Journal of Nuclear Medicine, published on May 14, 2015 as doi:10.2967/jnumed.114.149583

In vivo Evaluation of ^{18}F-SiFAlin modified TATE: A Potential Challenge for ^{68}Ga-DOTATATE, the Clinical Gold Standard for Somatostatin Receptor Imaging with Positron Emission Tomography (PET)

Word Count: 4970

Funding from the German Research Foundation (WA 2132/3-1), the Bayern-Quebec-Allianz and the Ministry of Science, Research and the Arts of the State of Baden-Württemberg (AZ 42-04HV.M1412(12)/1/1) is gratefully acknowledged. Research funding from the National Science and Engineering Research Council of Canada (NSERC) (Discovery Grants, Novel hydrophilic Silicon-Fluoride-Acceptors (SiFAs) for Positron Emission Tomography (PET)) was provided.

Sabrina Niedermoser[1], Joshua Chin[2], Carmen Wängler[3], Alexey Kostikov[2], Vadim Bernard-Gauthier[2,4], Nils Vogler,[5] Jean-Paul Soucy[2,6], Alexander J McEwan[4], Ralf Schirrmacher[2,4*], Björn Wängler[1*]

[1] Molecular Imaging and Radiochemistry, Department of Clinical Radiology and Nuclear Medicine, Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany

[2] McConnell Brain Imaging Centre, Montreal Neurological Institute, McGill University, Montreal, Canada

[3] Biomedical Chemistry, Department of Clinical Radiology and Nuclear Medicine, Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany

[4] Department of Oncology, Div. Oncological Imaging, University of Alberta, 11560 University Avenue, Edmonton, AB, Canada, T6G 1Z2

[5] Nuclear Medicine, Department of Clinical Radiology and Nuclear Medicine, Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany

[6] Concordia -PERFORM Centre, Concordia University, Montreal, Canada

* Ralf Schirrmacher and Björn Wängler share corresponding authorship

DR. RALF SCHIRRMACHER

Department of Oncology, Div. Oncological Imaging, University of Alberta, 11560 University Avenue, Edmonton, AB, Canada, T6G 1Z2
+1 780 248 1829
schirrma@ualberta.ca

Prof. Dr. BJÖRN WÄNGLER

Molecular Imaging and Radiochemistry, Department of Clinical Radiology and Nuclear Medicine, Medical Faculty Mannheim of Heidelberg University, Mannheim, Germany
+49 (0)621 383 5594
Bjoern.Waengler@medma.uni-heidelberg.de

Short Title: IN VIVO EVALUATION OF ^{18}F-SiFAlin

Radiolabeled peptides for tumor imaging with positron emission tomography (PET) that can be produced with kits are currently in the spot light of radiopharmacy as well as nuclear medicine. The diagnosis of neuroendocrine tumors in particular has been a prime example for the usefulness of peptides labeled with a variety of different radionuclides. Among those, ^{68}Ga and ^{18}F stand out because of the ease of radionuclide introduction (e.g. ^{68}Ga isotope) or optimal nuclide properties for PET imaging (slightly favoring ^{18}F isotope). In vivo properties of GMP-compliant, newly developed kit-like producible ^{18}F-SiFA- and ^{18}F-SiFA*lin*- (SiFA = silicon-fluoride acceptor) modified TATE derivatives were compared to the current clinical gold standard ^{68}Ga-DOTATATE for high quality imaging of somatostatin receptor-bearing tumors.

Methods:

SiFA- and SiFA*lin*-derivatized somatostatin analogs were synthesized and radiolabeled using cartridge-based dried ^{18}F and purified via a C18 cartridge (RCY 49.8 ± 5.9% within 20-25 min) without HPLC purification. Tracer lipophilicity and stability in human serum were tested in vitro. Competitive receptor binding affinity studies were performed using AR42J cells. The most promising tracers were evaluated in vivo in an AR42J xenograft mouse model by ex vivo biodistribution as well as in vivo PET/CT imaging studies for evaluation of their pharmacokinetic profiles and the results were compared to those of the current clinical gold standard ^{68}Ga-DOTATATE.

Results:

Synthetically easily accessible ^{18}F-labeled silicon-fluoride acceptor modified somatostatin analogs were developed. They exhibited high binding affinities to somatostatin receptor-positive tumor cells (1.88-14.82 nM). The most potent compound demonstrated comparable pharmacokinetics and an even slightly higher absolute tumor

accumulation level in ex vivo biodistribution studies as well as higher tumor SUVs in PET/CT imaging than ^{68}Ga-DOTATATE in vivo. The radioactivity uptake in non tumor tissue was higher than for ^{68}Ga-DOTATATE.

Conclusion:

The introduction of the novel SiFA building block SiFA*lin* as well as of hydrophilic auxiliaries enables a favorable in vivo biodistribution profile of the modified TATE peptides, resulting in high tumor-to-background ratios however still lower than those observed with ^{68}Ga-DOTATATE. Moreover, the SiFA-methodology enables a kit-like labeling procedure for ^{18}F-labeled peptides advantageous for routine clinical application.

Keywords: ^{68}Ga-DOTATATE, PET, kit-like ^{18}F labeling, ^{18}F-SiFA*lin*-TATE, Somatostatin Receptor Imaging

Radiolabeled peptides have emerged as valuable imaging tools for PET, although their clinical availability is still limited (*1*). These peptides are mostly labeled with radiometals such as gallium-68 (^{68}Ga) and copper-64 (^{64}Cu) but also with fluorine-18 (^{18}F) (*2-6*). Although ^{68}Ga-labeled peptides exhibit very favorable properties in clinical routine imaging, the resolution of the obtained PET images and thus sensitivity could be further improved using ^{18}F (*7*). Even more importantly, the wide-spread availability of ^{18}F would make such tracers more accessible. Furthermore, the availability and clinical utilization of ^{68}Ga based tracers has been less than optimal due to the limited availability of the generators and their uncertain regulatory status. As there is an urgent demand in clinical diagnostic oncology for tumor-specific radiolabeled compounds depicting even very small tumors and metastases to compensate for the shortcomings of [^{18}F]FDG *(8, 9*), the development of ^{18}F-labeled peptides is worth exploring. ^{18}F represents one of the most attractive radionuclides because of its accessibility and low positron energy allowing for high-resolution in vivo images. However, only very few clinical PET imaging sites routinely offer ^{18}F-radiolabeled peptides as a result of the typically difficult radiochemistry involved in peptide labeling with ^{18}F (*10*, *11*). Therefore, new labeling approaches have been developed, especially over the last decade (*12-16*). One of these approaches takes advantage of the thermodynamically favored formation of silicon-^{18}F bonds (Silicon-Fluoride-Acceptor (SiFA)-chemistry, **Figure 1**) (*17-23*). However, in vitro and in vivo studies revealed inherent difficulties for its in vivo applicability: the unalterably high lipophilicity of the silicon containing building block necessary for the in vivo stability of the Si-^{18}F-bond leads to changes in the pharmacokinetic properties of the accordingly derivatized peptides (*19*, *24*). Nevertheless, the radiolabeling of SiFA is

exceptionally mild, proceeds without forming side-products and can be performed by even untrained personnel (*17*). This renders this technique ideally-suited for a kit-labeling approach similar to ^{99m}Tc-radiochemistry for SPECT imaging.

In order to reduce the lipophilicity of SiFA-modified peptides, hydrophilic linkers and amino acids such as aspartic acid were introduced into the peptide sequence in this study together with a SiFA building block containing a permanent positive charge: SiFA*lin* **(Figure 1)** (*25*, *26*). Most recently, Perrin's group introduced ^{18}F-AMBF$_3$-TATE for somatostatin receptor imaging, proving that a supposedly "non-canonical" labeling methodology based on isotopic exchange on trifluoroborates delivers an imaging agent of high specific activity and promising in vivo characteristics **(Figure 2)** (*27*).

The main goal of our study was to prove the applicability of the SiFA approach for the labeling of clinically relevant peptides by developing a SiFA*lin*- derivatized Tyr^3-octreotate (TATE) that matches or surpasses the targeting ability of the current gold standard ^{68}Ga-DOTATATE (**Fig. 2**) for SSTR (somatostatin receptor) in vivo imaging with PET, thus overcoming ^{68}Ga limitations in terms of availability.

MATERIALS AND METHODS

Chemistry

Synthesis of Peptides by Solid Phase Peptide Synthesis. SiFA- and SiFA*lin*-derivatized somatostatin-analogs were synthesized as described earlier (*17*, *28*). Details can be found in the supplementary information.

[^{18}F]Fluoride Radiolabeling and Quality Control of the Peptides. The radiolabeling procedure was carried out as previously described (*17*). In brief, aqueous [^{18}F]F$^-$ was trapped on a cartridge (Waters Sep-Pak Accell Plus QMA Carbonate light (46 mg)),

dried with 20 mL air and then with dry acetonitrile. Subsequently, a lyophilized mixture containing 110 µmol Kryptofix 2.2.2® and 100 µmol KOH was dissolved in dry acetonitrile (500 µL). This freshly prepared solution was then used to elute the dried [^{18}F]F^{-} from the cartridge (*17*, *29*). To the ^{18}F-solution, 25 µL of a 1M solution of oxalic acid in dry acetonitrile and a solution of 25 nmol of the precursor peptide were added. The isotopic exchange radiolabeling reaction proceeded within 5 min at ambient temperature with 70-90% [^{18}F]fluoride incorporation. The reaction was quenched by addition of 9 mL of 0.1 M HEPES buffer (pH 2) in order to receive the product in an aqueous acidic solution of ~pH5 which was subsequently purified with a SepPak C18 cartridge. After trapping the product, reactants and solvent were removed by washing the cartridge with water for injection. After elution of the purified product peptide with 200-500 µL of ethanol, the solution was diluted with isotonic saline to a final ethanol concentration of ≤10% and sterile-filtered. Analytical radio-HPLC showed a radiochemical purity of ≥98%.

In Vitro Characterization of Radiotracers

Determination of Lipophilicities. 2 µL of the product solution were added to 800 µL n-octanol and 800 µL of phosphate buffer (0.05 M, pH 7.4). After vigorous shaking for 5 min, the phases were separated by centrifugation at 14,000×g for 2 min. Three aliquots of 100 µL of each phase were measured in a gamma-counter (*27*).

Determination of the Serum Stabilities. 200 µL of the injectable peptide solution were mixed with 500 µL of human serum and incubated over a period of 120 min at 37° C. After 10, 30, 60 and 120 min, aliquots of 5 µL were withdrawn and analyzed by

analytical radio-HPLC. The analyses indicated an unaltered radiochemical purity of all tracers of ≥98% after 120 min in human serum.

Competitive Receptor Binding Affinity Studies. A competitive receptor binding assay using viable AR42J cells (10^5 cells per well, cell culture is described in the **SI**) was performed. The cells were incubated with 0.5 nM ^{177}Lu-DOTATATE (preparation protocol is available in the **SI**) in presence of different concentrations of each SiFA-derivatized peptide (0 - 250 nM) dissolved in binding buffer (containing 10 mM $MgCl_2$, 1 mM $CaCl_2$, 25 mM HEPES, 0.5% BSA, pH 7.4) for 60 min at ambient temperature with gentle agitation. Removal of binding buffer and washing steps (2 x 100 µL and 1 x 200 µL ice-cold PBS) were carried out using a multiscreen vacuum manifold (Millipore). Subsequently, the cell-bound and internalized radioactivity was measured using a gamma-counter (Cobra Quantum Packard) and the inhibitory concentrations (IC_{50}-values) were calculated by using a nonlinear regression algorithm (GraphPad-Prism). The experiments were each performed in triplicate.

In Vivo Characterization of Radiotracers

All animal experiments respected German animal protection laws and protocols of the local committee.

Biodistribution Experiments. 50 µL injectable solutions containing 1.1 - 5.3 MBq of the respective radiolabeled peptide were injected intravenously. 4 - 10 animals per group bearing AR42J tumors (details can be found in the **SI**) were sacrificed after 60 or 90 min and the organs were dissected. After determination of organ weight, the activity of the tissues was measured using a gamma-counter (Wallac Wizard 1480) along with standards taken from the injected solution to determine the total injected activity. In

blocking experiments, 200 µg of DOTATATE was co-administrated together with the radiotracer.

Animal PET Imaging Studies. Nine tumor bearing mice were injected with 4-8 MBq of the respective radiolabeled peptide (^{18}F-**9**, n = 5, and ^{68}Ga-DOTATATE, n = 4). A dynamic scan over 90 min and a subsequent CT-acquisition were performed using a triple-modality Bruker Albira small animal PET/SPECT/CT scanner.

RESULTS

SiFA Labeling Chemistry

Former studies introducing the SiFA labeling chemistry (1st generation SiFA-TATE (*19*) (^{18}F-**1**) and a less lipophilic 2nd generation compound SiFA-Glc-PEG$_1$-TATE (*17*) (^{18}F-**3**), **SI**, **Figure S3**) for convenient, fast and facile peptide labeling of somatostatin-analogs already demonstrated a high ^{18}F-fluoride incorporation within 5 - 10 min at ambient temperature. In in vivo scans, however, a pronounced liver uptake of ^{18}F-**1** and hardly any radioactivity accumulation in tumor tissue was observed (**Figure 5A**) due to the highly lipophilic character of the SiFA-synthon. The introduction of a carbohydrate moiety (*30*) and a PEG$_1$-linker (*26*) did not fully compensate for the SiFA lipophilicity, although a much higher amount of the labeled ^{18}F-**3** was found in tumor tissue (**Fig. 5B**). In order to obtain more hydrophilic SiFA-bearing TATE derivatives better suitable for in vivo imaging of somatostatin-positive tumors due to more favorable pharmacokinetic properties, we introduced different hydrophilic auxiliaries into the peptide sequence and studied their influence on the tracer pharmacokinetics in vivo (*26*).

Chemical- and Radio-syntheses

The synthesis of the TATE derivatives was performed using standard Fmoc solid-phase peptide synthesis protocols (*31*) (**see SI for details**). Besides a carbohydrate moiety, we introduced additional hydrophilic auxiliaries such as PEGs (polyethylene glycols) of different lengths, one or two aspartic acids and a permanently positively charged SiFA*lin* synthon based on a previously described charged SiFA building block (*25*) (**Figure 1**).

The purification of the labeled peptides proved to be simple with a C18 cartridge purification. The ^{18}F-labeled peptides were obtained in high radiochemical yields of 49.8 ± 5.9% (n = 20) and chemical as well as radiochemical purities of ≥98% in only 20-25 minutes total synthesis time. This convenient one-step ^{18}F-fluoride labeling strategy for peptides, proceeding with high efficiency and reliability yields the ^{18}F-radiolabeled peptides with excellent specific activities of 44 - 63 GBq/µmol using starting activities of 3.3 - 6.7 GBq.

In Vivo Stability and Lipophilicity Determination

In accordance to previous studies, we found comparable results regarding the high stability of ^{18}F-labeled SiFA and SiFA*lin*-TATE derivatives in human serum at 37°C over 120 min, showing no degradation of the radiolabeled peptides. The $\log_D$ values (n-octanol/phosphate buffer pH 7.4) of the ^{18}F-labeled peptides were determined to gauge the influence of the hydrophilic auxiliaries. A significantly reduced overall lipophilicity was found for all newly developed SiFA and SiFA*lin* derivatized somatostatin-analogs compared to 1st and 2nd generation SiFA-TATE derivatives **1** and **3** (**SI**, **Figure S2**). Interestingly, the aspartic acids play the pivotal role in decreasing the lipophilicity since neither the different PEG spacers nor the permanently positive charge at the SiFA moiety decreased the overall lipophilicity significantly.

In Vitro Receptor Affinity Studies

Although former studies displayed no loss of SSTR affinity (*28*) upon chemical modification at the non-pharmacophoric *N*-terminus of the somatostatin-analog TATE, a competitive cell-based affinity study was performed on viable rat pancreas carcinoma AR42J cells, known to overexpress somatostatin receptors (*32*). The binding assay demonstrated excellent IC_{50} values in the low nanomolar range for the newly developed SiFA-TATE derivatives and thus a preserved binding affinity to SSTRs (**Figures 3** and **S3**).

Biodistribution Experiments of ^{18}F-SiFA-TATE Derivatives

To investigate the pharmacokinetic characteristics of the most promising ^{18}F-labeled candidates for in vivo tumor imaging, derivatives with two different PEG lengths (PEG_1 and PEG_5) and both SiFA-synthons (**4** and **7** bearing SiFA, **9** and **12** bearing SiFA*lin*) were chosen for a comparative biodistribution study in AR42J tumor-bearing xenograft mice. The obtained results were compared to the current clinical gold standard ^{68}Ga-DOTATATE.

Once tumor sizes reached 0.2 - 0.8 cm^3, solutions of the radiolabeled peptides ^{18}F-**4,** ^{18}F-**7,** ^{18}F-**9 and** ^{18}F-**12** were injected intravenously.

For biodistribution studies, the mice were sacrificed at 60 and 90 min post injection and radioactivity measurements of necropsy tissue samples were performed (results of 60 min p.i. see **SI**, **Figure S4**). The results showed significantly higher tumor-to-organ ratios for the positively charged SiFA*lin* derivatives ^{18}F-**9** and ^{18}F-**12** than for their SiFA counterparts ^{18}F-**4** and ^{18}F-**7** (**SI**, **Figure S6**). The same was found at 90 min p.i.:

Despite slightly lower absolute tumor accumulations as compared ^{18}F-**4** and ^{18}F-**7**, the SiFA*lin* derivatives ^{18}F-**9** and ^{18}F-**12** showed significantly higher tumor-to-background ratios (Table 1), thus rendering them better-suited for tumor visualization in vivo. Interestingly, the tracers containing a PEG_5-linker demonstrated an almost twofold higher uptake in physiologically SSTR-positive tissues although the tumor uptakes of PEG_1- and PEG_5-comprising peptide derivatives were comparable, thus making ^{18}F-SiFA*lin*-Glc-Asp_2-PEG_1-TATE (^{18}F-**9**) the most promising of the newly developed radiotracers.

When comparing ^{18}F-**9** to the clinically established gold standard ^{68}Ga-DOTATATE, evaluated under identical experimental conditions, ^{18}F-**9** demonstrated comparable pharmacokinetic properties (**Figure 4**), displaying a high and specific tumor uptake and low non-target organ-accumulations. Those results demonstrate that ^{18}F-**9** is an interesting ^{18}F-labeled alternative to ^{68}Ga-DOTATATE, being as easily synthetically accessible as the latter, exhibiting more favorable physical decay characteristics such as a longer half-life and a significantly shorter maximum range of positrons.

In order to evaluate the potential of peptide ^{18}F-**9** for in vivo tumor imaging, an additional animal PET study was performed. The outcomes of this study are shown in **Figure 5C**, illustrating the evolution of SiFA-modified ^{18}F-labeled TATE-derivatives with regard to their in vivo pharmacokinetics.

Furthermore, in order to directly compare the image qualities achievable using the clinical gold standard ^{68}Ga-DOTATATE and ^{18}F-**9,** a comparative small animal PET/CT study in SSTR-positive tumor-bearing animals was performed and the results are shown in **Figure 6**.

These results demonstrate the high potential of newly developed compound ^{18}F-**9** for clinical in vivo imaging of neuroendocrine tumors with PET.

DISCUSSION

^{18}F-SiFA*lin*-Glc-Asp$_2$-PEG$_1$-TATE (^{18}F-**9**) was identified from among a variety of new synthetically easily accessible SiFA-modified TATE derivatives being evaluated for ^{18}F-labeled peptide-based SSTR imaging (**SI**, **Figure S1**). ^{18}F-**9** demonstrated – as compared to the current clinical gold standard ^{68}Ga-DOTATATE – favorable in vivo pharmacokinetic properties with higher absolute tumor uptakes, very good tumor to normal tissues ratios, and excellent spatial resolution of the images. Nevertheless, higher binding by most normal tissue will likely decrease contrast with ^{18}F-9 in direct comparison to ^{68}Ga-DOTATATE.

Importantly, the radiosynthesis of ^{18}F-**9** was achieved within 20-25 min in a kit-like manner devoid of any special equipment or intricate purification procedures. The radiotracer was synthesized in radiochemical yields of 52.5% ± 4.9% with specific activities of 44 - 63 GBq/µmol (1200-1700 Ci/mmol).

Similar to Perrin's synthesis of ^{18}F-AMBF$_3$-TATE, the SiFA*lin* labeling approach maintained all the benefits of the trifluoroborate chemistry, again highlighting the advantages of ^{18}F labeling methods based on isotopic exchange (*27*). The introduction of aspartic acids into the TATE derivatives was the key to sufficiently reduce the overall lipophilicity of ^{18}F-**9** to a $\log_D$ of -1.21 ± 0.02. In addition, binding affinities of all SiFA*lin* and auxiliary-modified peptides were in the nanomolar range, thus allowing for a strong interaction with the target receptors. The introduction of the novel SiFA building block

SiFA*lin* **(Figure 1)**, characterized by a permanent positive charge, resulted in improved tumor-to-non-tumor-tissue ratios. These measures ultimately enabled a tumor-specific accumulation and renal clearance which is mandatory for the overall quality of the resulting PET images. For ^{18}F-**9**, only renal excretion was observed in the AR42J tumor-bearing xenograft mouse model (**Figures 5C** and **6B**) with a maximum radioactivity accumulation in the tumor of 18.51 ± 4.89% ID/g at 60 min p.i. compared to 14.10 ± 4.84% ID/g found for ^{68}Ga-DOTATATE under the same conditions as determined by ex vivo biodistribution experiments (**Figure 4**). As stated above, the higher accumulation of radioactivity in normal tissue might be detrimental to the overall image quality. It is also noteworthy that the in vivo resistance of ^{18}F-**9** to ^{18}F-hydrolysis is very high as can be deduced from the low observed bone uptake of only 1.31 ± 0.31% ID/g at 90 min p.i. (**Figure 4**).

Moreover, the SiFA*lin* structure seems to have a pronounced positive influence on the tumor-to-organ ratios as well as on the pathway of excretion. In the used xenograft tumor model, ^{18}F-**9** showed a promising biodistribution approaching the quality of ^{68}Ga-DOTATATE which is a prerequisite for human application. In addition, the radioisotope ^{18}F has noteworthy physical advantages over other common radioisotopes used in SSTR imaging yielding PET images of high resolution and quality, enabling a highly sensitive and efficient detection of neuroendocrine tumors. In studies where quantification of uptake might be required, this approach is likely superior to that using ^{68}Ga-labeled peptides because of the significantly higher maximum energy of ^{68}Ga positrons as compared to those from ^{18}F (1899 KeV and 644 KeV respectively) (*33*). The longer half-life of ^{18}F is also a major advantage, as it will allow for distribution of the

tracer over longer distances like ^{18}F-FDG, to reach PET centers not equipped with a ^{68}Ge/^{68}Ga generator, which could even locally perform ^{18}F-labelling.

The direct comparison of both tracers ^{68}Ga-DOTATATE and ^{18}F-**9** with PET/CT imaging of tumor-bearing animals (**Figure 6**) shows the excellent image quality which can be obtained using the ^{18}F-labeled compound ^{18}F-**9** not only in terms of image resolution but also absolute tumor uptake. However if transferred to a human imaging situation where the tumor is likely localized abdominally and not peripherally as in the animal tumor model, the background activity will indeed be a potential liability for accurate tumor visualization.

The fact that the synthesis of the radiolabeled compounds takes place at room temperature within 5 minutes, is devoid of any HPLC purification, can be made fully compliant with good manufacturing practice protocols and proceeds in a kit-like manner yielding the final ^{18}F-labeled peptide in high radiochemical yields within 20 to 25 min only, renders this new radiotracer highly attractive towards a clinical application. The final success of the atypical SiFA labeling methodology employing the optimized SiFA*lin* in a preclinical setting opens up new avenues for nuclear medicine diagnostic imaging based on ^{18}F-labeled peptide imaging agents.

For a new radiotracer to become a routine clinical tool, not only good science and clinical scale up is required, but also carefully conducted multi-center clinical trials, ubiquitous availability, regulatory approval, clinical acceptance funding by insurers and providers. These goals are likely easier to achieve with an ^{18}F labeled radiopharmaceutical with access to an extensive central radiopharmacy network than with a significantly shorter half-life generator produced tracer that is inevitably going to

be limited in use at larger centers. Our belief is that the ^{18}F-**9** tracer described in this manuscript is an important step to achieving the goals identified above. The kit-like formulation has the potential to make regulatory approval easierand ease acceptance by radiopharmacies. We propose to validate the safety and clinical efficacy of ^{18}F-**9** in future phase I/II clinical trials.

CONCLUSION

We presented the synthesis, in vitro and in vivo evaluation of an auxiliary-derivatized ^{18}F-SiFA*lin*-TATE for somatostatin receptor imaging in a preclinical AR42J mouse xenograft model. The synthesis was developed as a kit-like procedure yielding multiple doses of the tracer with high specific activity. In vitro binding assays confirmed the high binding affinity of the tracers towards somatostatin receptors. The direct comparison of tracer ^{18}F-**9** with ^{68}Ga-DOTATATE in PET/CT imaging of tumor-bearing animals (**Figure 6**) shows the excellent image quality which can be obtained using the ^{18}F-labeled compound ^{18}F-**9** in terms of image resolution and absolute tumor uptake.

Ex vivo as well as in vivo biodistribution data obtained with small animal PET/CT demonstrated the favorable pharmacokinetics and excellent tumor-to-non-target tissue ratios in the preclinical mouse model that warrants translation into human clinical trials.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Dr. Gerhard Glatting, Elisabeth Götz, Tobias Herzel, Sergej Losev, Dr. Flavia Molina, Dr. Nils Vogler, Dr. Mareike Roscher and Anne-Maria Suhr for their excellent technical support. Funding from the German Research Foundation (WA 2132/3-1), the Bayern-Quebec-Allianz and the Ministry of Science,

Research and the Arts of the State of Baden-Württemberg (AZ 42-04HV.M1412(12)/1/1) is acknowledged. Funding from the National Science and Engineering Research Council of Canada (NSERC) (Discovery Grant) was provided to R.S.

AUTHOR CONTRIBUTIONS

Sabrina Niedermoser performed chemical, radiochemical, in vitro and in vivo experiments and contributed to manuscript preparation; Joshua Chin performed chemical syntheses; Carmen Wängler contributed to animal experiments, study design and manuscript preparation; Alexey Kostikov performed radiochemical syntheses; Vadim Bernard-Gauthier performed chemical syntheses; Jean-Paul Soucy and Alexander J McEwan contributed to the manuscript preparation and gave their expert opinion as a nuclear medicine physicians; Nils Vogler analyzed the PET data, Ralf Schirrmacher contributed to study design and manuscript preparation and Björn Wängler set up the study design and prepared the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

REFERENCES

1. Smith G, Carroll L, Aboagye E. New frontiers in the design and synthesis of imaging probes for PET oncology: Current challenges and future directions. *Mol Imaging Biol.* 2012;14:653-666.

2. Decristoforo C, Pickett RD, Verbruggen A. Feasibility and availability of 68Ga-labelled peptides. *Eur J Nucl Med Mol Imaging.* 2012;39 Suppl 1:S31-40.

3. Ikotun OF, Lapi SE. The rise of metal radionuclides in medical imaging: copper-64, zirconium-89 and yttrium-86. *Future Med Chem*. 2011;3:599-621.

4. Coenen HH, Elsinga PH, Iwata R, et al. Fluorine-18 radiopharmaceuticals beyond [18F]FDG for use in oncology and neurosciences. *Nucl Med Biol.* 2010;37:727-740.

5. Choyke PL. Science to practice: Pilot study of FPPRGD2 for imaging αvβ3 integrin—how integral are integrins? *Radiology* 2011;260:1-2.

6. Li W, Meyer L, Anderson C. Radiopharmaceuticals for positron emission tomography imaging of somatostatin receptor positive tumors. In: Krause W, ed., *Contrast Agents III. Topics in Current Chemistry. 252.* Berlin : Springer; 2005:179-192.

7. Breeman WAP, de Blois E, Sze Chan H, Konijnenberg M, Kwekkeboom DJ, Krenning EP. 68Ga-labeled DOTA-peptides and 68Ga-labeled radiopharmaceuticals for positron emission tomography: Current status of research, clinical applications, and future perspectives. *Semin Nucl Med.* 2011;41:314-321.

8. Adams S, Baum R, Rink T, Schumm-Dräger P-M, Usadel K-H, Hör G. Limited value of fluorine-18 fluorodeoxyglucose positron emission tomography for the imaging of neuroendocrine tumours. *Eur J Nucl Med.* 1998;25:79-83.

9. Ambrosini V, Tomassetti P, Franchi R, Fanti S. Imaging of NETs with PET radiopharmaceuticals. *Q J Nucl Med Mol Imaging* 2010;54:16-23.

10. Wester HJ, Schottelius M. Fluorine-18 labeling of peptides and proteins. In: Schubiger PA, Lehmann L, Friebe M, ed., *PET Chemistry*. *Ernst Schering Research Foundation Workshop. 64.* Berlin: Springer; 2007:79-111.

11. Pretze M, Pietzsch D, Mamat C. Recent trends in bioorthogonal click-radiolabeling reactions using fluorine-18. *Molecules* 2013;18:8618-8665.

12. McBride WJ, Sharkey RM, Karacay H, et al. A novel method of F-18 radiolabeling for PET. *J Nucl Med.* 2009;50:991-998.

13. Wan W, Guo N, Pan D, et al. First experience of 18F-alfatide in lung cancer patients using a new lyophilized kit for rapid radiofluorination. *J Nucl Med*. 2013;54:691-698.

14. Smith GE, Sladen HL, Biagini SCG, Blower PJ. Inorganic approaches for radiolabelling biomolecules with fluorine-18 for imaging with positron emission tomography. *Dalton T.* 2011;40:6196-6205.

15. Ting R, Adam MJ, Ruth TJ, Perrin DM. Arylfluoroborates and alkylfluorosilicates as potential PET imaging agents: high-yielding aqueous biomolecular 18F-labeling. *J Am Chem Soc.* 2005;127:13094-13095.

16. Liu Z, Li Y, Lozada J, et al. Kit-like 18F-labeling of RGD-19F-arytrifluroborate in high yield and at extraordinarily high specific activity with preliminary in vivo tumor imaging. *Nucl Med Biol.* 2013;40:841-849.

17. Wängler C, Niedermoser S, Chin JS, et al. One-step F-18-labeling of peptides for positron emission tomography imaging using the SiFA methodology. *Nat Protoc.* 2012;7:1946-1955.

18. Wängler B, Kostikov AP, Niedermoser S, et al. Protein labeling with the labeling precursor [18F]SiFA-SH for positron emission tomography. *Nat Protoc.* 2012;7:1964-1969.

19. Schirrmacher R, Bradtmöller G, Schirrmacher E, et al. F-18-labeling of peptides by means of an organosilicon-based fluoride acceptor. *Angew Chem Int Edit.* 2006;45:6047-6050.

20. Mu LJ, Höhne A, Schubiger RA, et al. Silicon-based building blocks for one-step 18F-radiolabeling of peptides for PET imaging. *Angew Chem Int Edit.* 2008;47:4922-4925.

21. Dialer LO, Selivanova SV, Muller CJ, et al. Studies toward the development of new silicon-containing building blocks for the direct 18F-labeling of peptides. *J Med Chem*. 2013;56:7552-7563.

22. Schulz J, Vimont D, Bordenave T, et al. Silicon-based chemistry: An original and efficient one-step approach to [18F]-nucleosides and [18F]-oligonucleotides for PET imaging. *Chem Eur J.* 2011;17:3096-3100.

23. Kostikov AP, Chin J, Orchowski K, et al. Synthesis of [18F]SiFB: a prosthetic group for direct protein radiolabeling for application in positron emission tomography. *Nat Protoc.* 2012;7:1956-1963.

24. Höhne A, Mu L, Honer M, et al. Synthesis, F-18-labeling, and in vitro and in vivo studies of bombesin peptides modified with silicon-based building blocks. *Bioconj Chem.* 2008;19:1871-1879.

25. Kostikov AP, Iovkova L, Chin J, et al. N-(4-(di-tert-butyl[F-18]fluorosilyl)benzyl)-2-hydroxy-N, N-dimethylethylammonium bromide ([F-18]SiFAN(+)Br(-)): A novel lead

compound for the development of hydrophilic SiFA-based prosthetic groups for F-18-labeling. *J Fluorine Chem*. 2011;132:27-34.

26. Hosseinimehr SJ, Tolmachev V, Orlova A. Liver uptake of radiolabeled targeting proteins and peptides: considerations for targeting peptide conjugate design. *Drug Discov Today*. 2012;17:1224-1232.

27. Liu Z, Pourghiasian M, Menard F, Pan J, Lin K-S, Perrin DM. Preclinical evaluation of a high-affinity 18F-trifluoroborate octreotate derivative for somatostatin receptor imaging. *J Nucl Med*. 2014;55:1499-1505.

28. Wängler C, Waser B, Alke A, Iovkova L, Buchholz HG, Niedermoser S, et al. One-step F-18-labeling of carbohydrate-conjugated octreotate-derivatives containing a silicon-fluoride-acceptor (SiFA): In vitro and in vivo evaluation as tumor imaging agents for positron emission tomography (PET). Bioconj Chem 2010;21:2289-2296.

29. Wessmann SH, Henriksen G, Wester HJ. Cryptate mediated nucleophilic F-18-fluorination without azeotropic drying. Nuklearmedizin 2012;51:1-8.

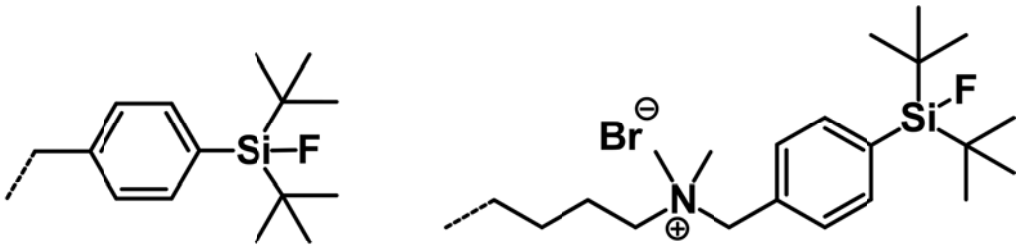
30. Schottelius M, Wester H-J, Reubi JC, Senekowitsch-Schmidtke R, Schwaiger M. Improvement of pharmacokinetics of radioiodinated Tyr3-octreotide by conjugation with carbohydrates. Bioconj Chem 2002;13:1021-1030.

31. Wellings DA, Atherton E. Standard Fmoc protocols. Methods Enzymol 1997;289:44-67.

32. Taylor JE, Theveniau MA, Bashirzadeh R, Reisine T, Eden PA. Detection of somatostatin receptor subtype-2 (Sstr2) in established tumors and tumor-cell lines - evidence for Sstr2 heterogeneity. Peptides 1994;15:1229-1236.

33. Kemerink GJ, Visser MGW, Franssen R, Beijer E, Zamburlini M, Halders SGEA, Brans B, Mottaghy FM, Teule GJJ. Effect of the positron range of 18F, 68Ga and 124I on PET/CT in lung-equivalent materials. Eur J Nucl Med Mol Imaging 2011; 38:940–948.

.



SiFA building block SiFA*lin* building block

Figure 1: Structures of Silicon-Fluoride-Acceptor building blocks SiFA and SiFA*lin*.

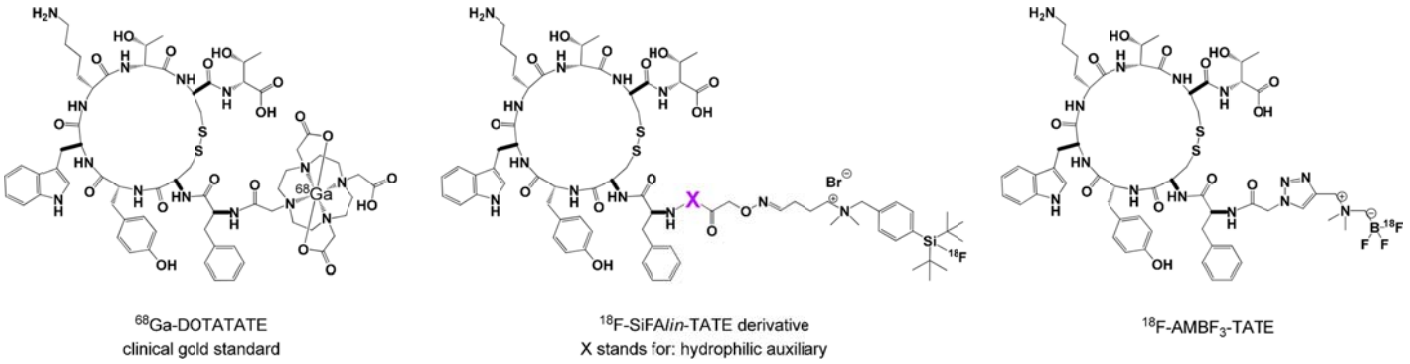


Figure 2: Structures of ^{68}Ga-DOTATATE, ^{18}F-trifluoroborate derivative ^{18}F-AMBF$_3$-TATE and ^{18}F-SiFA*lin*-analogs. ”X” indicates the site of introduction of hydrophilic auxiliaries for optimizing in vivo biodistribution properties.

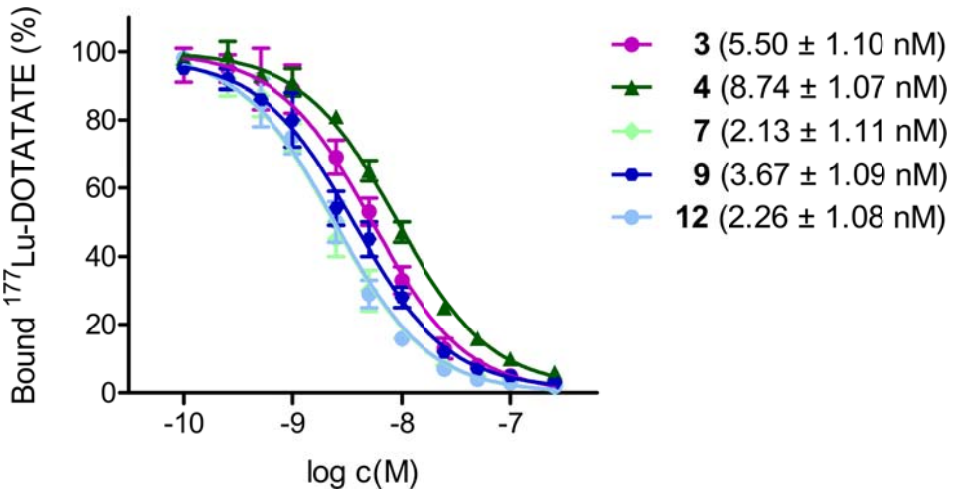


Figure 3: High tolerance of SSTR binding affinities of Tyr^3-octreotate towards hydrophilic chemical modifications without loss of bioactive potency. Binding affinities of 3rd generation SiFA- (green, PEG_1 **4** & PEG_5 **7**) and SiFA*lin*- (blue, PEG_1 **9** & PEG_5 **12**) derivatives compared to 2nd generation (magenta, **3**) were determined by competitive receptor binding affinity studies using AR42J cells and ^{177}Lu-DOTATATE as competitor. After incubation for 60 min in buffer at ambient temperature and successive washing steps, both cell-bound and internalized activity was measured using a gamma counter. All experiments were performed in triplicate (error bars: means ± s.d.).

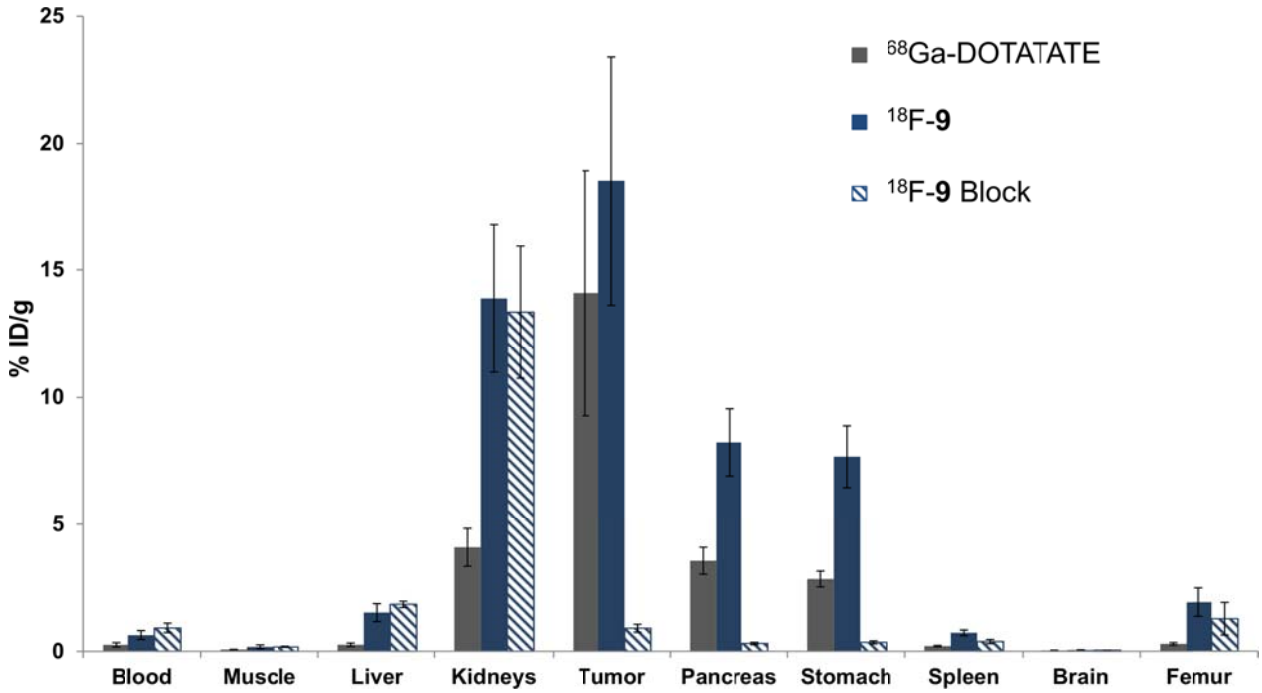


Figure 4: Results from ex vivo biodistribution studies comparing ^{68}Ga-DOTATATE (n = 5) to ^{18}F-**9** (n = 10) in AR42J tumor-bearing mice at 60 min p.i. The values are given as % ID/g. The most promising ^{18}F-SiFA*lin*-derivatized derivative ^{18}F-**9** demonstrates a highly specific and even slightly higher tumor uptake than gold standard ^{68}Ga-DOTATATE. For ^{18}F-**9**, a blocking experiment using 200 µg/mouse DOTATATE (n = 5) was performed (dashed blue columns), showing the specific binding of the tracer to the tumor and physiologically SSTR-positive tissues. ^{18}F-SiFA*lin*-derivatives enable tumor-to-background ratios comparable to ^{68}Ga-DOTATATE.

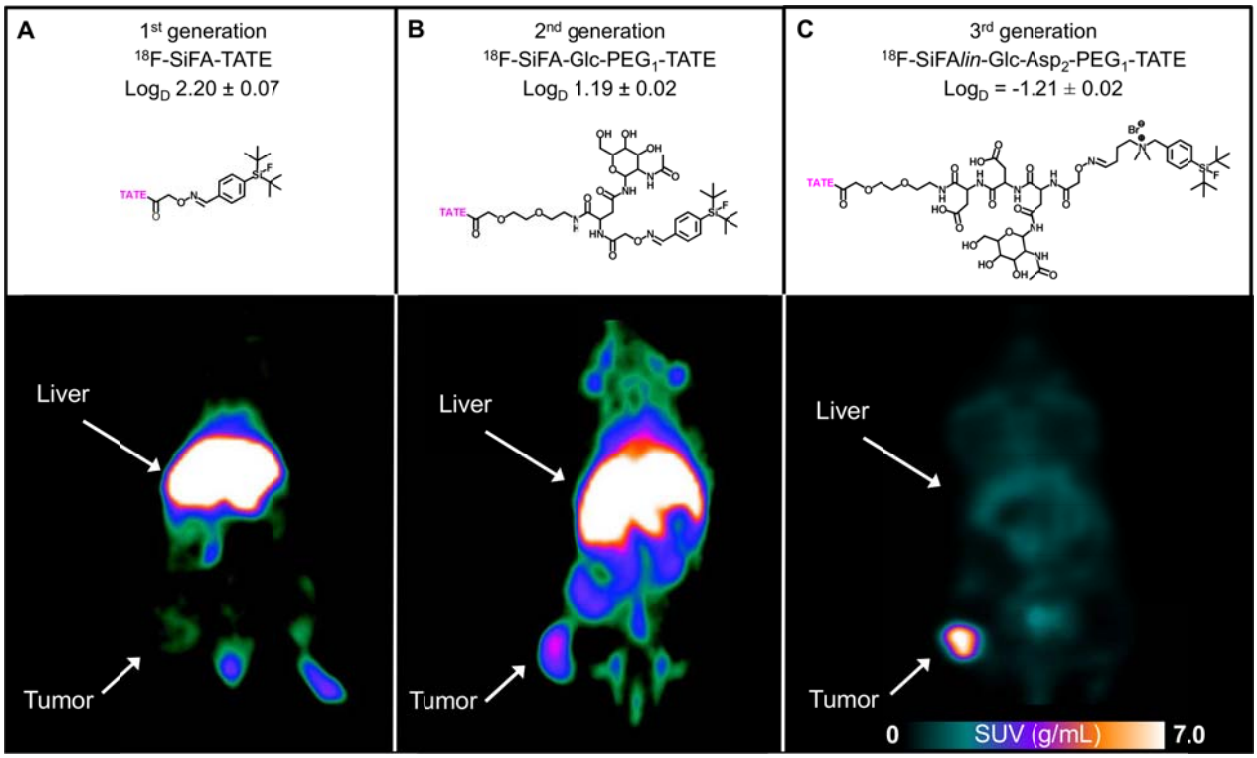


Figure 5: Small animal PET images of three generations of SiFA-derivatized somatostatin-analogs evaluated in AR42J tumor-bearing rodents. All images show coronal slices of the 50 to 90 min. p.i. time frame (**A**) ^{18}F-SiFA-TATE (^{18}F-]**1)** in vivo images were obtained using a Philips Mosaic small animal PET scanner. (**B**) ^{18}F-SiFA-Glc-PEG$_1$-TATE (^{18}F-**3)** was performed with a Siemens Inveon small animal PET-scanner. (**C**) Images of ^{18}F-SiFA*lin*-Glc-Asp$_2$-PEG$_1$-TATE (^{18}F-**9)** distribution were obtained using a Bruker Albira small imaging PET/SPECT/CT scanner indicating both a renal clearance and high activity accumulation in the somatostatin receptor-positive tumor tissue.

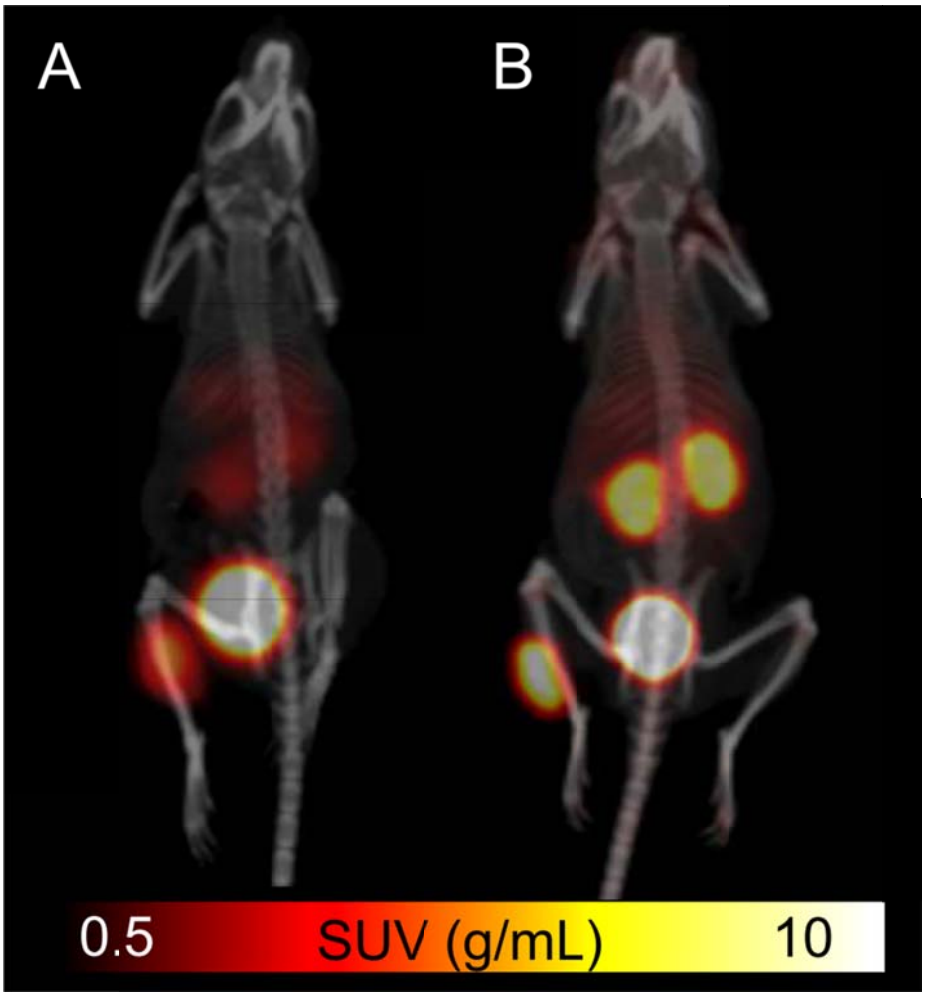


Figure 6: Comparative small animal PET/CT imaging of SSTR-positive tumor-bearing mice using ^{68}Ga-DOTATATE (A) and ^{18}F-**9** (B) with identical SUV scale for both images. The tumor accumulation SUVs in the last frame (80 - 90min p.i.; showing the highest accumulation of both tracers) are 5.50 for ^{68}Ga-DOTATATE and 7.80 for ^{18}F-**9**.

	^{18}F-**4**	^{18}F-**9**	^{18}F-**7**	^{18}F-**12**
tumor/blood ratio	4.30 ± 0.26	57.58 ± 35.89	12.31 ± 4.19	74.42 ± 20.37
tumor/muscle ratio	33.31 ± 11.94	211.05 ± 143.38	94.12 ± 26.37	256.49 ± 61.17

Table 1: Tumor-to-blood and tumor-to-muscle-ratios for the tested newly developed ^{18}F-labeled peptides ^{18}F-**4,** ^{18}F-**7,** ^{18}F-**9** and ^{18}F-**12** obtained from ex vivo biodistribution at 90min p.i..