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System A Amino Acid Transport-Targeted Brain and Systemic Tumor PET Imaging Agents 2-Amino-3-[¹⁸F]Fluoro-2-Methylpropanoic Acid and 3-[¹⁸F]Fluoro-2-Methyl-2-(Methylamino)propanoic Acid

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Abstract

Introduction—Amino acid based radiotracers target tumor cells through increased uptake by membrane-associated amino acid transport (AAT) systems. In the present study, four structurally related non-natural ¹⁸F-labeled amino acids, (R)- and (S)-[¹⁸F]FAMP **1** and (R)- and (S)-[¹⁸F]MeFAMP **2** have been prepared and evaluated *in vitro* and *in vivo* for their potential utility in brain and systemic tumor imaging based upon primarily system A transport with positron emission tomography (PET).

Methods—The transport of enantiomers of [¹⁸F]FAMP **1** and [¹⁸F]MeFAMP **2** was measured through *in vitro* uptake assays in human derived cancer cells including A549 (lung), DU145 (prostate), SKOV3 (ovary), MDA MB468 (breast) and U87 (brain) in the presence and absence of amino acid transporter inhibitors. The *in vivo* biodistribution of these tracers was evaluated using tumor mice xenografts at 15, 30, 60 and 120 min post injection.

Results—All four tracers showed moderate to high levels of uptake (1–9 %ID/5×10⁵ cells) by the cancer cell lines tested *in vitro*. AAT cell inhibition assays demonstrated that (R)-[¹⁸F]**1** and (S)-[¹⁸F]**1** entered these tumor cells *via* mixed AATs, likely but not limited to system A and system L. In contrast, (R)-[¹⁸F]**2** and (S)-[¹⁸F]**2** showed high selectivity for system A AAT. Similar to the results of *in vitro* cell studies, the tumor uptake of all four tracers was good to high and persisted over the 2 hours time course of *in vivo* studies. The accumulation of these tracers was higher in tumor than most normal tissues including blood, brain, muscle, bone, heart, and

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lung, and the tracers with the highest *in vitro* selectivity for system A AAT generally demonstrated the best tumor imaging properties. Higher uptake of these tracers was observed in the pancreas, kidney and spleen compared to tumors.

Conclusions—These preclinical studies demonstrate good imaging properties in a wide range of tumors for all four amino acids evaluated with (*R*)-[¹⁸F]2 having the highest selectivity for system A AAT.

Keywords

[¹⁸F]FAMP; [¹⁸F]MeFAMP; system A amino acid transport; tumor imaging; fluorine-18; PET

1. Introduction

Cancer is the 2nd leading cause of death in the United States, exceeded only by heart disease [1], and imaging plays key roles the clinical diagnosis, staging, and/or treatment monitoring of most human malignancies. Alterations in cellular metabolism including amino acid transport (AAT) and metabolism are hallmarks of cancer cells and can be targeted for diagnostic imaging and for therapy [2, 3]. Metabolic positron emission tomography (PET) imaging of cancer targets the altered metabolism of cancer cells, and the glucose analogue 2-[¹⁸F]fluoro-2-deoxy-D-glucose (FDG) has been successfully employed for the clinical imaging of a wide range of cancers based on the increased rates of glucose transport and glycolysis that occur in many human tumors. Despite its success, FDG has important limitations that potentially can be overcome with PET tracers targeting different aspects of tumor metabolism and biochemistry. Glycolysis reflects only one facet of tumor metabolism and may not be the most effective molecular imaging target for certain cancers and may not be adequate to predict and monitor response to certain metabolic and molecular therapies. Additionally, FDG has high uptake in inflammatory processes which limits its specificity [4-10]. Finally, certain types of cancer, including prostate cancer, have relatively low uptake of FDG which limits its sensitivity for detecting these neoplasms [11].

Radiolabeled α-amino acids (AAs) represent a diverse and useful class of PET tracers that target the increased rates of AAT exhibited by many cancer cells. AA-based radiotracers enter and accumulate in tumor cells through membrane-associated AAT systems. For molecular imaging, tumor uptake of radiolabeled AAs is primarily determined by AAT rather than protein synthesis [12, 13]. The most extensively evaluated AAs for human tumor imaging include L-[¹¹C]methionine ([¹¹C]MET) [14-17], *O*-(2-[¹⁸F]fluoroethyl)-L-tyrosine ([¹⁸F]FET) [18, 19] and 3,4-dihydroxy-6-[¹⁸F]-fluoro-L-phenylalanine ([¹⁸F]FDOPA) [20, 21]. All these amino acids are primarily system L (leucine-preferring) substrates and these studies have implicated increased system L expression in tumor cells. System L transport is active at the normal blood-brain barrier (BBB) which makes these system L tracers particularly well-suited for brain tumor imaging, but their performance for detecting other tumor types outside of the brain has been relatively poor in part due to low tumor to background ratios limiting the sensitivity of these tracers [12].

System A (alanine-preferring) AAT is an important target of oncogene action and a crucial regulator of cell growth based on its response to environmental stimuli, growth factors,

oncogenic transformation and the dependency of its synthesis on protein kinase C [22-30]. System A transporters have been shown to be upregulated in several human cancers including breast cancer, hepatocellular carcinoma and cholangiocarcinoma [29, 31, 32]. Unlike system L transporters, system A AAT can concentrate substrates in tumor cells through secondary active transport. This concentrative property of system A transport has the potential to provide higher tumor to normal tissue ratios and more persistent tumor uptake, leading to superior tumor visualization and detection. To date, there are relatively few preclinical/clinical studies involving system A substrates since the chemical structures of the system A substrates are restricted to α -AAs with short, neutral side chains. The reported PET tracer examples for system A are limited to carbon-11 labeled 2-amino isobutyric acid (AIB) and its *N*-methyl derivative 2-(methylamino)isobutyric acid (MeAIB) [9, 10, 33, 34]. The limited evaluation of [^{11}C]AIB/MeAIB has shown promise in clinical oncology for PET tumor imaging in patients with brain and systemic tumors. However the short-lived carbon-11 label ($T_{1/2}=20$ min) greatly limits their suitability for routine clinical use since an on-site cyclotron and radiosynthetic capabilities are needed to produce ^{11}C , and batch production for multiple patients and remote distribution is challenging.

Through our efforts to develop PET tracers targeting system A AAT with greater potential clinical utility, we have prepared fluorine-18 ($T_{1/2}=110$ min) labeled AIB analogs (*R*)- and (*S*)-2-amino-3-[^{18}F]fluoro-2-methylpropanoic acid ((*R*)- and (*S*)-[^{18}F]FAMP, **1**) and fluorine-18 labeled MeAIB analogs (*R*)- and (*S*)-3-[^{18}F]fluoro-2-methyl-2-*N*-(methylamino)propanoic acid ((*R*)- and (*S*)-[^{18}F]FMeAMP, **2**), which chemical structures are shown in Figure 1. In our initial study with a rat brain tumor model, these fluorinated AIB/MeAIB analogs showed high and selective *in vitro* uptake by the system A AAT, and demonstrated excellent tumor to normal brain ratios of 20:1 to 115:1 *in vivo* in part due to the lack of system A transport at the normal BBB [35, 36]. To test the tumor imaging potential of these tracers for a wider range of human cancers including prostate cancer, breast cancer, and non-small cell lung cancer, we further evaluated (*R*)- and (*S*)- FAMP **1** and (*R*)- and (*S*)- MeFAMP **2** through *in vitro* AA uptake assays and in tumor-bearing mice implanted with human derived cancer cell lines.

2. Materials and Methods

2.1. Synthesis of Tracers

Enantiomers of [^{18}F]FAMP **1** and [^{18}F]MeFAMP **2** used for *in vitro* and *in vivo* studies performed at Emory University were synthesized according to the method previously described [35, 36]. Briefly, the automated radio-syntheses of (*R*)-[^{18}F]**1**, (*S*)-[^{18}F]**1**, (*R*)-[^{18}F]**2** and (*S*)-[^{18}F]**2** were carried out with no-carrier-added (NCA) nucleophilic fluorination using the cyclic sulfamidate precursors (*S*)-**3**, (*R*)-**3**, (*S*)-**4**, and (*R*)-**4**, respectively (Scheme 1). The synthesis was completed within 90 min after the start of synthesis (SOS), with the average of $52 \pm 12\%$ ($n=10$), $56 \pm 12\%$ ($n=6$), $66 \pm 12\%$ ($n=10$) and $66 \pm 18\%$ ($n=8$) decay corrected yields (DCY) for (*R*)-[^{18}F]**1**, (*S*)-[^{18}F]**1**, (*R*)-[^{18}F]**2**, and (*S*)-[^{18}F]**2**, respectively, in over 99% radiochemical purity based on radiometric TLC [acetonitrile / water / methanol = 4:1:1 (v/v/v), $R_f = 0.4$ for **1**, $R_f = 0.5$ for **2**].

2.2. Cells and Culture

All tissue culture materials and reagents were provided by Dr. Olson at Emory University. The human derived cancer cells used in the study included A549 lung adenocarcinoma, MDA MB468 estrogen-independent breast carcinoma, DU145 androgen-independent prostate carcinoma, SKOV3 estrogen-positive ovarian adenocarcinoma and U87 glioblastoma tumor cell lines, which represent different malignant phenotypes. The tumor cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum, 100 units/mL penicillin and 100ug/mL streptomycin, maintained in T-150 tissue culture flasks under humidified incubator conditions (37 °C, 5% CO₂/95% air) with routinely passaged at confluence [37, 38]. Cells thus prepared would be used in cell uptake and inhibition assays and in mice tumor implantations.

2.3. Amino Acid Uptake and Inhibition

At the time of the experiments, the medium was exchanged to amino acid free Hank's balanced salt solution (HBSS) and cells were adjusted to a final concentration of 5×10^7 cells/mL. The following standard condition applied to each study (refer to Supplementary Data for optimization information). Approximately 5×10^5 cells were exposed to 0.185 MBq (*R*)-[¹⁸F]1, (*S*)-[¹⁸F]1, (*R*)-[¹⁸F]2 or (*S*)-[¹⁸F]2, respectively, in 0.1 mL of amino acid/serum-free HBSS in the absence (control condition) or presence of transport inhibitors for 30 minutes under incubator conditions (37 °C, 5% CO₂/95% air) in 1.5 mL conical tubes. 10 mM final concentrations of MeAIB were used as a system A AAT inhibitor. 2-amino-bicyclo[2.2.1]heptane-2-carboxylic acid (BCH) inhibits sodium-independent system L AAT as well as sodium-dependent system B⁰-like AATs, and 10 mM final concentrations of BCH were used to inhibit uptake mediated by these non-system A AATs. After incubation, cells were twice centrifuged (75 G for 5 minutes) and rinsed with ice-cold HBSS to remove residual activity in the supernatant. Each assay condition was performed in triplicates. The activity in tubes was counted in a Packard Cobra II Auto-Gamma counter, the raw counts decay corrected, and the activity per cell number determined. The data from these studies were expressed and normalized as percent uptake of the initial dose per 0.5 million cells (% ID/ 5×10^5 cells) $\pm$ standard deviation (SD).

2.4. Tumor Induction and Animal Preparation

All procedures of animal handling and experimentation were in accordance with the protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Emory University. SCID mice (C.B-17, either sex, Charles Rivers Laboratory, USA) were used for tumor subcutaneous transplantation at the age of four weeks. Tumor cells for implantation experiments were cultured and prepared the same way as the uptake and inhibition assays, and then were washed with phosphate buffer solution (PBS) and were made a final concentration of 1×10^7 in 0.1 mL of PBS per animal. Cells were injected subcutaneously into the left or right side flank of SCID mice using 1 mL syringes with 25-gauge $\times$ 1 inch needles. Typically, among 25 animals implanted with tumor cells, 20 would develop visible tumors to the size of 0.5-1 cm³ in approximately 2-4 weeks depending upon the cell line implanted, and were used in the biodistribution study.

2.5. Mice Biodistribution Studies

All biodistribution studies using SCID mice were performed at Emory University. The tissue distribution of radioactivity was measured in 16-20 tumor-bearing mice, which had developed tumors and were allowed food and water ad libitum before the experiment. Awake animals were used to avoid potential mortality and alterations in AAT due to prolonged anesthesia. The 16-20 mice were divided into 4 groups of 4-6 mice per time point. The animals each received approximately 0.185 MBq of radiotracer (*R*)-[¹⁸F]**1**, (*S*)-[¹⁸F]**1**, (*R*)-[¹⁸F]**2** or (*S*)-[¹⁸F]**2** in 0.1 mL of sterile normal saline *via* tail vein injection with the aid of a restraint device. The groups of mice were sacrificed at 15, 30, 60 and 120 minutes post injection, respectively, and the tumors and selected organs/tissues isolated through dissection, weighed and counted along with dose standards in a Packard Cobra II Auto-Gamma Counter. The raw counts were decay corrected, and the counts were normalized as the percent of total injected dose per gram of organ/tissue (%ID/g) $\pm$ SD.

2.6. Statistics

The data from these studies were presented as mean %ID/g $\pm$ SD, graphed using Microsoft Excel, and were statistically analyzed. When comparing data between 2 groups, F-tests were performed to determine the homogeneity of variance. Then 2-tailed t-tests with equal/unequal variances were carried out accordingly. One-way ANOVA was used to analyze data between more than 2 groups. In all cases, $P < 0.05$ was considered as significant.

3. Results and Discussion

3.1. *In vitro* Uptake and Inhibition

To measure the contribution of system A AAT to the uptake of (*R*)- and (*S*)-[¹⁸F]**1** and (*R*)- and (*S*)-[¹⁸F]**2** by human A549, DU145, U87, MDA MB468 and SKOV3 tumor cells, inhibition assays were performed using cultured tumor cells in the presence and absence of amino acid transporter inhibitors. MeAIB is a selective competitive inhibitor of system A while BCH is an inhibitor of system L AAT that also inhibits system B⁰-like AAT in the presence of sodium [39-41]. The transport assays were all performed in the presence of sodium ions, and thus the BCH condition was used to define non-system A AAT including system L AAT. The results of the uptake and inhibition studies are summarized in Table 1 and Figure 2. The uptake data was normalized and expressed as mean percent uptake relative to the control condition. The uptake of (*R*)-[¹⁸F]**1** and (*S*)-[¹⁸F]**1** in tumor cells after 30 minutes incubation was approximately 5-9 and 2-5 %ID/5 $\times$ 10⁵ cells, respectively without inhibitors. Both BCH and MeAIB inhibited uptake of (*R*)-[¹⁸F]**1** and (*S*)-[¹⁸F]**1** to certain degrees, with more inhibition by BCH (36-68%) than by MeAIB (10-32%) in most cell lines. There was no significant MeAIB inhibition observed for (*S*)-[¹⁸F]**1** in MDA MB468 cells. The exceptions were (*R*)-[¹⁸F]**1** in U87 and SKOV3 cells, in which cases MeAIB inhibited 67% and 50% of uptake vs. BCH inhibited 20% and 17% of uptake relative to control, respectively (Figure 2). In the case of (*R*)-[¹⁸F]**2** and (*S*)-[¹⁸F]**2**, uptake in tumor cells after 30 minutes incubation without inhibitors was at the similar level of approximately 1-7 %ID/5 $\times$ 10⁵ cells. MeAIB inhibited majority of uptake (50-91%), whereas no BCH

inhibition was observed in most cases, except for (S)-[^{18}F]2 in DU145 and SKOV3 cells, in which cases BCH inhibited 29% and 6% of uptake, respectively.

All four tracers showed good to high levels of uptake (1–9 %ID/ 5×10^5 cells) into these tumor cells *in vitro* (Table 1). In general, the tumor uptake of (R)-enantiomer was slightly higher than that of (S)-enantiomer for both FAMP 1 and MeFAMP 2 pairs; the tumor uptake of FAMP 1 was slightly higher than that of MeFAMP 2 ((R)-[^{18}F]1 vs. (R)-[^{18}F]2 and (S)-[^{18}F]1 vs. (S)-[^{18}F]2) in tested tumor cell lines. AAT cell inhibition assays demonstrated that (R)-[^{18}F]1 and (S)-[^{18}F]1 entered these tumor cells *via* mixed AATs, likely but not limited to system A and system L. (R)-[^{18}F]2 and (S)-[^{18}F]2 showed high selectivity for system A AAT as MeAIB inhibited majority of uptake whereas no or little BCH inhibition was observed for these tumor cells in most cases (Table 1). An important consideration when interpreting these studies is the use of AA-free media which is different than the *in vivo* setting which has endogenous AAs that can potentially compete for AA transport. This effect has been observed with the system A substrate AIB which undergoes some system L transport under AA-free conditions but does not undergo substantial system L transport in the presence of physiological concentrations of naturally-occurring system L substrates, presumably due to competitive inhibition [42].

3.2. *In vivo* Biodistribution

3.2.1 Tumor Uptake of Tracers—The biodistribution studies were performed using SCID mice xenograft tumor models of gliomas, prostate cancer, breast cancer, non-small cell lung cancer, and ovarian cancer. The results from these studies are shown in Figure 3 and in Tables 2 and 3. All four tracers demonstrated relatively high absolute uptake in tumors. The uptake of tracers in tumor was compared between (R)- and (S)-enantiomer pairs for both FAMP 1 and MeFAMP 2. The tumor uptake at 60 min post injection was 4.2 ± 0.3 — 9.0 ± 2.2 and 4.2 ± 0.7 — 8.3 ± 1.0 %ID/g for (R)-[^{18}F]1 and (S)-[^{18}F]1, respectively (Figure 3A and 3B). Tumor uptake of radioactivity for (R)-[^{18}F]1 and (S)-[^{18}F]1 varied from tumors and after injection time points under the same conditions. In general, the tumor uptake of (R)-[^{18}F]1 peaked quickly at 15 min post injection (4/5 cases) and slightly decreased over the time. The much higher uptake of (R)-[^{18}F]1 in SKOV3 tumor at 30 min was the exception, the reason of which is to be clarified. The highest tumor uptake of (S)-[^{18}F]1 was observed at 30 (3/5 cases) and 60 (2/5 cases) min post injection, which was slower and at the lower values than (R)-[^{18}F]1, and slightly decreased over the time.

The tumor uptake of MeFAMP 2 was varied at 15 and 30 min post injection and reached relative consistent values from 60 min post injection time point for both enantiomers, in the range of 1.5 ± 0.6 — 7.5 ± 1.8 %ID/g ($n=4-6$), with the exception of (S)-[^{18}F]2 in SKOV3 tumor, in which case the uptake was as high as 14.1 ± 3.1 %ID/g at 30 min post injection ($n=5$), see Figure 3C and 3D. Tumor uptake of radioactivity for (R)-[^{18}F]2 and (S)-[^{18}F]2 was similar for both tracers with slightly higher uptake of (R)-[^{18}F]2 than (S)-[^{18}F]2 in most cases (4/5 tumors at all time points), which may be due to greater system A transport selectivity for (R)-[^{18}F]2. Although the reasons of exceptionally high uptake of (S)-[^{18}F]2 in SKOV3 tumor at all time points need to be elucidated, the data could not be excluded from the studies since the standard deviations were within limits ($n=5$, $\text{SD}=0.9-3.3$).

Similar to the results of *in vitro* cell studies, the uptake of radioactivity by tumors *in vivo* was good to high and persistent at 2 hours after injection for all four tracers. In general, the patterns of the tumor uptake correlated to those of the tumor cell uptake, thus the uptake of (*R*)-enantiomer in tumors was slightly higher than that of (*S*)-enantiomer for both [¹⁸F]**1** and [¹⁸F]**2** which may be due in part to greater system A selectivity observed in the uptake assays. The one exception to this pattern occurred with (*S*)-[¹⁸F]**2** in SKOV3 tumor. However, the tumor uptake of (*R*)-[¹⁸F]**1** was typically slightly higher than that of (*S*)-[¹⁸F]**1**, (*R*)-[¹⁸F]**2** and (*S*)-[¹⁸F]**2** in tested tumors indicating that system A selectivity alone does not confer higher tumor uptake of this class of tracers as (*R*)-[¹⁸F]**2** would be predicted to have the higher absolute tumor uptake if system A selectivity alone determined absolute uptake. The comparison charts of tumor uptake time-activity curves for four tracers were shown in the “Supplementary Data” section. Tumor uptake of all four tracers was compared between groups of the same tumor at the same post injection time point using one-way ANOVA. There was no statistical difference of radioactivity uptake with U87 tumor at 15 min post injection time point between (*R*)-[¹⁸F]**1**, (*S*)-[¹⁸F]**1**, (*R*)-[¹⁸F]**2** and (*S*)-[¹⁸F]**2** ($P = 0.10$, $n = 4-6$). In all other cases, there was at least one statistically significant case for tumor uptake between these tracers ($P < 0.05$, $n = 4-6$). Tumor uptake of all four tracers was also compared each other using 2-tailed t-test. Refer to the “Supplementary Data” section for details.

3.2.2 Biodistribution of Tracers—The biodistribution of (*R*)-[¹⁸F]FAMP **1**, (*S*)-[¹⁸F]FAMP **1**, (*R*)-[¹⁸F]MeFAMP **2** and (*S*)-[¹⁸F]MeFAMP **2** in SCID mice xenografts were examined and compared. We observed that the normal tissue/organ distribution of the activity in this animal model was affected considerably by tracers but negligibly by tumor types. The uptake results with (*R*)-[¹⁸F]**1** and (*S*)-[¹⁸F]**1** in normal tissues of tumor-bearing mice are shown in Table 2, and the uptake results for (*R*)-[¹⁸F]**2** and (*S*)-[¹⁸F]**2** are shown in Table 3. For all four tracers, the pancreas typically had the highest uptake of activity, and the kidneys had the second highest uptake levels. These findings are similar to many other radiolabeled amino acids [37, 43-46]. With all tracers, the tissues studied including blood, heart, lung, bone and muscle showed relatively low uptake of radioactivity which decreased over the course of the two hour study (Tables 2 and 3). The activity in bone decreased from 15 min to 120 min in all cases demonstrated that all tracers in this study will expect to have significant stability to *in vivo* de-fluorination. Collectively, these data suggest that all four compounds have good tumor imaging properties based on high and persistent uptake in human cancer xenografts and relatively low uptake in most normal tissues other than the pancreas and kidneys.

Low brain uptake observed with all four amino acids, especially for the selective system A AAT substrates (*R*)- and (*S*)-[¹⁸F]**2**, is compatible with primarily system A transport *in vivo* which is absent at the luminal surface of the endothelium of the normal blood brain barrier (BBB). The *in vitro* inhibition of uptake of [¹⁸F]**1** by BCH indicates the existence of system L and non-system A transport components for these tracers. It is possible that these system L and non-system A AATs are responsible for the increased uptake of [¹⁸F]**1** into normal brain over the time *in vivo*. Because system L AAT occurs across the normal BBB, substrates for system L would be expected to show uptake in normal brain thus [¹⁸F]**1** demonstrated

higher brain uptake than [^{18}F]2. Although [^{18}F]1 showed an accumulation in brain tissue, the uptake values are still much lower compared to those of system L substrates in mice. For example, the values of brain uptake of activity at 60 min with A549 tumor mice were 1.1 ± 0.3 , 0.8 ± 0.1 , 0.4 ± 0.04 , and 0.2 ± 0.1 %ID/g for (R)-[^{18}F]1, (S)-[^{18}F]1, (R)-[^{18}F]2, and (S)-[^{18}F]2, respectively. In contrast, the brain uptake of [^{18}F]FET, a well-established system L AAT substrate, ranged from 2.4 ± 0.3 to 2.6 ± 0.4 %ID/g in nude mice under similar experimental conditions [46], representing 2.5–3.5 fold higher uptake than [^{18}F]1 and 7–14 fold higher uptake than [^{18}F]2. The low brain uptake of (R)- and (S)-[^{18}F]1 and (R)- and (S)-[^{18}F]2 is consistent with the earlier studies performed with [^{11}C]AIB [47] as well as with [^{18}F]1 and [^{18}F]2 in a rat orthotopic brain tumor model [35, 36]. Again in this rat model, the values of brain uptake of (R)-[^{18}F]1, (S)-[^{18}F]1, (R)-[^{18}F]2, and (S)-[^{18}F]2 at 60 min were 0.1 ± 0.02 , 0.06 ± 0.01 , 0.05 ± 0.02 , and 0.05 ± 0.01 %ID/g, respectively, and the brain uptake of system L substrate [^{18}F]FACBC was 0.26 ± 0.03 %ID/g [48], showing a good correlation with the present study.

3.2.3 Tumor to tissue ratios of tracer accumulation—Biodistribution studies with compounds (R)-[^{18}F]1, (S)-[^{18}F]1, (R)-[^{18}F]2 and (S)-[^{18}F]2 have shown high and persistent tumor uptake through two hour post injection with mice xenografts. To select the optimal tumor imaging agents that offer better tumor to background contrast, the ratios of tumor to the body structure tissues such as blood, muscle and bone, were compared with all four tracers. The ratios of tumor to normal lung tissue with A549 and MDA MB468 tumor xenografts were compared with all four tracers to assess their potential for detecting primary or metastatic lung and breast malignancies. In addition, the ratios of tumor to brain were also compared for potential brain tumor imaging agent selection. The ratios of tumor to background tissue for [^{18}F]FAMP 1 and [^{18}F]MeFAMP 2 are shown in Figures 4–6, respectively. The ratios of tumor to lung and to brain for [^{18}F]FAMP 1 and [^{18}F]MeFAMP 2 are shown in Figure 7 and Figure 8, respectively. The ratios of tumor to blood were increasing over the period of 15 min to 120 min for all four tracers in all tumor xenografts primarily due to the clearance of the activity from the blood, except for (R)-[^{18}F]1 in SKOV3 xenograft, in which case the ratios were peaked at 30 min and retained through 120 min (Figure 4). The ratios of tumor to muscle varied from tumors and time points for all tracers, and were above 2.5, 2, 4 and 2 for (R)-[^{18}F]1, (S)-[^{18}F]1, (R)-[^{18}F]2 and (S)-[^{18}F]2, respectively in most cases (Figure 5). The ratios of tumor to bone followed the similar patterns of the ratios of tumor to muscle for the corresponding tracers with the ratios above 2 in most cases (Figure 6). The ratios of tumor to lung were increasing over the time in most cases for all four amino acids (Figure 7) primarily due to the clearance of the activity from the lung. The values of the tumor to brain ratios were the highest for all four tracers (Figure 8) compared to the other tissues tested in the studies due to the low uptake of activity in the normal brain for system A AAT substrates. As a result, (R)-[^{18}F]2 and (S)-[^{18}F]2 yielded higher tumor to brain ratios than those of (R)-[^{18}F]1 and (S)-[^{18}F]1 due to (R)-[^{18}F]2 and (S)-[^{18}F]2 being more system A transport selective than (R)-[^{18}F]1 and (S)-[^{18}F]1. These data support the potential of using these tracers for imaging human tumors in a variety of locations. Although (R)-[^{18}F]1 typically showed the highest absolute tumor uptake in the studies, the highest tumor to tissue ratios, especially at later time points, were observed with (R)-[^{18}F]2, the most selective of the four compounds for system A transport.

Conclusion: The current study demonstrates that radiofluorinated amino acids (*R*)- and (*S*)-[¹⁸F]**1** and (*R*)- and (*S*)-[¹⁸F]**2** have high uptake in tumor cells *in vitro* and *in vivo*. The inhibition experiments showed that (*R*)- and (*S*)-[¹⁸F]**1** entered these tumor cells *in vitro* via mixed system A AAT (inhibited by MeAIB) and non-system A AAT (inhibited by BCH), likely including system L transport. Future studies are planned to better define the non-system A transport systems mediating the uptake of the enantiomers of [¹⁸F]**1**, particularly (*S*)-[¹⁸F]**1**. Both (*S*)- and (*R*)-[¹⁸F]**2** enter tumor cells *in vitro* primarily via system A transport with low levels of non-system A amino acid transport for (*S*)-[¹⁸F]**2**. Of these tracers, (*R*)-[¹⁸F]**2** was the most selective for system A transport. Biodistribution studies with all four amino acids demonstrated rapid and persistent retention of radioactivity in tumors xenograft models of gliomas, breast cancer, non-small cell lung cancer, prostate cancer, and ovarian cancer with good tumor to normal tissue ratios. The relatively low uptake of radioactivity of these compounds in most normal tissues with the exception of the kidney and pancreas, indicate that these tracers, particularly the most selective system A substrate (*R*)-[¹⁸F]**2**, are promising PET tracers for a range of human malignancies. Future preclinical imaging studies in animal models and clinical studies in human cancer patients will provide more detailed information regarding the biodistribution and kinetics of these tracers in normal and neoplastic tissues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Amino acid tracers [^{18}F]FAMP **1** and [^{18}F]MeFAMP **2** for system A amino acid transport.

Figure 2.

Percent uptake and inhibition of (R)-[¹⁸F] 1, (S)-[¹⁸F] 1, (R)-[¹⁸F] 2 and (S)-[¹⁸F] 2 in tumor cells relative to control condition.

Error bars indicate ± standard deviation (n=3).

The data were acquired through cell studies shown in Table 1.

P values represent comparisons of uptake with inhibitors to control uptake for each radiotracer using 2-tailed t-tests. * *P* < 0.05, † *P* = 0.05.

Figure 3.

Tumor uptake time-activity curves of **A:** (R)-[^{18}F]1, **B:** (S)-[^{18}F]1, **C:** (R)-[^{18}F]2 and **D:** (S)-[^{18}F]2 in SCID mice xenografts. Error bars indicate $\pm$ standard deviation (n=4-6). The data were acquired through biodistribution studies shown in Tables 2 and 3.

Figure 4. Tumor to blood ratios of tracers in tumor-bearing mice

Tumor to blood ratios were obtained with the PET tracers (R)-[¹⁸F]1, (S)-[¹⁸F]1, (R)-[¹⁸F]2, and (S)-[¹⁸F]2 in xenograft tumor-bearing SCID mice at 15, 30, 60, and 120 min after tracer injection (n=4-6 each time point). The data were acquired through biodistribution studies shown in Tables 2 and 3. Error bars indicate $\pm$ standard deviation (SD).

Figure 5. Tumor to muscle ratios of tracers in tumor-bearing mice

Tumor to muscle ratios were obtained with the PET tracers (R)-[¹⁸F]1, (S)-[¹⁸F]1, (R)-[¹⁸F]2, and (S)-[¹⁸F]2 in xenograft tumor-bearing SCID mice at 15, 30, 60, and 120 min after tracer injection (n=4-6 each time point). The data were acquired through biodistribution studies shown in Tables 2 and 3. Error bars indicate $\pm$ standard deviation (SD).

Figure 6. Tumor to bone ratios of tracers in tumor-bearing mice

Tumor to bone ratios were obtained with the PET tracers (R)-[¹⁸F]1, (S)-[¹⁸F]1, (R)-[¹⁸F]2, and (S)-[¹⁸F]2 in xenograft tumor-bearing SCID mice at 15, 30, 60, and 120 min after tracer injection (n=4-6 each time point). The data were acquired through biodistribution studies shown in Tables 2 and 3. Error bars indicate $\pm$ standard deviation (SD).

Figure 7. Tumor to lung ratios of tracers in tumor-bearing mice

Tumor to lung tissue ratios obtained with the PET tracers (R)-[¹⁸F]1, (S)-[¹⁸F]1, (R)-[¹⁸F]2 and (S)-[¹⁸F]2 in A549 and MDA MB468 xenograft tumor-bearing SCID mice at 15, 30, 60, and 120 min after tracer injection (n=4-6 each time point). The data were acquired through biodistribution studies shown in Tables 2 and 3. Error bars indicate $\pm$ standard deviation (SD).

Figure 8. Tumor to brain ratios of tracers in tumor-bearing mice

Tumor to brain ratios were obtained with the PET tracers (R)-[¹⁸F]1, (S)-[¹⁸F]1, (R)-[¹⁸F]2, and (S)-[¹⁸F]2 in xenograft tumor-bearing SCID mice at 15, 30, 60, and 120 min after tracer injection (n=4-6 each time point). The data were acquired through biodistribution studies shown in Tables 2 and 3. Error bars indicate $\pm$ standard deviation (SD).

Scheme 1.

Radiosynthesis of (R) - $[^{18}\text{F}]\mathbf{1}$, (S) - $[^{18}\text{F}]\mathbf{1}$, (R) - $[^{18}\text{F}]\mathbf{2}$ and (S) - $[^{18}\text{F}]\mathbf{2}$.

Table 1

Cell uptake of (R)-[¹⁸F]1, (S)-[¹⁸F]1, (R)-[¹⁸F]2 and (S)-[¹⁸F]2 at 30 minutes incubation in the presence or absence of the AAT inhibitors.

Tracer		Tumor cell line				
		A549	DU145	U87	MDA MB468	SKOV3
(R)-[¹⁸ F] 1	Control	5.1±0.3	5.1±0.1	6.0±0.3	5.7±0.3	9.2±0.2
	BCH	2.3±0.3 [*]	3.3±0.2 [*]	4.8±0.3 [*]	3.0±0.4 [*]	7.6±0.2 [*]
	MeAIB	4.6±0.5 [†]	3.6±0.2 [*]	2.0±0.3 [*]	4.4±0.2 [*]	4.6±0.1 [*]
(S)-[¹⁸ F] 1	Control	4.8±0.7	3.6±0.3	1.5±0.1	3.4±0.3	3.9±0.2
	BCH	1.5±0.1 [*]	1.6±0.1 [*]	0.8±0.1 [*]	2.2±0.1 [*]	1.7±0.1 [*]
	MeAIB	3.9±0.6 [†]	3.2±0.4 [†]	1.0±0.04 [*]	3.5±0.2 [†]	2.9±0.2 [*]
(R)-[¹⁸ F] 2	Control	2.4±0.2	1.5±0.2	2.7±0.3	1.4±0.1	3.9±0.6
	BCH	3.1±0.1 [†]	1.9±0.1 [*]	3.5±0.2 [†]	1.5±0.2 [†]	4.5±0.7 [†]
	MeAIB	0.4±0.04 [*]	0.4±0.1 [*]	0.6±0.1 [*]	0.5±0.2 [*]	0.5±0.2 [*]
(S)-[¹⁸ F] 2	Control	1.2±0.1	1.7±0.2	2.4±0.2	1.0±0.1	6.9±0.6
	BCH	1.3±0.1 [†]	1.2±0.04 [†]	2.5±0.3 [†]	1.1±0.02 [*]	6.5±0.5 [†]
	MeAIB	0.4±0.1 [*]	0.2±0.01 [*]	0.5±0.1 [*]	0.5±0.1 [*]	0.6±0.1 [*]

Values are reported as percent uptake of the initial dose per 0.5 million cells (% ID/5×10⁵ cells) ± standard deviation (SD) (n=3).

P values represent comparisons of uptake with inhibitors to control uptake for each radiotracer using 2-tailed t-tests.

^{*} P < 0.05,

[†] P = 0.05

Table 2Biodistribution of [^{18}F]FAMP 1 in tumor-bearing SCID mice.

Tumor Xenograft	Tissue (%ID/g)	(R)-[^{18}F] FAMP 1				(S)-[^{18}F]FAMP 1			
		15 min	30 min	60 min	120 min	15 min	30 min	60 min	120 min
<i>A549</i>	blood	2.9±0.4 *	2.0±0.2 *	1.3±0.1 *	1.3±0.9 *	3.9±0.6	3.1±0.6	2.3±0.1	2.0±0.4
	heart	4.4±0.10 *	3.5±1.0 *	3.5±0.9 *	3.9±1.5	3.2±0.7	3.3±0.4	3.0±0.4	2.7±0.8
	lung	5.0±0.9	3.1±1.0	2.3±0.5	1.9±0.6 *	5.5±2.0	4.3±1.7	2.8±1.3	3.1±0.9
	liver	4.8±0.5 *	5.7±1.2 *	5.3±1.1 *	7.1±2.4 *	3.7±0.5	3.8±0.6	3.5±0.4	2.8±0.4
	pancreas	54±14	43±11 *	28±6.3	20±7.1 *	42±8.4	39±8.2	33±6.1	29±2.3
	spleen	9.5±3.9	7.8±1.6	5.1±1.8 *	4.9±3.1 *	9.0±3.0	8.4±1.7	6.5±1.1	6.0±1.7
	kidney	18±3.5 *	10±1.9 *	6.6±1.0 *	5.5±3.1 *	29±5.7	21±6.9	17±1.4	14±1.8
	muscle	2.0±0.4 *	2.0±0.6	1.8±0.2 *	2.3±1.2 *	3.3±0.7	3.0±0.8	2.9±0.6	2.3±0.4
	brain	0.8±0.2 *	0.9±0.2 *	1.1±0.3 *	1.8±0.9 *	0.5±0.1	0.6±0.1	0.8±0.1	0.8±0.1
	bone	2.6±0.6	2.3±0.6	2.0±0.6	2.2±1.1 *	2.6±0.8	2.5±0.6	2.7±0.6	2.0±0.5
	Tumor	9.9±1.7 *	7.5±1.9	7.7±1.9	8.8±4.8 *	6.7±1.7	7.9±1.0	8.3±1.0	6.5±0.8
<i>DUI45</i>	blood	5.2±1.2	3.7±0.3	2.2±0.3	1.6±0.3	5.1±1.1	2.9±1.1	2.4±0.04	1.8±0.4
	heart	5.8±1.5 *	5.6±0.6 *	4.8±1.0 *	4.9±0.9 *	3.2±1.2	3.2±1.3	3.5±0.3	3.0±0.7
	lung	7.6±1.8	3.1±0.4	2.0±0.4 *	2.1±0.8	6.5±1.5	5.0±2.6	3.4±0.8	3.3±1.1
	liver	7.2±3.4	6.3±0.8 *	5.5±1.0 *	3.6±0.6	3.4±0.6	2.9±0.9	3.3±0.5	2.8±0.7
	pancreas	62±20	52±3.8	39±6.9	27±4.5	55±8.8	37±13	36±3.1	28±7.4
	spleen	17±7.6	13±1.4 *	11±2.9	6.0±0.9	7.9±2.3	6.6±1.1	9.2±5.1	5.3±1.8
	kidney	26±7.5 *	16±2.2	10±0.8 *	7.9±0.7 *	36±5.1	21±6.2	17±0.6	13±1.7
	muscle	2.9±0.7	3.5±0.5	3.0±0.7	2.7±0.8	4.0±0.8	2.9±0.2	2.5±0.3	1.7±0.5
	brain	1.0±0.3 *	1.3±0.1 *	1.5±0.3 *	1.6±0.2 *	0.6±0.1	0.5±0.2	0.6±0.1	0.6±0.1
	bone	3.9±1.0	4.0±0.3 *	3.2±0.7 *	2.6±0.5 *	2.4±1.1	2.2±0.5	2.1±0.2	1.6±0.4
	tumor	8.8±1.9 *	8.4±1.1 *	7.9±1.7 *	6.8±0.8 *	3.2±2.5	1.8±0.4	3.4±1.7	3.5±0.7
<i>U87</i>	blood	3.2±0.2	2.6±0.7 *	1.6±0.4	0.8±0.1	3.5±0.4	3.7±0.7	2.1±0.4	1.2±0.5
	heart	4.7±1.2 *	4.3±0.6 *	4.2±0.9	3.6±0.4 *	3.1±0.4	3.1±0.4	2.8±0.7	2.1±0.3
	lung	4.7±0.6	3.4±0.5	2.4±0.6	1.3±0.2	4.6±0.6	3.6±0.5	2.6±0.8	1.5±0.4
	liver	7.6±2.9	7.7±1.5	6.9±1.4 *	8.0±0.7 *	6.6±2.0	6.0±1.0	4.4±0.6	4.1±1.3
	pancreas	62±6.3	43±8.2	28±5.0	15.1±3.2	48±11	50±8.7	32±11	18±6.4
	spleen	19±6.4 *	13±2.4 *	7.0±1.4	3.5±0.4	11±2.7	10±1.0	6.1±1.1	3.4±1.1
	kidney	23±2.5 *	15±2.1 *	8.0±1.8 *	4.2±0.4 *	37±4.9	28±5.2	15±3.3	7.3±1.4
	muscle	2.2±0.5 *	2.2±0.5 *	2.9±0.6 *	1.6±0.2	3.3±0.7	3.3±0.3	2.3±0.3	1.3±0.3
	brain	0.8±0.2	0.9±0.2 *	1.2±0.2 *	1.2±0.1 *	0.5±0.1	0.6±0.1	0.6±0.1	0.7±0.2
	bone	3.2±1.5	3.5±0.5 *	3.2±0.8 *	1.5±0.2 *	2.4±0.7	2.1±0.5	2.0±0.1	1.0±0.3

Tumor Xenograft	Tissue (%ID/g)	(R)-[¹⁸ F] FAMP 1				(S)-[¹⁸ F]FAMP 1			
		15 min	30 min	60 min	120 min	15 min	30 min	60 min	120 min
	tumor	6.5±1.2	6.0±0.9	4.2±0.3	3.1±0.8	6.4±1.3	6.8±0.8	4.4±0.8	2.8±0.4
<i>MDA MB468</i>	blood	3.0±0.2	2.0±0.4 *	1.1±0.3 *	0.5±0.2 *	4.2±0.3	3.6±0.4	2.9±1.0	1.5±0.1
	heart	3.9±0.6	3.5±1.0 *	3.3±0.8	2.3±0.4	3.4±0.3	3.2±0.3	2.9±0.5	2.0±0.2
	lung	5.4±0.6	3.3±1.1	2.2±0.5	1.1±0.4	4.5±1.3	4.3±1.9	2.6±0.6	1.6±0.4
	liver	4.4±0.9	4.5±1.0	3.8±0.9	4.1±0.8 *	4.8±0.9	4.7±0.8	4.3±0.6	2.6±0.2
	pancreas	61±8.4	39±11	20±7.2 *	11±4.6 *	51±10	56±7.6	46±9.5	23±6.8
	spleen	8.7±2.2 *	6.5±1.1 *	4.1±1.3 *	2.2±1.1 *	14±2.8	12±4.2	7.1±0.9	5.6±2.2
	kidney	17±2.3 *	9.1±2.8 *	5.0±1.1 *	2.4±1.1 *	39±3.3	29±6.1	20±2.7	11±1.4
	muscle	2.2±0.8	2.0±0.6 *	2.0±0.6	1.6±0.6	3.9±0.7	3.4±0.4	2.7±0.6	1.3±0.5
	brain	0.7±0.2	0.8±0.1	0.8±0.1 *	0.9±0.1	0.6±0.1	0.8±0.2	0.7±0.1	0.8±0.1
	bone	2.7±0.6	2.2±0.6	1.7±0.5	1.1±0.5	2.5±0.3	2.4±0.6	2.2±0.6	1.0±0.6
	tumor	6.0±1.8	5.3±1.7	4.8±1.2	3.6±0.7	5.8±0.7	6.1±1.4	4.8±1.2	5.0±0.4
<i>SKOV3</i>	blood	2.9±0.7	2.7±0.3	2.1±0.1 *	1.5±0.2 *	3.5±0.8	3.1±0.7	2.1±0.6	2.9±5.3
	heart	4.0±2.1	4.9±0.1 *	4.9±1.1 *	3.6±0.6 *	2.8±0.8	2.8±0.5	2.6±0.7	2.6±1.4
	lung	3.9±1.3	3.1±0.3 *	2.5±0.3	1.8±0.3	4.8±1.3	4.4±0.7	3.2±1.1	2.3±0.8
	liver	4.6±2.5	5.0±0.7	4.5±0.3 *	2.7±0.5	3.6±1.0	3.9±0.6	3.6±1.1	3.2±0.7
	pancreas	38±18	41±7.4	34±4.7	19±2.4	44±15	40±13	36±8.6	22±3.6
	spleen	8.7±3.4	13±3.6 *	6.8±1.0 *	4.5±1.1	9.8±4.3	8.4±2.1	5.5±1.2	4.6±2.3
	kidney	17±4.5 *	14±2.6	10±0.8	7.0±0.8	31±6.8	22±4.9	15±4.8	11±5.0
	muscle	2.3±1.1	2.8±0.3	2.6±0.6 *	2.0±0.4 *	2.8±0.9	2.8±0.5	2.2±0.7	1.7±0.9
	brain	0.7±0.4	1.0±0.04 *	1.0±0.3 *	1.2±0.2 *	0.4±0.1	0.5±0.1	0.6±0.2	0.7±0.3
	bone	2.6±1.0	2.8±0.7	2.3±0.3 *	1.9±0.4 *	2.2±0.7	2.2±0.4	1.7±0.6	1.6±1.1
	tumor	10±4.8	14±1.1 *	9.0±2.2 *	6.2±0.5 *	6.2±4.1	7.3±3.0	6.2±3.1	5.1±1.6

Values are reported as mean percent uptake of the injected dose per gram of tissue (% ID/g) ± standard deviation (SD) (n=4-6).

P values represent comparisons of uptake with (R)-[¹⁸F]FAMP 1 vs. (S)-[¹⁸F]FAMP 1 under the same condition using 2-tailed t-tests.

* *P* < 0.05; all other, *P* ≥ 0.05.

Table 3Biodistribution of [^{18}F]MeFAMP 2 in tumor-bearing SCID mice.

Tumor Xenograft	Tissue (%ID/g)	(R)-[^{18}F]MeFAMP 2				(S)-[^{18}F]MeFAMP 2			
		15 min	30 min	60 min	120 min	15 min	30 min	60 min	120 min
<i>A549</i>	blood	3.2±1.2	1.9±0.3	1.1±0.1*	0.7±0.2	2.6±0.3	1.8±0.1	0.8±0.2	0.5±0.1
	heart	3.2±0.8	3.6±0.4*	3.5±1.0	3.7±0.6*	3.7±0.7	2.7±0.6	2.8±0.6	2.8±0.5
	lung	3.7±0.8	2.9±0.9	2.1±0.4*	1.7±0.2*	3.3±0.3	2.8±0.5	1.3±0.5	1.1±0.1
	liver	6.9±2.0	7.5±1.5*	5.2±0.3*	4.9±0.7*	5.6±0.9	4.6±0.3	2.8±0.8	2.3±0.9
	pancreas	56±16	50±4.9	31±7.6*	20±2.7*	57±10	38±10	18±2.7	11.2±3.2
	spleen	12±4.4	9.8±1.3	12±1.9*	6.2±0.4*	10±1.9	10±3.1	5.6±1.5	4.3±1.0
	kidney	31±3.8*	15±3.9	7.3±1.3*	4.1±0.9*	18±2.9	11±1.1	4.1±1.3	2.6±0.3
	muscle	1.4±0.6	1.2±0.3	1.0±0.1	1.6±0.7	2.1±0.9	1.8±0.7	1.5±0.7	1.4±0.5
	brain	0.4±0.1	0.4±0.1*	0.4±0.04*	0.4±0.1*	0.4±0.04	0.3±0.03	0.2±0.1	0.2±0.02
	bone	2.3±1.0	2.3±0.6	1.6±0.4	1.9±0.6	2.6±0.7	2.1±0.6	1.4±0.7	1.2±0.3
	Tumor	5.3±1.9	6.3±1.3*	5.1±1.3	5.6±1.3*	4.4±0.5	4.5±0.3	3.8±0.9	3.6±0.6
<i>DU145</i>	blood	2.2±0.4*	1.6±0.4	0.9±0.1*	0.6±0.1	3.1±0.3	2.0±0.2	1.1±0.1	0.7±0.2
	heart	2.7±0.9	2.9±1.0	2.6±0.4	3.5±0.9*	2.6±0.4	2.9±0.4	2.2±0.4	2.3±0.2
	lung	2.7±0.7*	2.3±0.7	1.4±0.4	1.5±0.4	3.6±0.3	3.0±0.5	1.7±0.3	1.3±0.1
	liver	7.8±1.2*	8.5±2.1*	4.9±0.6*	4.3±1.5	6.2±0.6	5.5±0.8	4.0±0.4	3.5±0.8
	pancreas	51±6.6	53±17	28±7.4	22±4.2	46±4.9	45±7.8	22±6.3	18±5.1
	spleen	13±1.0	15±5.4*	9.3±1.9*	7.4±1.6	11±3.3	7.9±0.9	6.5±0.9	5.7±1.8
	kidney	25±6.2	12±3.3	6.0±0.6	3.6±0.5	28±3.5	13±2.0	6.4±0.8	4.2±1.2
	muscle	0.8±0.2*	0.9±0.2*	0.7±0.1*	0.9±0.2	1.6±0.1	1.7±0.3	1.2±0.1	1.0±0.1
	brain	0.4±0.2	0.4±0.1	0.3±0.1	0.3±0.1*	0.3±0.03	0.3±0.03	0.2±0.03	0.2±0.04
	bone	1.7±0.7	2.0±0.6	1.5±0.4	1.8±0.5	2.3±0.2	2.2±0.2	1.9±0.2	1.6±0.3
	Tumor	5.2±1.2	5.1±0.7	4.5±0.6	4.0±1.0	3.9±0.8	4.4±0.8	4.1±1.1	4.3±0.6
<i>U87</i>	blood	2.4±0.4	1.7±0.3	1.1±0.1	0.5±0.1	2.6±0.3	1.8±0.2	1.0±0.4	0.6±0.3
	heart	4.4±1.1	4.5±1.7	4.5±1.0	3.9±0.6	3.5±0.4	3.4±1.3	3.0±1.4	3.3±1.0
	lung	4.1±0.7	3.1±0.2	2.5±0.4	1.9±0.3	3.7±0.4	2.8±0.9	1.9±0.8	1.5±0.2
	liver	9.3±3.5	8.9±1.6	6.8±1.3*	5.0±0.8	8.4±1.2	8.1±1.6	4.2±1.8	3.9±1.5
	pancreas	74±11*	54±5.7	34±7.3	16±2.1	57±10	54±17	32±12	18±4.1
	spleen	16±6.7	14±2.5	12±1.2	6.1±0.5	12±1.9	12±3.8	8.3±3.7	6.0±2.4
	kidney	27±4.8*	11±2.6	6.8±1.3*	3.3±0.4	18±2.7	9.2±2.1	4.2±1.7	2.8±1.0
	muscle	1.2±0.3	1.8±0.3*	1.4±0.2	1.4±0.5	1.5±0.2	1.4±0.3	1.1±0.4	1.1±0.1
	brain	0.6±0.2	0.5±0.1*	0.5±0.1*	0.5±0.1*	0.5±0.1	0.4±0.03	0.3±0.1	0.3±0.1
	bone	4.2±1.0	4.3±1.2	4.0±1.0*	2.3±0.5	3.3±0.8	3.5±0.6	1.9±1.0	2.0±0.4
	Tumor	5.6±0.7	5.9±1.1	5.8±0.8*	5.1±1.1*	4.9±1.0	4.8±0.9	2.8±1.0	2.9±0.7

Tumor Xenograft	Tissue (%ID/g)	(R)-[¹⁸ F]MeFAMP 2				(S)-[¹⁸ F]MeFAMP 2			
		15 min	30 min	60 min	120 min	15 min	30 min	60 min	120 min
MDA MB468	blood	2.3±0.3	1.5±0.1*	1.0±0.2	0.5±0.1	2.7±0.3	2.0±0.3	1.0±0.3	0.5±0.1
	heart	3.1±0.6	3.6±0.7	3.9±0.7*	3.9±0.5*	3.3±0.4	2.7±0.9	2.7±0.8	2.8±0.9
	lung	3.2±0.4	2.0±0.5	2.0±0.3*	1.1±0.3	3.7±1.1	2.3±0.7	1.5±0.3	1.0±0.3
	liver	6.9±1.7	6.5±0.8*	4.5±0.4*	5.0±0.9*	5.1±0.7	4.2±1.5	3.3±0.4	1.8±0.3
	pancreas	59±3.3*	38±4.3	27±4.5	19±2.5*	50±4.4	42±12	25±4.3	12±1.5
	spleen	12±1.7*	10±1.3	9.3±1.3*	6.6±0.8*	9.0±2.1	8.6±1.9	7.3±1.1	4.7±1.4
	kidney	24±1.7	11±1.5	5.9±0.8*	3.6±0.4*	21±2.2	14±2.6	4.8±0.8	2.7±0.5
	muscle	1.1±0.1*	1.2±0.3*	1.3±0.2	0.9±0.1	1.6±0.2	2.3±0.9	1.3±0.4	1.3±0.5
	brain	0.4±0.03	0.3±0.04	0.3±0.01*	0.3±0.1*	0.4±0.1	0.3±0.1	0.2±0.04	0.2±0.03
	bone	2.2±0.2	1.9±0.7	1.5±0.4	1.9±0.4	2.1±0.1	2.4±0.9	1.3±0.4	1.3±0.5
	Tumor	4.3±0.5	3.9±0.5*	3.9±0.6*	4.1±0.7*	3.4±0.6	2.3±0.5	2.0±0.2	1.5±0.6
SKOV3	blood	1.4±0.3*	1.1±0.3*	0.7±0.1*	0.4±0.1*	3.1±0.8	2.3±0.8	1.2±0.2	0.8±0.1
	heart	2.3±0.6	2.4±0.6*	2.6±0.1	2.3±0.6	2.7±0.4	3.2±0.3	2.7±0.3	2.4±0.7
	lung	2.2±0.6	1.9±0.3*	1.3±0.2	0.9±0.1*	3.2±1.0	2.9±0.6	1.6±0.3	1.2±0.2
	liver	4.7±0.9	4.0±1.2	2.8±0.4	2.5±0.7	4.2±0.7	5.1±0.9	3.0±0.3	2.2±0.3
	pancreas	32±9.8	30±11	21±2.3*	14±3.6*	42±2.8	38±3.8	27±2.1	20±1.6
	spleen	14±0.7*	13±2.5*	11±3.2	6.8±0.6	8.5±3.0	9.3±1.2	8.9±2.1	6.2±0.9
	kidney	18±3.4*	11±3.6	5.1±0.8*	2.8±0.5*	25±3.9	12±3.2	6.9±0.8	4.3±0.5
	muscle	0.9±0.2*	0.8±0.2*	0.7±0.1*	0.7±0.2*	1.3±0.2	1.5±0.3	1.3±0.2	1.2±0.2
	brain	0.3±0.1	0.3±0.1	0.2±0.03	0.2±0.1	0.3±0.1	0.3±0.1	0.3±0.04	0.2±0.04
	bone	3.2±0.7*	2.8±0.8	2.3±0.4	2.0±0.4	2.3±0.5	2.5±0.4	2.3±0.2	2.0±0.5
	Tumor	6.4±1.7	6.4±3.3*	7.5±1.8*	6.9±3.3*	8.5±3.3	14±3.1	13±2.3	13±0.9

Values are reported as mean percent uptake of the injected dose per gram of tissue (% ID/g) ± standard deviation (SD) (n=4-6).

P values represent comparisons of uptake with (R)-[¹⁸F]MeFAMP 2 vs. (S)-[¹⁸F]MeFAMP 2 under the same condition using 2-tailed t-tests.

* $P < 0.05$; all other, $P \geq 0.05$.