Supplementary Information

Automated cassette-based production of high specific activity [$^{203/212}$Pb]peptide-based theranostic radiopharmaceuticals for image-guided radionuclide therapy for cancer.

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Running Title: Efficient automated synthesis of $^{203/212}$Pb bioconjugates

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Nuclear and Decay Data

Half lives and all nuclear data (decay energies and branching ratios) were obtained from the National Nuclear Data Center, Brookhaven National Laboratory, and are based on the Evaluated Nuclear Structure Data File available through www.nndc.bnl.gov.

Cyclotron Production of ^{203}Pb

Reactions: ^{203}Pb for the current studies was produced on Lantheus cyclotrons using natural Tl (^{nat}Tl) targets at an approximate beam energy of 26.5MeV. There are two naturally occurring stable isotopes of Tl (Tl-203; ^{203}Tl and Tl-205 ^{205}Tl), with abundances of ~29.5% and ~70.5% respectively.

The primary reaction of interest in the manufacture of ^{203}Pb is:

(1) ^{205}Tl (p, 3n) ^{203}Pb ($T_{1/2}$ = 51.88 hours)

While co-produced stable metals can be removed easily *via* the pre-concentration step described herein, co-production of stable Pb isotopes must be controlled as excess stable Pb is an interference to the radiolabeling and ultimate specific activity of the radiopharmaceuticals prepared. Along these lines, important reactions to consider in the production ^{203}Pb for radiopharmaceuticals include:

(2) ^{203}Tl (p, 3n) ^{201}Pb ($T_{1/2}$ = 9.33 hours)

(3) ^{205}Tl (p, 2n) ^{204m}Pb ($T_{1/2}$ = 1.12 hours)

Each of these Pb isotopes decays by electron capture (**ec)** or internal transition (**it)** to its corresponding Pb) or Tl isotope according the following schemes:

(4) ^{203}Pb ($T_{1/2}$ = 51.92 hours) → ^{203}Tl Stable

(5) ^{201}Pb ($T_{1/2}$ = 9.33 hours)→ ^{201}Tl ($T_{1/2}$ = 72.91 hours)

(6) ^{204m}Pb ($T_{1/2}$ = 1.12 hours) → ^{204}Pb Stable

^{201}Pb produced *via* **(2)** is the primary radio-contaminant; and a hold time to allow for decay (approximately 90 h) is used to allow decay of Pb-201 **(5)**. ^{204m}Pb **(3, 6)** is the primary metallic contaminant produced on the cyclotron and leads to the production of a small quantity of stable ^{204}Pb; and the relationship of ^{203}Pb production to co-production of ^{204m}Pb is under evaluation for a future publication.

Targetry and Purification: Highly pure ^{nat}Tl (>99.99%) is purchased as thalium nitrate (Alfa-Aesar). The nitrate is dissolved using pure water. Plating additives (proprietary) are added to the dissolved Tl to create the plating bath. The solution is transferred into the plating cell. The Cu cyclotron target is serially numbered and flashed with Ni prior to Tl plating. The target is plated uninterrupted until bath depletion. The target is removed from the bath, dried, visually inspected and weighed. Acceptable targets masses are 2.5-9 grams with no visual damage or voids. ^{nat}Tl target irradiations are performed on an Cyclone 30 (IBA RadioPharma Solutions) or C-28 Cyclotron (Cyclotron Inc.) for 6-24 h. The irradiated targets are transferred to high energy processing cells and held for approximately 90 hours to allow decay of ^{201}Pb **(5)**. The Tl metal target material is then dissolved from the cyclotron target using hot 1M HNO_3. The solution is filtered through a glass wool column and pH adjusted using concentrated NH_4OH and/or 1 M HNO_3 to a final value between pH 5-6. The adjusted solution is passed though a Chelex 100 (Bio Rad) ion exchange column (14 mL resin bed, ammonium form) and washed with 150-175 mL of 1 M NH_4NO_3 to remove residual Tl metal. Cu, Ni, Fe, Zn and ^{203}Pb are retained on the column. The ^{203}Pb is removed from the column using ~75 mL of hot (~85°C) 0.5 M NH_4OH. The ^{203}Pb hydroxide solution is pH adjusted between 5-6 using 1

M HNO_3 and/or concentrated NH_4OH to a final value between pH 5 and pH 6. The adjusted solution is loaded onto a second Chelex 100 column. The column is washed with 150-175 mL of 1 M $NaNO_3$. The ^{203}Pb is removed from the column using ~75 mL of hot (~85°C) 0.5 M NaOH. The ^{203}Pb hydroxide solution is pH adjusted approximately 5-6 using 1 M HNO_3 and/or concentrated NH_4OH to a final value between pH 5.6. The column is washed with 150-175 mL of 1 M NH_4NO_3 followed by a 60 mL of pure water. The ^{203}Pb is then stripped from the column with 10-20 mL increments of 1 M HNO_3 until the ^{203}Pb is removed from the column. The ^{203}Pb strip solution is reduced to dryness in a beaker on a hotplate. Approximately 10 mL in 2-3 mL increments of 0.5 M HCl is used to remove the ^{203}Pb activity from the beaker and the stock is transferred to a 10 mL vial for final assay by dose calibrator (Capintec) to determine concentration according to the manufacturer's instructions.

Analytical Testing/Quality Control: Two samples for analytical testing are prepared from the assayed stock solution. The first sample is a serially diluted sample containing approximately 370 kBq of ^{203}Pb is analyzed on NIST traceable high purity germanium detector (HPGe) (Canberra) for radio-nuclidic identification and purity. The other sample is prepared for inductively coupled plasma analysis (ICP-OES) (Varian) for trace metal and specific activity determination.

Radiochemical purity and HPLC isolation of radiopeptides from unlabeled precursors.

Instant thin layer chromatography (iTLC) imaging was also applied to confirm the achievable radiolabeling efficiency and radiochemical purity. For ^{212}Pb analysis, 2µl of ^{212}Pb labeled peptide and free $^{212}Pb^{2+}$ positive control was spotted on the iTLC strip (10cm×2cm) that was dried at 90°C for at least 2h before using. The sample strips were developed in the mobile phase 0.2M NaOAc with 20mM EDTA. After overnight equilibration, the strips were analyzed with a Typhoon FLA7000 phosphor-imager. The radiochemical purity was calculated by integrating the radioactivity counts at the solvent front (Rf=1) and comparing to the integrated counts at the origin (Rf=0). Radio-HPLC was used to determine radiochemical purity of $^{203/212}$Pb labeled peptides using HPLC: Agilent 1200 Series; Radioactivity detector: IN/US system β-RAM Model 4, using a linear 16%-26% acetonitrile gradient in 20mM HCl over 20 minutes on a Vydac 218TP C18 column (4.6×150mm, 5µm) with 1mL min^{-1} flow rate. By using the same radio-HPLC gradient, both radiolabeled peptides (*i.e.*, DOTATOC and DOTA-PEG4-VMT-MCR1) were separated from excess unlabeled peptide to enhance the specific activity of final product. This separation was verified by co-injecting [^{203}Pb]peptide with 10µg bulk unlabeled peptide and monitoring both UV and radio-detector traces. Theoretical specific activity was calculated as follows: The amount of purified radio-peptide from HPLC collection was estimated from measured radioactivity and λ for Pb-203. Stable Pb isotope impurity was referred from QC technical data sheet from manufacture. Final

specific activity was calculated by A/([^{203}Pb+^{stable}Pb]peptide) and is expressed as MBq/nmol).

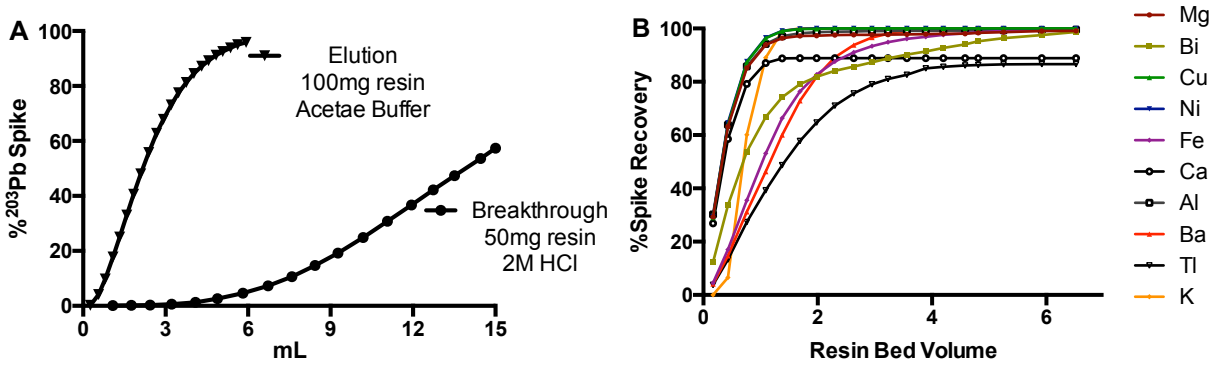

Figure S1. Elution and breakthrough of **(A)** Pb (as ^{203}Pb); and **(B)** metallic interferences. A smaller (50 mg) resin bed was used to find breakthrough volumes; and 100 mg resin bed was used to understand the elution behavior. These figures suggest 5 mL of HCl load/rinse solution can be passed over the column before breakthrough of Pb would occur; Similarly, 5 mL of NaOAc buffer is sufficient to elute 100% of the Pb. Metal interferences can be removed with a 2-column-volume rinse. Solutions were collected dropwise and counted by gamma counter.

Table S1. Mass spectroscopy analysis of ^{224}Ra/^{212}Pb generator eluate before and after Pb-resin purification.

Metal	Before Pb-resin (µg)	After Pb-resin (µg)
Cu	0.3	0.2
Fe	46.2	0.74
Pb	1.74	1.7
Se	0.42	0.2