Rhenium-186-Labeled Chimeric Antibody NR-LU-13: Pharmacokinetics, Biodistribution and Immunogenicity Relative to Murine Analog NR-LU-10

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A mouse-human chimeric monoclonal antibody (NR-LU-13), with the same pancarcinoma antigen recognition site as a previously studied murine monoclonal antibody (NR-LU-10), was radiolabeled with $^{188}$Re using a bifunctional chelate. Nine patients (ages 31–81 yr) with metastatic adenocarcinoma received $^{188}$Re NR-LU-13. A single intravenous dose of 42 mg NR-LU-13 labeled with 25 mCi/m$^2$ (two patients) or 60 mCi/m$^2$ (seven patients) was administered. Mean serum disappearance halftime values for the chimeric $^{188}$Re antibody were alpha, 2.6 hr; beta, 36.5 hr; and whole-body, 112 hr (compared with 5.1, 26.5 and 66 hr, respectively, for $^{188}$Re NR-LU-10). Fifty percent of the radiolabel was excreted in the urine by 8 days. Tumor localization was demonstrated by gamma camera imaging in seven of nine patients. The percent injected dose per gram in a single tumor biopsy specimen was 0.003% at 72 hr postinjection. Absorbed dose to bone marrow was 1.5 ± 0.7 rads/mCi and resulted in reversible myelosuppression in five of six evaluable patients who received 60 mCi/m$^2$; median WBC nadir = 2500/μl; median platelet nadir = 85,500/μl. Low grade fever, nausea, slight elevations of liver function tests and mild allergic reactions were seen in some patients. The chimeric antibody elicited low levels of anti-NR-LU-13 antibody in six of eight evaluable patients (75%), in contrast to NR-LU-10 which elicited higher levels of human anti-mouse antibody in all patients. This pilot study demonstrates the ability of the chimeric antibody to target tumors with reduced (but not absent) immunogenicity and delayed clearance relative to the murine monoclonal.

J Nucl Med 1993; 34:2111–2119

In recent years, numerous investigators have performed trials exploring the feasibility of radioimmunotherapy in man using murine monoclonal antibodies (Mabs). A major limitation of these trials has been the inability to administer repetitive doses of murine antibodies because of the development of human anti-mouse antibodies (HAMA) (1,2).

Development of HAMA has frustrated attempts to fractionate doses of radioimmunotherapy which would likely result in decreased hematopoietic toxicity (3). One approach to decreasing the immunogenicity of murine Mabs has been to develop mouse-human chimeric antibodies (4,5). These genetically engineered immunoglobulin molecules contain the murine variable domain and antigen recognition site but the majority of the molecule consists of human immunoglobulin constant regions. One such chimeric antibody is NR-LU-13, a mouse-human chimeric Mab with the same antigen recognition site as the pancarcinoma murine Mab NR-LU-10 (6,7).

Rhenium-186 was selected for radioimmunotherapy because of its 3.7-day half-life which is compatible with the pharmacokinetics of tumor localization and clearance of murine antibodies (8). It has a medium-energy beta particle (91% abundance) with maximum energy of 1.07 MeV. This emission is particularly suitable for radioimmunotherapy because 90% of the energy from a point source is delivered within 2 mm of the source ($X_{90}$) (9). In addition, $^{188}$Re has a 137-keV gamma photon which is ideal for gamma camera imaging even at high doses. The low energy and low abundance (9%) of the 137-keV gamma photon and the very small fraction (0.05%) of higher energy gamma photons (>600 keV) result in minimal radiation exposure to medical personnel compared with $^{131}$I.

In this report, we describe the results of our pilot Phase I clinical trial of $^{188}$Re-labeled NR-LU-13 in patients with advanced cancer. We investigated the pharmacokinetics, biodistribution, immunogenicity, radiation absorbed dose and toxicity of $^{188}$Re NR-LU-13 given intravenously.

METHODS

Patients

Nine patients (ages 31–81 yr, median 45 yr) with adenocarcinoma of the colon (four patients), gastroesophageal junction (two patients), and lung, breast and unknown primary (one each) were included in this pilot study. All patients were at least 4 wk from their most recent chemotherapy or radiation therapy and had Karnofsky performance status of greater than 60%, no other se-
rious concurrent illnesses, creatinine less than 1.8 mg/dl, bilirubin less than 2 mg/dl, platelet count greater than 150,000/μl, white blood count greater than 3,500/μl, and no prior known exposure to murine antibody. Tumor extent and volume were determined by CT scan prior to therapy. This study was conducted under an application with the Washington State Board of Pharmacy and was approved by the Institutional Review Board of the Virginia Mason Medical Center. Patients granted informed consent after thorough explanation of the study.

**Chimeric Antibody NR-LU-13**

Antibody NR-LU-10 is a murine IgG2b Mab that recognizes a 40 kD glycoprotein antigen expressed by epithelial tumors including carcinomas of the lung, colon, ovary and breast (7). The target antigen for NR-LU-10 has not been fully characterized. Clinically, cross-reactivity of NR-LU-10 with renal tubules and thyroid has been observed (6). NR-LU-13 is a murine-human chimeric antibody that has been genetically engineered by substituting a human IgG1 constant region for the IgG2b constant region of the murine Mab NR-LU-10 (Fig. 1). The chimeric antibody is composed of the variable heavy and light chain regions of murine NR-LU-10 and the constant regions of the heavy and kappa light chains of human immunoglobulin IgG1. The recombiant chimeric antibody genes were constructed as previously described (10) and transferred into cell line Sp2/0-ag 14 by protoplast fusion (Damon Biotech, Needham, MA). Subclones of this cell line (after transfer of the expression vector) were passaged multiple times and then assayed for antibody production. A high producing clone was selected and expanded to produce sufficient amounts of NR-LU-13 for clinical testing. The chimeric antibody was purified and then tested for lack of pyrogenicity, sterility and absence of contamination by mycoplasma, viruses and polynucleotides. The antibody was aseptically vialed and stored at pH 7 in phosphate buffered saline.

**Radiolabeling**

The 99mTc labeling of the Fab fragment of the NR-LU-10 antibody (used for imaging in some patients) was performed as previously described (11). Rhenum-186 labeling of NR-LU-13 was done using tetrafluorophenyl S-ethoxethyl-mercaptoacetylglu-cylglycy1-gamma-amino butyrate (MAG-GABA) to form an N₃S amide thiolate complex of rhenium which was conjugated to the antibody by active ester acylation of protein amines (6). All preparations were made at a rhenium-to-antibody final molar incorporation ratio of approximately 3:1, and the radiolabeled immunoconjugate was then purified by chromatography. Prior to patient injection, immunoconjugate preparations were assayed as previously described (6) for endotoxin (all had less than 0.125 IU/ml), for radiochemical purity by instant thin-layer chromatography (mean 93.9%) and for percent of radioactivity associated with monomeric NR-LU-13 by size exclusion chromatography (mean 98.3%). The major peak from the size exclusion column also had a nonintegrating trailing shoulder which typically accounted for less than 10% of the monomeric peak area. The identity of the lower molecular weight components in the trailing shoulder has not been established but additional tests indicated that they did not bind to target cells in the cell binding assay. Mean immuno-reactivity of 186Re NR-LU-13 clinical preparations determined in the cell-binding assay was 71%, or 89% of the immunoreactivity of 125I NR-LU-13 (80%).

**Study Design**

All patients were administered a single intravenous dose of 42 mg of antibody NR-LU-13. The amount of NR-LU-13 was determined by the amount needed for radiolabeling over the anticipated millicurie dose range to ensure that the resulting ratio of 186Re-to-antibody was maintained below a level resulting in loss of immunoreactivity or targeting potential. The first two patients received 25 mCi/m² of 186Re (46 and 42 mCi total) and the subsequent seven patients received 60 mCi/m² of 186Re (95–129 mCi, median 118 mCi).

Five patients also received 10 mg of 99mTc NR-LU-10 Fab for tumor imaging 1–7 days before injection of 186Re NR-LU-13 in order to select patients with positive tumor uptake (6). We used 99mTc NR-LU-10 Fab to detect positive tumor uptake in patients because of extensive previous experience with this immunoconjugate (6,11,12), only weak immunogenicity of the Fab antibody fragment (12), identical variable domains (antigen recognition sites) of NR-LU-10 and NR-LU-13 and the availability of a clinical diagnostic kit for 99mTc NR-LU-10 Fab (11,12).

Patients were followed for 6 days after infusion of 186Re NR-LU-13 to determine 186Re serum, whole-body and organ clearance and cumulative 186Re excretion in the urine. Gamma camera imaging was performed to assess tumor localization and to determine the activity in source organs at each time point for calculation of radiation absorbed dose. Quantitation of activity in the whole body, liver and lungs was estimated by the conjugate view method; kidney and tumor quantitation was estimated from regions of interest on one view with attenuation correction factors based on depth (13,14). Radiation absorbed doses were estimated by the Medical Internal Radiation Dose (MIRD) method (15). Marrow dose was estimated from serum clearance activity (14,16) since marrow activity was not detected on the gamma camera images (except for a single patient, see footnote to Table 2). Patients were followed serially for 6 wk to assess toxicity and...
tumor response using standard criteria as previously described (6). Serum was also obtained every 2 wk to investigate the serologic immune response to the immunoconjugate.

Standard parametric statistical techniques were used to compile standard deviations and compare means. Data obtained in this study are compared to similar data obtained previously in 15 patients who received intact murine antibody ¹⁸⁶Re NR-LU-10 (6). These two trials were similar in terms of patient population, mode of immunoconjugate administration and doses of ¹⁸⁶Re administered.

**Antiglobulin Measurement**

HAMA was measured in patient sera using an ELISA assay format as previously described (6, 17). The Fab fragment of NR-LU-10 was used as the target antigen. In order to be categorized as a positive HAMA response, two criteria were required: first, post-treatment HAMA levels needed to be at least twofold higher than pretreatment levels; and second, the post-treatment levels needed to exceed a response threshold level of 4.6 normal serum (NS) units. The threshold level is two standard deviations above the geometric mean HAMA level from a pool of untreated normal individuals (6).

Serum levels of human antichimera antibody (HACA) were also measured using a sandwich ELISA format. The assay used is a variation of the method of LoBuglio et al. (18) converted to a conventional ELISA format using a peroxidase-labeled chimeric antibody as the detection molecule in place of iodine-labeled antibody. NR-LU-13 was absorbed onto the wells of a 96-well polystyrene microtiter plate. Test serum was then added and unbound material was washed away. Peroxidase-labeled NR-LU-13 was then added to each well. Following a wash step, chromogenic reagent and hydrogen peroxide were added, and the level of color development was determined in a spectrophotometer at a wavelength of 492 nm. Goat anti-human IgG was used as a HACA-positive standard and control. Optimization of assay parameters, e.g., NR-LU-13 coating concentration, peroxidase chimeric (detector) concentrations, etc., was established for the ELISA assay. HACA units for patient specimens are reported in µg/ml relative to the absorbance value for the standard. No detectable levels of HACA were found in pretreatment serum samples.

The units used in the HAMA and HACA assays are based on different standards and no attempt has been made to normalize the data. The relationship between HAMA and HACA units is unknown, so only relative trends should be interpreted from the data. Human anti-chelate (anti-ligand) antibody was not measured in either the HAMA or HACA assays since unconjugated antibody was used as the target antigen in each assay.

**RESULTS**

**Pharmacokinetic Analysis**

Rhenium-186 activity was followed to determine serum clearance (disappearance) half-times of chimeric NR-LU-13. Results in nine patients are shown in Table 1 along with similar data previously obtained in 15 patients who received intact murine antibody ¹⁸⁶Re NR-LU-10 (6). As assessed by instant thin-layer and size exclusion chromatography, more than 98% of the ¹⁸⁶Re detected in the serum was associated with a protein that had chromatographic properties identical to NR-LU-13.

The primary route of excretion of ¹⁸⁶Re from administration of ¹⁸⁶Re NR-LU-13 was urinary. By 144 hr after injection, 50% ± 12% of the radioactivity had been excreted via the urine. The primary route of excretion of NR-LU-10 was also urinary; 65% ± 12% of the radioactivity appeared in the urine within 144 hr of administering ¹⁸⁶Re NR-LU-10 (p = 0.02) (6). As was true for intact NR-LU-10 (6), the radiolabeled material excreted in the urine after administration of ¹⁸⁶Re NR-LU-13 consisted of low molecular weight catabolites of the antibody fragment (data not shown). The lysine adduct of the ¹⁸⁶Re-MAG3-GABA complex appeared in the urine by 1-2 hr and was the major catabolite at all times. In addition, small amounts (<10%) of the N-acetylated lysine adduct, free acid and perrhenate were found. Fecal excretion was measured in two patients who received ¹⁸⁶Re NR-LU-13. At 120 hr after injection, 4.2% and 27.6% of the injected dose was recovered in the feces.

**Gamma Camera Imaging**

Biological (i.e., decay-corrected) disappearance of ¹⁸⁶Re NR-LU-13 from normal organs determined from gamma camera imaging is shown in Figure 2A and comparable data from patients given murine antibody ¹⁸⁶Re NR-LU-10 are shown in Figure 2B. Note that disappearance from the liver was comparable for both antibodies, but that clearance of ¹⁸⁶Re NR-LU-13 from whole body and lungs was delayed approximately twofold and clearance from the kidneys was delayed approximately fivefold. After administration of ¹⁸⁶Re NR-LU-13, the kidneys were evident immediately and became relatively more prominent through Day 7 (the final day of imaging); the liver was visualized immediately and was still visible through Day 7; intestinal tract radioactivity was usually seen on Day 2 and progression of activity through the large bowel was observed through Day 7; the thyroid was visualized initially on Day 3 after ¹⁸⁶Re NR-LU-13 and remained prominent through Day 7. The pituitary gland was visualized in only one patient (7) and marrow activity was observed in only one patient (5).

Tumor localization was noted in seven patients given ¹⁸⁶Re NR-LU-13 and is illustrated in Figure 3. Tumor was visualized in four of five patients with hepatic metastases, in three of four patients with nodal metastases and in individual patients in skin, soft tissue, chest wall, bone and series.
and that the most striking differences are observed in estimated dose to the kidney and lung. It was possible to estimate the radiation dose in seven tumors in five patients treated with Re NR-LU-13. Tumor volume was particularly difficult to estimate from CT scan in Patients 5 and 9, and thus these estimates of tumor dose are especially uncertain. With these limitations, tumor absorbed doses ranged from 0.9 to 7.5 rads/mCi (mean = 3.5 ± 2.5 [s.d.], median = 1.9 rads/mCi). Patient 2 had a subcutaneous nodule biopsied from the left chest area 72 hr after Re NR-LU-13 administration. The specimen was counted and found to contain 0.003% of the injected dose per gram.

**Clinical Observations**

Nonhematologic toxicity for the nine patients who received Re NR-LU-13 is detailed in Table 3. Three patients experienced mild acute adverse events immediately after receiving Re NR-LU-13. Patient 1 experienced adrenal metastases. Maximum visualization was noted 1–3 days after injection of Re NR-LU-13. Somewhat greater difficulty was experienced in visualizing tumors with Re NR-LU-13 than with Re NR-LU-10. In part, this difference appeared to be related to higher background activity because of slower disappearance of the chimeric antibody from serum and normal tissues. Given the limited number of patients studied, however, the ability of NR-LU-13 to localize to tumor was similar to that previously noted for NR-LU-10.

Mean radiation dose estimates to normal organs and tumors for patients receiving Re NR-LU-13 are shown in Table 2 with comparable data for patients given Re NR-LU-10 (6). Note that estimates of absorbed radiation dose in most organs are greater for patients who received Re NR-LU-13 than for those treated with Re NR-LU-10,

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**FIGURE 2.** Mean biological (i.e., decay-corrected) disappearance curves of Re determined from gamma camera imaging from whole body, liver, kidneys and lungs after administration of Re-labeled immunoconjugate: (A) Re Re-LU-13 (chimeric); (B) Re Re-LU-10 (murine, reference 6).

**FIGURE 3.** Gamma camera image and computed tomogram from Patient 5 who had metastatic non-small-cell lung cancer involving left axillary lymph nodes (A) and a malignant pleural effusion (PE). Localization of Re Re-LU-13 in the left axilla (A) and left hemithorax (PE) can be appreciated in the upper gamma camera image (posterior view) obtained 68 hr after administration of the immunoconjugate. The lower computed tomogram (oriented to be comparable to the gamma camera image with the patient's left side to the left) also shows the enlarged left axillary nodes (A) and PE.
shaking chills starting approximately 1 hr after administration of the imunoconjugate and lasting for 3 hr. This patient also developed fever (102.1°F maximum) which began 1 hr after antibody infusion and lasted intermittently for 3 days. Patient 4 experienced an allergic reaction (hives) approximately 20 min after the end of the infusion. He also complained of pain in the area of his lower sternum and developed elevated liver function tests (alkaline phosphatase and serum glutamic oxaloacetic transaminase) 24 hr after infusion. Patient 7 experienced an allergic reaction (itching and a single hive) minutes after the end of the antibody infusion. Note that all nonhematologic toxicities were mild (Grade I or II) and transient. Liver function tests were altered frequently (eight of the nine patients) but were elevated in all patients to no more than 2.5 times normal and returned to normal within 3 wk. Similar alterations in liver function were also observed after 186Re NR-LU-10 (6); the mechanism of these alterations has not been established.

Hematologic toxicity observed following administration of 186Re NR-LU-13 is also detailed in Table 3. The time course of hematologic toxicity (shown by white count and platelet count expressed as percent of baseline) for patients who received 60 mCi/m² of 186Re NR-LU-13 is shown in Figures 4A and 4B. Also shown in Figure 4 are similar data previously obtained in patients given the same dose of 186Re NR-LU-10 (6). Greater hematologic toxicity was observed in patients given 186Re NR-LU-13 compared with 186Re NR-LU-10, an observation consistent with the slower whole-body and serum clearance (Table 1 and Fig. 2) and the subsequent greater radiation dose delivered to marrow (Table 2). None of the hematologic or nonhematologic toxicities observed was dose-limiting or severe. All were managed conservatively and resolved with time without sequelae.

No anti-tumor responses were seen. Patient 5 died of progressive disease 3 wk after receiving 186Re NR-LU-13. Patients 1 and 2 had stable disease and the other six patients had progressive disease when evaluated 6 wk after 186Re NR-LU-13.

**Human Antibody Response**

Details of the HAMA and HACA immune responses in the eight evaluable patients are given in Table 4. Patients 1–4 (Group A, Fig. 5) received only 186Re NR-LU-13; thus the immunogen in these patients was chimeric antibody alone. Patients 5–9 (Group B) underwent a 99mTc NR-LU-10 Fab imaging study prior to receiving 186Re NR-LU-13; thus in these patients the immunogen was the Fab fragment of NR-LU-10 plus NR-LU-13, the chimeric antibody.

**TABLE 2**

Normal Organ and Tumor Dosimetry After 186Re Imunoconjugates

<table>
<thead>
<tr>
<th>Site</th>
<th>NR-LU-13 (rads/mCi)</th>
<th>NR-LU-10 (rads/mCi)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal organs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mean)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole body</td>
<td>0.8 ± 0.2*</td>
<td>0.6 ± 0.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Marrow</td>
<td>1.3 ± 0.3*</td>
<td>1.0 ± 0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>3.6 ± 0.9</td>
<td>1.9 ± 0.5</td>
<td>ns</td>
</tr>
<tr>
<td>Kidney</td>
<td>12.6 ± 3.1</td>
<td>5.7 ± 3.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lung</td>
<td>3.3 ± 1.3</td>
<td>1.4 ± 0.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 5 axilla</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 6 liver</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 7 liver</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 8 liver</td>
<td>4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 9 neck</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.5 ± 2.5*</td>
<td>6.3 ± 4.8</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Mean ± s.d.

Patient 2 had specific imunoconjugate localization in marrow. Marrow dose estimate based on ROI data was 3.2 rads/mCi. This patient's marrow dose estimate was not included in mean value given in table.

ns = Not significant, i.e., p > 0.10.

**TABLE 3**

Toxicity of 186Re NR-LU-13

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose level (mCi/m²)</th>
<th>Absorbed whole-body dose (rads)</th>
<th>Hematologic</th>
<th>Nonhematologic (all Grade I or II)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nadir counts ×10³/µl</td>
<td>WBC</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>26</td>
<td>4.4</td>
<td>118</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>45</td>
<td>3.3</td>
<td>181</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>76</td>
<td>1.9</td>
<td>114</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>101</td>
<td>1.4</td>
<td>84</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>84</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>97</td>
<td>3.1</td>
<td>38</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>39</td>
<td>7.3</td>
<td>162</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>80</td>
<td>3.8</td>
<td>87</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>101</td>
<td>1.2</td>
<td>15</td>
</tr>
</tbody>
</table>

N.D. = Not determined because the patient died; LFT = elevation of liver function tests; and N/V = nausea and/or vomiting.
Five of the eight $^{186}$Re NR-LU-13 patients were classified as HAMA responders to the Fab fragment of NR-LU-10 (one of four in Group A; all four in Group B). As shown in Figure 5, however, the magnitude of the HAMA responses was generally 10- to 1,000-fold lower than the HAMA responses seen in the 15 patients treated with the $^{186}$Re NR-LU-10 murine Mab (Group C, Fig. 5) (6). None of the patients had detectable levels of HACA prior to treatment with $^{186}$Re NR-LU-13, but six of the eight patients developed detectable HACA levels following treat-

![Table 4: Human Antibody Response](chart)

<table>
<thead>
<tr>
<th>Group</th>
<th>Patient no.</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HAMA</td>
<td>HACA</td>
<td>HAMA</td>
<td>HACA</td>
<td>HAMA</td>
<td>HACA</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>0.17</td>
<td>DL</td>
<td>0.55</td>
<td>DL</td>
<td>0.56</td>
<td>DL</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>0.07</td>
<td>DL</td>
<td>0.10</td>
<td>DL</td>
<td>0.35</td>
<td>DL</td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>0.05</td>
<td>DL</td>
<td>0.22</td>
<td>DL</td>
<td>2.09</td>
<td>DL</td>
</tr>
<tr>
<td>A</td>
<td>4</td>
<td>0.10</td>
<td>DL</td>
<td>0.30</td>
<td>DL</td>
<td>3.47</td>
<td>1.45</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>0.67</td>
<td>DL</td>
<td>19.61</td>
<td>0.78</td>
<td>10.7</td>
<td>0.47</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>2.71</td>
<td>DL</td>
<td>9.18</td>
<td>0.10</td>
<td>1446.0</td>
<td>4.35</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>0.20</td>
<td>DL</td>
<td>0.32</td>
<td>DL</td>
<td>19.81</td>
<td>DL</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>0.32</td>
<td>DL</td>
<td>0.12</td>
<td>DL</td>
<td>2.00</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*Patients in Group A received only NR-LU-13; those in Group B received NR-LU-10 Fab as an imaging agent 1-7 days before receiving NR-LU-13 (see text).

†Patient 5 died 3 wk after receiving NR-LU-13 and is thus not evaluable.

HAMA = human antimouse antibody against NR-LU-10 Fab; NS units (see text); HACA = human anti-chimera antibody against NR-LU-13; $\mu$g/ml relative to positive control (see text); and DL = below detection limit of assay.

Responder values shown in BOLD type.
ment. Two patients, both in Group A and thus given only $^{186}$Re NR-LU-13, never developed significant titers of either HAMA or HACA.

**DISCUSSION**

The primary rationale for the development of chimeric mouse-human Mabs has been to lessen the immunogenicity of immunoglobulin tumor-targeting molecules in human patients. Development of HAMA is frequent in patients with solid tumors who receive murine antibodies (1, 2, 6, 19–21). Although HAMA formation is a somewhat lesser problem in patients with hematologic malignancies because of the immunosuppression associated with these diseases, it is nevertheless still a concern (22–24). Attempts to lessen the immunogenicity of the immunoglobulin molecule itself have included the preparation of genetically engineered antibody constructs, such as those with only minimal portions of the murine immunoglobulin variable region, known as humanized antibodies (25), or those with somewhat larger portions of the murine immunoglobulin variable region, i.e., chimeric mouse-human antibodies (4, 5, 26).

In this report, we have described the pharmacokinetics, biodistribution, dosimetry, immunogenicity and clinical features of chimeric antibody $^{186}$Re NR-LU-13. The NR-LU-13 mouse-human chimeric Mab was less immunogenic than its murine counterpart. Decreased immunogenicity was demonstrated both by a substantial reduction of HAMA titer (Fig. 5) and by a reduced likelihood of HAMA development (Table 4). Nevertheless, some degree of immunogenicity of the murine portion of the mouse-human chimeric antibody remained when tested against the murine Fab, i.e., HAMA. Furthermore, construction of the chimeric antibody results in the development of unique amino acid sequences associated with the “splice region” of the murine and human antibody. Antibodies against these portions for the immunoglobulin molecule or idiotypic antibodies against the human portion of the NR-LU-13 antibody can be detected only by using the chimeric antibody itself as the test reagent in an antibody response designated as HACA. Since the HACA assay can also detect reactivity against the murine portion of the chimeric antibody, the detection of HACA does not determine whether the response is to murine or human epitopes. As shown in Table 4, HACA could be demonstrated in six of eight patients and was detected in two of the four patients who received only the chimeric antibody (i.e., Patients 2 and 4). Thus, although the chimeric antibody NR-LU-13 is less immunogenic than its murine counterpart NR-LU-10, human antibody responses could still be detected. Other chimeric antibodies have shown considerable variability in immunogenicity, emphasizing the need to evaluate each chimeric antibody individually. For example, chimeric antibodies ch 17-1A and ch L6 have low immunogenicity, while chimeric antibody ch B72.3 has considerable immunogenicity in man (27–30). Furthermore, it is important to recognize that we have not, as yet, tested for human anti-body responses which might have occurred against the metal chelate (ligand).

Elimination of human immune responses to Mab radiolabeled immunoconjugates may also require manipulations in addition to use of genetically engineered antibody constructs. For example, immunogenicity of murine antibodies may be, in part, related to antibody size, i.e., fragments of antibodies are less immunogenic than intact antibodies (6) and fragments of chimeric antibodies may be especially attractive as radionuclide immunoconjugates (31). Frequency of antibody administration also affects the likelihood of HAMA formation, i.e., there is increased likelihood of significant HAMA titer following multiple compared to single injections (6, 28). The likelihood of HAMA formation is also influenced by concurrent administration of chemotherapy, i.e., there is a lesser likelihood of HAMA formation in patients administered immunosuppressive anti-tumor chemotherapy shortly following the administration of murine antibody fragments (12). One approach to reducing the likelihood of HAMA formation has been the administration of immunosuppressive agents such as cyclosporin (2, 32). The lesser magnitude of human anti-tibody responses to at least some chimeric antibodies, including NR-LU-13, suggests that it may be possible to suppress more easily or more completely development of these weaker immune responses using immunosuppressive agents.

The studies of the pharmacokinetics and biodistribution of the chimeric antibody $^{186}$Re NR-LU-13 suggest a more rapid initial distribution phase, or alpha half-time compared to the murine antibody $^{186}$Re NR-LU-10 (Table 1). In our experience with clinical trials, however, the alpha half-time measurement is associated with less precision than either the beta or monoexponential half-time. This is most likely the result of infusion (instead of bolus) administration and patient to patient differences in infusion times which impact the determination of the precise t = 0 serum concentration. The large relative standard deviation and the p values of only 0.04 are consistent with no convincing difference in the alpha half-time of the chimeric and murine antibodies.

In contrast, the overall pharmacokinetic behavior of the two antibodies was significantly different. The beta, monoexponential and whole-body half-lives of the chimeric antibody were significantly longer than those of the murine antibody $^{186}$Re NR-LU-10 (Table 1). The validity of these differences is reflected in the small p values for the half-time measurements and is consistent with the visual interpretation of the images. These data for NR-LU-13 are similar to those reported for two other chimeric antibodies, though the prolongation of beta and whole-body half-lives reported for chimeric antibodies ch 17-1A and ch B72.3 (27, 28, 30) relative to their respective parent murine Mabs were relatively greater (5–10 times) than that observed in this study for NR-LU-13 (1.5–2 times greater). In contrast, however, serum clearance of murine and chimeric L6 were similar (29).
The relatively slower appearance of the chimeric antibody \(^{186}\)Re NR-LU-13 in the urine compared to the murine antibody \(^{186}\)Re NR-LU-10 was presumably related to the prolonged serum clearance. We were somewhat surprised, however, to find that the disappearance of kidney activity from the images was extremely slow (Fig. 2A). The prolonged retention in the kidney for the chimeric antibody compared to the murine antibody (Fig. 2B) cannot be explained simply on the basis of prolongation of serum disappearance and, in fact, is substantially longer than the relative prolongation of whole-body disappearance, i.e., 112 hr for the chimera versus 66 hr for the murine antibody. We have previously observed weak in vitro reactivity of the NR-LU-10 antibody with renal tubular epithelial cells (unpublished observations), suggesting that the antibody may remain bound to the kidney for a prolonged period of time. We also have shown that the uptake of \(^{186}\)Re NR-LU-13 (or \(^{186}\)Re NR-LU-10) by the kidney and thyroid is related to the immunologic reactivity of this antibody and not to \(^{186}\)Re dissociating from the immunoconjugate since \(^{186}\)Re conjugated antibody NR-CO-02 does not show localization to either kidney or thyroid (6).

The dosimetry estimates of \(^{186}\)Re NR-LU-13 (Table 2) reflect the extended residence times observed for whole body and normal organs (Fig. 2A) and, for the lungs and kidneys, a greater initial percentage uptake of the injected dose. The dosimetry estimates to the marrow were slightly higher with the chimeric than with the murine antibody. The validity of this estimate is confirmed by the observed hematologic toxicity (Fig. 4) which is greater for the chimeric antibody than for the same dose of murine antibody. Grade III or IV hematologic toxicity was observed in patients receiving more than 120 rads to marrow or 95 rads to the whole body. Renal toxicity has not been observed to date in patients who received 60 mCi/m² of \(^{186}\)Re NR-LU-13 in spite of mean estimated kidney dose of 1300 rads and a 9-mo follow-up.

We had hoped that prolonged serum and whole-body residence of the chimeric antibody would result in greater tumor uptake, improved imaging of tumors and greater tumor radiation doses. The somewhat prolonged serum disappearance of \(^{186}\)Re NR-LU-13, however, resulted in greater difficulty imaging tumors because of higher background activity, an observation previously reported in studies of chimeric antibody ch 17-1A (27). Tumor radiation doses estimated by gamma camera (Table 2 and Fig. 6) or by biopsy, however, were comparable to similar observations in patients given murine antibody (6). Thus, an increase of approximately 50% in serum circulation time was of no observed benefit in terms of specific tumor uptake. This may relate, in part, to the observation that tumor uptake is maximal 24–48 hr after immunoconjugate administration (6 and unpublished data). During this period, \(^{186}\)Re NR-LU-13 and \(^{186}\)Re NR-LU-10 serum concentrations were similar, perhaps accounting for comparable tumor uptake. Hence, based neither on imaging nor therapy characteristics, did we observe any clinical advantage in the administration of chimeric compared to murine antibody. On the other hand, we did demonstrate reduced immunogenicity of the chimeric antibody. In this study, however, we made no attempt to administer repeat doses of antibody and thus did not attempt to take advantage of this lesser immunogenicity. Future studies, perhaps including concurrent administration of immunosuppressive drugs, will therefore be designed incorporating multiple doses of chimeric antibody.

Further studies are also needed to improve the therapeutic ratio associated with radioimmunotherapy using \(^{186}\)Re-labeled Mabs. These studies should continue to address problems associated with immunoconjugate immunogenicity. They must also attempt to increase the percent of the injected dose localizing to tumor, thereby resulting in increased radiation dose to tumor, and to decrease the circulation time and normal organ uptake of these radiolabeled molecules, thereby resulting in less nonspecific radiation dose and less toxicity.

ACKNOWLEDGMENT

The authors thank S. Wolf, RN, A.B. Einstein, MD and the many other individuals at Virginia Mason Medical Center who contributed to the clinical care and study of these patients; J-L Vanderheyden, PhD, F-M Su, T. Dodd and J. Bugaj for radiolabeling; M. Maurer for patient laboratory studies; J. Reno, B. Bottino, R. McIntyre and R. Klein for antibody manufacturing; G. Ehrlhardt and M. Evans of the Radiosotope Application Group of the University of Missouri Research Reactor for production of \(^{186}\)Re; J. Durham, PhD of Battelle Pacific Northwest Laboratories for dosimetry calculations; and Jane Lopez for word processing.


Financial support provided by the NeoRx Corporation.

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