Preclinical evaluation of $^{89}$Zr-labeled anti-CD44 monoclonal antibody RG7356 in mice and cynomolgus monkeys
Prelude to Phase 1 clinical studies

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RG7356 is a humanized antibody targeting the constant region of CD44. RG7356 was radiolabeled with $^{89}$Zr for preclinical evaluations in tumor xenograft-bearing mice and normal cynomolgus monkeys to enable study of its biodistribution and the role of CD44 expression on RG7356 uptake.

Studies with $^{89}$Zr-RG7356 were performed in mice bearing tumor xenografts that differ in the level of CD44 expression (CD44+ or CD44-) and RG7356 responsiveness (resp or non-resp): MDA-MB-231 (CD44+, resp), PL45 (CD44-, non-resp) and HepG2 (CD44-, non-resp). Immuno-PET whole body biodistribution studies were performed in normal cynomolgus monkeys to determine normal organ uptake after administration of a single dose.

At 1, 2, 3, and 6 days after injection, $^{89}$Zr-RG7356 uptake in MDA-MB-231 (CD44+, resp) xenografts was nearly constant and about 9 times higher than in HepG2 (CD44-, non-resp) xenografts (range 27.44 ± 12.93 to 33.13 ± 7.42% ID/g vs. 3.25 ± 0.38 to 3.90 ± 0.58% ID/g). Uptake of $^{89}$Zr-RG7356 was similar in MDA-MB-231 (CD44+, resp) and PL45 (CD44-, non-resp) xenografts. Studies in monkeys revealed antibody uptake in spleen, salivary glands and bone marrow, which might be related to the level of CD44 expression. $^{89}$Zr-RG7356 uptake in these normal organs decreased with increasing dose levels of unlabeled RG7356.

$^{89}$Zr-RG7356 selectively targets CD44+ responsive and non-responsive tumors in mice and CD44+ tissues in monkeys. These studies indicate the importance of accurate antibody dosing in humans to obtain optimal tumor targeting. Moreover, efficient binding of RG7356 to CD44+ tumors may not be sufficient in itself to drive an anti-tumor response.

Introduction

CD44 is a multifunctional receptor involved in cell-cell and cell-extracellular matrix (ECM) interactions, cell trafficking, lymph node homing, presentation of chemokines and growth factors to traveling cells, and transmission of growth signals.1,2 CD44 also participates in the uptake and intracellular degradation of hyaluronic acid (HA), as well as in transmission of signals mediating hematopoiesis and apoptosis.2,3 Many types of cancer and their metastases express high levels of CD44.2,4 RG7356 (also referred as RO5429083 and ARH460–16–2) is a humanized IgG1 antibody targeting the constant region of CD44 that shows antitumor efficacy in mice implanted with CD44+ tumors, such as the MDA-MB-231 breast cancer cell line.5 RG7356 demonstrated therapeutic effectiveness in CD44+ and HA+ MDA-MB-231 tumor xenografts, while no therapeutics effects were found in CD44+ and HA+ PL45 tumor xenografts. Therefore, the primary mode of action of RG7356 is postulated to be disruption of the CD44-HA interaction, which results in anti-tumor effects. In addition, RG7356 treatment has been shown to modulate the MAPK pathway in the responsive model (MDA-MB-231).5

Anti-CD44 antibodies such as bivatuzumab mertansine were investigated in patients with solid tumors with low to moderate success.6 Radiolabeled bivatuzumab was also investigated in patients in imaging and radioimmunotherapy studies.7,8 Unlike bivatuzumab, which binds to CD44v6 and has no intrinsic anti-tumor activity, RG7356 binds to the constant and/or standard region of CD44 (CD44s) and has intrinsic anti-tumor activity.5 CD44v6 is primarily overexpressed in squamous cell carcinoma, whereas CD44s is overexpressed in numerous solid tumor types and hematological malignancies.3,4,9,10 In addition to expression in malignancies, CD44 is also expressed on normal circulating...
blood cells and plays a physiological role in normal tissues and in inflammatory processes.\textsuperscript{1,12} CD44 has been shown to be overexpressed in human epithelial tissues such as lung, skin, mammary gland, prostate gland, salivary gland and urinary bladder.\textsuperscript{13}

The knowledge of distribution and expression levels of a given receptor is critical to the successful development of receptor-targeted cancer therapy. Analysis of biopsied specimens ex vivo by various biochemical and immunohistochemistry assays is the most straight-forward approach to confirm the presence of target receptors. However, not all lesions can be biopsied and biopsied samples or samples of resected primary tumor may not represent all metastases within a single patient, or samples of previously resected tumor may not be available at all. Non-invasive in vivo imaging techniques may allow for comprehensive detection of given targets and permit subsequent monitoring of therapy progress.

Immunoo-positron emission tomography (immuno-PET) can potentially be applied to assess the extent and distribution of target in the whole body over time. Additionally, immuno-PET with a therapeutic antibody can confirm whether the antibody reaches the target tissue of interest, the dose required to saturate the target, cross-reactivity with normal organs and inter-individual variation.

To better understand the role of ubiquitous expression of CD44 on uptake of RG7356 in normal organs and tumors, and to evaluate the suitability of RG7356 for clinical therapeutic application, RG7356 was radiolabeled with \(^{89}\text{Zr}\) for preclinical evaluations in tumor bearing mice and normal cynomolgus monkeys. Biodistribution studies were performed in the responsive CD44\(^{+}\) and HA\(^{-}\) MDA-MB-231 tumor model, the non-responsive CD44\(^{-}\) and HA\(^{-}\) PL45 tumor model and the CD44\(^{-}\) HepG2 tumor model.\textsuperscript{2} Because of the lack of cross-reactivity of RG7356 to murine CD44, immuno-PET studies were performed in cynomolgus monkeys to assess uptake of the antibody by normal organs and tissues. Human and monkey CD44 have over 90\% homology in their gene sequences.\textsuperscript{14-16}

**Results**

**Conjugation, radiolabeling, and quality control**

RG7356 was premodified with \(N\)-succinyl-desferrioxamine (\(N\)-succ-DFO), which under the applied stoichiometry resulted in an average of 0.9 ± 0.1 \(N\)-succ-DFO groups per RG7356 molecule (\(n = 5\)). Subsequent radiolabeling with \(^{89}\text{Zr}\) resulted in 83–95\% labeling efficiency and radiochemical purity as determined by high-performance liquid chromatography (HPLC) and instant thin-layer chromatography (iTLC) always exceeded >96\% after purification with PD10. Immunoreactivity was shown to be 73–81\% at the highest cell concentration.

**Biodistribution study in mice**

**CD44 targeting study with \(^{89}\text{Zr-RG7356}\)**

For assessment of the specificity of tumor targeting by RG7356, two different xenografts were evaluated in tumor xenograft-bearing mice: MDA-MB-231 (CD44\(^{+}\)) vs. HepG2 (CD44\(^{-}\)). A low dose of 25 \(\mu\)g \(^{89}\text{Zr-RG7356}\) was injected and the biodistribution of the radiolabeled antibody was evaluated at 1, 2, 3, and 6 d after injection (Fig. 1). The blood content of \(^{89}\text{Zr-RG7356}\) in the CD44\(^{-}\) xenografts was slightly higher than that of the CD44\(^{+}\) xenografts, but only at 1 d after injection was this finding significant (\(P < 0.05\)). The CD44\(^{+}\) tumor xenografts showed increased tumor uptake of 31.2 ± 5.4\% ID/g at 24 h after injection, and this was constant up to at least 144 h after injection (33.1 ± 7.4\% ID/g). On the other hand, CD44\(^{-}\) tumor xenografts showed tumor uptake of 3.6 ± 0.5\% ID/g 24 h post injection, which also did not increase further (Fig. 1; Table S1) and was as low as uptake in skin, tongue and muscle. The tumor-to-blood ratios of the CD44\(^{+}\) xenografts were significantly higher than that of the CD44\(^{-}\) xenografts (4.03 ± 1.95 vs. 0.21 ± 0.02 at 1 d post-injection and 8.71 ± 3.18 vs. 1.19 ± 1.17 at 6 d post-injection, respectively, Fig. S2). Some mice showed faster blood clearance rates and this resulted in relatively large standard deviations. This phenomenon has been described previously for other humanized IgG1 antibodies.\textsuperscript{17}

**Antibody dose escalation study with \(^{89}\text{Zr-RG7356}\)**

For the determination of the dependency of tumor targeting in mice on the dose level of administered mAb, a dose escalation study was performed in MDA-MB-231 xenografts (CD44\(^{+}\)), which received 25, 50, 200 and 500 or 1000 \(\mu\)g RG7356 co-injected with tracer amounts of \(^{89}\text{Zr-RG7356}\). A preblocking study was also performed by injecting 1000 \(\mu\)g RG7356 24 h before \(^{89}\text{Zr-RG7356}\). Biodistribution of the mAb was assessed at 2, 3, and 6 d post injection and is summarized in Figure 2 and Table S2. At higher mAb dose levels, uptake in the tumors decreased from 27.80 ± 10.95\% ID/g for 25 \(\mu\)g, 27.06 ± 4.01\% ID/g for 50 \(\mu\)g mAb, 24.51 ± 5.85\% ID/g for 200 \(\mu\)g, 20.70 ± 3.71\% ID/g for 500 \(\mu\)g to 15.62 ± 4.55\% ID/g for 1 mg mAb at 2 d after injection. The same trend was observed for the other biodistribution time points. For healthy organs and blood, no dose dependency was observed and the %ID/g was comparable for the different biodistribution time points. The %ID/g measurements in different organs showed, however, smaller standard deviations with higher mAb dose, which can be explained by the fast blood clearance and high liver and spleen uptake of some young mice that received a relatively low mAb dose (25 or 50 \(\mu\)g).\textsuperscript{17}

Tumor-to-blood ratios decreased with increasing mAb doses and increased over time as summarized in Figure S2, due to the fact that the %ID/g in the tumor was almost constant over time, while the %ID/g in the blood decreased over time.

**CD44 targeting of RG7356 in responding and non-responding xenografts**

To compare the levels of tumor targeting, RG7356 was evaluated in mice with CD44\(^{+}\) responding (MDA-MB-231) and non-responding (PL45) xenografts.

The PL45 tumor xenograft-bearing mice showed significantly lower \(^{89}\text{Zr-RG7356}\) tumor uptake (16.8 ± 2.11\% ID/g vs. 23.17 ± 4.88\% ID/g, \(P < 0.05\)) at 1 d post-injection compared with the MDA-MB-231 tumor xenograft-bearing mice and a comparable uptake at 3 d post-injection (21.32 ± 3.41\% ID/g vs. 22.61 ± 2.75\% ID/g).

**Immuno-PET study in cynomolgus monkeys**

Immuno-PET studies in normal monkeys with \(^{89}\text{Zr-RG7356}\) revealed high uptake in the liver, spleen and the bone marrow.
With increasing doses of unlabeled RG7356, the blood pool radioactivity of $^{89}$Zr-RG7356 increased, whereas the radioactivity in the liver, spleen, salivary gland, and bone marrow decreased (Fig. 5). Dose-dependent decreases in spleen:blood, salivary gland:blood and bone marrow:blood ratios were observed at day 2 and 5 after injection (Fig. 5). At 2 d after injection, the spleen:blood ratio of $1.99 \pm 0.01$ (n = 2) at 0.1 mg/kg dose of $^{89}$Zr-RG7356 (tracer dose) was 2.9 times greater than spleen:blood ratio of $0.68$ (n = 1) when 0.1 mg/kg of $^{89}$Zr-RG7356 was co-injected with 20 mg/kg of the unlabeled RG7356. A similar trend was observed with bone marrow and salivary glands at 2 d. The bone marrow:blood ratio of $1.15 \pm 0.04$ (n = 2) at tracer dose was 2.6 times greater than the bone marrow:blood ratio of $0.43$ (n = 1), while the salivary glands:blood ratio of $0.93 \pm 0.08$ (n = 2) at tracer dose was 2.4 times greater than the bone marrow:blood ratio of 0.39 when the radiolabeled antibody was co-injected with 20 mg/kg of the unlabeled antibody. A high liver:blood ratio was obtained at tracer dose, but no clear dose-dependence was observed with increasing doses of the unlabeled antibody. The brain:blood, muscle:blood, kidney:blood and lung:blood ratios were similar across all doses (Fig. 5). The serum area under the curve (AUC) for radiolabeled antibody that was co-injected with 20 mg/kg of the unlabeled antibody was 1.3 times greater than the AUC for the radiolabeled antibody alone, indicative of target-mediated disposition and saturation of the sink with higher doses of the unlabeled antibody (Fig. S3). Due to the small sample size, statistical analyses were not performed on these data.

Single doses of $^{89}$Zr-RG7356 and RG7356 (up to 20 mg/kg) were well tolerated in all cynomolgus monkeys during the observation period of 2 wk.

**Discussion**

For any targeted therapy, it is important to understand target engagement at tumor tissue and normal organs and its relationship to dose and time. Radiolabeled antibodies have been used to confirm target expression at the site of interest (e.g., tumor tissue) and to assess whole body biodistribution, including determination of binding sites in physiologically normal organs.\(^{18-20}\) In addition to diagnostic applications, radiolabeled antibodies are used to gain better insights into the in vivo behavior and efficacy of therapeutic antibodies in patients.\(^{21-23}\)

The utility of immuno-PET in the development of an anti-CEACAM6 antibody drug conjugate (ADC) was described by Strickland et al. In non-human primates, antigen-dependent
toxicity of the anti-CEACAM6 ADC consisted of dose-dependent and reversible depletion of granulocytes and their precursors. This was associated with preferential and rapid localization of the antibody in bone marrow, as determined by sequential in vivo immune-PET imaging of the ⁶⁴Cu-radiolabeled anti-CEACAM6. Localization of the radiolabeled tracer could be attenuated by pre-dosing with unlabeled antibody confirming specific accumulation in this compartment.²⁴ Clinical utility of this technology was demonstrated in the early assessment of epidermal growth factor receptor (EGFR) as a target and cetuximab as a therapeutic antibody. The study revealed dose-dependent uptake in liver and tumor lesions, with optimum tumor targeting observed at 120 mg and 300 mg with partial saturation of the expression of EGFR in the liver.¹⁹

Considering recent advances in the development of ⁹⁹Zr for antibody imaging and immuno-PET,²⁵-²⁸ we radiolabeled

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**Figure 2.** Dose escalation study of ⁸⁹Zr-RG7356 in MDA-MB-231 xenograft bearing nude mice at 2 d (A), 3 d (B), and 6 d (C) after injection. A total mAb dose of 25, 50, 200, 500 and 1000 μg (latter predose) was injected and data are presented as %ID/g ± SD.
RG7356 with $^{89}$Zr to study whole body biodistribution in CD44$^+$ and CD44$^-$ tumor xenograft-bearing mice and normal cynomolgus monkeys. At 24 h after injection, $^{89}$Zr-RG7356 uptake in CD44$^+$ MDA-MB-231 tumors was nearly 9 times greater than that observed in CD44$^-$ HepG2 tumors (31.2 ± 5.4% ID/g vs. 3.6 ± 0.5% ID/g). The uptake of $^{89}$Zr-RG7356 remained consistent throughout the study period of 144 h in CD44$^+$ MDA-MB-231 tumors, and there was no significant difference between tumor uptake at 24 h compared with 144 h (31.2 ± 5.4% ID/g vs. 33.1 ± 7.4% ID/g). Decreased tumor uptake was observed when $^{89}$Zr-RG7356 was co-injected with increasing doses of the unlabeled antibody due to in vivo competition of the binding sites. However, in some mice we observed fast clearance of $^{89}$Zr-RG7356, which could be attributed to low endogenous IgG levels at the time of experimentation due to the immature immune system found in young $nu/nu$ mice. The immaturity of the immune system of these animals results in an excess of free Fc-binding receptors. These receptors bind to and clear the administered humanized monoclonal antibodies upon injection, as previously observed.17

The uptake of $^{89}$Zr-RG7356 in CD44$^+$ MDA-MB-231 (RG7356 therapy responsive tumors) did not increase from 1 d to 3 d, while the uptake in CD44-expressing PL45 (RG7356 therapy non-responsive tumors) did increase significantly ($P < 0.05$). These observations highlight the complex nature of tumor targeting and resultant efficacy. Tumor targeting is influenced not only by antigen density at the tumor tissue, but also by interstitial fluid pressure, tumor convection, and spatial variation in extravasation of the tumor. In addition, antigen density in normal tissues can greatly influence the availability of antibody to tumor tissue. Tumor uptake levels of antibodies are thus determined by the complex interplay between dose, affinity, diffusivity, antigen concentration, transport, and clearance. Mechanistic studies may be required to evaluate the correlation between tumor targeting and anti-tumor effects as well.

The studies in tumor xenograft-bearing mice demonstrated specific accumulation of $^{89}$Zr-RG7356 in CD44$^+$ tumors. RG7356 does not cross-react with murine CD44; therefore, the studies performed in human tumor xenograft-bearing mice, although informative with respect to tumor targeting, do not provide any information regarding accessible binding sites in physiologically normal organs. To circumvent the limitation of cross-reactivity to murine CD44, we performed additional studies in cynomolgus monkeys, which is a cross-reactive species. This represents the first report of $^{89}$Zr-immuno-PET imaging in cynomolgus monkeys. Immuno-PET studies in cynomolgus monkeys revealed dose-dependent uptake of the radiolabeled antibody in spleen, salivary glands and bone marrow. The expression of CD44 in these organs was confirmed by immunohistochemistry in another study performed with chimeric version of RG7356.29 The observed biodistribution of $^{89}$Zr-RG7356 in the spleen, salivary glands and bone marrow is of particular interest as CD44 is expressed on granulocytes and stem cells. High spleen uptake observed in monkeys may partially be attributed
to the interaction of the antibody with the host immune system, which is not observed in mice due to lack of cross-reactivity, as well as absence of an intact immune system in athymic nude mice. Furthermore, spleen and bone marrow are “sink” organs that can potentially influence tumor targeting at lower doses of mAb.

The preclinical studies in tumor-bearing mice and cynomolgus monkeys facilitated the clinical translation of $^{89}$Zr-RG7356 in patients with CD44-expressing solid tumors, which may enable better decision making in the clinical development of RG7356. In particular, the findings from the study reported helped us in the design of the clinical imaging study with respect to selection of time-points for imaging and doses for assessments. RG7356 and $^{89}$Zr-RG7356 are currently being evaluated in a Phase 1 study of patients with solid tumors (ClinicalTrials.gov Identifier: NCT01358903).

Materials and Methods

Antibody, cell lines, and radioactivity

RG7356, a full-length recombinant human mAb (150 kDa) of the immunoglobulin G1 (IgG1) kappa subclass was obtained from Discovery Oncology, Roche Diagnostics GmbH, Penzberg, Germany. For these studies, RG756 was produced using Chinese hamster ovary cell line as host cell line.

The human breast cancer cell line MDA-MB-231, the pancreatic adenocarcinoma epithelial cell line PL45 and the human hepatocellular carcinoma cell line HepG2 were obtained from American Type Culture Collection (ATCC). Cell lines were grown as monolayers at 37 °C in a humidified atmosphere with 5% CO₂. Cells were cultured in Dulbecco Minimal Essential Medium with 25 mM Hepes (Lonza Verviers), supplemented with 2 mM glutamine (200 mM solution, Lonza Verviers) and 5% fetal bovine serum (Heat inactivated, South American origin, Lonza Verviers). The CD44 expression profiles of these cell lines were previously described in the publication demonstrating therapeutic efficacy of RG7356.26

Radiolabeling, analyses, and stability studies

$^{89}$Zr-N-succinyl-desferrioxamine-RG7356 (hereafter designated $^{89}$Zr-RG7356) was prepared using previously described methods.26 Radiochemical purity was assessed using iTLC, HPLC and sodium dodecylsulfate-PAGE followed by phosphor imager analyses. In vitro binding characteristics of the radiolabeled RG7356 were determined in an immunoreactivity assay using a serial dilution of 2% paraformaldehyde-fixed MDA-MB-231 cells and a fixed amount of radiolabeled RG7356 (10 ng).30

Biodistribution studies in mice

Nude mice bearing xenografts obtained after subcutaneous injection of MDA-MB-231, PL45 or HepG2 cells were used. Female mice (athymic nu/nu, 21–31 g; Harlan CPB), were 7–12 wk old at the time of the experiments. All animal experiments were performed according to the National Institute of Health principles of laboratory animal care and Dutch national law (“Wet op de proefdieren.” Stb 1985, 336). CD44 positivity of MDA-MB-231 and PL45 tumors was confirmed by immunohistochemistry as described in the supplementary materials and methods.

CD44 targeting study with $^{89}$Zr-RG7356

In the first experiment, mice bearing MDA-MB-231 xenografts (n = 20) were injected via the retroorbital plexus with 185 kBq $^{89}$Zr-RG7356 in 100 μL of buffer. Unlabeled RG7356 was added to the injection mixture to bring the total mAb dose to 25 μg (0.8–1.0 mg/kg) per mouse. At 1, 2, 3 and 6 d after injection, five mice were anesthetized, bled, killed, and dissected. After blood, tumor, and normal tissues had been weighed, the amount of radioactivity in each sample was measured in a gamma-well counter. Radioactivity uptake was calculated as the percentage of the injected dose per gram of tissue (%ID/g).

In a second experiment, mice bearing HepG2 xenografts (n = 20) were injected via the retroorbital plexus with 185 kBq $^{89}$Zr-RG7356 in 100 μL of buffer. Unlabeled RG7356 was added to the injection mixture to bring the total mAb dose to 25 μg (0.8–1.0 mg/kg) per mouse. At 1, 2, 3, and 6 d after injection, five mice were anesthetized, bled, killed, and dissected. After blood, tumor, and normal tissues had been weighed, the amount of radioactivity in each sample was measured in a gamma-well counter. Radioactivity uptake was calculated as the percentage of the injected dose per gram of tissue (%ID/g).

Antibody dose escalation study with $^{89}$Zr-RG7356

Mice bearing MDA-MB-231 xenografts (n = 60) were injected via the retroorbital plexus with 185 kBq $^{89}$Zr-RG7356 in 100 μL. Unlabeled RG7356 was added to the injection mixture to bring the total mAb dose to 25, 50, 200, or 500 μg per mouse (12 mice per group). A fifth group also received 25 μg of $^{89}$Zr-RG7356, but was pre-dosed 2 d before with 1000 μg of unlabeled RG7356. At 2, 3 and 6 d after injection, four mice per group were anesthetized, bled, killed, and dissected, with further processing according to the above procedure.
CD44 targeting of RG7356 in responding and non-responding xenografts

Mice bearing MDA-MB-231 xenografts (n = 10) or PL45 xenografts (n = 10) were injected via the retroorbital plexus with 185 kBq 89Zr-RG7356. Unlabeled RG7356 was added to the injection mixture to bring the total mAb dose to 50 μg per mouse. At 1 and 3 d after injection, five mice were anesthetized, bled, killed, and dissected, with further processing according to the above procedure (Fig. 3).

Immuno-PET study in cynomolgus monkeys

Immuno-PET studies were performed in naive adult male cynomolgus macaques, aged 2–3 y and weighing approximately 1.5–2 kg at the time of the study. All procedures in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

Animal preparation was performed under propofol anesthesia (2.5–10 mg/kg) and consisted of insertion of an intravenous catheter in the right cephalic or saphenous vein distally for the injection of the radiolabeled antibody and endotracheal intubation for administration of anesthesia. Monkeys were positioned supine in a PET compatible pediatric restraint on the scanner couch, and placed under isoflurane anesthesia (1–2%). On a single occasion, 15 MBq of 89Zr-RG7356 (0.1 mg/kg) was co-injected with 0, 0.5, 2, 5, and 20 mg/kg of the unlabeled RG7356 antibody, one dose for each separate subject. Static three-dimensional (3D) PET scans were performed on a General Electric Discovery VCT whole body scanner; 35 simultaneous slices with an axial field of view of 15.7 cm. Animals were scanned for 20 min at each of three bed positions covering the snout to the knees on day 2. On day 5, the animals were scanned for 40 min in each of the three bed positions. During the study, the animal was moved successively axially in 15 cm increments to image the head, chest, abdomen, and pelvis. Transmission scans, for attenuation correction and image co-registration, were performed at all levels. The exact scan time was recorded along with the time of administration of the radiolabeled test substance. The 3D emission scan data were corrected for attenuation, dead-time, decay, scatter, randoms and were normalized. The 141 image planes of 3.27 mm were Fourier rebinned and reconstructed using a fully 3D iterative reconstruction algorithm taking into account detector geometry, off-center reconstruction and partial volume effects, giving a resolution of ~4.0 mm Full Width Half Maximum. The reconstructed volume consisted of 141 image planes of 256 × 256 voxels, with each voxel equaling 0.78 × 0.78 × 3.27 mm. These were fused into a single 3D data set (Fig. 4). Standardized uptake values (SUV) were derived by drawing 3D isocountour volumes of interest enclosing the tissue of interest using PMOD 3.2 software.

Figure 5. Tissue to blood ratios at 2 d (A) and 5 d (B) after injection of 89Zr-RG7356 in cynomolgus macaques injected with 15 MBq 89Zr-RG7356 and 0.5, 2, 5, or 20 mg/kg unlabeled RG7356.
(PMOD technology Ltd). In addition to imaging, safety assessments were made by measuring vital signs, clinical chemistry and hematology parameters.

**Statistical analysis**

Statistical analysis was performed on pharmacokinetics, tissue uptake and tumor-to-blood ratios between different groups of mice with the Student *t* test (SPSS) for paired data. Two-sided significance levels were calculated and *P* ≤ 0.05 was considered as statistically significant.

**Disclosure of Potential Conflicts of Interest**

Vugs D was supported by the Roche Postdoctoral fellowship Program. Nayak TK and Bergstrom M were employees of F Hoffmann La Roche at the time of the study, and Weigand S is an employee of Roche Diagnostics GmbH. The study was funded by F Hoffmann La Roche.

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**Supplemental Materials**

Supplemental materials may be found here:

www.landesbioscience.com/journals/mabs/article/27415
28. Nayak TK, Garmestani K, Milenic DE, Brechbiel MW. PET and MRI of metastatic peritoneal and pulmonary colorectal cancer in mice with human epidermal growth factor receptor 1-targeted 89Zr-labeled panitumumab. J Nucl Med 2012; 53:113-20; PMID:22213822; http://dx.doi.org/10.2967/jnumed.111.094169

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