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Pharmacokinetics and Tumor Targeting of ^{131}I-Labeled F(ab′)$_2$ Fragments of the Chimeric Monoclonal Antibody G250: Preclinical and Clinical Pilot Studies

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ABSTRACT

Introduction: *Clinical and animal studies of chimeric monoclonal antibody G250 (moAb cG250) for the targeting of clear-cell renal cell carcinoma (RCC), to date, have been with the intact IgG form. To determine whether F(ab′)$_2$ fragments are more suited for radioimmunotherapy (RIT) than intact IgG, biodistribution experiments in nude mice were performed, and a pilot study in RCC patients was carried out. In these studies, the biodistribution, pharmacokinetics, and tumor-targeting characteristics of ^{131}I-cG250-F(ab′)$_2$ fragments were determined.* ***Methods:*** *The biodistribution of intact IgG and F(ab′)$_2$ fragments (moAb cG250) was directly compared in mice with subcutaneous (s.c.) RCC xenografts that were coinjected with ^{125}I-cG250-IgG and ^{131}I-cG250-F(ab′)$_2$ fragments. Groups of 5 mice were dissected at 1, 2, 3, 5, and 7 days postinjection (p.i.). The activity in tumor and normal tissues was expressed as the percentage of the injected dose per gram (%ID/g). Five (5) patients with evidence of primary RCC on computed tomography (CT) and scheduled for nephrectomy received a diagnostic infusion of 150 MBq ^{131}I-cG250-F(ab′)$_2$. At various time points after injection of the antibody preparation (5 minutes, 3 hours, and 1, 2, 3, and 4 days), whole-body gamma camera images were acquired. After surgery, histology was determined and immunohistochemistry was performed. The scintigraphic images were analyzed visually and quantitatively. Radioactivity in whole-body, normal tissues and primary RCC was calculated and expressed as %ID.* ***Results:*** *In mice, ^{131}I-cG250-F(ab′)$_2$ fragments cleared faster from the blood and other tissues, and absolute uptake in tumor (3.4 ± 0.9 %ID/g at 24 hours p.i.) and normal tissues was considerably lower compared to intact ^{125}I-cG250. However, the tissue-to-blood ratios for both antibody preparations were similar for most tissues and at most time points. The results in patients corresponded with the results of the studies in mice. The ^{131}I-cG250-F(ab′)$_2$ fragments cleared rapidly from the blood and body. The half-life of the distribution and elimination phase ($t_{1/2}\alpha$ and $t_{1/2}\beta$) in blood of RCC patients were 4.8 ± 0.9 hours and 29.0 ± 3.3 hours, respectively. At 4 days p.i., whole-body activity was 20%ID. Faint visualization of tumor was observed in only 2 of 5 patients.* ***Conclusions:*** *In mice, the tissue-to-blood ratios were similar for intact IgG and the ^{131}I-cG250-F(ab′)$_2$ fragments for most tissues and at*

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most time points, although absolute uptake in all tissues was considerably lower for the F(ab′)$_2$ fragments. In patients with primary RCC, tumorous kidney tissue was faintly visualized with ^{131}I-cG250-F(ab′)$_2$ fragments. The intact IgG form of cG250 appears to be more suitable than cG250-F(ab′)$_2$ fragments for targeting clear-cell RCC.

Key words: renal cell carcinoma, monoclonal antibody cG250, F(ab′)$_2$ fragments

INTRODUCTION

For the successful use of monoclonal antibodies (moAbs) in radioimmunoscintigraphy (RIS) and/or radioimmunotherapy (RIT), it is important that the antibody has optimal antigen-binding capacities, optimal penetration into tumor tissue, and rapid clearance from normal tissues. This should lead to high and specific tumor targeting.[1] The first radiolabeled moAb preparations used to visualize or treat malignancies consisted of whole IgG. Later, F(ab′)$_2$ and Fab or Fab′ fragments were tested for the same applications. Intact IgG is composed of four polypeptide chains, two heavy and two light chains, linked by disulfide bonds. Enzymatic degradation of the intact IgG with pepsin results in the formation of F(ab′)$_2$ fragments that can be reduced to Fab′ fragments, while enzymatic degradation of IgG with papain results in the formation of Fab fragments. F(ab′)$_2$ fragments still contain two antigen binding sites (bivalent), while Fab and Fab′ fragments are monovalent, resulting in a reduced affinity for the target cell.

In experimental studies, there have been contradictory reports with respect to the optimal antibody form for RIT. Compared to fragments, intact IgG has a much longer residence time in the blood (IgG > F(ab′)$_2$ > Fab).[1–4] Because high and sustained blood levels of the antibody are the driving force for tumor uptake, intact IgG has a higher uptake (%ID/g) in the tumor. For the same reason, however, whole IgG delivers a higher radiation-absorbed dose to the bone marrow, the dose-limiting organ in RIT.[2,4] Advantages of the fragments over intact IgG are rapid clearance from the blood and normal tissues, generally resulting in higher tumor-to-normal tissue ratios, and a reduced radiation-absorbed dose to bone marrow.[5] In addition, tumor penetration may be better for the smaller antibody fragments, leading to a more homogeneous intratumoral distribution.[3] Furthermore, the antibody fragments are believed to be less immunogenic as a result of their reduced residence time in the blood.[6,7] On the other hand, the antibody fragments may show increased renal uptake resulting from reabsorption by the renal tubular cells. This, in turn, may lead to radiation-induced nephrotoxicity, if a radiometal is used instead of radioiodine.[8]

In several preclinical reports, F(ab′)$_2$ fragments were favored for RIT, arguing that the reduced absolute uptake in the tumor can be compensated by the increased total amount of radioactivity that can be administered.[2,9] In contrast, better antitumor efficacy of intact IgG because of a higher radiation-absorbed dose delivered to the tumor by whole IgG has been observed.[4] The hypothesis that monovalent antibody fragments, with their reduced antibody affinity, are less suitable for RIT purposes than the bivalent antibodies has also been questioned.[2,3] In mice, in a direct comparison, monovalent Fab fragments outperformed intact IgG regarding antitumor efficacy, presumably because of their homogeneous and rapid uptake and high maximum tolerated activities, resulting in higher dose rates.[3]

In the majority of RIT studies in humans, intact IgG has been used.[10,11] In a few clinical studies, whole IgG was compared to a fragment form, but it remains unclear which antibody form is most suited for RIT.[1,12,13] The conclusions from various studies range from reaching the same percentage of injected dose per gram (%ID/g) in metastases[13] to a substantial advantage for F(ab′)$_2$ fragments, compared to intact IgG in RIT.[12] In a direct comparison between intact cMOv18-IgG and its F(ab′)$_2$ fragments in ovarian cancer patients, the tumor-to-normal tissue ratios for both antibody forms were similar, suggesting a similar performance for therapeutic application.[1] It appears that the most optimal antibody form has to be defined specifically for each antibody-antigen system.

In our previous studies, the IgG form of moAb cG250 was used.[14–17] MoAb cG250 is directed against the G250/MN CA IX antigen on RCC cells.[18,19] Almost all (>95%) clear-cell RCCs express the G250 antigen on the cell surface.[20] Approximately 75% of RCCs are of the clear-cell

type.[21] For cG250 RIT in RCC patients, the most optimal antibody form has not yet been established.

To directly compare cG250-F(ab′)$_2$ fragments to cG250-IgG, we performed a dual-label, dual-isotope biodistribution study in mice bearing subcutaneous (s.c.) RCC xenografts. Furthermore, we carried out a pilot study in RCC-bearing patients to determine the pharmacokinetics and kinetics of tumor targeting of ^{131}I-labeled cG250-F(ab′)$_2$ fragments to address the maximal uptake (%ID) of F(ab′)$_2$ fragments in a tumoros kidney.

MATERIALS AND METHODS

F(ab′)$_2$ Fragments of Monoclonal Antibody cG250

F(ab′)$_2$ fragments of chimeric moAb G250 were produced from clinical-grade cG250 (Centocor Europe BV, Leiden, The Netherlands). Briefly, 0.25 g of cG250 (5 mg/mL) was digested with 5 mg of pepsin in 0.1 M of citrate buffer, at a pH of 3.8. After 4 hours at 37°C, the digestion was stopped by adding 10 mL 1.0 M Tris. Subsequently, the cG250 F(ab′)$_2$ material was purified on a cation exchange column (MONO-S 16/10, Amersham Pharmacia Biotech, Uppsala, Sweden) eluted with 40 mM of acetate buffer, at a pH of 5.2, with a $0 \rightarrow 400$ mM LiCl gradient. The F(ab′)$_2$ containing fractions were buffer-changed by ultrafiltration, and the F(ab′)$_2$ fragments were vialed aseptically in 20-mL vials (5.0 mg/mL, 1.2 mL/vial). The cG250-F(ab′)$_2$ fragments met the following release criteria:

(1) sterility: no microbial contamination
(2) endotoxin: $<$40 pg/mL, and
(3) protein composition: 85% F(ab′)$_2$ fragments, $<$1% IgG and $<$1% Fab′ fragments, and pepsin below the detection limit ($<$0.2%), using the quantitative analysis of SDS-polyacrylamide gelelectrophoresis (SDS-PAGE).

The immunoreactive fraction of the radioiodinated cG250 F(ab′)$_2$ preparation was $>$95% immediately postlabeling and $>$90% after 4 hours of incubation in serum, essentially as described by Lindmo et al., with minor modifications.[14,22,23] Scatchard analysis revealed that the affinity of ^{125}I-cG250 F(ab′)$_2$ was 2.6×10^9 M^{-1}, while the affinity of parental ^{125}I-cG250-IgG1 was 2.3×10^9 M^{-1} in the same, simultaneously run assay.

Radiolabeling and Quality Control

For the biodistribution experiments, intact IgG-cG250 was radiolabeled with ^{131}I and cG250-F(ab′)$_2$ fragments, with ^{125}I as described previously (specific activity: 2 μCi/μg).[24] For clinical use, the cG250-F(ab′)$_2$ fragments were radioiodinated with ^{131}I (MDS Nordion, Fleurus, Belgium), according to the IodoGen method using a remote system, as described previously (specific activity: 30 MBq/mg).[14,15,25] The radiochemical purity of radiolabeled cG250-F(ab′)$_2$ fragments was determined by instant thin-layer chromatography (ITLC) using ITLC silica gel strips (Gelman Sciences, Inc., Ann Arbor, MI) using 0.15 M of citrate buffer, at a pH of 5.0, as the mobile phase (release criterion: $<$5% free radioiodine). Also, prior to each administration of cG250-F(ab′)$_2$ fragments, the immunoreactive fraction at infinite antigen excess was determined on freshly trypsinized SK-RC-52 RCC cells, as described by Lindmo et al.[22]

The stability of the cG250 F(ab′)$_2$ fragments in the circulation was investigated by incubating radioiodinated cG250 F(ab′)$_2$ in serum at 37°C (10^6 cpm/mL). Samples were drawn at 1, 2, 3, 4, and 24 hours after incubation and analyzed on SDS-PAGE.

Biodistribution Experiments in Mice

The *in vivo* characteristics of the radioiodinated cG250 F(ab′)$_2$ preparation were determined in nude mice with s.c. SK-RC-52 human renal cell carcinoma xenografts and compared to the performance of radioiodinated intact IgG cG250.[23] SK-RC-52 cells were cultured in RPMI medium (Life Technologies, Breda, The Netherlands) and supplemented with 10% fetal calf serum (FCS) at 37°C in a humidified atmosphere with 5% CO_2. Cells were washed in saline, trypsinized, washed in RPMI + 10% FCS, and 2×10^6 cells (volume 0.2 mL) were injected subcutaneously (s.c.) into the right flank of 6–8-week-old BALB/c nu/nu mice. The biodistribution experiments were initiated 2 weeks after the inoculation of the tumor cells. RCC tumor-bearing mice were randomly divided into 5 groups of 5 mice. Mice were intravenously (i.v.) coinjected with 10 μCi ^{131}I-cG250-IgG and 10 μCi ^{125}I-cG250-F(ab′)$_2$. Mean tumor weight was 0.039 g, with a range of 0.012–0.080 g, and all mice received a protein dose of 5 μg intact IgG and 5 μg F(ab′)$_2$-cG250 (total injection volume 200 μL/mouse). Groups of mice were killed at 1, 2, 3, 5, and 7 days postin-

jection (p.i.), and the biodistribution of both radiolabels was determined. The tumor and normal tissues (blood, muscle, lung, spleen, kidney, liver, and small intestines) were dissected, weighed, and counted in a gamma counter (1480 Wizard 3", PerkinElmer Life Sciences, Boston, MA). To correct for radioactive decay, injection standards were counted simultaneously. The activity in samples was expressed as the percentage of injected dose per gram of tissue (%ID/g). All animal experiments were approved by the Animal Experiments Committee of the University Medical Center Nijmegen and were performed in accordance with their guidelines.

Patient Characteristics

Five (5) patients, ages between 41 and 76, with clinical diagnoses of primary renal cell carcinoma were included (Table 1). Patients had to have a Karnofsky performance status >70%, and had to be over 18 years of age. Furthermore, they were excluded when they were pregnant or lactating, or had been previously injected with any type of monoclonal antibody of murine origin. Also, they could not participate in the case of untreated hypercalcemia, liver, and/or renal failure, central nervous system dysfunction, severe ischemic myocardial disease, or known arrhythmia. The study was approved by the Medical Ethical Committee of the University Medical Center Nijmegen. Prior to study entry, written, informed consent was obtained from all patients.

Clinical Study Design and Radioimmunoscintigraphy

Within 30 days before study entry, a baseline computed tomography (CT) scan of the abdomen of each patient was obtained for adequate measurement of the primary tumor. Prior to the administration of the ^{131}I-cG250-F(ab′)$_2$ fragments, the medical history was taken, a physical examination was performed, and baseline blood samples were drawn for routine blood chemistry, hematology, and thyroid function.

To prevent ^{131}I uptake in the thyroid, patients received 100 mg of potassium iodide twice-daily and 200 mg of potassium perchlorate 4 times daily, starting on the day of the F(ab′)$_2$ administration. This regimen was continued for 1 week.

Patients received a diagnostic i.v. infusion of 5 mg cG250-F(ab′)$_2$ fragments labeled with 150 MBq ^{131}I (total volume 10 mL), followed by the acquisition of 6 whole-body scans at 5 minutes, 3 hours, 1, 2, 3, and 4 days p.i. Simultaneously, aliquots of dose were scanned to allow for a quantitative analysis of the images. The images were recorded using a double-headed gamma camera (Multispect 2, Siemens Inc., Hoffman Estates, IL), equipped with parallel-hole, high-energy collimators (symmetric 15% window over 364 keV, scan speed 10 cm/min [5 minutes and 3 hours, 1 and 2 days p.i.]) and 5 cm/minutes [3 and 4 days p.i.]) and stored digitally in a 256 × 1024 matrix. At the same time points, except for the last 2 days, planar images of the upper abdomen were recorded with a preset count number of 1,000,000 counts.

Table 1. Patient Characteristics, Size, and Location of Primary Tumor, Pathology, and Immunohistochemical G250 Staining

Patient no.	*Age at study entry*	*Gender*	*Maximum size (height × width × length [cm]) and location of primary kidney tumor*	*Nephrectomy*	*Pathology, tumor (T) classification of UICC*	*Immunohistochemical G250 staining*
1	63	M	7 × 7 × 8, right	Yes	Clear cell RCC, pT3[b]	+++[b]
2	41	M	6 × 7 × 8, left	Yes	Clear cell RCC, pT3[a]	n.d.[c]
3	60	M	4.5 × 4.5 × 4.5, right	Yes	Clear cell RCC, pT1	+++
4	71	M	15 × 12 × 17, right	No	Clear cell RCC, pTx[a]	n.d.[d]
5	76	M	6 × 5 × 5, right	Yes	Clear cell RCC, pT3[b]	+++

UICC, International Union Against Cancer; n.d., not determined.

[a]The size of the primary RCC could not be established, as the lesions were irresectable. The pathological diagnosis of clear cell RCC was established from a metastatic lesion from the omentum.

[b]More than 95% of cells stained positive for G250.

[c]No representative frozen tissue available (only necrotic tissue).

[d]No frozen tissue available.

Pulse rate, blood pressure, and temperature were checked once every hour up to 4 hours p.i., and, thereafter, once-daily until the last scanning day. At 4 days p.i., the chemistry and hematological parameters were checked again. Approximately 3 months later, blood was sampled to reevaluate thyroid function. Once a nephrectomy had been performed, the clinical diagnosis was confirmed by routine pathology of the kidney tumors, and the stage was determined using the international union against cancer (UICC) Tumor-Node-Metastases (TNM) and Heidelberg classification systems.[21,26]

Pharmacokinetics and Quantitative Analysis of the Scintigraphic Images

To determine the pharmacokinetics of ^{131}I-cG250-F(ab′)$_2$ fragments in the blood of patients, blood samples were drawn at 5 minutes and 0.5, 1, 2, 4, 8, and 12 hours p.i., and at 1, 2, 3, and 4 days p.i. The samples were counted in a well-type gamma counter (1480 Wizard 3", PerkinElmer Life Sciences, Boston, MA). To correct for radioactive decay, injection standards were counted simultaneously. The activity in these samples was expressed as the percentage of injected dose per gram (%ID/g). The half-life of the disappearance from plasma (t $^1/_2$) was calculated by nonlinear, least-square regression analysis (α and β phase).

Serial whole-body images were used to quantitate the uptake of ^{131}I-cG250-F(ab′)$_2$ fragments in the whole body, heart, liver, kidney, primary kidney tumor, and the nonaffected kidney, as has been described previously.[27–29] Briefly, regions of interest (ROIs) were drawn on the anterior and posterior whole-body images.[29] Absolute activity in tumors and organs was calculated, using the conjugated-view method with partial background subtraction, according to Buijs et al.[27,28] Subsequently, the activity in tissues was corrected for physical decay and attenuation, and expressed as %ID, setting the number of counts in the whole-body image recorded immediately after injection at 100%. Uptake in normal kidney and primary kidney tumors was also expressed as %ID/g, assuming that 1 mL of tissue equals 1 g. The volume of the kidney tumor was derived from CT measurements, using the formula π(length $\times$ width $\times$ height)/6. The weight of a normal kidney was derived from a standard adult male phantom and was assumed to be 150 g.

Immunohistochemistry

Immunohistochemistry on frozen tissues was carried out, as described previously.[14] Briefly, 4-μm cryostat sections were acetone-fixed, dried, and washed. Sections were incubated with 100 μL of 10 μg/mL murine antibody G250 for 1 hour at room temperature and washed. Subsequently, sections were incubated with rabbit antimouse IgG conjugated to horseradish peroxidase (RAMPO, DAKO, Carpentina, CA), washed, and developed with 3-3′-diaminobenzidine/0.03% hydrogen peroxide.

Statistical Analysis

Statistical analysis was performed using the unpaired Student's t test. Differences were considered significant when $p < 0.05$, and two-sided. All values are expressed as mean $\pm$ standard deviation (SD), unless stated otherwise.

RESULTS

Radiolabeling and Quality Control

The labeling efficiency of the radioiodination of the clinical-grade F(ab′)$_2$ fragments was always between 85% and 90%. After purification, more than 98% of the ^{131}I-activity in the antibody preparation was protein-bound, as determined by ITLC. The immunoreactive fraction of ^{131}I-cG250-F(ab′)$_2$ ranged between 88% and 94% (mean: 90%).

The autoradiogram of the gel showed that the F(ab′)$_2$ fragments were stable in serum during at least 24 hours and that no monovalent Fab′-fragments were formed (Fig. 1).

Biodistribution Experiments in Mice

The results of the biodistribution experiments are shown in Figure 2. In all tissues analyzed, the uptake of ^{125}I-cG250-F(ab′)$_2$ fragments was significantly lower when compared to the uptake of intact ^{131}I-cG250-IgG (p between < 0.0001 and < 0.05) (Fig. 2A). Maximum tumor uptake of cG250-F(ab′)$_2$ fragments was already reached at 1 day p.i., whereas the maximum uptake of intact radiolabeled cG250 was reached 3 days p.i. (Fig. 2A). Tumor-to-blood ratios were only significantly different at 1 and 2 days p.i., with the highest ratio for the ^{125}I-cG250-F(ab′)$_2$ fragments (2.7 ± 0.6) at 1 day p.i. (Fig. 2B). There was no

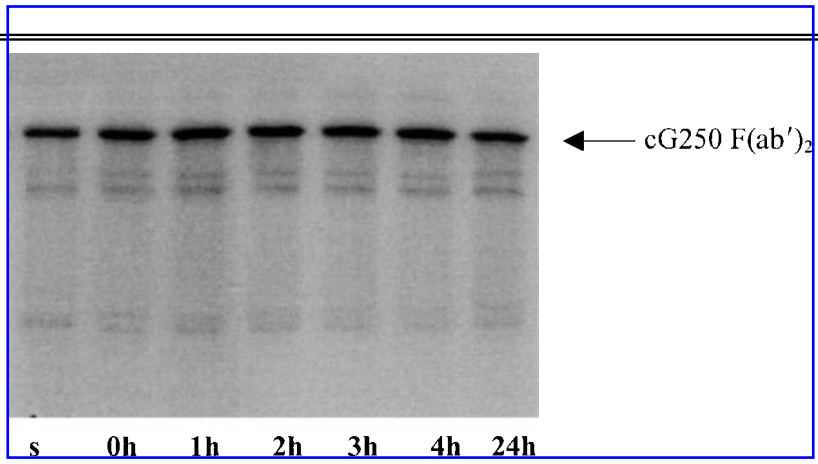

Figure 1. Autoradiogram of the SDS-acrylamide gel, with the samples of ^{125}I-cG250 F(ab′)$_2$ fragments incubated in serum at 37°C during 0–24 hours. In the first lane, a sample of the ^{125}I-cG250 F(ab′)$_2$ preparation was run as a reference.

difference in maximum tumor-to-blood ratio reached at 7 days p.i. (approximately 3 for both preparations) (Fig. 2B). Most of the tissue-to-blood ratios in the other tissues were similar for the F(ab′)$_2$ fragments and intact IgG, although a few significant differences were noted, e.g., kidney-to-blood and liver-to-blood at 1, 2, and 5 days p.i. (Fig. 2B).

To estimate the radiation dose that could be guided to the tumor with ^{131}I-labeled F(ab′)$_2$ and IgG, for both antibody forms the area under the curve (AUC) was calculated for the tumor and the blood. For intact IgG, the $AUC_{tumor} = 26.6$ and $AUC_{blood} = 18.6$, while for F(ab′)$_2$ the $AUC_{tumor} = 4$ and $AUC_{blood} = 2.1$, suggesting that, in this mouse model, a higher radiation dose (1.4-fold) can be guided to the tumor with ^{131}I-cG250 F(ab′)$_2$, compared to ^{131}I-cG250 IgG. In these estimations, the effects of the different dose rates are not accounted for.

Clinical Observations and Radioimmunoscintigraphy

All ^{131}I-cG250-F(ab′)$_2$ fragment injections were well tolerated by the patients. No significant changes in vital signs, hematological or blood chemistry parameters, or thyroid function were observed (2 patients were lost to follow-up for thyroid evaluation). After the 5-day protocol had been completed, all patients underwent surgery (Table 1). In 4 patients, a nephrectomy was performed; in 1 patient (patient 4), the tumor turned out to be irresectable during surgery and was embolized. The findings of routine pathology of the kidney tumors that were removed are documented in Table 1. In 3 of 4 tumor tissues obtained after nephrectomy, immunochemistry showed that 95% of the RCC cells stained positive for G250. One (1) frozen tissue sample (patient 4) consisted mainly of necrotic tissue, and, therefore, the G250 expression of the frozen sample could not be reliably assessed (Table 1).

In 2 patients (3 and 4), faint tumor targeting was observed from 48 hours p.i. onwards (Fig. 3). Significant targeting of ^{131}I-cG250-F(ab′)$_2$ fragments to the primary RCC tumors was observed in none of the patients during the time period in which gamma-camera scans were recorded. Immediately after injection, the primary tumor appeared as photopenic areas in posterior scintigraphies in the majority of patients (1–4) (Fig. 4).

Pharmacokinetics and Quantitative Analysis of the Scintigraphic Images

Clearance of the radiolabel from the blood after an i.v. injection of radioiodinated cG250-F(ab′)$_2$ fragments was characterized by a rapid distribution phase, with a half-life ($t\,^1/_2\,\alpha$) ranging from 4.1 to 6.3 hours (mean: 4.8 hours), and a slower elimination phase, with a half-life ($t\,^1/_2\,\beta$) ranging from 25.8 to 34.1 hours (mean: 29.0 hours). The total ^{131}I activity in the body of 5 RCC patients during the first 4 days after ^{131}I-cG250-F(ab′)$_2$ administration is shown in Figure 5A. For comparison, historical data showing the activity in whole body and relevant organs at 96 hours after injection of intact ^{131}I-labeled cG250-IgG is

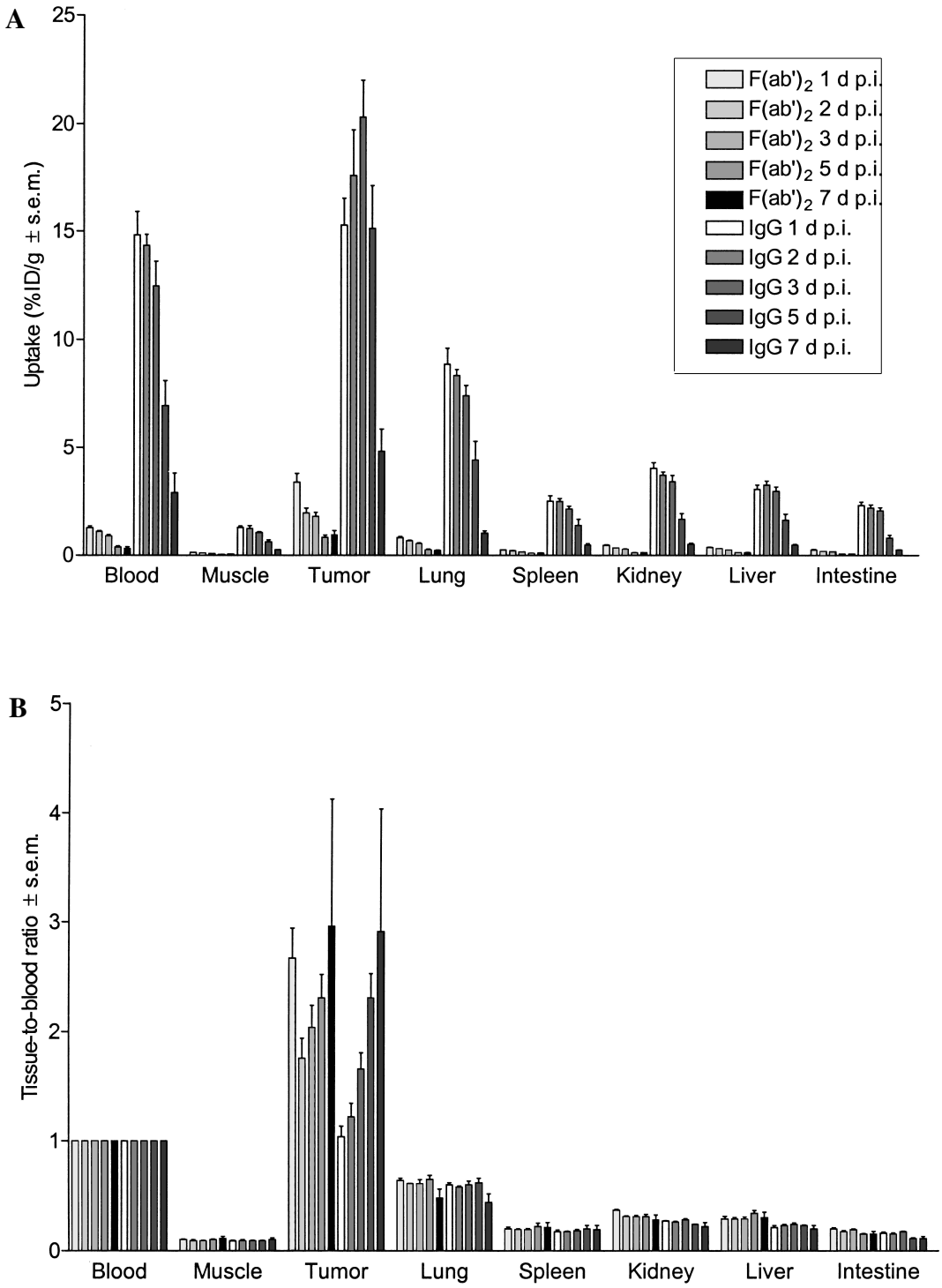

Figure 2. Biodistribution of ^{125}I-cG250 F(ab′)$_2$ and ^{131}I-cG250-IgG in nude mice with s.c. SK-RC-52 human renal cell carcinoma xenografts. Groups of 5 mice were killed at 1, 2, 3, 5 and 7 days p.i. Uptake (%ID/g) (**A**) and tissue-to-blood ratios (**B**) in various tissues. s.e.m., standard error of the mean.

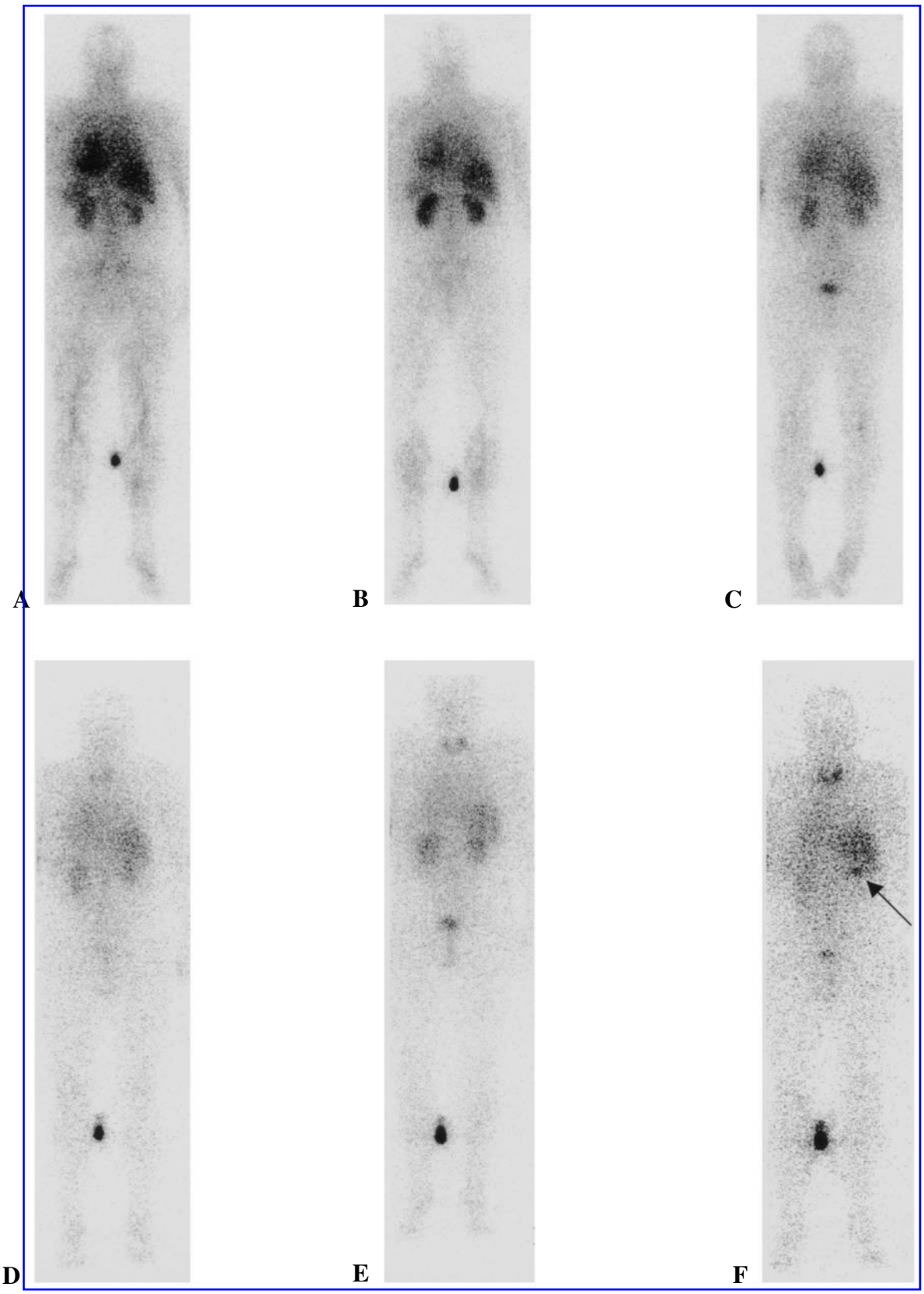

Figure 3. Posterior whole-body images of patient 3 after an i.v. injection of ^{131}I-cG250-F(ab′)$_2$ fragments at: 5 minutes (**A**); 3 hours (**B**); and 1 (**C**), 2 (**D**), 3 (**E**), and 4 days p.i. (**F**). At later time points, a faint uptake was noticed in the region of the primary RCC tumor in the upper pole of the right kidney (arrow in F).

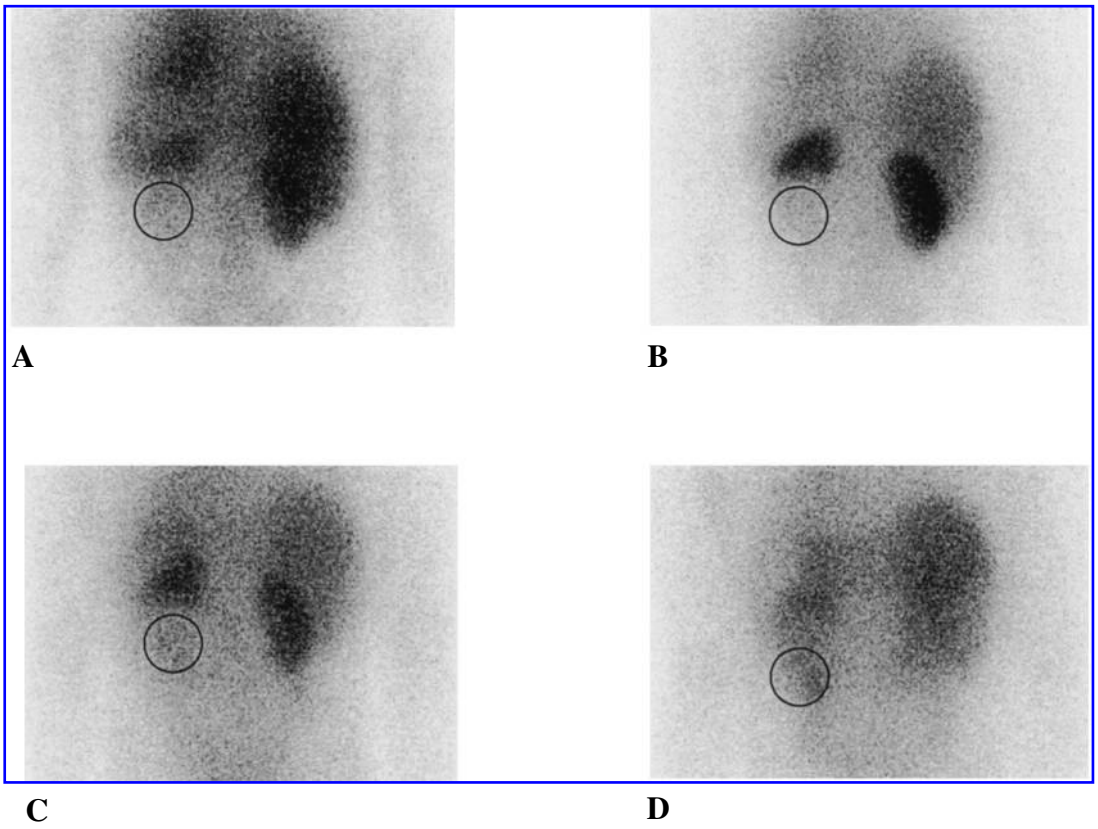

Figure 4. Posterior images of the abdomen of patient 2 after an i.v. injection of ^{131}I-cG250-F(ab′)$_2$ fragments at: 5 minutes (**A**); 3 hours (**B**); and 1 (**C**) and 2 days p.i. (**D**). The primary kidney tumor was situated at the lower pole of the left kidney (circle).

also depicted.[29] F(ab′)$_2$ fragments cleared significantly faster from the body compared to intact IgG; at 96 h p.i., only 20 %ID of the F(ab′)$_2$ fragments was still present in the body versus approximately 60 %ID for intact cG250 (Fig. 5A). The same pattern was observed for the activity in relevant organs (Fig. 5B). The activity in the heart after an injection of ^{131}I-cG250-F(ab′)$_2$ fragments decreased with time, whereas the activity in the other organs reached a maximum uptake at 3 hours p.i. Thereafter, the activity decreased (Fig. 5B). The mean activity (%ID/g) in the primary RCC was never higher than the mean activity (%ID/g) in the normal contralateral kidney (Fig. 5C). Thus, specific accumulation of radioiodinated F(ab′)$_2$ fragments in primary RCCs could be quantitatively demonstrated in zero of 5 patients.

DISCUSSION

This pilot study describes the results of the application of ^{131}I-labeled cG250-F(ab′)$_2$ fragments in mice and patients. In mice, our results with chimeric moAb G250 were in line with previous studies by Van Dijk et al., who compared murine G250-F(ab′)$_2$ fragments to murine G250-IgG in the same SK-RC-52 tumor model.[30] In mice, the accumulation of ^{131}I-cG250-F(ab′)$_2$ fragments in tumor and normal tissues was considerably lower and clearance of the radiolabel from all tissues was faster, compared to radioiodinated intact cG250-IgG. Nevertheless, the tumor-to-blood ratios at the first time points analyzed were higer for the F(ab′)$_2$ fragments, indicating a potential advantage of F(ab′)$_2$ in RIS and RIT. From 3 days p.i. onwards, tumor-to-blood ratios were similar for F(ab′)$_2$ and IgG. Despite similar biokinetics, the use of bivalent fragments might be advantageous because of improved tumor penetration and reduced immunogenicity.

In patients, i.v. injection of radioiodinated cG250-F(ab′)$_2$ fragments did not result in the clear visualization of primary RCC tumors. The tumor was faintly visualized in only 2 patients

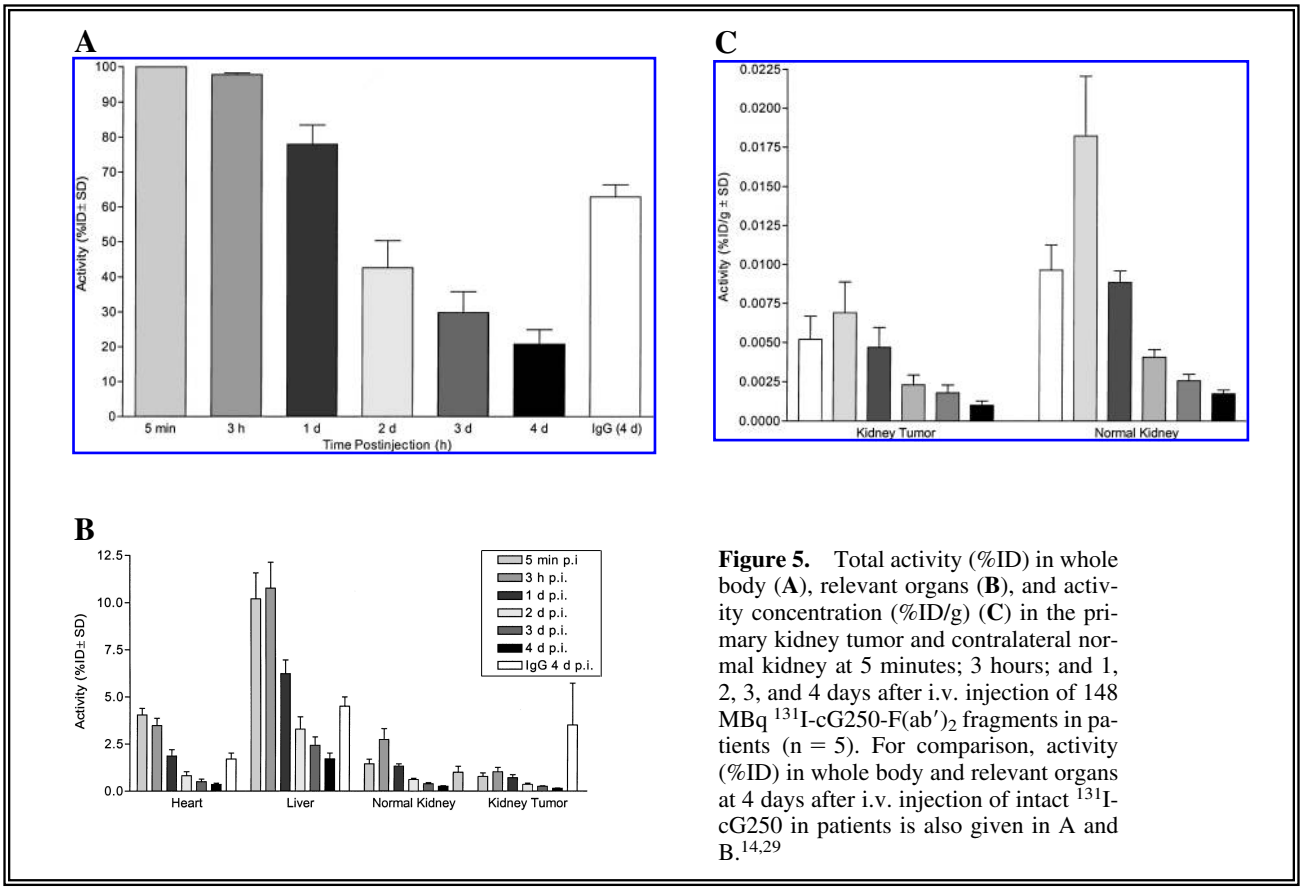

Figure 5. Total activity (%ID) in whole body (**A**), relevant organs (**B**), and activity concentration (%ID/g) (**C**) in the primary kidney tumor and contralateral normal kidney at 5 minutes; 3 hours; and 1, 2, 3, and 4 days after i.v. injection of 148 MBq ^{131}I-cG250-F(ab′)$_2$ fragments in patients (n = 5). For comparison, activity (%ID) in whole body and relevant organs at 4 days after i.v. injection of intact ^{131}I-cG250 in patients is also given in A and B.[14,29]

from 2 days p.i. onwards. Because of the poor localization of the tumor, specific targeting of the ^{131}I-cG250-F(ab′)$_2$ fragments in the tumor could not be quantitatively demonstrated. Relatively high uptake in normal kidney was observed for the F(ab′)$_2$ fragments, most likely the result of reabsorption of F(ab′)$_2$ fragments by the renal tubular cells. These results are in contrast with previous observations after the injection of intact ^{131}I-cG250 in RCC patients with *in situ* primary tumors.[14] In the latter study, excellent visualization of G250 antigen-positive tumors was obtained, usually from 1 day p.i. onwards. Because of the clearance of the radiolabeled moAb—lowering background levels—image quality improved with time, up to 7 days p.i..[14] Furthermore, there was no physiologic uptake in the normal kidney at 4 days p.i. with intact ^{131}I-cG250.[16]

The limited accretion of the F(ab′)$_2$ fragments in RCC tumors could be the result of the instability of the F(ab′)$_2$ fragments *in vivo*. However, this was ruled out by checking the immunoreactivity and by analyzing the ^{131}I-cG250 F(ab′)$_2$ preparation on SDS-PAGE before and after incubation in the blood. These data demonstrated that the immunoreactivity of the preparation was preserved and that the radiolabel in the blood remained associated with F(ab′)$_2$ fragments, and there was no indication of formation of labeled degradation products (e.g., monovalent Fab′). Furthermore, the clinical pharmacokinetic data do not support the degradation of the F(ab′)$_2$ preparation in the circulation. The clearance of the radiolabel from the blood is in line with clearance rates observed for F(ab′)$_2$ fragments in other studies.[1,31] The t $^1/_2$ β of Fab′ fragments is reported to be considerably shorter (t $^1/_2 < 20$ h).[32]

It could also be the result of G250 antigen-negative kidney tumors. This explanation was ruled out by the results of routine pathology (all tumors were clear-cell RCCs) and the immunohistochemistry data (in 3 of 4 tissues, >95% of the tumor cells stained positive for G250). One (1) tissue sample consisted mainly of necrotic tumor tissue, and, therefore, immunohistochemistry

could not be performed. Even though large, necrotic tumors might have hampered tumor targeting and visualization with ^{131}I-cG250-F(ab′)$_2$, the presence of necrotic tissue did not previously hamper uptake of intact ^{131}I-cG250 in the tumor and visualization of the tumor.[14]

One might postulate that the protein dose of F(ab′)$_2$ fragments was suboptimal. The protein dose of the F(ab′)$_2$ fragments (5 mg) was derived from the optimal protein dose (5–10 mg) for intact ^{131}I-cG250.[14] Average tumor uptake of cG250-IgG in the tumor was approximately 0.01 %ID/g.[14] With cG250-IgG, antigen saturation was observed only at protein doses exceeding 10 mg of intact cG250.[14] Thus, 5-mg radioiodinated cG250-F(ab′)$_2$ fragments likely did not saturate the tumors. Conversely, at relatively low protein doses of 2 mg cG250-IgG, enhanced uptake in the liver was observed, which was saturable and, presumably, the result of the known G250 antigen expression on the larger bile ducts.[14] In the present study, uptake in liver immediately postinjection of ^{131}I-cG250 F(ab′)$_2$ fragments was approximately 10%ID, which is in the same range as the observed hepatic uptake of approximately 12%ID immediately after the injection of ^{131}I-cG250-IgG.[29] Thus, underdosing of the F(ab′)$_2$ fragments is also an unlikely explanation.

Lastly, there might be a concern of the appropriatness of the radiolabel used. Although we recently showed that, with a residualizing radionuclide such as ^{111}In, more metastatic RCC lesions could be visualized, and a higher uptake of ^{111}In-radiolabeled cG250 could be achieved in these metastases, compared to ^{131}I-cG250, the reported high uptake in primary RCCs (up to 0.52%ID/g) with ^{131}I-cG250 by Steffens et al., demonstrated that radioiodinated G250 can visualize RCC lesions very well.[14,29]

Thus, based on the observations reported in our current study, it is concluded that the intact IgG form of moAb cG250 that has been used, up to now, is currently the most appropriate antibody form for RIS and RIT in patients with RCC.

CONCLUSION

In conclusion, the tissue-to-blood ratios in mice were similar for intact IgG and the ^{131}I-cG250-F(ab′)$_2$ fragments for most tissues and at most time points, although absolute uptake in all tissues was considerably lower for the F(ab′)$_2$ fragments. In patients with their primary RCC tumor *in situ,* scintigraphic detection and tumor targeting of tumorous kidney tissue was inadequate with ^{131}I-cG250-F(ab′)$_2$ fragments. These results differ from the findings in a previous patient study with the ^{131}I-labeled intact IgG moAb cG250, in which clear tumor targeting was observed from 1 day p.i. onwards. Apparently, sustained high blood levels of intact cG250-IgG are a prerequisite for the effective accumulation of the antibody in RCC tumor tissue. Thus, the intact IgG form of moAb cG250 that has been used up to now is more suitable than cG250-F(ab′)$_2$ fragments for targeting RCC tumors.

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