Phase II Trial of Yttrium-90-DOTA-Biotin Pretargeted by NR-LU-10 Antibody/Streptavidin in Patients with Metastatic Colon Cancer[1]

Susan J. Knox,[2] Michael L. Goris, Margaret Tempero, Paul L. Weiden, Louis Gentner, Hazel Breitz, Gregory P. Adams, Donald Axworthy, Sally Gaffigan, Kyle Bryan, Darrell R. Fisher, David Colcher, Ivan D. Horak, and Louis M. Weiner

Stanford University School of Medicine, Department of Radiation Oncology [S. J. K.], and Department of Diagnostic Radiology, Division of Nuclear Medicine [M. L. G.], Stanford, California 94305; University of Nebraska Medical Center, UNMC/Eppley Cancer Center [M. T., D. C.], Omaha, Nebraska 68198; Virginia Mason Medical Center, Medical Oncology, Seattle, Washington 98101 [P. L. W., H. B.]; Janssen Research Foundation, Titusville, New Jersey 08560 [L. G., I. D. H.]; Fox Chase Cancer Center, Department of Medical Oncology, Philadelphia, Pennsylvania 19111 [G. P. A., L. M. W.]; NeoRx Corporation, Seattle, Washington 98119 [D. A., S. G., K. B.]; and Pacific Northwest National Laboratory, Richland, Washington 99352 [D. R. F.]

ABSTRACT

A Phase II study of yttrium-90-tetra-azacyclododecanetetra-acetic acid-biotin (^{90}Y-DOTA-biotin) pretargeted by NR-LU-10 antibody/streptavidin (SA) was performed. The primary objectives of the study were to evaluate the efficacy and safety of this therapy in patients with metastatic colon cancer. Twenty-five patients were treated with a single dose of 110 mCi/m^2 (mean administered dose, 106.5 ± 10.3 mCi/m^2) of ^{90}Y-DOTA-biotin. There were three components of the therapy. Patients first received NR-LU-10/SA on day 1. A clearing agent (biotin-galactose-human serum albumin) was administered ~48 h after the NR-LU-10/SA to remove residual circulating unbound NR-LU-10/SA. Lastly, 24 h after administration of clearing agent, patients received biotin-DOTA-labeled with 110 mCi/m^2 ^{90}Y. All three components of the therapy were administered i.v. Both hematological and nonhematological toxicities were observed. Diarrhea was the most frequent grade 4 nonhematological toxicity (16%; with 16% grade 3 diarrhea). Hematological toxicity was less severe with 8% grade 3 and 8% grade 4 neutropenia and 8% grade 3 and 16% grade 4 thrombocytopenia. The overall response rate was 8%. Two partial responders had freedom from progression of 16 weeks. Four patients (16%) had stable disease with freedom from progression of 10–20 weeks. Despite the relatively disappointing results of this study in terms of therapeutic efficacy and toxicity, proof of principle was obtained for the pretargeting approach. In addition, valuable new information was obtained about normal tissue tolerance to low-dose-rate irradiation that will help to provide useful guidelines for future study designs.

INTRODUCTION

Patients with metastatic adenocarcinoma of the colon and rectum are incurable with standard chemotherapy agents, and new therapies are needed for more effective treatment of these diseases. Although encouraging results have been obtained in clinical studies using MABs[3] or RIT for the treatment of patients with recurrent B-cell lymphoma (1), results of clinical RIT trials in solid tumors have been relatively disappointing. RIT trials for solid tumors have resulted in low response rates of shorter duration than observed in B-cell lymphoma (2). The efficacy of RIT in solid tumors using directly labeled MABs has been limited by a number of factors (2) including: (*a*) relatively low level and heterogeneous deposition of MAB in tumors; (*b*) relatively low and heterogeneous distribution of absorbed radiation doses in tumors; and (*c*) radioresistance of many solid tumors, resulting in the inability to deliver sufficient tumor doses with acceptable toxicity.

Pretargeting of RIT is a promising approach that has the potential to increase achievable tumor doses, improve tumor: normal tissue ratios, and therefore to increase the therapeutic index of pretargeted RIT compared with the use of directly labeled MABs. Pretargeting approaches dissociate the delivery of unlabeled antibody from the delivery of the radionuclide. This is potentially advantageous because the antibody that is administered first is not radiolabeled and therefore does not expose normal organs to radiation. The unlabeled antibody localizes in tumor and is then cleared from the circulation with a clearing agent. The radionuclide-hapten complex is then administered that reacts with the pretargeted antibody, thereby concentrating the radionuclide in the tumor. Pretargeting ap-

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[2] To whom requests for reprints should be addressed, at Stanford University Medical Center, Department of Radiation Oncology (A-093), Stanford, CA 94305. Phone: (650) 723-5832; Fax: (650) 725-8231.

[3] The abbreviations used are: MAB, monoclonal antibody; RIT, radioimmunotherapy; SA streptavidin; DOTA, tetra-azacyclododecanetetra-acetic acid; MTD, maximum tolerated dose; GI, gastrointestinal; HAMA, human antimouse antibody; HASA, human antistreptavidin antibody; HACA, human anticonjugate antibody; LAL, *Limulus* amebocyte lysate; MIRD, Medical Internal Radiation Dose; FFP, freedom from progression; PR, partial response.

proaches use either bifunctional antibodies (3–5), a biotin-streptavidin approach, or similar high-affinity ligand systems (6–7).

The optimal timing of administration of the radionuclide is achieved when the tumor:background ratio of unlabeled antibody is maximum. This is optimized by accelerated clearance of unlabeled antibody from circulation using a clearing agent based on the biotin-avidin or streptavidin system (8). Advantages of this system include the small molecular weight of biotin, which can quickly circulate throughout the body, and the high-binding affinity between biotin and avidin or streptavidin (10^{15} M^{-1}). Because the hapten-radionuclide complex is relatively small, it is cleared by the kidneys, and unbound radionuclide is cleared rapidly. Because biotin is a tetravalent molecule, the availability of four binding sites per streptavidin molecule also multiplies radionuclide deposition in tumor. Studies using both two- and three-step approaches have demonstrated that these pretargeting approaches permit the administration of much higher doses of radionuclide with acceptable toxicity than is possible with RIT using directly labeled antibodies (2, 9).

In the Phase II study reported here, a three-step pretargeting approach was used in which streptavidin-conjugated NR-LU-10 MAB was administered and allowed to localize in tumor. Next, a biotin-containing clearing agent was administered, followed by administration of yttrium-90-biotin. The murine MAB NR-LU-10 recognizes a noninternalizing M_r 40,000 glycoprotein antigen (Ep-CAM) expressed on several epithelial tumors, such as carcinomas of the lung, colon, breast, prostate, and ovary (10), as well as on some normal tissues including gastrointestinal epithelium. NR-LU-10 possesses two desirable characteristics for pretargeting: reactivity with a high percentage of tumor cells in a broad range of adenocarcinomas (11), and efficient *in vivo* tumor cell localization in both animal models and in humans (12).

NR-LU-10 linked to SA was administered as a first step and allowed to localize in tumor. Next, biotin-galactose-human serum albumin was administered as the clearing agent. This biotinylated protein, administered when the peak MAB uptake in the tumor had occurred, rapidly and quantitatively complexes circulating NR-LU-10/SA, which is then removed from the circulation by hepatocytes in the liver. Removal of circulating antibody conjugate prior to administration of the radionuclide reduces the amount of circulating antibody by ≥95% (13), which if radiolabeled would be a source of nonspecific dose deposition that would result in additional toxicity. In addition, because the clearing agent is cleared rapidly, it has little opportunity to bind to the tumor-localized NR-LU-10/SA and thereby compromise the binding of the radiolabeled biotin to tumor.

Lastly, the therapeutic radionuclide (third component of therapy) was administered after confirmation of NR-LU-10/SA clearance from the blood. ^{90}Y was selected for therapy because it is a pure β emitter with a half life of 64 h, a maximum energy of 2.28 MeV, an average energy of 0.935 MeV, and a mean range in tissue of ~2.5 mm. The pathlength over which 90% of the emitted energy is absorbed is 5.3 mm (14–16). ^{90}Y was linked to biotin by the linker known as DOTA. Previously, studies have shown that DOTA binds to ^{90}Y with a favorably high level of stability so that leaching (*in vitro*) is minimized (17).

The three steps of this pretargeting regimen (including doses of the components and the timing of their administration) were optimized in patients to maximize tumor:normal tissue ratios (13). A Phase I dose escalation trial was performed using this optimized regimen. The MTD was determined to be 110 mCi/m^2. The dose-limiting toxicity at 140 mCi/m^2 was GI toxicity. Two of three patients experienced grade 4 diarrhea requiring hospitalization and i.v. hydration. Two of four patients at the 120-mCi/m^2 dose level also experienced grade 3/4 GI toxicity. One patient experienced grade 4 diarrhea requiring hospitalization, and another patient experienced grade 3 diarrhea requiring i.v. hydration. The onset of diarrhea occurred between 5 and 14 days after treatment with ^{90}Y. Six subjects were treated at the 110-mCi/m^2 cohort, with one grade 3 diarrhea unrelated to study medication. For this reason, the dose of 110 mCi/m^2 was chosen to be the dose used in the Phase II study (18).

In the Phase II study reported here, patients with metastatic colorectal cancer were treated with 110 mCi/m^2 of ^{90}Y-DOTA-biotin pretargeted by NR-LU-10/SA. The primary objective of the study was to evaluate the efficacy and safety of this therapy in this patient population. Secondary objectives included: evaluation of the duration of tumor responses; time to tumor progression; quality of life; and the incidence and titer of HAMAs, HASAs, and HACAs after a single dose of murine NR-LU-10/SA.

MATERIALS AND METHODS

Patients. Twenty-five patients were treated in the study at four different sites. Patients included 13 men and 12 women, ranging in age from 37 to 74 (mean, 57.6) years. The clinical profile of patients, including their histories of prior therapy, is summarized in Table 1. Before entry in the study, patients met the following eligibility criteria: (*a*) pathologically confirmed colon cancer with metastases; (*b*) age, ≥18 years; (*c*) bidimensionally measurable disease; (*d*) no prior cancer therapy for 4 weeks; (*e*) WBCs ≥3000/mm^3, platelets ≥100,000/mm^3, absolute neutrophil count ≥1500/mm^3; (*f*) creatinine ≤1.5 mg/dl, serum aspartate aminotransferase and serum alanine aminotransferase <3 × upper limit of normal and total bilirubin <1.5 × upper limit of normal; (*g*) baseline Eastern Cooperative Oncology Group performance status 0 or 1; (*h*) no prior high-dose chemotherapy requiring stem cell support or radiation to >25% of the bone marrow; (*i*) no known HIV positivity; (*j*) no concurrent malignancy (except basal cell or T_{0-2} N_0M_0 squamous cell carcinoma of the skin); (*k*) no history of previous malignancy or treatment for any cancer in the last 5 years, except for colon cancer and skin carcinoma; (*l*) HAMA/HASA/HACA results <2 SDs above the geometric mean of a control population with no prior history of MAB administration for imaging or therapy; (*m*) no current treatment with another investigational drug; (*n*) no prior mitomycin C or nitrosoureas; (*o*) at least one prior standard therapy; (*p*) life expectancy >3 months; (*q*) no active and untreated brain metastases; (*r*) no serious illnesses that would, in the opinion of the investigator, preclude the patient from participation or study completion; and (*s*) no administration of Metastron (strontium-90) or Quadramet (sumarium-153) within 12 weeks of trial entry. All patients had prior abdominal/pelvic surgery, and all had failed at least one

Table 1 Patient profile

Patient	Age/Sex	Dosage[a]		Prior chemotherapy[b]	Prior radiation (fields)	Disease sites[c]
		mCi/m^2	Total mCi			
1	53/M	93	186	A	No	L[d]
2	47/M	110	223	A	No	L, P
3	52/F	110	163	E	Yes (pelvis)	L, P
4	74/M	127	248	A	No	P[d], AD[d]
5	51/M	75	159	A	No	L, TA
6	65/M	106	244	A	No	P, TA
7	60/F	111	243	A, C	No	L, P
8	54/M	102	191	A	No	L, AD, AA
9	45/M	110	185	A × 2, C	Yes (pelvis, L2–L4)	P
10	66/F	111	214	A, C	No	L[d], P, AA, S, BO
11	51/F	100	176	A, F	No	L, P
12	59/M	110	214	G, C, A, D	No	L[d], PA
13	73/F	97	169	G, A, H, C	No	L, P, TA[d]
14	37/F	86	150	A	Yes (proximal femur/hip)	L, P, PM
15	55/F	110	176	G, A, F	No	L[d]
16	46/F	104	200	A, F	No	L, AM
17	56/F	114	214	A	Yes (pelvis)	L[d], P, PM, BO
18	63/F	117	182	A, C	Yes (pelvis, PALN)[e]	L[d], AA
19	57/M	109	212	A, G, C	No	L
20	72/F	110	215	A, C, D	No	L[d], P, TA
21	74/F	109	187	A, G	No	L[d]
22	67/M	111	194	A	No	L[d], P
23	52/M	109	219	B	No	PM, AM
24	61/M	108	236	A, C	No	PM, AA
25	50/M	113	193	A, C	No	L, P, PM

[a] Administered activity.
[b] Specific regimens (all are systemic unless otherwise stated; excludes other investigational agents, vaccines, and vitamins): A, 5-fluorouracil (5-FU) + leucovorin; B, 5-FU + levamisole; C, irinotecan; D, mitomycin-C; E, 5-FU; F, oxaliplatin, 5-FU + leucovorin; G, intrahepatic 5-fluro-2-deoxyuridine (FUDR) ± leucovorin ± decadron; H, cyclophosphamide.
[c] L, liver; P, pulmonary; TA, thoracic adenopathy; AA, abdominal adenopathy; PA, pelvic adenopathy; AM, abdominal mass; PM, pelvic mass; S, splenomegaly; AD, adrenal mass; BO, bone (probably underreported because bone scans were not required by the study).
[d] >5.0 cm in at least one dimension.
[e] PALN, para-aortic lymph-nodes.

course of conventional chemotherapy, with most having had two courses of prior therapy. Five patients had received prior radiation therapy. Additional eligibility criteria added after study initiation for safety reasons included: (*a*) no medical history of irritable bowel syndrome; (*b*) no ≥ grade 1 diarrhea at baseline; (*c*) no prior external beam radiotherapy to the pelvis; and (*d*) no uncorrected hypokalemia. In addition, all patients had to agree to comply with the protocol stipulations and sign the informed consent form prior to any pretrial testing. Patients were instructed not to take vitamins containing biotin during the study, and females of child-bearing potential had to use an accepted method of birth control for the duration of the study.

Study Design. This was an open-label, multicenter Phase II trial of a single 110 mCi/m^2 dose of ^{90}Y-DOTA-biotin administered as part of a pretargeting approach using murine NR-LU-10/SA in patients with advanced colon cancer. There were three components of the therapy for this study: murine NR-LU-10/SA, biotin-galactose-human serum albumin (clearing agent), and ^{90}Y-DOTA-biotin (treatment agent). Patients received NR-LU-10/SA (calculated to be 125 μg/ml of plasma volume) on an outpatient basis on day 1. NR-LU-10/SA dosing was based on plasma volume because antibodies initially distribute into a plasma-like volume and because of the observation in Phase I imaging trials that higher sustained serum concentrations of NR-LU-10/SA resulted in higher tumor uptake of radiolabeled -DOTA-biotin. In this study, gender-specific plasma volumes were used for dosing to achieve more consistent initial serum concentrations of NR-LU-10/SA and subsequent concentration-time gradients. Plasma volumes, which directly correlate with body surface area, were calculated and corrected to account for gender differences using the method described by Pearson *et al.* (19). The NR-LU-10/SA was given i.v. in up to 100 ml of saline as a single bolus infusion over 15 min. Patients were then observed for acute toxicities and had vital signs monitored immediately prior to and at 10, 30, and 60 min after administration. Patients were then admitted to the hospital for the administration of clearing agent, i.v. hydration, and subsequent ^{90}Y-DOTA-biotin administration. The clearing agent (biotin-galactose-human serum albumin) was administered ~48 h after the NR-LU-10/SA administration. The dose of the clearing agent was calculated to be 1.04 times the dose of NR-LU-10/SA. The clearing agent was given i.v. in up to 150 ml of saline as a single-bolus injection over a period of 5 min. Patients were observed for acute toxicities and had vital signs monitored immediately prior to and at 10, 30, and 60 min and 2 and 4 h after administration. Patients were hydrated from ~18 h prior to DOTA-biotin administration to ~6 h post-DOTA-Biotin with 1 liter of 5% dextrose in water with one-half normal saline plus 20 mEq KCl plus 10 mg of Furosemide every 6 h as tolerated.

Seventy-two h after administration of NR-LU-10/SA and

24 h after administration of clearing agent, patients received 0.5 mg of ^{90}Y-DOTA-biotin labeled with 110 mCi/m^2 ^{90}Y. The ^{90}Y-DOTA-biotin was given in up to 60 ml of saline as a single, rapid i.v. bolus injection (15–20 s). Patients were again observed for acute toxicities and had vital signs monitored immediately prior to and 10, 30, and 60 min after administration of ^{90}Y-DOTA-biotin and then as clinically indicated. Stools were tested for occult blood when clinically indicated. Patients were discharged when stable and when their level of emitted radiation met revised Nuclear Regulatory Commission release guidelines. They were discharged with Imodium and antiemetics to use at the first sign of any GI toxicity. Any patient experiencing $\geq$ grade 2 diarrhea was seen by a physician, and patients with grade 3 or 4 GI toxicity were referred to a gastroenterologist for evaluation.

Antibody and Radioimmunoconjugate Preparation. ^{90}Y was obtained from the United States Department of Energy's isotope production program at the Pacific Northwest National Laboratory (Richland, Washington). Patients were treated with ^{90}Y-DOTA-Biotin (110 mCi/m^2) pretargeted by NR-LU-10/SA under BB-IND-5247. The NR-LU-10 was conjugated to SA and tested for general safety, sterility, pyrogenicity, polynucleotides, *Mycoplasma*, and adventitious virus contamination. The ^{90}Y-DOTA-biotin was prepared as follows. The ^{90}Y (1.3 $\times$ patient dose in mCi) was buffered with ammonium acetate and mixed. Ascorbic acid (0.05 ml) was then added to the reaction vial and mixed. Ammonium acetate buffer (0.8 ml) was subsequently added to the DOTA-biotin vial and mixed. Next, 0.25 ml of the diluted DOTA-biotin vial was added to the vial and mixed. The shielded ^{90}Y reaction vial was incubated in a water bath at 80°C for 60 min. After removal from the water bath, 0.06 ml from the diethylene triamine-pentaacetic acid vial was added to the reaction vial as a precautionary measure to scavenge any unchelated ^{90}Y. The final dilution for patient administration was prepared by transferring the contents of the ^{90}Y reaction vial into a 30-ml syringe containing 8 ml of PBS and 1.0 ml of ascorbic acid. Flushing of the vial with 15 ml of PBS ensured that the transfer was complete. The entire preparation was filtered through a 0.2 μm filter.

Quality control assays were then performed on a 0.3-ml aliquot from the 30-ml syringe as follows. Three release assays (LAL testing, determination of percentage of binding, and radiochemical purity) were performed on site prior to the release of the ^{90}Y-DOTA-biotin. One safety assay (Relative Biotin Binding) was performed before the patient was injected with ^{90}Y-DOTA-biotin. The LAL gel-clot method is a qualitative test for Gram-negative endotoxin. Gram-negative bacterial endotoxin catalyzes the activation of a proenzyme in the LAL. The initial rate of activation is determined by the concentration of endotoxin present. The activated enzyme (coagulase) hydrolyzes specific bonds within a clotting protein (coagulation) also present in LAL. Once hydrolyzed, the resultant coagulin self-associates and forms a gelatinous clot. This assay was performed using a positive product control for each sample tested. Typically, the assay sensitivity of the LAL kit (BioWhittaker, Inc., Walkersville, MD) ranged between 0.25 and 0.125 endotoxin units/ml, and the ^{90}Y-DOTA-biotin was always less than this level of endotoxin. Determination of the percentage of binding was accomplished by measuring the percentage of binding of SA to biotin in a mixture of sample to its binding partner coated on agarose beads. Using radiolabeled samples, the percentage of the activity associated with the beads compared with the total activity was determined by a gamma counter. Briefly, 0.2 ml of avidin-coated beads were washed twice with 500 μl of PBS and reconstituted with 500 μl of PBS. The patient preparation (10 μl of a 1:1000 dilution) was added to each of two Microfilterfuge tubes (one with the beads and one with 500 μl of PBS only), incubated for 10 min at room temperature, and centrifuged in a microcentrifuge for 20–30 s. The beads were washed twice with PBS. Then 900 μl of each filtrate were removed into separate test tubes and counted in the gamma counter. The percentage of binding was determined by comparing the cpm of the Microfilterfuge tube section associated with the beads to the total cpm of the sample:

$$\% \text{ binding} = \frac{\text{Total cpm} - \text{free cpm}}{\text{Total cpm}} \times 100$$

Results were adjusted for radiolabeled purity by dividing the percentage of binding by the percentage of instant TLC of the radiolabeled protein, which was determined using an equilibrated Sep Pak C-18 column as follows. A sample of radiolabeled DOTA-biotin diluted in water (0.5 ml containing 300–500 μCi) was placed on the equilibrated Sep Pak C-18 column. The column was washed with 5 ml water, and ^{90}Y that was not DOTA-biotin chelated was collected in the wash. ^{90}Y chelated to DOTA-biotin was eluted with 5 ml of 50% methanol/water. The percentage of ^{90}Y chelated to DOTA-biotin was determined from the ratio of radioactivity in the "wash" *versus* the "eluted" solutions. The protocol required radiochemical purity to be $\geq$90% for patient administration, and the mean purity of the ^{90}Y-DOTA-biotin administered to patients was 98.4 $\pm$ 2.6%. A relative biotin binding assay was performed to determine the biotin binding capacity of SA-conjugated antibodies remaining in circulation after *in vivo* administration of the clearing agent. This assay is used as a safety measure, by determining the amount of biotin capable of binding to SA conjugate in patient serum prior to the administration of radiolabeled biotin derivatives. The relative biotin binding (percentage bound) was calculated as [1 −(mean unbound/mean total counts)] $\times$ 100%. For patient administration, it was required that binding for 40 μg/ml control be >80% and that the patient serum contain <5 μg/ml of biotin binding conjugate. For the patients treated in this study, their serum contained a mean level ($\pm$SD) of biotin binding conjugate of 1.4 $\pm$ 1.6 μg/ml, with a range of 0–4.5 μg/ml, and no detectable biotin binding conjugate in the serum of 10 patients after administration of the clearing agent, prior to administration of the ^{90}Y-DOTA-biotin. This represented clearance of $\geq$95% of the circulating antibody after clearing agent administration.

Dosimetry. Dosimetric studies were not required by the protocol and were not routinely performed in this Phase II study. However, in three patients treated at Virginia Mason Medical Center, dosimetry studies were performed using ^{111}In-DOTA-biotin, as described previously (20), in which absorbed radiation doses were estimated for normal organs and tissues, the whole body, and for tumor masses using methods that are consistent with those recommended by the MIRD Committee of The

Society of Nuclear Medicine (21–23). These methods account for both the penetrating gamma and the nonpenetrating β radiation emitted by radioactivity distributed throughout the body. Dosimetry calculations were based on gamma-camera measurements of ^{111}In-labeled-biotin in the major source organs, tumors, blood serum, and in the total body at various times after administration using methodology described previously (20, 24–26). These calculations were used to estimate the range of doses to normal organs and tissues delivered by ^{90}Y-DOTA-biotin at the MTD in these patients to try to better understand the observed toxicity.

The *S* values (the absorbed dose per unit cumulated activity in cGy per μCi-h, or Gy per Becquerel-s) used for these calculations were the same as those that were used previously in the International Commission on Radiological Protection Publication 30 (27) and implemented in MIRDOSE2 computer software (Oak Ridge Associated Universities, Oak Ridge, TN). *S* values for tumors were estimated by extrapolation using normal organs of similar size and location in the body. Published *S* values are not available for mucosal tissue of the small and large intestines. Therefore, we calculated the *S* values from first principles using a mathematical model of the intestinal wall, mucosa of the wall, and lumen (bowel contents). These *S* values were calculated (28) for ^{90}Y activity deposited in the mucosa, the wall, or lumen using a Monte Carlo code (EGS4, Stanford Linear Accelerator, Palo Alto, CA). The small and large intestines were modeled as parallel-packed cylinders (>30 cm length) for these calculations. We assumed a wall thickness for small intestine of 0.35 cm, which includes a mucosa of 0.06-cm thickness. We assumed a small intestine luminal diameter of 0.57 mm. Radiation absorbed doses to mucosa were then obtained by multiplying the calculated *S* values by the cumulated activities, $\tilde{A}$, that were obtained from ^{111}In gamma camera measurements for ^{90}Y in the small or large intestines.

Clinical Parameters Monitored. After treatment, a number of parameters were followed. These included blood counts, chemistry panels, thyroid function tests, pancreatic enzyme levels, urine analyses, carcinoembryonic antigen levels, and HAMA/HASA/HACA tests. Patients were seen for follow-up examinations at least every 2 weeks for the first 3 months and then monthly until disease progression was documented. The exact timing of these visits was determined in part by the patient's clinical status. A toxicity assessment, with special emphasis on gastrointestinal toxicity, was performed three times/week for 3 weeks after ^{90}Y administration. An electrocardiogram was performed at baseline and at the time patients went off study. The European Organization for Research and Treatment of Cancer Quality of Life assessment was performed on day 1, weeks 4, 8, and 12, and then every 3 months as long as the patients remained on study. Restaging was performed at weeks 4–6 and 8–10 after treatment and then every 2 months until progression and was based on physical examination and a variety of radiographic studies including chest, abdominal, and pelvic computed tomography scans. Standard response criteria were used and defined as follows: a complete response was defined as disappearance of all clinical evidence of tumor by physical examination, roentgenography, and computed tomography scans for a minimum of 4 weeks. A PR was a 50% or greater decrease in the sum of the product of the diameters of the measurable sentinel lesions for a minimum of 4 weeks without any increase in size of other lesions and the appearance of no new lesions. Stable disease was any change in the size of the sentinel lesions not meeting the criteria of a complete response or PR or progression. Progressive disease was a 25% or greater increase in the sum of the product of the diameters of the measurable sentinel lesions and/or the appearance of a new lesion. The duration of response was the number of days between the first documentation of a PR or complete response and the first documentation of progression of disease.

HAMA/HASA/HACA Response. Patients were monitored for the production of HAMA, HASA, and HACA. Antiglobulin levels were measured in patient sera using an ELISA as described previously (29). Briefly, streptavidin, NR-LU-10, or NR-LU-10/SA was used as a capture antigen for HASA, HAMA, and HACA, respectively. In each case, antigen was coated on 96-well polyvinyl microtiter plates (Falcon Plastics, Oxnard, CA) in PBS (Sigma Chemical, St. Louis, MO). Patient sera was added in 4-fold dilutions to wells in PBS containing 0.5% Tween and 4% chicken serum (PCT buffer). After washing unbound sera components, peroxidase-labeled goat antihuman (heavy and light chain) antibody was added in PCT for each of the three assays. After additional washes, the chromogen substrate, 2,2′ azino-bis-3-ethylbenzothiozoline-6-sulfonic acid, was added, and color development was monitored spectrophotometrically. Relative reactivity was determined by measuring the HASA, HAMA, and HACA immune response relative to a pooled serum source of untreated normal individuals. To be considered a positive response, posttreatment levels needed to be at least 2-fold higher than pretreatment levels.

RESULTS

Clinical Responses. The clinical responses are summarized in Table 2. The actual dose administered is shown for each patient, as well as the subsequent best response and FFP in months. The response rate was 8% with two PRs in patients 20 and 21 with FFP of 16 weeks. Four patients (16%) had stable disease with FFP of 10–20 weeks, as determined by tumor measurements made at the study sites.

Toxicity. The nonhematological toxicity observed after administration of ^{90}Y-DOTA-biotin is summarized in Table 3. Diarrhea was the most frequent grade 4 toxicity. Twenty-two of the 25 patients (88%) had diarrhea of ≥grade 1 toxicity, 4 patients (16%) had grade 3, and 4 patients (16%) had grade 4 toxicity. Of the patients with grade 3 or 4 diarrhea, 50% had received prior radiation therapy to a field including bowel. Of the two patients with grade 4 diarrhea that had not received prior radiation therapy, one had a history of diverticulitis and one had an underlying partial small bowel obstruction. One patient with severe diarrhea, dehydration, and hypokalemia died of a cardiac arrest after refusing hospitalization for 2 consecutive days immediately prior to her death. Although autopsy revealed severe underlying cardiac disease, it is possible that diarrhea with hypokalemia contributed to this death. Nausea and vomiting occurred in 76% and 60% of patients, respectively, with all but one patient experiencing only grade 1 or 2 toxicity. Fatigue (grades 1–3) occurred in 84% of patients, with 68% of patients with grade 1 or 2 toxicity. Anorexia also occurred in 68% of

Table 2 Clinical responses

Patient	Dosage Total mCi	Best response[a]	FFP (weeks)
1	186	Pd	6
2	223	Pd	6
3	163	Sd	20
4	248	Pd	6
5	159	Pd	6
6	244	Pd	9
7	243	Sd	14
8	191	Pd	5
9	185	Pd	10
10	214	Pd[b]	0
11	176	Pd	7
12	214	Pd	0
13	169	Pd	7
14	150	Sd	12
15	176	Pd	7
16	200	Sd[c]	10
17	214	Pd	7
18	182	Pd	9
19	212	Pd	8
20	215	PR	16
21	187	PR	16
22	194	Pd	6
23	219	Pd	6
24	236	Pd	7
25	193	Pd	7

[a] Pd, progressive disease; Sd, stable disease.
[b] Expired 2 weeks after treatment (grade 5 toxicity).
[c] Minor response with >25% but <50% tumor shrinkage.

Table 3 No. of patients with acute nonhematological toxicity by grade

	Grade 1	Grade 2	Grade 3	Grade 4
Symptoms				
Nausea	11	7	0	1
Cough	3	0	3	0
Diarrhea	4	10	4	4 (1 grade 5)
Vomiting	8	6	0	1
Tachycardia	1	3	0	0
Anorexia	6	8	1	2
Fever	0	4	1	0
Fatigue	8	9	4	0
Dyspnea	0	0	3	0
Dehydration	2	1	0	2
Headache	1	2	0	0
Taste changes	3	3	0	0
Stomatitis	0	1	0	1
Edema/ascites	0	0	1	0
Back pain	2	0	0	0
Abdominal cramping	4	0	0	1
Abdominal pain	1	2	0	1
Laboratory abnormalities[a]				
Elev. BUN[b]	4	0	0	0
Elev. Creatinine[c]	2	2	0	1[d]
Hypokalemia	2	8	3	1 (grade 5)[e]
LDH[f]	0	0	0	0
Alkaline phosphatase	12	4	4	0
SGOT[g]	11	3	2	0
SGPT[h]	8	3	1	0
GGT[i]	10	2	2	0
Bilirubin	2	1	2	3[j]

[a] Exclusive of baseline elevations (e.g., preexisting grade 1 toxicity).
[b] Blood urea nitrogen.
[c] Acute toxicity only [excludes two patients (no. 2 and 3) with elevated creatinine 7–8 months after treatment].
[d] Unrelated to study drug.
[e] Patient 10.
[f] Lactate dehydrogenase.
[g] Serum aspartate aminotransferase.
[h] Serum alanine aminotransferase.
[i] Gamma glutamyl transferase.
[j] Patients 5, 14, and 23.

Table 4 No. of patients with hematological toxicity by grade

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Leukopenia	7	8	4	0
Granulocytopenia	6	4	2	2[a]
Thrombocytopenia	10	7	2	4[b]
Anemia	1	11	2	0

[a] Mean duration of grade 4 granulocytopenia was 1 week.
[b] Mean duration of grade 4 thrombocytopenia was 2 weeks (range, 1–3 weeks).

patients, with a 56% incidence of grade 1 or 2 toxicity. Other nonhematological toxicities occurred less frequently. The other grade 4 toxicities observed included: two patients with dehydration secondary to diarrhea, nausea, vomiting, and anorexia; one patient with jaundice with elevated creatinine with end-stage hepatorenal syndrome thought to be unrelated to the RIT (patient had progressive disease and post-operative complications after surgery for a bowel obstruction); one patient with stomatitis; and one patient with both abdominal cramping and abdominal pain secondary to small bowel obstruction from progressive disease. The number of patients with elevated liver function tests is shown in Table 3. Grade 3 or 4 abnormalities in alkaline phosphatase, transaminases, and total bilirubin were observed in 32% of patients. In 50% of the patients with grade 3 or 4 elevated liver function tests, these elevations were transient and reversible, and in 50% of patients, these abnormalities persisted and were attributable to progressive disease. Grade 1 or 2 only liver function test toxicities were observed in 60% of patients. Again, these abnormalities were either transient and reversible in 27% of patients or persistent and apparently secondary to progressive disease in 73% of patients. Of concern, two patients (patients 2 and 3) developed significant elevations in serum creatinine levels 7–8 months after treatment to 3.1 and 6.8, respectively, that cannot be explained by progressive disease.

Hematological toxicity was less severe and is summarized in Table 4. The numbers of patient with grades 1–4 leukopenia, granulocytopenia, thrombocytopenia, and anemia are shown. There were more patients with grade 4 thrombocytopenia than grade 4 neutropenia. Mean nadir counts are based on absolute nadir counts or the lowest count that occurred prior to the initiation of granulocyte-colony stimulating factor in three patients or platelet transfusions in three patients. Nadirs generally occurred between 5 and 6 weeks after treatment and usually resolved by approximately 6–8 weeks after treatment (Table 5).

Human Antimouse, SA, and Conjugate Responses. All patients had a positive antibody response to the mouse antibody (HAMA), SA (HASA), and conjugate (HACA), de-

Table 5 Blood count nadirs (mean ± SD)

	Pretreatment counts × 10^3/mm^3	Count	Nadir onset (wk)[a]	Resolution[b]
WBC	7.1 ± 2.6	3.2 ± 1.6	5.8 ± 1.8	8.1 ± 1.6
Platelets	243.2 ± 92.6	77.4 ± 72.7	5.2 ± 1.4	6.8 ± 1.3

[a] Weeks after treatment at which nadir occurred.
[b] Weeks after treatment at which counts had returned to pretreatment levels or to within the normal range.

fined as an increase ≥2 SD above the mean of a control population in measured normal human serum units. Positive antibody responses were observed in 70–80% of patients by 2 weeks after treatment and in all patients by 4–5 weeks after treatment.

Dosimetry. Three patients treated at Virginia Mason Medical Center on the Phase II study underwent dosimetry studies with estimated doses to the small intestine (standard MIRD calculation), kidney, and bone marrow of 2102 ± 591 cGy, 2864 ± 840 cGy, and 33 ± 8 cGy, respectively. Tumor doses were estimated for two of these patients at 479 cGy (patient 6, lung mass) and 2885 cGy (patient 8, liver lesion). A tumor dose was not calculated for the third patient (patient 7) because of poor imaging of disease in that patient.

DISCUSSION

Pretargeting approaches for RIT of solid tumors provide an opportunity to significantly increase the therapeutic index of pretargeted RIT compared with the use of directly labeled MABs. Unfortunately, the results of the Phase II trial described here are disappointing because of the inability to deliver sufficiently high doses to tumor and because of dose-limiting normal tissue toxicity secondary to reactivity of the antibody with normal tissues. Nevertheless, proof of principle was demonstrated for the pretargeting approach, with clearance of ≥95% of circulating antibody to serum levels <5 μg/ml of biotin bonding conjugate, and useful information was obtained that will allow for improved RIT using similar approaches in the future.

The response rate in the Phase II study was similar to that observed in the Phase I study for patients treated at ≥80 mCi/m^2, with PR rates of 8% (current study) and 9% (Phase I study), respectively. However, only 16% of patients in the Phase II study had stable disease compared with 54% of patients in the Phase I study treated with ≥80 mCi/m^2 ^{90}Y-DOTA-biotin, who achieved either a minor response (2 patients) or stable disease (16 patients). Furthermore, the mean FFP for responses was shorter in the current study. The discrepancy in responses between the two studies may be attributable in part to the histology and associated natural history of the disease types studied, because only 22% of the patients in the Phase I study had colorectal cancer (29% had prostate cancer and 22% had ovarian cancer, with the remaining 27% comprised of breast, kidney, lung, cervix, and endometrial cancers).

The incidence and severity of GI and hematological toxicity at the MTD, determined by the previous Phase I trial (18), were surprising and not predicted by the results of that study. In the Phase I trial, 40 patients with advanced adenocarcinoma of a variety of sites were treated in a dose escalation study (dose increments 5–20 mCi/m^2), with total doses ranging from 25 to 140 mCi/m^2 ^{90}Y-DOTA-biotin. In an interim analysis, grade 3/4 diarrhea, nausea/vomiting, thrombocytopenia, and neutropenia occurred in 4, 3, 7, and 4 of 40 patients, respectively. The dose-limiting toxicity in this Phase I trial was diarrhea at a dose level of 140 mCi/m^2 ^{90}Y. The reason for the discrepancy in the toxicity results of the Phases I and II studies is unclear but could be attributable in part to small numbers of patients at each dose level in the Phase I trial as well as to the heterogenicity of tumor types in the Phase I trial as compared with the Phase II trial. The patients in the Phase II study were not more unfavorable than those treated in the Phase I study in terms of the extent of prior myelosuppressive therapy or the presence of risk factors for GI toxicity (prior abdominal or pelvic radiation therapy and/or laparotomy). The observed renal toxicity was also not predicted by the prior Phase I study, perhaps because there were only small numbers of patients treated at high doses, with many not surviving long enough to develop late toxicities of treatment. Of note, one of the patients treated previously in the Phase I study with 140 mCi/m^2 now has a diminished creatinine clearance. In the Phase II study, the timing of onset of elevated serum creatinine levels is consistent with radiation-induced nephritis (30).

Estimated doses to bowel, kidney, and bone marrow from the Phase I study were 10.6 ± 3.9 cGy/mCi, 11.5 ± 4.2 cGy/mCi, and 0.15 ± 0.06 cGy/mCi, respectively. Similarly, doses estimated for small intestine (standard MIRD calculation), kidney, and bone marrow for three patients in the Phase II study were 2102 ± 591 cGy, 2864 ± 840 cGy, and 33 ± 8 cGy, respectively. Because dosimetry studies were not performed routinely as part of this Phase II study, doses to these normal tissues were also estimated by extrapolation from the Phase I experience. For patients receiving 110 mCi/m^2, assuming a body surface area of 2.0 m^2, the average estimated dose to the kidney and bone marrow could have ranged between 1606 and 3454 (mean, 2530) cGy and 11–55 (mean, 33) cGy, respectively. Using a new model for calculating radiation absorbed dose to intestinal tissues for the three Phase II study patients above (28), Fisher *et al.* (28) have estimated the dose to the wall of the small intestine in the GI tract to be 59 ± 2.0 cGy/mCi (compared with 9.4 ± 2.5 cGy/mCi as predicted by standard MIRD calculations for the small intestine, which assumes that the activity resides in the bowel contents as compared with the tissue itself), with doses for the large intestine slightly >50% of the dose to the small intestine (because of the greater mass of the large intestinal wall). Therefore, these patients in the Phase II study received on average a small intestinal wall dose of 13,334 cGy. This is because of cross-reactivity of the NR-LU-10 antibody with the bowel epithelium and not because of GI excretion of the ^{90}Y-DOTA-biotin. NR-LU-10 also cross-reacted with kidney tubules; therefore kidney doses were secondary to both renal excretion of ^{90}Y as well as to the targeting of bound NR-LU-10/SA by ^{90}Y-DOTA-biotin. Given that conservative estimates of tolerable whole-organ doses from conventional (high-dose rate) radiation therapy are 1500–1700 cGy for kidneys and 4000–4500 cGy for the small bowel (31), the doses to these organs in this study may have greatly exceeded these "tolerable" ranges in many patients. These dose estimates are more than sufficient to explain the observed toxicity. If these

estimates are accurate, it is in fact surprising that more toxicity was not observed and demonstrates the impact of dose rate effects on toxicity. RIT results in continuous exponentially decreasing low-dose-rate radiation. Little has been known about normal organ tolerance to low-dose-rate radiation, and these observations provide new insight into the radiobiology and toxicity of this form of therapy. It is important to emphasize, however, that dose estimates for radioimmunotherapy lack the precision of the dosimetric methodology used to calculate tumor and normal tissue doses from conventional external beam radiation therapy. It is possible that the imprecision associated with estimating doses in this study could have resulted in overestimation of doses to normal tissue, which would affect our interpretation of the findings with regard to expected toxicity as a function of dose rate.

In this study, proof of principle was obtained for the pretargeting approach used with documentation of excellent clearance of circulating antibody. New information about normal tissue tolerance to low-dose-rate irradiation was obtained that will help to provide useful guidelines for future study designs. Clearly, future studies should use antibodies directed to a different antigenic target because reactivity of the NR-LU-10 MAB with normal GI epithelium and collecting tubules in the kidney clearly contributed to toxicity. Ideally, the targeted tumor antigen should have highly restricted expression in normal tissues. Efficacy may be further improved by using less immunogenic agents (*e.g.,* chimeric or humanized MABs) that may allow for multidose fractionated RIT. The tumor-targeting vehicle should be multivalent and highly tumor avid, yet small enough to penetrate into tumors from the vasculature and rapidly clear from normal organs. If this is possible, a formal clearance step may not be necessary. The high-affinity interactions between the tumor-targeting vehicle and the radionuclide should be mediated by nonimmunogenic proteins, again ideally of human origin, and the high-affinity interactions should not involve potential cross-reactivity with host elements, such as endogenous biotin. Pretargeted RIT remains a promising area of clinical investigation that merits further study. Modifications of the pretargeting strategy, such as those described above, will enable pretargeted RIT to achieve its full potential.

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REFERENCES

1. Davis, T., and Knox, S. J. Radioimmunoconjugate therapy of non-Hodgkin's lymphoma. *In:* L. Grossbard (ed.), Monoclonal Antibody-based Therapy of Cancer, pp. 113–136. New York: Marcel Dekker, Inc., 1998.

2. Meredith, R. F., and Knox, S. J. Radioimmunotherapy. *In:* J. E. Tepper and L. L. Gunderson (eds.), Clinical Radiation Oncology, Chapter 15. Edinburgh, Scotland: Churchill-Livingstone, in press, 2000.

3. Stickney, D. R., Slater, J. B., Kirk, G. A., Ahlem, C., Chang, C. H., and Frincke, J. M. Bifunctional antibody: ZCE/CHH indium-111 BLEDTA-IV clinical imaging in colorectal cancer. Antib. Immunoconjug. Radiopharm., *2:* 1–13, 1989.

4. Bosslet, K., Steinstraesser, A., Hermentin, P., Kuhlman, L., Broynck, A., Magerstaedt, M., Seeman, G., Schwarz, A., and Sedlacek, H. H. Generation of bispecific monoclonal antibodies for two-phase radioimmunotherapy. *In*: A. A. Epenetos (ed.), Monoclonal Antibodies: Applications in Clinical Oncology, pp. 167- 178. London: Chapman and Hall, 1991.

5. Chatal, J. F., Faivre-Chauvet, A., and Bardies, M. Bifunctional antibodies for radioimmunotherapy. Hybridoma, *14:* 125–128, 1995.

6. Paganelli, G., Magnani, P., Siccardi, A. G., and Fazio, F. Clinical application of the avidin-biotin system for tumor targeting. *In*: D. M. Goldenberg (ed.), Cancer Therapy with Radiolabeled Antibodies, pp. 239–254. Boca Raton: CRC Press, Inc., 1995.

7. Paganelli, G., Magnani, P., and Fazio, F. Monoclonal antibody pretargeting techniques: the avidin-biotin system in clinical oncology. *In*: A. A. Epenetos (ed.), Monoclonal Antibodies 2: Applications to Clinical Oncology, pp. 171–178. London: Chapman and Hall, 1993.

8. Sinitsyn, V. V., Mamontova, A. G., Checkneva, Y. Y., Shnyra, A. A., and Domogatsky, S. P. Rapid blood clearance of biotinylated IgG after infusion of avidin. J. Nucl. Med., *30:* 66–69, 1989.

9. Breitz, H. B., Weiden, P. L., Fritzberg, A. R., Reno, J. M., and Abrams, P. G. Radiolabeled monoclonal antibodies for localization and treatment of metastatic cancer. *In*: R. K. Oldham (ed.), Principles of Cancer Biotherapy, Ed. 3, pp. 348–368. Dordrecht, the Netherlands: Kluwer Academic Publishers, 1998.

10. Okabe, T., Kaizu, T., Fujisawa, J., Watanabe, J., Kojima, K. Yamashita, T., and Takaku, F. Monoclonal antibodies to surface antigens of small cell carcinoma of the lung. Cancer Res., *44:* 5273–5278, 1984.

11. Pearson, J. W., Sivan, G., Manger, R., Wiltrout, R. H., Morgan, A. C., Jr., and Longo, D. Enhanced therapeutic efficacy of an immunotoxin in combination with chemotherapy against an i.p. human tumor xenograft in athymic mice. Cancer Res., *49:* 4990–4995, 1989.

12. Beaumier, P. L., Venkateasan, P., Vanderheyden, J-L., Burgua, W. D., Kunz, L. L., Fritzberg, A. R., Abrams, P. G., and Morgan, A. C. ^{186} Re radioimmunotherapy of small cell lung carcinoma xenografts in nude mice. Cancer Res., *51:* 676–681, 1991.

13. Breitz, H. B., Weiden, P. L., Beaumier, P. L., Axworthy, D. B., Seiler, C., Su, F-M., Graves, S., Bryan, K., and Reno, J. M. Clinical optimization of pretargeted radioimmunotherapy (PRIT™) with antibody-streptavidin conjugate and ^{90}Y-DOTA-biotin. J. Nucl. Med., in press, 2000.

14. Press, O. W., Eary, J. F., Appelbaum, F. R., and Bernstein, I. D. Radiolabeled antibody therapy of lymphomas. *In*: V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Biological Therapy of Cancer, pp. 1–13. Philadelphia: J. B. Lippincott Company, 1994.

15. Simpkin, D. J., and Mackie, T. R. EGS4 Monte Carlo determination of the β dose kernel in water. Med. Phys. (NY), *17:* 179–186, 1990.

16. Prestwich, W. V., Nunes, J., and Kwok, C. S. Beta dose point kernels for radionuclides of potential use in radioimmunotherapy. J. Nucl. Med., *30:* 1036–1046, 1989.

17. Meares, C. F., Moi, M. K., Diril, H., Kukis, D. L., McCall, M. J., Deshapnde, S. V., DeNardo, S. J., Snook, D., and Epenetos, A. A. Macrocyclic chelates of radiometals for diagnosis and therapy. Br. J. Cancer, *62* (Suppl. X)*:* 21–26, 1990.

18. Murtha, A., Weiden, P., Knox, S. J., Breitz, H., Goris, M., Axworthy, D., Seiler, C., Beaumier, P., Bryan, K., and Reno, J. Phase I dose escalation trial of pretargeted radioimmunotherapy (PRIT) with ^{90}Yttrium. Proc. Am. Soc. Clin. Oncol., *17:* 438a, 1998.

19. Pearson, T. C., Guthrie, D. L., Simpson, J., Chinn, S., Barosi, G., Ferrant, A., Lewis, S. M., and Najean, Y. Interruption of measured red cell mass and plasma volume in adults: Expert Panel on Radionuclides of the International Council for Standardization in Hematology. Br. J. Haematol., *89:* 748–756, 1995.

20. Breitz, H. B., Fisher, D. R., Goris, M. L., Knox, S., Ratliff, B., Murtha, A. D., and Weiden, P. L. Radiation absorbed dose estimation for ^{90}Y-DOTA-biotin with pretargeted NR-LU-10/SA. Cancer Biother. Radiopharm., *14:* 381–395, 1999.

21. International Commission on Radiation Units and Measurements (ICRU). Methods of assessment of absorbed dose in clinical use of radionuclides. ICRU Report No. 32. Washington, DC: International Commission on Radiation Units and Measurements, 1979.

22. National Council on Radiation Protection and Measurements (NCRP). The experimental basis for absorbed dose calculations in medical uses of radionuclides. NCRP Report No. 83, Bethesda, Maryland: National Council on Radiation Protection and Measurements, 1985.

23. Loevinger, R., Budinger, T. F., and Watson, E. E. MIRD primer for absorbed dose calculations. New York: The Society of Nuclear Medicine, Inc., 1988.

24. Fisher, D. R., Badger, C. C., Breitz, H., Eary, J. F., Durham, J. S., Hui, T. E., Hill, R. L., and Nelp, W. B. Internal radiation dosimetry for clinical testing of radiolabeled monoclonal antibodies. Antib. Immunoconjug. Radiopharm., *4:* 655–664, 1991.

25. Siegel, J. A., Wessels, B. W., Watson, E. E., Stabin, M. G., Vriesendorp, H. M., Bradley, E. W., Badger, C. C., Brill, A. B., Kwok, C. S., Stickney, D. R., Eckerman, K. F., Fisher, D. R., Buchsbaum, D. J., and Order, S. E. Bone marrow dosimetry and toxicity for radioimmunotherapy. Antib. Immunoconjug. Radiopharm., *3:* 213–233, 1990.

26. Breitz, H. B., Fisher, D. R., and Wessels, B. W. Marrow toxicity and radiation absorbed dose estimates from rhenium-186-labeled monoclonal antibody. J. Nucl. Med., *39:* 1746–1751, 1998.

27. International Commission on Radiological Protection. Limits for intakes of radionuclides by workers. ICRP Publication 30. Oxford: Pergamon Press, 1979.

28. Fisher, D. R., Rajon, D., Bolch, W. E., and Breitz, H. D. Dosimetry model for radiolabeled antibodies in gastro-intestinal mucosa. Cancer Biother. Radiopharm., *13:* 301, 1998.

29. Breitz, H. B., Weiden, P. L., Vanderheyden, J-L., Appelbaum, J. W., Bjorn, M. J., Fer, M. F., Wolf, S. B., Ratliff, B. A., Seiler C. A., and Foisie, D. C. Clinical experience with Re-186-labeled monoclonal antibodies for radioimmunotherapy: results of Phase I trials. J. Nucl. Med., *33:* 1099–1112, 1992.

30. Rubin, P., Constine, L. S., and Williams, J. P. Late effects of cancer treatment: radiation and drug toxicity. *In:* C. A. Perez and L. W. Brady (eds.), Principles and Practice of Radiation Oncology, Ed. 3, pp. 155–211. New York: Lippincott-Raven, 1998.

31. Hoppe, R. T. The non-Hodgkin's lymphomas: pathology, staging and treatment. Curr. Prob. Cancer, *6:* 364–447, 1987.

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Phase II Trial of Yttrium-90-DOTA-Biotin Pretargeted by NR-LU-10 Antibody/Streptavidin in Patients with Metastatic Colon Cancer

Susan J. Knox, Michael L. Goris, Margaret Tempero, et al.

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