Targeting of Renal Cell Carcinoma With Iodine-131–Labeled Chimeric Monoclonal Antibody G250


Purpose: Pharmacokinetics, biodistribution, immunogenicity, and imaging characteristics of iodine 131 (131I)-labeled chimeric monoclonal antibody (mAb) G250 (cG250) were studied in patients with renal cell carcinoma (RCC) to determine the therapeutic potential of this antibody.

Patients and Methods: Sixteen patients with RCC received a single intravenous (IV) infusion of 6 mCi 131I-labeled cG250. Five protein dose levels were investigated (2 to 50 mg). Planar scintigraphic images were acquired, and normal tissue biopsies and tumor samples were obtained at surgery (7 days postinjection). The immunogenicity of cG250 was investigated using a sandwich enzyme-linked immunosorbent assay (ELISA) and dosimetric analysis was performed.

Results: In all patients with antigen-positive tumors (n = 13), the primary tumors and all known metastases were clearly visualized. Overall uptake, expressed as the percentage of the injected dose (%ID), in the primary tumors ranged from 2.4 to 9.0. Focally, 131I-cG250 uptake was highly heterogeneous. Approximately 30% to 40% of all patients present with metastatic disease at the time of diagnosis. The treatment of choice for RCC patients without clinical apparent metastatic disease is radical nephrectomy. However, about one third of these patients develop metastases after surgery, usually within 1 year. For these patients, as well as for patients with advanced disease, the 5-year survival rate is less than 10% and the median survival time is less than 2 years. Given such a poor prognosis, there is need for a more effective treatment for RCC patients following radical nephrectomy.

Since the development of hybridoma technology by Köhler and Milstein, therapeutic approaches using monoclonal antibodies (mAbs) have been investigated intensely. Nevertheless, their therapeutic promise still needs fulfillment. In patients with primary RCC, excellent tumor targeting of iodine 131 (131I)-labeled murine mAb G250 (mG250) was shown. In a subsequent activity dose-escalation study with 131I-mG250 in patients with metastasized RCC, a few minor responses were observed. Although mG250 seemed a suitable vehicle for radioimmunotherapy, the occurrence of human antimouse antibodies (HAMA) in all patients studied hampered the use of mG250 for multiple-treatment radioimmunotherapy, which was possibly necessary to obtain durable responses. To minimize the occurrence of HAMA, a chimerized version of mAb G250 (cG250) has been developed. In this study, we report on a clinical phase I protein dose-escalation trial with 131I-labeled cG250 performed to determine the safety of a single intravenous (IV) infusion. The in vivo behavior in terms of pharmacokinetics, biodistribution, immunogenicity, and imaging characteristics of this chimerized antibody as compared with its murine progenitor was studied in patients with primary RCC. In addition, dosimetry was performed to estimate the radiation absorbed dose that was guided to RCC lesions and organs at risk to determine the therapeutic potential of 131I-cG250.

Conclusion: 131I-cG250 tumor uptake is among the highest reported in clinical studies with antitumor antibodies in solid tumors. Since tumor-sterilizing levels may be guided to the tumor when high doses 131I-cG250 are administered (>100 mCi) and cG250 appears to be immunosilent, cG250 is a promising vehicle for radioimmunotherapy in RCC.


PATIENTS AND METHODS

Patient Characteristics

Sixteen patients with a clinical diagnosis of primary RCC were studied: 10 men, age 46 to 80 years (mean, 59), and six women, take as high as 0.52% ID/g was observed. However, intratumoral uptake was highly heterogeneous. 131I-cG250 uptake in nontumorous tissues remained low. Dosimetric analysis showed that up to 0.48 Gy/mCi was guided to the primary tumors. Selected “hot areas” within these tumors received up to 0.72 Gy/mCi. A bone metastasis received 0.23 Gy/mCi and regional lymph node metastases received 0.20 Gy/mCi. Minimal human antichimeric antibody (HACA) levels were detected in two of 16 patients.
age 40 to 74 years (mean, 61). All patients underwent a radical tumor nephrectomy 1 week after infusion of the antibody as part of their treatment plan. Patient characteristics are listed in Table 1. The study protocol and consent forms were approved by the institutional review board (IRB) of the University Hospital Nijmegen. Before participating, all patients reviewed and signed informed consent.

mAb cG250

The generation, characteristics, and reactivity of mAb mG250 have been described earlier. In summary, mAb G250 is reactive with the antigen G250, expressed in all clear cell RCCs and the majority of non-clear cell RCCs. Expression in normal organs is restricted to the gastric mucosal cells and the larger bile ducts. mAb cG250 immunoglobulin G1 (IgG1) has been produced by DNA recombinant technology as described by Velders et al. Essentially, the constant regions of heavy and light chains of mAb mG250 were substituted by their human analogs.

The cG250 antigen-binding specificity was studied in vitro in a competitive binding assay. Briefly, 131I-labeled mG250 was incubated with 10^6 G250-expressing RC-SK-52 cells (a human RCC cell line kindly provided by Dr L.J. Old, Memorial Sloan-Kettering Cancer Center, New York, NY) and increasing amounts of either unlabeled cG250 or unlabeled mG250. Cells were incubated for 4 hours at room temperature. Subsequently, the cell suspension was centrifuged and the activity associated with the cell pellet measured. The first activity peak eluted from the PD 10 column was collected and cold cG250 was added to obtain the desired amount cG250 protein labeled with 6 mCi 131I. Instant thin-layer chromatography (TTLA) was used to determine the presence of free 131I and the immunoreactive fraction of each preparation was determined on freshly trypsinized SK-RK-52 cells as described previously.

Study Design

Before the antibody injection, the medical history of each patient was taken and a physical examination was performed. To block 131I uptake in the thyroid, patients received 100 mg potassium iodide two times daily and 200 mg potassium perchlorate four times daily, starting 24 hours before administration of the radiolabeled antibody. This regimen was continued until surgery. Five protein dose levels of cG250 were investigated (2, 5, 10, 25, and 50 mg) with at least three patients per dose level. 131I-cG250 in 0.9% sodium chloride (NaCl) (total volume, 10 to 20 mL) was administered IV over a 20-minute period. Vital signs were measured frequently and blood samples were drawn just before the antibody administration and at later time points until surgery. Whole-body images were recorded 1, 24, 48, 96, or 120 and 144 or 168 hours postinjection using a dual-headed gamma camera (Multispect 2; Siemens Inc, Hoffman Estates, IL) equipped with a high-energy collimator. At surgery (1 week postinjection), a tumor nephrectomy was performed and biopsies of normal tissue (skin, fat, muscle, peritoneum, and liver) were obtained. All primary tumors were cut in slices of 1-cm thickness. The slices were imaged directly on a single-head gamma camera (Orbiter; Siemens Inc) equipped with a high-energy collimator. Using the images, tumor samples (1 cm^3), including high and low uptake areas, were selected from a tumor slice. Tissues were weighed and the 131I content was measured in a gamma counter. 131I-cG250 blood levels and tumor and normal tissue uptake were expressed as the percentage of the injected dose per gram of tissue (%ID/g). The half-life of disappearance from the blood was calculated by nonlinear least-square regression analysis.

Immunohistochemistry

Antigen expression was determined by immunohistochemistry on a number of selected tumor samples. Briefly, 4-μm cryostat sections of snap-frozen tumor biopsies were acetone-fixed, washed, incubated for 1 hour at room temperature with 100 μL of 10 μg/mL mAb mG250, washed, reacted with peroxidase-labeled rabbit antimonue Ig (RAMPO; DAKO, Carpentina, CA), washed, and developed with 3,3'-diaminobenzidine/0.03% hydrogen peroxide (H_2O_2). Antigen expression was scored as follows: -, less than 5% cells positive; +, 5% to 50% cells positive; ++, 50% to 95% cells positive; and ++++, greater than 95% cells positive.

Determination of Human Anti-Chimeric Antibody Responses

The immunogenicity of cG250 was investigated in serum samples of all patients obtained before and 1, 3, 6, and 12 weeks after infusion. A sandwich enzyme-linked immunosorbent assay (ELISA) was developed in which cG250 was used as capture antibody and biotinylated cG250 as tracer antibody. Biotinylation of cG250 was performed according to the manufacturer's instructions (Pierce Europe, Oud Beijerland, the Netherlands). Wells of 96-well microtiter
plates (Costar, Cambridge, MA) were coated with cG250 by incubation overnight at 4°C with 100 µL of 2.5-µg/mL cG250. After blocking with 1% bovine serum albumin (BSA) in PBS (1 hour at 37°C), serial dilutions of patient sera were added (100 µL/well, starting undiluted, 1 hour at 37°C), rinsed (PBS/1% BSA, three times), and incubated with 100 µL of 1.25-µg/mL biotinylated cG250 (1 hour at 37°C). After another wash (PBS/1% BSA, three times), wells were incubated with streptavidin-biotinylated horseradish peroxidase complex for 30 minutes at 37°C (Amersham, Little Chalfont, United Kingdom). Wells were washed (PBS/1% BSA, three times) and developed with 3,3',5,5'-tetramethylbenzidine/H₂O₂ (100 µL, 20 minutes at room temperature). The reaction was stopped by adding 100 µL 2.5-mol/L sulfuric acid (H₂SO₄). Optical density was measured at 450 nm. Proven human antimouse antibody (HAMA)-positive sera from patients who participated in previous trials with mG250 were used as positive controls (a kind gift of Dr N.H. Bander, New York Hospital, New York, NY). Serial dilutions of antiidiotype antibody NUH-82 IgG1 were used to obtain a standard curve. The detection limit of the assay was 25 ng NUH-82/mL.

To define further the epitopes of the cG250 IgG1 molecule against which a human anti-chimeric antibody (HACA) response might be directed, sera that were positive in the sandwich ELISA as described earlier were investigated in two similar ELISAs, as follows: (1) mG250 IgG1 as catcher/tracer antibody to determine any response against the murine part of cG250, and (2) chimeric M0V1 (a mAb reactive with an ovarian cancer-associated antigen) with the same human constant regions as cG250 was used as catcher antibody and biotinylated cG250 as tracer antibody to detect reactivity directed against the human part of cG250.

**Dosimetry**

Dosimetric analysis was performed in 10 patients (two patients at each dose level). The conjugated views counting technique for the whole-body images was used to quantitate ¹³¹I-cG250 uptake in selected organs. Briefly, regions of interest (ROI) over the tumor, whole body, chest, total abdomen, pelvis, liver, spleen, and extremities were drawn on anterior and posterior view images recorded at all five time points. The differences in attenuation between the thorax, extremities, and abdomen were determined using a ¹³¹I transmission flood source. The geometric mean of anterior and posterior counts was calculated after correction for differences in attenuation. After background subtraction and correction for physical decay, absolute retention of activity in a ROI was calculated via comparison with the activity of the total-body scintigram directly after infusion. Data were expressed as a percentage of the total injected dose (%ID). Radiation-absorbed doses to the tumor and to organs of interest were calculated with the MIRDose3 program.

**RESULTS**

**Affinity, Immunoreactivity, Radiolabeling, and Quality Control**

To evaluate whether chimerization of mAb G250 had affected the binding characteristics of the antibody, competitive binding assays were performed. Competition analysis showed that unlabeled cG250 was at least equally effective in displacing ¹²⁵I-mG250 as unlabeled mG250. The IC₅₀ of mG250 was 1,100 pmol/L and the inhibition concentration₅₀ (IC₅₀) of cG250 was 700 pmol/L (data not shown). The affinity constants (Kₐ) of both mG250 and cG250 as determined in a Scatchard analysis were identical with a Kₐ = 4 x 10⁹ mol/L⁻¹.

The efficiency of the radioiodination reaction, ie, the percentage of ¹³¹I added to the reaction that was bound to the antibody, varied between 65% and 80%. After PD 10 elution, ITLC showed that greater than 99% of the pooled radioactivity was protein-bound (release criterium = 95%). The mean immunoreactive fraction of the ¹³¹I-cG250
preparations was 95% ± 5%. In two patients, the immunoreactive fraction of plasma $^{131}$I activity was monitored up to 1 week postinjection and at least 80% of the circulating $^{131}$I activity was shown to be immunoreactive for the investigated period (0 to 7 days postinjection).

Clinical Observations

The injection of the radiolabeled antibody was tolerated well by all patients and no side effects were seen. No significant changes in vital signs, hematologic, or blood chemistry parameters were observed.

Pharmacokinetics

The mean blood clearance curves from patients with G250-positive tumors at the various dose levels are shown in Fig 1. Curves fitted a two-compartment model. The half-life of the distribution phase ($t_{1/2\alpha}$) was similar at all protein dose levels ($t_{1/2\alpha} = 3.8 \pm 2.0$ hours). In contrast, the half-life of the elimination phase ($t_{1/2\beta}$) differed significantly between the 2-mg and the higher protein dose levels. At the 2 mg protein dose level, $t_{1/2\beta}$ was significantly lower ($t_{1/2\beta} = 39.6 \pm 9.6$ hours) as compared with the other, higher protein dose levels ($t_{1/2\beta} = 68.5 \pm 13.5$ hours) ($P < .005$). Mean overall cumulative excretion in the urine in 7 days was 59.9% ± 12.9% ID, which indicates that the primary route of radiolabel excretion was via the kidneys.

Imaging

Excellent visualization of primary tumors was obtained in all 13 patients with G250 antigen-positive tumors. In

Fig 2. Whole-body scans recorded 7 days postinjection. (A) patient no. 2, 2-mg dose; (B) patient no. 9, 10-mg dose. Images were printed at the same grey scale. Arrows: primary tumors in both patients. Note difference in relative liver uptake between the 2 dose levels. Arrowhead: metastasis of the ischiadic bone. Ant, anterior view; Post, posterior view.
general, tumors lesions were visualized from 1 day postinjection onwards. Due to the background clearance of $^{131}$I-cG250, image quality improved with time. In some cases studied at the lower protein dose levels (2 and 5 mg), bowel activity complicated interpretation of the images. All metastatic lesions (five) as identified by ultrasound, computed tomography, or x-ray, were visualized: one bone (patient no. 2, Fig 2), one pulmonary (patient no. 4), one vaginal (patient no. 9), and multiple regional lymph node metastases (patient no. 8, Fig 3).

At higher protein dose levels (25 and 50 mg), tumor accumulation was less pronounced, and as a consequence, tumor images were less clear as compared with images at the lower protein dose levels. Nevertheless, tumors were still well delineated at the higher protein dose levels.

As was observed with mG250, liver uptake was more pronounced at the 2-mg dose level as compared with the higher protein dose levels (Fig 2).

**Biodistribution**

Extensive tumor sampling and sampling of selected normal tissues 8 days postinjection was performed to determine the absolute uptake of $^{131}$I-cG250 (Table 2). The overall uptake in the tumorous kidneys (including the remaining normal kidney tissue and perirenal fat) ranged from 2.4% to 9.0% ID. Most of these tumorous kidneys were large, with a weight greater than 1,000 g (range, 300 to 1,700 g), and some contained large areas with extensive necrosis. Therefore, 1-cm$^3$ samples were taken from viable tumor areas and the radioactivity in each sample was determined separately. In most antigen-positive tumors, considerable differences in regional tumor uptake, up to two orders of magnitude, were observed (Fig 4). In three tumors (patients no. 1, 9, and 16), focal tumor uptake was greater than 0.1% ID/g. The highest focal tumor uptake was found in the primary tumor of patient no. 16 (5-mg dose): 0.5233% ID/g. In the primary tumors of patients who received 25 or 50 mg cG250, tumor uptake was not greater than 0.0170% ID/g, while at the lower protein doses, higher focal uptake was found. Uptake in a cluster of tumor-positive lymph nodes (patient no. 8) was 0.0136% ID/g. Uptake in necrotic areas remained low and was not greater than 0.0015% ID/g. cG250 uptake in antigen-negative tumors was not greater than 0.0040% ID/g (range, 0.0005% to 0.0040% ID/g; Table 2).

Activity measurements of the liver biopsies (all patients with antigen-positive tumors) confirmed the enhanced liver uptake, as noted on the images, at the lower protein dose levels (0.0034% ± 0.0020% ID/g at 2-mg dose and 0.0017% ± 0.0002% ID/g at 5-mg dose) as compared with the liver uptake at the higher protein dose levels (0.0009% ± 0.0003% ID/g). Assuming a total liver mass of 1.5 kg, liver uptake at the 2-mg dose accounted for 5.1% of the total injected dose, while at the higher protein dose levels (10, 25, and 50 mg), liver uptake accounted for 1.4% of the total injected dose.

**HACA Responses**

In two patients (no. 7 and 8), a HACA response could be detected in the sera obtained at 12 weeks postinjection as determined by sandwich ELISA with cG250 as catcher/tracer antibody. HACA responses were not detectable in sera obtained earlier than 12 weeks postinjection and HACA levels in these two patients were low, with a maximum equivalent of 300 ng/mL NUH-82, whereas the positive control sera (from patients who participated
### Table 2. Biodistribution of $^{131}$I-cG250 mAb

<table>
<thead>
<tr>
<th>Patient No./Dose</th>
<th>RCC Samples</th>
<th>Tissues (uptake in %ID $\times 10^{-3}$/g)</th>
<th>Normal Kidney</th>
<th>Liver</th>
<th>Peritoneum</th>
<th>Fat</th>
<th>Muscle</th>
<th>Skin</th>
<th>Blood</th>
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</thead>
<tbody>
<tr>
<td>1/2 mg</td>
<td>342.0</td>
<td>102.0</td>
<td>80.0</td>
<td>11.0</td>
<td>4.0</td>
<td>2.1</td>
<td>0.2</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>2/2 mg</td>
<td>76.0</td>
<td>52.0</td>
<td>21.0</td>
<td>0.4</td>
<td>3.7</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>3/2 mg</td>
<td>7.0</td>
<td>6.0</td>
<td>5.0</td>
<td>0.5</td>
<td>1.3</td>
<td>1.1</td>
<td>0.2</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>4/5 mg</td>
<td>42.0</td>
<td>36.0</td>
<td>28.0</td>
<td>0.9</td>
<td>1.8</td>
<td>1.7</td>
<td>2.4</td>
<td>0.5</td>
<td>2.0</td>
</tr>
<tr>
<td>5/5 mg*</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.7</td>
<td>2.6</td>
<td>2.9</td>
<td>0.7</td>
<td>0.3</td>
<td>2.2</td>
</tr>
<tr>
<td>6/5 mg*</td>
<td>4.0</td>
<td>2.0</td>
<td>1.5</td>
<td>0.9</td>
<td>2.5</td>
<td>2.0</td>
<td>5.2</td>
<td>1.3</td>
<td>2.4</td>
</tr>
<tr>
<td>7/10 mg</td>
<td>28.0</td>
<td>27.0</td>
<td>26.0</td>
<td>3.6</td>
<td>0.9</td>
<td>1.5</td>
<td>0.2</td>
<td>0.8</td>
<td>1.7</td>
</tr>
<tr>
<td>8/10 mg</td>
<td>23.0</td>
<td>22.0</td>
<td>21.0</td>
<td>1.4</td>
<td>1.2</td>
<td>3.7</td>
<td>0.2</td>
<td>0.2</td>
<td>1.2</td>
</tr>
<tr>
<td>9/10 mg</td>
<td>169.0</td>
<td>131.0</td>
<td>130.0</td>
<td>0.6</td>
<td>1.1</td>
<td>1.7</td>
<td>0.2</td>
<td>0.4</td>
<td>1.8</td>
</tr>
<tr>
<td>10/25 mg*</td>
<td>1.7</td>
<td>1.5</td>
<td></td>
<td>2.2</td>
<td>1.9</td>
<td>1.5</td>
<td>0.8</td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>11/25 mg</td>
<td>60.2</td>
<td>30.3</td>
<td>17.2</td>
<td>1.0</td>
<td>0.9</td>
<td>0.9</td>
<td>0.3</td>
<td>0.3</td>
<td>1.4</td>
</tr>
<tr>
<td>12/25 mg</td>
<td>3.6</td>
<td>3.1</td>
<td>2.6</td>
<td>2.3</td>
<td>0.7</td>
<td>0.4</td>
<td>1.1</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>13/50 mg</td>
<td>6.3</td>
<td>5.7</td>
<td>4.8</td>
<td>4.6</td>
<td>1.2</td>
<td>1.1</td>
<td>1.6</td>
<td>0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>14/50 mg</td>
<td>12.0</td>
<td>12.0</td>
<td>11.9</td>
<td>1.6</td>
<td>1.2</td>
<td>2.5</td>
<td>0.6</td>
<td>0.8</td>
<td>2.9</td>
</tr>
<tr>
<td>15/50 mg</td>
<td>9.9</td>
<td>9.1</td>
<td>8.2</td>
<td>7.7</td>
<td>0.7</td>
<td>0.6</td>
<td>1.1</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>16/5 mg*</td>
<td>523.3</td>
<td>393.2</td>
<td>324.7</td>
<td>96.1</td>
<td>0.8</td>
<td>1.5</td>
<td>0.9</td>
<td>0.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Patients with an antigen-negative tumor.
†Extra patient at 5-mg dose level.

In trials with mG250) showed HAMA levels up to 36,000 ng/mL NUH-82. Follow-up serum samples, drawn at 20 weeks postinjection, showed that the HACA responses in these two patients did not increase. Further analysis of these HACA responses with the ELISAs using (1) murine G250 as catcher/tracer antibody and (2) chimeric MOv18 as catcher/cG250 as tracer antibody showed that these responses were not directed against the human part of the cG250 IgG molecule.

In the preinjection serum sample of patient no. 9, preexisting antibodies reactive with both mG250 and cG250 were detected (300 ng NUH-82/mL).

**Dosimetry**

Dosimetric analysis performed in 10 patients (two patients per dose level) showed that the radiation-absorbed dose to the G250-positive primary tumors ranged from .06 (patient no. 1, 2-mg dose) to .48 Gy/mCi (patient no. 16/5 mg).
16, 5-mg dose). As considerable tumor heterogeneity was observed, which was partially a reflection of tumor necrosis, radiation-absorbed dose estimates were also calculated for selected “hot areas” within these primary tumors. This analysis showed that considerably higher radiation-absorbed doses were guided to these areas (Table 3). The highest amount of radioactivity was delivered to a tumor thrombus, which received .72 Gy/ mCi (patient no. 16, 5-mg dose, Fig 5). More importantly, dosimetric analysis of metastatic lesions showed that a bone metastasis received .23 Gy/mCi (patient no. 2, 2-mg dose) and regional lymph node metastases received .20 Gy/mCi (patient no. 8, 10-mg dose).

Calculation of the mean radiation-absorbed dose to organs at risk indicated that .17 ± .08 Gy/mCi was delivered to the thyroid, the liver received .04 ± .01 Gy/mCi, and the bone marrow received .011 ± .003 Gy/mCi.

### DISCUSSION

A phase I protein dose-escalation study was performed to determine the pharmacokinetics, toxicity, immunogenicity, and imaging characteristics of 131I-labeled mAb cG250 in patients with RCC. Previous studies have indicated that mAb mG250 may have considerable therapeutic potential as a vehicle for radioimmunotherapy,45 provided the strong HAMA responses can be circumvented. Multiple doses are probably needed to produce any lasting responses in radioimmunotherapy. Chimerization resulted in a major decrease of the immunogenicity of this antibody: minimal HACA responses were detected in only two patients as determined by cG250 sandwich ELISA. No measurable immune responses were detected against the human part of cG250. The minimal, presumably clinically nonrelevant HACA responses, observed in two of 16 patients, illustrate the highly reduced immunogenicity of cG250 as compared with its murine progenitor. Therefore, multiple treatments with cG250 seem feasible. These observations are in accordance with the results of other studies: Meredith et al43 showed that chimerization of mAb 17-1A highly reduced the immunogenicity and Buist et al44 found similar results with chimeric mAb MOv18.

The in vitro binding characteristics of cG250 were similar to those of mG250, which demonstrates that chimerization of the antibody did not affect specificity, affinity, or avidity. In general, the in vivo behavior, including the half-life ($t_{1/2}$), of cG250 was comparable to mG250 ($t_{1/2}$ cG250 68.5 hours $v$ $t_{1/2}$ mG250 47 hours). The first chimerized antibodies, eg, chimeric mAb 17-1A13 and chimeric mAb B72.3,15 showed much longer half-lives in patients than their murine progenitors, which resulted in relatively poor tumor/nontumor ratios, considered a disadvantage in radioimmunotherapy. However, other chimerized antibodies, eg, chimeric mAb MOv18,16 chimeric anticarcinoembryonic antigen (anti-CEA) antibodies,17 and chimeric mAb LL2,18 have shown half-lives similar to their murine counterpart, as was observed for mAb cG250.

Antigen-mediated tumor uptake of 131I-cG250 was demonstrated by the difference in uptake between antigen-positive versus antigen-negative tumors: uptake in samples of antigen-negative tumors (7 days postinjection) was not greater than 0.0040% ID/g (blood 7 days postinjection, 0.0042% ID/g), while uptake in antigen-positive tumors was as high as 0.5233% ID/g (blood, 0.0028% ID/g). Extensive sampling of the primary tumors showed that regional differences in tumor uptake were as high as two orders of magnitude. This observed heterogeneity was much more pronounced at the 2-, 5-, and 10-mg dose levels as compared with the 25- and 50-mg dose levels. In addition, tumor uptake greater than 0.1% ID/g was observed only at the 2-, 5-, and 10-mg dose levels, while maximum uptake at the 25- and 50-mg dose levels was 0.0170% ID/g and 0.0120% ID/g, respectively. These ob-

### Table 3. Dosimetric Analysis

<table>
<thead>
<tr>
<th>Patient No./Dose</th>
<th>Whole Tumor</th>
<th>ROI Primary Tumor</th>
<th>Metastases</th>
<th>Liver</th>
<th>Thyroid</th>
<th>Bone Marrow</th>
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<tbody>
<tr>
<td>1/2 mg</td>
<td>0.06</td>
<td>0.42</td>
<td>0.06</td>
<td>0.17</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>2/2 mg</td>
<td>0.09</td>
<td>0.28</td>
<td>0.23 (bone)</td>
<td>0.06</td>
<td>0.34</td>
<td>0.009</td>
</tr>
<tr>
<td>4/5 mg</td>
<td>0.10</td>
<td>0.19</td>
<td>0.06</td>
<td>0.13</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>16/5 mg</td>
<td>0.48</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/10 mg</td>
<td>0.15</td>
<td>0.31</td>
<td>0.20 (lymph nodes)</td>
<td>0.04</td>
<td>0.13</td>
<td>0.010</td>
</tr>
<tr>
<td>9/10 mg</td>
<td>0.16</td>
<td>0.53</td>
<td>0.06</td>
<td>0.18</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>11/25 mg</td>
<td>0.09</td>
<td>0.13</td>
<td></td>
<td>0.05</td>
<td>0.26</td>
<td>0.010</td>
</tr>
<tr>
<td>12/25 mg</td>
<td>0.07</td>
<td>0.09</td>
<td>0.01 (brain)</td>
<td>0.03</td>
<td>0.11</td>
<td>0.011</td>
</tr>
<tr>
<td>14/50 mg</td>
<td>0.15</td>
<td>0.18</td>
<td></td>
<td>0.05</td>
<td>0.12</td>
<td>0.018</td>
</tr>
<tr>
<td>15/50 mg</td>
<td>0.12</td>
<td>0.16</td>
<td></td>
<td>0.02</td>
<td>0.09</td>
<td>0.013</td>
</tr>
<tr>
<td>Overall</td>
<td>0.04 ± 0.01</td>
<td>0.17 ± 0.08</td>
<td>0.011 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ROI, region of interest.
Fig 5. Whole-body scan (patient no. 16, 2-mg dose) recorded 7 days postinjection. Arrows: primary tumor; arrowheads: tumor thrombus extending into the caval vein. The primary tumor (without the tumor thrombus) received a dose of .48 Gy/mCi, and the tumor thrombus received a dose of .72 Gy/mCi.

Observations suggest a saturation of accessible G250 epitopes in the tumor at the higher protein doses. In the study with mG250, a similar relative decrease in tumor uptake with increasing protein dose was observed. In contrast, in investigations with other antitumor antibodies, doses of 10 mg/kg or greater have been administered without any indication of tumor saturation.

Saturable, antigen-mediated liver uptake was observed with cG250, similar to mG250. This liver uptake is in accordance with the known antigen expression on the larger bile ducts. Assuming a liver weight of 1,500 g, mean liver uptake at the 2-mg dose level accounted for 5.1% of the total injected dose, while at the higher protein doses (10, 25, and 50 mg) mean liver uptake accounted for 1.4% of the total injected dose. At the 2-mg protein dose level, blood clearance was faster as compared with the other protein dose levels. This might be the result of enhanced hepatic uptake at this lower protein dose level, since the hepatic compartment absorbed a relatively high amount of cG250 at 2 mg protein as compared with higher protein dose levels. Furthermore, higher excretion of $^{131}$I in the bile as a result from this hepatic uptake may also explain the observed bowel activity at the lower protein dose levels.

All antigen-positive primary tumors, as well as all metastatic lesions, as identified by conventional imaging techniques, were visualized. Bander et al reported 90% successful imaging of primary RCC sites with $^{131}$I-mG250. Additionally, occult lesions, confirmed at surgery were visualized. In our study, no additional lesions were detected. Larger studies are needed to evaluate the diagnostic potential of mAb G250 as an imaging agent.

Radioimmunotherapy is most likely to be effective in metastasized patients with a relatively low tumor burden. In RCC patients with recurrent or metastasized disease, radioimmunotherapy might be applied as a second-line therapeutic modality, since no other effective therapy is available. Dosimetric analyses indicated that radiation-absorbed doses ranging from .06 to .48 Gy/mCi were guided to the primary tumors. More importantly, a number of regional lymph node metastases received .20 Gy/mCi and a bone metastasis received .23 Gy/mCi. In solid tumors, responses of radioimmunotherapy can be expected when radiation-absorbed doses greater than 50 Gy are delivered to the tumor lesions. For bone marrow, 2 Gy is considered to be the maximum-tolerated radiation dose in radioimmunotherapy. Assuming the bone marrow to be the dose-limiting organ, doses as high as 200 mCi $^{131}$I-cG250 can be administered safely. Thus radiation-absorbed doses close to tumor-sterilizing levels seem achievable. Furthermore, single high-dose radioimmunotherapy with bone marrow support or multiple dosing, either with or without bone marrow support, may be necessary to achieve major responses. With both approaches, considerably higher radioactivity doses can be administered.

In conclusion, cG250 seems to be a good candidate for radioimmunotherapy of RCC. The highly reduced immunogenicity opens the possibility of multiple-treatment therapy. Further studies with cG250 are warranted to investigate whether this approach will lead to an effective therapy for patients with metastasized RCC.

ACKNOWLEDGMENT

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REFERENCES


