Phase I Radioimmunotherapy of Metastatic Renal Cell Carcinoma with $^{131}$I-labeled Chimeric Monoclonal Antibody G250


Abstract
Clinical tumor targeting studies with chimeric monoclonal antibody G250 (cG250) in renal cell carcinoma (RCC) patients indicated the potential use of this antibody for radioimmunotherapy. Here we report on a phase I activity dose escalation study to determine the safety, the maximum tolerable dose (MTD), and the possible therapeutic potential of $^{131}$I-labeled cG250 in patients with progressive metastatic RCC. All patients ($n = 12$) received a diagnostic i.v. infusion of 5 mg of cG250 labeled with a high dose of $^{131}$I. If accumulation of the antibody in metastatic lesions was observed, patients were hospitalized and a second, therapeutic, i.v. infusion of 5 mg of cG250 labeled with a high dose of $^{131}$I was administered ($n = 8$). Three patients per dose level were entered, starting at 1665 MBq/m$^2$. If no dose-limiting toxicity occurred, the study continued at the next dose level (555 MBq/m$^2$ to 2775 MBq/m$^2$). Most patients experienced mild nausea without vomiting. No other complaints were reported during hospitalization. In two of two patients who received a dose of 2775 MBq/m$^2$, grade IV hematological toxicity was observed, which was defined as dose limiting. Thus, the MTD was set at 2220 MBq/m$^2$. In one patient (2220 MBq/m$^2$), stable disease (lasting 3-6 months) was achieved, whereas another patient (2220 MBq/m$^2$) showed a partial response that is ongoing (> 9 months). The minor responses observed in this phase I trial in patients with an advanced stage of RCC are encouraging and warrant further study in a phase II setting at the MTD to determine the efficacy of radioimmunotherapy for metastatic RCC.

Introduction
RCC, the most common malignancy of the adult kidney, accounts for approximately 3% of all adult cancers (1). Roughly one-third of all patients present with metastatic disease at the time of diagnosis (2). An additional 40% of the patients treated for their primary tumor will ultimately relapse with metastatic disease. For these patients, the 5-year survival is <10% and the median survival time is <2 years (2). Chemotherapy and radiotherapy have limited value, and the overall response rate in most immunotherapy trials does not exceed 20% (3). Hence, the prognosis of metastatic RCC is very poor, and there is a definite need for more effective treatment modalities.

Radioimmunotherapy using monoclonal antibodies could lead to selective delivery of high radiation doses to tumors. Although complete and lasting responses have been achieved with radioimmunotherapy in patients with relapsed hematological malignancies despite chemotherapy (4-6), its promise for the treatment of solid tumors still needs fulfillment (7).

Previous clinical tumor targeting studies with murine as well as mAb cG250 demonstrated the ability of this antibody to guide high doses of radioactivity to RCC lesions (8, 9). Uptake of this antibody in primary renal cell carcinomas (up to 0.52% of the injected dose per gram of tumor tissue) was among the highest ever reported in human solid tumors. Furthermore, dosimetric analysis indicated that radiation doses as high as 1.9 cGy/MBq could be delivered focally to primary RCC tumors (9). Additionally, in a phase I/II radioimmunotherapy trial with murine mAb G250, several minor responses were observed in patients with advanced metastatic RCC (10).

The occurrence of HAMA responses, observed in all patients who received murine mAb G250, hampered the use of this antibody for multiple treatment radioimmunotherapy (8). Therefore, a chimerized version of this antibody, with the same specificity, affinity, and binding characteristics, has been produced. mAb cG250 appeared to be immunosilent, thus potentially allowing multiple dosing treatment (9). Here we report on a phase I radioimmunotherapy trial to determine the safety, the MTD, and the possible therapeutic potential of $^{131}$I-labeled mAb cG250.

Patients and Methods

Patient Characteristics. Twelve patients with metastatic RCC were studied: 10 men, ages 44-77 years (median age, 59 years); and 2 women, ages 44 and 50 years. All patients had undergone a tumor nephrectomy in the past and had measurable progressive disease at the time of treatment. To exclude the

---

1 Presented at the “Seventh Conference on Radioimmunodetection and Radioimmunotherapy of Cancer,” October 15-17, 1998, Princeton, NJ. This study was supported by the Dutch Cancer Society/KWF Grant 94-738.
2 To whom requests for reprints should be addressed, at Department of Nuclear Medicine, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Phone: 31-24-3613813; Fax: 31-24-3618942; E-mail: O.Boerman@nugen.azn.nl.

---

2 The abbreviations used are: RCC, renal cell carcinoma; mAb, monoclonal antibody; cG250, chimeric G250; HAMA, human antimouse antibody; MTD, maximum tolerable dose; CT, computed tomography; HACA, human antichimeric antibody.
possible effects of other treatments, participation in this trial was only allowed at least 3 months after the last course of any other therapy. Patient characteristics are listed in Table 1. The study protocol and consent forms were approved by the institutional review board of the University Hospital Nijmegen. Before participating, all patients reviewed and signed informed consent.

mAb cG250. The generation, characteristics, and reactivity of mAb cG250 and its murine progenitor have been described elsewhere (9, 11). mAb cG250 has been produced by DNA recombinant technology as described by Velders et al. (12). Briefly, the constant regions of heavy and light chains of murine mAb G250 were substituted by their human analogues. mAb cG250 is reactive with the antigen G250, which is expressed in all clear-cell RCC and in the majority of non-clear-cell RCC (13). Expression in normal organs is restricted to the gastric mucosal cells and the larger bile ducts. Recently, the G250 antigen has been cloned and was found to be homologous to the MN antigen expressed in cervical carcinomas (14).

Radiolabeling and Quality Control. Sterile vials with purified and pyrogen-free cG250 were generously provided by Centocor Europe BV (Leiden, The Netherlands). mAb cG250 was labeled according to the IodoGen method (15), using a remote system as described by Weadock et al. (16). The remote system was assembled in a lead-shielded, ventilated glove box. Briefly, 1.0 ml of cG250 (5 mg/ml) and 100 μl of 0.5 M sodium phosphate (pH 7.2) were added directly to an IodoGen-coated vial (200 μg of IodoGen; Pierce Chemical Company, Rockford, IL). Na131I (Nordion, Fleurus, Belgium) was added via the remote system, and the total reaction volume was adjusted to 4 ml with 50 mM sodium phosphate (pH 7.2). After 15 min of incubation (stirring) at room temperature, 2.0 ml of a 20% anion exchange resin suspension (AG 1-X8; Bio-Rad Laboratories, Hercules, CA) was applied to the reaction mixture. After 5 min of incubation, the reaction mixture was filtered through a 0.22 μm Milli-Fil GS filter unit (Millipore, Bedford, MA) and collected in a sterile glass vial containing 20 ml of PBS with 20% human serum albumin. The desired amount of radioactivity was collected in a sterile glass vial containing 20 ml of PBS with 20% human serum albumin. The desired amount of radioactivity was obtained from the collection vial, and cold cG250 was added to obtain a final protein dose of 5 mg.

Instant TLC was used to determine the presence of free radiiodine, using Gelman ITLC-SG strips (Gelman Sciences, Inc., Ann Arbor, MI) and 0.15 M sodium citrate (pH 5.5) as the mobile phase (release criterion, <5% free radiiodine).

To exclude the formation of antibody aggregates as a result of radiolysis, the high-activity dose-labeled mAb cG250 preparations were analyzed by high-performance liquid chromatography at several time points on a gel filtration column (Biosep SEC 3000; Phenomenex, Torrance, CA), using 0.1 M Tris-0.15 M NaCl-0.001 M EDTA (pH 7.2) as the eluent.

Prior to the administration of the radiolabeled antibody, a baseline CT scan or planar X-rays were obtained for adequate measurement of metastases. To block 131I uptake in the thyroid, patients received 100 mg of potassium iodide twice daily and 200 mg of potassium perchlorate four times daily, starting 24 h prior to the administration of the radiolabeled antibody. This regimen was continued for 1 week after the first administration of mAb cG250 and for 2–3 weeks after the second administration of mAb cG250, depending on the remaining total body activity. All patients received a diagnostic i.v. infusion of 5 mg of cG250 labeled with 220 MBq of 131I followed by acquisition of five whole-body scans at 1, 24, 48, 120, and 144 h post infusion, using a dual-headed gamma camera (Multispect 2; Siemens Inc., Hoffman Estates, IL). If accumulation of the antibody in metastatic lesions was visualized, patients were hospitalized on the ward for radionuclide therapy where the second, therapeutic, i.v. infusion of 5 mg of cG250 labeled with a high dose of 131I was administered. Patients remained hospitalized until the total dose of 131I in the body had dropped below 370 MBq. Additional whole-body radioimmunoscintigrams were obtained 1, 2, and 3 weeks (if feasible) after the second antibody administration. Three patients per dose level were entered, starting at 1665 MBq/m² of body surface area. Toxicity was monitored up to 10

---

### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Prior therapy</th>
<th>Metastases visualized</th>
<th>Activity dose level (MBq/m²)</th>
<th>131I-labeled cG250 activity dose (MBq)</th>
<th>Whole body toxicity grade (WHO)</th>
<th>Therapy outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>Male</td>
<td>No</td>
<td>Yes</td>
<td>1665</td>
<td>3034</td>
<td>1.2</td>
<td>1 Progression</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>Male</td>
<td>IFN-α, IL-2</td>
<td>Yes</td>
<td>1665</td>
<td>3182</td>
<td>1.0</td>
<td>2 Progression</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>Male</td>
<td>No</td>
<td>Yes</td>
<td>1665</td>
<td>3515</td>
<td>1.1</td>
<td>1 Progression</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>Male</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>Male</td>
<td>IFN-α, vinblastine</td>
<td>Yes</td>
<td>2220</td>
<td>4070</td>
<td>1.4</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>Male</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>1 Stable disease</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>Male</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>55</td>
<td>Male</td>
<td>No</td>
<td>Yes</td>
<td>2220</td>
<td>4329</td>
<td>1.6</td>
<td>2 Minor response</td>
</tr>
<tr>
<td>9</td>
<td>61</td>
<td>Male</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>Female</td>
<td>IFN-α, IL-2</td>
<td>Yes</td>
<td>2220</td>
<td>3663</td>
<td>1.7</td>
<td>3 Progression</td>
</tr>
<tr>
<td>11</td>
<td>44</td>
<td>Male</td>
<td>No</td>
<td>Yes</td>
<td>2775</td>
<td>5550</td>
<td>2.0</td>
<td>4 Progression</td>
</tr>
<tr>
<td>12</td>
<td>44</td>
<td>Female</td>
<td>IFN-α, IL-2</td>
<td>No</td>
<td>2775</td>
<td>5106</td>
<td>2.0</td>
<td>4 Progression</td>
</tr>
</tbody>
</table>

* IFN-α, interferon-α; IL-2, interleukin-2; NA, not applicable.

---

In April 18, 2017. © 1999 American Association for Cancer Research.
weeks p.i. according to the WHO toxicity criteria. If no dose-limiting toxicity was observed, three other patients were entered at the next dose level (555 MBq/m² increase). Dose-limiting toxicity was defined as (a) the occurrence of grade IV hematological toxicity (<25 × 10⁹ platelets/liter, <1.0 × 10⁹ leukocytes/liter) in two patients at a certain dose level, or (b) the occurrence of one grade IV or two grade III nonhematological toxicities (WHO toxicity criteria) at a certain dose level. MTD was defined as the dose level below the one at which dose-limiting toxicity was observed. A repeat CT scan and/or planar X-rays were obtained 10–12 weeks after the therapeutic mAb ¹³¹I-cG250 injection and compared to the baseline situation. The outcome of the therapy was evaluated according to the WHO response criteria.

**Determination of HACA Responses.** The immunogenicity of mAb cG250 (dose range, 2–50 mg) has been tested previously in a series of 16 patients with primary RCC (9). To further determine the immunogenicity of this antibody, humoral HACA responses to the mAb cG250 injection were determined in all patients. Serum samples were obtained before and 1, 3, 6, and 10 weeks after infusion. The serum samples were tested in a sandwich ELISA as described previously (9). In brief, cG250 was coated on microtiter plates and used as capture antibody. After incubation with the serum samples, biotinylated cG250 was used as tracer antibody. Proven human HAMA-positive sera from patients who participated in previous trials with murine G250 were used as positive controls (a kind gift of Dr. N. H. Bander, New York Hospital, New York, NY). Serial dilutions of
Anterior Posterior Anterior Posterior

Fig. 2 Radioimmunoscintigrams of patients 2 (A) and 3 (B) obtained 1 week p.i. Arrows, two bone metastases (patient 2; A) and a soft-tissue metastasis (patient 3; B) that were not visualized by planar X-ray. A, arrowheads indicate uptake of 131I-labeled cG250 in a lymph node metastasis (left neck), left lung (massive tumor infiltration), and a thoracic vertebra metastasis. In this patient, uptake of 131I-labeled cG250 was also noted in the left upper abdominal region (nonspecific gastric and colonic activity). B, arrowheads indicate uptake of 131I-labeled cG250 in metastases of the right lung.

Results

Radiolabeling and Quality Control. The overall labeling efficiency of the remote radioiodination method ranged between 75 and 90%. After purification, more than 97% of the pooled radioactivity was protein bound. The high-performance liquid chromatograms indicated that less than 2% of the radioactivity was associated with high molecular weight aggregates (Mr >250,000). The immunoreactivity of the 131I-labeled cG250 preparations was 95 ± 5%.

Immunoscintigraphy. After the diagnostic injection of 131I-labeled cG250, metastatic RCC lesions were visualized adequately in 9 of 12 patients. In general, metastatic tumors lesions were visualized from 1 to 2 days p.i. onward. Due to the background clearance of 131I-labeled cG250, image quality improved with time.

One patient (patient 4) showed good visualization of metastases but did not receive a second, high-activity dose of 131I-labeled cG250 (Table 1). This patient developed neurological complaints due to a bone metastasis in the base of the skull, which required external beam irradiation. Afterward, his clinical condition worsened, not allowing reentry into the study. Thus, eight patients received a second injection.

The immunoscintigrams obtained after the first injection (7 days p.i.) were almost identical to the immunoscintigrams obtained after the second injection (7, 14, and 21 days p.i.). This confirmed that the distribution of the therapeutic injection could be predicted accurately on the basis of the scans obtained after administration of the tracer dose (Fig. 1). The thyroid uptake indicated the release of some radiolabel from the antibody. In one patient (patient 5), a hot spot in the brain was found on the 131I-labeled cG250 immunoscintigrams that was not visualized with a subsequent CT scan of the brain (Fig. 1A, arrow). In two other patients (patients 2 and 3) a total of three suspected lesions (two bone and one soft tissue), which were not visualized by planar X-ray (Fig. 2) were found. All four suspected lesions were confirmed by follow-up, either radiologically (bone and brain metastases) or clinically (progressive soft tissue metastasis).

Toxicity and Clinical Observations. Both injections of the radiolabeled antibody were well tolerated by all patients, and
no direct side effects were observed. During hospitalization, most patients complained of mild nausea and fatigue. The maximum duration of hospitalization (2775 MBq/m² dose level) was 11 days. All patients were released in good condition. Two to 3 weeks after injection of the high-dose ¹³¹I-labeled cG250, a drop in thrombocyte and leukocyte counts was observed in all patients, with a nadir between 4 and 6 weeks (Fig. 3). At the 1665 MBq/m² dose level, grade II hematological toxicity was observed in one patient (thrombocytopenia and leukocytopenia). At the 2220 MBq/m² dose level, one patient showed grade II thrombocytopenia and leukocytopenia, whereas another patient showed grade II thrombocytopenia and grade III leukocytopenia. Both patients treated at 2775 MBq/m² showed grade IV hematological toxicity (thrombocytopenia and leukocytopenia) and required two platelet infusions. Nonhematological toxicity did not exceed grade I (nausea without vomiting, fatigue without decrease in daily activities). On the basis of the observations in these eight patients, the MTD was set at 2220 MBq/m².

Antitumor Response. Two of eight patients who received a therapeutic injection of ¹³¹I-labeled cG250 showed an antitumor response, whereas the other six patients showed progression of disease. Both patients who showed a response were treated at the 2220 MBq/m² dose level. In the first patient (patient 5), stable disease was achieved, lasting 3–6 months. In the second patient (patient 8), a partial response (>50% reduction in size of tumor lesions) was observed that is ongoing (>9 months).

HACA Responses. In one patients (patient 9), a positive HACA response was detected in the serum prior to the first administration of ¹³¹I-labeled cG250 as well as subsequently. This patient showed more rapid whole-body clearance as determined on the radioimmunoscintigrams. Four months prior to participation in the current trial, this patient had participated in another clinical study in which RCC patients with a primary tumor had received two injections of 5 mg of mAb cG250 given 4 days apart. At that time, the patient showed positive uptake of

Fig. 3 Thrombocyte (top) and leukocyte (bottom) counts of all patients at several time points after the therapeutic injection. All patients showed a nadir in thrombocyte counts at 4–5 weeks and a nadir in leukocyte counts at 5–6 weeks after the therapeutic injection. Two patients (patients 11 and 12) showed a grade IV hematological toxicity, which was dose limiting.
mAb cG250 in his primary tumor. No HACA responses were detected in the sera of other patients up to 10 weeks after the second administration of 131I-labeled cG250.

Dosimetry. The estimated whole-body doses (Gy) of the patients who received a therapeutic injection are summarized in Table 1.

Discussion

A phase I radioactivity dose escalation study was performed to determine the safety, the MTD, and the therapeutic potential of mAb cG250 labeled with a high dose of 131I in patients with progressive metastatic RCC. Previous clinical studies have shown that high doses of radioactivity can be delivered to RCC tumor lesions with this antibody (8, 9). Antitumor responses have been observed in a radioimmunotherapy trial with murine mAb G250 (10). However, strong positive HAMA responses occurred in all patients after a single dose of murine mAb G250 (8), which hampered repetitive treatment. Chimerization of murine mAb has resulted in a major decrease in immunogenicity, and mAb cG250 appeared to be immunosilent (9). Although it has been described that chimeric antibodies can still elicit an immune response, several clinical studies have shown a major reduction in the immunogenicity of antibodies after chimerization (23, 24). In the present study, a HACA response was found in patient 9. In this patient, the diagnostic dose was cleared from the blood more rapidly, most likely because of this HACA response. Patient 9 had participated in a previous trial with mAb cG250 3 months prior to the current inclusion. During this previous study this patient had received two injections of 5 mg of cG250. In the present study, the preinjection serum sample was positive for HACA, indicating that the previous exposure to mAb cG250 had induced the immune response. The absence of any detectable HACA responses in the sera of other patients emphasized the low immunogenicity of chimeric mAb cG250.

A remarkable difference between radioimmunotherapy with mAb cG250 and murine mAb G250 is the absence of nonhematological toxicity. In the previous trial with murine mAb G250, all patients showed transient hepatic dysfunction, which sometimes led to transient icterus (10). In contrast to the radioimmunotherapy studies with murine mAb G250, in the present study patients received a diagnostic dose of cG250 (5 mg of 6 mCi 131I-labeled cG250) prior to the therapeutic dose. As described previously, the absence of liver toxicity in the present study may be due to saturation of the antigenic sites in the liver by the diagnostic dose of cG250, which reduced hepatic uptake of the therapeutic administration (8, 9). Another possible explanation may be the higher hepatic uptake of murine mAb G250 compared with mAb cG250. At equal doses, liver uptake of murine mAb G250 (8) was 2–3 times higher than the liver uptake of mAb cG250 (9).

Another difference between the murine 131I-labeled G250 and the 131I-labeled cG250 is the lower MTD of 131I-labeled cG250. The hematological toxicity encountered with murine 131I-labeled G250 indicated an MTD of 3330 MBq/m² (10), whereas MTD in the current study was 2220 MBq/m². The lower MTD of chimeric 131I-labeled cG250 can be explained by the longer circulation time of chimeric mAb cG250 compared with murine mAb G250 (t1/2B, 69 h versus 47 h, respectively). The longer circulation time causes an enhanced radiation dose to the bone marrow, and thus hematological toxicity is encountered at lower activity doses.

One partial response and one stabilization of disease was observed in RCC patients with documented progressive disease prior to study entry. These results warrant further study in a phase II setting at MTD. However, to achieve complete and lasting responses, more intensive dosing is required. Bone marrow support may allow the administration of higher doses of radioactivity. It has been shown in several clinical radioimmunotherapy studies that support with peripheral blood progenitor cells or autologous bone marrow transplantation allows the administration of much higher doses of radioactivity (2–3 times higher; Refs. 25, 26).

Another approach to enhance the therapeutic window may be to aim for reduction of the radiation dose to the bone marrow. Such a reduction might be achieved by using a F(ab')2 fragment, which has a more rapid blood clearance, instead of the whole mAb cG250 IgG1. Therefore, a dual-label study investigating the tumor-targeting capacity and the pharmacokinetic behavior of both mAb cG250 F(ab')2 and whole mAb cG250 IgG1 in patients with primary RCC will be initiated in the near future.

In conclusion, cG250 is a very suitable candidate for radioimmunotherapy of RCC. Several strategies, necessary to increase the radiation dose to tumors, are available, and future studies will focus on optimization of this therapeutic approach for patients with metastasized RCC.

Acknowledgments

We thank Prof. Dr. S. O. Warnaar (Centocor Europe BV, Leiden, The Netherlands) for generously providing the chimeric mAb G250.

References


Phase I Radioimmunotherapy of Metastatic Renal Cell Carcinoma with $^{131}$I-labeled Chimeric Monoclonal Antibody G250


*Clin Cancer Res* 1999;5:3268s-3274s.

Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/5/10/3268s

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.