Phase I/II Radioimmunotherapy Trial with Iodine-131-labeled Monoclonal Antibody G250 in Metastatic Renal Cell Carcinoma


ABSTRACT

This Phase I/II radioimmunotherapy study was carried out to determine the maximum tolerated dose (MTD) and therapeutic potential of 131I-G250. Thirty-three patients with measurable metastatic renal cell carcinoma were treated. Groups of at least three patients received escalating amounts of 131I (30, 45, 60, 75, and 90 mCi/m²) labeled to 10 mg of mouse monoclonal antibody G250, administered as a single i.v. infusion. Fifteen patients were studied at the MTD of activity. No patient had received prior significant radiotherapy; one had received prior G250. Whole-body scintigrams and single-photon emission computed tomography images were obtained in all patients. There was targeting of radioactivity to all known tumor sites that were ≥2 cm. Seventeen of 33 patients had stable disease. There was no correlation between the amount of 131I administered or hepatic absorbed radiation dose (median, 0.073 Gy/mCi) and the extent or nature of hepatic toxicity. Two of the first six patients at 90 mCi/m² had grade ≥3 thrombocytopenia; the MTD was determined to be 90 mCi/m² 131I. Hematological toxicity was correlated with whole-body absorbed radiation dose. All patients developed human antimouse antibodies within 4 weeks posttherapy; retreatment was, therefore, not possible. Seventeen of 33 evaluable patients had stable disease. There were no major responses. On the basis of external imaging, 131I-labeled mouse monoclonal antibody G250 showed excellent localization to all tumors that were ≥2 cm. Seventeen of 33 patients had stable disease, with tumor shrinkage observed in two patients. Antibody immunogenicity restricted therapy to a single infusion. Studies with a nonimmunogenic G250 antibody are warranted.

Received 5/7/98; revised 8/20/98; accepted 8/24/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by National Cancer Institute Grant CA-33049.
2 To whom requests for reprints should be addressed, at Nuclear Medicine Service, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-2459; Fax: (212) 717-3263; E-mail: divgi@mskcc.org.
3 Present address: Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Austin and Repatriation Medical Centre, Heidelberg, Victoria, 3084, Australia.
4 Present address: Department of Urology, Academic Hospital, 6500 HB, Nijmegen, the Netherlands.

INTRODUCTION

RCC is the most common renal malignancy in adults, accounting for 3% of all malignancies and 80–90% of all primary kidney neoplasms (1). The prognosis for these patients is poor; ~30–40% of all patients present with metastatic disease at the time of diagnosis (2). In the United States, an estimated 28,000 new cases are diagnosed each year, and ~11,500 deaths occur per year (3).

Despite attempts to develop treatment strategies for RCC, the prognosis for these patients remains unsatisfactory. Five-year survival is <10% (4, 5), and for those with distant metastases or stage IV RCC, the median survival is <2 years. The tumor is resistant to radiation therapy (6) and chemotherapy (7). Biological response modifiers have, thus far, shown only minimal efficacy in patients with RCC (8–15).

The development of technologies to generate mAbs (16) created the possibility of new treatment modalities for cancer. A range of novel cell surface antigens expressed by human cancer have been defined by mAbs, and a number of antibody-based therapies targeting such antigens are being developed (17). The different therapeutic strategies under exploration include: (a) antibody-mediated tumor cell killing via complement activation or antibody-dependent cellular cytotoxicity (18); (b) antibody-mediated interference of cell growth by neutralizing growth factors or blocking growth factor receptors (19); and (c) antibody delivery systems targeting radioactive isotopes (20), toxins (21), or chemotherapeutic agents (22) to the tumor site. The

The abbreviations used are: RCC, renal cell carcinoma; mAb, monoclonal antibody; mG250, mouse mAb G250; IRB, Institutional Review Board; MTD, maximum tolerated dose; MTDₐ, MTD of activity; CT, computed tomography; NRS, normal rabbit serum; cG250, chimeric G250.
many technical challenges confronting successful application of antibody therapies, e.g., immunogenicity of mouse antibodies, antigen expression by normal tissues, low levels of antibody uptake by tumors, and heterogeneity in antigen expression by tumor cells, have become clear from initial clinical trials, and progress is being made in overcoming these limiting features.

Efforts to identify and select antigens expressed in renal carcinomas led to the development of mG250. G250 detects an antigen that is expressed in virtually all clear cell carcinomas of the kidney but does not react with normal kidney tissue. G250 reactivity in normal tissues was found to be restricted to the gastric epithelium, the large biliary ducts in the liver, and some pancreatic acini.

The G250 antigen has recently been characterized by expression cloning, and the complete G250 sequence and organization of the coding gene have been characterized (25). The G250 antigen is closely related or identical to the MN antigen, an antigen originally detected by a mouse antibody against HeLa cells and extensively analyzed as a marker for cervical dysplasia and carcinoma (26). The G250/MN sequence has a domain that is homologous to carbonic anhydrase and a putative helix-loop-helix DNA-binding sequence (26).

Presurgical clinical studies were conducted using 131I labeled to escalating mass amounts of mG250 to determine tumor uptake and biodistribution (24). Tumor biopsy samples showed a high level of mG250 localization, with levels up to 0.1% of the injected dose per gram of tumor 7 days postinfusion. As expected from the expression of G250 in bile ducts, there was liver uptake of 131I-mG250, which decreased with higher doses, suggesting saturation of G250 sites by antibody. On the basis of these ex vivo observations, the optimal protein dose was estimated to be 10 mg of mG250 per infusion.

Here, we present the results of a Phase I/II radioimmunotherapy study in patients with measurable metastatic RCC, designed to determine the maximum dose of i.v. administered 131I labeled to mG250, radioisotope retention in tumors, quality of imaging, and assessment of organ toxicity.

PATIENTS, MATERIALS, AND METHODS

Study Design

Prior to antibody i.v. infusion, a physical examination was performed and the medical history of each patient reviewed, including histopathological confirmation of RCC. Starting before administration of the radiolabeled antibody, patients received 10 drops of a saturated potassium iodide solution three times daily for 2 weeks to block 131I uptake in the thyroid.

Five levels (30, 45, 60, 75, and 90 mCi/m2) of 131I labeled to 10 mg of mG250 were investigated. Radiolabeled antibodies were administered i.v. over 60 min using a Pancretec 5000 pump (Pancretec, San Diego, CA). A minimum of three patients formed the cohort for treatment at each dose level. Patients occupied single rooms under strict radiation isolation procedures and were monitored by the Radiation Protection Service of Memorial Hospital until discharge from the hospital when their measured radiation exposure rates decreased to <5 mR/h at 1 m. Additional measurements were taken 1 week later. Patients were not retreated.

Escalating activities of 131I-mG250 were prepared with a constant mass amount of mG250, until the MTDX had been established. An additional nine patients were studied at the MTDx to monitor any possible therapeutic effects.

Vital signs were measured before and at the end of the infusion. Hematological and serum hepatic function parameters were measured at least weekly for 6 weeks or until recovery from toxicity. For determination of serum 131I content, blood samples were drawn just before antibody administration, at the end of the infusion, daily until hospital discharge, and 1 and 2 weeks postinjection. Serum samples were analyzed for radioactivity in a gamma counter (LKB Wallac, Piscataway, NJ).

Patient Eligibility

The Phase I/II radioimmunotherapy study was conducted under an Investigational New Drug Application (BB-IND-3154) with a protocol and consent form approved by the IRB at Memorial Sloan-Kettering Cancer Center (92-71), according to the principles of the Declaration of Helsinki. Before participation, the patients provided written informed consent.

Patients who had prior radiotherapy to the entire pelvis or lumbosacral spine were excluded from the study, as were patients with clinically significant heart disease (New York Heart Association Class III/IV), evidence of central nervous system tumor involvement, any infection requiring antibiotics, or illness requiring steroids. Pregnant or lactating women were not eligible for the study.

Histological slides from all patients were reviewed in the Department of Pathology, Memorial Hospital Sloan-Kettering Cancer Center, for confirmation of RCC. Male and female patients were eligible for the study if they had measurable metastatic clear cell renal carcinoma, had a survival expectancy of ≥ 6 weeks, and were ambulatory with an adequate performance status ( Karnofsky score, ≥ 70%). Clinical laboratory measurements required: a WBC count of ≥ 3500/mm3; a platelet count of ≥ 100,000/mm3; a prothrombin time ≤ 1.3 times that of control; a serum creatinine level of ≤ 2 mg/dl; a serum bilirubin level of ≤ 2 mg/dl; and a serum calcium level of ≤ 12.5 mg/dl.

mAb: Preparation and Radiolabeling

The generation, characteristics, and reactivity of mG250 have been described (23, 24). For each individual patient, 10 mg of mG250 were labeled with an appropriate amount of 131I (New England Nuclear, Boston, MA) to prepare the requisite therapeutic administration (in mCi/m2) using the chloramine T method (27). 131I-mG250 was chromatographed over Sephadex G25 (Pharmacia LKB, Piscataway, NJ) and fractions with peak radioactivity were pooled and passed through a 0.22-μm sterile filter. These freshly prepared clinical samples of 131I-mG250 had a median immunoreactivity of 68% (range, 53–89%) with >95% of 131I precipitable in 10% trichloroacetic acid.

Radioimmunoscintigraphy and Pharmacokinetics

Tumor typing was not performed for G250 antigen because the majority of RCCs express the antigen (23, 24). Indirect evidence for G250 antigen expression was obtained by correlation of 131I-mG250 targeting, assessed by imaging, with known tumor sites.

Patients remained in the hospital between 1 and 4 days. The
number of days in hospital varied depending on the activity administered. Anterior and posterior whole-body $^{131}$I images (General Electric, Milwaukee, WI; ADAC Laboratories, Milpitas, CA) were obtained prior to discharge from the hospital and 1 and 2 weeks after infusion. Single-photon emission CT images of relevant areas were obtained at 1 and, if possible, at 2 weeks postinfusion.

**Determination of Human Antimouse IgG Antibodies**

Terasaki plates (WVR Scientific, West Chester, PA) were coated with mG250 (250 μg/ml in 1% NRS-0.05 m carbonate buffer, pH 9.5) and allowed to adhere overnight at 37°C. Negative control wells were similarly coated with 1% NRS-carbonate without mG250, and positive control wells were coated with 1% normal human serum in 1% NRS-carbonate buffer. To block nonspecific binding, 10% NRS in PBS was added to the wells. Serial dilutions of patient sera, starting at dilutions of 1:10, were then added to the wells and incubated for 1 h at room temperature. Afterward, plates were washed and incubated with rabbit antimouse immunoglobulin-alkaline phosphatase (1:100; DAKO, Carpenteria, CA) in 1% NRS-PBS. Substrate (p-nitrophenyl phosphate; Sigma Chemical Co., St. Louis, MO) at 1 mg/ml was added to the wells, and plates were incubated at 37°C for 30 min. Absorbance was read at 405 nm. Pretreatment patient sera were used as an internal negative control. A positive result was defined as posttreatment serum that gave an absorbance reading at 405 nm above the reading obtained for the pretreatment sample at the same dilution.

**Radiation Dosimetry**

**Whole Body.** Serum clearance was derived from blood samples and expressed as the decay-corrected percentage of the administered activity per liter.

The derivation of whole-body clearance kinetics has been described elsewhere (28). Briefly, clearance curves were obtained by combining survey meter measurements, taken during patient isolation, with planar imaging data collected subsequently. A monoexponential clearance function was fitted to whole body activity and an effective half-time calculated. Cumulated whole-body activity was derived from the area under the clearance curve. Mean absorbed radiation doses to the whole body were calculated using standard MIRD (29) methods by multiplying the cumulated activity in the whole body by a mass-adjusted S factor for $^{131}$I.

**Liver.** The modified conjugate view method (30) was used on planar whole-body images to quantitate $^{131}$I-mG250 uptake in the liver. Briefly, gamma camera sensitivity was derived from $^{131}$I standards of known activity that were imaged simultaneously with the patients. Regions of interest were drawn around the liver at each time point, and the total activity was determined. Liver volumes were obtained from baseline CT scans. Monoexponential clearance functions were fitted to activity (mCi/g) in the liver, and cumulated activities were derived. Absorbed doses to liver were calculated using values of 4.1 gGy/mCi/h for the mean $\beta$ energy emitted per unit cumulated activity (absorbed fraction = 1.0) and 6.3 gGy/mCi/h for the mean photon energy emitted per unit cumulated activity. Absorbed fractions for photons were derived by interpolation, on the basis of liver mass, from the data presented in MIRD pamphlet 3 (31) for monoenergetic 364-keV photons.

**Safety and Toxicity**

Weekly blood samples, obtained from all patients, were analyzed for complete blood counts and laboratory values until all values returned to baseline. Thyroid function tests (serum tri-iodothyronine, total thyroxine, and thyroid-stimulating hormone) were also obtained at baseline and at 6-week intervals until the end of the study. The National Cancer Institute Common Toxicity Criteria were used to grade the severity of toxicity and abnormal laboratory values. All grade 4 toxicities were reported in writing within 24 h to the IRB.

The MTD$_{A}$ was defined as the activity per m$^2$ at which not more than one-third of the total number of patients had grade 3 or 4 toxicity. The response proportions for combination chemotherapy in this patient population ranges from 10 to 20%. Hence, it was decided that, for the Phase II portion of the study, efficacy of <15% would not be worthy of further consideration. We, therefore, decided to accrue 15 patients at the MTD$_{A}$ and to study 15 more patients only if two or more major responses were seen in the initial cohort of 15 patients. Estimation of the response rate within ±17% with 95% confidence was expected.

CT scans of the chest, abdomen, and pelvis were obtained for evaluation of disease at baseline and for determination of possible response. Scans were obtained = 2 weeks prior to radioimmunotherapy and between 6 and 8 weeks after therapy.

All pre- and postradioimmunotherapy CT scans were evaluated by a radiologist. Responses were graded as follows. Complete response was defined as disappearance of all clinical evidence of active disease (tumor and symptoms) for a minimum of 1 month. Partial response was defined as a decrease of ≥50% in the sum of the products of the diameters of all measurable lesions for a minimum of 1 month. Also, there could be no simultaneous increase in size of any lesion or any new lesions. Stable disease was defined as a <50% decrease or no objective change in any disease parameter throughout treatment. Also, there could be no new lesions or worsening of symptoms, and this state had to persist for a minimum of 3 months. Progression was defined as an unequivocal increase in size (>25%) of any lesion(s) or the appearance of any new lesion.

**RESULTS**

Thirty-three patients (24 men and 9 women) with a mean age of 59 years (range, 29–79 years) were entered. Patient characteristics are shown in Table 1. All patients had RCC, confirmed by histopathology, with measurable metastatic disease.

**Hematopoietic Toxicity**

Hematopoietic toxicity (Tables 2 and 3) was not seen in the three patients at the lowest dose (30 mCi/m$^2$). Three (patients 6, 8, and 9) of six patients at 45 mCi/m$^2$ and one (patient 11) of three at 60 mCi/m$^2$ had grade 2 thrombocytopenia, which occurred 28–35 days after infusion. At 75 mCi/m$^2$, one (patient 15) of six patients had grade 4 thrombocytopenia 35 days after infusion, and two (patients 16 and 18) had grade 2 thrombocy-
Radioimmunotherapy with $^{131}$I-G250 mAb in RCC

Severe toxicity did not last more than a week in any patient. Thus, 90 mCi/m² was administered and the extent or nature of hepatic toxicity. Abnormal values did not last more than a week in any patient. No patient required platelet transfusion. RBC counts were unchanged after therapy.

Blood counts for the first six patients (patients 19–24) who received the MTD_A in the Phase I part of the study are illustrated in Fig. 1. Platelet levels declined to their lowest levels ($<25 \times 10^9$ platelets/liter) ~4 weeks postinfusion and then returned to baseline by 8 weeks postinfusion. Similarly, granulocyte counts declined to levels of $1-3 \times 10^9$ platelets/liter, rebounding to baseline levels within 8 weeks postinfusion.

There were no changes in serum thyroid function tests in any patient.

### Hepatic Toxicity

The majority of patients (27 of 33) exhibited transient elevation in serum bilirubin and hepatic enzyme (alkaline phosphatase and aspartate transaminase) levels (Table 2). Fig. 2 illustrates the values for three patients at the lowest and six patients at the highest $^{131}$I doses. Abnormal values did not last longer than 2 weeks, and they returned to baseline within 3 weeks in all patients. There was no correlation between the dose of radioactivity administered and the extent or nature of hepatic toxicity. The majority of patients (27 of 33) exhibited transient elevation in serum bilirubin and hepatic enzyme (alkaline phosphatase and aspartate transaminase) levels (Table 2). Fig. 2 illustrates the values for three patients at the lowest and six patients at the highest $^{131}$I doses. Abnormal values did not last longer than 2 weeks, and they returned to baseline within 3 weeks in all patients. There was no correlation between the dose of radioactivity administered and the extent or nature of hepatic toxicity. The majority of patients (27 of 33) exhibited transient elevation in serum bilirubin and hepatic enzyme (alkaline phosphatase and aspartate transaminase) levels (Table 2). Fig. 2 illustrates the values for three patients at the lowest and six patients at the highest $^{131}$I doses. Abnormal values did not last longer than 2 weeks, and they returned to baseline within 3 weeks in all patients. There was no correlation between the dose of radioactivity administered and the extent or nature of hepatic toxicity.

Table 1  Patient characteristics and distribution within dosage groups

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Body surface area (m²)</th>
<th>Primary cancer</th>
<th>Prior therapy</th>
<th>Extent of metastatic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mCi/m² $^{131}$I</td>
<td>30</td>
<td>65</td>
<td>F</td>
<td>1.7</td>
<td>Nephrectomy</td>
<td>IL-2 sq</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>57</td>
<td>M</td>
<td>2.0</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>67</td>
<td>M</td>
<td>2.1</td>
<td>In place</td>
<td>RT bone</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>46</td>
<td>M</td>
<td>1.8</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>76</td>
<td>F</td>
<td>1.5</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>49</td>
<td>F</td>
<td>1.6</td>
<td>Nephrectomy</td>
<td>IL-2 sq, IFN-γ</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>60</td>
<td>M</td>
<td>1.6</td>
<td>Nephrectomy</td>
<td>IL-2 sq, IFN-γ</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>76</td>
<td>F</td>
<td>1.5</td>
<td>In place</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>60</td>
<td>M</td>
<td>1.8</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>65</td>
<td>M</td>
<td>1.7</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>53</td>
<td>M</td>
<td>2.4</td>
<td>In place</td>
<td>IL-2 sq</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>65</td>
<td>M</td>
<td>1.8</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
<td>60</td>
<td>M</td>
<td>1.9</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>46</td>
<td>M</td>
<td>2.1</td>
<td>Nephrectomy</td>
<td>5-FU, IFN-γ</td>
</tr>
<tr>
<td>17</td>
<td>18</td>
<td>49</td>
<td>M</td>
<td>1.9</td>
<td>In place</td>
<td>None</td>
</tr>
<tr>
<td>19</td>
<td>20</td>
<td>54</td>
<td>F</td>
<td>2.2</td>
<td>In place</td>
<td>IL-2 i.v., IFN-γ</td>
</tr>
<tr>
<td>21</td>
<td>22</td>
<td>66</td>
<td>F</td>
<td>2.6</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>23</td>
<td>24</td>
<td>57</td>
<td>F</td>
<td>2.2</td>
<td>Nephrectomy</td>
<td>IL-2 sq</td>
</tr>
<tr>
<td>25</td>
<td>26</td>
<td>65</td>
<td>M</td>
<td>1.7</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>27</td>
<td>28</td>
<td>63</td>
<td>F</td>
<td>1.7</td>
<td>In place</td>
<td>None</td>
</tr>
<tr>
<td>29</td>
<td>30</td>
<td>72</td>
<td>M</td>
<td>2.0</td>
<td>In place</td>
<td>RT bone</td>
</tr>
<tr>
<td>31</td>
<td>32</td>
<td>42</td>
<td>M</td>
<td>2.0</td>
<td>Nephrectomy</td>
<td>IL-2 i.v., IFN-γ, 5-FU</td>
</tr>
<tr>
<td>33</td>
<td>34</td>
<td>40</td>
<td>M</td>
<td>2.0</td>
<td>Nephrectomy</td>
<td>cis-RA, IL-2 sq</td>
</tr>
<tr>
<td>35</td>
<td>36</td>
<td>51</td>
<td>M</td>
<td>2.0</td>
<td>In place</td>
<td>None</td>
</tr>
<tr>
<td>37</td>
<td>38</td>
<td>75</td>
<td>F</td>
<td>1.6</td>
<td>Nephrectomy</td>
<td>IFN-γ</td>
</tr>
<tr>
<td>39</td>
<td>40</td>
<td>60</td>
<td>M</td>
<td>2.0</td>
<td>Nephrectomy</td>
<td>IFN-γ, IL-2 sq</td>
</tr>
<tr>
<td>41</td>
<td>42</td>
<td>57</td>
<td>M</td>
<td>2.2</td>
<td>In place</td>
<td>None</td>
</tr>
<tr>
<td>43</td>
<td>44</td>
<td>79</td>
<td>M</td>
<td>2.1</td>
<td>Nephrectomy</td>
<td>RT bone</td>
</tr>
<tr>
<td>45</td>
<td>46</td>
<td>55</td>
<td>M</td>
<td>2.2</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
<tr>
<td>47</td>
<td>48</td>
<td>67</td>
<td>F</td>
<td>1.7</td>
<td>Nephrectomy</td>
<td>None</td>
</tr>
</tbody>
</table>

* IL-2, interleukin-2; sq., s.c.; RT, radiation therapy.
* IFN-γ, γ-interferon; cis-RA, cis-retinoic acid; 5-FU, 5-fluouracil.
* Bone/marrow.
* Lung.
* Liver.
* Renal.
* Renal.
* Bone.
* Malignant.
* Infarction.
* 1 cm disease in lung.
* None.
* 1 cm disease.

---

Clinical trials of radioimmunotherapy with $^{131}$I-G250 mAb in RCC

2732 Radioimmunotherapy with $^{131}$I-G250 mAb in RCC
Table 2  Relationship of hematotoxicity and hepatotoxicity to absorbed dose

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Total radioactivity (mCi/131I)</th>
<th>Whole-body absorbed dose (cGy)</th>
<th>Grade</th>
<th>Hematotoxicity (day postinfusion)</th>
<th>Hepatic absorbed dose (cGy)</th>
<th>Grade</th>
<th>Hepatotoxicity (day postinfusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mCi/m²</td>
<td>30 mCi/m²</td>
<td>50 ND</td>
<td>0 NA</td>
<td>310</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>45 mCi/m²</td>
<td>45 mCi/m²</td>
<td>85 ND</td>
<td>0 NA</td>
<td>620</td>
<td>3</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>60 mCi/m²</td>
<td>60 mCi/m²</td>
<td>100 ND</td>
<td>0 NA</td>
<td>550</td>
<td>1</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>75 mCi/m²</td>
<td>75 mCi/m²</td>
<td>143 ND</td>
<td>1 35</td>
<td>770</td>
<td>0</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>90 mCi/m²</td>
<td>90 mCi/m²</td>
<td>200 ND</td>
<td>2 35</td>
<td>2000</td>
<td>3</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

Table 3  Numbers of patients with toxicity at each dose

<table>
<thead>
<tr>
<th>Dose (mCi/m²)</th>
<th>No. of patients per dose</th>
<th>Hematotoxicity (no. of patients per grade)</th>
<th>Hepatotoxicity (no. of patients per grade)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>30 mCi/m²</td>
<td>3</td>
<td>3 3 1 1</td>
<td>1 1 1</td>
</tr>
<tr>
<td>45 mCi/m²</td>
<td>6</td>
<td>3 3 3 1</td>
<td>1 1 4</td>
</tr>
<tr>
<td>60 mCi/m²</td>
<td>3</td>
<td>2 1 3 1</td>
<td>2 1 2</td>
</tr>
<tr>
<td>75 mCi/m²</td>
<td>6</td>
<td>3 2 3 1</td>
<td>2 2 2</td>
</tr>
<tr>
<td>90 mCi/m²</td>
<td>6</td>
<td>1 3 1 3</td>
<td>1 3 2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>33</td>
<td>8 11 7 4 3</td>
<td>6 7 5 15 0</td>
</tr>
</tbody>
</table>

patients. No patient exhibited delayed elevation of liver enzymes or evidence of chronic hepatic dysfunction. The median time of follow-up was 7 months.

The hepatotoxicity was evaluated in the following manner. The grade 3 hyperbilirubinemia observed in patient 1 who had metastatic liver disease was considered disease related. Thus, patients were added to the next dose level, 45 mCi/m². During recruitment into this cohort, a protocol modification was approved by the IRB and the Food and Drug Administration considering hepatic toxicity dose limiting only when it persisted.
for ≥2 weeks. Six patients were entered into this cohort while they awaited approval of the modification. No patient had prolonged hepatic toxicity, and this transient toxicity was, therefore, not considered dose limiting.

Radiation Dosimetry

Whole Body. Absorbed dose estimates to the whole body could be made for 17 patients (Table 2). Estimates ranged from 0.6 to 1.7 Gy with a median value of 0.95 Gy, corresponding to 0.0033–0.011 Gy/mCi, with a median value of 0.0063 Gy/mCi. There was a marked relationship between whole-body absorbed dose and hematological toxicity. No grade 3 or 4 toxicities were observed in patients whose whole-body absorbed dose was <0.95 Gy. In addition, all patients with whole-body doses of >1.2 Gy experienced a grade 3 or 4 toxicity.

Liver. Absorbed dose estimates to liver could be made for 23 patients (Table 2). Estimated absorbed doses ranged from 3.1 to 21 Gy with a median value of 13.0 Gy, corresponding to 0.014 to 0.15 Gy/mCi, with a median value of 0.073 Gy/mCi. Patients with hepatic metastases tended to receive greater absorbed doses to liver (median of 16 Gy compared with 10 Gy for patients without hepatic involvement). However, there was no correlation between the presence or absence of hepatic metastases and toxicity. Of 23 patients with liver absorbed dose estimates, 5 had liver involvement. Three of 5 had grade ≥3 toxicity, as compared to 10 of 18 patients without hepatic metastases.

Immunogenicity

Human antimouse immunoglobulin antibody was detected in all patients by 4 weeks after infusion.

Scintigraphy

Scintigraphy was carried out within the first week after infusion, usually by day 2 or 3. Prior to that time, the patient was in the radiation isolation unit of the hospital. Known tumors were visualized between day 2 and 4 after infusion. All lesions that were ≥2 cm in diameter were imaged.

Posttreatment 131I-mG250 radionuclide scans showed normal blood pool visualization of major organs without increased concentration of isotope in the liver. Fig. 3 is a whole-body 131I-mG250 scan of a representative patient (patient 9). The patient had lesions in the liver, multiple nodal groups, both lungs, and multiple s.c. nodules. Serial anterior and posterior whole-body images demonstrated all disease sites beginning 3 days after 131I-G250 infusion. As radioactivity cleared from normal organs, visualization of the lesions improved; all lesions were clearly visible by day 7 with retention of 131I in tumor evident on day 10, despite 131I decay.

Most patients had metastatic disease predominantly in the liver, the lung and lymph nodes, or the skeleton. Fig. 4 displays representative whole body scans obtained a week after 131I-G250 infusion and grouped according to the predominant location, type, and extent of metastatic lesions. Patients 15, 22, 24, and 25 had predominantly liver metastases and are presented in Fig. 4A. The posterior whole-body image of patient 22 shows diffuse liver involvement with a right renal primary tumor. Patient 15 had a large necrotic lesion in the liver, visible as a “doughnut” in the anterior view. Anterior views of patients 24 and 25 show multiple liver metastases.

Patients 23, 27, and 29 had predominantly lung and lymph node metastases (Fig. 4B). Multiple lung nodules and nodal metastases are visible in the posterior whole-body image of patient 29. The anterior view of patient 23 shows diffuse liver disease and bilateral lung lesions. Patient 27 had right pleural effusion and numerous s.c. lesions, one of which, a 4-mm lesion of the lower lip, is clearly visible in the anterior view.

Patients 3, 8, 11, and 17 had predominantly bone metasta-
Fig. 2. Hepatic function tests in patients 1–3, who received the lowest dose (30 mCi/m²), and patients 19–24, who received the highest dose (90 mCi/m²) during Phase I. A and B, bilirubin; C and D, aspartate transaminase (AST); E and F, alkaline phosphatase.

s (Fig. 4C). The posterior whole-body image of patient 8 shows the primary right renal tumor and a L5 vertebral lesion. The posterior view of patient 11 shows the primary left renal tumor and a right iliac lesion with an L1 vertebral lesion. Patient 17 had a right renal primary, initially detected during evaluation for a pathological fracture of the left femur, as well as a rib lesion. The anterior view of patient 3 showed the right renal primary lesion, and known right iliac and lumbar vertebral lesions. The superior mediastinal and right rib lesions were not seen on a standard pretreatment CT, and were confirmed by additional thin-section (5-mm) thoracic CT.

On the basis of CT estimates of tumor diameter, 27 patients had clearly measurable disease with lesions that were ≥2 cm in diameter; 4 patients had lesions between 1 and 2 cm in diameter that were not as well visualized; and 2 patients (patients 2 and 12) had lesions that were <1 cm in diameter. Most s.c. lesions that were <2 cm in diameter were visualized. In some patients, increased uptake was seen in areas that were normal on standard imaging modalities, including CT scans. In patient 16, increased uptake in the humerus and in the para-aortic nodes could not be confirmed by bone scan or thin-section CT.

Safety and Clinical Observations

Thirty-two patients survived through the end of the study. One patient (patient 6) died of progressive disease during the course of the study.

Patients exhibited no treatment-related changes in their general physical status during the course of the clinical study. The injection of the radiolabeled antibody was well tolerated. No significant changes in vital signs were observed.

Seventeen of 33 patients had stable disease for 2 months after treatment. Patient 27 had complete resolution of a right pleural effusion and of the s.c. lesion of the lower lip. His lung lesion did not increase in size, and all other lesions remained the same. Patients 29 and 31 had a 30–35% reduction in the sum of greatest diameters of lung metastases without the appearance of any new lesions. The responses lasted for ~3 months at which time patients were transferred to other therapy.

Pharmacokinetics

The results of the pharmacokinetic analysis are described in detail elsewhere (28). Monoexponential fits to whole-body and serum clearances yielded biological half-lives of 69 h (SE = 5 h) for whole body and 22 h (SE = 2 h) for serum.

DISCUSSION

This Phase I/II radioactivity-escalation study was performed to determine the targeting and toxicity characteristics of $^{131}$I-G250. Murine $^{131}$I-G250 targeted RCC and its metastases and was retained by these tissues in sufficient amounts and lengths of time to demonstrate high-quality imaging of the lesions. The MTD$_A$ was established at 90 mCi/m², with dose-limiting toxicity being thrombocytopenia. Transient elevation in hepatic enzymes occurred in most patients and was unrelated to administered activity or absorbed dose to liver. Due to its...
reversible nature, hepatic toxicity was not considered dose limiting unless it persisted for ≥2 weeks. In all 27 patients with elevated bilirubin and/or enzymes, values returned to pretreatment levels within 4 weeks of therapy.

The high fraction of G250 antigen-expressing tumors in patients with clear cell morphology subset of RCC is confirmed by the large number of lesions demonstrating selective radioactive antibody uptake. This is particularly noteworthy because patients in the study were not preselected for antigen expression by immunohistochemical analysis.

All lesions that were ≥2 cm in size, independent of location, were visualized by scintigraphy by the first imaging scan, between 2 and 4 days after administration of $^{131}$I-G250. Smaller s.c. lesions were visualized, probably due to their superficial location and increased relative uptake. Bone lesions were visualized in three patients (patients 3, 11, and 24) who had previously been treated with external beam radiation, indicating remaining antigen-positive tumor. Targeting to lesions was rapid, and lesion contrast improved with time. This excellent targeting suggests that G250 has potential as a diagnostic agent, linked to more optimal imaging radionuclides, such as $^{99m}$Tc.

The MTD of radioactivity was established as 90 mCi/m$^2$. Similar studies using other $^{131}$I-labeled antibodies have shown that the MTD was 75 mCi/m$^2$ in patients who had received prior chemotherapy, including mitomycin and/or nitrosourea (20, 32), whereas the MTD in patients who had not received these agents was 90 mCi/m$^2$. In this study none of the patients had been treated with either mitomycin or alkylating agents and were able to tolerate activities up to 90 mCi/m$^2$.

Because the dose-limiting toxicity associated with radioimmunotherapy is transient and not life threatening, the MTD has frequently been considered that dose at which dose-limiting toxicity occurs in not more than a third of patients, by us and by others (32, 33). Yu et al. (33) considered only grade ≥3 hematopoietic toxicity lasting for 2 weeks or longer to be dose limiting and also defined the MTD as that at which not more than a third of patients had dose-limiting toxicity. In this study, 6 A. M. Scott, C. R. Divgi, N. Kemeny, F. Daghighian, J. Schlom, and S. M. Larson. Phase I radioimmunotherapy with $^{131}$I-CC49 in metastatic colon carcinoma: influence of prior chemotherapy upon myelotoxicity, manuscript in preparation.
Fig. 4  Typical whole body images of patients who had primarily liver (A), lung and lymph node (B), or skeletal (C) metastases recorded during the first week after infusion. Posterior views are presented first and scans are grouped by type and extent of lesions. Arrows, specific visible lesions. Arrow style is consistent among lesion types for Figs. 3 and 4. Anterior, anterior view; Posterior, posterior view.
severe hematopoietic toxicity did not last for longer than a week in any patient.

The calculated whole-body absorbed radiation dose was a better predictor of hematopoietic toxicity than administered radioactivity. Of 16 evaluable patients, none with <0.95 Gy to whole body had severe (grade >3) hematopoietic toxicity, whereas 5 of 7 patients who received more than 1 Gy had severe hematopoietic toxicity (34).

Abnormalities in serum hepatic function tests followed infusion of $^{131}$I-G250 in most patients. Hepatotoxicity was unrelated to administered activity or absorbed dose to liver. It was transient and resolved within 2–3 weeks without any detectable long-term abnormalities. The cause of the hepatic toxicity is unclear, but it is probably related to radiation injury from $^{131}$I-G250 bound to bile duct epithelium. The rapid onset and resolution of toxicity suggests that this may be a form of inflammatory response rather than a consequence of stem cell sterilization in biliary epithelium. In addition, it is uncertain whether the serum abnormalities reflect toxicity to the large bile duct cells alone or extend to adjacent normal hepatocytes. Whether this toxicity can be reduced or modified, e.g., through the use of steroids, has not been determined but may be important when repeated dosing becomes possible with less immunogenic G250 constructs. Another possible strategy to reduce toxicity would be to predose patients with unlabeled G250 to saturate hepatic antigen sites and thus reduce bile duct binding of radiolabeled G250.

Vessella et al. (35) treated four patients with metastatic renal cancer with 50 mCi $^{131}$I-labeled mAb A6H and reported tumor doses (in 12 tumors studied) of up to 13 Gy (range, 1–26 cGy/mCi; mean, 16 cGy/mCi). Preliminary analysis indicates that, in our series, radiation dose to tumor was also highly variable, ranging from 2 to 149 Gy. The lack of major responses is not surprising, given that most patients had considerable disease burden and most large tumors received relatively low radiation doses.

eCG250 has been constructed (36). A presurgical trial showed excellent targeting of $^{131}$I-cG250 to RCC, with the optimal dose being 5 mg. There was no evidence of an immune response to the cG250 in these patients (37). Oosterwijk et al. are now carrying out a radioimmunotherapy trial in the Netherlands to determine the MTA and therapeutic efficacy of a single large administration of $^{131}$I-cG250 in patients with metastatic RCC.

Experimental data suggest that multiple administrations of radiolabeled antibody may have greater therapeutic effect than a single infusion (38, 39). A Phase I/II radioimmunotherapy trial is being initiated at the Memorial Sloan-Kettering Cancer Center to evaluate the utility of multiple, outpatient administrations of $^{131}$I-cG250 in patients with measurable metastatic RCC. Both the single- and the multiple-administration studies use an initial pretherapy administration of $^{131}$I-cG250 to evaluate dosimetry and targeting. These trials represent continuing steps in the evaluation of the therapeutic potential of G250 for RCC.

ACKNOWLEDGMENTS

We are grateful to the Clinical Immunology Research Nurses for their assistance in the infusions and monitoring of patients and to Naomi Mendelsohn for assistance in manuscript preparation.

REFERENCES


Phase I/II radioimmunotherapy trial with iodine-131-labeled monoclonal antibody G250 in metastatic renal cell carcinoma.


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/4/11/2729

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.