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REVIEW ARTICLE

Development of ^{225}Ac Radiopharmaceuticals: TRIUMF Perspectives and Experiences

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Abstract: ***Background:*** The development of radiopharmaceuticals containing ^{225}Ac for targeted alpha therapy is an active area of academic and commercial research worldwide.

Objectives: Despite promising results from recent clinical trials, ^{225}Ac-radiopharmaceutical development still faces significant challenges that must be overcome to realize the widespread clinical use of ^{225}Ac. Some of these challenges include the limited availability of the isotope, the challenging chemistry required to isolate ^{225}Ac from any co-produced isotopes, and the need for stable targeting systems with high radiolabeling yields.

Results: Here we provide a review of available literature pertaining to these challenges in the ^{225}Ac-radiopharmaceutical field and also provide insight into how performed and planned efforts at TRIUMF - Canada's particle accelerator centre - aim to address these issues.

Keywords: Actinium-225, targeted alpha therapy, isotope production, radiochemistry, radiolabeling, TRIUMF.

1. INTRODUCTION

Targeted radionuclide therapy using alpha-emitting isotopes combined with disease-specific targeting vectors (antibodies or peptides) has the potential to treat metastatic disease by delivering a source of cytotoxic radiation directly to targeted cells [1-8]. Given specific targeting, the short range and high cytotoxicity of alpha particles result in the destruction of nearby diseased cells with limited harm to healthy tissues [9]. Due to this potential, the development of alpha-emitting radiopharmaceuticals for Targeted Alpha Therapy (TAT) is an active area of academic and commercial research worldwide. Though one alpha-emitting radiopharmaceutical, Xofigo ($^{223}RaCl_2$), is approved for clinical use, the clinical approval of an alpha-emitting isotope combined with a disease-targeting biomolecule has yet to occur and these radiopharmaceuticals remain in the development stages. Several candidate isotopes for TAT are currently under clinical and preclinical evaluation, including ^{149}Tb, ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{224}Ra, ^{227}Th, and ^{225}Ac. ^{225}Ac is one promising candidate isotope for TAT due to its 9.9 day half-life - suitable for targeting with antibodies - and the emission of four alpha particles in its decay chain (it decays to stable ^{209}Bi via four alpha- and two beta-decays - Fig. **1**). ^{225}Ac can also be used as a generator of ^{213}Bi ($t_{1/2}$ = 45.6 min), itself a promising TAT isotope.

While several clinical trials have demonstrated the potential of ^{225}Ac or ^{213}Bi radiopharmaceuticals to treat advanced cancers [10-13], the development of these drugs faces many challenges that have slowed progress, including: 1) the limited supply of ^{225}Ac - currently only 63 GBq (1.7 Ci) globally annually - which could treat fewer than 1000 patients per year; 2) the need for adequate chemical purification processes required to separate ^{225}Ac from co-produced stable or unstable isotopes during production; and 3) the need for stable targeting systems with high radiolabeling yields and appropriate pharmacodynamics, the development of which is hindered by a limited understanding of actinium chemistry and complicated by the need to also successfully retain ^{225}Ac progeny isotopes at the target site despite recoiling daughter nuclei and changing chemistry as the decay chain progresses [14].

Each section of this review addresses one of these challenges. For each, we summarize the current literature including the current standard methods and any proposed alternatives, and also discuss how performed, planned, and potential activities at TRIUMF attempt to address these challenges facing the development of ^{225}Ac-radiopharmaceuticals.

2. PRODUCTION OF ^{225}AC

2.1. Existing ^{225}Ac Supplies

Current sources of ^{225}Ac are primarily derived from the build-up of ^{229}Th through the decay of ^{233}U stockpiles (see Fig. (**1**) of the ^{233}U decay chain). The majority of this ^{233}U ($t_{1/2}$ = 1.6 × 10^5 y) was produced between 1954 and 1970 *via* neutron irradiation of ^{232}Th while being investigated for its use in nuclear weapons and reactors that were never fully deployed [15]. Between 1995 and 2005, ^{229}Th ($t_{1/2}$ = 7340 y)

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generated from ^{233}U decay was extracted from stockpiles stored at Oak Ridge National Laboratory (ORNL, Oak Ridge, TN). This ^{229}Th now exists in two sources: one at ORNL (~5.55 GBq (150 mCi), or ~704 mg) [16] and another (1.7 (46 mCi), or 215 mg) [17] transferred to the Institute for Transuranium Elements (ITU, Karlsruhe, Germany). A third ^{229}Th source (5.55 GBq (150 mCi), 704 mg) obtained from Russia ^{233}U stockpiles exists at the Leipunskii Institute for Physics and Power Engineering (IPPE, Obninsk, Russia) [18]. These three sources serve as generators of ^{225}Ac and its parent ^{225}Ra ($t_{1/2}$ = 14.9 d) and act as the major ^{225}Ac sources worldwide, producing approximately 26.6 GBq (720 mCi) (ORNL) [19] and 13.1 GBq (350 mCi) (ITU) [17] of ^{225}Ac annually. While the IPPE source contains as much ^{229}Th as the ORNL source, reported values indicate ^{225}Ac production from this source is sporadic [18, 20]. Overall, the accepted global annual production from ^{229}Th is 63 GBq (1.7 Ci) [21-25].

While a key advantage of this production method is an ^{225}Ac product free of other actinium isotopes, 63 GBq (1.7 Ci) is insufficient to meet the current global demand for researchers and the development of new agents and will be even more inadequate should any ^{225}Ac therapies become deployed clinically [19]. From research into fundamental ^{225}Ac chemistry [9] to the most promising clinical trials [10], the development of ^{225}Ac radiopharmaceuticals is slowed by the small supply and resulting high cost that makes ^{225}Ac inaccessible to many researchers.

2.2. Leveraging Unique Facilities to Supply ^{225}Ac Research

Due to the high cost of ^{225}Ac, ^{225}Ac-radiopharmaceutical development at TRIUMF currently relies on internal production using the Isotope Separator and Accelerator (ISAC) Facility [26]. Commissioned in 2000, ISAC produces beams of rare isotopes for experiments primarily studying nuclear structure and nuclear astrophysics. Irradiation of uranium or thorium targets with protons at 480 MeV results in the production of a number of isotopes that are extracted into a heterogeneous ion beam. Isotope Separation On-Line (ISOL) mass-separates these isotopes to produce a homogeneous isobaric ion beam [27]. Isolation of mass 225 produces an ion beam containing ^{225}Ra and ^{225}Ac that is directed onto an aluminum target in which the isotopes are deposited at a depth of 20 nm (as determined by SRIM [28]). Etching of the aluminum post-implantation followed by separation of ^{225}Ra and ^{225}Ac on a solid phase extraction (DGA) resin provides both a primary ^{225}Ac fraction for immediate studies, as well as a number of subsequent ^{225}Ac batches isolated through the decay of ^{225}Ra. When eluted at the optimal time (every 17.5 days) this generator produces ^{225}Ac with an activity equal to 44.4% of the ^{225}Ra activity present at the previous elution[1]. Since 2015, ^{225}Ac production at ISAC has enabled radiolabeling and preclinical studies at TRIUMF. ISOL methods have also been applied at ISOLDE (CERN, Geneva) to produce ^{225}Ac for radiopharmaceutical development [29].

Though ISOL provides isotopically pure ^{225}Ac sources, yields remain insignificant compared to quantities available from ^{229}Th generators. At TRIUMF, the maximum measured ISAC beam intensities of 1.3×10^8 ions/s for ^{225}Ac and 1.6×10^8 ions/s for ^{225}Ra could theoretically produce up to 370 MBq (10 mCi) of ^{225}Ac per month[2], ISAC does not operate as a dedicated medical isotope production facility and ^{225}Ac production occurs within the overarching context of the laboratory's research program. Total ^{225}Ac production for 2016 was only 44.4 MBq (1.2 mCi).

In order to better accommodate medical isotope research, the European Organization for Nuclear Research (CERN) has launched the Medical Isotopes Collected from ISOLDE facility (MEDICIS), which plans to produce mass-separated isotope beams from offline targets starting in 2017 and with uranium targets for ^{225}Ac production available in late 2018 [29, 30]. As a dedicated medical isotope production facility, MEDICIS suggests the capacity to produce up to about 112 MBq (3 mCi) of ^{225}Ac per month[3].

2.3. The Need for New ^{225}Ac Production Methods

Estimates of current demand for ^{225}Ac are less than 185 GBq (5 Ci) per year, however, this is likely significantly tempered by both supply constraints and cost. While predicting future demand is difficult, it can be estimated to grow by about 200 to 400 GBq per year (about 5 to 10 Ci per year) for each ^{225}Ac-based therapy that is approved for clinical use[4]. Should efforts to develop ^{213}Bi-radiopharmaceuticals also increase, ^{225}Ac demands will be even higher.

While facilities like ISAC and MEDICIS can facilitate radiopharmaceutical development by providing access to medical isotopes that are otherwise challenging to obtain, their application will remain limited to medical isotopes in the development stages. Even with potential proposed upgrades to the MEDICIS facility that could enable monthly production of up to 1.7 GBq (45 mCi) of ^{225}Ac [29], and potential upgrades to ISAC that could increase yields by a factor of ~1000[5], these facilities are not expected to meet existing ^{225}Ac demand, are not commercially viable production sources, and could not

[1] 1 MBq of ^{225}Ra produces 1/(1-0.444) = 0.80 MBq of ^{225}Ac from the generator if it is eluted every 17.5 days. Calculations in this publication convert ^{225}Ra production values to ^{225}Ac production values using this conversion factor.

[2] For three 10-day implantations, ^{225}Ac yield = $3\times[1.3\times10^8(1-e^{-\lambda_{Ac}\times10d}) + 0.8\times1.6\times10^8(1-e^{-\lambda_{Ra}\times10d})]$ = 370 MBq (10 mCi).

[3] While no specific monthly production estimate is explicitly stated by Dos Santos Augusto *et al.* [29], the reported estimated activity from one target is 28 MBq (0.75 mCi) from targets exchanged on a "weekly basis", which is 112 MBq (3 mCi) monthly.

[4] Assuming four rounds of 1 μCi/kg doses for 10 000 patients per year per therapy, and one half-life for processing and transport.

[5] 10× from increasing proton beam current from 10 to 100 μA, 6× from increasing target thickness from 10 to 60 g/cm^2, 2× more efficient beam extraction, and 8.39× from replacing uranium carbide targets with thorium targets [31].

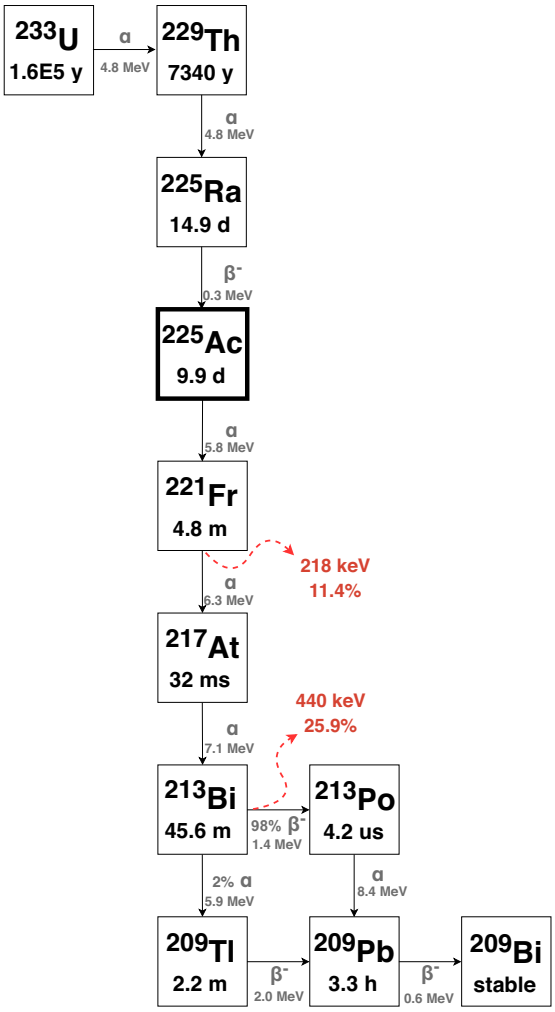


Fig. (1). Decay schematic showing the decay and production pathways for ^{225}Ac. Gamma emissions useful for quantification of ^{225}Ac are shown in red. *(The color version of the figure is available in the electronic copy of the article).*

supply enough ^{225}Ac to support the widespread use of a clinically approved therapy. These facilities should instead be viewed as valuable research enablers whose utility comes from their ability to provide quick access to a range of high-purity medical isotopes so that the feasibility of a given isotope's applications can be explored before having to build dedicated large-scale, isotope-specific production infrastructure.

While harnessing untapped ^{229}Th supplies has the potential to more significantly impact ^{225}Ac availability, in 2005 the U.S. Congress ordered the Department of Energy (DOE) to cease extraction of ^{229}Th from ^{233}U stockpiles and to instead begin down-blending (dilution with ^{238}U to a non-weaponizable ^{233}U concentration) and permanent disposal of the two tonnes of stockpiled ^{233}U [15]. Petitions to recover ^{229}Th before ^{233}U disposition have been denied [20], and completion of these efforts is scheduled for 2018 [15]. From the high- and intermediate-purity ^{233}U sources within the inventory [32], this represents the loss of 32.6 g (~260 GBq or 7 Ci) of ^{229}Th or a potential 1.5 TBq (40.5 Ci) of annual ^{225}Ac production. Other estimates suggest this is a loss of 37 g (~8 Ci) of ^{229}Th [16] and suggest a loss of 2.2 TBq (60 Ci) of annual ^{225}Ac production [20]. Without new ^{229}Th sources, ^{225}Ac production from current DOE ^{229}Th generators could increase by only 20% if the current elution schedule is optimized for ^{225}Ac production instead of unit cost [19]. While quantities of additional ^{229}Th-containing ^{233}U sources may exist in other countries (ex. Russia), quantities - to the best of our knowledge - are unknown.

Without the existence of significant additional ^{229}Th sources, the use of ^{225}Ac or ^{213}Bi in multiple approved therapies will require the development of new ^{225}Ac production methods. The remainder of this section aims to present a comprehensive list of alternative ^{225}Ac production options. The potential of each method to meet projected ^{225}Ac demand and the practical challenges associated with each method will be discussed. Possible production methods proposed in the literature are summarized in Table **1**, while Table **2** summarizes other nuclear reactions capable of producing ^{225}Ac but that are considered impractical at this time. When not derived from original sources, details of calculations are provided in footnotes.

2.4. Potential for ^{225}Ac Production in Nuclear Reactors

While the majority of medical isotopes today are sourced from nuclear reactors [33], the potential for reactor-based ^{225}Ac production is limited. The parent isotope, ^{225}Ra, can be produced in reactors via the ^{226}Ra(n, 2n) ^{225}Ra reaction, however, this reaction requires an intense source of high (>6.4 MeV) neutrons found only near the tail end of a typical breeder reactor neutron energy spectrum. Given that significantly more lower energy neutrons would be present, these irradiations would be dominated by the co-production of ^{227}Ac, a long-lived ($t_{1/2}$ = 21.8 y) and highly toxic actinium isotope, the presence of which in significant quantities may prevent the clinical approval of a pharmaceutical. To the best of our knowledge, this method has not been investigated experimentally or thoroughly modelled. However, rough estimates suggest this method could produce MBq to GBq (μCi to mCi) amounts of ^{225}Ac per month per gram of ^{226}Ra target material at a single reactor facility[6].

The potential to increase ^{229}Th stocks using reactors has also been investigated [36, 37]. Results suggest the irradiation of ^{226}Ra targets at a single reactor could produce 100 MBq (2.7 mCi) of ^{229}Th per month per gram of ^{226}Ra target material [36]. Other results have suggested this value may be closer to 59 MBq (1.6 mCi) [37]. While larger specific yields were seen when using ^{228}Ra and ^{227}Ac target materials (352 and 600 MBq (9.5 and 16.2 mCi) of ^{229}Th per month per gram, respectively), these isotopes cannot be supplied in sufficient quantities [36]. The ^{228}Th(n,γ)^{229}Th reaction is impractical for the same reason. For example, 2.5 thousand tonnes of natural thorium would have to be processed to produce a single gram of ^{228}Ra ($t_{1/2}$ = 5.8 y), which could potentially be used to slowly generate ^{228}Th ($t_{1/2}$ = 1.9 y).

Given the challenges and costs associated with safely handling large ^{226}Ra sources (see Section 2.7) and the result-

[6] Assuming two 15-day irradiations per month, 1 gram ^{226}Ra target, average cross-section of 2 barns over the 6.4 to 16.4 MeV range [34], and average neutron flux of 10^{12} n/s/cm^2 over the same energy range [35] produces 52 GBq (1.4 Ci) of ^{225}Ra or 44 GBq (1.2 Ci) of ^{225}Ac per month.

Table 1. Summary of current and potential future ^{225}Ac production methods. Production values for current sources list current production levels, while values for potential sources list estimates of maximum possible production at sample of existing and operational facilities that have dedicated stations for large-scale medical isotope production. Details of calculations or references to cited values can be found in the text. Values listed for ^{226}Ra targets assume a target mass of 1 g.

	Production Method	Facility	Capabilities	Monthly ^{225}Ac Production [GBq (Ci)]
Current Sources	**^{229}Th generator**	ORNL	0.704 g (150 mCi) of ^{229}Th	2.2 (0.06)
		ITU	0.215 g (46 mCi) of ^{229}Th	1.1 (0.03)
		IPPE	0.704 g (150 mCi) of ^{229}Th	2.2 (0.06)
Potential	**^{232}Th(p, x)^{225}Ac**	TRIUMF	500 MeV, 120 μA	11266.5 (304.05)
		BNL	200 MeV, 173 μA	2675.84 (72.32)
		INR	160 MeV, 120 μA	1002.0 (27.08)
		Arronax	70 MeV, 2×375 μA	462.1 (12.49)
		LANL	100 MeV, 250 μA	444.0 (12.00)
		iThemba LABS	66 MeV, 250 μA	127.7 (3.45)
Future Sources	**^{226}Ra(p, 2n)^{225}Ac**	20 MeV, 500 μA cyclotron		3983.1 (107.65)
		15 MeV, 500 μA cyclotron		1157.4 (31.28)
	ISOL	TRIUMF (existing)		0.37 (0.01)
		TRIUMF (potential upgrades)		190.6 (5.15)
	^{226}Ra(γ, n)^{225}Ra	medical linac	18 MeV, 26 μA	48.1 (1.3)
		ALTO	50 MeV, 10 μA	55.5 (1.5)
	^{226}Ra(n, 2n)^{225}Ra	fast breeder reactor		~37 (1)

Table 2. Possible yet impractical methods for ^{225}Ac production.

Production Method	Comments
^{226}Ra(p, pn)^{225}Ra	Yields insignificant compared to ^{226}Ra(p, n) ^{225}Ac production (10^5× less according to FLUKA simulation)
^{232}Th(p,4n)^{229}Pa	Low cross-section
^{nat}U(p, x)^{225}Ac	Produces ~10× less ^{225}Ac and ^{225}Ra compared to thorium spallation, creates fissile ^{239}Pu and ^{235}U, can handle less beam current than thorium spallation targets
^{232}Th(n, γ)^{233}U	Would take decades for ^{229}Th to build up
^{230}Th(γ, n)^{229}Th	^{230}Th not available in sufficient quantities
Reactor production of ^{229}Th	Potential target materials ^{228}Ac, ^{228}Ra, ^{228}Th, and ^{230}Th not available in sufficient quantities. Production yields from ^{226}Ra irradiation (110 MBq/month/g, or 3 mCi/month/g) too low considering cost and difficulty of ^{226}Ra source production.

ing low ^{225}Ra or ^{229}Th yields, reactor production of sufficient ^{225}Ac quantities would likely be prohibitively expensive.

2.5. Potential for ^{225}Ac Production Using Electron Accelerators

The use of the ^{226}Ra(γ, n)^{225}Ra reaction for ^{225}Ac production has been experimentally explored [38] and modelled [39]. These works have explored irradiating old radium needles on electron linear accelerators (linacs) found in most modern cancer centres. These linacs typically use electron beams incident on tungsten targets to produce bremsstrahlung x-rays for external beam radiation therapy. Experimental results measured the production of 2.44 MBq (66 μCi) of ^{225}Ac after a single-hour irradiation by 18 MV x-rays of a 20 mg of ^{226}Ra source located 12.5 cm from the tungsten target and with an incident electron beam of 26 μA average current [38]. This scales to a potential 48 GBq (1.3 Ci) of ^{225}Ac per month for a 1 g ^{226}Ra source, which could be potentially increased by irradiation parameter optimization. This method has the advantage of producing ^{225}Ac without contamination from other actinium isotopes. While co-production of ^{224}Ra occurs for photons above 12 MeV, this should not impact the desired ^{225}Ra/^{225}Ac generator as ^{224}Ra ($t_{1/2}$ = 3.7 d) decays to inert ^{220}Rn and does not result in the production of any Ac isotopes.

While many medical linacs capable of this ^{225}Ac production method may exist, these facilities are used for patient care and, to our knowledge, none are currently equipped with the infrastructure required for safe large-scale isotope pro-

duction and processing. Again, the 48 GBq (1.3 Ci) of ^{225}Ac per month would likely be cost prohibitive given the challenges (see Section 2.7) associated with a 1 g ^{226}Ra target. It has been estimated that linac-based ^{225}Ac production could be increased by up to a factor of 16, although accompanied by an increase in target mass [40].

Given this low yield, sufficient ^{225}Ac production via the ^{226}Ra(γ, n)^{225}Ra reaction would require the use of a facility with significantly higher electron beam current. Though none are dedicated medical isotope production facilities, some such facilities exist for which ^{225}Ac production values determined by scaling experimental medical linac irradiation results by electron beam current can be found in Table **1**. In addition, TRIUMF's planned Advanced Rare IsotopE Laboratory (ARIEL) facility will use a 35 MeV, 10 mA electron beam to produce intense high-energy x-rays for radioisotope production by photofission [26]. While the ARIEL electron target is intended for operation as an ISOL facility for fundamental research - not a medical isotope production facility - scaling experimental results for ^{225}Ra production on medical linacs to account for the higher current and different irradiation geometry suggests ARIEL could theoretically produce up to 74 TBq (2000 Ci) of ^{225}Ac per month from a 1 g ^{226}Ra target. However, how an isotope production target could survive a 100 kW beam is another unsolved problem - current designs for ARIEL ISOL targets only consider 50 kW. Other lower current electron accelerators, such as the existing 50 MeV, 10 μA ALTO electron accelerator (Orsay, France) [41], could theoretically produce up to 56 GBq (1.5 Ci) per month.

2.6. Potential for ^{225}Ac Production using Low Energy Proton Accelerators

The promising use of the ^{226}Ra(p,2n)^{225}Ac reaction to produce ^{225}Ac on low-energy proton accelerators was first demonstrated in 2005 by Apostolidis *et al.* [42]. This reaction has a high (710 mb) cross-section peak at 16.8 MeV and could thus be performed on the many low energy cyclotrons already in use worldwide for medical isotope production. An estimated >550 of these cyclotrons have an energy over 16 MeV, some of which operate at up to 500 μA [43]. Another advantage of this approach is that it would not coproduce ^{227}Ac. While the (p, n) reaction is expected to produce some ^{226}Ac ($t_{1/2}$ = 29.4 h), measurements of co-production of ^{226}Ac have not been reported from experiments found in the literature [42]. A simple FLUKA [44,45] simulation approximating the Apostolidis *et al.* experiment suggests an ^{226}Ac activity at end of bombardment (EOB) equal to ~11% the ^{225}Ac activity. However, unlike with ^{227}Ac contaminants, the ratio of expected ^{226}Ac to ^{225}Ac activity would decrease over time due to the differences in half-lives. The co-production of ^{225}Ra via the ^{226}Ra(p,pn)^{225}Ra reaction is expected to be negligible at the optimal energies required for direct ^{225}Ac production [46].

Given the high cross-section, large-scale production of ^{225}Ac via the (p,2n) reaction would be capable of meeting long-term demand for ^{225}Ac with only a single production site. Combining available cross-section data [42] with stopping power for ^{226}Ra [28] suggests a single 20 MeV, 500 μA proton beam incident on a ^{226}Ra target (~1 g) could produce a theoretical maximum of 4 TBq (108 Ci) of ^{225}Ac per month[7].

The use of low energy proton irradiation of ^{232}Th to produce ^{229}Pa, which decays to ^{229}Th, has also been explored [47]. The peak measured cross-section for this ^{232}Th(p, 4n)^{229}Pa reaction is 162 mb for a proton energy of 29.8 MeV. However, yields for this production method are low, with a potential to produce only 7.4 MBq (0.2 mCi) of ^{229}Th per month on a 50 MeV, 500 μA cyclotron.

2.7. Challenges Associated with ^{226}Ra Targets

All alternative ^{225}Ac production methods discussed so far have involved the use of ^{226}Ra as a target material. Cost-effective isotope production requires the use of stable or naturally occurring and long-lived target materials, and as the closest such isotope to ^{225}Ac, ^{226}Ra is one of only a few options - spallation reactions on naturally occurring ^{232}Th and ^{238}U being the only other possibilities. Other potential target materials such as ^{230}Th, ^{228}Th, ^{228}Ac, and ^{228}Ra are not available in large enough quantities to be of practical use.

Despite its potential, the use of ^{226}Ra targets poses significant challenges due to the availability of the isotope and safety hazards that complicate the target manufacturing, irradiation, processing, and recycling. Part of the ^{238}U decay chain, ^{226}Ra ultimately decays to stable ^{206}Pb and is typically found in equilibrium with most isotopes in its decay chain. ^{226}Ra was the first radioactive isotope discovered and was produced in large quantities from the 1920s for use in a number of medical and industrial applications until production stopped in 1960 [48]. Due to its high radiotoxicity, reactivity with water and air, and decay to the noble alpha-emitting ^{222}Rn gas, ^{226}Ra sources typically contained radium salts encapsulated in platinum [49]. The internal production of helium from the five alpha decays in the ^{226}Ra chain caused most of these sources to rupture, after which ^{222}Rn gases are released and ^{226}Ra salts can leak out. Even when sealed, the high energy gamma rays present from ^{226}Ra progeny present external radiation hazards, with a dose rate of 8.1 mSv/h at 1 m from a 37 GBq (1 Ci, 1 g) ^{226}Ra source.

While the use of ^{226}Ra sources declined after the health effects of radiation exposure became known and safer reactor-based isotopes became available, many ^{226}Ra sources remained in storage - primarily in hospitals - for decades. The hazards associated with the presence of ^{226}Ra sources lead many governments to push for the elimination of ^{226}Ra inventories and in 1996 the International Atomic Energy Agency (IAEA) established guidelines for the disposal of ^{226}Ra sources in long-term geological repositories [49]. This limits the availability of large ^{226}Ra quantities, with the IAEA estimating only a few kilograms of ^{226}Ra exist among

[7] Activity produced by a target completely stopping the incident proton beam can be calculated using energy-dependent values for stopping power, S(E), and cross-section, σ(E), using the following equation: $A(t) = \rho\phi(1 - e^{-\lambda t})$, given target density ρ, proton fluence ϕ and initial energy E_0, irradiation time time t, and product isotope decay constant λ. For this equation, a ^{226}Ra target density of 5 g/cm^2 was assumed, as well as an irradiation schedule of three 10-day irradiations to get a monthly production value. The integral was performed using fitted data in MATLAB.

these sources worldwide [49]. Typical medical sources contained <100 mg of ^{226}Ra, with some industrial sources containing up to 1000 mg quantities. For this reason, calculations in Table **1** assume 1 g as a reasonable upper limit on the size of potential ^{226}Ra targets.

New ^{226}Ra sources could be extracted from the waste of current uranium mining operations. Approximately 50 thousand tonnes of uranium ore is mined each year [50], from which ^{226}Ra is separated and disposed of as waste. With the potential to extract 257 mg of ^{226}Ra from each tonne of U_3O_8 [39], this amounts to about 12.85 kg of ^{226}Ra waste per year.

Whether obtained using old sources or through uranium mine tailings, the manufacturing of ^{226}Ra targets for ^{225}Ac production would require infrastructure beyond what is typically used to make medical isotope production targets. ^{226}Ra regulatory and safety issues - specifically those associated with ^{222}Rn - would also require additional infrastructure during the target irradiation, processing, and the necessary recycling of irradiated ^{226}Ra material. While ^{226}Ra targets have the greatest potential for ^{225}Ac production per gram of target material, difficulties and costs associated with these targets is a significant disadvantage of the ^{226}Ra(p,2n)^{225}Ac and ^{226}Ra(γ, n)^{225}Ra production methods.

2.8. Potential for ^{225}Ac Production via High-Energy Proton Spallation of Thorium

An alternative ^{225}Ac production method that avoids the use of ^{226}Ra targets involves the irradiation of natural thorium targets with protons above 70 MeV. This ^{232}Th(p, x)^{225}Ac reaction produces ^{225}Ac through a number of reaction pathways, though the total cross-section peaks ~40 times lower than for ^{226}Ra(p, 2n) ^{225}Ac production (for details on cross-sections, the reader is referred to: [51]). Thorium metal is the preferred chemical form for post-irradiation processing of the target, and isolation of MBq to GBq (μCi to mCi) quantities of ^{225}Ac from irradiated thorium metal has been demonstrated by both American and Russian research groups at Brookhaven National Laboratory (BNL, Brookhaven, NY), Los Alamos National Lab (LANL, Los Alamos, NM), and the Institute for Nuclear Research of the Russian Academy of Sciences (INR) [20, 22, 23, 51-58]. Unlike ^{226}Ra (3.7×10^{10} Bq/g, or 1 Ci/g), ^{232}Th (4.1×10^{3} Bq/g, 110 nCi/g) is not prohibitively radioactive, poses fewer radiological hazards and is readily available as a target material. Tens of kilograms are known to exist in stockpiles within a number of countries, and more thorium metal is able to be produced in bulk quantities from thorium oxide or thorium nitrate, hundreds of tonnes of which are produced annually worldwide as a by-product of rare-earth mining [50]. This availability means recycling of irradiated ^{232}Th target material may not be necessary. Another advantage of this method is that facilities already exist with demonstrated ability to perform target fabrication, irradiation, and processing. Examples of accelerator facilities capable of producing large amounts of ^{225}Ac via proton spallation are listed in Table **1**.

While spallation of naturally occurring ^{238}U will also produce ^{225}Ac, ^{232}Th irradiation is preferred for a number of reasons. The ^{238}U(p,x)^{225}Ac reaction cross-section is ~10 times lower (as modelled using FLUKA and GEANT4), and due to the higher density and lower melting point of uranium, thorium targets could more safely handle the higher heat load induced by higher beam currents. The co-production of fissile ^{239}Pu and ^{235}U is also avoided by ^{232}Th irradiation.

The spallation of thorium produces a number of isotopes other than ^{225}Ac. While this may provide an opportunity for recovery of other useful isotopes, it also complicates target processing by requiring the separation of dozens of elements. An overview of the different methods for isolating ^{225}Ac from irradiated thorium is described in Section 3.2.

Concerns exist in the field that the amount of ^{227}Ac co-produced from thorium spallation will prevent its use as a method for clinical-grade ^{225}Ac production. An ^{227}Ac to ^{225}Ac activity ratio of 0.1-0.2% is typically found in irradiated targets at end of bombardment. However, potential exists for current target processing methods to be modified to produce ^{225}Ac quantities that are free of ^{227}Ac by isolation of an radium-actinium generator [59]. Most methods already isolate radium from the irradiated thorium matrix, and if this is done days after EOB, only ^{228}Ra, ^{226}Ra , ^{225}Ra , ^{224}Ra, and ^{223}Ra will be present because of the length of their half-lives ($t_{1/2}$ = 5.7 y, 1600 y, 14.9 d, 3.6 d, and 11.4 d, respectively). Of these, ^{228}Ra and ^{225}Ra beta-decay to actinium isotopes, while the others alpha-decay to radon isotopes. Use of this mixture as a radium-actinium generator will produce ^{225}Ac free of ^{227}Ac. While ^{228}Ac ($t_{1/2}$ = 6.2 h) will be present after ^{225}Ra/^{225}Ac separation, the ratio of ^{228}Ac to ^{225}Ac activity (~0.88% at the time of optimal ^{225}Ac elution [59]) will decrease with time. After sufficient ^{228}Ac decay, ^{225}Ac could then be removed from the ^{228}Th ($t_{1/2}$ = 1.9 y) produced by ^{228}Ac decay to obtain a final ^{225}Ac product with significantly reduced radioactive impurities when compared to the directly produced ^{225}Ac fraction. While the total ^{225}Ac yield from this method will be reduced by a factor of about 10, it does not prevent the use of the directly produced, ^{227}Ac-containing batch of ^{225}Ac from being used for research or for use in ^{225}Ac/^{213}Bi generators.

Only a few existing accelerators can produce proton beams with a current and energy sufficient for large-scale ^{225}Ac production. A list of some of these facilities is given in Table **1** along with estimates of the maximum amount of ^{225}Ac each could produce per month. These values only include directly produced ^{225}Ac and exclude ^{225}Ac produced from ^{225}Ra generators that would increase production for each by roughly 10% to 20%. Without knowing details of each institutions's target irradiation facilities, all are compared based on their maximal yield estimates that assume a target station capable of handling a thorium target thick enough to completely stop the proton beam (the same assumption was made for ^{226}Ra (p,2n)^{225}Ac maximal yields)[8]. As a result, practical yields will be lower. For example, while TRIUMF's 500 MeV, 120 μA beam could theoretically produce 11.2 TBq (304 Ci) of ^{225}Ac per month, 3 TBq (82 Ci) of monthly ^{225}Ac production is a more practical limit given the existing target station's size and cooling capacity.

[8] Calculated using the equation in Footnote vii, using a target density of 11.72 g/cm^2 and three 10-day irradiations. Thorium stopping power was obtained from SRIM [28], thorium spallation cross-sections were obtained from EXFOR. [60].

Similarly, practical estimates for production at BNL and LANL are 165 GBq (4.5 Ci) per month [58].

2.9. TRIUMF Perspective on ^{225}Ac Production

Given the costs and challenges associated with ^{226}Ra targets, the existing facilities already capable of thorium target spallation, and the large number of successful thorium target irradiations described in the literature, we believe that the development of ^{225}Ac production via proton spallation of thorium is the fastest way to reliably meet the current global demand for ^{225}Ac and support the widespread clinical use of any future therapies.

To this end, TRIUMF is working towards the development of routine ^{225}Ac production via the irradiation of thorium metal targets on its primary beamline (BL1A), which delivers up to 500 MeV, 120 μA protons to the currently under-utilized legacy 500 MeV Isotope Production Facility (IPF). Since IPF is located directly before the BL1A beam dump, IPF routinely receives >100 μA of beam. Progress so far has included the low-level (2 μAh) irradiation of a prototype thorium oxide target in December 2016. The irradiation of thorium metal targets followed by processing on-site to isolate 370 MBq (10 mCi) of ^{225}Ac is anticipated for late 2017, followed by 3700 MBq (100 mCi) production in 2018.

3. RADIOCHEMICAL ISOLATION OF ^{225}AC

The most established production method is the ^{229}Th generator, which currently provides the main source of ^{225}Ac and ^{225}Ac/^{213}Bi generators used in preclinical or clinical trials. In addition, radiochemical procedures for several alternative strategies for ^{225}Ac production from thorium and radium targets are also discussed. Details on radiochemical aspects of ^{225}Ac/^{213}Bi generators are beyond the scope of this review, and the reader is referred to a review by Mogenstern *et al.* for additional insight into this area [24].

3.1. Isolation of ^{225}Ac from ^{229}Th Generators

Separation of ^{225}Ac and ^{225}Ra from ^{229}Th (Fig. **1**) is routinely performed at Oak Ridge National Laboratory (ORNL, TN, USA) [16], Institute for Transuranium Elements (JRC-ITU, Karlsruhe, Germany) [17, 61], and Leipunskii Institute for Physics and Power Engineering (IPPE, Obninsk, Russia) [62]. At ORNL, ^{229}Th (5.6 BGq, 150 mCi) stock is divided into several batches and separation occurs every three weeks. A four-step chemical recovery procedure is used, including the combination of anion and cation exchange columns in nitric and hydrochloric media [16]. An anion exchange resin is first used to remove the bulk thorium mass. A 5×60 cm^2 (1.2 L) column filled with MP1 (250±50 mesh) resin, allows the sorption of up to 30 g of thorium in 8 M HNO_3, while ^{225}Ac and ^{225}Ra pass through the column. This process is repeated using a second MP1 column (2×10 cm^2, 30 mL) in order to separate residual thorium quantities. The fraction containing Ac and Ra is evaporated to dryness and re-dissolved in 10 M HCl and then loaded onto the next anion exchange (MP1) resin, which allows purification of actinium and radium from iron and uranium micro-impurities. Final separation of ^{225}Ac from radium is performed on two cation exchange (AG50x4) columns in nitric acid media. Average recovery yield of ^{225}Ac is 80% over one campaign with radionuclidic purity of over 99%. The use of isolated ^{225}Ra as an additional ^{225}Ac source occurs by storing ^{225}Ra on a cation exchange resin for ^{225}Ac accumulation and subsequent separation.

At ITU, a similar strategy using anion exchange column for ^{225}Ac and ^{225}Ra separation from ^{229}Th is employed [17]. Due to the smaller ^{229}Th (1.7 GBq, 46 mCi) stock compared to ORNL, the production of clinically relevant ^{225}Ac quantities requires separation once every nine weeks after maximum ^{225}Ac buildup is achieved. Separation from thorium is performed on two anion exchange (AG1X8) columns (0.5 L and 80 mL) in 8 M HNO_3, where ^{225}Ac and ^{225}Ra first pass through without sorption and ^{229}Th is later eluted from the resin in 0.05 M HNO_3. The ^{229}Th fraction is evaporated to near dryness and re-dissolved in 8 M HNO_3 and mixed with anion exchange resin for storage until the next elution. The Ra and Ac fraction is subject to further purification from residual thorium via solid phase extraction chromatography, using three columns filed with diamyl, amylphosphonate (UTEVA) resin. Separation of Ra from Ac is then performed inoctyl(phenyl)-*N,N*-diisobutylcarbamoyl-methylphosphine oxide (RE-resin) resin in nitric acid. The reported recovery yield for ^{225}Ac is higher than 95% with a de-contamination factor from thorium of about 10^2. The same group also reports alternative separation of ^{225}Ra and ^{225}Ac via *N*,*N*,*N*',*N*"-tetrakis-2-ethylhexyldiglycolamide (DGA) based resin in nitric acid [61].

At IPPE, separation of ^{225}Ac and ^{225}Ra from ^{229}Th stock (5.6 BGq, 150 mCi) occurs on an anion exchange column in nitric acid followed by Ra/Ac separation on a cation exchange column in diluted nitric acid [18]. The final ^{225}Ac fraction is additionally purified with a combination of cation and anion exchange columns. A portion of the stock of ^{229}Th is loaded on an anion exchange column (Dowex 1X8, 0.5 L) in 8 M HNO_3. As previously described, Ac and Ra pass through the column, while ^{229}Th is retained. The column is additionally washed with 1 L of 8 M HNO_3 for complete Ac/Ra elution. ^{229}Th is then stripped with 2.2 L of 0.05 M HNO_3 and converted to 8 M HNO_3 (250-300 mL) for future use. The eluted Ac and Ra fractions are evaporated to dryness and re-dissolved in 0.5 M HNO_3 and passed through a cation exchange column (Dowex 50X8, 6 mL) on which ^{225}Ac is retained while ^{225}Ra passes through. The column is further washed with 1.5 M HNO_3 to remove residual ^{225}Ra isotopes. Actinium is desorbed with 8 M HNO_3 and converted to 10 M HCl for further purification. Finer purification of the ^{225}Ac solution is performed using anion and cation exchange columns in hydrochloric and nitric acids, respectively. The final actinium product is eluted in 10-12 mL of 8 M HNO_3. ^{225}Ac separation yield varies from 85-95% with $^{229, 228}$Th impurities below 10^{-5} of ^{225}Ac by activity.

Additionally, several alternative methods were previously reported for isolation of research quantities of ^{225}Ac and ^{225}Ra from ^{229}Th [63,64]. One purifies ^{225}Ac from ^{229}Th by chelation with citric acid at various pH on a cation exchange column [63]. The final separation scheme includes sorption of Ac, Ra, and Th on a cation exchange (Aminex A-5, 4×40 mm^2) column after loading with 0.25 M citrate (pH <1). Th was further eluted with ammonia citrate (pH 2.3) and Ac and Ra were eluted with ammonia citrate pH 4.5 and 4 M HNO_3, respectively. Another separation strategy based on

the combination of cation and anion exchange chromatography with mixed HNO_3-methanol solution was also tested [64]. This strategy provides a tandem system for consequent separation of ^{225}Ac from ^{229}Th followed by loading of the ^{225}Ac fraction on a generator for ^{213}Bi.

3.2. ^{225}Ac Isolation from Thorium Targets

As described in Section 2.8, the irradiation of thorium metal with protons above 100 MeV can produce large quantities of ^{225}Ac. The main challenges associated with the chemical isolation of ^{225}Ac from these targets are a limited number of facilities capable of handling targets containing large ^{225}Ac quantities (>10 GBq, or >0.3 Ci) and the challenging chemistry required for separating actinium from multiple grams of thorium and several hundreds of co-produced fission products. As discussed in Section 2.8, another drawback is the co-production of other actinium isotopes, most of which have relatively short half-lives (≤30 hours). One exception is the longest-lived Ac isotope, ^{227}Ac ($t_{1/2}$ = 21.8 y), co-produced at a rate of 0.1-0.2% when compared to ^{225}Ac activity. While the production of ^{225}Ac without ^{227}Ac is possible via the isolation of a ^{225}Ra/^{225}Ac generator (see Section 2.8), this further complicates the required chemical processing.

Strategies similar to those described in Section 3.1 that use anion exchange columns to retain thorium while eluting Ac and Ra can also be applied to the processing of thorium targets. However, this method requires a large volume of resin to remove the many grams of thorium used as target material - compared to the mg quantities used in ^{229}Th generators - as well as additional purification steps to remove other spallation products. While several strategies were developed and tested for separation of actinium and radium from thorium [54, 65], none of these provide actinium with the minimal impurities required for radiolabeling of biomolecules and clinical application.

More recently, two alternative separation methods were developed and tested for isolation of ^{225}Ac suitable for radiopharmaceutical application. Both methods are practically suitable for masses of thorium of up to 100 grams - larger ^{232}Th targets will require larger volumes of extraction and chelation agents. The first, described by Aliev *et al.* [23], uses a three-step procedure that includes liquid-liquid and solid phase extraction chromatography [23, 57, 66]. First thorium metal target was dissolved in nitric acid with a small portion of HF. Further, two extractions with Di-(2-ethylhexyl)phosphoric acid (HDEHP) were performed, where the bulk thorium mass was extracted into in the organic phase while actinium, radium and most spallation products were retained in aqueous phase. Following phase separation, the aqueous phase is passed through *N,N,N',N''*-tetrakis-2-ethylhexyldiglycolamide (DGA) column for separation of actinium and lathanides from fission products and radium in nitric acid media. The Ac and lanthanide fraction is then processed using TRU resin in 3 M HNO_3. Reported recovery yields for actinium are higher than 85% and the process was adopted to a hot cell environment for remote manipulation [23]. A modified strategy has also been applied to co-extract ^{223}Ra along with ^{225}Ac [67].

The second method was the result of an multi-institutional collaboration within the US DOE that included Los Alamos, Oak Ridge and Brookhaven National Laboratories [22, 68, 69]. This two-step procedure first removes the bulk thorium mass by chelating thorium and spallation products in 1 M oxalic acid at pH 2. At these conditions, all cations with charge 4+ and higher form negatively charged complexes, while cations with lower charge form positively charged complexes. Therefore, positively charged actinium and radium complexes are retained on the cation exchange resin while the bulk mass of thorium and majority of the fission products pass through the column without absorption. A similar strategy had also been previously used for ^{229}Th/^{225}Ac generators [63]. Ac and Ra are then eluted in 6 M HNO_3 and directly loaded onto a solid phase extraction chromatography column (*N,N,N',N''*- tetrakis-2-ethylhexyldiglycolamide (DGA branched). At loading conditions, 2+ charged cations (e.g. Ra and Ba) pass through the column without sorption and actinium and lanthanides are retained. Actinium can be separated from lanthanides by specifically eluting with 10 M HNO_3 from the DGA column, while lanthanides are retained under such conditions. Reported recovery yield of actinium is higher than 98% with high radionuclidic and radiochemical purity suitable for radiolabeling or generator application.

This strategy has been further improved by additional steps for the co-extraction of other valuable medical radionuclides - including $^{225, 224, 223}$Ra, ^{230}Pa, ^{103}Ru and ^{111}Ag - without disturbing the actinium purification process [70].

3.3. ^{225}Ac Isolation from ^{226}Ra Targets

Irradiation of radium with low energy protons and photons also results in ^{225}Ac production (see Sections 2.5 and 2.6). From a radiochemical separation standpoint, ^{225}Ac needs to be isolated from macroscopic amounts of radium isotopes and their daughters. Previously published work suggests the use of a two-step procedure for ^{225}Ac purification [42]. Irradiated ^{226}Ra targets are dissolved in 0.01 M HCl, followed by purification of Ac from the bulk Ra mass (plus small Po and Pb quantities) by sorption of ^{225}Ac on di(2-ethylhexyl) orthophosphoric acid (HDEHP) resin (LN-resin) in 0.01 M HCl. The column is washed with 0.1 M HCl and actinium was eluted with 2 M HCl. The second purification step uses 4,4'(5')-di-t-butylcyclohexano 18-crown-6 (crown ether) resin (Sr-resin) for removal of residual amounts of radium and its daughters. The actinium fraction was passed through the Sr-resin column in 2 M HCl. No radiochemical yields or specific values for radiochemical purities have been reported for this method reported, though radiochemical purity is stated to be similar to ^{225}Ac derived from ^{229}Th generators.

3.4. Status at TRIUMF

At TRIUMF, several production routes for ^{225}Ac are currently in use and under consideration. The collection of ^{225}Ra and ^{225}Ac ion beams at ISAC (see Section 2.2), is followed by a radiochemical separation consisting simply of one DGA column in nitric acid. However, chemical purity of ^{225}Ac is still under evaluation due to the possible stable impurities originating from the implantation target. As mentioned in Section 2.9, ^{225}Ac production via irradiation of sealed tho-

rium metal targets is also planned at TRIUMF's 500 MeV Isotope Production Facility, located on beamline 1A. While isolation of actinium and radium isotopes can be performed with one of the previously described strategies, any potential future full utilization of the high proton beam energy will require irradiation of large targets (>100 g). Therefore, alternative separation strategies are under evaluation. The upcoming ARIEL facility [26, 71] will also provide additional opportunity for irradiation of thorium targets with protons (480 MeV, 10-100 μA) as well irradiation of radium targets with photons. Design of radium targets and radiochemical separation of actinium from bulk radium are currently under consideration by our Life Sciences group.

4. TARGETING DESEASE WITH ^{225}Ac

4.1. Chemistry of ^{225}Ac and Radiolabeling Challenges

The lack of any stable actinium isotopes has restrained the advancement of actinium chemistry, and as a consequence the chemistry of Ac(III) is virtually unknown [72, 73]. Only very recently have some studies begun to elucidate the fundamental coordination chemistry of this highly radioactive element [74, 75]. Actinium isotopes are typically +3 ions with a documented ionic radius of 112 pm (CN 6) [76]; its large size is likely suited to large polydentate chelators of high denticities, since most commonly used chelates for Ac(III) range between 8-12 coordinate [72]. Actinium is similar to other actinides and rare earth elements, and can undergo hydrolysis in solution in the absence of a chelating agent to form $[Ac(OH)_{3-x}]^{x-}$; the sub-picomolar concentrations of ^{225}Ac will cause the hydroxide species in turn to form radiocolloids that bind to surfaces such as reaction vessels [77].

The emission of multiple alpha-particles in the ^{225}Ac decay chain (Fig. **1**) makes ^{225}Ac a particular effective isotope to kill cancer cells, y*et al*so makes the directed delivery of the nuclide and its decay daughters a challenge. Due to the conservation of momentum, the emission of an energetic alpha particle (energies shown in Fig. (**1**)) imparts a recoil energy to the daughter nucleus often >100 keV, 1000 times larger than the binding energy for any chemical bond [14]. This results in release of the daughter nuclide from the original delivery vector (Fig. **2**). The subsequent redistribution of the alpha- emitting daughter nuclides *in vivo* can cause substantial harm to untargeted healthy tissues and reduce the therapeutic effect. Consequently, renal toxicity induced by ^{213}Bi is considered to be a major constraint to the application of ^{225}Ac in a large number of clinical trials [78]. There are three main strategies for limiting the toxicity of recoil daughters in the literature: fast uptake and internalization of the alpha emitters in the target tissue, encapsulation of the nuclide in a nanoparticle, or local administration of radioactivity directly into the target site via injection [14]. Herein, a literature review of the first two strategies is included.

4.2. Chelating Agents for ^{225}Ac(III)

The discovery of a chelating agent that binds Ac(III) with sufficient stability and that also controls the release of its daughter nuclides remains a challenge. Moreover, limited global availability of ^{225}Ac and the absence of a stable surrogate nuclide has limited the study of this isotope to a handful of institutions around the world that have secured a reliable ^{225}Ac supply. The majority of initial ^{225}Ac chelation studies have focused on screening a variety of commercially available polydentate macrocyclic or acyclic ligands for their ability to bind ^{225}Ac and form stable complexes *in vitro* or *in vivo*. Despite the unique coordination preferences of the large +3 actinide, very few studies investigating new ligands specifically designed to coordinate Ac(III) can be found throughout the literature. A brief summary of ligands tested with ^{225}Ac can be found in Table **3**.

Early studies by Davis *et al*. Deal *et al*. and McDevitt *et al*. screened a library of ligands for their ability to coordinate ^{225}Ac and tested the resultant complex's *in vitro* or *in vivo* stability [77, 79, 80]. In the study presented by Davis *et al*., ligands EDTA (ethylenediaminetetraacetic acid, N_2O_4), CHX-A"-DTPA (cyclohexyl-diethylenetriaminepentaacetic acid, N_3O_5), and PEPA (1,4,7,10,13-pentaazacyclopentadecane-*N,N',N",N"',N""*-pentaacetic acid, N_5O_5) were radiolabeled with ^{225}Ac with radiochemical yields (RCY) of 80-90%. Biodistribution profiles over the course of 8 days for each of the purified ^{225}Ac-complexes were assessed by injecting 92 kBq (2.5 μCi) of each complex, and compared to the ^{225}Ac-acetate biodistribution as a control. Since uncomplexed ^{225}Ac accumulates predominantly in the liver with small amounts in the bone, kidney, and heart, high liver uptake of an ^{225}Ac-chelate indicates an unstable complex *in vivo*. CHX-A"-DTPA and PEPA reduced liver uptake of the ^{225}Ac-complex by more than 5.5 times compared to ^{225}Ac-acetate, and although their data suggests ^{225}Ac-CHX-A"-DTPA to be the most effective tested chelator complex with regard to its *in vivo* stability, improvements can still be made to further reduce non-target tissue accumulation [77]. As such, CHX-A"-DTPA provides inadequate chelation of

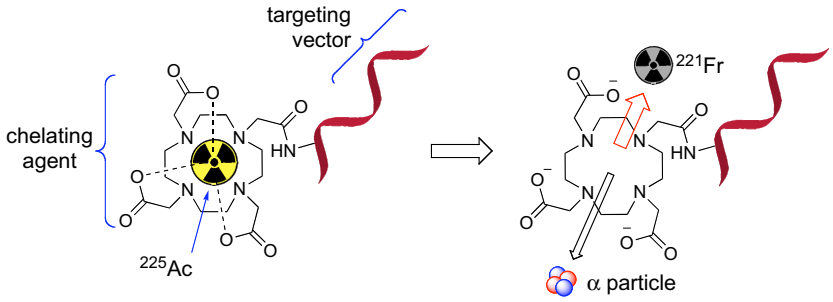


Fig. (2). Depiction of the recoil effect associated with α-decay of ^{225}Ac. Daughter isotope ^{221}Fr is released from the chelating agent due to the 100 keV recoil energy associated with the alpha emission of ^{225}Ac. ^{221}Fr and its decay daughters are consequently free to migrate within in the body.

Ac(III). Another important finding of the initial *in vivo* study was that the maximum tolerated dose of ^{225}Ac-CHX-A"-DTPA was less than 185 kBq (5 μCi), since at doses of 185 kBq (5 μCi) or higher, severe tissue damage was observed as early as 1 hr post-injection (p.i.), which ultimately lead to death causing 100% mortality by day 8 p.i. [77].

In search of a better ^{225}Ac chelator, Deal *et al.* developed a novel dodecadentate (coordination number, CN = 12) chelator with an extended macrocyclic ring to accommodate the large Ac^{3+} ion, HEHA (1,4,7,10,13,16-hexaazacyclohexadecane-*N,N',N",N"',N"",N""'*-hexaacetic acid, N_6O_6), and compared its *in vivo* stability to ^{225}Ac labeled EDTA, CHX-A"-DTPA, DOTA, and PEPA [80]. ^{225}Ac-HEHA demonstrated the highest complex stability evidenced by the low uptake of the complex in all tissues; essentially all radioactivity was excreted within 1 hour. Despite this perceived *in vivo* stability, the authors suggested the predicted -3 charge at physiological pH of the ^{225}Ac-HEHA complex may be mediating the fast excretion of the complex, giving it the appearance of stability since the radiometal ion doesn't have time to dissociate within the time frame of excretion [80]. Given these initial promising results, efforts towards the preparation of a bifunctional HEHA analogue were undertaken [81]. A C-functionalized isothiocyanate HEHA derivative was successfully prepared and conjugated to three monoclonal antibodies (mAbs): BL-3, T101, and CC49. One-step labeling of ^{225}Ac to HEHA-mAb incubated for 30 min at 37 °C, pH 7.0 produced moderate to high RCYs of 60-85% with specific activities of 7.4-14.8 MBq/mg (200-400 μCi/mg) (for ligand:mAb ratios > 1.0); these bioconjugates were sufficient for animal therapy studies. *In vitro* serum stability revealed the ^{225}Ac-HEHA-BL-3 mAb conjugate to be only 50% stable in serum after 24 h, suggesting that the HEHA system may very well not be a suitable chelator for sequestering ^{225}Ac [81].

McDevitt *et al.* screened the ^{225}Ac radiolabeling efficiency and *in vitro* stability of several polydentate chelators including DTPA (diethylenetriaminepentaacetic acid, N_3O_5), DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, N_4O_4), TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid, N_4O_4), DOTPA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrapropionic acid, N_4O_4), TETPA (1,4,8,11- tetraazacyclotetradecane-1,4,8,11-tetrapropionic acid, N_4O_4), and DOTMP (1,4,7,10- tetraazacyclododecane-1,4,7,10-tetramethylene-phosphinic acid, N_4O_4) [79]. Of the six ligands tested, only DOTA and DOTMP showed any complexation of ^{225}Ac after 2 h at 37 °C with RCYs of >99 and 78%, respectively. Subsequent *in vitro* stability assays in serum suggested that the ^{225}Ac-DOTA complex was robust, remaining >90% intact after 10 days, while the ^{225}Ac-DOTMP complex rapidly dissociated.

The initial promising *in vitro* stability of ^{225}Ac-DOTA motivated the authors to prepare conjugates of DOTA with antibodies HuM195 (anti-CD33), mJ591 and huJ591 (anti-PSMA), B4 (anti-CD19), and 3F8 (anti-GD2). The elevated temperatures required to achieve adequate labeling yield of Ac-DOTA were not amenable to antibody conjugates since such reaction conditions would denature proteins and result in loss of function. Consequently, a two-step labeling process was employed which required ^{225}Ac radiolabeling of the bifunctional DOTA-NCS ligand first, followed by mAb conjugation (pH 8.7, 37 °C for 52 min). Despite low overall radiochemical yields of only 9.8 ± 4.5%, reasonable specific activity (4.1 ± 2.6 GBq/g, or 0.11 ± 0.07 Ci/g) was achieved which would allow for preclinical therapeutic studies. Low yields were attributed to the first ^{225}Ac labeling step of DOTA-NCS which required heating and, consequently, degradation of the isothiocyanate linker resulting in poor mAb conjugation in the following step.

Attempts to increase the 2-step labeling yields of ^{225}Ac-DOTA-mAb conjugates via the modification of a more robust DOTA-linker chelate system have yielded some improvements [82]. Antczak *et al.* established a 2-step labeling protocol for thiol-based DOTA-linkers which provided a marked improvement compared to the DOTA- NCS 2-step method, with chelation yields of 95-99% and labeling yield up to 40%. In the same study, the authors incorporated an enzymatically cleavable linker which could minimize the toxicity associated with long-circulating mAbs to normal tissues by allowing the release of a small molecular weight radiometal-chelate complex from the mAb to promote fast clearance of the therapeutic nuclide. However, constructs resulted in high accumulation of ^{225}Ac in the liver in small animal models, indicative of ^{225}Ac release from the chelate [82].

Perhaps the most noteworthy improvement to ^{225}Ac radiolabeling to date was presented by Maguire *et al.* [83], which offered for the first time an efficient 1-step radiolabeling procedure for ^{225}Ac-DOTA-antibody constructs. Typical radiolabeling proceeded in 2 M tetramethyl ammonium acetate buffer (pH 7.5) with the addition of L- ascorbic acid as radioprotectant to the addition of DOTA-antibody construct and $^{225}Ac^{3+}$ with a typical final reaction pH of 5.8. Heating to 37 °C for 2 hours allowed a 10-fold increase in radiochemical yield (80%) compared to previous 2-step methods (6-12%), and resulted in the preparation of bioconjugates with up to 30-fold higher specific activities (120 GBq/g compared to 3.7-14.8 GBq/g) [83]. The highest specific activity achieved was equivalent to 1 actinium for every 25 antibodies. These results will likely have great implications in preclinical and clinical uses of ^{225}Ac labeled antibodies, since the ease of synthesis can more easily be translated in a clinical setting and the significant reduction of ^{225}Ac losses during labeling can save the cost of this rare and valuable isotope.

4.3. Bioconjugates for ^{225}Ac Labelling

Despite often requiring elevated temperatures and extended reaction times, promising stability in serum and efficient labeling of ^{225}Ac-DOTA complexes has cemented the commercial chelator and its bifunctional analogues as the most exploited chelator for ^{225}Ac thus far. With the relatively limited scope of chelates tested with ^{225}Ac to date (see Table **3**), and very few examples of specifically tailored Ac-chelates in the literature, it seems apparent there is much room for improvement in the field of Ac-chelation, particularly as more powerful spectroscopic and computational techniques evolve that will continue to help elucidate the

Table 3. Tested ^{225}Ac chelates coupled with targeting vectors for *in vitro* or *in vivo* application and their radiolabeling yields (RCY) and stabilities. Ligands rating system: red = not suitable for use; orange = adequate labeling, but likely unstable complex, or needs improvement; green = sufficient labeling and complex stability *in vitro* and *in vivo*, recommended for use.

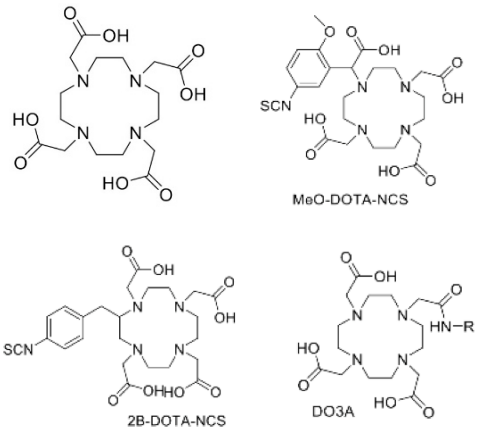
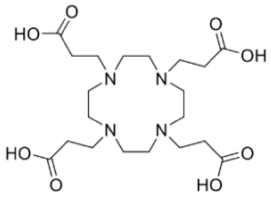
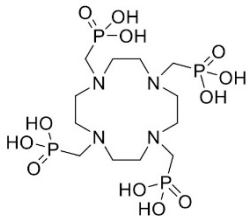
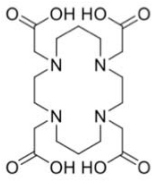
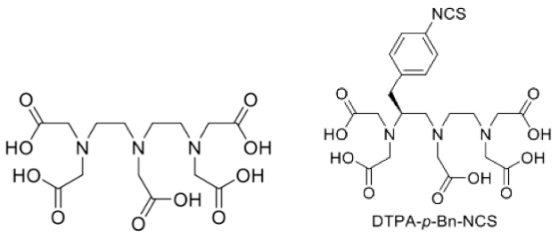
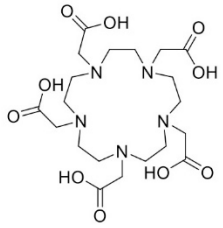
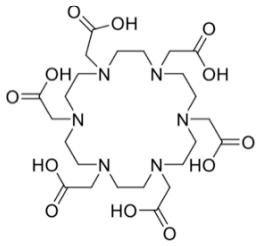
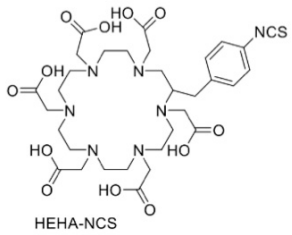
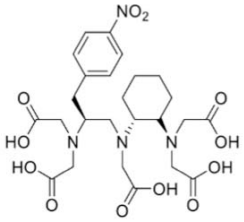
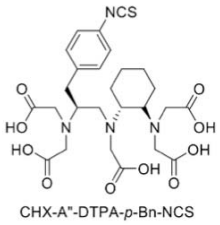
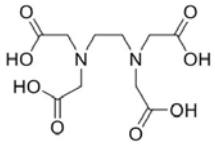
Chelate (and Corresponding Tested Bifunctional Analogues)	Donor Set (CN#)	Grade	Radiolabeling Conditions & RCY	*In vitro* or *In vivo* Stability	Ref.
DOTA 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid 	N_4O_4 CN = 8	Green-orange	0.02 M ligand, NH_4Ac pH 6, 37 ◦C, 2 h, RCY = 99%	*In vitro* human stability, 37 ◦C remained 90% intact after 10 days.	[79]
DOTPA 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrapropionic acid 	N_4O_4 CN = 8	Red	0.02 M ligand, NH_4Ac pH 6, 37 ◦C, 2 h, RCY = 0%	n/a	[79]
DOTMP 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylene-phosphinic acid 	N_4O_4 CN = 8	Red	0.02 M ligand, NH_4Ac pH 6, 37 ◦C, 2 h, RCY = 78%	*In vitro* human serum stability - rapid decom-plexation	[79]
TETPA 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetrapropionic acid 	N_4O_4, CN = 8	Red	0.02 M ligand, NH_4Ac pH 6, 37 ◦C, 2 h, RCY = 0%	n/a	[79]
DTPA diethylenetriaminepentaacetic acid 	N_3O_5, CN = 8	Red	0.02 M ligand, NH_4Ac pH 6, 37 ◦C, 2 h, RCY = 0%	n/a	[79]

Table (3) contd….

Chelate (and Corresponding Tested Bifunctional Analogues)	Donor Set (CN#)	Grade	Radiolabeling Conditions & RCY	*In vitro* or *In vivo* Stability	Ref.
PEPA 1,4,7,10,13-pentaazacyclopentadecane-*N,N',N'',N''',N''''*-pentaacetic acid 	N_5O_5, CN = 10	Red	0.01 M ligand, NH_4OAc pH 5.8, 40 ◦C, 30 min, RCY = 80%	inadequate stability *in vivo*	[80]
HEHA 1,4,7,10,13,16-hexaazacyclohexadecane-N,N',N'',N''',N'''',N''''',N'''''-hexaacetic acid   HEHA-NCS	N6O6, CN = 12	Orange	M ligand, NH4OAc pH 5.8, 40 ◦C, 30 min, RCY = >95%, or >98% after 2 h	Rapid whole body clearance in vivo, facilitated by -3 charge. Low liver uptake suggests stability over short time in body	[80, 81]
CHX-A''-DTPA   CHX-A''-DTPA-*p*-Bn-NCS	N3O5, CN = 8	Red	0.01 M ligand, NH4OAc pH 5.8, 40 ◦C, 30 min, RCY = >95%	In vivo decomplexation indicated by high liver uptake	[77, 80]
EDTA ethylenediaminetetraacetic acid  EDTA	N2O4, CN = 6	Red	0.01 M ligand, NH4OAc pH 5, 40 ◦C, 30 min, RCY = 80-90%	In vivo decomplexation indicated by high liver uptake	[77]

unique chemical differences between Ac^{3+} and other +3 actinides and lanthanides [74]. Nonetheless, ^{225}Ac labeled DOTA-small molecule [10], peptide [84-86], and antibody [78, 87, 88] conjugates have been tested in a variety of *in vitro* and *in vivo* preclinical studies, and a select few have seen clinical successes. Specifically, the ^{225}Ac-labeled humanized anti-CD33 (HuM195) mAb is in clinical trials for the treatment of patients with advanced myeloma [89]. Most recently a brief communication by Kratochwil *et al.* [10] reported remarkable treatment success in two patients with metastasized castration-resistant rostate cancer (mCRPC) who had extensive pretreatments and showed resistance to other therapies including beta-emitting radiopharmaceuticals. The small molecule ^{225}Ac-PSMA-617 (Fig. **3**) was administered bi-monthly at doses of 100 kBq/kg (2.7 μCi/kg); patient 2 saw complete remission after 3 treatment cycles.

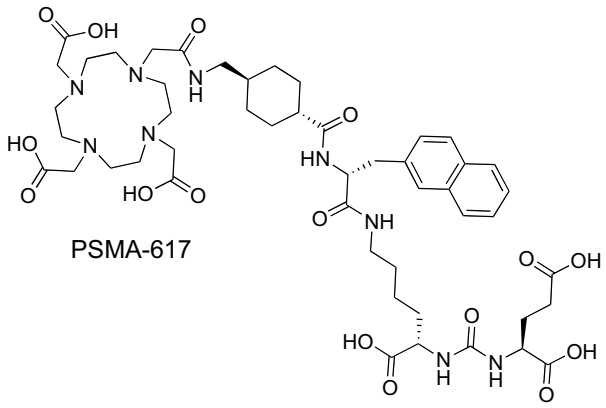


Fig. (3). DOTA-urea conjugate PSMA-617 used for targeting of prostate-specific membrane antigen.

4.4. ^{225}Ac Labelled Nanoparticles

To circumvent the inevitable loss of ^{225}Ac daughters after alpha decay from an actinium-chelate complex, researchers have sought to encapsulate the highly potent alpha-emitter into a nanoparticle structure. It is hypothesized that the $^{225}Ac^{3+}$ ion and its decay daughters can be retained within the cavity of the nanoparticle structure, while the alpha particles are released and able to deposit their therapeutic dose at the intended target site. However, the use of nanoparticles as a platform to affix radionuclides or other biomolecular targeting vectors comes with several limitations. The biodistribution of nanoparticles is dominated by their large size and ability to take advantage of the enhanced permeability and retention (EPR) effect of cancer cells, where "leaky" vessels of poorly vascularized tumours allow for the uptake and retention of large macromolecules [72]. Moreover, the relatively large particles are often primarily excreted through the hepatic pathway which can cause unwanted high liver uptake. The accumulation of a highly toxic alpha-emitter in the liver may cause damage to the organ. Much of the available literature describing ^{225}Ac-labeled nanoparticles provides *in vitro* data only [90-95]. Nonetheless, a brief overview of some strategies to prepare ^{225}Ac radiolabeled nanoparticles is found below.

Work by Matson *et al.* [94] investigated the encapsulation of ^{225}Ac 3+ ions in single-walled carbon nanotubes (SWNTs) by co-encapsulation of Gd^{3+} in an ion cluster. Although the Gd^{3+} ions remained inside the SWNTs, continual leakage of the $^{225}Ac^{3+}$ ions was seen when challenged with serum. McLaughlin *et al.* [90] employed a multilayered nanoparticle structure which can contain the recoiling daughters of the *in vivo* alpha generator at the centre cavity, while coupling the outer layer to antibodies and without preventing the release of emitted alpha-particles. The shells included a radiation resistant lanthanide phosphate crystal doped with ^{225}Ac and layered with a magnetic $GdPO_4$ layer, plus a gold outer shell for the attachment of targeting vectors. Polymer vesicles (polymersomes) composed of poly(butadiene-b-ethylene oxide) have also been used to encapsulate ^{225}Ac [92]. Preliminary *in vitro* studies in cells showed that smaller particles were absorbed by the cells and gathered around the cell nucleus. However, experiments and simulation indicated that larger polymerases are needed to attain satisfactory retention of recoiling daughters [92]. PEGylated liposomes loaded with ^{225}Ac and labeled with mouse antihuman PSMA J951 antibody or with the A10 PSMA aptamer were tested *in vitro* for their targeting, internalization, and cytotoxicity on a prostate cancer cell line [91, 95]. These studies demonstrated anti- PSMA targeted liposome loaded with ^{225}Ac can selectively bind, become internalized, and kill PSMA-expressing cells. Similarly, a ^{225}Ac-loaded lipid-based nanocarrier was labeled with a PSMA targeting antibody or small molecule urea-based agent, and the targeting selectivity and cytotoxicity were compared to the radiolabeled antibody on its own [95]. It was found that the loaded lipid vesicles improved the killing efficacy 3-fold compared to the same levels of activity per cell when delivered by the PSMA-targeting antibody.

4.5. Assessing the Biodistribution of the ^{225}Ac Decay Chain

While this discussion so far has been limited to the biodistribution of ^{225}Ac itself, assessment of the biodistribution of each alpha-emission in the decay chain is necessary when evaluating the performance of ^{225}Ac-radiopharmaceuticals. As previously mentioned in Section 4.1, the retention or redistribution of ^{221}Fr, ^{217}At, and ^{213}Bi at the target site impacts the efficacy and toxicity of the radiopharmaceutical. While the half-life of ^{217}At is short enough that its biodistribution can be assumed to be effectively identical to ^{221}Fr, the short ^{221}Fr half-life makes accurately determining its biodistribution - and also independently determining the biodistribution of its ^{213}Bi granddaughter - a challenge using conventional *ex vivo* counting methods. Speedy harvesting and counting of organs is essential, since while successive measurements of the same *ex vivo* tissue samples over time can be used to estimate the amount of ^{221}Fr or ^{213}Bi present at the time of sacrifice, the uncertainty in these estimates increases the longer after sacrifice the first measurements are made [14].

Imaging-based methods can also be used to assess the biodistribution of the radionuclides *in vivo*, and quantitative SPECT imaging of ^{225}Ac progeny isotopes has been demonstrated on small-animal SPECT/CT systems for ^{213}Bi alone [96], and for both ^{221}Fr and ^{213}Bi simultaneously, via their 218 keV and 440 keV gamma lines, respectively [97]. Unfortunately, quantitative imaging of the high energy ^{213}Bi photopeak (440 keV) requires the use of a high energy collimator not available on most imaging systems. However, qualitative SPECT imaging of ^{213}Bi has been performed clinically, as has qualitative ^{221}Fr SPECT in preclinical settings [11, 12, 90, 98, 99]. The use of Cerenkov imaging has also been demonstrated *in vivo* for the ^{225}Ac decay chain [86], though this imaging modality is incapable of quantitative biodistribution measurements and cannot distinguish between individual ^{225}Ac decay chain components.

While quantitative SPECT imaging of ^{221}Fr and ^{213}Bi with sub-millimeter spatial resolution has the potential to assess the retention of ^{225}Ac progeny within the tumour and determine uptake within whole organs [97], the short range (<100 μm) of alpha particles mean that information regarding the sub-organ biodistribution - a level of detail not provided by current *in vivo* imaging modalities - is necessary for alpha-particle dosimetry [100, 101]. While *ex vivo* imaging using alpha-cameras can determine ^{225}Ac biodistributions with spatial resolutions sufficient for dosimetry [102-106], alpha particle dosimetry itself faces additional challenges that currently limit the translation of preclinical dosimetric data to biological outcomes in the clinic [100, 101], a discussion of which is beyond the scope of this review.

4.6. Progress at TRIUMF

By leveraging our existing infrastructure TRIUMF has established ~37 MBq (1 mCi) annual production of ^{225}Ac via our ISOL facility (Section 2.2). This ^{225}Ac has enabled TRIUMF to conduct a variety of preclinical radiolabeling, complex stability, and imaging studies [97]. In particular, the apparent lack of chelating agents available to complex ^{225}Ac under conditions commensurate for "kit" type preparation of radiopharmaceuticals has motivated our researchers to search for alternate ligands which can quantitatively sequester the radioactive metal under mild, room temperature conditions with low ligand concentrations while forming thermodynamically stable and kinetically inert radiometal-chelate complexes. To this end, we are currently screening a wide

variety of macrocyclic and acyclic chelates for their potential as ligands in ^{225}Ac radiopharmaceutical development. Ligand candidates which display promising ^{225}Ac radiolabeling efficiencies and high *in vitro* stability will be conjugated to selected antibody or peptide targeting vectors, and small animal *in vivo* studies can be conducted.

CONCLUSION

With some recent and remarkable clinical results in the treatment of late-stage cancers using beta- and alpha-emitting radiopharmaceuticals, the community has experienced a surge in interest in certain therapeutic isotopes. Supply challenges, especially for isotopes such as ^{225}Ac, have limited the development of existing and emerging targeted radiotherapeutics, and pose a significant challenge in the discovery of new agents. That said, there exists a substantial amount of untapped production potential across a number of facilities.

Discussed within are the different ^{225}Ac production routes which span the spectrum of neutron (reactor), electron (gamma) and proton-induced reactions. At this time, the most promising short-term approach to greater availability of ^{225}Ac would be to irradiate thorium metal with high energy (>70 MeV) protons. By enable existing facilities with access to protons in this energy range to produce ^{225}Ac, it will allow for a focus on addressing unanswered questions on a specific activity, radiochemical and/or radionuclidic purity. There exists the possibility of exploiting the co-production of ^{225}Ra to assemble and distribute ^{225}Ra/^{225}Ac generators - a route that could help reduce the amount of ^{227}Ac in the ^{225}Ac used for radiopharmaceutical preparation. ^{225}Ac produced during the irradiation itself could be used for manufacturing ^{225}Ac/^{213}Bi generator. Beyond this, safety challenges will continue in the handling and processing of irradiated Th targets given their extensive radionuclide content, which can be mitigated by leveraging the experience and repurposing equipment from facilities designed to handle large quantities of radioactive material from reactor fuel and waste processing.

Given the widespread availability of lower energy (16-24 MeV) medical cyclotrons, there also exists the tantalizing possibility of large-scale, decentralized production across the fleet of the hundreds of medical cyclotrons in operation across the globe today. The key challenge, in this case, would be more on the procurement of sufficient quantities of ^{226}Ra, not to mention the safety implications of a failed irradiation in a hospital-based setting.

Adequate radiopharmaceuticals will also require the optimization of chelate design, with several advances having been recently reported. Enhanced supply will allow for the study and identification of compounds with the most suited pharmacokinetic profiles for therapy with minimized bystander organ dose or side effects. With clinical interest increasing, the most efficient way forward would be to enable increased production quantities by building or enabling existing facilities to produce isotopes such as ^{225}Ac at the bulk-scale. By using these facilities to supply sufficient and routine quantities of ^{225}Ac to advance existing clinical trials for compounds in clinical development - and should the early results seen to date represent results anticipated from a larger patient pool - there will undoubtedly be an increase in demand for more alpha-therapeutics toward more indications.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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