SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

Radiolabeling

Concentration and purification of $^{18}F^-$

$^{18}F^-$ solution in enriched water ($H_2{}^{18}O$, BV Cyclotron, VU, Amsterdam, The Netherlands) was purified from metal impurities and concentrated before use. Briefly, a CM cartridge (weak cation-exchange cartridge, Waters, Sep-Pak Accell Plus CM, 130 mg) and a QMA cartridge (strong anion-exchange cartridge, Waters, Sep-Pak Waters Accell Plus QMA Plus Light, 130 mg) were pre-washed with 10 mL metal-free ultrapure water (Milli-Q, Millipore). The $^{18}F^-$ solution (8-15 GBq) was pushed slowly through the CM cartridge connected to the QMA cartridge, followed by 6 mL wash with metal-free ultrapure water. Finally, the CM cartridge containing cationic metal impurities was disposed of and $^{18}F^-$ was eluted from the QMA cartridge with a small volume (150-200 µL) of saline.

Cold labeling of JMV4168 and JMV5132 with ^{nat}Ga

2 µL of a $Ga(NO_3)_3$ solution (0.2 M) was added to 10 µL of 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (1 M, pH 3.6), followed by addition of quenchers (methionine, gentisic acid and ascorbic acid, 5 mM) and peptide (100 nmol). Reaction mixture was heated for 10 min at 95°C. Reverse-phase high performance liquid chromatography (RP-HPLC) analysis was performed as described in the section “Quality Control”, showing complete incorporation of ^{nat}Ga; no further purification was needed.

Cold labeling of JMV5132 with ^{nat}F

10 μL of a $AlCl_3$ solution (0.1 M) was mixed with 5 μL NaF (1 M) and 0.5 μL sodium acetate (NaAc, 2.5 M), followed by addition of 25 μL ethanol and 10 μL JMV5132 (5 mM). Reaction mixture was heated for 15 min at 106°C. $Al^{nat}F$-JMV5132 was purified by RP-HPLC.

Quality Control

Peptide synthesis

Purification of JMV5132 was accomplished by a preparative HPLC (Waters Delta Preparative, Waters 4000 system controller) with a C18 column (40 mm × 100 mm, Waters Delta Pack, column II). The final product was characterized by RP-HPLC (Beckman, LC-126) on a reverse phase-18 Chromatolith Speed ROD column (50 mm × 4.6 mm, Merck, column I) and ESI/MS (Waters micromass ZQ, Waters 2695 Separation Module).

Radiolabeling

Labeling efficiency and colloid formation were assessed by instant thin layer chromatography using silica gel coated paper (Agilent Technologies, Malvern, PA) and 0.1 M ammonium acetate (NH_4OAc) pH 5.5: 0.1 M ethylenediaminetetraacetic acid (1:1), or 1 M NH_4OAc:methanol (1:3), respectively. Radiochemical purity of labeled peptides was analyzed by RP-HPLC on an Agilent 1200 system (Agilent Technologies). A C-18 column (Onyx monolithic, 4.6 mm x 100 mm; Phenomenex) was used at a flow rate of 1 mL/min with the following buffer system: buffer A, 0.1% v/v trifluoroacetic acid in water; buffer B, 0.1% trifluoroacetic acid in acetonitrile; with a gradient as follows: 97% buffer A (0-5 min), 97 to 76% buffer A (5-8 min), 76 to 75% buffer A (8-13 min), 75% buffer A (13-25 min). The radioactivity of the eluate was monitored using an in-line NaI radiodetector (Raytest GmbH, Straubenhardt, Germany). Elution profiles were analyzed using Gina-star software (version 2.18; Raytest GmbH).

Cell culture and Competitive Cell Binding Assay

Tissue sections were pre-incubated for 5 min in ice-cold binding buffer (5 mM $MgCl_2$, 167 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 7.6), followed by incubation for 1 h in binding buffer containing 1% w/v BSA, [125I-Tyr4]BBN (5.10-10 M) and JMV4168, JMV5132, ^{nat}Ga-JMV4168 or ^{nat}Ga-JMV5132 in increasing concentrations (range of 10-12 M to 10-6 M). After incubation, the sections were washed successively with ice-cold binding buffer with 0.25 % w/v bovine serum albumin (BSA) for 5 min, binding buffer without BSA for 5 min, and ice-cold Ultrapure water for 5 seconds. Dried sections were placed in apposition to phosphor screens (PerkinElmer, Super Resolution) for 1 day. Radioactivity was assessed using a phosphor imager system (Cyclone, Packard, model A431201) and quantified using Optiquant software (PerkinElmer, Waltham, MA).

Small-animal PET/CT and Biodistribution Studies

Mice were scanned in prone position on a small animal PET/CT scanner (Inveon; Siemens Preclinical Solutions, Knoxville, TN). PET emission scans were acquired for 30-60 min, followed by a CT scan (spatial resolution 113.15 µm; 80 kV; and 500 µA). Scans were reconstructed using Inveon Acquisition Workplace software (version 1.5; Siemens Preclinical Solutions), using a 3-dimensional ordered-subset expectation maximization/maximization a posteriori algorithm with the following parameters: matrix, 256 x 256 x 161; pixel size, 0.40 x 0.40 x 0.796 mm; and β-value, 1.5, with uniform variance and FastMAP. After scanning, blood, tumor, and relevant organs and tissues were collected, weighed and counted in a γ-counter (1480 WIZARD automatic gamma counter, PerkinElmer, Waltham, MA) with a counting time of 60 seconds per sample, isotope-specific energy window and an error not exceeding 5 %.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5.01 (San Diego, CA, USA). IC_{50} values were determined with datasets from 3 independent experiments, each in duplicate. Data are represented as percentage of total binding (normalized), with SEM and 95% confidence band. An extra sum-of-squares F test was used to compare two best-fit values, and the level of significance was set at $P < 0.05$. Biodistribution data are represented as the mean %ID/g ± SD, with group sizes of 3 mice, except at 1 h p.i.: n = 2 for Al^{18}F-JMV5132 and at 2 h p.i.: n = 5 for ^{68}Ga-JMV4168, Al^{18}F-JMV5132. Statistical analysis of biodistribution data was performed using a 1-way ANOVA with a Bonferroni post-hoc test, and the level of significance was set at $P < 0.05$.