dose). CT scans of a comparable level through the liver: (D) prior to ^{186}Re-NR-CO-02; (E) 4 wk following first treatment; and (F) 4 wk following second treatment.

Mabs (*6,7,11*) suggests that these abnormalities may be related to metabolism of the MAG_2-GABA ligand. Other nonhematological toxicities were sporadic and appear most likely to have been immunologically mediated.

In the NR-CO-02 study, there were sufficient patients to determine that the MTD in patients who were heavily pretreated with radiation and/or chemotherapy was 120 mCi/m^2. In 17 patients with minimal prior therapy, the MTD as defined was not reached at 200 mCi/m^2, although toxicity was seen in some patients. These data suggest that patients without prior therapy may tolerate a dose of radioimmunoconjugate at least 80 mCi/m^2 higher than those with intensive prior therapy.

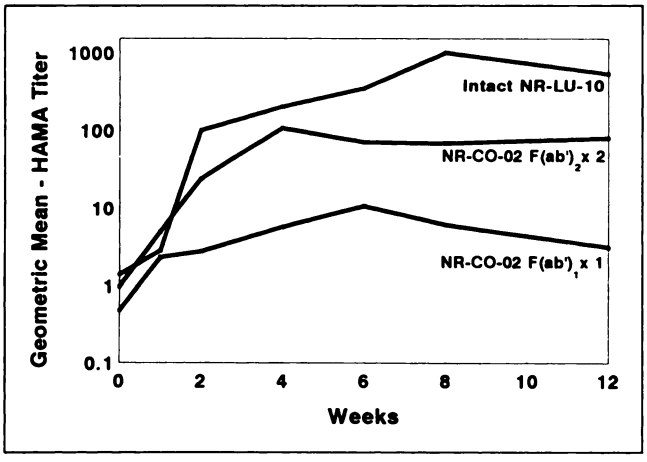

FIGURE 6. Mean HAMA responses following ^{186}Re-immunoconjugates. Rhenium-186-NR-LU-10 was preceded by ^{99m}Tc-Fab NR-LU-10. Threshold = 4.6 NS units. NR-CO-02 F(ab')$_2$ × 1 were patients undergoing ^{99m}Tc imaging study only. NR-CO-02 F(ab')$_2$ × 2 were patients given both ^{99m}Tc- and ^{186}Re-NR-CO-02 F(ab')$_2$. Threshold = 10.7 NS units.

Iodine-131 has been the most commonly employed beta-emitting radionuclide for radioimmunotherapy. The MTD following ^{131}I-labeled intact antibody has typically been 150 mCi, similar to ^{186}Re-NR-LU-10. However, ^{131}I has several disadvantages: (a) chloramine-T or iodogen labeling methods result in de-iodination of ^{131}I from the protein and (b) the 8-day half-life and abundant high energy gamma photons deliver significant doses of radiation to non-tumor sites, including the thyroid and bone marrow, and also result in significant radiation exposure to medical personnel. Yttrium-90 antibodies are associated with marrow toxicity at low administered doses, approximately 10–20 mCi/m^2 (*10*), because of instability of the chelate and uptake of released ^{90}Y in bone. By using a chelating agent systemically, the MTD of ^{90}Y has been increased to 30 mCi (*34*). The administered ^{186}Re activity may therefore be 4–6-fold higher than that of ^{90}Y.

Experience from external beam therapy suggests that radiation nephritis occurs with increasing frequency following exposure to 1500 rads or higher (*35*). Thus, the kidneys may be the second organ of dose-limiting toxicity (Table 2). NR-LU-10 cross-reacts with the collecting tubules of the renal medulla (*20,21*), in part accounting for the greater dose to the kidney after NR-LU-10 than after

NR-CO-02 (Table 2). This cross-reactivity most likely results in a heterogeneous distribution of radioactivity in the kidney and may cause less toxicity to renal function than the uniform, high dose rate of external beam therapy in which the radiosensitive glomeruli receive a dose identical to the remainder of the renal tissue. Renal function studies have shown no evidence of renal dysfunction 18 mo following treatment of one patient (#46) in whom we estimated a kidney dose of 1940 rads. Ligand modifications that reduce renal exposure have been studied in animals (*36*) and may be beneficial to humans.

The similar chemistry of technetium and rhenium did allow the successful use of similar chelation methods to conjugate these metals to antibodies. Technetium-99m- and ^{186}Re-immunoconjugates behaved similarly in vivo and ^{99m}Tc imaging could be used to successfully select appropriate candidates for ^{186}Re-antibody therapy. The 137 keV gamma photon of ^{186}Re resulted in good quality images for assessing tumor uptake, and, with few exceptions, tumor uptake of ^{186}Re immunoconjugate paralleled that of the ^{99m}Tc immunoconjugates.

The incidence of HAMA formation and HAMA titers were higher in patients receiving intact NR-LU-10 antibody compared to patients receiving NR-CO-02 F(ab')$_2$. Both the incidence of HAMA formation after NR-CO-02 F(ab')$_2$ and the magnitude of the HAMA titers were influenced by the number of antibody administrations. Sixty-one percent of those patients receiving only a single dose of NR-CO-02 F(ab')$_2$ formed HAMA, whereas 86% of those receiving two doses formed HAMA. The mean antibody titer was higher after two doses (Fig. 6).

Tumor responses in these Phase I studies in patients with advanced, refractory tumors were not expected following the delivery of less than 2000 rads to tumors, even though animal models have shown that doses to tumor of 2000–3000 rads can cure nude mice of small (18 mm^3) tumors (*2*). A definite anti-tumor response occurred in one patient in this Phase I trial (Fig. 5). Prominent activity was seen in hepatic metastases on this patient's images as early as 3 hr. Dosimetry was obtained in four tumors. One tumor received 2100 rads following the first infusion and 700 rads following the second infusion. Three other tumors received 700, 500 and 700 rads, respectively, average 4.4 rads/mCi. Dose to whole liver mass including tumors following each injection was 2.9 and 2.4 rads/mCi, respectively. With radioimmunotherapy, it appears that 500–2100 rads delivered by an isotope conjugated to a tumor-reactive Mab may be sufficient to result in a radiation-mediated tumor response. We cannot exclude the possibility that in this uniquely responding patient, other mechanisms, such as complement-mediated or antibody-dependent cellular cytotoxicity may have contributed to tumor response. We did investigate whether the response might be related to the intra-arterial route of administration, but in none of the four other patients treated intra-arterially did we observe an objective tumor response. The fall in serum CEA in nine additional patients is also consistent with a biologic effect of the immunoconjugate upon tumors, but one that is too small to be sustained or detected applying standard criteria.

The therapeutic ratio of radiolabeled immunoconjugates could be increased by decreasing hematopoietic toxicity. Reinfusion of cryopreserved autologous marrow or concurrent administration of hematopoietic growth factors (GM-CSF, G-CSF, IL-3, etc.) may permit marrow recovery after doses of radioimmunoconjugates substantially higher than the MTDs identified here. Dose fractionation (*37*) requiring suppression of the HAMA response, by use of less immunogenic, chimeric antibodies (*38,39*) or by concurrent administration of immunosuppressive medications, e.g., cyclosporin A (*40*) may also improve the therapeutic ratio. Hematological toxicity might be decreased by removal of circulating, non-tumor localized antibody by extracorporeal immunoabsorption (*41*) or administration of an "anti-antibody" to increase serum clearance of non-localized antibody (*42,43*).

We administered a relatively small, constant antibody dose, approximately 40 mg in order to conserve antibody. Larger doses with higher antibody mass might improve tumor penetration (*9*). Also multiple doses or treatment of smaller, less refractory tumors might result in detectable tumor responses.

In other studies (*44*), we have shown targeting of NR-LU-10 to small-cell lung cancer, a relatively radiation responsive tumor. We therefore plan to continue the dose escalation of ^{186}Re-NR-LU-10 using cryopreserved autologous marrow re-infusion to reverse marrow toxicity in patients with small-cell lung cancer to continue testing the efficacy of ^{186}Re-NR-LU-10 radioimmunotherapy.

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