Supplementary information

Supplementary Methods

General reagents and consumables

Solvents and reagents were purchased from Sigma Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) unless stated otherwise. 9-Fluorenylmethoxycarbonyl (Fmoc)-protected L-amino acids ("Fmoc-amino acids"), NovaSyn TGR resin, and O-(7-azabenzotriazol-1-yl)-*N,N,N',N*'-tetramethyluronium hexafluorophosphate (HATU) were purchased from MilliporeSigma (Burlington, MA); amino-acid side-chain protections were 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for arginine, *tert*-butyl ester (O*t*Bu) for aspartic acid, tert-butyl (*t*Bu) for threonine, trityl (Trt) for asparagine and glutamine. Monodisperse Fmoc-amino-PEG-propionic acid ("Fmoc-PEG-COOH"; FW = 1544.8 g/mol) was purchased from Polypure (Oslo, Norway); 4-fluorobenzoic acid was purchased from Acros/VWR (Radnor, PA). Sep-Pak C18 Plus cartridges and Sep-Pak Dry Sodium Sulfate Plus were purchased from Waters (Milford, MA); CHROMAFIX 30-PS-HCO3 cartridges were purchased from ABX (Radeberg, Germany). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), Penicillin-Streptomycin-Glutamine (PSG), and phosphate buffered saline (PBS) were purchased from Gibco/Thermo Fisher (Waltham, MA).

Reagents for clinical production of [^{18}F]$\alpha_v\beta_6$-BP

Suppliers were as described above, unless noted otherwise. Sodium chloride (0.9%, USP, preservative free, for injection) was purchased from Baxter (Deerfield, IL) and Hospira (Lake Forest, IL) and ethanol (absolute, USP) was purchased from Koptec/Decon Labs (King of Prussia, PA). PETNET Solutions (Sacramento, CA) supplied the [^{18}F]fluoride ion in ^{18}O-water. HPLC grade water and HPLC grade acetonitrile were purchased from EMD/MilliporeSigma; acetonitrile (99.9%, extra dry, over molecular sieves), dimethyl sulfoxide (DMSO, 99.7%, extra dry, anhydrous); diisopropylethylamine (DIPEA);

tetrapropylammonium hydroxide (25% in water), and *O-(N*-succinimidyl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TSTU, 97%) were purchased from Acros/VWR. 4-(Ethoxycarbonyl)-*N,N,N*-trimethylbenzenaminium triflate ([^{18}F]fluorobenzoic acid precursor) and 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix) were purchased from ABX. Phosphoric acid (85%, ACS grade) was purchased from Amresco (Solon, OH) and acetic acid (glacial, USP) was purchased from J.T.Baker/Avantor (Center Valley, PA).

Mass spectrometry

Mass spectrometry analysis was performed at the UC Davis Mass Spectrometry Facility using a matrix assisted laser desorption ionization time of flight (MALDI TOF) spectrometer (UltraFlextreme; Bruker, Billerica, MA; 4700 Mass Spectrometer; Applied Biosystems/Thermo Fisher) in positive ionization mode using a α-cyano-4-hydroxycinnamic acid or sinapinic acid matrix.

Chromatography

Reversed-phase high pressure liquid chromatography (HPLC) was used to purify and analyze the peptides. HPLC systems were serially equipped with a UV absorbance detector (UV, 220 nm) and a radioactivity detector (photomultiplier tube, PMT). HPLC columns were purchased from Phenomenex (Torrance, CA).

HPLC for preclinical production of [^{18}F]$\alpha_v\beta_6$-BP

HPLC were performed using a water/trifluoroacetic acid (TFA) 0.05% v/v (solvent A) / acetonitrile (solvent B) solvent system.

Semi-preparative HPLC of the non-radioactive peptide was performed using a Jupiter Proteo 10 µm 90 Å column (250 × 10 mm). A flow rate of 3 mL/min using solvent B isocratic 9% for 2 min, followed by a linear gradient to 81% over 30 min. Semi-preparative HPLC of the radioactive peptide was performed using a

Jupiter C18 10 µm 90 Å column (250 × 10 mm), at a flow rate of 3 mL/min using solvent B isocratic 9% for 2 min, followed by a linear gradient to 81% over 30 min.

Analytical HPLC was performed using a Jupiter Proteo 4 µm 90 Å column (250 × 4.6 mm) at a flow rate of 1.5 mL/min using solvent B isocratic 9% for 2 min, followed by a linear gradient to 81% over 30 min.

HPLC of precursor peptide H_2N-$\alpha_v\beta_6$-BP for clinical production of [^{18}F]$\alpha_v\beta_6$-BP

Semi-preparative HPLC of H_2N-$\alpha_v\beta_6$-BP was performed using a water/TFA 0.05% v/v (solvent A) / acetonitrile (solvent B) solvent system on a Jupiter Proteo 10 µm 90 Å column (250 × 10 mm). At a flow rate of 3 mL/min solvent B was kept isocratic 9% for 2 min, followed by a linear gradient to 39.5% ACN at 42 min.

HPLC for clinical production of [^{18}F]$\alpha_v\beta_6$-BP

Semi-preparative HPLC was performed using a Jupiter Proteo 10 µm 90 Å column (250 x 10 mm) at a flow rate of 2.5 mL/min using a saline (solvent A) / ethanol (solvent B) gradient system with a linear gradient from 9% to 35% solvent B at 0.5 min, followed by a linear gradient to 50% solvent B over 30 min. Analytical HPLC was performed using the same solvents on a Jupiter Proteo 4 µm 90 Å column (250 x 4.6 mm) at a flow rate of 1 mL/min using solvent B isocratic 30% for 1 min, followed by a linear gradient to 50% over 19 min.

HPLC for serum stability of clinical [^{18}F]$\alpha_v\beta_6$-BP

Serum samples were analyzed using a water/TFA 0.05% v/v (solvent A) / acetonitrile (solvent B) solvent system. Analytical HPLC was performed using a Jupiter Proteo 4 µm 90 Å column (250 × 4.6 mm) at a flow rate of 1.5 mL/min using solvent B isocratic 9% for 2 min, followed by a linear gradient to 81% over 30 min.

GLP synthesis of precursor peptide H_2N-$\alpha_v\beta_6$-BP

The precursor peptide for clinical use was synthesized manually under GLP conditions on NovaSyn TGR resin using solid-phase peptide synthesis Fmoc chemistry: Fmoc-amino acids or Fmoc-PEG-COOH were

pre-activated in situ with HATU in the presence of DIPEA in *N*,*N*-dimethylformamide (DMF) before addition to the H_2N-peptidyl-resin. Fmoc-amino acids were coupled at ambient temperature (20-24° C) for a minimum of 1.5 h using an Fmoc-amino acid/HATU/DIPEA ratio of 5/4.75/10 vs the resin loading; Fmoc-PEG-COOH was double-coupled at ambient temperature for 2 h and overnight, using an Fmoc-PEG-COOH/HATU/DIPEA ratio of 1/0.95/2 vs the resin loading per coupling. After coupling, the resin was washed with DMF (5×). Removal of the Fmoc protecting group was accomplished with 4-methylpiperidine (20% w/w solution in DMF) for 2 × 15 min, followed by washes with DMF (5 ×), methanol (5 ×), and DMF (5 ×). Completeness of coupling and Fmoc-removal were evaluated colorimetrically, either by picrylsulfonic acid or chloranil test. The assembled Fmoc-peptide was stored frozen as dried, fully protected peptidyl-resin until use. Following Fmoc-removal, the peptidyl-resin was dried under vacuum and the fully assembled precursor peptide, H_2N-$\alpha_v\beta_6$-BP, was liberated from the resin with concomitant removal of side-chain protecting groups by a cleavage mixture consisting of TFA/water/triisopropylsilane 95/2.5/2.5 (v/v/v) at ambient temperature for 3 h. The solution was evaporated to dryness under a stream of nitrogen gas, taken up in water, washed with diethyl ether (3 ×), and lyophilized. HPLC purification was done and pooled fractions containing pure H_2N-$\alpha_v\beta_6$-BP were lyophilized and stored frozen; samples were used for analysis.

Single dose acute toxicity study of [^{19}F]$\alpha_v\beta_6$-BP in CD-1 mice

A single dose, 14-day good laboratory practice (GLP) acute toxicology study in male and female CD-1 mice was performed with [^{19}F]$\alpha_v\beta_6$-BP doses exceeding 100 x the human equivalent dose, 1012 µg/kg (based on body surface area, human weight 60 kg: mouse weight: 20 g). The testing facility was the Division of Laboratory Animal Medicine (DLAM), David Geffen School of Medicine, UCLA. Animals were observed over 14 days with necropsy on day 1 (*n* = 10/group) and day 14 (*n* = 6/group). Body weights, clinical signs, clinical chemistries, hematology and histopathology were measured and no abnormal events were observed.

First-in-human clinical study with [^{18}F]$\alpha_v\beta_6$-BP

The inclusion criteria were: (i) Men and women age ≥18 yrs; (ii) diagnosed with primary or metastatic cancer in one or more of the following locations: breast, colorectal, lung and pancreas; (iii) ECOG performance score of 0-1; (iv) ability to understand and sign the IRB-approved consent form; (v) able to remain motionless for up to 30-60 minutes per scan. Prior to commencement of the study, all female volunteers were required to have a documented negative pregnancy test. The exclusion criteria were: (i) creatinine > 2 × upper limit of normal; (ii) AST/ALT >2 × upper limit of normal; (iii) life expectancy <3 mo; (iv) women who are pregnant or breast-feeding; (v) patients who cannot undergo PET/CT scanning because of weight limits; (vi) lack of availability for follow-up assessments; (vii) participation in another clinical trial involving an investigational agent within 4 weeks of enrollment. Bloods samples were obtained 24 hours prior to imaging for full CBC, chemistry and liver function tests, including: WBC count, RBC count (with differential), hemoglobin, hematocrit, platelet count, sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, serum creatinine, glucose, calcium, protein, albumin, alkaline phosphatase, aspartate transaminase, total bilirubin, alanine transaminase, and estimated glomerular filtration rate.