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TITLE First-in-Human Study of 68 Ga-DOTA-Siglec-9, PET Ligand Targeting Vascular Adhesion Protein 1

YEAR 2020, August

DOI https://doi.org/10.2967/jnumed.120.250696

VERSION Final Draft (AAM)

CITATION Riikka Viitanen, Olli Antero Moisio, Petteri Lankinen, Xiang-Guo Li, Mikko Koivumäki, Sami Suilamo, Tuula Tolvanen, Kirsi Taimen, Markku Mali, Ia Kohonen, Ilpo Koskivirta, Vesa Oikonen, Helena Virtanen, Kristiina Santalahti, Anu Autio, Antti Saraste, Laura Pirilä, Pirjo Nuutila, Juhani Knuuti, Sirpa Jalkanen and Anne Roivainen
Journal of Nuclear Medicine August 2020, jnumed.120.250696; DOI: https://doi.org/10.2967/jnumed.120.250696

Journal of Nuclear Medicine, published on August 28, 2020 as doi:10.2967/jnumed.120.250696

First-in-Human Study of ^{68}Ga-DOTA-Siglec-9, PET Ligand Targeting Vascular Adhesion Protein 1

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Word count: 5000; abstract word count 279

Running title: ^{68}Ga-DOTA-Siglec-9 first-in-human trial

Conflict of interest: Sirpa Jalkanen owns stocks in Faron Pharmaceuticals Ltd. The remaining authors have no conflicts of interest to disclose.

Financial support: The study was conducted within the Finnish Centre of Excellence in Cardiovascular and Metabolic Diseases supported by the Academy of Finland, University of Turku, Turku University Hospital, and Åbo Akademi University. The study was financially supported by grants from the Academy of Finland, the State Research Funding of Turku University Hospital, Business Finland, the Sigrid Jusélius Foundation, the Jane and Aatos Erkko Foundation, the Finnish Foundation for Cardiovascular Research, the Finnish Cultural Foundation, and the Drug Research Doctoral Programme of the University of Turku Graduate School.

ABSTRACT

Sialic acid-binding immunoglubulin-like lectin 9 (Siglec-9) is a ligand of vascular adhesion protein 1 (VAP-1). A gallium 68-labeled peptide of Siglec-9, ^{68}Ga-DOTA-Siglec-9, holds promise as a novel PET tracer for imaging of inflammation. This first-in-human study investigated the safety, tolerability, biodistribution, and radiation dosimetry of this radiopharmaceutical. **Methods:** Six healthy males underwent dynamic whole-body PET/CT. Serial venous blood samples were drawn from 1–240 min after intravenous injection of 162 ± 4 MBq of ^{68}Ga-DOTA-Siglec-9. In addition to gamma counting, the plasma samples were analyzed by high-performance liquid chromatography to detect intact tracer and radioactive metabolites. Radiation doses were calculated using the OLINDA/EXM 2.2 software. In addition, a patient with early rheumatoid arthritis was studied with both ^{68}Ga-DOTA-Siglec-9 and ^{18}F-FDG PET/CT to determine the ability of the new tracer to detect arthritis. **Results:** ^{68}Ga-DOTA-Siglec-9 was well tolerated by all subjects. ^{68}Ga-DOTA-Siglec-9 was rapidly cleared from blood circulation and several radioactive metabolites were detected. The organs with the highest absorbed doses were the urinary bladder wall (0.38 mSv/MBq) and kidneys (0.054 mSv/MBq). The mean effective dose was 0.022 mSv/MBq (range 0.020–0.024 mSv/MBq). Most importantly, however, ^{68}Ga-DOTA-Siglec-9 was able to detect arthritis comparable to ^{18}F-FDG. **Conclusion:** Intravenous injection of ^{68}Ga-DOTA-Siglec-9 was safe and biodistribution is favorable for testing of the tracer in larger group of patients with rheumatoid arthritis planned in the next phase of clinical trials. The effective radiation dose of ^{68}Ga-DOTA-Siglec-9 was within the same range as those of other ^{68}Ga-labeled

tracers. Injection of 150 MBq of ^{68}Ga-DOTA-Siglec-9 would expose a subject to 3.3 mSv. These findings support the possible repeated clinical use of ^{68}Ga-DOTA-Siglec-9, e.g., in trials aiming to elucidate the treatment efficacy of novel drug candidates.

Key Words: dosimetry; gallium-68; kinetics; PET; vascular adhesion protein 1; whole-body distribution

INTRODUCTION

Early detection of inflammatory foci is critical for effective treatment of patients with a variety of diseases. Quantitative positron emission tomography/computed tomography (PET/CT) imaging provides a valuable tool for diagnosing and monitoring the effects of therapeutic interventions. The glucose analog 2-deoxy-2-^{18}F-fluoro-*D*-glucose (^{18}F-FDG) is the gold standard radiopharmaceutical for inflammation using PET, but it is not specific. Also, ^{68}Ga-citrate and ^{89}Zr-transferrin can detect inflammation due to increased expression of transferrin receptor 1 on mononuclear cells, but the receptor is also expressed in cancer cells (*1*). Accordingly, radiopharmaceuticals more specific than the above-mentioned are needed to assess inflammation and its consequences for various pathologies.

Vascular adhesion protein 1 (VAP-1); also known as amine oxidase, copper containing 3 is an inflammation-inducible endothelial cell molecule that mediates leukocyte trafficking from blood to sites of inflammation. Although VAP-1 plays important roles in early phases of inflammation, its luminal expression on the endothelium remains constant if inflammation continues, making it a promising target for molecular imaging of inflammation. We previously showed that sialic acid-binding immunoglobulin-like lectin 9 (Siglec-9) is a VAP-1 ligand, and that the gallium 68-labeled 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid-conjugated peptide containing residues 283–297 of Siglec-9 (^{68}Ga-DOTA-Siglec-9) can be used for PET imaging of inflammation in various experimental models (*2*-*9*).

We are currently engaged in the clinical evaluation phase of the ^{68}Ga-DOTA-Siglec-9 radiopharmaceutical. The purpose of this first-in-human study

was to obtain information on the safety, tolerability and whole-body biodistribution and kinetics of ^{68}Ga-DOTA-Siglec-9 after single intravenous injection in healthy volunteers and in a patient with rheumatoid arthritis (RA). The study sought to assess the effective radiation dose and radiation exposure in critical target organs in order to evaluate the safety of this ^{68}Ga-labeled PET ligand. In addition, we evaluated the *in vivo* stability and pharmacokinetics of ^{68}Ga-DOTA-Siglec-9, which will be important for quantifying VAP-1 receptor density in future clinical trials of patients with inflammatory diseases. Finally, we tentatively compared the ability of ^{68}Ga-DOTA-Siglec-9 PET/CT to detect RA by comparing it to ^{18}F-FDG.

MATERIALS AND METHODS

Chemicals and Reagents

Good Manufacturing Practice (GMP) grade precursor DOTA-Siglec-9 was obtained from ABX Advanced Biomedical Compounds GmbH. All other reagents were purchased from commercial suppliers and were either synthesis or analytical grade.

Preparation of ^{68}Ga-DOTA-Siglec-9

Radiosynthesis was performed as previously described using a fully automated synthesis device (Modular Lab PharmTracer; Eckert & Ziegler); the process complied with GMP requirements (*10*). ^{68}Ga was obtained from a ^{68}Ge/^{68}Ga-generator (IGG-100, 1.85 GBq, Eckert & Ziegler) by eluting the generator with 6 mL of 0.1 M HCl and passing the eluate through a Strata-XC cation exchange

cartridge. Bound $^{68}GaCl_3$ was eluted with acidified acetone (0.8 mL, containing 3.25% water and 0.02 M HCl) into a reaction vial preloaded with a mixture of DOTA-Siglec-9 (30 µg, 12 nmol in 60 µL water), sodium acetate buffer (2.0 mL, 0.2 M, pH 4.0), and absolute ethanol (0.2 mL). The reaction mixture was incubated at 65°C for 6 min and then diluted with saline (4 mL, 0.9 mg/mL). The crude product was purified by loading onto a C18 cartridge (SepPak Light C18; Waters) and washing the cartridge with saline (2×8 mL). ^{68}Ga-DOTA-Siglec-9 was eluted with ethanol (1.3 mL, 70% [*v/v*]) through a non-pyrogenic 0.22 µm filter into the sterile final product vial. The product was formulated in physiological saline, and the final volume of the end product was 10 mL. The total synthesis time was 25 min.

The radiochemical purity of the product was evaluated by high-performance liquid chromatography (radio-HPLC; LC-20A Prominence HPLC System, Shimadzu; on-line radioactivity detector Flow-Count, Bioscan Inc.) using an analytical Kinetex C18 column (2.6 µm, 100 Å, 75×4.6 mm; Phenomenex) at a flow rate of 1.0 mL/min and a gradient of 0.16% trifluoroacetic acid (TFA) in water (A) and 0.16% TFA in acetonitrile (B) (gradient from 18% B to 50% B over 12 min).

The *in vitro* stability of ^{68}Ga-DOTA-Siglec-9 was tested in the formulation solution (saline) by incubation for 2 h at room temperature, followed by radio-HPLC analysis as described above.

Healthy Subjects

Six healthy male volunteers (age, 37 ± 9 years; weight 80 ± 4 kg; height 181 ± 8 cm) were studied for the whole-body distribution kinetics of intravenously (*i.v.*) administered ^{68}Ga-DOTA-Siglec-9 using PET/CT imaging. One catheter was inserted into an antecubital vein for injection of ^{68}Ga-DOTA-Siglec-9, and another into the contralateral arm for blood sampling.

The study was approved by the joint Ethics Committee of the University of Turku and Turku University Hospital, and by the Finnish Medicines Agency. Each subject gave informed consent before entering the study. The study was registered as a clinical trial (NCT03755245).

Absence of significant medical, neurological, and psychiatric history, and of history of alcohol or drug abuse, was assessed using questionnaires. In addition, a review of medical history, routine blood tests, electrocardiography, and a physical exam were performed for each subjects. During imaging, vital signs were monitored, including 12-lead electrocardiography, and blood and urine analyses were performed before and after PET/CT.

Rheumatoid Arthritis Patient

Gadolinium-enhanced magnetic resonance imaging (MRI), ^{68}Ga-DOTA-Siglec-9 and ^{18}F-FDG PET/CT imaging were performed on a 49-year-old male patient with RA (duration of disease 3.5 weeks, rheumatoid factor positive, anti-citrullinated peptide antibodies >340 U/mL, erythrocyte sedimentation rate 5 mm/h, C-reactive protein <1 mg/L). The patient fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism Rheumatoid Arthritis Classification Criteria for his diagnosis. Disease activity was assessed

clinically at screening, four weeks before PET studies, when DAS28 was 3.3 and the tender and swollen joints counts were 5.

PET/CT

The biodistribution of ^{68}Ga-DOTA-Siglec-9 was imaged using a Discovery 690 whole-body PET/CT scanner (General Electric Medical Systems). Low-dose CT (120 kV, 14 mA) for attenuation correction was performed before PET.

In healthy subjects, ^{68}Ga-DOTA-Siglec-9 (162 ± 4 MBq, 4.2 ± 0.9 mL, 13.6 ± 3.0 μg) formulated in saline was injected *i.v.*, and whole-body PET scanning started at 1, 10, 20, 40, 100, and 200 min post-injection. Acquisition times per bed position were 30, 60, 120, 180, 300, and 360 sec. Scanning at 100 min post-injection included 14 bed positions, covering the range from head to toes. All other scans used only eight bed positions, covering the range from head to mid thighs.

The patient with RA was first subjected to a ^{18}F-FDG PET/CT whole-body scan. After a fast of 6 h, PET scan started 46 min after 198 MBq injection of ^{18}F-FDG. An immobilization system was used for the hands and wrists to ensure the same positioning for subsequent PET/CT. The patient was scanned from head to toes, with a 2-min acquisition time for each bed position. Then, on the next day, the patient was *i.v.* injected with 175 MBq of ^{68}Ga-DOTA-Siglec-9 and a 30-min dynamic PET/CT (time frames 4×30 s, 3×60 s, 5×180 s, 2×300 s) was performed on hands followed by whole-body scan (3 min per bed position).

PET images were reconstructed using a 3D VUE Point algorithm with two iterations, 24 subsets, and a 3 mm full-width at half-maximum post-filter. Scatter

correction, random counts, and dead-time corrections were all incorporated into the reconstruction algorithm.

Distribution Kinetics and Dose Estimates

Whole-body image data were quantified in accordance with the Radiation Dose Assessment Resource method for internal dose estimation (*11*). Time-activity values were determined for brain, bone (cortical and trabecular), heart contents (left ventricle), heart wall, kidneys, liver, lungs, muscle, pancreas, red bone marrow, salivary glands, spleen, and urinary bladder. Volumes of interests (VOIs) covered the whole organ or representative volume of the organ. Urinary clearance and biological half-life were estimated using measurements of urinary voids.

The resultant kinetic data were modeled using the sums of exponentials to determine number of disintegrations (residence times) of the source organs. A urinary bladder voiding interval of 3.5 h was used in calculation of bladder residence time. Radiation absorbed doses were determined using these residence times and the Organ Level INternal Dose Assessment/EXponential Modeling (OLINDA/EXM) 2.2 software (*12*). An adult male (~70 kg) reference model was utilized.

Assessment of Arthritis

VOIs were defined on the area of three inflamed finger joints (proximal interphalangeal joints PIP2, PIP3, distal interphalangeal joint DIP3) of the right hand, the time-activity curves of ^{68}Ga-DOTA-Siglec-9 were determined and

standardized uptake values (SUV) of ^{68}Ga-DOTA-Siglec-9 and ^{18}F-FDG were compared.

Blood Analyses

Venous blood samples were collected into heparinized tubes at 2, 3, 5, 6, 7, 10, 15, 20, 30, 60, 90, 180, and 240 min the post-injection of ^{68}Ga-DOTA-Siglec-9.

The radioactivity of whole blood was measured with an automatic gamma counter (1480 Wizard 3"; EG&G Wallac). Plasma was separated by centrifugation (2,100×*g* for 5 min at 4°C), and plasma radioactivity was measured. Then, 500 µL plasma was mixed with 700 µL acetonitrile to precipitate plasma proteins. The ratio of radioactivity in plasma vs. blood (plasma-blood ratio) and the percentage of radioactivity bound to plasma proteins were calculated as previously described (*13*).

To examine the stability of ^{68}Ga-DOTA-Siglec-9 *in vivo*, 600 µL plasma was mixed with 600 µL 10% sulfosalicylic to precipitate plasma proteins. The protein-free plasma supernatants obtained after centrifugation were analyzed by radio-HPLC. Radio-HPLC was performed using a semi-preparative Kinetex C18 column (5 µm, 300 Å, 150×10 mm; Phenomenex) at a flow rate of 5 mL/min and a gradient of 0.16% TFA in water (A) and 0.16% TFA in acetonitrile (B) as follows: 0−11 min, solvent B from 0% to 50%; 11−12 min, solvent B from 50% to 100%; 12−14 min, solvent B 100%; and 14−15 min, solvent B from 100% to 0%. The radio-HPLC system consisted of a LaChrom Instruments HPLC system (Hitachi) and a Radiomatic 150TR flow-through radioisotope detector (Packard).

Urine samples were analyzed using the same HPLC procedure as for the plasma samples.

To estimate the plasma concentration of unchanged tracer, the fraction of ^{68}Ga-metabolites in plasma was subtracted from the total radioactivity. The metabolite-corrected plasma concentration was used to calculate the pharmacokinetic parameters. Prior to any pharmacokinetic evaluation, all radioactivity values in blood and plasma were decay-corrected using the ^{68}Ga physical half-life of 68 min. Pharmacokinetic parameters were obtained using monoexponential fitting of the tail and were adjusted for injected radioactivity dose.

Soluble VAP-1

The activity of sVAP-1 in the plasma samples was measured using an assay described in detail in (*14*). The levels of sVAP-1 in the heparin samples were measured with an in-house enzyme-linked immunosorbent assay as previously described (*15*).

Statistical Analyses

Arithmetic mean values were calculated from the individual measurements and expressed at a precision of 1 standard deviation (mean ± SD).

RESULTS

Preparation of ^{68}Ga-DOTA-Siglec-9

The radiopharmaceutical ^{68}Ga-DOTA-Siglec-9 was obtained at high yield (89.7 ± 2.0%, n = 6). Radioactivity concentration and molar activity at the end of synthesis were 56.7 ± 10.6 MBq/mL and 43.2 ± 8.0 GBq/μmol, respectively. Radiochemical purity was ≥95% in all batches, and the tracer remained radiochemically stable for 2 h (longer times were not tested) in sterile saline at room temperature. Quality control data from three representative batches of ^{68}Ga-DOTA-Siglec-9 are shown in Supplemental Table 1.

Safety and Tolerability

^{68}Ga-DOTA-Siglec-9 was well tolerated by all subjects. We observed no adverse or clinically detectable pharmacologic effects in any subject. Moreover, we observed no significant changes in vital signs or in the results of laboratory studies or electrocardiograms. Healthy subject #6 was nauseous and got a headache at the end of PET/CT imaging, but it turned out that this subject had been fasting without water, contrary to the instructions, for a long period of time. Hematology, serology, and clinical chemistry of the study subjects are shown in Supplemental Table 2 and 3.

Whole-Body Distribution and Radiation Dose

Whole-body PET/CT imaging of five healthy volunteers revealed that the ^{68}Ga-radioactivity after *i.v.* injection of ^{68}Ga-DOTA-Siglec-9 was rapidly excreted through kidneys to the urinary bladder (Fig. 1). Maximum peak uptake of total injected radioactivity was observed in the urinary bladder contents, with SUV 114 (Fig. 2 and Supplemental Fig. 1). Subject #6 differed from the other

participants, exhibiting even higher urinary bladder radioactivity, peaking at SUV 242. Average biological half-life of ^{68}Ga-DOTA-Siglec-9 was 191 ± 33 min.

The normalized number of disintegrations (residence times) of the source organs and the remainder of the body are listed in Table 1. The largest mean residence times for ^{68}Ga-DOTA-Siglec-9 were in urinary bladder contents (0.33 h) and the remainder of the body (0.73 h).

Absorbed doses, reported in Table 2, were estimated based on an adult male weighing 70 kg. The organs with the highest absorbed doses were the urinary bladder wall (383.2 μSv/MBq) and the kidneys (54.4 μSv/MBq). The mean effective dose (International Commission on Radiological Protection [ICRP] publication 103) (*16*) was 0.022 mSv/MBq. Thus, the effective dose from 150 MBq of injected radioactivity was 3.3 mSv.

Assessment of Arthritis

High regional uptake of both ^{68}Ga-DOTA-Siglec-9 and ^{18}F-FDG was observed at the site of inflamed joints as compared with the unaffected joints. The time course at the site of arthritis revealed very fast uptake of ^{68}Ga-DOTA-Siglec-9, which reached a plateau after 10 minutes (Fig. 3).

Metabolism and Pharmacokinetics

Following administration of ^{68}Ga-DOTA-Siglec-9, the concentration of radioactivity in venous plasma declined rapidly, and several other peaks in addition to the intact tracer were detected by radio-HPLC. Typical radio-HPLC

chromatograms are shown in Fig. 4 and Supplemental Fig. 2. The retention time of ^{68}Ga-DOTA-Siglec-9 was 9.3 ± 0.1 min. According to the radio-HPLC analysis, the mean ± SD values of intact ^{68}Ga-DOTA-Siglec-9 were 79.2% ± 5.3% and 4.3% ± 1.6% at 1, and 10 min post-injection, respectively. Two radiometabolite peaks, with retention times of 3.2 ± 0.1 min and 4.6 ± 0.1 min, were still detectable in plasma at 90 min post-injection. Analysis of urine revealed only one radiometabolite peak (retention time, 4.6 ± 0.1 min) in all patients.

The pharmacokinetic parameters determined for total radioactivity and the intact tracer are summarized in Supplemental Table 4. Time-activity curves revealed rapid clearance from the blood circulation (Fig. 5A), and the PET image-derived curves from heart left ventricle closely correlated with radioactivity concentration measured from venous blood (Supplemental Fig. 3). The mean plasma half-life of total radioactivity was 106.9 min, and total clearance was 0.18 mL/min. For the intact tracer, total clearance was estimated to be 3.3 mL/min (Fig. 5B).

The plasma-blood ratio of radioactivity was 1.7 ± 0.04 throughout the 240 min duration of the PET imaging, indicating that most of the radioactivity was in the plasma. Mean plasma protein binding of radioactivity was 25.9% ± 8.8%; the value slowly increased during PET imaging, ranging from 13.3% to 42.7% (Fig. 5C).

Soluble VAP-1

Because VAP-1 is also present in a soluble form in plasma, we also analyzed the concentration of sVAP-1 protein and its enzymatic activity in plasma. On the day of PET imaging, sVAP-1 levels in plasma were 874.0 ± 139.9 ng/mL, and enzymatic activity levels were 10.9 ± 2.2 nmol/L/h, as determined from four healthy subjects.

DISCUSSION

^{68}Ga-DOTA-Siglec-9 is a promising novel PET tracer for the imaging of inflammation. Here, we report the whole-body distribution and radiation exposure of ^{68}Ga-DOTA-Siglec-9. We also evaluated its metabolic fate and pharmacokinetics in healthy humans. Most importantly, however, we show for the first time that ^{68}Ga-DOTA-Siglec-9 PET/CT clearly delineates arthritis in a pilot patient with early RA.

Recently, we set up a well-validated radiosynthesis protocol for the production of GMP grade ^{68}Ga-DOTA-Siglec-9 (*10*). This protocol is highly reproducible, and to date the successful production rate in our lab has been 100%. This has paved the way for evaluation of ^{68}Ga-DOTA-Siglec-9 in the clinical setting. Accordingly, following the same radiosynthesis protocol, in this study we produced six batches of ^{68}Ga-DOTA-Siglec-9 with high radiochemical yield, radiochemical purity, and high molar activity. Prior to clinical trials, we performed safety studies of DOTA-Siglec-9 in rats complying with the requirements of GMP and Good Laboratory Practice, and using a 1000-fold excess of the planned clinical dose (40 μg per subject) (*17*). In routine

radiosynthesis, we managed to use even less precursor (30 μg) in each batch due to the efficiency of the synthesis protocol; the actual clinical dose (13.6 ± 3.0 μg per subject) is far below the estimated clinical dose used in the safety evaluations. Therefore, the potential clinical risks (if any) related to the test item itself were further reduced for this first-in-human trial.

I.v. administered ^{68}Ga-DOTA-Siglec-9 exhibited fast renal clearance; this is presumably connected to the hydrophilic properties and small size of the peptide. The highest radioactivity uptake was detected in urinary bladder, in line with the previous preclinical studies with Siglec-9 peptide (*7*), which could be reduced with frequent bladder voids. The radioactive metabolites of ^{68}Ga-DOTA-Siglec-9 may contribute to uptake in kidneys and urinary bladder, although the extent of such uptake remains unknown.

Results of image quantification and radiation dose estimates were consistent across the subject population, except for one subject who had significantly higher urinary bladder uptake than the others. Notably, this subject had been fasting without water, contrary to the instructions, for a long period of time. Although ^{68}Ga has been used extensively for the labeling of synthetic peptides, data for human dose estimates are available only for a few, e.g., for peptide analogs that bind to somatostatin receptors, bombesin, or $\alpha_v\beta_3$ integrin. The effective dose of ^{68}Ga-DOTA-Siglec-9 reported here (0.022 mSv/MBq according to ICRP-103) is within the range for ^{68}Ga-DOTATOC (0.023 mSv/MBq), ^{68}Ga-DOTANOC (0.025 mSv/MBq), and ^{68}Ga-NOTA-RGD (0.022 mSv/MBq) (*18-20*).

Although only one patient has been studied so far, the most intriguing finding in this study is the ability of ^{68}Ga-DOTA-Siglec-9 PET/CT to detect early RA. The observed SUVs of ^{68}Ga-DOTA-Siglec-9 (0.70−1.05) were slightly lower than those of ^{18}F-FDG (1.08−1.46) but it remains to be seen how optimization of the imaging protocol would affect ^{68}Ga-DOTA-Siglec-9 results.

^{68}Ga-DOTA-Siglec-9 exhibited rapid blood clearance, which usually occurs due to fast renal excretion. In addition to intact ^{68}Ga-DOTA-Siglec-9, several additional peaks were observed in plasma radio-HPLC chromatograms, but we did not further identify the radiometabolites of plasma or investigate their extent at the inflammatory sites. Radiometabolism and plasma protein binding are consistent with values reported previously in pigs (*6,21*).

Blood radioactivity concentration was not correlated with sVAP-1 level. Soluble VAP-1 levels in the plasma of healthy subjects varied from 729 to 1082 ng/mL, with a median of 874 ng/mL. These results are within the range previously reported in a Finnish prospective cohort study (*15*). The enzymatic activity levels of sVAP-1 reported here are slightly lower than those of previous studies. The lower values can be explained by our use of heparin-plasma samples, which have been shown to interfere with sVAP-1 enzymatic activity in comparison to serum samples (*14*).

Radiation burden and biodistribution were comparable with earlier results extrapolated from rats (*7*). The difference between the effective dose derived from rat data (0.024 mSv/MBq) and the human dosimetry (0.022 mSv/MBq) is small and in practice within the limits of measurement accuracy.

CONCLUSION

Intravenously injected ^{68}Ga-DOTA-Siglec-9 was safe and rapidly cleared from the blood by renal excretion. The radiation burden from ^{68}Ga-DOTA-Siglec-9 was relatively low and comparable to other ^{68}Ga-peptides. Frequent urination is recommended to reduce the radiation dose to the bladder. From a radiation safety perspective, ^{68}Ga-DOTA-Siglec-9 imaging is feasible for applied clinical studies and could be performed in the same subject multiple times per year. Such repeatability would be useful, for example, in trials aiming to clarify the treatment efficacy of novel drug candidates. Based on these results, we conclude that ^{68}Ga-DOTA-Siglec-9, which targets VAP-1, represents a promising new PET radiopharmaceutical for imaging inflammation in such diseases as RA.

FINANCIAL DISCLOSURE

SJ owns stock in Faron Pharmaceuticals. The other authors declare that they have no conflicts of interest to disclose.

ACKNOWLEDGMENTS

We greatly appreciate the professional assistance of the staff at Turku PET Centre, and we thank Timo Kattelus for preparing the figures.

KEY POINTS

QUESTION: Is the new radiopharmaceutical ^{68}Ga-DOTA-Siglec-9 safe and how is it distributed in healthy humans and patient with RA?

PERTINENT FINDINGS: Six healthy men and a patient with RA were studied by whole-body PET/CT imaging with safety monitoring and analyses of blood and urine. ^{68}Ga-DOTA-Siglec-9 was safe and exhibited rapid clearance from the blood circulation via renal excretion into the urine. ^{68}Ga-DOTA-Siglec-9 clearly detected arthritic joints. The highest radiation exposure was to the urinary bladder wall and kidneys. The effective dose 0.022 mSv/MBq was comparable to those of other ^{68}Ga-tracers.

IMPLICATIONS FOR PATIENT CARE: The characteristics of ^{68}Ga-DOTA-Siglec-9 are favorable for planned patient studies.

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FIGURES AND FIGURE LEGENDS

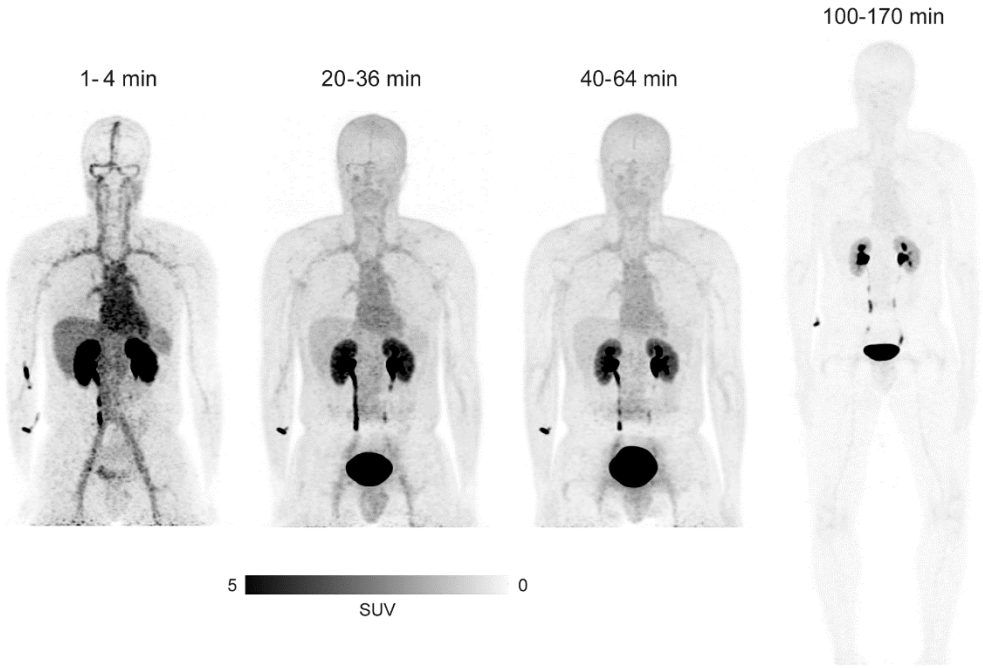

FIGURE 1. Whole-body coronal PET images of a healthy volunteer (male, 29 yrs, 74 kg, 183 cm) after intravenous injection of 167 MBq of ^{68}Ga-DOTA-Siglec-9. Distribution of ^{68}Ga-radioactivity 1-4 min after injection, based on imaging for 30 sec per bed position, revealed high uptake, predominantly in the heart, liver, kidneys, and ureter. At 20-36 min after injection and subsequent times, uptake was mainly in kidneys, ureter, and urinary bladder.

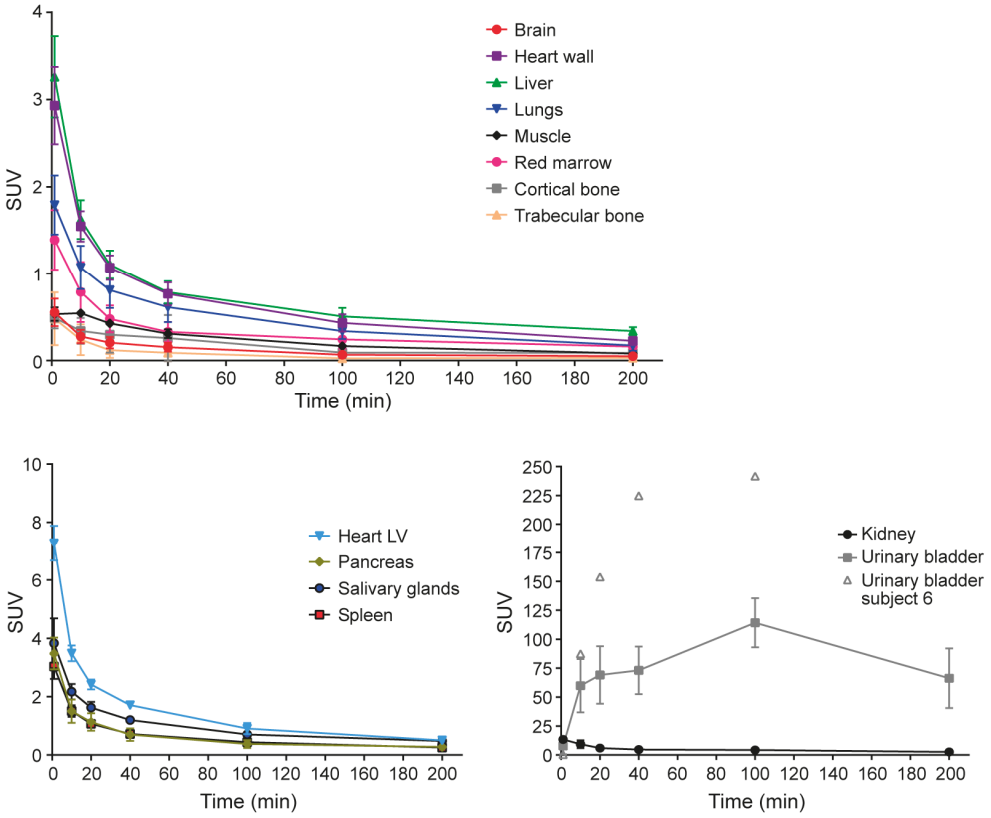

FIGURE 2. Decay-corrected time-activity curves of main organs. The urinary bladder result in subject #6 was an outlier, so it is presented separately. SUV=standardized uptake value; LV=left ventricle.

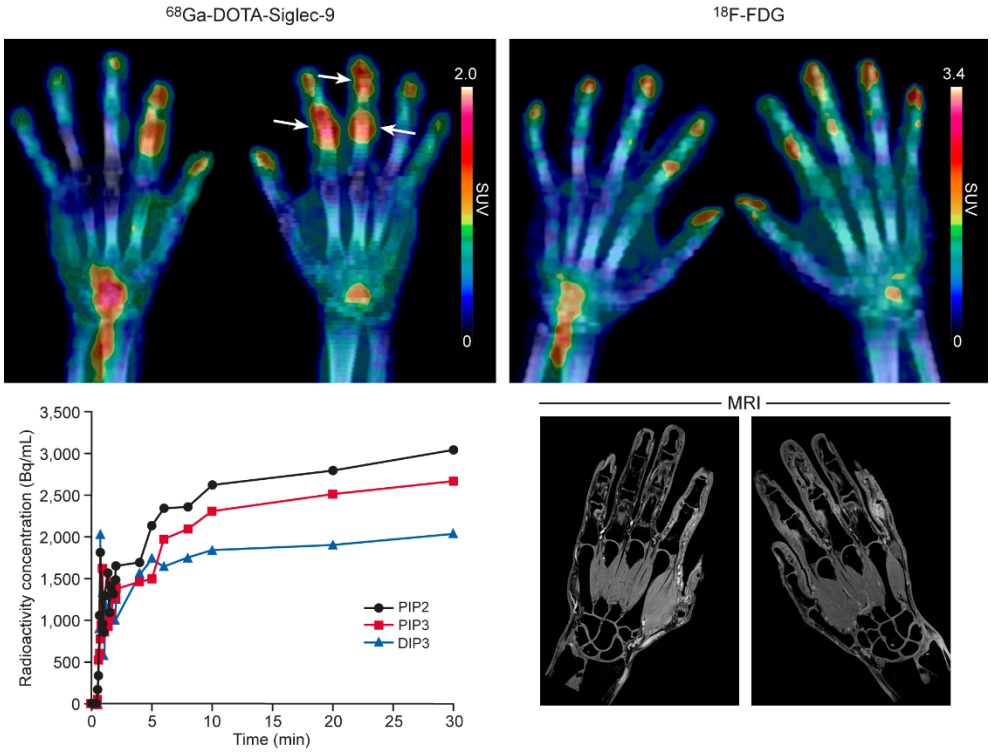

FIGURE 3. ^{68}Ga-DOTA-Siglec-9 and ^{18}F-FDG PET/CT and MR images of the hands of a 49-year-old patient with early RA. White arrows denote proximal interphalangeal joints PIP2, PIP3 and distal interphalangeal joint DIP3 whose time-activity curves are in the lower left panel. Corresponding SUVs were 1.05, 0.92 and 0.70 with ^{68}Ga-DOTA-Siglec-9 and 1.46, 1.22 and 1.08 with ^{18}F-FDG, respectively.

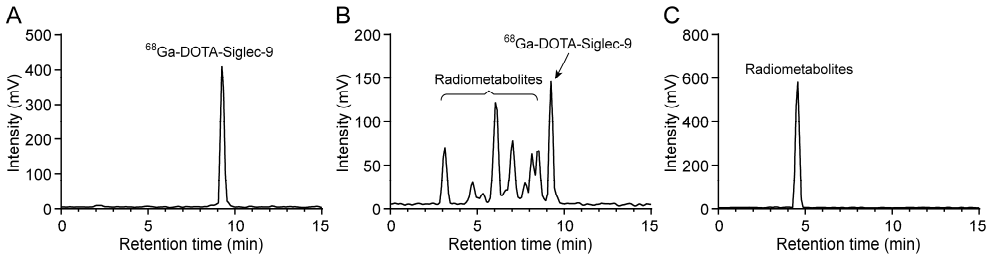

FIGURE 4. Representative radio-HPLC chromatograms of (A) intact ^{68}Ga-DOTA-Siglec-9, (B) human plasma 5 min after injection, and (C) urine at 40 min after injection. In addition to parent tracer, several other peaks were detected in plasma whereas in the urine the radioactivity was entirely from the radiometabolite.

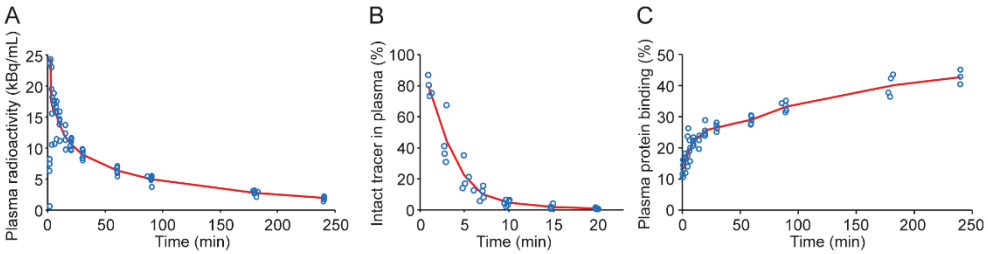

FIGURE 5. (A) Total radioactivity as well as (B) proportion of parent tracer decreased rapidly in blood circulation. (C) On average, 26% of radioactivity was bound to plasma proteins.

TABLE 1

Normalized Number of Disintegrations (Hours) in Source Organs After Injection of ^{68}Ga-DOTA-Siglec-9

	Mean	SD	Minimum	Maximum
Bone, cortical	0.0260	0.0045	0.0195	0.0312
Bone, trabecular	0.0039	0.0017	0.0025	0.0064
Brain	0.0047	0.0013	0.0033	0.0069
Heart contents	0.0033	0.0003	0.0030	0.0036
Kidneys	0.0359	0.0104	0.0231	0.0485
Liver	0.0377	0.0055	0.0266	0.0409
Lungs	0.0133	0.0033	0.0105	0.0176
Pancreas	0.0017	0.0004	0.0012	0.0021
Red marrow	0.0099	0.0019	0.0069	0.0127
Salivary gland	0.0005	0.0001	0.0005	0.0006
Spleen	0.0033	0.0004	0.0029	0.0040
Urinary bladder	0.3252	0.0426	0.2662	0.3831
Remainder of the body	0.7257	0.0906	0.5861	0.8501

TABLE 2

Dose Equivalent Estimates (μSv/MBq) and Effective Dose (mSv/MBq) After Injection of ^{68}Ga-DOTA-Siglec-9

	Mean	SD	Minimum	Maximum
Adrenals	12.2	1.4	10.7	14.2
Brain	2.4	0.5	1.9	3.3
Colon, left	8.3	0.8	7.2	9.3
Colon, right	8.4	0.8	7.3	9.4
Esophagus	6.9	0.8	5.9	8.0
Eyes	5.9	0.7	4.8	6.9
Gallbladder wall	8.7	0.9	7.7	9.8
Heart wall	11.5	1.3	10.0	13.3
Kidneys	54.4	14.9	36.0	72.5
Liver	12.7	1.6	9.4	13.7
Lungs	6.8	1.2	5.8	8.5
Osteogenic cells	9.9	1.2	8.4	11.7
Pancreas	8.5	1.4	6.8	10.0
Prostate	17.4	0.5	16.7	17.9
Rectum	14.1	0.1	14.0	14.3
Red marrow	8.6	0.8	7.7	10.0
Salivary glands	4.5	0.3	4.0	5.0
Small intestine	9.5	0.6	8.5	10.3
Spleen	12.8	1.2	11.5	14.6
Stomach wall	7.5	0.8	6.4	8.7
Testes	8.8	0.5	8.0	9.5
Thymus	6.8	0.8	5.7	8.0
Thyroid	6.7	0.8	5.5	7.8
Urinary bladder wall	383.2	48.5	316.0	449.0
Whole body	10.1	0.5	9.4	10.7
Effective dose*	0.022	0.002	0.020	0.024

* ICRP 103 (*16*).

SUPPLEMENTAL DATA

First-in-Human Study of ^{68}Ga-DOTA-Siglec-9, PET Ligand Targeting Vascular Adhesion Protein 1

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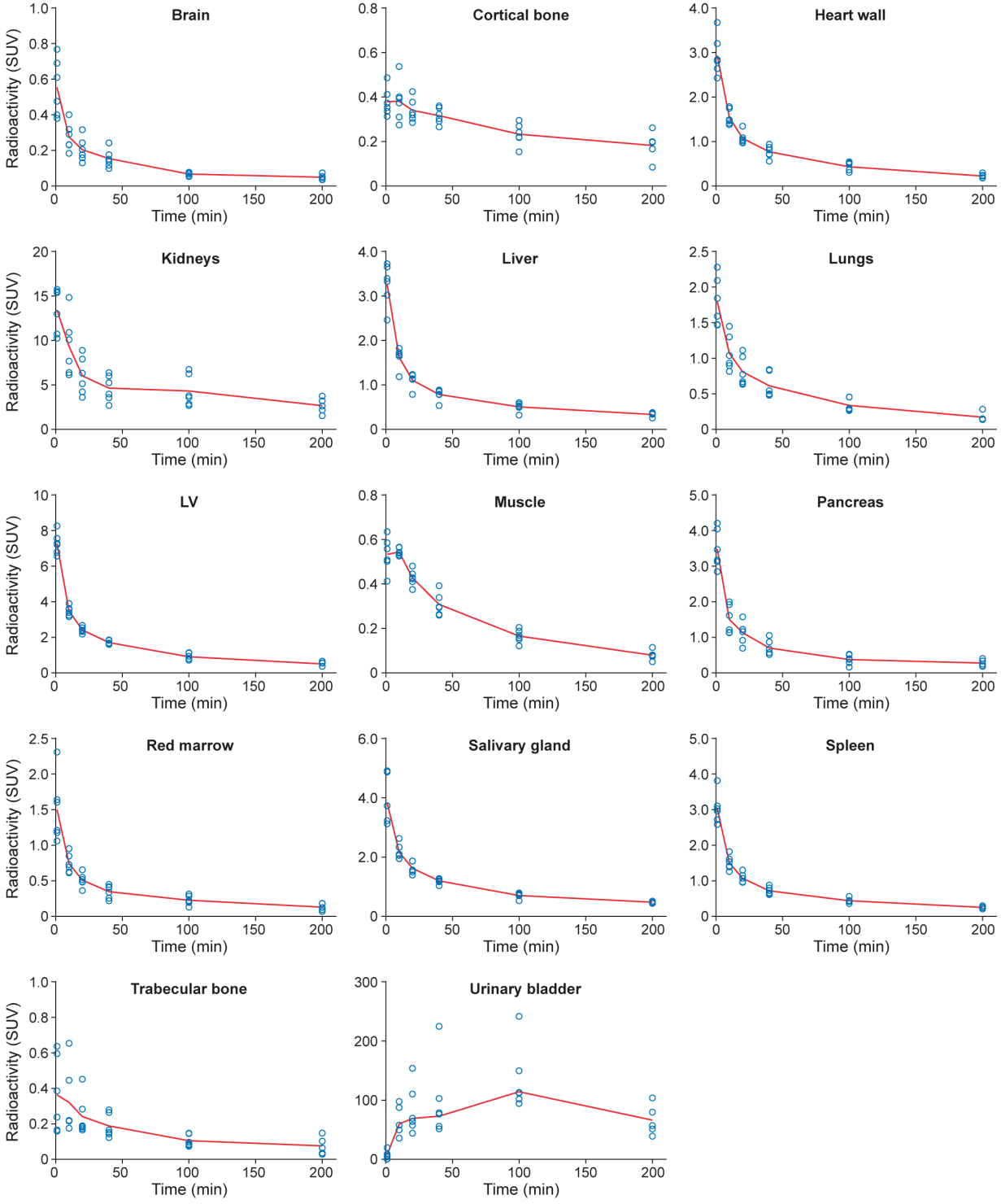
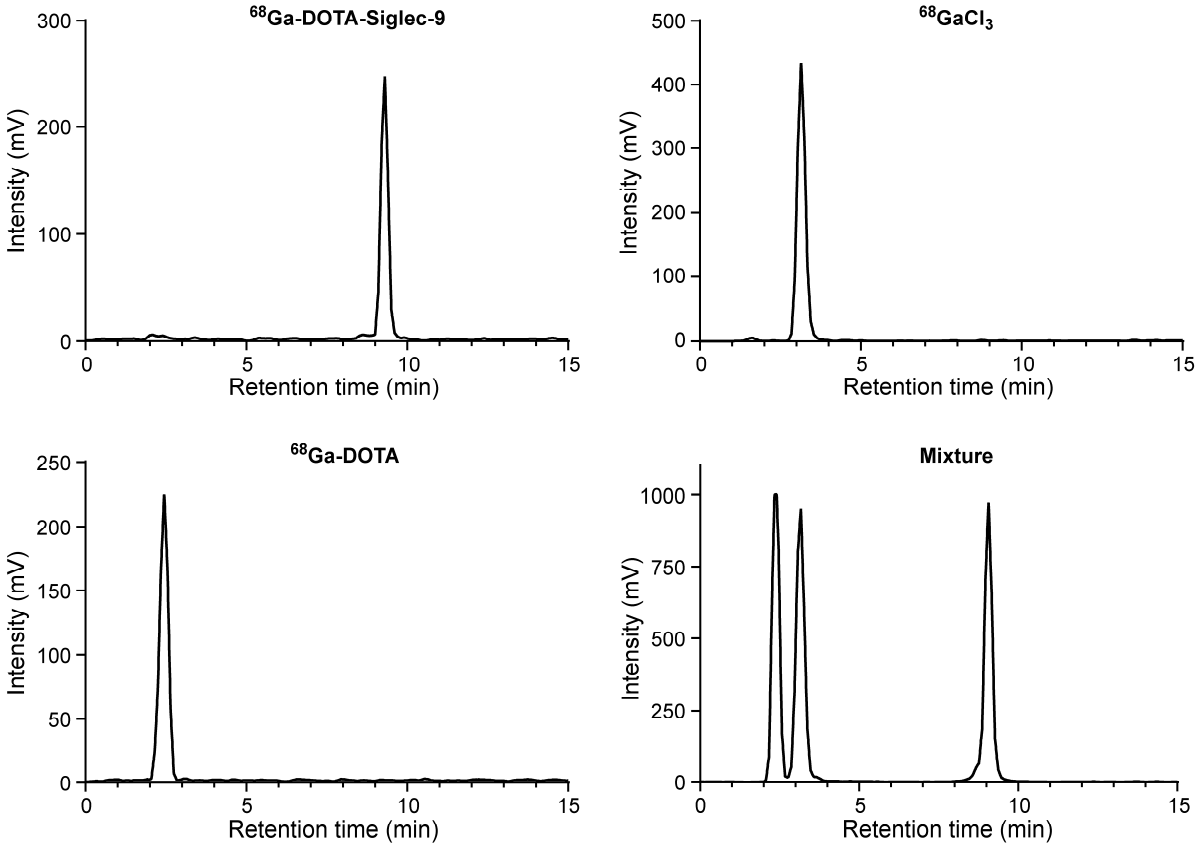
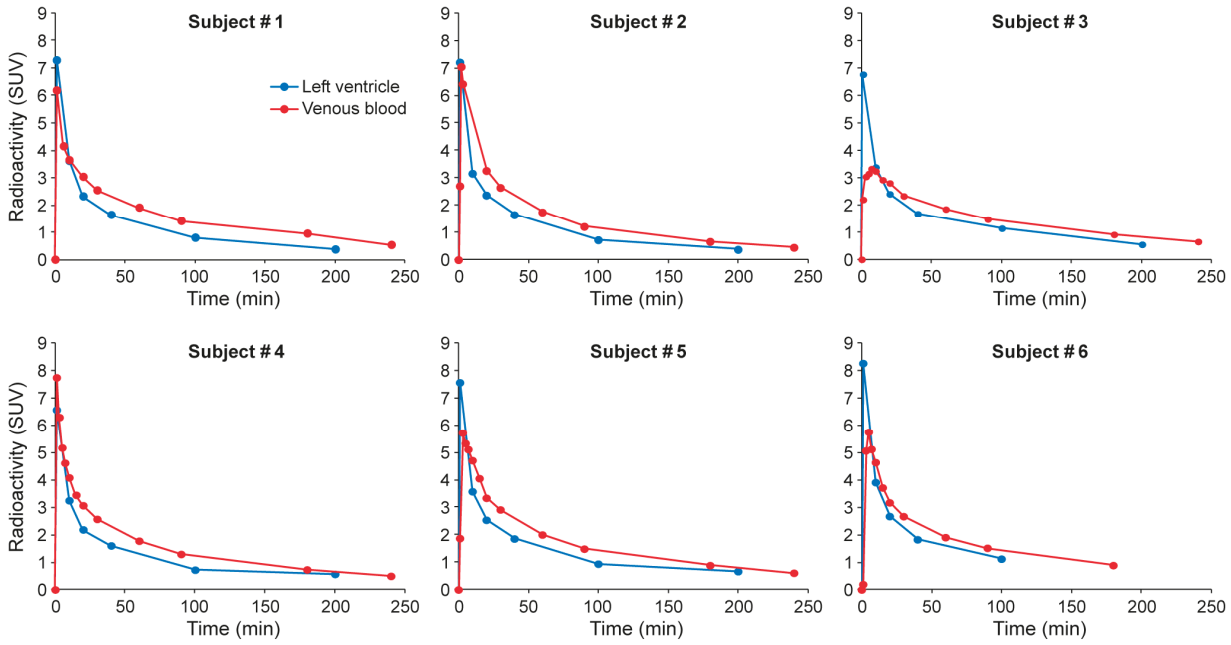


SUPPLEMENTAL FIGURE 1. Decay-corrected time-activity curves of main organs of six healthy subjects. The open circles represent each individual and red line is the average. SUV = standardized uptake value; LV = heart left ventricle.



SUPPLEMENTAL FIGURE 2. Representative radio-HPLC chromatograms of authentic standards. Mixture = *in vitro* mixed ^{68}Ga-DOTA-Siglec-9, $^{68}GaCl_3$, and ^{68}Ga-DOTA.



SUPPLEMENTAL FIGURE 3. PET image-derived time-activity curve from heart left ventricle closely correlates with radioactivity concentration of venous blood measured with a gamma counter. SUV = standardized uptake value.

SUPPLEMENTAL TABLE 1

Quality Control Data from Three Representative Batches of ^{68}Ga-DOTA-Siglec-9

Product specifications	Limits for product release	Results
Appearance	Clear and colorless solution, free of particles	Pass
pH	4.0 – 8.0	5.0 ± 0.0
Radionuclidic identity	68 min ± 3 min	67.7 ± 0.6 min
Identity	Retention time of ^{68}Ga-DOTA-Siglec-9 complies with the retention time of reference standard (± 1.0 min)	Pass*
Radiochemical purity	≥ 91% by HPLC	96.0 ± 1.0%
	≥ 91% by iTLC	97.7 ± 0.6%
Sum of unknown chemical impurities	≤ 4.1 µg/mL based on DOTA-Siglec-9	Pass
Content of DOTA-Siglec-9	≤ 4.1 µg/mL based on DOTA-Siglec-9	Pass
Radionuclidic purity	≥ 99.9% ^{68}Ga	Pass
	≤ 0.001% ^{68}Ge	Pass
Content of ethanol	≤ 10%	7.1 ± 0.7%
Residual solvents (acetone)	≤ 0.5%	0.0 ± 0.0%
Sterile filter integrity	≥ 3.45 bar	3.6 ± 0.1 bar
Sterility	Sterile (no viable organisms detected)	Pass
Bacterial endotoxins	≤ 17.5 endotoxin units/mL	Pass†

* Retention time of reference standard was 7.6 ± 0.1 min, and retention time of ^{68}Ga-DOTA-Siglec-9 was 7.7 ± 0.1 min.

† In each of the three batches of product, the level of bacterial endotoxins was < 0.1 units/mL.

SUPPLEMENTAL TABLE 2

Hematology, Serology, and Clinical Chemistry of Study Subjects

Parameter	Screening	Before PET/CT	After PET/CT	Reference value
White blood cell count (10^9/L)	5.8 ± 1.2	6.1 ± 1.1	7.7 ± 0.8	3.4 – 8.2
Red blood cell count (10^{12}/L)	5.1 ± 0.2	5.0 ± 0.2	4.8 ± 0.1	4.3 – 5.7
Hemoglobin (g/L)	154 ± 6.9	147 ± 2.9	145 ± 2.7	134 – 167
Hematocrit	0.45 ± 0.02	0.43 ±0.01	0.42 ± 0.01	0.39 – 0.50
Mean corpuscular volume (fL)	87 ± 2.3	87 ± 1.3	87 ± 0.7	82 – 98
Mean corpuscular hemoglobin (pg)	30 ± 0.8	30 ± 0.5	30 ± 0.3	27 – 33
Platelet count (10^9/L)	212 ± 41	202 ± 22	196 ± 15	150 – 360
Neutrophil count (10^9/L)	3.0 ± 1.1	3.5 ± 1.0	5.1 ± 0.9	1.5 – 6.7
Lymphocyte count (10^9/L)	2.0 ± 0.3	1.8 ± 0.1	1.9 ± 0.1	1.3 – 3.6
Monocyte count (10^9/L)	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.2 – 0.8
Eosinophil count (10^9/L)	0.23 ± 0.17	0.18 ± 0.05	0.14 ± 0.05	0.03 – 0.44
Basophil count (10^9/L)	0.05 ± 0.03	0.05 ± 0.01	0.04 ± 0.06	0.0 – 0.1
Absolute neutrophils (%)	51 ± 9.6	54 ± 5.3	64 ± 4.6	41 – 81
Absolute lymphocytes (%)	35 ± 9.4	32 ± 5.0	26 ± 3.1	20 – 45
Absolute monocytes (%)	9.3 ± 1.2	9.8 ± 0.9	7.5 ± 1.0	1 – 11
Absolute eosinophils (%)	3.8 ± 2.2	3.6 ± 1.2	2.2 ± 0.8	1 – 5
Absolute basophils (%)	0.8 ± 0.4	1.0 ± 0.3	0.5 ± 0.2	0 – 1
Erythrocyte sedimentation rate (mm/h)	3.7 ± 2.9	3.2 ± 0.7	2.0 ± 0.0	< 15
C-reactive protein (mg/L)	1.2 ± 0.4	1.0 ± 0.0	1.0 ± 0.0	< 10
Potassium (mmol/L)	4.1 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.3 – 4.8
Sodium (mmol/L)	142 ± 2.1	142 ± 0.5	142 ± 0.7	137 – 144
Creatinine (µmol/L)	88 ± 13	82 ± 5.5	80 ± 5.2	60 – 100
Alkaline phosphatase (U/L)	70 ± 12	65 ± 5.2	59 ± 5.0	35 – 105
Alanine aminotransferase (U/L)	44.7 ± 21.0	36.2 ± 7.6	35.7 ± 6.6	< 50
Rheumatoid factor (IU/mL)	< 10	ND	ND	< 14
Citrullinated peptide antibody (U/mL)	< 7	ND	ND	< 7

ND = not determined

SUPPLEMENTAL TABLE 3

Hematology, Serology, and Clinical Chemistry of a Patient with Rheumatoid Arthritis

Parameter	Before PET/CT	After PET/CT	Reference value
White blood cell count (10^9/L)	4.6	6.3	3.4 – 8.2
Red blood cell count (10^{12}/L)	4.50	4.23 *	4.3 – 5.7
Hemoglobin (g/L)	147	138	134 – 167
Hematocrit	0.41	0.39	0.39 – 0.50
Mean corpuscular volume (fL)	91	92	82 – 98
Mean corpuscular hemoglobin (pg)	33	33	27 – 33
Platelet count (10^9/L)	118 *	122 *	150 – 360
Neutrophil count (10^9/L)	3.02	4.67	1.5 – 6.7
Lymphocyte count (10^9/L)	0.96 *	1.03 *	1.3 – 3.6
Monocyte count (10^9/L)	0.52	0.50	0.2 – 0.8
Eosinophil count (10^9/L)	0.05	0.05	0.03 – 0.44
Basophil count (10^9/L)	0.03	0.03	0.0 – 0.1
Absolute neutrophils (%)	66	74	41 – 81
Absolute lymphocytes (%)	21	16 *	20 – 45
Absolute monocytes (%)	11	8	1 – 11
Absolute eosinophils (%)	1	1	1 – 5
Absolute basophils (%)	1	1	0 – 1
Erythrocyte sedimentation rate (mm/h)	5	7	< 15
C-reactive protein (mg/L)	< 1	< 1	< 10
Potassium (mmol/L)	3.9	4.0	3.3 – 4.8
Sodium (mmol/L)	139	140	137 – 144
Creatinine (µmol/L)	77	72	60 – 100
Alkaline phosphatase (U/L)	37	33 *	35 – 105
Alanine aminotransferase (U/L)	17	16	< 50
Rheumatoid factor (IU/mL)	19 *	ND	< 14
Anti-citrullinated peptide antibodies (U/mL)	> 340 *	ND	< 7

ND = not determined, * Not within the reference value

SUPPLEMENTAL TABLE 4

Plasma Pharmacokinetic Parameters After Intravenous Administration of ^{68}Ga-DOTA-Siglec-9

Parameter	Total radioactivity				Intact tracer	
	k_{el} (1/min)	$t_{1/2}$ (min)	$AUC_{(0\text{-}tlast)}$ (kBq*min/mL)	Cl_T (mL/min)	$AUC_{(0\text{-}tlast)}$ (kBq*min/mL) †	Cl_T (mL/min) †
Arithmetic mean	0.0065	106.9	915.9	0.1783	53.6	3.3221
Arithmetic SD	0.0004	7.1	86.3	0.0144	17.1	1.1450
Coefficient of variation (%)*	6.6	6.6	9.4	8.1	31.8	34.5

Injected dose of ^{68}Ga-DOTA-Siglec-9 was 162 ± 4 MBq (total mass, 13.6 ± 3.0 µg). Six experiments were performed.

k_{el} = elimination rate constant; $t_{1/2}$ = plasma half-life; AUC = area under curve; Cl_T = total clearance.

* SD/mean×100.

† Values have to be regarded as estimates because sampling time was not sufficient to adequately describe the terminal elimination phase.