Structural Optimization Method to Improve the Theranostic Performance of Peptide Receptor-Targeted Radionuclide Therapy for Cancers

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

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CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application Serial No. 62/967,497, filed on January 29, 2020, the disclosure of which is hereby incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Neuroendocrine neoplasms (NENs) are a heterogeneous group of neoplasm, the incidence of which has been constantly increasing over the decades (*1,2*). NENs are commonly subcategorized into well-differentiated (low to intermediate grade) neuroendocrine tumors (NETs) and poorly-differentiated (high grade) neuroendocrine carcinomas (NECs) by histological, biological, and pathological differences (*2,3*). In many cases, the well-differentiated NETs are less aggressive than the poorly-differentiated NECs, and they respond to several targeted forms of therapies (*2*). The majority (>80%) of NENs express somatostatin receptors (*3*), and among them, somatostatin receptor subtype 2 (SSTR2) is a well-known target for a specific therapy called peptide receptor radionuclide therapy (PRRT). The current developments of the SSTR2-targeted PRRT are based on beta-particle emitters, Yttrium-90 (^{90}Y) and Lutetium-177 (^{177}Lu) (*4-8*). Especially, ^{177}Lu-labeled DOTA-tyr^3-octreotate (^{177}Lu-DOTATATE; Lutathera) is the only US Food and Drug Administration (FDA)-approved radiotherapeutic drug for the well-differentiated NETs (*9*). The drug had shown a therapeutic benefit by tumor response and increased progression-free survival (PFS) of the patients (*6-8*). However, the benefit was limited to partial response – and complete responses are rarely reported.

Alpha-particle emitters are alternatives to the conventional beta-particle emitters bringing significantly higher radiation doses (up to a few hundred fold) in cells and tumor metastases from decays (*10*) as well as higher relative biological effectiveness (RBE) arising from the high linear energy transfers (LETs) of the alpha particles (*11*). Multiple studies demonstrated that the alpha-particle emitters have the promise to treat the cancer patients who

were refractory to the beta-particle emitters (*12,13*). Lead-212 (^{212}Pb) is an attractive alpha-particle emitter that has a favorable half-life (10.64 h) for clinical application (*14*) and is well-matched with the biological half-lives (few hours) of peptides in vivo. Also, ^{212}Pb has the diagnostic pair, lead-203 (^{203}Pb), which is available for single-photon emission computed tomography (SPECT) by 279 keV photons (81 % intensity) (*15*). The half-life (51.87 h) of ^{203}Pb is long enough to monitor the biodistribution and pharmacokinetics of each patient by serial imaging up to 4–5 half-lives of ^{212}Pb (*14*). In addition, the theranostic pair shares the same chemistry for radiolabeling, and has a similar binding affinity and pharmacokinetics when labeled with the same peptides, which is critical for precise dosimetry.

Changes in peptide structure can shift the binding affinity, pharmacokinetics, and biodistribution of radiopeptides significantly. Thus, structural changes to the peptide can potentially improve therapeutic outcomes of the peptide-based therapies, by improving on these parameters. Approaches to manipulation of the ultimate performance of peptides for this application include modifications to the cyclization method, insertion of appropriate size and composition of the linker that connects the chelator to the peptide backbone, and development of radionuclide-specific chelators. Rhenium-coordinated peptide cyclization (*16*) and "click"-cyclization as well as a further optimization with glycine-glycine (GG) linker (*17*) have been evaluated in melanoma models targeting melanocortin receptor subtype 1 (MC1R), suggesting the potential of improved tumor targeting and pharmacokinetics and biodistribution by the approaches. Many other investigations implicated that radiopeptides can be optimized for optimal tumor targeting with improved in vivo performance by different linker insertions (*17-19*) and chelator modifications (*20-22*).

In this study, modifications in peptide structure with various strategies were made based on Tyr^3-octreotide (TOC). A new chelator composition, 1,4,7,10-tetraazacyclododecane-7-acetamide-1,4,10-triacetic acid (herein, called Pb-specific chelator or PSC) was introduced for Pb isotopes and other 2+ charged radionuclides. The structure was further optimized with the additions of polyethylene glycol (PEG) linkers between the chelator and TOC. DOTATOC, PSCTOC, PSC-PEG$_2$-TOC, and PSC-PEG$_4$-TOC were synthesized by the standard Fmoc-based solid phase peptide synthesis. The performance of each peptide was evaluated comprehensively by radiolabeling efficiency, binding affinity, cellular uptake, and biodistribution, and the lead compound was used for ^{203}Pb SPECT imaging and ^{212}Pb therapy/toxicity studies.

SUMMARY OF THE INVENTION

As noted above, the present invention is directed to a new chelator which, in one embodiment, is 1,4,7,10-tetraazacyclododecane-7-acetamide-1,4,10-triacetic acid. The chelator

is specific to 2+ charged radionuclides, including Pb isotopes. The structure includes a polyether linker between the chelator and Tyr^3-octreotide (TOC) or other peptide, with a polyethylene glycol (PEG) linker being preferred. The invention is primarily used to target any cancers that express the somatostatin receptor subtype 2 (SSTR2) which include, but are not limited to, neuroendocrine tumors, small cell lung cancer, meningioma, neuroblastoma, medulloblastoma, paraganglioma, and pheochromacytoma.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides in certain embodiments a carcinoma-targeting conjugate comprising Formula I:

T-L-X

wherein T is a SST2R targeting ligand,

L is a linker, and

X is a chelator.

for the therapeutic treatment of cancer.

In certain embodiments, the radiolabeled SST2R-targeted ligand is a peptide, or antibody or antibody fragment, or a small molecule.

In certain embodiments, T is Tyr^3-octreotide.

In certain embodiments, the SST2R-targeted ligand is radiolabeled with a radionuclide that is chemically bound to the chelator (X) and used for medical imaging and/or therapy of the cancerous tumors.

In certain embodiments, the radionuclide is Ga-68; In-111; Pb-203; F-18; C-11; Zr-89; Sc-44; Tc-99m or other medical radionuclide used for imaging.

In certain embodiments, the radionuclide is Y-90; Pb-212; Bi-212; Bi-213; At-211; Lu-177; Re-188; or other medical radionuclide used to treat the cancerous tumors.

In certain embodiments, L is a chemical linker that is inserted into a position between the peptide backbone that recognizes the SST2R protein and the chelator that is used to radiolabel the composition using radionuclides for therapy and/or diagnostic imaging; and the linker improves the binding and/or internalization of the composition into cells; improves the retention of the composition in tumors; and improves the clearance of residual composition through other excretion pathways for more precise delivery of radiation to the cancerous tissue, while minimizing radiation exposure to other organs (for example, kidneys).

In certain embodiments, L is a polyether linker comprising up to 4 carbons consisting of an aliphatic carbon chain that connects the chelator to the peptide backbone.

In certain embodiments, L is PEG_n, wherein n is 1-4. In certain embodiments, n is 2 or 4.

In certain embodiments, X is radiolabeled with a radionuclide that is used for medical imaging and/or therapy of the cancerous tumors.

In certain embodiments, the radionuclide is Ga-68; In-111; Pb-203; Cu-64 or other Cu isotopes; F-18; C-11; Zr-89; Sc-44; Tc-99m or other medical radionuclide used for imaging.

In certain embodiments, the radionuclide is Y-90; Pb-212; Cu-67; or other Cu isotopes; Bi-212; Bi-213; At-211; Lu-177; Re-188; or other medical radionuclide used to treat the cancerous tumors.

In certain embodiments, the chelating agent is based on 1,4,7,10-tetraazacyclododecane-7-acetamide-1,4,10-triacetic acid or other chelator that is used to bind the radionuclide for diagnostic imaging or therapy for cancer or other disease.

The present invention provides in certain embodiments a conjugate consisting of PSC-PEG_2/PEG_4-TOC.

In certain embodiments, the agent is administered orally or parenterally.

In certain embodiments, the agent is administered subcutaneously.

In certain embodiments, the conjugate is administered orally or parenterally.

In certain embodiments, the method further comprises administering an anti-cancer composition.

In certain embodiments, the conjugate is administered in a single dose.

In certain embodiments, the conjugate is administered in multiple doses.

In certain embodiments, the conjugate is administered sequentially daily for several days.

In certain embodiments, the conjugate is administered once per week for 1 month. In certain embodiments, the conjugate is administered once per week for up to 6 months.

In certain embodiments, the conjugate is administered in a dose of 1 mCi for medical imaging.

In certain embodiments, the conjugate is administered in a dose of up to 10 mCi for medical imaging.

In certain embodiments, the conjugate is administered in a dose of up to 50 mCi for medical imaging.

In certain embodiments, the conjugate is administered in a dose of 0.1 mCi for medical treatment of the cancerous tumors.

In certain embodiments, the conjugate is administered in a dose of up to 1 mCi for medical treatment of the cancerous tumors.

In certain embodiments, the conjugate is administered in a dose of up to 10 mCi for medical treatment of the cancerous tumors.

In certain embodiments, the conjugate is administered in a dose of up to 100 mCi for medical treatment of the cancerous tumors.

In certain embodiments, the conjugate is administered for more than a month.

In certain embodiments, the conjugate is administered for more than a year.

In certain embodiments, the conjugate is administered at a dosage of at least 0.05 μg/day.

The present invention provides in certain embodiments a use of the conjugate described above wherein:

a) the conjugate is administered simultaneously with the one or more anti-cancer agents; or

b) the conjugate and the one or more anti-cancer agents are administered sequentially; or

c) administration of the one or more anti-cancer agents begins about 1 to about 10 days before administration of the conjugate; or

d) administration of the conjugate thereof begins about 1 to about 10 days before administration of the one or more anti-cancer agents; or

e) administration of conjugate and administration of the one or more anti-cancer agents begins on the same day.

In certain embodiments, the ligand is a peptide.

In certain embodiments, the peptide is radiolabeled.

In certain embodiments, the SST2R-targeted ligand is a peptide that binds to the somatostatin receptor subtype 2.

In certain embodiments, the peptide is radiolabeled.

In certain embodiments, the agent that increases expression of SST2R is administered separately, sequentially or simultaneously with the SST2R-targeted ligand.

In certain embodiments, the agent that increases expression of SST2R is administered from about one day to about 6 months before the administration of the SST2R-targeted ligand.

In certain embodiments, the agent is administered orally or parenterally.

In certain embodiments, the agent is administered subcutaneously.

In certain embodiments, the SST2R-targeted ligand is administered orally or parenterally.

In certain embodiments, the administration of the agent begins about 1 to about 10 days before administration of the SST2R-targeted ligand.

In certain embodiments, the administration of the agent and administration of the SST2R-targeted ligand begin on the same day.

In certain embodiments, the method further comprises administering an anti-cancer composition.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided to the Office upon request and payment of the necessary fee.

FIGURES 1A-1C. Structures of SST2R-targeted ligands DOTATOC (Fig. 1A), PSCTOC (Fig. 1B), and PSC-PEG_2/PEG4-TOC (Fig. 1C). The peptides were synthesized by the standard Fmoc-based solid phase peptide synthesis. They were based on tyr^3-octreotide (TOC), and DOTA or a new Pb-specific chelator (PSC) were conjugated. For the peptides with linkers, two different sizes of polyethylene glycol (PEG), PEG_2 and PEG_4, were inserted between PSC and the peptide backbone.

FIGURES 2A-2B. Excellent radiolabeling efficiency of SST2R-targeted ligands DOTATOC and the PSC-conjugated peptides with ^{203}Pb (A) and ^{212}Pb (B). 18.5 MBq of ^{203}Pb or 14.1 MBq of ^{212}Pb was reacted with 10 nmol peptides in 0.5 M sodium acetate (NaOAc) buffer (pH=5.4, 1 ml reaction volume). The reaction was conducted at various temperatures (25, 50, or 85°C) and reaction time (10, 20, or 30 min) for the ^{203}Pb labeling. DOTATOC and PSCTOC were selected for the ^{212}Pb labeling, and the reaction was conducted at a fixed temperature (85°C) with increasing time (up to 30 min).

FIGURE 3. Competitive inhibition of ^{125}I-tyr^3-octreotide (^{125}I-TOC) binding to SSTR2-positive AR42J cells by TOC, DOTATOC, and the PSC-conjugated peptides. IC_{50} values, TOC: 3.1 ± 1.1 nM, DOTATOC: 11.3 ± 1.3 nM, PSC-TOC: 6.2 ± 1.1 nM, PSC-PEG_2-TOC: 5.3 ± 1.2 nM, PSC-PEG_4-TOC: 9.4 ± 1.3 nM (at least n=6 from at least three biological replicates for DOTATOC and PSCTOC; n=4–6 from two biological replicates for TOC, PSC-PEG_2-TOC, and PSC-PEG_4-TOC).

FIGURE 4. Cellular uptake of ^{203}Pb-labeled DOTATOC, PSCTOC, and PSC-PEG_2-TOC in AR42J cells. 200,000 CPM of HPLC-purified ^{203}Pb-labeled peptides were incubated with AR42J SST2R expressing cells at 37 °C up to 120 min, and the cellular uptake of each radiotracer was measured. The data are shown as mean percentage of cellular uptake relative to incubated acitivties ± SD (n=4).

FIGURES 5A-5B. Biodistribution of ^{203}Pb-labeled SST2R-targeted DOTATOC, PSCTOC, and PSC-PEG_2-TOC in AR42J tumor bearing athymic nude mice. Biodistribution was observed at 1, 3, and 24 h post-injection after the i.v. injections of 37 kBq ^{203}Pb-labeled peptides **(A)**, and percent injection dose per gram of tissue (%ID/g) over time for tumor and kidneys as well as tumor-to-kidney ratio were shown **(B)**. The data is shown in mean percent injected dose per gram of tissue (%ID/g) or relative mean tumor-to-kidney ratio ± S.D. (n=3).

FIGURES 6A-6C. ^{203}Pb SPECT/CT images in AR42J-tumor-bearing athymic nu/nu female mice. **(A)** AR42J-tumor-bearing mice were imaged at 3h and 24h post-injection after the administrations of 11.1 MBq ^{203}Pb-DOTATOC and ^{203}Pb-PSC-PEG$_2$-TOC. 30 nmol of unlabeled peptide was co-injected for the blocking imaging to confirm the tumor specificity. **(B)** Tumor-to-kidney ratio over time analyzed from the obtained images using the Inveon research workplace software. **(C)** The mice were euthanized at 30h post-injection and biodistribution was obtained.

FIGURE 7. Stability of PSC-PEG$_2$-TOC in water and human serum. PSC-PEG$_2$-TOC was radiolabeled with 50 MBq (1.34 mCi) of ^{203}Pb, and 9 MBq (0.24 mCi) of purified radiopeptide was added into 3 ml water or human serum and incubated at 37 °C for up to 24 h. The peptide degradation was monitored by a radio-HPLC system (Agilent 1200 Series connected with an IN/US β-RAM Model 4 radio-detector) after 8h and 24h.

FIGURE 8. Clinically-relevant high specific activity ^{203}Pb radiolabeling of PSC-PEG$_2$-TOC. The radiolabeling was conducted with high activities of ^{203}Pb at either 90 MBq/nmol DOTATOC for reference **(A)**, 90 MBq/nmol PSC-PEG$_2$-TOC **(B)**, or 120 MBq/nmol PSC-PEG$_2$-TOC **(C)** in 0.5 M Sodium Acetate (NaOAc) buffer (pH=5.4, 1–2 ml reaction volume) at 85 ºC for 30 min.

FIGURE 9. Biodistribution of ^{203}Pb/^{212}Pb-labeled PSC-PEG$_2$-TOC in AR42J tumor bearing athymic nude mice at 3 h post-injection. 74 kBq of ^{212}Pb-PSC-PEG$_2$-TOC (specific activities, 3.7 MBq/nmol) was injected via tail vein and the biodistribution was obtained at 3 h post-injection (n=4). This data was directly compared to the biodistribution of ^{203}Pb-PSC-PEG$_2$-TOC obtained previously (specific activity, 22.2 MBq/nmol; Fig. 5).

FIGURES 10A-10B. Reduced renal accumulation of ^{203}Pb-PSC-PEG$_2$-TOC by DL-lysine co-injection, and specific tumor binding of the radiopeptide in AR42J bearing nude mice informed by tumor blocking with co-injection of excess unlabeled peptide. **(A)** Biodistribution of ^{203}Pb-PSC-PEG$_2$-TOC at 3h post-injection in AR42J-tumor-bearing nude mice with lysine co-injection (400 mg/kg), without lysine co-injection, or with unlabeled peptide co-injection (for tumor blocking; 10 nmol PSC-PEG$_2$-TOC). **(B)** Complete biodistribution of ^{203}Pb-PSC-PEG$_2$-TOC with DL-lysine co-injection (400 mg/kg) at 1, 3, 6, and 24h post-injection. Results are in percent injected dose per gram of tissue (%ID/g) ± S.D. (n=3) .

FIGURES 11A-11C. Therapeutic outcomes of initial ^{212}Pb-PSC-PEG$_2$-TOC therapy studies in mice bearing AR42J-SST2R-expressing tumors at 30 days post-therapy. ^{212}Pb-PSC-PEG$_2$-TOC therapy was initiated when the average tumor size became around 150 mm^3. 0.37 MBq (10 μCi) and 1.85 MBq (50 μCi) of ^{212}Pb-PSC-PEG$_2$-TOC were injected via tail vein with DL-lysine (400 mg/kg) co-injection to block the kidney uptake of the radiotherapeutic.

FIGURE 12A-12F. Dosimetry and toxicities of escalated doses (up to 150 μCi) of ^{212}Pb-PSC-PEG$_2$-TOC in CD-1 Elite (SOPF) male mice. **(A)** Body weight change after the injection of ^{212}Pb-PSC-PEG$_2$-TOC. After an initial decrease in body weight in first days, the body weight of the treated mice increased gradually in which the increase of the body weight is dose-dependent. **(B)** ^{212}Pb-PSC-PEG$_2$-TOC biodistribution in CD-1 Elite (SOPF) male mice. ^{212}Pb is used for the study to include the impact of potential demetallation of ^{212}Pb and redistribution in bone marrow. **(C)** Estimated renal dose arising from the escalating doses of ^{212}Pb-PSC-PEG$_2$-TOC based on the biodistribution in CD-1 Elite (SOPF) male mice. Organ level internal dose assessment (OLINDA) V2.1 was used for dose estimation in mice using 30g mouse voxel phantom model. **(D-E)** The levels of renal toxicity markers arising from the escalating doses of ^{212}Pb-PSC-PEG$_2$-TOC assessed by urine neutrophil gelatinase-associated lipocalin (uNGAL; D) at day 1 and day 3 post-administration and blood urea nitrogen (BUN; E) at 3 months post-administration. **(F)** A reversible hematologic toxicity indicated by complete blood count (CBC) at week 1, 2, and 4 post-administration.

The following example is intended to further illustrate the invention. It is not intended to limit the invention in any manner.

MATERIALS AND METHODS

Peptide synthesis

DOTATOC, PSCTOC, PSC-PEG$_2$-TOC, and PSC-PEG$_4$-TOC were synthesized by the standard Fmoc-based solid phase peptide synthesis. The linear peptide, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr(ol) was synthesized on the resin at 100 μmol scale using an automated peptide synthesizer (AAPPTEC Apex 396) and the N-terminus of the linear peptide was deprotected by 25% piperidine (PIP) at the end of automated synthesis. The manual addition of the PEG linker (PEG$_2$ or PEG$_4$) was followed for PSC-PEG$_2$-TOC or PSC-PEG$_4$-TOC. The peptide-resin was suspended with N, N-dimethylformamide (DMF), and 5 equivalence (equiv.) of Fmoc-NH-PEG$_2$/PEG$_4$-propionic acid (purchased from AAPPTEC), 2-(7-aza-1H -benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), and 1-hydroxybenzotriazole (HOBt), and 10 equiv. of N, N-diisopropylethylamine (DIPEA) were added and reacted while being mixed at 37 °C for 2h. The Fmoc on the N-terminus of the peptide-resin were then manually deprotected by 25% piperidine (in DMF) with mild mixing at 25 °C for 10 mins, and wash with DMF/Dichloromethane (DCM)/Methanol and repeated the process. The linear peptides with open N-terminus on the resin were then resuspended in DMF, and 5 equiv. of either DOTA-tris(tert-butyl ester) or PSC-bis(tert-butyl ester), HATU, and HOBt, and 10 equiv. of DIPEA were added and reacted at 37 °C while being mixed overnight. The success of each step of

coupling/deprotection was verified by the Kaiser test and the process repeated until successful. The linear peptides were then cyclized by iodine oxidation. Iodine (I_2; 20 equiv.) was dissolved in 6 ml DMF and added to the peptide-resin and allowed reaction to proceed trityl deprotection from cysteine and concomitantly promote disulfide formation via oxidation for 3 h. The resin and protecting groups were then cleaved from the cyclized peptides by adding 3 mL cleavage cocktail (93% trifluoroacetic acid, 3% triisopropylsilane, 4% water) for 2 h at room temperature, followed by ether precipitation on ice for at least 4 h. The crude peptides were then purified by semi-preparative high performance liquid chromatography (HPLC) with a C-18 column (Vydac 10 × 250 mm, 10 μm; Grace, Deerfield, IL). The collected samples were concentrated by rotary evaporation, and lyophilized. The purified peptides were characterized by a mass spectrometer.

^{203}Pb/^{212}Pb radiolabeling efficiency

DOTATOC and the PSC-conjugated peptides were radiolabeled with ^{203}Pb and ^{212}Pb. 18.5 MBq of ^{203}Pb or 14.1 MBq of ^{212}Pb was reacted with 10 nmol peptides in 0.5 M Sodium Acetate (NaOAc) buffer (pH=5.4, 1 ml reaction volume). The reaction was conducted at various temperatures (25, 50, or 85 °C) and reaction time (10, 20, or 30 min) for the ^{203}Pb labeling. DOTATOC and PSCTOC were selected for the ^{212}Pb labeling, and the reaction was conducted at a fixed temperature (85 °C) with increasing time (up to 30 min). After the reaction, the resultant was spotted on pre-dried instant thin layer chromatography (iTLC) strips and developed by 10 mM diethylenetriaminepentaacetic acid (DTPA) in 0.1 M NaOAc buffer. The strips were then cut by half and the radio-activities of each portion (top, free ^{203}Pb/^{212}Pb; bottom, ^{203}Pb/ ^{212}Pb labeled to the peptides) of the iTLC strips were measured by the isotope-specific gamma peaks (^{203}Pb, 279 keV; ^{212}Pb, 239 keV) using a NaI detector.

^{125}I-TOC competitive binding assay

TOC was labeled with iodine-125 (^{125}I) by conventional chloramine T method as described elsewhere (*23*). 1.0x10^5 AR42J rat pancreatic acinar cells were plated into poly-D-lysine-coated 24-well plates. After 3 days, the cells were incubated with 30,000 CPM of ^{125}I-TOC in binding medium (RPMI 1640 supplemented with 0.2% bovine serum albumin; 0.3 mM 1, 10-phenanthroline) with TOC, DOTATOC, PSCTOC, PSC-PEG_2-TOC, or PSC-PEG_4-TOC of increasing concentration (10^{-11} to 10^{-6} M) for 2 hours at 37 °C. The cells were then washed twice with ice-cold PBS and lysed with 0.5 N NaOH, and the radioactivity was measured via a gamma counter. The half maximal inhibitory concentration (IC_{50}) was determined using GraphPad Prism V8.0.

Internalization and efflux of ^{203}Pb-labeled peptides

37 MBq of ^{203}Pb was labeled with 10 nmol of DOTATOC, PSCTOC, and PSC-PEG_2-TOC, and the labeled peptides were separated from the unlabeled by high performance liquid chromatography (HPLC) based on differential retention times of the labeled and the unlabeled peptides by the previously developed separation method (*15*). The HPLC-separated radiopeptides were then purified by a C-18 cartridge. The AR42J cells that were plated with a density of $2.0x10^5$ cells 2 days before were incubated with 200,000 CPM of the HPLC-purified ^{203}Pb-labeled peptides at 37 °C for up to 120 min. The cells were then washed twice with ice-cold PBS, and the membrane-bound radioactivity was washed off by 50 mM acidic (pH=4) sodium acetate buffer and collected. The remaining cells were lysed by adding 0.5N NaOH for 5 min. The radioactivity of each portion (membrane-bound and internalized) was counted by a 310 Cobra II gamma counter (PerkinElmer, Freemont, CA). For the efflux assay, the cells were incubated with 200,000 CPM of the HPLC-purified ^{203}Pb-labeled peptides at 37 °C for 120 min. The cells were then washed twice with ice-cold PBS and replenished with the binding medium. After 60 min and 120 min, the radioactivities of the effluxed (into the medium), the membrane-bound and the internalized (harvested by the same way as in the internalization assay) were counted.

Biodistribution of ^{203}Pb-labeled peptides

37 kBq of ^{203}Pb-labeled DOTATOC, PSCTOC, and PSC-PEG_2-TOC (specific activity: 22.2 MBq/nmol) were injected into female AR42J tumor-bearing athymic nu/nu mice via tail vein. The mice were euthanized at 1, 3, and 24 h post-injection by cervical dislocation under isoflurane anesthesia. Tumor and organs/tissues of interest were harvested and the weights of the collected organs/tissues were measured. The radioactivities of the samples were measured by the PerkinElmer 310 Cobra II gamma counter (PerkinElmer, Freemont, CA).

Tumor and kidney dosimetry

The Particle and Heavy Ion Transport code System (PHITS) was used for dosimetry analysis. For the kidney dosimetry, DigiMouse voxel phantom model was used, and the voxel size of the model was adjusted so that the volume of the kidneys became identical to the average volume of the kidneys of female athymic nude mice (288.7 +- 41.4 mg; 28 mice) from the biodistribution study (AR42J bearing; 8-10 weeks). The elemental composition of the kidney and the mass density was assumed to be identical as the human reference adults' values obtained from the International Commission on Radiation Units and measurements (ICRU) report 46. For tumor dosimetry, a spherical volume was constructed based on the average tumor mass (156.9

+-0.096 mg) of the 28 mice. The elemental composition (adenoidcystic carcinoma)and mass density (1.04 g/cm^3) of the tumor was adapted from Maughan *et al.* 1997 *Med Phys* 24(8):1241-4 and RM Thomson *et al.* 2013 *Phys. Med. Biol.* 58:1123-50. At least 1 million particles were transported for the Monte Carlo simulations to reduce the statistical uncertainties less than 1%.

Serial SPECT/CT imaging of ^{203}Pb-DOTATOC vs. ^{203}Pb-PSC-PEG$_2$-TOC

1.85 GBq (50 mCi; 61.7 MBq/nmol) of ^{203}Pb was labeled with DOTATOC and PSC-PEG$_2$-TOC. 11.1 MBq of each ^{203}Pb labeled peptide was injected into AR42J bearing mice via tail vein, and the mice was imaged at 3h and 24h post-injection. Separately, the same activity of ^{203}Pb-PSC-PEG$_2$-TOC was co-injected with 30 nmol of unlabeled PSC-PEG$_2$-TOC for the blocking study to confirm the tumor specificity of the radiotracer. The images were reconstructed and analyzed with the same parameter setting using the Inveon research workplace software. Standardized uptake values corrected by body weight (SUVbw) were analyzed, and the biodistributions of the mice were obtained at 30 h post-administration.

Stability of ^{203}Pb-PSC-PEG$_2$-TOC in water and human serum

As the identified lead compound, PSC-PEG$_2$-TOC was further evaluated in various aspects. PSC-PEG$_2$-TOC was radiolabeled with 50 MBq (1.34 mCi) of ^{203}Pb and purified by C-18. 9 MBq (0.24 mCi) of purified radiopeptide was added into 3 ml water or human serum and incubated at 37 °C for up to 24 h. After incubation, the serum samples with ^{203}Pb-PSC-PEG$_2$-TOC were transferred to Amicon Ultra Centrifugal Filter (3K; Millipore) and centrifuged by a Beckman Coulter Avanti J-25I centrifuge. The penetrates by centrifugation (serum sample) or the samples in water were analyzed by a radio-HPLC system (Agilent 1200 Series connected with an IN/US β-RAM Model 4 radio-detector) to monitor the degree of peptide degradation.

Clinically-relevant high specific activity ^{203}Pb radiolabeling of PSC-PEG$_2$-TOC

PSC-PEG$_2$-TOC were radiolabeled with ^{203}Pb at high specific activities of either 90 MBq/nmol or 120 MBq/nmol. DOTATOC was also labeled in 90 MBq/nmol for reference. The reactions were conducted in 0.5 M Sodium Acetate (NaOAc) buffer (pH=5.4, 1–2 ml reaction volume) at 85 ºC for 30 min. 2 µl of reaction product containing ^{203}Pb-labeled peptide was spotted on an instant thin layer chromatography (iTLC) strip. The sample strip was developed in the mobile phase (0.2 M sodium acetate with 20 mM EDTA) and then imaged with a phosphor imager (Typhoon FLA7000). The strip was cut by half and the radioactivity of each side of strip was measured by the NaI detector by ^{203}Pb gamma peak (279 keV) to determine the radiolabeling efficiency.

Biodistribution of ^{212}Pb-PSC-PEG$_2$-TOC in AR42J bearing nude mice

74 kBq of ^{212}Pb-PSC-PEG$_2$-TOC (specific acitivity, 3.7 MBq/nmol) was injected into AR42J bearing athymic nude mice via tail vein and the biodistribution was obtained at 3h post-injection (n=4). This data was directly compared to the biodistribution of ^{203}Pb-PSC-PEG$_2$-TOC obtained previously (specific activity, 22.2 MBq/nmol; Fig. 5). The data determined the adequacy of ^{203}Pb-PSC-PEG$_2$-TOC as the imaging and dosimetry surrogate for ^{212}Pb-PSC-PEG$_2$-TOC.

Biodistribution of ^{203}Pb-PSC-PEG$_2$-TOC in AR42J bearing nude mice with lysine co-infusion

37 kBq of ^{203}Pb-PSC-PEG$_2$-TOC (specific activity: 22.2 MBq/nmol) was injected into AR42J tumor-bearing nude mice via tail vein with and without co-injection of DL-lysine (400 mg/kg; 8 mg/animal) to observe if lysine co-injection could reduce the non-specific renal uptake of the radiotracer. Also, a separate group was added for tumor blocking to verify the specificity of tumor targeting by co-injecting 10 nmol of unlabeled peptide (without lysine) with 37 kBq of ^{203}Pb-PSC-PEG$_2$-TOC. These mice were then euthanized at 3 h post-injection and biodistribution was assessed (n=3 for each group). In a separate study, comprehensive biodistribution was obtained at 1, 3, 6, and 24 h post-injection with co-injection of DL-lysine to acquire complete pharmacokinetic data for further dosimetry studies.

^{212}Pb-PSC-PEG$_2$-TOC therapy

5.0x10^6 AR42J rat pancreatic acinar cells were implanted on the left shoulder of female athymic nu/nu mice. After 10 days, when the average tumor size became around 150 mm^3, 274 MBq (7.4 mCi) ^{212}Pb were reacted with 30 nmol PSC-PEG$_2$-TOC (9.1 MBq/nmol) in the presence of ascorbic acid (1 mg/ml) for 20 min at 85 °C. After reaction, the radio-peptide were purified by C-18 and resuspended with saline containing ascorbic acid (1 mg/ml). 0.37 MBq (10 μCi) and 1.85 MBq (50 μCi) of ^{212}Pb-PSC-PEG$_2$-TOC were injected via tail vein. DL-lysine (400mg/kg) was co-injected to block the kidney uptake of the radiotherapeutic.

^{212}Pb-PSC-PEG$_2$-TOC toxicity studies

Escalating doses (0, 0.37, 1.85, 3.33, and 5.55 MBq or 0, 10, 50, 90, and 150 μCi) of ^{212}Pb-PSC-PEG$_2$-TOC were administered to tumor-free CD-1 Elite (SOPF) male mice (n=4 for each group). Body weight was measured 2 times a week by 3 weeks post-injection and 1 time a week afterwards. Urine samples were collected (via metabolic case) at day 1 and day 3 post-administration to evaluate acute tubular toxicities in kidneys. The urine samples were

centrifuged, and the levels of urine neutrophil gelatinase-associated lipocalin (uNGAL) were measured using a mouse NGAL ELISA kit (Kit 042; BIOPORTO Diagnostics) according to the manufacturer's manual. At 3 months post-injection, the serum samples were collected by tail vein nicking, sent to IDEXX Laboratories, inc., and analyzed for comprehensive blood chemistry including blood urea nitrogen (BUN). Further follow-up will be made at 6–7 months for the comprehensive blood chemistry test and kidney histopathology analysis. The hematological toxicity was assessed by complete blood counts (CBC) using an automated veterinary hematology analyzer (ADVIA 120, Siemens Healthineers) at week 1, 2, and 4 post-administration. In addition, ^{212}Pb-PSC-PEG$_2$-TOC biodistribution study was conducted at 1, 3, 6, and 24h (including bone marrow), to support dosimetry analyses that can correlate with toxicity profile in critical organs/tissues including kidneys and bone marrow. Dose estimation was performed in Organ Level Internal Dose Assessment (OLINDA, V2.1) software using 30g mouse voxel phantom model.

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It should be appreciated that minor dosage and formulation modifications of the composition and the ranges expressed herein may be made and still come within the scope and spirit of the present invention.

Having described the invention with reference to particular compositions, theories of effectiveness, and the like, it will be apparent to those of skill in the art that it is not intended that the invention be limited by such illustrative embodiments or mechanisms, and that modifications can be made without departing from the scope or spirit of the invention, as defined by the appended claims. It is intended that all such obvious modifications and variations be included within the scope of the present invention as defined in the appended claims. The claims are meant to cover the claimed components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates to the contrary.

The foregoing description has been presented for the purposes of illustration and description. It is not intended to be an exhaustive list or limit the invention to the precise forms disclosed. It is contemplated that other alternative processes and methods obvious to those skilled in the art are considered included in the invention. The description is merely examples of embodiments. It is understood that any other modifications, substitutions, and/or additions may be made, which are within the intended spirit and scope of the disclosure. From the foregoing, it can be seen that the exemplary aspects of the disclosure accomplishes at least all of the intended objectives.