Development and evaluation of interleukin-2 derived radiotracers for PET imaging of T-cells in mice

Elly L. van der Veen¹, Frans V. Suurs¹, Frederik Cleeren², Guy Bormans², Philip H. Elsinga³, Geke A.P. Hospers¹, Marjolijn N. Lub-de Hooge³,⁴, Elisabeth G.E. de Vries¹, Erik F.J. de Vries³, Inês F. Antunes³

¹Department of Medical Oncology, University of Groningen, University Medical Center Groningen, The Netherlands, ²Laboratory for Radiopharmaceutical Research, Department of Pharmacy and Pharmacology, University of Leuven, Belgium, ³Department of Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Center Groningen, The Netherlands, ⁴Department of Clinical Pharmacy and Pharmacology, University of Groningen, University Medical Center Groningen, The Netherlands.

Address of correspondence:
Inês Farinha Antunes, PhD
Department of Nuclear Medicine and Molecular Imaging
University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands
Tel.+31-50-3615779;Fax.+31-50-3611687
E-mail: i.farinha.antunes@umcg.nl

First author:
Elly L. van der Veen, PharmD / PhD student
Department of Medical Oncology
University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen,
The Netherlands
Tel.+31-50-3610871;Fax.+31-50-3611687
E-mail: e.l.van.der.veen@umcg.nl

Short running title: Interleukin-2 radiotracers for PET

Word count: 4993

Financial support: The research leading to these results received funding from the Innovative Medicines Initiatives 2 Joint Undertaking under grant agreement No 116106 (TRISTAN). This Joint Undertaking receives support from the European Union’s Horizon 2020 research and innovation program and EFPIA.

This research received support from Research Foundation – Flanders (FWO) (G0D8817N). Frederik Cleeren is a Postdoctoral Fellow of FWO (12R3119N).
ABSTRACT

Recently, N-(4-18F-fluorobenzoyl)-interleukin-2 (18F-FB-IL2) was introduced as PET tracer for T-cell imaging. However, production is complex and time-consuming. Therefore, we developed two radiolabeled interleukin-2 (IL-2) variants, namely aluminum 18F-fluoride-(restrained complexing agent)-IL-2 (18F-AlF-RESCA-IL2) and 68Ga-gallium-(1,4,7-triazacyclononane-4,7-diacetic acid-1-glutaric acid)-IL-2 (68Ga-Ga-NODAGA-IL2) and compared their in-vitro and in-vivo characteristics with 18F-FB-IL2. Methods: Radiolabeling of 18F-AlF-RESCA-IL2 and 68Ga-Ga-NODAGA-IL2 was optimized and stability was evaluated in human serum. Receptor binding was studied with activated human peripheral blood mononuclear cells (hPBMCs). Ex-vivo tracer biodistribution in immunocompetent BALB/cOlaHsd (BALB/c) mice was performed at 15, 60 and 90 min after tracer injection. In-vivo binding characteristics were studied in severe combined immune-deficient (SCID) mice inoculated with activated hPBMCs in Matrigel. Tracer was injected 15 min after hPBMCs inoculation and a 60-min dynamic PET scan was acquired, followed by ex-vivo biodistribution studies. Specific uptake was determined by co-injection of tracer with unlabeled IL2 and by evaluating uptake in a control group inoculated with Matrigel only. Results: 68Ga-Ga-NODAGA-IL2 and 18F-AlF-RESCA-IL2 were produced with radiochemical purity >95% and radiochemical yield of 13.1±4.7% and 2.4±1.6% within 60 and 90 min, respectively. Both tracers were stable in serum, with >90% being intact tracer after 1h. In-vitro, both tracers displayed preferential binding to activated hPBMCs. Ex-vivo biodistribution studies in BALB/c mice showed higher uptake of 18F-AlF-RESCA-IL2 than 18F-FB-IL2 in liver, kidney, spleen, bone and bone marrow. 68Ga-Ga-NODAGA-IL2 uptake in liver and kidney was higher than 18F-FB-IL2 uptake. In-vivo, all tracers revealed uptake in activated hPBMCs in SCID mice. Low uptake was seen after a blocking dose of IL2 or in the Matrigel control group. In addition, 18F-AlF-RESCA-IL2 yielded highest contrast PET images of target lymph nodes.
Conclusions: Production of $^{18}$F-AlF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2 is simpler and faster than $^{18}$F-FB-IL2. Both tracers showed good *in-vitro* and *in-vivo* characteristics with high uptake in lymphoid tissue and hPBMC xenografts.

**Keywords:** PET imaging, T-cells, immunotherapy, interleukin-2, radiopharmaceuticals
INTRODUCTION

Molecular imaging of immune cells for diagnosis and therapy evaluation in inflammatory and infectious diseases has been performed for decades (1,2). Now, interest in visualizing the immune response in cancer is also growing, as a result of the introduction of cancer immunotherapies. Immune checkpoint inhibitors have anti-tumor effects across several tumor types. However, not all patients respond and some patients experience serious immune-related side effects. Therefore, strategies to select patients and optimize therapy are needed. Molecular imaging of immune cells might be a suitable method for patient selection, response prediction and treatment evaluation in this context (2).

T-cells are major players in both immune-related diseases and the tumor-immune response. During an immune response, T-cells secrete the protein interleukin-2 (IL2). IL2 binds to the IL2 receptor (IL2-R), consisting of three subunits: IL2-Rα (CD25), IL2-Rβ (CD122) and IL2-Rγ (CD132) (3-6). Binding of IL2 to this complex leads to T-cell activation and differentiation (3). The IL2-R is not only expressed on activated cytotoxic T-cells, but also on other subpopulations, such as regulatory T-cells. Specific binding of radiolabeled IL2 to T-cells could be exploited for molecular imaging and might provide insight in immune responses.

Recombinant human IL2 binds to human and murine IL2-R and has been radiolabeled previously with isotopes for single-photon emission computed tomography (SPECT) like technetium-99m (99mTc) and iodine-123 (123I) (7-9). As positron emission tomography (PET) offers better resolution compared to SPECT, the fluor-18 (18F) labeled PET tracer N-(4-18F-fluorobenzoyl)-interleukin-2 (18F-FB-IL2) was developed. 18F-FB-IL2 PET imaging detected subcutaneously (s.c.) injected activated T-cells and tumor infiltration of T-cells in response to radiotherapy and immunization (10-12). Clinical trials with 18F-FB-IL2 are ongoing. However, radiotracer production is complex, requiring 2.5h and several synthesis modules. Moreover, the
production yields sufficient radiotracer for only one or two patients (13). Therefore, we aimed to develop other radiolabeled IL2 PET tracers. First, we developed a simplified method for radiolabeling with $^{18}$F, requiring less synthesis steps. Secondly, we used another PET isotope, gallium-68 ($^{68}$Ga), which is a generator-produced radioisotope. Here we present the development of $^{18}$F-AlF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2 (Fig. 1). To determine their potential for clinical use, \textit{in-vitro} and \textit{in-vivo} characteristics were evaluated and compared to $^{18}$F-FB-IL2. For this purpose, \textit{in-vitro} binding assays with activated human peripheral blood mononuclear cells (hPBMCs) and \textit{ex-vivo} biodistribution studies in immunocompetent BALB/cOlaHsd (BALB/c) mice were performed. In addition, PET imaging and \textit{ex-vivo} biodistribution studies were executed in severe-combined immunodeficient (SCID) mice s.c. inoculated with activated hPBMCs.

METHODS

Production Of $^{18}$F-FB-IL2

The production method of $^{18}$F-FB-IL2 has been described previously (10). Adaptations to this production method were made to improve tracer yields (13).

Production Of $^{18}$F-AlF-RESCA-IL2

We developed a simplified method to label IL2 with $^{18}$F, using the indirect $^{18}$F-AlF-RESCA (REStrained Complexing Agent) methodology, combining chemical advantages of a chelator-based radiolabeling method with the unique physical properties of $^{18}$F (14-16). $^{18}$F-AlF was allowed to react with the RESCA-tetrafluorophenol ester (±)-H$_3$RESCA-TFP (Leuven University, Belgium). Subsequently, the $^{18}$F-AlF-RESCA complex was conjugated with IL2. The complete synthesis is described in the supplementary methods.
Production Of $^{68}$Ga-Ga-NODAGA-IL2

Our second strategy was radiolabeling of IL2 with $^{68}$Ga. Due to the short half-life of $^{68}$Ga, less synthesis steps and synthesis time are allowed. First, 2,2′-(7-(1-carboxy-4-((2,5-dioxopyrrolidin-1-yl)oxy)-4-oxobutyl)-1,4,7-triazonane1,4-diyl)diacetic-acid (NODAGA-NHS) was conjugated to IL2, followed by radiolabeling with $^{68}$Ga. We used two synthesis methods. In method 1, $^{68}$Ga was eluted from the PS-H$^+$ cartridge in the cationic form (0.1M hydrochloric acid, HCl). However, large amounts of HCl are needed to efficiently elute all $^{68}$Ga, leading to low concentrations of $^{68}$Ga. Thus, for obtaining high concentrations of $^{68}$Ga in a non-fractionated elution, we implemented method 2, in which $^{68}$Ga was eluted from a PS-HCO$_3$ cartridge with deionized water (17). The complete synthesis methods are described in the supplementary methods.

In-Vitro Binding Assays

*In-vitro* binding to IL2-Rs was determined by performing binding assays with hPBMCs, isolated from peripheral blood from healthy volunteers by Ficoll-Paque Plus separation (GE Healthcare). Cells were kept in RPMI-1640 medium (GIBCO) supplemented with 10% fetal calf serum. Isolated hPBMCs were activated by incubation with 5 µg/mL of phytohemagglutinin (PHA, Sigma-Aldrich) in a 5% CO$_2$ atmosphere at 37°C for 48h (10). As control, non-activated hPBMCs were incubated 48h at 37°C and 5% CO$_2$. On the day of the experiment, another batch of hPBMCs were isolated, to compare CD25 (IL2-R$_\alpha$) expression on these freshly isolated cells with expression on 48h incubated cells.

Approximately 5x10$^5$ cells were incubated with 50 µL tracer solution at 37°C for 30 min. Cells were washed twice with 1 mL ice-cold phosphate buffered saline (PBS) containing 1%
human serum albumin (HSA, Sanquin). Activity in the cell fraction was measured in a gamma-counter (Wizard² 2480-0019, SW 2.1, PerkinElmer). Tracer uptake was corrected for the number of viable cells, counted manually using trypan blue. Uptake was expressed as percentage of cell-associated radioactivity per 500,000 cells. For $^{18}$F-FB-IL2 and $^{18}$F-AIF-RESCA-IL2 four independent experiments were performed, each experiment in triplicate, for $^{68}$Ga-Ga-NODAGA-IL2 two independent experiments were performed, each experiment in triplicate.

**In-Vitro Stability Studies**

The stability of $^{18}$F-AIF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2 was evaluated by adding 50 μL tracer to 200 μL of human serum. The mixture was vortexed and incubated at 37°C for 60 and 120 min. After incubation, radiochemical purity was determined by a trichloroacetic acid (TCA) precipitation assay (18,19).

**Animals Studies**

Animal studies were performed according to Dutch Regulations for Animal Welfare (IvD number 16395-01-007). The protocol was approved by the animal ethical committee of the University of Groningen. Animals were randomly assigned to different groups. A power calculation has been performed to calculate the experimental group size. With an expected variation coefficient of 10%, a meaningful effect size of 25%, a confidence interval of 95% and a power of 90%, a minimum of 5 animals per group is required. Therefore 5-6 animals were included in each group. Exclusion criteria were abnormal behavior, signs of sickness, reduction of body weight >15% and death.
Biodistribution Studies And In-Vivo Stability In BALB/c mice. Ex-vivo biodistribution studies were performed in 5-8 weeks old, immunocompetent BALB/c mice (Envigo). Weights of animals were comparable. Tracer (150 μl) was injected via the penile vein: 4.20±0.8 MBq (0.75±0.22 μg) \[^{18}\text{F} \]FB-IL2; 1.21±0.95 MBq (0.25±0.24 μg) \(^{18}\text{F} \)AlF-RESCA-IL2; 0.55±0.13 MBq (0.32±0.18 μg) \(^{68}\text{Ga} \)Ga-NODAGA-IL2 (method 1). Mice were sacrificed at the following time-points post-injection (p.i.): 15, 60 and 90 min (n=6 mice per time-point). Organs were dissected and counted in a gamma-counter. Bone marrow was isolated from the hind limb long bones (femur and tibiae) via centrifugation. Uptake in organs was calculated as percentage of injected dose per gram of tissue (%ID/g). Total injected dose was determined by measuring activity in the syringe before and after injection. In-vivo stability was determined in plasma samples by a TCA precipitation assay.

PET Imaging And Ex-Vivo Biodistribution In SCID Mice With A hPBMC Xenograft. PET studies were performed in 5-8 weeks old Fox Chase severe combined immunodeficient (SCID) beige mutant mice (Envigo). Mice were s.c. implanted with 10x10^6 48h PHA-activated hPBMCs in 300 μL 1:1 PBS:Matrigel (Corning) in the right shoulder. As negative control group, mice were s.c. inoculated with Matrigel only. Tracer was injected via the penile vein 15 min after inoculation: 1.17±0.62 MBq (0.15±0.11 μg) \(^{18}\text{F} \)FB-IL2; 2.10±2.41 MBq (0.18±0.12 μg) \(^{18}\text{F} \)AlF-RESCA-IL2; 0.44±0.18 MBq (0.26±0.13 μg) \(^{68}\text{Ga} \)Ga-NODAGA-IL2 (method 2). For blocking experiments, a third group inoculated with 10x10^6 PHA activated hPBMCs, received a co-injection of tracer with a blocking dose unlabeled IL2 (100 μg). Directly after tracer injection a 60-min dynamic PET scan was made using a Focus 220 PET scanner (CTI Siemens), followed by a 15 min transmission scan with a \(^{57}\text{Co} \) point source to correct for tissue attenuation, random coincidences and scatter. After the scan, mice were sacrificed and organs were dissected and
counted in a gamma-counter. During all scans and invasive procedures mice were anesthetized with isofluorane/medical air inhalation anesthesia (5% induction, 2.5% maintenance).

**PET Reconstruction**

PET data was reconstructed into six frames and corrected for radioactive decay, random coincidences, scatter, and attenuation. Reconstructed images were analyzed using PMOD software (version 3.9, PMOD technologies LCC). Three-dimensional regions of interest (ROI) were drawn around the site of cell inoculation. For other organs a fixed-sizes sphere was drawn in representative parts of the organs. PET data is presented as %ID/g.

**FACS Analysis**

The CD25 (IL2-Rα) expression on hPBMCs was determined by fluorescence-activated cell sorting (FACS) analysis. For *in vitro* experiments hPBMCs were washed once with 3 mL ice-cold PBS and resuspended in 500 µL PBS. To select viable cells, 5 µL Zombie Aqua (Biolegend) was added and incubated in the dark at room temperature for 15 min. Cells were washed with PBS containing 5% fetal calf serum and resuspended in PBS containing 2% fetal calf serum (FACS buffer), to a concentration of 1x10⁶ cells/mL. To 0.1 mL cell suspension, either 5 µL FITC-conjugated mouse anti-human antibody CD25 (ImmunoTools) or mouse FITC-IgG (BD Biosciences) as a control was added. Then 5 µL PE-conjugated anti-human CD3 antibody (eBioscience) was added and cells were incubated for 45 min on ice. Thereafter cells were washed twice with 3 mL cold FACS buffer and resuspended in 0.1 mL FACS buffer. FACS measurements were performed on a BD FACSVersa apparatus (BD Biosciences). FACS data were analyzed using Flow-Jo software (version 10). Cells were gated for living cells, followed by
CD3-positive cells. In this population the percentage of CD25-positive cells was selected. For \textit{in-vivo} experiments hPBMC activation was confirmed by FACS analysis of CD25 only.

\textbf{Statistical Analysis}

Data are presented as mean±standard deviation (SD). Statistical analyses between two groups were performed using an unpaired two-tailed t-test (\textit{in-vitro} binding data and stability data) or a Bonferroni corrected Mann-Whitney U-test (PET imaging and biodistribution data) (Graphpad Prism 7.0). P-values \leq 0.05 were considered statistically significant.

\textbf{RESULTS}

\textbf{Production Of $^{18}$F-FB-IL2}

$^{18}$F-FB-IL2 was obtained with a radiochemical yield of 1.0±0.4%, a molar activity of 342±385 GBq/μmol and a radiochemical purity >95% within 150 min.

\textbf{Production Of $^{18}$F-AlF-RESCA-IL2}

In the first step, the $^{18}$F-AlF-RESCA-TFP complex was formed at room temperature with high yield (radiochemical yield >80%). The subsequent conjugation of IL2 with $^{18}$F-AlF-RESCA-TFP provided the final product, $^{18}$F-AlF-RESCA-IL2, with a radiochemical yield of 2.4±1.6%, a molar activity of 910±927 GBq/μmol and a radiochemical purity >95% within 90 min.

\textbf{Production Of $^{68}$Ga-Ga-NODAGA-IL2}

The conversion of NODAGA-IL2 into $^{68}$Ga-Ga-NODAGA-IL2 according to method 1 (as described in method section) resulted in almost quantitative yields (85±29%) when small
volumes (100-200 μL, 20-100 MBq) of freshly eluted $^{68}$Ga-Cl$_3$ were used. When large volumes of $^{68}$Ga-Cl$_3$ (1 mL, 200-600 MBq) were used, the yields dropped to 6.0±6.6%.

Labeling of NODAGA-IL2 with small volumes (200 μL, 200-600 MBq) of freshly eluted $^{68}$Ga-Cl$_3$ according to method 2 resulted in $^{68}$Ga-NODAGA-IL2 with a radiochemical yield of 13.1±4.7%, a molar activity of 76±34 GBq/μmol and a radiochemical purity >91% within 60 min.

**In-Vitro Binding Assays**

$^{18}$F-AlF-RESCA-IL2 uptake in activated hPBMCs (73±27.6%) was substantially higher than $^{18}$F-FB-IL2 (4.8±2.8%, P=0.015) and $^{68}$Ga-Ga-NODAGA-IL2 uptake (12.7±0.1%, P=0.019) (Fig. 2A). When comparing ratios of activated/non-activated, it was found that the new tracers were equally selective as $^{18}$F-FB-IL2 (Fig. 2B). All tracers showed a reduction in cell binding in fresh and non-activated hPBMCs compared to uptake in activated hPBMCs. In the activated hPBMCs, more CD3-positive T-cells (Supplemental Fig. 1) expressed CD25 than in the incubated non-activated hPBMCs (32.1±6.0% versus 3.4±2.1%, P=0.002) and freshly isolated hPBMCs (3.1±1.2%, P=0.001).

**In-Vitro Stability Studies**

$^{18}$F-AlF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2 showed comparable high stability in human serum, with >90% of both tracers remaining intact after 1h and 2h (Fig. 3). For comparison, $^{18}$F-FB-IL2 showed slightly higher stability (100% at 1h and 2h, as described earlier (10,11).
Animals Studies

*Ex-Vivo Biodistribution Studies In Immunocompetent Mice.* Ex-vivo biodistribution studies showed high uptake of $^{18}$F-FB-IL2 at sites of renal excretion (Fig. 4; Supplemental Table 1). After 15 min kidney uptake of $^{18}$F-AlF-RESCA-IL2 is higher compared to $^{18}$F-FB-IL2 ($P=0.009$) (Supplemental Table 1). However, activity found in urine is higher for $^{18}$F-FB-IL2 ($P=0.004$), indicating faster renal excretion. At 60 and 90 min p.i., $^{18}$F-AlF-RESCA-IL2 showed higher uptake than $^{18}$F-FB-IL2 in liver, kidney, spleen, bone and bone marrow. $^{68}$Ga-Ga-NODAGA-IL2 showed a comparable biodistribution pattern to $^{18}$F-AlF-RESCA-IL2, with high uptake in liver and kidneys. Moreover, $^{68}$Ga-Ga-NODAGA-IL2 uptake in the kidneys was higher than both $^{18}$F-FB-IL2 and $^{18}$F-AlF-RESCA-IL2 at all time-points (Fig. 4). $^{18}$F-AlF-RESCA-IL2 showed highest uptake in lymphoid organs, such as spleen, lymph nodes and bone marrow, at 60 min and 90 min.

*In-vivo* stability of $^{18}$F-AlF-RESCA-IL2 was comparable to $^{18}$F-FB-IL2 at 90 min p.i. ($^{18}$F-FB-IL2 81%±9% versus $^{18}$F-AlF-RESCA-IL2 72±9%, $P=0.058$) (Supplemental Fig. 2). $^{68}$Ga-Ga-NODAGA-IL2 was less stable compared to $^{18}$F-FB-IL2 at 90 min p.i. ($^{68}$Ga-Ga-NODAGA-IL2 65%±5%, $P=0.003$).

*PET Imaging And Ex-Vivo Biodistribution In SCID Mice With A hPBMC Xenograft.* Activated hPBMC xenografts inoculated in SCID mice were visualized with all tracers by dynamic PET imaging. No uptake was observed in the Matrigel control group or after co-injection of tracer with a blocking dose unlabeled IL2. PET images of all tracers showed high uptake in liver and kidney, which did not substantially change over time (data not shown).

With $^{18}$F-FB-IL2 PET, lymph nodes could be detected in only three out of five mice (Fig. 5A), while $^{18}$F-AlF-RESCA-IL2 PET could clearly visualize lymph nodes in all mice at 60 min.
p.i. (Fig. 5B). With $^{68}$Ga-Ga-NODAGA-IL2 PET, lymph nodes could be detected as well, although not as clear as with $^{18}$F-AlF-RESCA-IL2 PET (Fig. 5C).

The PET results were confirmed by ex-vivo studies with higher uptake in activated PBMCs than in Matrigel control for all tracers (Fig. 6, Supplemental Table 2). A blocking dose of unlabeled IL2 reduced tracer uptake in PBMCs, with the largest reduction for $^{18}$F-AlF-RESCA-IL2 ($^{18}$F-FB-IL2=43.2%; $^{18}$F-AlF-RESCA-IL2=67.5%; $^{68}$Ga-Ga-NODAGA-IL2=26.9%).

**DISCUSSION**

We developed two radiolabeled IL2 tracers, namely $^{18}$F-AlF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2. Both tracers could be produced consistently with a shorter production time compared to $^{18}$F-FB-IL2. Although labeling of $^{18}$F-AlF-RESCA-IL2 gives similar decay-corrected radiochemical yields as $^{18}$F-FB-IL2, practical amounts of $^{18}$F-AlF-RESCA-IL2 produced are 50% higher due to shorter production time. In addition, the production requires only one synthesis module. This is an advantage for clinical use, as more production runs could be planned and thus, more patients can be scanned. The radiochemical yield of $^{68}$Ga-Ga-NODAGA-IL2 strongly depends on the volume in which $^{68}$GaCl3 can be obtained. An advantage of this tracer could be that no cyclotron is needed for radioisotope production, which allows production at sites without a cyclotron.

RESCA was chosen as chelator for the incorporation of Al$^{18}$F, since the complex can be formed at room temperature, preventing potential degradation of the IL2 protein at higher temperatures. Although RESCA was successfully conjugated to IL2, most of the direct radiolabeling attempts did not yield any product, most likely due to the substantial loss of RESCA-IL2 conjugate in the tC2 cartridge during purification (data not shown). Therefore, it was decided to use an indirect radiolabeling method. This approach provided the desired product,
although at low radiochemical yield of 2.4±1.6%. Nevertheless, starting with less than 50 GBq allows production of sufficient $^{18}$F-AlF-RESCA-IL2 for several patients (1375±791 MBq; recommended injected dose 200 MBq).

For the $^{68}$Ga-based IL2 tracer NODAGA was chosen as chelator, because it possesses a smaller coordination pocket forming more stable complexes with $^{68}$Ga. Moreover, it allows labeling with $^{68}$Ga at room temperature (20,21). During $^{68}$Ga-Ga-NODAGA-IL2 production, addition of higher amounts of $^{68}$Ga-Cl$_3$ led to yields <10%. A plausible explanation for this is the low amounts of protein remaining after conjugation, as most of the conjugated NODAGA-IL2 is lost during tC2 purification. With these low radiochemical yields and a maximum amount of 1 GBq eluted from the $^{68}$Ge/$^{68}$Ga-generator, $^{68}$Ga-Ga-NODAGA-IL2 becomes less suitable for clinical use, since it would be difficult to reliably obtain a patient dose. Therefore, in order to use $^{68}$Ga-Ga-NODAGA-IL2 in clinical studies, further optimization is warranted.

Our study showed that $^{18}$F-AlF-RESCA-IL2 had highest in-vitro uptake in hPBMCs and highest in-vivo uptake in target tissues of BALB/c mice, such as lymph nodes, spleen and bone marrow. Since IL2 is injected in a sub-pharmacological dose, no adverse effects are expected. In addition, in the first clinical study with $^{18}$F-FB-IL2, no adverse effects due to radiation burden were observed (data not shown). Therefore, we do not expect additional toxicity from the new analogues. In contrast to $^{18}$F-FB-IL2 and $^{68}$Ga-Ga-NODAGA-IL2, which mainly have a renal clearance, $^{18}$F-AlF-RESCA-IL2 has higher hepatobiliary clearance. This can be attributed either to presence of the RESCA moiety, which introduces an additional charge, or to presence of degradation products, such as unconjugated $^{18}$F-AlF-RESCA or free $^{18}$F-AlF (16,22). These high uptake values obtained in excretory organs are in the same range as obtained with previous IL2-based tracers, where no renal or hepatic toxicity was found (23-25). A limitation for clinical use of $^{68}$Ga-Ga-NODAGA-IL2 is high retention in kidneys, which can lead to radiation burden due to
its higher positron energy compared to $^{18}$F-fluorine. Therefore, $^{18}$F-AlF-RESCA-IL2 might be preferred for clinical molecular imaging studies. PET images obtained from $^{18}$F-AlF-RESCA-IL2 showed less background and therefore, better contrast images, compared to the other tracers. In addition, lymph nodes could be visualized with $^{18}$F-AlF-RESCA-IL2 in all animals, while with $^{18}$F-FB-IL2 PET lymph nodes were only visible in some animals, indicating good targeting characteristics of $^{18}$F-AlF-RESCA-IL2.

Previous SPECT and PET imaging studies have shown that IL2-derived tracers could detect T-cells in inflammatory and infectious diseases, as well as different tumor types (7-9, 23-28). In a small clinical pilot study in only five patients with melanoma, $^{99m}$Tc-IL2 SPECT could detect metastases (29). Currently, also other tracers targeting immune cells are being developed, such as targeting T-cells via CD8 specific tracers (30-33). Another method to detect immune cells is radiolabeling of immune checkpoint targeting monoclonal antibodies, with long half-life isotopes such as zirconium-89 ($^{89}$Zr). Compared to these antibody-based tracers, radiolabeled IL2 tracers have the advantage that with shorter half-lives, imaging can be performed shortly after tracer injection. Thus, in a fast, non-invasive manner, information about the presence and dynamics of immune cells can be gained. Our results indicate good in-vitro and in-vivo characteristics of $^{18}$F-AlF-RESCA-IL2 and the potential to use it as a PET tracer for imaging of T-cells.

**CONCLUSION**

We developed two radiolabeled IL2 tracers for imaging of immune cells: $^{18}$F-AlF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2. Production of the tracers was faster than the previously developed $^{18}$F-FB-IL2, potentially being an advantage for clinical use. $^{18}$F-AlF-RESCA-IL2 showed highest practical production yield and highest in-vitro binding to activated
hPBMCs. Moreover, in-vivo studies showed high uptake of $^{18}$F-AlF-RESCA-IL2 in PBMCs xenografts and lymphoid tissues like spleen, bone marrow and lymph nodes. This supports the potential to use this tracer in future studies for detection of CD25-positive immune cells.

**DISCLOSURE**

**Competing Interests**

E.G.E.d.V reports grants from IMI TRISTAN (GA no.116106), during the conduct of the study; consulting and advisory role for NSABP, Daiichi Sankyo, Pfizer, Sanofi, Merck, Synthon Biopharmaceuticals; grants from Amgen, Genentech, Roche, Chugai Pharma, CytomX Therapeutics, Nordic Nanovector, G1 Therapeutics, AstraZeneca, Radius Health, Bayer, all made available to the institution, outside the submitted work; E.F.J.d.V. reports grants from ZonMW (grant numbers 95104008 and 95105010), during the conduct of the study; grant from Dutch Cancer Foundation (RUG2015-7235); contract research studies were performed with Rodin Therapeutics, Lysosomal Therapeutics Inc., Hoffmann-La Roche Ltd and Ionis Pharmaceuticals, made available to the institution outside the submitted work; G.A.P.H reports consulting and advisory role for Amgen, Roche, MSD, BMS, Pfizer, Novartis; grants from BMS, Seerave; made available to the institution outside the submitted work. F.C. is a Postdoctoral Fellow of FWO (12R3119N). All remaining authors have declared no conflicts of interest.

**Funding**

The research leading to these results received funding from the Innovative Medicines Initiatives 2 Joint Undertaking under grant agreement No 116106 (TRISTAN). This Joint Undertaking receives support from the European Union’s Horizon 2020 research and innovation
program and EFPIA. This research received support from Research Foundation – Flanders (FWO) (G0D8817N).

**KEY POINTS**

**Question**

What are the *in-vitro* and *in-vivo* characteristics of two radiolabeled IL2 variants, $^{18}$F-AlF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2, compared to $^{18}$F-FB-IL2?

**Pertinent Findings**

Production of the tracers is faster than previous developed $^{18}$F-FB-IL2. $^{18}$F-AlF-RESCA-IL2 shows best *in-vitro* binding characteristics. *In-vivo* studies showed high $^{18}$F-AlF-RESCA-IL2 uptake in PBMCs and lymphoid organs like spleen, bone marrow and lymph nodes.

**Implications For Patient Care**

Our results indicate the potential to use $^{18}$F-AlF-RESCA-IL2 in future molecular imaging studies for detection of CD25-positive immune cells.
REFERENCES


FIGURES AND TABLES

FIGURE 1. Structures of the radiolabeled interleukin-2 PET tracers.
FIGURE 2. In-vitro binding assay in human PBMCs for $^{18}$F-FB-IL2, $^{18}$F-AlF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2. Four independent experiments were performed for $^{18}$F-FB-IL2 and $^{18}$F-AlF-RESCA-IL2, in triplicate, for $^{68}$Ga-Ga-NODAGA-IL2 two independent experiments were performed, in triplicate. **A** ) Data expressed as percentage of cell-associated radioactivity per 500,000 cells. **B** ) Data expressed as ratio activated to non-activated cells. Data are mean±SD; *P≤0.05.
FIGURE 3. In-vitro stability studies in human serum for $^{18}$F-AlF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2. Data expressed as percentage of intact tracer, after 60 and 120 min incubation. Data expressed as mean±SD.
FIGURE 4. Comparison of ex-vivo biodistribution in immunocompetent BALB/c mice 60 min p.i. between $^{18}$F-FB-IL2, $^{18}$F-AlF-RESCA-IL2 and $^{68}$Ga-Ga-NODAGA-IL2 (n=6 per tracer). Uptake is expressed as percentage of injected dose (%ID/g). Data expressed as mean±SD; *P≤0.05, **P≤0.01.
FIGURE 5. Representative PET images in immunodeficient SCID mice inoculated with activated PBMCs or Matrigel control in the right shoulder 15 min before tracer injection for A) $^{18}$F-FB-IL2, B) $^{18}$F-ALF-RESCA-IL2 and C) $^{68}$Ga-Ga-NOGAGA-IL2. Clear lymph node uptake is depicted by red arrows. Upper panel transaxial view, lower panel coronal view. LN = lymph node; L = liver.
FIGURE 6. *Ex-vivo* uptake (%ID/g) of the three radiolabeled IL2 variants in mice inoculated with activated PBMCs or Matrigel control in the right shoulder 15 min before tracer injection. For blocking an excess of unlabeled IL2 was co-injected with radiolabeled IL2 (PBMC block). $^{18}$F-FB-IL2: PBMC n=4, PBMC block n=6, Matrigel control n=5; $^{18}$F-AIF-RESCA-IL2: PBMC n=5, PBMC block n=6, Matrigel control n=6; $^{68}$Ga-Ga-NODAGA-IL2: PBMC n=5, PBMC block n=6, Matrigel control n=5. Data expressed as mean±SD; *P*≤0.05.
SUPPLEMENTARY METHODS

Production of $^{18}$F-AlF-RESCA-IL2

First, aqueous $^{18}$F-fluoride was produced by irradiation of $^{18}$O-water with a cyclotron (Cyclon 18 Twin, IBA) via the $^{18}$O(p,n)$^{18}$F nuclear reaction. The $^{18}$F-fluoride solution was passed through a QMA Sep-Pak Light anion exchange cartridge (Waters Chromatography Division, Millipore Corp, preconditioned with 3 mL metal free water) to recover the $^{18}$O-water. The QMA was then washed with 10 mL of metal free water and 10 mL of air. The $^{18}$F-fluoride was then eluted from the cartridge with 400 µL 0.9% sodium chloride (NaCl) (B. Braun). An $^{18}$F-AlF solution was freshly prepared by adding ~10 GBq $^{18}$F-fluoride in 400 µL 0.9% NaCl to 25 µL aluminum chloride (2 mM, 50 nmol) in 100 µL sodium acetate buffer 0.1 M, pH 4.5 (NaOAc, Sigma-Aldrich), and allowed to react at room temperature (RT) for 5 min. To the $^{18}$F-AlF solution, 40 µL of the restrained complexing agent-tetrafluorophenol ester ((±)-H₃RESCA-TFP (50 nmol; NaOAc buffer, 0.1 M, pH 4.5; Leuven University, Belgium) was added. After 15 min of reaction at RT, the reaction mixture was diluted with 10 mL of water and transferred to an HLB cartridge (Waters Chromatography Division, Millipore Corp). $^{18}$F-AlF-RESCA-TFP was eluted from the cartridge with 0.6 mL ethanol and 0.7 mL sodium acetate (pH 8.5) into a vial containing 100 µL IL2 (17 nmol) in dimethyl sulfoxide (DMSO, Sigma-Aldrich). The conjugation occurred at 50°C for 15 min, after which the reaction was quenched with 25 µL 25% phosphoric acid (H₃PO₄) and 48 µL 10% sodium dodecyl sulfate (SDS, Sigma-Aldrich). The product was diluted in 10 mL of water for injections (WFI) and passed through a tC2 Sep-Pak cartridge (Waters Chromatography Division, Millipore Corp), which was preconditioned with 5 mL ethanol (EtOH, Merck KGaA) followed by 5 mL of a solution of 5% EtOH containing 12 µL
of 2.5% \( \text{H}_{3}\text{PO}_{4} \) and 10 mL of air). The cartridge was washed three times with 2 mL 50% aqueous EtOH containing 23 \( \mu \text{L} \) 0.25% \( \text{H}_{3}\text{PO}_{4} \). \(^{18}\text{F}\)-AIF-RESCA-IL2 was eluted from the cartridge with 0.8 mL 100% EtOH containing 5 \( \mu \text{L} \) 0.25% \( \text{H}_{3}\text{PO}_{4} \) and 3.5 mL 5% glucose (Baxter) and collected in a vial containing 1.5 mL 5% glucose, 0.1% SDS and 0.5% human serum albumin (HSA, Sanquin) solution.

Radiochemical identity and purity were assessed by instant thin-layer chromatography (iTLC), eluted in a solution of 75% aqueous acetonitrile (\( R_f^{18}\text{F}\)-AIF-RESCA-TFP=1 and \( R_f^{18}\text{F}\)-AIF-RESCA-IL2=0) and by UPLC-ESI-HRMS using a Dionex Ultimate 3000 UPLC System (Thermo Fisher Scientific, Sunnyvale, USA) coupled in series to a UV detector and a radioactivity detector (Berthold FlowStar LB513, Mx50–6 flow cell. The identity of the product was confirmed using native IL2 as reference material.

**Production Of \(^{68}\text{Ga}\)-Ga-NODAGA-IL2**

*Synthesis of NODAGA-IL2.* To a solution of IL2 in DMSO (100 \( \mu \text{L} \), 14 nmol), a 4-fold molar excess of a solution of NODAGA-NHS ester in DMSO (40 \( \mu \text{L}\)–55 nmol, Chematech) was added, followed by 5 \( \mu \text{L} \) N,N-diisopropylethylamine (pH 8.5). This mixture was incubated for 2 h at RT with slow stirring. The reaction was quenched with 25 \( \mu \text{L} \) 25% \( \text{H}_{3}\text{PO}_{4} \) and 48 \( \mu \text{L} \) 10% SDS. The product was diluted in 10 mL of WFI and purified with a tC2 cartridge, as described above. The final product was eluted with 0.5 mL 100% EtOH, containing 5 \( \mu \text{L} \) 0.25% \( \text{H}_{3}\text{PO}_{4} \), and 0.5 mL 100% EtOH. The conjugate was kept at -80°C until the day of radiolabeling.

*Radiolabeling of \(^{68}\text{Ga}\)-Ga-NODAGA-IL2 (Method 1).* \(^{68}\text{Ga}\)-Cl\(_{3}\) was eluted from a \( >\) 9 month old GMP 1110 MBq grade \(^{68}\text{Ge}\)/\(^{68}\text{Ga}\)-generator was used (Eckert & Ziegler). \(^{68}\text{Ga}\)\(^{3+}\) was trapped in a PS-H\(^{+}\) cartridge (ABX) and subsequently eluted from this cartridge with 1.5 mL 5M sodium
chloride (NaCl). To the defrosted solution of NODAGA-IL2, 100 μL $^{68}$Ga-Cl$_3$ (20-100 MBq) and 300 μL 1.5M HEPES buffer (ABX) were added (pH between 3-5). After 15 min of conjugation at 50°C, the mixture was quenched with 25 μL 25% H$_3$PO$_4$ and 48 μL 10% SDS. The product was purified with a tC2 cartridge as described above. $^{68}$Ga-Ga-NODAGA-IL2 was eluted from the cartridge with 0.8 mL 100% EtOH, containing 5 μL 0.25% H$_3$PO$_4$, and 3.5 mL of 5% glucose and collected in a vial containing 1.5 mL of 5% glucose, 0.1% SDS and 0.5% HSA solution. The final product was analyzed by iTLC eluted with a 0.1M citric acid solution ($R_f$ $^{68}$Ga-Cl$_3$=1 and $R_f$ $^{68}$Ga-NODAGA-IL2=0) and by ultra-performance liquid tomography (UPLC).

*Radiolabeling of $^{68}$Ga-Ga-NODAGA-IL2 (Method 2).* $^{68}$Ga-Cl$_3$ was eluted from a $^{68}$Ge/$^{68}$Ga-generator with 6 mL 0.1M hydrochloric acid (HCl, Rotem Industries) into a vial containing 4 mL 37% HCl to form a final HCl concentration of 4 M. The eluate was then passed through a PS-HCO$_3^-$ cartridge (Synthra®), preconditioned with successively 5 M HCl, 1 M HCl, WFI and again 5 M HCl). The cartridge was washed with 2 mL 4 M HCl and dried under a strong flow of nitrogen to eliminate the excess 4 M HCl. $[^{68}\text{Ga}]$Cl$_3$ was subsequently eluted with 300 μL water (100-360 MBq) into an Eppendorf (metal free) containing 75 mg HEPES and 10 μL of 25% ammonia (Merck KGaA). This solution (pH 3-4) was added to the solution of NODAGA-IL2 and the pH was adjusted to 4-5 with 25% ammonia. After 15 min of conjugation at 50°C, the mixture was quenched with 25 μL H$_3$PO$_4$ 25% and 48 μL 10% SDS. The product was purified, formulated and analyzed as mentioned above.
SUPPLEMENTARY FIGURES AND TABLES

SUPPLEMENTARY FIGURE 1. FACS analysis for in vitro experiments. Expression of CD25+ was determined in lymphocyte, CD3 positive, population of activated (A) and non-activated (NA) hPBMCs. Data expressed as mean±SD; *P≤0.05, **P≤0.01.

SUPPLEMENTARY FIGURE 2. Stability in vivo determined by TCA precipitation assay in plasma samples of immunocompetent BALB/c mice collected 15, 60 and 90 min after tracer injection (n=6 per time point for each tracer). Data expressed as mean±SD.
**Supplementary TABLE 1** *Ex vivo* biodistribution of three radiolabeled IL2 tracers. 15, 60 and 90 min post-injection in BALB/c mice.

<table>
<thead>
<tr>
<th>Organs</th>
<th>$^{18}$F-FB-IL2 (%ID/g)</th>
<th>$^{18}$F-ALF-RESCA-IL2 (%ID/g)</th>
<th>$^{68}$Ga-Ga-NODAGA-IL2 (%ID/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>60 min</td>
<td>90 min</td>
</tr>
<tr>
<td>Whole blood</td>
<td>7.7 ± 2.1</td>
<td>3.1 ± 2.0</td>
<td>2.3 ± 1.4</td>
</tr>
<tr>
<td>Plasma</td>
<td>10.0 ± 1.8</td>
<td>4.0 ± 2.6</td>
<td>2.9 ± 1.5</td>
</tr>
<tr>
<td>Heart</td>
<td>6.5 ± 2.5</td>
<td>3.1 ± 2.1</td>
<td>2.8 ± 2.2</td>
</tr>
<tr>
<td>Lung</td>
<td>13.7 ± 5.2</td>
<td>6.9 ± 4.8</td>
<td>8.8 ± 6.7</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>1.5 ± 0.5</td>
<td>1.1 ± 0.7</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Thymus</td>
<td>2.1 ± 0.6</td>
<td>1.8 ± 1.6</td>
<td>1.4 ± 1.0</td>
</tr>
<tr>
<td>Liver</td>
<td>25.9 ± 8.2</td>
<td>9.6 ± 7.8</td>
<td>4.2 ± 2.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>18.4 ± 5.9</td>
<td>18.9 ± 9.4</td>
<td>14.4 ± 4.3</td>
</tr>
<tr>
<td>Urine</td>
<td>33.7 ± 13.3</td>
<td>111.6 ± 62.3</td>
<td>432.0 ± 325.6</td>
</tr>
<tr>
<td>Bladder</td>
<td>5.9 ± 3.0</td>
<td>27.2 ± 23.9</td>
<td>126.9 ± 102.5</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.7</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.4 ± 0.5</td>
<td>1.4 ± 0.9</td>
<td>1.4 ± 1.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>22.4 ± 11.8</td>
<td>13.9 ± 10.0</td>
<td>9.2 ± 6.3</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1.8 ± 0.6</td>
<td>3.2 ± 2.4</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>Colon</td>
<td>1.3 ± 0.4</td>
<td>1.1 ± 0.7</td>
<td>1.8 ± 2.9</td>
</tr>
<tr>
<td>Lymph node (axillary)</td>
<td>1.9 ± 1.6</td>
<td>3.0 ± 2.5</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>Lymph node (mesenteric)</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.9</td>
<td>3.5 ± 5.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Skin</td>
<td>0.7 ± 0.1</td>
<td>1.0 ± 0.7</td>
<td>2.6 ± 2.3</td>
</tr>
<tr>
<td>Brain</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Bone</td>
<td>2.1 ± 1.0</td>
<td>0.7 ± 0.4</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>38.7 ± 19.7</td>
<td>8.8 ± 8.6</td>
<td>14.3 ± 24.8</td>
</tr>
</tbody>
</table>
**Supplementary TABLE 2** Ex vivo biodistribution of three radiolabeled IL2 tracers in immunodeficient SCID mice, inoculated with human activated PBMCs, 15 min before tracer injection. After tracer injection a 60 min dynamic PET scan was made, followed by ex vivo biodistribution studies. Activity in each organ was measured and the percentage of the injected dose (%ID/g) was calculated.

<table>
<thead>
<tr>
<th>Organs</th>
<th>$^{18}$F-FB-IL2 (%ID/g)</th>
<th>$^{18}$F-ALF-RESCA-IL2 (%ID/g)</th>
<th>$^{68}$Ga-Ga-NODAGA-IL2 (%ID/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBMC</td>
<td>PBMC block</td>
<td>Matrigel</td>
</tr>
<tr>
<td>Whole blood</td>
<td>6.4 ± 2.9</td>
<td>3.8 ± 2.6</td>
<td>3.6 ± 1.4</td>
</tr>
<tr>
<td>Plasma</td>
<td>7.8 ± 2.8</td>
<td>3.2 ± 2.0</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>Heart</td>
<td>8.9 ± 4.7</td>
<td>8.2 ± 6.4</td>
<td>6.6 ± 6.4</td>
</tr>
<tr>
<td>Lung</td>
<td>13.6 ± 5.5</td>
<td>23.0 ± 19.9</td>
<td>8.3 ± 6.8</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>2.1 ± 0.7</td>
<td>1.5 ± 1.0</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>Thymus</td>
<td>5.9 ± 3.6</td>
<td>4.2 ± 1.6</td>
<td>2.9 ± 1.1</td>
</tr>
<tr>
<td>Liver</td>
<td>23.1 ± 14.2</td>
<td>16.4 ± 12.5</td>
<td>14.3 ± 7.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>29.9 ± 9.2</td>
<td>15.9 ± 14.1</td>
<td>17.7 ± 4.6</td>
</tr>
<tr>
<td>Urine</td>
<td>499.3 ± 383.7</td>
<td>253.7 ± 122.7</td>
<td>231.0 ± 160.7</td>
</tr>
<tr>
<td>Bladder</td>
<td>39.7 ± 22.9</td>
<td>52.1 ± 21.9</td>
<td>134.1 ± 136.5</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.3 ± 1.1</td>
<td>1.4 ± 0.5</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2.2 ± 0.7</td>
<td>2.0 ± 0.5</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>18.6 ± 13.4</td>
<td>20.9 ± 15.5</td>
<td>10.5 ± 5.9</td>
</tr>
<tr>
<td>Small intestine</td>
<td>4.7 ± 0.9</td>
<td>2.5 ± 1.2</td>
<td>3.3 ± 1.6</td>
</tr>
<tr>
<td>Colon</td>
<td>2.2 ± 0.9</td>
<td>1.9 ± 1.0</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Lymph node (axillary)</td>
<td>4.4 ± 4.4</td>
<td>1.6 ± 0.9</td>
<td>1.9 ± 1.9</td>
</tr>
<tr>
<td>Lymph node (mesenteric)</td>
<td>2.6 ± 1.1</td>
<td>2.8 ± 2.0</td>
<td>2.9 ± 1.4</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.9 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Skin</td>
<td>2.4 ± 1.6</td>
<td>1.0 ± 0.4</td>
<td>0.9 ± 0.7</td>
</tr>
<tr>
<td>Brain</td>
<td>0.3 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>1.9 ± 0.8</td>
<td>1.1 ± 0.6</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1.7 ± 0.7</td>
<td>1.1 ± 0.5</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>15.7 ± 5.9</td>
<td>11.2 ± 6.4</td>
<td>10.3 ± 5.7</td>
</tr>
<tr>
<td>PBMCs</td>
<td>2.0 ± 0.5</td>
<td>1.2 ± 0.4</td>
<td>0.9 ± 0.3</td>
</tr>
</tbody>
</table>
Development and evaluation of interleukin-2 derived radiotracers for PET imaging of T-cells in mice

Elly L. van der Veen, Frans V. Suurs, Frederik Cleeren, Guy Bormans, Philip H. Elsinga, Geke A.P. Hospers, Marjolijn N. Lub-de Hooge, Elisabeth G.E. de Vries, Erik F.J. de Vries and Ines F. Antunes

J Nucl Med.
Published online: February 28, 2020.
Doi: 10.2967/jnumed.119.238782

This article and updated information are available at: http://jnm.snmjournals.org/content/early/2020/02/27/jnumed.119.238782

Information about reproducing figures, tables, or other portions of this article can be found online at: http://jnm.snmjournals.org/site/misc/permission.xhtml

Information about subscriptions to JNM can be found at: http://jnm.snmjournals.org/site/subscriptions/online.xhtml

JNM ahead of print articles have been peer reviewed and accepted for publication in JNM. They have not been copyedited, nor have they appeared in a print or online issue of the journal. Once the accepted manuscripts appear in the JNM ahead of print area, they will be prepared for print and online publication, which includes copyediting, typesetting, proofreading, and author review. This process may lead to differences between the accepted version of the manuscript and the final, published version.