A Phase I Trial of Humanized Monoclonal Antibody A33 in Patients with Colorectal Carcinoma: Biodistribution, Pharmacokinetics, and Quantitative Tumor Uptake


Abstract

Purpose: To determine the in vivo characteristics of huA33, a CDR-grafted humanized antibody against the A33 antigen, we have conducted an open-label, dose escalation, biopsy-based phase I trial of huA33 in patients with colorectal carcinoma.

Experimental Design: Patients with colorectal carcinoma were infused with $[^{131}I]huA33$ (400 MBq: 10 mCi) and $[^{125}I]huA33$ (40 MBq: 1 mCi) 1 week before surgery. There were four huA33 dose levels (0.25, 1.0, 5.0, and 10 mg/m$^2$). Adverse events, pharmacokinetics, biodistribution, tumor biopsies, and immune responses to huA33 were evaluated.

Results: There were 12 patients entered into the trial (6 males and 6 females; age range, 39-66 years). No dose-limiting toxicity was observed. The biodistribution of huA33 showed excellent uptake of $[^{131}I]huA33$ in metastatic colorectal carcinoma. Pharmacokinetic analysis showed no significant difference in terminal half-life ($T_{1/2}$) between dose levels (mean ± SD, 86.92 ± 22.12 hours). Modeling of colon uptake of huA33 showed a $T_{1/2}$ of elimination of 32.4 ± 8.1 hours. Quantitative tumor uptake ranged from 2.1 × 10$^{-3}$ to 11.1 × 10$^{-3}$ %ID/g, and tumor/nor- mal tissue and tumor/serum ratios reached as high as 16.3:1 and 4.5:1, respectively. Biosensor analysis detected low-level human anti-human antibody responses in four patients following huA33 infusion.

Conclusions: huA33 shows selective and rapid localization to colorectal carcinoma in vivo and penetrates to the center of large necrotic tumors, and colon elimination half-life of huA33 is equivalent to basal colonocyte turnover. The excellent targeting characteristics of this humanized antibody indicate potential for the targeted therapy of metastatic colorectal cancer in future trials.

Colorectal cancer is one of the most common cancers in the western world and is the second most common cause of cancer-related mortality (1–3). Adjuvant chemotherapy, radiation therapy, and immunotherapy are used in the treatment of primary colorectal carcinoma and its more advanced stages (4–9); however, agents with superior antitumor activity are needed to progress the treatment of colorectal cancer.

The selective targeting of tumors with monoclonal antibodies (mAb) has emerged as an important therapeutic approach in cancer therapy (7). The antigen target is a critical element in the success of this approach, and one of the most promising targets in colorectal cancer is the A33 antigen: a novel glycoprotein with a molecular weight of 43 kDa, with homology to the immunoglobulin superfamily (10–12). A33 consists of two extracellular immunoglobulin domains, a single transmembrane domain, and a short intracellular tail containing four acylation sites proximal to the transmembrane domain (11, 12). Extensive immunohistochemical analysis of malignant and normal tissues has shown that the antigen is homogeneously expressed by >95% of colon cancers and in the normal intestinal mucosa but not in other epithelial tissues (13, 14).

The localization of a murine mAb against the A33 antigen has been studied previously in patients with colorectal carcinoma (15). Phase I trials with $[^{131}I]$ and $[^{125}I]$ murine A33 in colon carcinoma patients showed excellent localization to colorectal cancer and some evidence of tumor response; however, human anti-mouse antibody precluded repeat dosing (15–18). Clinical observations and preclinical data from a radioimmunotherapy model in the nude mouse have also...
shown that the antitumor effects of radiolabeled mAb A33 can be significantly enhanced by chemotherapeutic agents (19, 20).

In view of the long retention time of mAb A33 in tumors, high tumor uptake, and minimal gut toxicity observed in these trials, a humanized version of mAb A33 was constructed to enable repeated dosing without immunogenicity (21). Two phase I trials with huA33 have been conducted, with huA33 alone and huA33 with chemotherapy in patients with colorectal carcinoma (22, 23). Neither of these trials examined the pharmacokinetics of huA33 or the quantitative tumor uptake of huA33. Moreover, the uptake and turnover kinetics in normal bowel of a construct targeting the A33 antigen has not been examined previously. This information is critical in determining the potential therapeutic strategies that can be explored with a humanized construct against this antigen target, in view of the expression of the A33 antigen in this organ. Therefore, to define the targeting characteristics and the serum and colon compartmental kinetics of a humanized form of mAb A33 (huA33), we conducted a phase I, open-label, dose escalation imaging and biopsy-based study of radiolabeled huA33 in patients with colorectal carcinoma, and the results of this study are presented.

Materials and Methods

**huA33 production.** The construction, production, and preclinical testing of the humanized CDR-grafted A33 IgG1 (huA33) have been described previously (21–25). This study was conducted according to the Food and Drug Administration (FDA) regulations and as part of an Investigatory New Drug application to the FDA and was also approved by the Human Research Ethics Committee of the Austin Hospital.

**Trial design.** The trial was an open-label, dose escalation, biopsy-based phase I study. The primary objectives were to establish the safety of i.v. administered radiolabeled huA33 in patients with colorectal carcinoma and to determine the biodistribution, pharmacokinetics, and immunogenicity of huA33 in these patients. A single infusion of huA33, labeled with [131I]huA33 (400 MBq: 10 mCi) and [125I]huA33 (40 MBq: 1 mCi), was administered 7 days before scheduled surgery. Each infusion was administered in 100 mL of 5% human serum albumin/normal saline over a 30-minute period. The uptake of radio-iodine in the thyroid and other organs was blocked by saturated solution of KI 10 drops orally thrice daily, commencing before antibody administration and continuing to the day of surgery. Three patients were entered at each of four dose levels of huA33 (0.25, 1, 5, and 10 mg/m²). Standard Common Toxicity Criteria were used for evaluation of toxicity.

Whole body gamma camera imaging was done on the day of infusion (day 0) and on at least three further occasions up to the day of surgery. Blood samples for pharmacokinetics were obtained before the infusion, immediately after infusion, at 15, 30, 45, and 60 minutes and 2 and 4 hours after infusion, and on three to four further occasions up to the day of surgery.

![Four-compartment model representing the kinetics of huA33. The compartments are (1) serum, (2) tissue, (3) colonic epithelium, and (4) tumor. The rate of uptake by the colonic epithelium is k₁₃, whereas the rate of elimination due to colonocyte turnover is k₃₀.](image)

### Table 1. Patient characteristics and sites of disease

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Karnofsky performance status at study entry</th>
<th>Dose level (mg/m²)</th>
<th>Prior treatment</th>
<th>Sites of disease*</th>
<th>Sites of [131I]huA33 uptake†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63</td>
<td>M</td>
<td>90</td>
<td>0.25</td>
<td>Hemicolecotomy, chemotherapy, hepatic lobectomy, chemotherapy</td>
<td>Liver metastasis</td>
<td>Liver metastasis</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>F</td>
<td>90</td>
<td>0.25</td>
<td>Anterior resection, abdominoperineal resection for recurrence</td>
<td>Lung metastasis</td>
<td>Lung metastasis</td>
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<tr>
<td>3</td>
<td>56</td>
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<td>90</td>
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<td>Liver metastasis</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>M</td>
<td>90</td>
<td>1</td>
<td>Anterior resection, chemotherapy, radiotherapy</td>
<td>Liver metastasis</td>
<td>Liver metastasis</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>F</td>
<td>100</td>
<td>1</td>
<td>Abdominoperineal resection</td>
<td>Liver metastasis</td>
<td>Liver metastasis</td>
</tr>
<tr>
<td>6</td>
<td>66</td>
<td>M</td>
<td>90</td>
<td>1</td>
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<td>Liver metastasis</td>
<td>Liver metastasis</td>
</tr>
<tr>
<td>7</td>
<td>62</td>
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<td>Colon tumor — ¹</td>
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</tr>
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<td>8</td>
<td>48</td>
<td>F</td>
<td>100</td>
<td>5</td>
<td>Hemicolecotomy, chemotherapy</td>
<td>Liver metastasis</td>
<td>Liver metastasis</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>F</td>
<td>100</td>
<td>5</td>
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<td>F</td>
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<td>Hemicolecotomy</td>
<td>Liver metastasis</td>
<td>Liver metastasis</td>
</tr>
<tr>
<td>11</td>
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<td>M</td>
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<td>10</td>
<td>Hemicolecotomy</td>
<td>Liver metastasis</td>
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<td>12</td>
<td>49</td>
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<td>100</td>
<td>10</td>
<td>Hemicolecotomy</td>
<td>Liver metastasis</td>
<td>Liver metastasis</td>
</tr>
</tbody>
</table>

* Determined by CT scan or colonoscopy.
† Sites of disease identified on planar or single photon emission CT imaging.
¹ No specific uptake identified at primary tumor sites (note tumor < 1.5 cm size).
At day 7, patients proceeded to scheduled surgery, where biopsies were obtained to allow assessment of A33 antigen expression, quantitative localization of \(^{[131I]}\)huA33 to tumor, histologic evaluation, and autoradiographic analysis of \(^{[125I]}\)huA33 localization in tumor.

**Patients.** Eligibility criteria for entry into the trial were as follows: patients must have histologically proven colorectal cancer; be candidates for surgery for resection of primary and/or metastatic colorectal cancer or candidates for intrahepatic artery pump insertion for liver metastases of colorectal cancer; no treatment with chemotherapy, radiotherapy, or immunotherapy for 4 weeks before study entry; ambulatory with a Karnofsky performance status of at least 70%; serum creatinine <0.2 mmol/L; serum bilirubin <20 \(\mu\)mol/L; WBC count >1.5 \(\times 10^9\)/L; platelet count >150 \(\times 10^9\)/L; prothrombin time <1.3 times upper limit normal; age >18 and <70 years; and ability to provide informed consent. Exclusion criteria included the following: clinically significant cardiac disease (New York Heart Association class III/IV); serious infection requiring treatment with antibiotics or other serious illness; illness requiring the use of steroids or other anti-inflammatory agents; pregnancy or lactation or risk of becoming pregnant; survival expectancy of <6 weeks; evidence of central nervous system tumor involvement; prior administration of mouse mAb or antibody fragment; and positive human anti-mouse antibody titre.

**Radiolabeling of huA33.** Sterile technique and pyrogen-free glass or plasticware were used in all labeling steps. huA33 was radiolabeled with \(^{131I}\) and \(^{125I}\) as described previously (25). The pooled, radiolabeled huA33 was measured for total radioactivity and then passed through a 0.2 \(\mu\)m filter before administration. Radiochemical purity, immunoreactivity, and \(in\) \(vivo\) stability of labeled antibodies were analyzed as described previously (25), with a class-matched IgG1 hu3S193 used as a control (26).

**Gamma camera imaging/biodistribution.** Whole body images of \(^{[131I]}\)huA33 biodistribution were obtained in all patients on day 0 after infusion of \(^{[131I]}\)huA33 and on at least three further occasions up to day 7 following infusion. Single photon emission computed tomography (CT) images of a region of the body with known tumor were also obtained on at least one occasion during this period. All gamma camera images were acquired on a dual-headed gamma camera (Trionix Research Laboratories, Twinsburg, OH). Whole body images were done as sweeps in a 1,024 \(\times\) 256–bit matrix, and a standard of known \(^{131I}\) activity was included in the field of view to allow dosimetry calculations.

**Pharmacokinetics.** Serum obtained from patients following infusion of radiolabeled huA33 was aliquoted in duplicate and counted in a gamma scintillation counter (Packard Instruments, Melbourne, Victoria, Australia). Triplicate standards prepared from the injected material were counted for \(^{131I}\) at each time point with serum samples to enable calculations to be corrected for the isotope physical decay. The results of the serum were expressed as % injected dose per liter (%ID/L). Pharmacokinetic calculations were done on serum data using a curve-fitting program (WinNonlin, Pharsight Co., Mountain View, CA). A two-compartment model was used to calculate pharmacokinetic variables of \(C_{\text{max}}\) (maximum serum concentration), AUC (area under the serum concentration curve extrapolated to infinite time), CL (total serum clearance), \(T_{1/2a}\) and \(T_{1/2b}\) (half-lives of the initial and terminal phases of disposition, respectively), \(A\) and \(B\) (0-time intercepts of the initial and terminal phases of disposition,

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**Fig. 2.** Biodistribution of \(^{[131I]}\)huA33 in patient 4. Anterior (A) and posterior (B) whole body gamma camera image 6 days after infusion. Uptake in metastatic lesions in the posterior right lobe of liver (arrows) is clearly seen. Some uptake in normal colon is also evident. C, single photon emission CT transverse image of the liver. D, corresponding CT scan slice.
Histologic analysis of biopsies and quantitative tumor uptake. Biopsy specimens from patients were obtained on day 7 after the infusion of radiolabeled huA33. Sections of tumor from surgical specimens were obtained from different areas of the tumor (periphery and central), and portions of each site were allocated for histology, autoradiography, and quantitative measurements. Specimens were examined by an anatomical pathologist in all cases.

The uptake of $[^{131}I]$huA33 was calculated based on tumor, normal tissue, and standard counts using a gamma scintillation counter and expressed as % injected dose per gram (%ID/g) of tissue. Dry film and emulsion autoradiography of tumor samples was also done from frozen tissue, and paraffin sections.

Regions of interest were drawn around the bowel on gamma camera images, using regions of interest around bowel on sequential images, with application of background and attenuation correction.

The kinetics of the huA33 antibody was represented by a four-compartment model consisting of serum, normal tissue, colonic epithelium, and tumor compartments (Fig. 1). The normal shedding of colonocytes was modeled as an exponential loss from the colon compartment.

The amount of antibody in the colonic epithelium is given by:

$$q_3(t) = \frac{k_{13}A}{k_{30} - \beta} (e^{-\alpha t} - e^{-\beta t}) + \frac{k_{13}B}{k_{30} - \beta} (e^{-\beta t} - e^{-\beta t_0^i})$$  \hspace{1cm} (A)

where $A, B, \alpha$, and $\beta$ are the usual serum pharmacokinetic variables and $k_{13}$ and $k_{30}$ are the rate of uptake and elimination of antibody by colon, respectively. These were estimated by fitting Eq. A to the amount of protein in colon by nonlinear least squares.

Human anti-human antibody measurement. Measurement of immune responses to huA33 in a patient’s serum was done using a BIACore 2000 biosensor (BIACore AB, Uppsalas, Sweden), and with an isotype-matched control IgG1 (26), using a method described previously (23, 27). Serum for human anti-human antibody (HAHA) measurement was obtained up to 6 months after infusion of huA33.

Statistical analysis. Statistical analysis was done on pharmacokinetic and tumor localization data obtained from all patients. Any differences in tumor localization, or pharmacokinetics of radiolabeled huA33 ($[^{131}I]$huA33) in serum, or whole body clearance, between the four dose levels were assessed by a one-way ANOVA. Any correlation between tumor volume and pharmacokinetics, whole body clearance, or mean tumor uptake of $[^{131}I]$huA33 was done by linear regression analysis. The analysis of uptake and elimination results of huA33 from colon was done using a Kruskal-Wallis rank test.

### Results

**Patients.** Twelve patients (6 males and 6 females) entered and successfully completed the clinical trial. The mean age was 56.9 ± 8.5 years (age range, 39-66 years). The patient demographics, including prior treatment and sites of disease on study entry, are detailed in Table 1. Ten patients had surgery for resection of hepatic metastases or insertion of intrahepatic gamma camera images, using regions of interest around bowel on sequential images, with application of background and attenuation correction.

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### Table 2. Dosage, tumor volume, serum pharmacokinetics, and whole body clearance of $[^{131}I]$huA33

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>BSA (m²)</th>
<th>Dose level (mg/m²)</th>
<th>Dose (mg)</th>
<th>Tumor volume (cm³)</th>
<th>Serum $T_1/2$ (h)</th>
<th>Serum $T_1/2$ (h)</th>
<th>WBC (h)</th>
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<tbody>
<tr>
<td>1</td>
<td>1.90</td>
<td>0.25</td>
<td>0.38</td>
<td>270</td>
<td>8.60</td>
<td>69.38</td>
<td>161.48</td>
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<tr>
<td>2</td>
<td>1.75</td>
<td>0.25</td>
<td>0.42</td>
<td>37</td>
<td>8.27</td>
<td>75.55</td>
<td>143.92</td>
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<tr>
<td>3</td>
<td>2.23</td>
<td>0.25</td>
<td>0.48</td>
<td>48</td>
<td>16.17</td>
<td>82.93</td>
<td>126.70</td>
</tr>
<tr>
<td>4</td>
<td>1.90</td>
<td>1</td>
<td>1.45</td>
<td>132</td>
<td>7.25</td>
<td>63.67</td>
<td>131.22</td>
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<td>1.86</td>
<td>1</td>
<td>1.82</td>
<td>26</td>
<td>16.19</td>
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<td>1</td>
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<td>16.77</td>
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<td>5</td>
<td>6.96</td>
<td>4</td>
<td>17.98</td>
<td>137.72</td>
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<td>5</td>
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<td>1,770</td>
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<td>78.40</td>
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<td>9.60</td>
<td>135</td>
<td>15.26</td>
<td>82.90</td>
<td>126.70</td>
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<td>14.97</td>
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<td>1.86</td>
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<td>12.76</td>
<td>131</td>
<td>7.00</td>
<td>71.08</td>
<td>132.43</td>
</tr>
</tbody>
</table>

* Dose of huA33 infused.

1 Denotes volume of tumor identified and measured on CT scan or pathology (patient 7).

2 Serum pharmacokinetic results from infusion of $[^{131}I]$huA33.

3 WBC, whole body clearance of $[^{131}I]$huA33 ($T_1/2$ biological).
pump, 1 patient had surgery for resection of lung metastases, and 1 patient with known multiple primary colon tumors underwent surgery for a total colectomy. Surgery was done 7 days after infusion of radiolabeled huA33 in all patients. All patients completed the trial successfully.

**Adverse events.** There were no adverse events related to huA33 observed for any patient entered into the trial. No dose-limiting toxicity was observed, and maximum tolerated dose was not reached.

**Radiolabeling and biodistribution of huA33.** The mean radiochemical purity of $[^{131}I]huA33$ and $[^{125}I]huA33$ was $>99\%$ and the mean immunoreactivity was $>65\%$ for all infusions. $[^{131}I]huA33$ and $[^{125}I]huA33$ were physicochemically stable in serum in vivo up to 7 days after infusion, with retention of high radiochemical purity and immunoreactivity (data not shown).

Biodistribution images of $[^{131}I]huA33$ showed blood pool distribution only on day 0. There was distinct uptake of $[^{131}I]huA33$ and $[^{125}I]huA33$ were physicochemically stable in serum in vivo up to 7 days after infusion, with retention of high radiochemical purity and immunoreactivity (data not shown).

Table 3. Mean (SD) serum pharmacokinetic variables of $[^{131}I]huA33$ based on total radioactivity

<table>
<thead>
<tr>
<th>Dose group (mg/m²)</th>
<th>n</th>
<th>$C_{\text{max}}$ (µg/mL)</th>
<th>CL (mL/h)</th>
<th>$T1/2\alpha$ (h)</th>
<th>$T1/2\beta$ (h)</th>
<th>AUC (µg -h/mL)</th>
<th>%AUC3</th>
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<tr>
<td>0.25</td>
<td>3</td>
<td>0.12 (0.04)</td>
<td>49.21 (17.52)</td>
<td>11.01 (4.47)</td>
<td>82.41 (17.50)</td>
<td>9.48 (3.48)</td>
<td>92.72 (2.19)</td>
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<tr>
<td>1.0</td>
<td>3</td>
<td>0.52 (0.15)</td>
<td>46.30 (16.55)</td>
<td>11.74 (4.78)</td>
<td>89.36 (28.60)</td>
<td>39.27 (9.07)</td>
<td>89.67 (6.89)</td>
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<td>5.0</td>
<td>3</td>
<td>1.80 (0.26)</td>
<td>58.38 (20.95)</td>
<td>16.20 (1.54)</td>
<td>99.67 (33.02)</td>
<td>151.23 (46.50)</td>
<td>85.94 (1.42)</td>
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<tr>
<td>10.0</td>
<td>3</td>
<td>4.03 (0.98)</td>
<td>40.36 (0.17)</td>
<td>12.01 (4.37)</td>
<td>76.23 (4.55)</td>
<td>290.84 (63.49)</td>
<td>90.18 (4.02)</td>
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</table>

Pharmacokinetics. The serum clearance data of $[^{131}I]huA33$ are shown in Table 2. The initial and terminal half-lives of huA33 ($T1/\alpha$ and $T1/\beta$) showed no significant differences between dose levels, with a mean $\pm$ SD of 12.74 $\pm$ 4.03 hours ($P = 0.429$) and 86.92 $\pm$ 22.12 hours ($P = 0.667$), respectively. Total serum clearance (CL) also showed no significant differences between dose levels ($P = 0.597$), with a mean $\pm$ SD clearance of 48.56 $\pm$ 12.22 mL/h (Table 3). $C_{\text{max}}$ and AUC increased in a dose-dependent fashion (Table 3). The data fitting yielded two-compartment kinetics, with 86% to 93% of AUC attributable to the elimination phase (Table 3).

**Histologic analysis of biopsies.** Histologic examination of biopsy specimens showed metastatic colorectal carcinoma in all patient biopsy samples, except for patient 7, who had three primary colon tumors identified in a colectomy specimen. A33 expression was uniform (>90%) in colorectal carcinoma tumor cells in all biopsies examined and negative in all normal tissue biopsied, except for normal colon (patient 7). Tumor volumes ranged from 4 to 2,099 cm³ (Table 2).

**Quantitative tumor and normal tissue uptake.** Mean quantitative uptake of $[^{131}I]huA33$ in tumor biopsies ranged from $2.1 \times 10^{-3}$ to $11.1 \times 10^{-3}$ %ID/g tumor tissue (Table 4), and individual biopsies reached as high as $14.2 \times 10^{-3}$ %ID/g (Fig. 3). There was no definite trend observed for localization of $[^{131}I]huA33$ in tumor between dose levels (Fig. 3) and no

Table 4. Analysis of $[^{131}I]huA33$ uptake in tumor and normal tissue and relationship to tumor volume

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose level (mg/m²)</th>
<th>Tumor volume (cm³)</th>
<th>Site of biopsy</th>
<th>Tumor uptake ($\times 10^{-5}$ %ID/g)</th>
<th>T:NT ratio</th>
<th>T:S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
<td>270</td>
<td>Liver metastasis</td>
<td>4.7 $\pm$ 1.9</td>
<td>3.7 $\pm$ 1.5</td>
<td>1.9 $\pm$ 0.8</td>
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<tr>
<td>2</td>
<td>0.25</td>
<td>37</td>
<td>Lung metastasis</td>
<td>2.1 $\pm$ 0.6</td>
<td>1.1 $\pm$ 0.3</td>
<td>0.5 $\pm$ 0.1</td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td>48</td>
<td>Liver metastasis</td>
<td>3.5 $\pm$ 1.1</td>
<td>2.5 $\pm$ 0.8</td>
<td>0.8 $\pm$ 0.2</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>132</td>
<td>Liver metastasis</td>
<td>10.0 $\pm$ 3.3</td>
<td>9.1 $\pm$ 3.1</td>
<td>2.4 $\pm$ 0.8</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>26</td>
<td>Liver metastasis</td>
<td>5.0 $\pm$ 1.9</td>
<td>5.4 $\pm$ 2.1</td>
<td>1.5 $\pm$ 0.6</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>2,099</td>
<td>Liver metastasis</td>
<td>2.7 $\pm$ 0.8</td>
<td>6.4 $\pm$ 2.0</td>
<td>2.4 $\pm$ 0.8</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>4</td>
<td>Colon primary</td>
<td>5.9 $\pm$ 1.0</td>
<td>14.4 $\pm$ 2.3</td>
<td>1.0 $\pm$ 0.2</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>1,770</td>
<td>Liver metastasis</td>
<td>3.4 $\pm$ 1.2</td>
<td>5.4 $\pm$ 2.0</td>
<td>1.6 $\pm$ 0.6</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>135</td>
<td>Liver metastasis</td>
<td>4.3 $\pm$ 0.8</td>
<td>5.8 $\pm$ 1.1</td>
<td>1.5 $\pm$ 0.3</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>80</td>
<td>Liver metastasis</td>
<td>2.2 $\pm$ 1.8</td>
<td>2.8 $\pm$ 2.3</td>
<td>0.5 $\pm$ 0.4</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>39</td>
<td>Liver metastasis</td>
<td>11.1 $\pm$ 1.9</td>
<td>14.5 $\pm$ 2.5</td>
<td>3.4 $\pm$ 0.6</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>131</td>
<td>Liver metastasis</td>
<td>5.5 $\pm$ 1.8</td>
<td>7.1 $\pm$ 2.3</td>
<td>1.2 $\pm$ 0.4</td>
</tr>
</tbody>
</table>

*Total volume of tumor identified on CT scan or pathology (patient 7).

† Site of tumor from which biopsy specimen was obtained for quantitative $[^{131}I]huA33$ uptake analysis.

‡ Mean $\pm$ SD ($[^{131}I]huA33$ uptake from biopsies of central and peripheral portions of tumor sample.

§ T:NT, tumor/normal tissue ratio from surgical tissue samples (mean $\pm$ SD).

‖ Normal tissue = liver for all samples, except for patient 7, where normal tissue sample was fat.

¶ T:S, tumor/serum tissue ratio on day of surgery (mean $\pm$ SD).
significant differences between the dose levels ($P = 0.475$). In one patient (patient 7), samples of normal terminal ileum and colon were obtained for measurement of $\left[{^{131}}I\right]$huA33 uptake. The uptake did not substantially differ between bowel regions and was measured as $5.1 \pm 2.0 \times 10^{-3} \%$ID/g tissue (mean $\pm$ SD).

**Autoradiography of tumor specimens.** Autoradiography showed excellent correlation between localization of radiolabeled huA33 and cellular components of tumor in all biopsies (Fig. 4). There was clear demonstration of penetration of radiolabeled huA33 to the central portion of metastatic lesions and minimal uptake in necrotic or stromal regions by dry film autoradiography. Emulsion autoradiography showed selective uptake and retention in cellular components of tumor, with minimal uptake in stromal or necrotic areas or normal tissue (e.g., liver; Fig. 4).

**Whole body clearance.** The whole body clearance of $\left[{^{131}}I\right]$huA33 ($T_{1/2}$ biological) was calculated to be $143.9 \pm 16.8$ hours (mean $\pm$ SD; Table 2). There were no significant differences between dose levels for calculated values ($P = 0.766$).

**Relationship of tumor volume to pharmacokinetics, whole body clearance, and tumor uptake of $\left[{^{131}}I\right]$huA33.** There was no significant difference in pharmacokinetics (serum terminal half-life) of $\left[{^{131}}I\right]$huA33 in patients with high tumor burden (>1,000 g; $n = 2$) compared with the remaining patients ($P = 0.243$). When comparing all dose levels, there was no linear correlation between tumor volume and pharmacokinetics, whole body clearance, or tumor uptake of $\left[{^{131}}I\right]$huA33.

**Normal colon compartment kinetics.** The average colon huA33 uptake ($k_{13}$) half-life was $197 \pm 74.6$ hours, whereas the average colon elimination ($k_{30}$) half-life was $32.4 \pm 8.1$ hours. There were no significant differences in the $k_{13}$ and $k_{30}$ half-lives between dose levels ($P = 0.22$ and 0.32, respectively; Kruskal-Wallis rank test).

**Human anti-human antibody measurement.** The prestudy BIACore measurements of a patient’s serum showed a mean $\pm$ SD of $14.3 \pm 2.9$ response units (RU), therefore providing a cutoff value for HAHA of 23 RU (mean $\pm$ 3 SD). Four of the 12 patients had detectable HAHA following infusion of huA33. These levels were all low, peaking at <90 RU. Patient 6 had a peak measurement of 85.2 RU at 14 days after infusion, patient 7 had a peak RU of 45.2 at 42 days after infusion, patient 8 had a peak at 49.7 RU, and patient 9 had a peak RU of 35.3 at 48 days after infusion of $\left[{^{131}}I\right]$huA33. Patients 7, 8, and 9 had HAHA measurements returned to normal levels within 3 months; no follow-up values were available for patient 6. No symptoms related to $\left[{^{131}}I\right]$huA33 infusion, or symptoms of serum sickness, were observed in any patient entered into the study.

**Discussion**

This study has shown that radiolabeled huA33 can selectively target primary and metastatic colorectal tumors and penetrate to the center of large necrotic metastatic lesions. Importantly, this study has also shown that huA33 has an elimination $T_{1/2}$ from bowel consistent with normal colonocyte shedding kinetics (28). The uptake of huA33 in tumor, huA33 pharmacokinetics, and huA33 colon uptake and elimination...
kinetics were not affected by huA33 protein dose. No adverse events related to a single infusion of huA33 were observed in any patient entered into the study.

Sequential biodistribution images of [131I]huA33 showed high uptake of antibody in sites of metastatic colorectal carcinoma >1.5 cm in all patients, often as early as day 2 after infusion, indicating excellent and rapid tumor uptake (Fig. 2). The intratumoral distribution of [131I]huA33 was also clearly shown by detailed autoradiographic studies of biopsies, with penetration to central portions of tumor despite the presence of bulky disease and, in some cases, extensive necrosis. This is an important observation, as the barriers to antibody penetration in tumors due to interstitial pressure, and vascular supply, are often stated to hinder potential efficacy. In the current study, huA33 was clearly able to penetrate to the center of large necrotic tumors to tumors cell nests.

Similar to previous studies with murine A33 mAb, uptake of [131I]huA33 in normal colon was observed in all patients and showed a changing pattern with time, consistent with gradual clearance (16–18). The colon compartment kinetics calculations confirmed the elimination T1/2 to be consistent with the turnover time of normal colonocytes (28). There are little published data on the uptake and elimination kinetics of antibodies that target antigens expressed on normal bowel, and this study therefore provides novel data on this important variable. The lack of gastrointestinal symptoms in patients receiving radioimmunotherapy with murine mAb A33 in prior trials is likely to be attributable to this observed colonocyte turnover (transcytosis) in colon mucosa, which reduces the potential cumulative radiation exposure to the gut. The importance of our observation of colonocyte turnover of huA33 is particularly relevant in view of the gastrointestinal toxicity reported with other antibodies that target antigens that are also expressed on normal gut epithelium (29, 30). In addition, the marked differences in retention of [131I]huA33 in normal bowel compared with tumor highlights the importance of the biological properties of cognate antigen in tumor-targeting strategies with recombinant antibodies.

The pharmacokinetics of [131I]huA33 showed a typical serum clearance pattern for IgG, and the 3- to 4-day terminal half-life of [131I]huA33 is at the lower range for published studies of humanized antibodies (31–37). The lack of effect of protein dose on pharmacokinetics is an important finding, in view of the small dose therefore required to saturate normal colon (which expresses the A33 antigen and shows uptake of [131I]huA33). This is in marked distinction to other antigen systems (e.g., CD20, epidermal growth factor receptor, and HER-2/neu), where large protein doses are required to saturate the normal tissue antigen pool (33, 34, 38–40). In view of the lack of difference in pharmacokinetics, whole body clearance, quantitative tumor uptake, and tumor penetration between dose levels, further protein dose escalation was not done above 10 mg/m². The results of this study indicate that 5 to 10 mg/m² represents an optimal tumor-targeting dosage of huA33. The escalation of huA33 protein dose to define the biological (Fc function) activity of unconjugated huA33 and to further define the toxicity of huA33 at higher dose levels has been explored in separate clinical trials (22, 23).

In published studies, humanized antibodies have shown substantially reduced immunogenicity compared with previous murine antibody trials (27, 29–38, 41). Analysis of patient immune responses following radiolabeled huA33 infusion showed low-level HAHA detectable in 4 of 12 patients. In all of these patients, HAHA levels were low (<90 RU), did not increase with time, and, although follow-up data were limited, in three patients quickly fell to the reference range. The biological significance of these findings is uncertain, as all patients received only one infusion of huA33, and changes in pharmacokinetics with repeat infusions were not evaluable.
In conclusion, radiolabeled huA33 when given as a single infusion is safe at the doses used and shows selective and rapid localization to colorectal carcinoma in vivo, with penetration to tumor cell clusters within the center of necrotic tumors. Our study has also shown that huA33 has an elimination T1/2 from normal colon that is consistent with normal colonic turnover, which correlates with the lack of gastrointestinal toxicity observed in trials with mAb against the A33 antigen. The excellent targeting characteristics of this humanized antibody indicate clear potential for the targeted therapy of colorectal cancer, and trials of radioimmunotherapy with [131I]huA33, including combination studies of [131I]huA33 with capricetamine, are currently under way.

References

A Phase I Trial of Humanized Monoclonal Antibody A33 in Patients with Colorectal Carcinoma: Biodistribution, Pharmacokinetics, and Quantitative Tumor Uptake

Andrew M. Scott, Fook-Thean Lee, Robert Jones, et al.