^{119}Sb: Production, Radiochemical Separation, and Chelation of a Promising Candidate for Targeted Radionuclide Therapy

by Jenasee Mynerich[1], Thomas I. Kostelnik, Aeli P. Olson, Atanaska Marinova, Dmitry Filosofov, Paul Schaffer, Jonathan W. Engle, Cornelia Hoehr, and Valery Radchenko

TRIUMF, Canada's national accelerator centre located in Vancouver, B.C., is home to the world's largest cyclotron as well as multiple smaller cyclotrons, advanced laboratories, and equipment. With these resources at hand, the Life Science Division at TRIUMF has developed a research program centred on the production and medical application of various radioisotopes including the treatment of cancer.

Cancer incidence continues to increase globally, with a staggering projection of 26 million new cases and 17 million cancer deaths per year by 2030 [1-3]. Clearly, there is a need for improvements to current treatments and development of new ones to address limitations. For the most common cancers, including prostate cancer in men and breast cancer in women, surgery is the primary method of treatment, often supported by radiotherapy or chemotherapy [4]. Surgery and traditional radiotherapy (external beam therapy) are most effective when the tumour is large or localised; however, as metastases form elsewhere in the body, these treatment options are rendered ineffective — not to mention impractical. In these cases, the use of Targeted Radionuclide Therapy (TRT) can be extremely effective.

TRT is based on the idea of selectively delivering dose to target cells while minimizing radiation exposure to healthy tissues [5]. This is commonly achieved by "radiolabeling" a targeting vector (e.g., peptide or antibody) with a therapeutic radionuclide. The direct radiolabeling of unmodified targeting vectors is seldom permissible for subsequent *in vivo* use, thus small organic molecules known as "bifunctional chelators" are typically attached to targeting vectors to permit safe delivery of the radioactive cargo [5]. Since the therapeutic capacity of so-called "radiopharmaceuticals" is directly linked to the decay properties of the employed radionuclide, selecting the ideal therapeutic nuclide is imperative. The choice is based on a number of factors including its emission type, range/energy of emission, and half-life [6,7].

For TRT, useful emissions include alpha particles, beta particles, and Auger electrons. Each of these interact differently in the body based on their Linear Energy Transfer (LET), or the amount of energy deposited per unit length (keV/µm). Alpha particles and Auger electrons have a high LET, meaning they deposit their energy over a short tissue range, ultimately producing dense regions of ionization capable of inducing cell death [7]. In contrast, beta particles have a much lower LET and are less likely to cause irreparable damage to target cells (via double-strand DNA breaks).

Particle energy and LET will determine how far the particle travels in biological medium. Beta particles have a typical range of 1-10 mm in media, while alpha particles will travel no more than 100 µm, or a few cell widths, as depicted in Fig. 1 [7]. Accordingly, beta particles are better suited to treat larger tumours, while alpha particles will be more effective in treating smaller tumours (notably metastases). Of current interest are Auger electrons, which have an even shorter tissue range of just 0.01-10 µm [8]. This extremely short tissue range promises targeting specificity at the scale of a single metastatic cell's nuclear DNA, which could prevent the spread of solid tumours elsewhere in the body.

Summary

Cyclotron production, separation chemistry, and proof-of-principle chelation applicable to the Auger-electron emitter ^{119}Sb ($t_{1/2}$ 38.1 h) has been explored to promote further development of this radionuclide as a candidate for targeted radionuclide therapy for cancer.

1 Jenasee was the winner of the TRIUMF Student Symposium (X2). She received a travel bursary to attend the Targeted Alpha Theory Symposium (TAT11), April 1-4, 2019, in Ottawa, ON. She also presented this work as an invited talk at the Canadian Organization of Medical Physicists conference in Kelowna, BC, Sept. 24-27, 2019.

Jenasee Mynerich <mynericj@mcmaster.ca> Nuclear Medicine Research Assistant, Life Science Division, TRIUMF/ Honours Medical & Health Physics Co-op Student, McMaster University, 4004 Wesbrook Mall, Vancouver, B.C. V6T 2A3

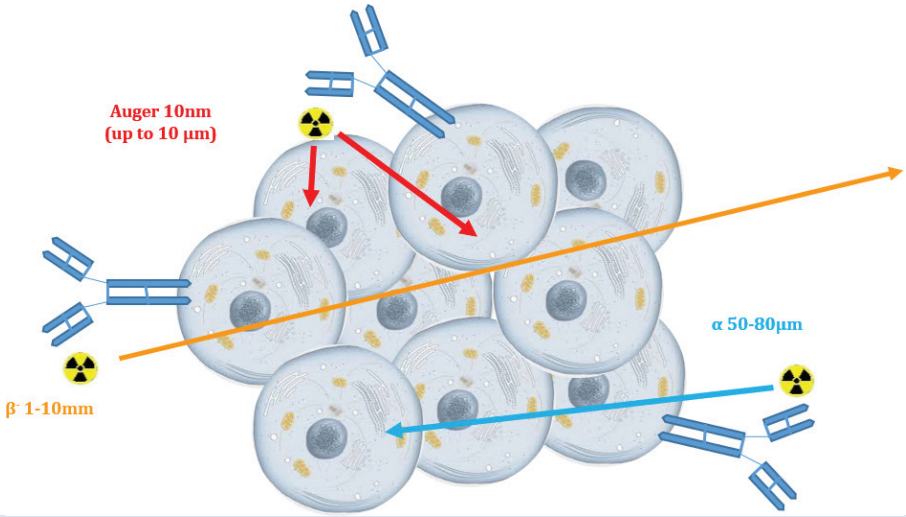

Fig. 1 Representation (not to scale) of the range of various charged particles in tissue.

Despite the therapeutic potential of Auger emitters, they have been the focus of limited research. The major deterrent is the common belief that their short decay range necessitates transport inside the cell to exert a significant therapeutic effect. Furthermore, initial calculations predicted only one single-strand DNA break per decay of most Auger emitters [8]. More recent work, however, has shown through Monte Carlo simulations that multiple single strand and double strand DNA breaks are possible due to indirect effects of Auger cascades, including creation of radical species that can damage DNA [9].

In 2001, Bernhardt *et al.* identified five Auger-emitting radionuclides as ideal candidates for TRT, based on their low photon-to-electron ratio, electron energy, half-lives, production capabilities, and chemical properties [10]. Among these candidates was ^{119}Sb, with a half-life of 38.1 h and 100% decay through electron capture. This enables ample time for delivery of the radionuclide and the lack of photon emission reduces dose to surrounding tissue [11]. Antimony-119 can be easily produced via the ^{119}Sn(p,n)^{119}Sb reaction on small medical cyclotrons with proton energies between 6-13 MeV. Estimated cross-sections are 400-600 mb for 10-13 MeV protons [11]. Despite favourable decay properties and a feasible production route, little research has been focused on the use of ^{119}Sb.

This article will briefly describe the recent efforts of a collaboration between the Life Sciences Division at TRIUMF, the Medicinal Inorganic Chemistry Group at the University of British Columbia (UBC) Chemistry Department, the University of Wisconsin-Madison, and the Joint Institute for Nuclear Research (JINR, Dubna) on the production of Sb isotopes from natural Sn targets, subsequent separation chemistry, and proof-of-principle chelation (radiolabeling).

TABLE 1

NATURAL ABUNDANCES OF STABLE ISOTOPES OF TIN. [12]

ISOTOPE	NATURAL ABUNDANCES (%)
Sn-112	0.900(3)
Sn-114	0.61(1)
Sn-115	0.350(6)
Sn-116	14.07(8)
Sn-117	7.54(3)
Sn-118	23.98(3)
Sn-119	8.620(3)
Sn-120	33.03(12)
Sn-122	4.78(1)
Sn-124	6.110(6)

METHODS

Cyclotron Production

Antimony radioisotopes were produced by a 60 min irradiation of a 0.1 mm thick natural Sn foil with approximately 13 MeV protons and 5 μA current. The natural abundance of Sn isotopes is shown above in Table 1 [12]. This leads to the production of a variety of radio-Sb isotopes including ^{117}Sb ($t_{1/2}$ 2.8 h), ^{118}Sb ($t_{1/2}$ 5.0 h), ^{119}Sb ($t_{1/2}$ 38.1 h), and ^{120m}Sb ($t_{1/2}$ 5.76 d). Of interest to us were ^{117}Sb which decays to ^{117m}Sn ($t_{1/2}$ 14 d) and ^{120m}Sb, which we used as tracers of Sb and Sn in radiochemical processes. These radionuclides have longer half-lives, enabling more time to work with produced material and better gamma emissions for detection with a high-purity

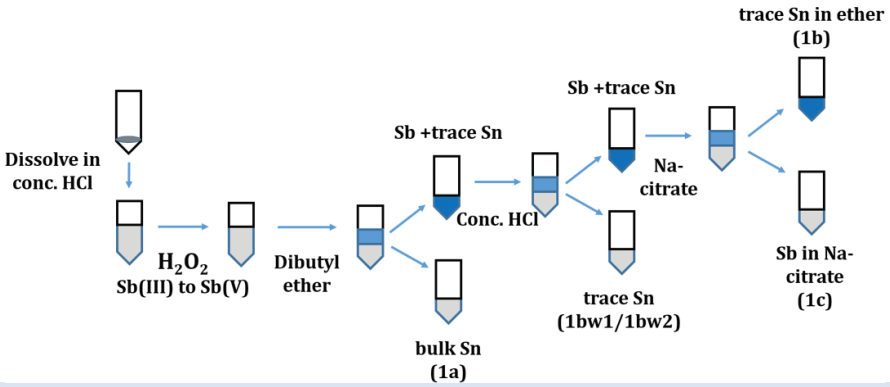

Fig. 2 Schematic of liquid-liquid extraction procedure to separate Sb from Sn target material after dissolution in concentrated hydrochloric acid.

germanium (HPGe) detector. A typical run produced about 1.5 MBq of ^{120m}Sb at end of bombardment (EOB).

While irradiation of natural tin targets is sufficient for research purposes, an enriched ^{119}Sn target would be ideal in future production of ^{119}Sb radiopharmaceuticals, to maximise ^{119}Sb yield and minimise the presence of impurities. Our collaborators at the University of Wisconsin-Madison have been investigating the production of thick enriched ^{119}Sn targets through electrodeposition from simple acidic solutions, as well as recovery of target material post-irradiation. At present, they have achieved successful deposition of natural tin with mass exceeding 500 mg/cm^2 [13]. Targets of this mass are thick enough to stop a 16 MeV proton beam, which is important for maximising production, and have withstood up to 40 μA of current. For these irradiation parameters, a 95% enrichment of ^{119}Sn is expected to produce a tenfold increase in the yield of ^{119}Sb, a significant amount suitable for radiopharmaceutical production [13].

Chemical Separation

To chemically separate the radio-Sb from bulk Sn, our process requires dissolution of the foil target in concentrated hydrochloric acid (HCl). Initial work involved establishing and standardizing a liquid-liquid extraction procedure, whose groundwork was developed by collaborators at the JINR in Dubna, Russia. Later work involved transferring to an ion-exchange separation strategy to improve reproducibility of separations and reduce the amount of hands-on work (and thus hand dose) involved.

Liquid-Liquid Extraction

Liquid-liquid extraction is a routine separation technique in chemistry. The general principle is to separate substances based on their solubility in two immiscible liquids (i.e., an organic (non-polar) and aqueous (polar) solvent). Non-polar substances will be more soluble in the organic solvent while polar substances will be soluble in the aqueous solvent. This was the basis for the liquid-liquid extraction procedure developed. It should be noted that while our current understanding of this established process has allowed us to form a number of hypotheses around the observed phenomenon, we have yet to empirically confirm exact chemical speciation. Below is an account of our current understanding based on qualitative observations.

In this procedure, Sb was extracted from the HCl target solution first into an ether solution, then back-extracted into an aqueous solution, as subsequent radiolabeling is preferred in aqueous solvents over organic solvents. Figure 2 shows a schematic of the separation procedure. To begin, hydrogen peroxide (H_2O_2) was added to the target solution to oxidize Sb from Sb(III) to Sb(V), and Sn from Sn(II) to Sn(IV). After oxidation, dibutyl ether was added in equal volume to the target solution. After sufficient mixing, the phases were allowed to settle so that the ether rested above the HCl. The ether, containing Sb(V) and trace Sn(IV), was transferred to another vial. Two washes with equal volumes of concentrated HCl removed the remaining Sn(IV). After the second wash, dilute sodium citrate (Na-citrate) was added in equal volume to the ether to back-extract Sb(V) into aqueous Na-citrate, with any trace Sn(IV) remaining in the ether.

To track the movement of Sb and Sn during the separation, aliquots were taken from the target solution, the first HCl extract (1a), the first HCl wash (1bw1), the second HCl wash (1bw2), the ether after back-extraction (1b), and Na-citrate after back-extraction (1c). These aliquots were each placed in a HPGe detector and counted to identify which radionuclides were present. The 197.3 keV gamma line from ^{120m}Sb (γ = 0.87) and 158.6 keV gamma line from ^{117m}Sn (γ = 0.86) were used to quantify the amount of each isotope present. Five sequential runs resulted in an average yield of 81.7 ± 0.1% of ^{120m}Sb in the final product.

Ion-Exchange Extraction

While the liquid-liquid extraction resulted in several successful separations, the process relied heavily on the user and the distinction between the organic and aqueous phases, which was

often inconsistent. If there was greater overlap in the phases, less of the Sb-containing phase was taken to prevent additional contamination with removed Sn, resulting in a reduced activity of ^{120m}Sb in the final product. Ion-exchange extraction offered a more reproducible and robust method of separation.

Solid-phase extraction involves passing the initial solution through a resin upon which the desired compound is absorbed while all other contaminants pass through. Once adsorbed, the desired compound can then be eluted from the column with another solvent. A particular type of solid-phase extraction is ion-exchange chromatography, in which the resin adsorbs certain species based on their charge. In cation-exchange chromatography, the resin adsorbs positively charged species, while anion-exchange does the opposite.

For the separation of Sb from Sn, a cation-exchange resin was used based on results reported by Kraus *et al.* [14]. This method requires the oxidized states of Sb and Sn, as Sb(V) will be slightly better retained than Sn(IV) on the resin. Figure 3a shows a schematic of the separation using cation exchange. All solutions passing through the column were collected in 1 mL fractions that were later counted in the HPGe detector to follow the movement of ^{120m}Sb and ^{117m}Sn. Similar to the liquid-liquid extraction procedure, H_2O_2 was used to oxidize Sb(III) to Sb(V) and Sn(II) to Sn(IV). The target solution was then loaded onto the cation exchange column which was preconditioned with various concentrations of HCl and deionized distilled water. The loaded target solution was allowed to drip through by gravity. The column was then washed with concentrated HCl to remove any trace Sn(IV) from the resin. Finally, the Sb(V) was eluted with sodium hydroxide (NaOH). The results of one run are shown in the elution curve in Figure 3b. Initial runs were very promising with greater than 95% of ^{120m}Sb in the NaOH fractions, and no detectable ^{117m}Sn.

Chelation

As mentioned previously, TRT relies on the chelation of a radioisotope with an organic molecule, known as the (bifunctional) chelator or ligand, which can then be attached to a bio-molecule of some kind. Identifying a chelator that can securely bind the radioisotope can be difficult, and depends on the charge, size, and chemical properties of the radioisotope, as well as reaction conditions such as time, temperature, pH, and concentration. To quantify radiolabeling yields, radio-thin-layer-chromatography can be used. Radio-TLC is similar to solid-phase extraction where a thin sheet (usually aluminum or paper) with a thin layer of adsorbent material (usually silica gel) is spotted with a small amount of a mixture solution and placed in a developing solvent. The components of the mixture will migrate different distances up the sheet based on their interaction with the adsorbent material and solvent. In radio-TLC migration of the radioisotope is tracked using a gas-proportional counter that can scan the plate and detects any betas/gammas emitted as a function of distance on the plate. The complexed radioisotope will often remain at the baseline whereas the free radioisotope will be carried up the plate.

To attempt to chelate Sb, a trithiol ligand (provided by Dr. Sylvia Jurisson and Yutien Feng from the University of Missouri) was used. This ligand had been used to successfully complex As(III), which sits just above Sb on the periodic table. For testing of chelation, the final back-extract from the liquid-liquid extraction containing radio-Sb(V) in a Na-citrate solution was used. Initial experiments in which Sb(V) was directly labelled were unsuccessful. Radiolabeling following reduction of Sb(V) to Sb(III) produced promising results. The first run yielded 66% complexed ^{120m}Sb, calculated by dividing the counts under the complexed peak by the total counts in the spectrum.

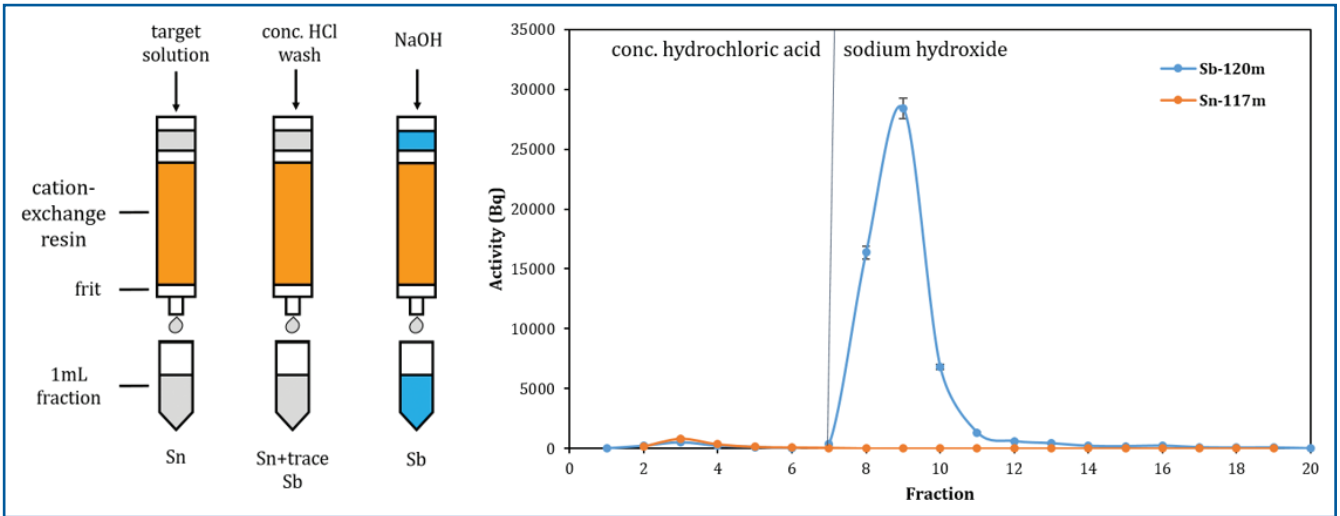

Fig. 3(a) Schematic of separation of Sb from Sn using a cation exchange resin after dissolution of target in concentrated hydrochloric acid. Sb was eluted with sodium hydroxide. (b) Elution curve showing activity of tracers ^{117m}Sn and ^{120m}Sb in each fraction collected. Fractions 1-6 contained the target solution and washes in hydrochloric acid. Fractions 7-20 contained the sodium hydroxide elutions.

FUTURE DIRECTION

After demonstrating production, various potential separation strategies, and chelation of ^{120m}Sb as a tracer of ^{119}Sb, future work will focus on optimizing these procedures. Work on the electrodeposition and recycling of Sn for future enriched ^{119}Sn targets continues as a priority at the University of Wisconsin-Madison to increase yields of ^{119}Sb. Experiments for the separation of Sb from Sn via cation exchange will look towards establishing a system that elutes the majority of the Sb from the column in a small volume of a solvent that is suitable for radiolabelling. While initial tests were encouraging, there is much work to be done on the chelation front, in terms of optimizing radiolabeling parameters (pH, time, temperature) with the trithiol ligand and exploring other potential chelators. Stability of the labelled complex and bi-functionalization of chelators can be examined for eventual *in vitro* and *in vivo* studies. This work will be the foundation to potentially bring ^{119}Sb radiopharmaceuticals into the clinic for cancer treatment.

ACKNOWLEDGEMENTS

Thanks to Prof. Silvia Jurisson and Yutian Feng from the University of Missouri for providing the trithiol ligand. Thanks to Linda Graham and David Prevost, the TR13 operators, for the irradiation and removal of countless targets.

TRIUMF receives funding from the National Research Council of Canada.

REFERENCES

1. M.J. Thun, J.O. Delancey, M.M. Centre, A. Jemal, and E.M. Ward, "The global burden of cancer: priorities for prevention", *Carcinogenesis*, **31**, 100-110 (2009).
2. P. Boyle, and L. Bernard, *World cancer report,* IARC Press, 2008.
3. A. Jemal, M.M. Center, C. DeSantis, and E.M. Ward, "Global patterns of cancer incidence and mortality rates and trends", *Cancer Epidemiology and Prevention Biomarkers*, **19**, 1893-1907 (2010).
4. K.D. Miller, R.L. Siegel, C.C. Lin, A.B. Mariotto, J.L. Kramer, J.H. Rowland, K.D. Stein, R. ALteri, and A. Jemal, "Cancer treatment and survivorship statistics", *CA: A Cancer Journal for Clinicians*, **66**, 271-289 (2016).
5. T.I. Kostelnik, and C. Orvig, "Radioactive main group and rare earth metals for imaging and therapy", *Chemical Reviews*, **119**, 902-956.
6. E. Devrim, I. Doddamane, and D. Cheng, "Targeted radionuclide therapy", *Cancers*, **3**, 3838-3855 (2011).
7. D.E. Milenic, E.D. Brady, and M.W. Brechbiel, "Antibody-targeted radiation cancer therapy", *Nature Reviews Drug Discovery*, **3**, 488 (2004).
8. A. Kassis, "Cancer therapy with Auger electrons: are we almost there?", *Journal of Nuclear Medicine*, **44**, 1479-1481 (2003).
9. S. Ftániková, and R. Böhm, "Monte Carlo calculations of energy deposition in DNA for Auger emitters", *Radiation Protection Dosimetry,* **92**, 269-278 (2000).
10. P. Bernhardt, E. Forssell-Anderson, L. Jacobsson, and G. Skarnemark, "Low-energy electron emitters for targeted radiotherapy of small tumours", *Acta Oncologica*, **40**, 602-608 (2001).
11. S. Mahdi, and M. Enferadi, "Nuclear medicine calculations on the production of 119Sb via various nuclear reactions", *Annals of Nuclear Energy*, **38**, 825-834 (2011).
12. J.R. White, and A.E. Cameron, "The natural abundance of stable elements", *Physical Review*, **74**, 991-1000 (1948).
13. A.P. Olson, P.A. Ellison, V. Radchenko, T.E Barnhart, and J.W. Engle, *Electrodeposition and recovery of Sn targets for 119Sb production*, 17th Workshop on Targetry and Target Chemistry [Abstract], 2018.
14. K.A. Kraus, D.C. Michelson, and F. Nelson, "Adsorption of negatively charged complexes by cation exchangers", *Journal of the American Chemical Society,* **81**, 3204-3207 (1959).