Phase I Trial of ^{131}I-huA33 in Patients with Advanced Colorectal Carcinoma

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Abstract

Purpose: Humanized monoclonal antibody A33 (huA33) targets the A33 antigen which is expressed on 95% of colorectal cancers. A previous study has shown excellent tumor-targeting of iodine-131 labeled huA33 (^{131}I-huA33). Therefore, we did a phase I dose escalation trial of ^{131}I-huA33 radioimmunotherapy.

Experimental Designs: Fifteen patients with pretreated metastatic colorectal carcinoma each received two i.v. doses of ^{131}I-huA33. The first was an outpatient trace-labeled "scout" dose for biodistribution assessment, followed by a second "therapy" dose. Three patients were treated at 20, 30, and 40 mCi/m^2 dose levels, and six patients at 50 mCi/m^2 to define the maximum tolerated dose.

Results: Hematologic toxicity was ^{131}I dose-dependent, with one episode of grade 4 neutropenia and two episodes of grade 3 thrombocytopenia observed at 50 mCi/m^2. The maximum tolerated dose was determined to be 40 mCi/m^2. There were no acute infusion-related adverse events, and gastrointestinal toxicity was not observed despite uptake of ^{131}I-huA33 in bowel. Seven patients developed pruritus or rash, which was not related to ^{131}I dose. There was excellent tumor-targeting of ^{131}I-huA33 shown in all patients. The serum T1/2β of ^{131}I-huA33 was (mean $\pm$ SD) 135.2 $\pm$ 46.9 hours. The mean absorbed tumor dose was 6.49 $\pm$ 2.47 Gy/GBq. Four patients developed human anti-human antibodies. At restaging, 4 patients had stable disease, whereas 11 patients had progressive disease.

Conclusion: Radioimmunotherapy using ^{131}I-huA33 shows promise in targeting colorectal tumors, and is deliverable at a maximum tolerated dose of 40 mCi/m^2. Further studies of ^{131}I-huA33 in combination with chemotherapy are planned.

Radioimmunotherapy is coming of age as a treatment modality for cancer. Patients with hematologic malignancies have been the first to benefit from treatment with radiolabeled monoclonal antibodies (mAb; refs. 1, 2). Yet, there is much potential for the application of radioimmunotherapy to epithelial malignancies as well. Colorectal cancer is a significant public health problem and is the leading cancer by incidence in Australia and the U.S. (3). Unfortunately, ~50% of all patients develop metastatic disease, for which curative treatment is not yet available (4).

The A33 antigen is a 43 kDa membrane bound glycoprotein present on the basolateral surfaces of normal colon and small bowel epithelial cells in mice and humans (5–7). It is also homogeneously expressed in >95% of human colorectal cancers and ~50% of gastric cancers (5). Approximately 50% of pancreatic cancers show heterogeneous expression. The A33 antigen is not detectably shed into the extracellular space or the bloodstream, in contrast to other tumor antigens such as carcinoembryonic antigen.

The A33 antigen has been sequenced and comprises three structural domains: an extracellular region containing two immunoglobulin-like domains, a hydrophobic transmembrane domain, and a highly polar intracellular tail (6–9). It is therefore regarded as part of a subfamily within the immunoglobulin superfamily, together with the transmembrane proteins CTX (corticothymocyte marker in *Xenopus*), CTM/CTH (mouse and human homologues of CTX) and CAR (Coxsackie and adenovirus receptor; ref. 7). Its structure is consistent with a putative role as a cell adhesion molecule or a novel cell surface receptor.

Previous radioimmunotherapy studies using iodinated murine A33 mAb (muA33) have shown the therapeutic potential of the A33 antigen-antibody system in patients with metastatic

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Received 11/14/04; revised 1/28/05; accepted 1/31/05.

Grant support: National Health and Medical Research Council Program grant nos. 280912 and 98/1905.

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colorectal cancer (10–12). A phase I/II study of treatment doses of ^{131}I-muA33 enrolled 23 patients with advanced unresectable colon cancer. Reversible hematologic toxicity was dose-limiting and the maximum tolerated dose was determined to be 75 mCi/m^2. No significant bowel toxicity was observed. Minor evidence of tumor response was seen, with three patients demonstrating mixed responses to treatment. Another phase I trial examined the effects of escalating doses of ^{125}I-muA33 in patients with advanced colon cancer (12). Twenty-one patients were enrolled and treated with ^{125}I-muA33 at doses ranging from 50 to 350 mCi/m^2. Dose-limiting toxicity was not observed and maximum tolerated dose was therefore not reached. One patient had a mixed tumor response and 12 patients had stable disease. This series of muA33 radioimmunotherapy trials showed safety, tolerability, and specific tumor-targeting in patients with advanced colorectal cancer. Hints of antitumor efficacy were also seen, justifying further clinical studies; however, the development of human anti-mouse antibodies in all patients prevented repeat treatment. In order to reduce immunogenicity, a CDR grafted, humanized mAb (huA33) was therefore developed (13). Clinical trials using multiple dose schedules of huA33 have shown safety and possible efficacy of huA33 mAb alone and combined with chemotherapy (14, 15).

As a prelude to ^{131}I-huA33 radioimmunotherapy, a phase I pharmacokinetic and tumor-targeting study accrued patients with colorectal carcinoma who were scheduled for surgical resection of one or more tumors. One week prior to surgery, patients were infused with ^{131}I and ^{125}I labeled huA33. Surgical tumor biopsies were analyzed for ^{131}I-huA33 uptake and microdistribution. Favorable biodistribution and tumor-targeting data were obtained,[4] thus a phase I trial using radioimmunotherapy doses of ^{131}I-huA33 was conducted and is reported here.

Patients and Methods

Patients. Patients with metastatic colorectal cancer who had received at least one line of chemotherapy for metastatic disease were eligible for this trial if they fulfilled all of the following inclusion criteria: histologically proven colorectal cancer; measurable or evaluable disease, Karnofsky performance status ≥70%; expected survival ≥4 months; serum creatinine <0.15 mmol/L; serum bilirubin <34 μmol/L; neutrophil count >1.5 × 10^9/L; platelet count >150 × 10^9/L; prothrombin time <1.3 × control; age ≥18 years; ability to give written informed consent. Patients who fulfilled any of the following exclusion criteria were ineligible for study treatment: active central nervous system metastases; exposure to chemotherapy, radiotherapy or immunotherapy within 4 weeks of study entry; not fully recovered from surgery; metastatic disease involving >50% of liver mass; positive huA33 human anti-human antibodies (HAHA) titer in the context of previous huA33 treatment; serious concurrent illness; pregnancy or lactation; concurrent treatment with systemic corticosteroids/immunosuppressants. All patients gave witnessed written informed consent to participate in this study. The protocol was approved by the Human Research Ethics Committee of the Austin Hospital, Melbourne, Australia.

A phase I dose escalation design was used where only the therapy ^{131}I dose was escalated between dose levels; the huA33 protein dose was held constant at 10 mg/m^2. Three patients were initially treated per ^{131}I dose level. Toxicity was defined as adverse events which were possibly, probably, or definitely related to the study agent, i.e., ^{131}I-huA33. Dose-limiting toxicity was defined as grade 3 nonhematologic toxicity, or grade 4 hematologic toxicity as defined by the National Cancer Institute Common Toxicity Criteria version 2.0, 1999. If dose-limiting toxicity was experienced by any patient at a particular dose level, three more patients were to be accrued to that dose level. The maximum tolerated dose was defined as the ^{131}I dose below that where two or more patients out of six experienced dose-limiting toxicities.

Within 2 weeks of study treatment, pretreatment assessments were done to confirm eligibility. Included were complete medical history and physical examination, documentation of measurable or evaluable tumor by computerized tomography, serum biochemistry, and hematology. A baseline serum sample for HAHA determination was taken in all patients. Assessments of cardiorespiratory function were also done as a baseline for monitoring of possible delayed radiotoxicity.

All patients commenced treatment with an i.v. trace-labeled "scout" dose of ^{131}I-huA33 in order to assess tumor-targeting, dosimetry, and biodistribution. All patients received 5 mg (fixed dose) huA33 conjugated to 5 to 8 mCi (200-320 MBq) ^{131}I, using an established labeling technique as previously described (16). Each patient was directly observed for evidence of anaphylactoid reactions during the entire 1-hour infusion period and for 1 hour after completion of the infusion. Vital signs were measured once during and immediately following the infusion, and 2, 3, and 4 hours after the completion of infusion. All patients received saturated solution of potassium iodide prior to the scout ^{131}I-huA33 infusion and continued for 3 weeks in total at 10 drops orally thrice daily.

Each patient received an i.v. therapy dose of ^{131}I-huA33 7 ± 2 days following the scout infusion. Patients were hospitalized in a self-contained radiation-shielded room for this dose. All patients received 10 mg/m^2 huA33 conjugated to 20, 30, 40, or 50 mCi/m^2 ^{131}I depending on assigned dose level. Automatic monitoring of blood pressure, pulse, and oxygen saturation was done during this time using a programmable patient monitoring device (Agilent Technologies, Andover, MA). Patients remained confined to this room until emitted radiation at 2 m fell to <25 μSv/hour or <25% of the initial reading. Geiger counter measurements were done daily while patients were hospitalized. All radiation safety precautions were fully compliant with Australian Radiation Protection and Nuclear Safety Agency guidelines and were supervised by the Radiation Safety Officer of the Austin Hospital.

For 6 weeks following the therapy dose, patients were reviewed weekly in order to conduct assessments of adverse events and to determine their relationship, if any, to the study agent. Blood was drawn at each weekly visit for biochemistry, hematology, and HAHA measurement. Clinical and investigational adverse events were recorded in case report forms and were coded according to the Common Toxicity Criteria.

Six weeks after administration of the therapy ^{131}I-huA33 dose, an end of study evaluation for each patient was done. This included clinical assessment of adverse events, as well as clinical and radiological tumor reassessment. Tumor response or progression was defined according to WHO criteria.

In order to detect possible delayed toxicity from ^{131}I-huA33 therapy, all patients were followed, where possible, at 3, 6, and 12 months following completion of the initial study period. Follow up assessments included clinical assessment, biochemical assessment of renal and thyroid function, chest X-rays, respiratory function tests, and gated cardiac blood pool scans.

Pharmacokinetics. Blood samples for analysis of pharmacokinetics of ^{131}I-huA33 (scout infusion) were drawn immediately prior to the infusion (day 0), 5 minutes after completion of ^{131}I-huA33 infusion, and 1, 2, 4, and 20 ± 2 hours (days 2 or 3, 4 or 5, and prior to the therapy infusion). The serum obtained was aliquoted in duplicate and counted in a gamma scintillation counter (Packard Instruments, Melbourne,

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Australia). Triplicate standards prepared from the injected material were counted for ^{131}I at each time point with serum samples to enable calculations to be corrected for the isotope's physical decay. The results of the serum were expressed as the percentage of injected dose per liter (%ID/L) and converted to micrograms per milliliter based on actual protein dose infused. Population pharmacokinetics used normalized %ID/L (expressing subsequent %ID/L values as a percentage of the initial value for each patient) in order to compare clearance patterns between different patients.

Pharmacokinetic calculations were done for serum data using a curve-fitting program (WinNonlin, Pharsight Co., Mountain View, CA). A two-compartment model was used to calculate pharmacokinetic results. The following variables were calculated from the model: C_{max} (maximum serum concentration); AUC (area under the serum concentration curve extrapolated to infinite time); CL (total serum clearance); T1/2α and T1/2β (half lives of the initial and terminal phases of disposition); and V1 (central compartment distribution).

Biodistribution of ^{131}I-huA33. Whole-body gamma camera scans were done 1 to 4 hours post-scout ^{131}I-huA33 infusion, and then on days 1, 2 or 3, and 4 or 5. A standard of known activity was included in the field of view to allow quantitation. Single-photon emission computed tomography imaging of areas of known tumor was done on day 4 or 5. Analysis of biodistribution images was done following the scout ^{131}I-huA33 dose to ensure that no abnormal distribution of ^{131}I-huA33 had occurred prior to administration of the therapy ^{131}I-huA33 dose.

Whole-body clearance and dosimetry of ^{131}I-huA33. Dosimetric analysis was done on the series of gamma camera whole-body planar images acquired in all patients following scout infusion. Whole body, lungs, liver, spleen, kidney, and thyroid regions of interest were defined for each time point on both anterior and posterior images. Organ radioactivity content was estimated from the geometric mean of anterior and posterior regions of interest counts. The counts for each organ were corrected for background using regions of interest drawn adjacent to each organ. Correction for attenuation of individual organs was estimated using an analytic technique as described by Liu et al. (17). Resultant counts were converted to activity using a camera sensitivity factor calculated from a gamma camera standard of known activity which was scanned at the same time.

A time-activity curve was generated from the derived activity of each imaging time point for each organ. The time-activity curves were decay-corrected to time of injection and fitted with a single component exponential clearance expression. These fits were used to determine biological clearance half-life, T_{bio}, and initial fraction of injected activity, A_0, for the whole body and various normal organs. The AUC was analytically determined and expressed as the residence time of the organ and remainder tissue (18). Residence times for the lungs, liver, spleen, kidney, thyroid, and remainder tissue were entered into the MIRDOSE3 computer software program (19) to calculate the estimated radiation absorbed dose to these specific organs per unit of injected activity (mGy/MBq). This value was multiplied by the total activity delivered to the patient to determine the absorbed radiation dose for each organ.

For tumor dosimetry calculations, a region of interest was drawn around all of or part of the tumor and a time-activity curve derived using similar methodology to normal organ dose. Tumor volumes were calculated from computerized tomography scans. Due to prolonged retention of ^{131}I-huA33 in tumor, additional imaging was done up to 28 days following therapy infusion in 5 of 15 patients to more accurately determine tumor uptake and dose. Tumor mean absorbed dose was calculated as follows:

$$\check{D} = \tilde{A}S$$

where $\check{D}$, mean absorbed dose to target; $\tilde{A}$, cumulated activity in target; S, absorbed dose in target per unit cumulated activity.

For ^{131}I, S values were obtained from predefined tables corresponding to the calculated tumor mass [MIRDOSE3, Nodule Module; ref. (19)].

To extrapolate therapy absorbed dose to tumor, the scout absorbed dose was normalized by the injected activity and then multiplied by the therapy injected activity.

Red marrow dosimetry calculations. Calculation of red marrow dose for ^{131}I-huA33 was done using a patient-specific marrow dosimetry methodology (20). (A) Serum clearance calculations of ^{131}I-huA33 were used to calculate serum cumulated activity concentration, and marrow cumulated activity concentration (μCi $-$ h/gm). (B) Whole body cumulated activity concentration (μCi $-$ h/gm) was calculated as previously described. The calculation of whole-body clearance for scout doses for red marrow dose was based on imaging data. As there were fewer imaging time points following the therapy infusion, red marrow–absorbed doses for therapy infusions were calculated using the whole body clearance determined from handheld dose rate monitor readings (UMo LB 123, EG&G Berthold, Germany).

Red marrow absorbed dose (rad/mCi) was then calculated with the formula (ref. 20):

$$\frac{\text{Red marrow absorbed dose (rad/mCi)} = (0.313 \times A + 0.456 \times B)}{\text{injected dose (mCi)}}$$

Biacore HAHA analyses. Blood samples for HAHA assessment were taken prior to each ^{131}I-huA33 infusion, then at weekly intervals until the end-of-study visit (6 weeks after therapy dose). Antibody responses against humanized antibodies (HAHA) induced after treatment of patients with huA33 mAb were analyzed by surface plasmon resonance technology using a Biacore 2000 instrument as previously described (21–23). Patient serum was considered HAHA-positive if the RU value at a serum dilution of 1:100 exceeded a cutoff value, defined as the mean interpatient baseline RU value +3 SD of pretreatment sera at a serum dilution of 1:100, provided that such an increase was absent in the isotype-matched control antibody channel (24).

Results

Fifteen patients were entered and treated according to the study protocol. Table 1 shows patient age, gender, assigned dose level, Karnofsky performance status and site of primary tumor. The median age was 63 (range, 39-77). The male/female ratio was 11:4. Median performance status was 90 (range, 80-100). Sites of metastatic disease ranged from liver, lung, retroperitoneal/mediastinal lymph nodes, pelvic, and peritoneum (Table 2). All patients had received previous therapy for metastatic colorectal cancer (Table 2). The median number of lines of previous chemotherapy was two (range, 1-5). Three patients had been previously exposed to humanized antibodies as part of clinical trials, but there was no evidence of HAHA to huA33 prior to study entry.

Toxicity. Overall, ^{131}I-huA33 was well-tolerated at dose levels up to the maximum tolerated dose. No acute infusion-related toxicities were observed. Hematologic toxicity was observed at all dose levels and increased with increasing dose level. Table 3 shows the maximum grade of hematologic toxicity by Common Toxicity Criteria experienced by each patient. Because of concerns about hematologic toxicity, in particular, thrombocytopenia, the 50 mCi/m^2 dose level was expanded to six patients. There was one episode of hematologic dose-limiting toxicity observed, which was grade 4 neutropenia in patient 14. This patient also experienced grade 3 thrombocytopenia and the longest time to complete hematologic recovery (10 weeks). No episodes of febrile neutropenia or thrombocytopenia requiring platelet transfusion were observed at any dose level.

Unexpected, but clinically significant, cutaneous toxicity was observed in a total of seven patients. The frequency of cutaneous events was similar at all ^{131}I dose levels. Two patients (nos. 7 and 14) experienced grade 3 extensive pruritic, erythematous, urticaria-like rashes requiring treatment with systemic corticosteroids. There was no evidence that the rash was associated with ^{131}I-dose; it was most likely due to A33 protein. In the other patients, symptoms related to rashes were treated with oral antihistamines or no specific treatment. There was no clear relationship between development of rash and/or pruritis and the development of HAHA. Table 4 shows the occurrence of cutaneous events, the presence of HAHA, and ^{131}I-huA33 dose levels. Skin biopsies from patient 7 were taken 3 weeks post-therapy ^{131}I-huA33 infusion, and histologic features were consistent with a nonspecific spongiform dermatitis and further immunostains for immune complexes/complement were negative.

No clinically significant gastrointestinal toxicity was observed, either in the initial study follow-up or in the long-term follow-up phases. Patient 13 experienced a "serum sickness"–like clinical syndrome in association with Biacore evidence of HAHA formation. Symptoms of myalgias, chills, and fatigue were prominent and commenced 1 week following the ^{131}I-huA33 therapy dose. Biodistribution following the scout infusion was normal and excellent tumor-targeting was evident.

Maximum tolerated dose. The maximum tolerated dose was determined to be 40 mCi/m^2 of ^{131}I. This was based on the occurrence of two episodes of dose-limiting toxicity (grade 4 neutropenia and grade 3 liver function test abnormalities) across six patients treated at the 50 mCi/m^2 dose level.

Evaluation of delayed toxicity. Eight patients were available for at least one follow-up assessment. The other seven patients were not available because of death due to tumor progression, other treatment, or withdrawal of consent for further follow-up. One patient developed biochemical hypothyroidism on long-term follow-up in the context of previous treatment with trace ^{131}I-labeled mAb as part of a previous clinical trial. The patient remained clinically euthyroid and oral thyroid hormone replacement was commenced with subsequent improvement in biochemical thyroid function. No significant deterioration in cardiac, respiratory, renal, or liver function attributable to ^{131}I-huA33 was detected on long-term follow-up on available patients.

Pharmacokinetics. Pharmacokinetic analysis of scout infusion data showed consistent results across all dose levels, with T1/2α (mean ± SD) 23.0 ± 12.5 hours and T1/2β 135.2 ± 46.9 hours. The calculated (mean ± SD) C_{max} was 1.40 ± 0.23 mg/L, CL was 0.035 ± 0.010 L/hour, AUC was 144.4 ± 41.8 hour × mg/L, and V1 3.48 ± 0.64 L.

Biodistribution and dosimetry of ^{131}I-huA33: Biodistribution. All patients had normal ^{131}I-huA33 biodistribution following the scout infusion, with initial images demonstrating ^{131}I-huA33 blood pool distribution, followed by tumor uptake and bowel localization (Fig. 1). Colon activity gradually resolved with blood clearance of ^{131}I-huA33. Excellent uptake of ^{131}I-huA33 in tumor was seen in all patients, with persisting high uptake visualized up to 4 weeks after the ^{131}I-huA33 therapy infusion (Fig. 2). Post-therapy images showed essentially identical biodistribution and tumor uptake of ^{131}I-huA33 compared with scout infusions in all patients.

Whole body clearance. Whole body clearance [biologic half-life (T1/2)] of ^{131}I-huA33 scout dose was calculated to be 227.52 ± 46.15 hours (mean ± SD).

Whole body and normal organ dosimetry. The results for individual organs and whole-body doses were similar between patients at the four dose levels. Target organs with the highest doses were lungs, liver, spleen, kidney, and thyroid, consistent with the blood pool appearance on gamma camera images and minor thyroid uptake from free ^{131}I (Fig. 1). Mean ± SD values were for kidney (0.23 ± 0.07 cGy/MBq), liver (0.17 ± 0.06 cGy/MBq), lungs (0.13 ±

Table 1. Baseline patient demographics

Patient no.	Dose level (mCi/m^2)	Therapy ^{131}I dose administered		Age at study entry (years)	Sex	Karnofsky performance status	Site of primary tumor
		(mCi)	(GBq)				
1	20	32.7	1.21	65	M	90	rectum
2	20	34.8	1.29	65	M	100	colon
3	20	37.3	1.38	63	M	90	colon
4	30	55.4	2.05	50	M	90	rectum
5	30	68.0	2.52	63	M	80	colon
6	30	63.4	2.35	39	F	90	rectum
7	40	77.0	2.85	53	M	90	colon
8	40	72.2	2.67	68	M	100	rectum
9	40	69.0	2.55	73	M	100	rectum
10	50	75.5	2.80	67	F	90	colon
11	50	94.7	3.50	56	M	100	rectum
12	50	85.5	3.16	77	M	90	colon
13	50	81.6	3.02	62	F	90	colon
14	50	70.8	2.62	54	F	100	colon
15	50	84.1	3.11	74	M	100	colon

Table 2. Patient treatment history and response to ^{131}I-huA33

Patient no.	Adjuvant radiotherapy	Chemotherapy for metastatic disease	Other treatment for metastatic disease	Sites of tumor at study entry	Tumor response to ^{131}I-huA33
1	yes	5-FU, I, O, R	RFA of liver metastases, huA33	liver, lung, LN	PD
2	no	5-FU	—	liver	SD
3	no	IHA 5-FU, 5-FU	resection of liver metastases, alcohol injection, huA33	liver, lung, LN, sacrum	PD
4	no	O/5-FU, I, R	—	liver, LN, presacral	PD
5	no	5-FU, I	—	liver, lung, LN	PD
6	yes	5-FU, I	—	liver, lung, LN	SD
7	no	5-FU, I	resection of pelvic and left iliac fossa metastases, pelvic radiotherapy	liver, lung, pelvic, LN	SD
8	yes	5-FU, I	resection of liver metastases	liver, lung, LN	PD
9	yes	O/5-FU	Sibrotuzumab	liver	SD
10	no	5-FU, I	right lung lower lobectomy	liver, lung	PD
11	yes	IHA 5-FU, I/5-FU	—	liver, lung	PD
12	no	I	—	liver, abdominal wall	PD
13	no	O/5-FU	—	liver, lung	PD
14	no	O/5-FU	—	liver	PD
15	no	I,O	—	lung, LN, ascites	PD

Abbreviations: I, irinotecan; O, oxaliplatin; R, raltitrexed; IHA, intrahepatic artery; RFA, radiofrequency ablation; sibrotuzumab, clinical trial monoclonal antibody; LN, lymph nodes; PD, progressive disease; SD, stable disease.

0.04 cGy/MBq), spleen (0.26 ± 0.06 cGy/MBq), and thyroid (0.39 ± 0.16 cGy/MBq). The whole-body dose (effective dose equivalent) was calculated to be 0.80 ± 0.11 mSv/MBq (mean ± SD).

Red marrow absorbed dose. The total red marrow radiation absorbed doses was similar between scout and therapy doses across all patients (0.056 ± 0.012 versus 0.054 ± 0.012 cGy/MBq). Total red marrow absorbed dose increased in proportion to therapy dose levels, with the mean ± SD values at the 20 mCi/m^2 dose level (69.00 ± 5.05 cGy), 30 mCi/m^2 dose level (96.68 ± 12.19 cGy), 40 mCi/m^2 dose level (122.46 ± 13.12 cGy), and 50 mCi/m^2 dose level (153.49 ± 46.80 cGy).

Tumor dosimetry. The mean specific absorbed tumor dose across all dose levels was 6.49 ± 2.47 Gy/GBq (mean ± SD). Total tumor dose increased with dose level, and was 19.26 ± 11.68 Gy at the 40 mCi/m^2 dose level (range 12.04-32.73 Gy), and was 21.19 ± 8.17 Gy at the 50 mCi/m^2 dose level (range 11.73-31.81 Gy).

Immunogenicity. There was Biacore evidence of HAHA formation in four patients (nos. 4, 5, 7, and 13). Only one patient had clinical manifestations probably due to HAHA

Table 3. Study agent – related toxicity (maximum Common Toxicity Criteria grade)

Patient no.	Dose level (mCi/m^2)	Hemoglobin	Leukocytes	Neutrophils	Platelets
1	20	0	0	0	1
2	20	0	1	1	0
3	20	0	0	0	1
4	30	0	0	0	1
5	30	0	0	0	2
6	30	0	0	0	1
7	40	0	2	2	1
8	40	0	2	2	0
9	40	0	1	2	2
10	50	0	0	0	1
11	50	0	1	1	2
12	50	0	2	2	3
13	50	0	0	0	0
14	50	0	4	4	3
15	50	0	3	2	2

Table 4. Occurrence of cutaneous events, presence of HAHA, and ^{131}I-huA33 dose level

Patient no.	Dose level (mCi/m^2)	Rash (Common Toxicity Criteria grade)	Pruritus (Common Toxicity Criteria grade)	HAHA
1	20	0	1	no
2	20	0	0	no
3	20	2	2	no
4	30	0	0	yes
5	30	0	2	yes
6	30	0	0	no
7	40	3	2	yes
8	40	0	1	no
9	40	0	0	no
10	50	0	0	no
11	50	0	0	no
12	50	2	2	no
13	50	0	0	yes
14	50	3	3	no
15	50	0	0	no

NOTE: All listed cutaneous events were considered to be related to the study agent.

(see Toxicity). Onset of Biacore-detected HAHA was generally 2 to 3 weeks following the scout ^{131}I-huA33 dose, with peak values within a week of onset. The exception was patient 13, whose HAHA was Biacore-detectable 1 week after the scout dose, peaking 1 week later. Biacore responses gradually declined after peaking, although in almost all affected patients, HAHA was still detectable 7 weeks after the scout infusion.

Tumor response. On WHO computerized tomography-based criteria, 4 patients had stable disease at the end of study visit (6 weeks after ^{131}I-huA33 therapy dose) and 11 patients had progressive disease (Table 1). Patients with more bulky disease tended to develop progressive disease while in the study, compared with patients with relatively low volume tumors who were more likely to have stable disease 6 weeks after treatment. In most cases, patients had clinical symptoms or signs of tumor progression in addition to computerized tomography evidence.

Discussion

This phase I radioimmunotherapy study reinforces our current understanding of the A33-huA33 mAb system and improves our ability to apply this to patients with advanced colorectal cancer. Like muA33, huA33 has been shown to be well-tolerated when given by i.v. infusion. Tumor-targeting and biodistribution with ^{131}I-huA33 is comparable to radiolabeled muA33. HuA33 has a longer serum half-life compared with muA33 with a T1/2β of 135 versus 38.5 hours (10). The incidence of HAHA is clearly less than the frequency of human anti-mouse antibody generated with the murine mAb.

The temporal pattern and magnitude of hematologic toxicity observed was consistent with previous radioimmunotherapy trials, including studies with ^{131}I-muA33 (11). In our study, two episodes of dose-limiting toxicity were observed at 50 mCi/m^2 dose level: one episode of grade 4 neutropenia was clearly due to ^{131}I-huA33, and it was not possible to exclude an acute radiation-induced hepatitis as the cause of the grade 3 liver function test abnormalities in an additional patient. The maximum tolerated dose in the ^{131}I-muA33 study was 75 mCi/m^2, which is somewhat higher than the maximum tolerated dose of 40 mCi/m^2 observed for ^{131}I-huA33. This can be readily explained by the longer half-life of the humanized mAb labeled with a moderately long-lived radioisotope, and the resultant red marrow dose.

Non–infusion-related cutaneous toxicity has not been shown in any of the preceding muA33 or huA33 clinical studies. Thus, the 47% incidence of rash and/or pruritis in this huA33 radioimmunotherapy study was unexpected. The etiology of these symptoms remains unclear; however, they were almost certainly related to the huA33 protein rather than the radioisotope. The lack of correlation between incidence and severity of cutaneous reactions and ^{131}I dose level supports this hypothesis. One might expect the mechanism to relate to HAHA formation, however, there was no correlation between skin toxicity and HAHA based on Biacore data.

Clinically significant gastrointestinal toxicity was not observed in any of the muA33 or huA33 radioimmunotherapy trials. Despite the presence of A33 antigen on normal colonic epithelium, and showed targeting of this in patients based on autoradiography and gamma imaging, clinical sequelae have not been observed. There are two potential reasons for this: the most likely explanation is that the radiation dose given was too low to cause radiation colitis. The other plausible explanation (which may act in tandem with the first) is that the normal physiologic turnover of colonic epithelial cells was protective against effects of radiation-induced apoptosis. This is consistent with the calculation of the average colon elimination (k_{30}) half-life of ^{131}I-huA33 to be 32.4 $\pm$ 8.1 hours from a previous biopsy-based huA33 trial.[4] The selection of ^{131}I rather than other β emitters (e.g., ^{90}Y or ^{177}Lu) labeled to huA33 was based

in part on the bowel uptake of huA33, and our concern regarding potential bowel toxicity such as has been reported with other ^{90}Y-labeled mAbs that also target an antigen expressed in normal bowel (25). The choice of ^{131}I has been vindicated by the absence of clinically significant bowel toxicity in this trial. The lack of bowel toxicity, combined with the excellent uptake in tumor of ^{131}I-huA33, indicates that radioimmunotherapy with huA33 is a highly attractive strategy for therapy of colorectal cancer.

Hypothyroidism is a known potential complication of ^{131}I-based radioimmunotherapy (26). The standard preventive procedure of oral iodine administration has been shown to reduce thyroid uptake of ^{131}I to <1% of the injected activity in patients receiving radioimmunotherapy with ^{131}I-labeled mAbs (27). Interestingly, the only patient to become biochemically hypothyroid only received 5.2 Gy to the thyroid gland—well below the mean experienced by the study population. It is possible that preexisting mild thyroid dysfunction at study entry progressed despite seemingly adequate oral iodine blockade.

In this study, four patients had stable disease at restaging in response to ^{131}I-huA33, all of whom had previously received two or fewer agents for advanced colon cancer. Eleven patients had progressive disease at restaging, of whom eight patients had previously received two or fewer agents for advanced colon cancer. Although this might suggest an inverse relationship between the number of prior therapies and response to ^{131}I-huA33, the numbers are too small to draw any definitive conclusions.

Other ^{131}I labeled humanized mAbs have been tested in trials and reported in humans with colorectal cancer. hMN-14 is a humanized mAb targeting carcinoembryonic antigen (28) and a phase II radioimmunotherapy trial using ^{131}I-hMN-14 has been recently reported (29). A dose of 60 mCi/m^2 was studied in 30 patients with small volume metastatic colorectal cancer. Moderate and reversible myelosuppression was the dominant toxicity, although one patient developed grade 4 thrombocytopenia. The pattern of myelotoxicity reported in this ^{131}I-hMN-14 trial was very consistent with that observed in our ^{131}I-huA33 radioimmunotherapy study. Normal organ and tumor dosimetry for ^{131}I-hMN-14 has been shown to be similar to that for ^{131}I-huA33, considering the differences in mAb specificity and radioisotope dose (28, 29). The prolonged retention of ^{131}I-huA33 in tumor (at least 4 weeks) is unique, however, and is likely due to the cellular location of the A33 antigen in tumor cells and lack of trafficking of A33

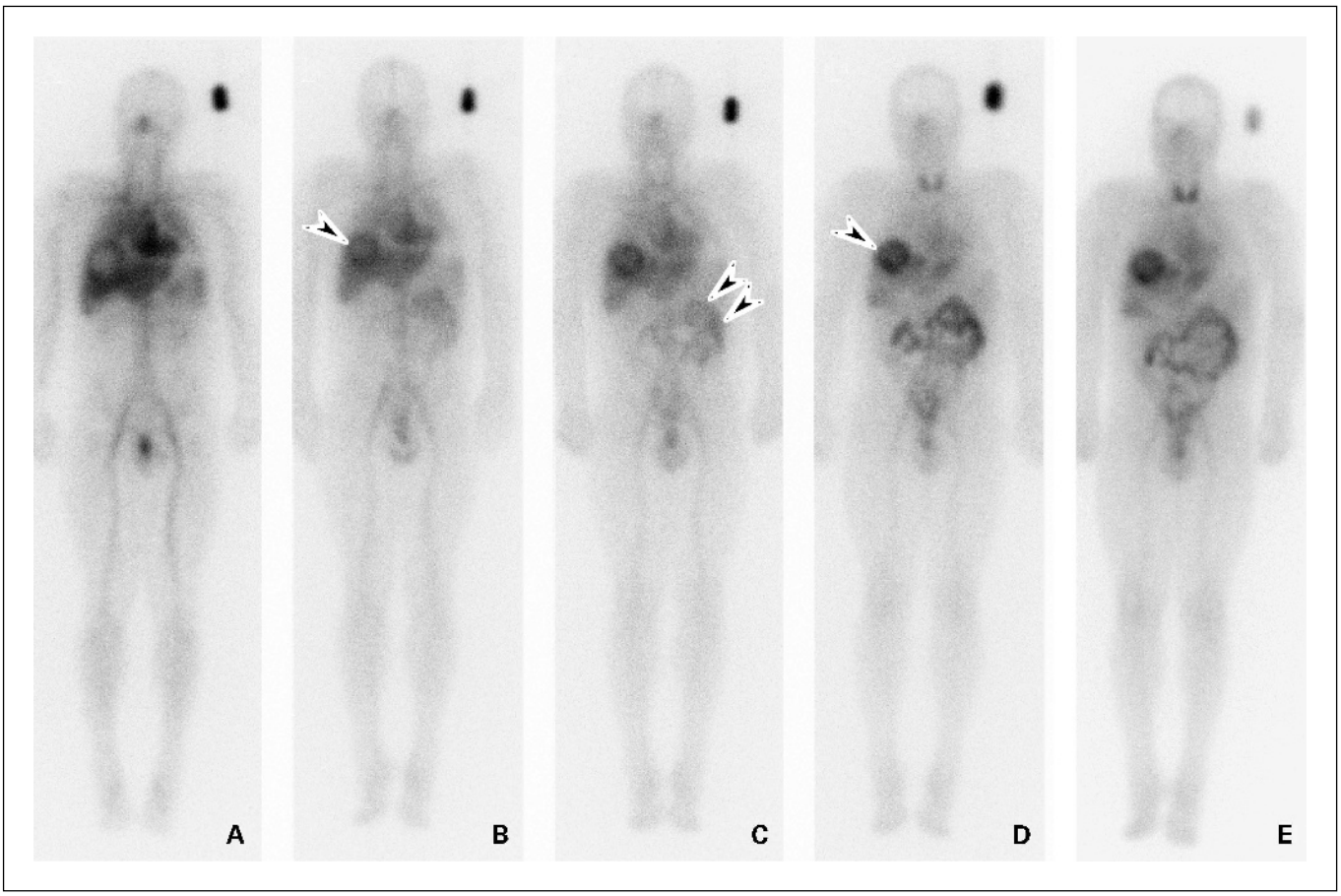

Fig. 1. Anterior whole body gamma camera images following infusion of ^{131}I-huA33 in patient 4 (30 mCi/m^2 dose level) are shown: (*A*) day 0, (*B*) day 1, (*C*) day 2, and (*D*) day 5 post-scout infusion, and (*E*) day 6 post-therapy infusion. A standard for quantitation of ^{131}I-huA33 uptake is present adjacent to the left shoulder. *A*, Initial (day 0) images show blood pool appearance only, with a metastatic lesion in the liver demonstrating an initial hypovascular appearance. *B*, excellent targeting of the metastatic lesion in the liver by ^{131}I-huA33 is clearly seen (*arrow*), as early as day 1, and (*C-D*) increasing rapidly with time. *D*, some central necrosis in the tumor is also evident (*arrow*). Gradual bowel uptake (*double arrow*) of ^{131}I-huA33 is also seen. *E*, the post-therapy image shows identical biodistribution and tumor uptake of ^{131}I-huA33 compared to the scout dose. *F*, computerized tomography scan of the metastatic lesion in the liver in this patient.

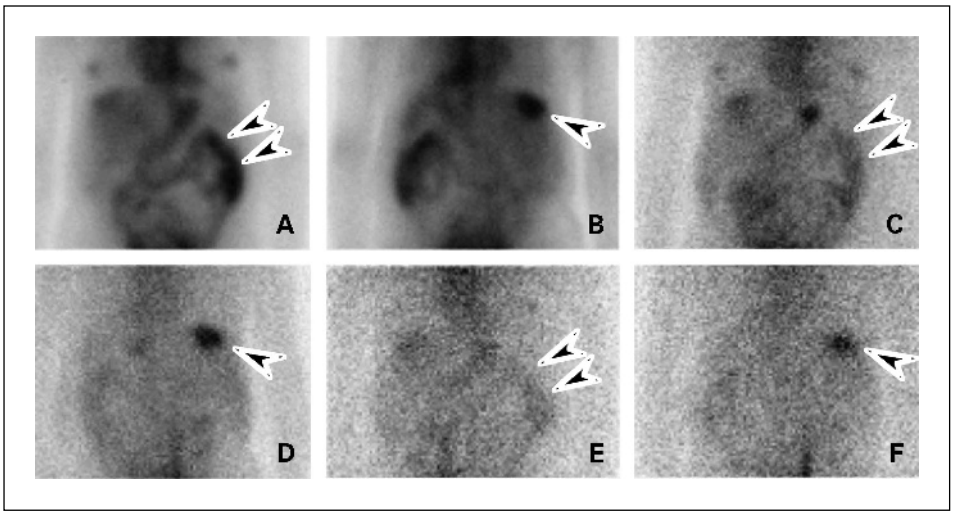

Fig. 2. Prolonged tumor localization following therapy ^{131}I-huA33 infusion in patient 12 (50 mCi/m^2). Gamma camera images of the upper abdomen (*A*) anterior and (*B*) posterior (day 7 post-therapy infusion); (*C*) anterior (*D*) posterior (day 21 post-therapy infusion); (*E*) anterior and (*F*) posterior (day 28 post-therapy infusion) are shown. Excellent uptake of ^{131}I-huA33 in a metastatic lesion in the posterior right lobe of liver (*arrow*) is shown up to day 28 post-therapy infusion. Bowel activity (*double arrow*) reduces with time, in contrast to the prolonged tumor retention of ^{131}I-huA33.

antigen/antibody complex to intracellular lysosomes. Our estimates of tumor retention and dose are therefore likely to be underestimates of the true radiation dose to tumor with this therapeutic approach.

Radioimmunotherapy has yet to establish itself as a standard part of therapeutic strategy for epithelial malignancies. In this respect, its application is not nearly as advanced as radioimmunotherapy for hematologic malignancies. The role of ^{131}I-huA33 mAb is also not as yet clearly defined, despite clear evidence of antitumor efficacy in animal models, and the excellent tumor-targeting properties and stable disease in 4 of 15 patients shown in this trial. The dose to tumor at maximum tolerated dose in this trial ranged from 12 to 33 Gy, indicating that a single dose of ^{131}I-huA33 may not be sufficient to achieve tumoricidal effects alone. Improved dose to tumor could be achieved with higher LET β emitters (e.g., ^{90}Y or ^{177}Lu); however, the problem of potential bowel toxicity will require careful evaluation in future trials.

Combined modality therapy is a currently favored route of drug and therapeutic development. Preclinical data have shown enhanced radiation sensitivity of tumor cells pretreated with cytotoxics such as paclitaxel (30). As a result, the combination of chemotherapy and radiotherapy has become standard treatment for a number of epithelial tumors over the last 10 years. The synergy of trastuzumab with cisplatin or paclitaxel observed in animal models of breast cancer has also translated into clinical practice, and Bevacizumab in combination with 5-fluorouracil (5-FU)/folinic acid has shown greater tumor response rates than 5-FU/folinic acid alone (31). There is also animal model data suggesting synergy between ^{131}I-huA33 and 5-FU for colorectal tumors (32), and a trial of ^{90}Y-anti-carcinoembryonic antigen chimeric T84.66 with 5-FU has shown the tolerability of this approach (33). Importantly, an orally bioavailable 5-FU prodrug, capecitabine, has been found to produce similar tumor response rates to i.v.-administered 5-FU, and also has been shown to improve response rates when given in conjunction with irradiation (34). The rationale for the combination of ^{131}I-huA33 radioimmuotherapy and capecitabine is therefore compelling, and we intend to evaluate this therapeutic approach in future trials.

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Clin Cancer Res 2005;11:4818-4826.