BNL-223911-2023-JAAM

Current State of 44Ti/44Sc Radionuclide Generator Systems and Separation Chemistry

C. E. Schmidt, V. A. Sanders

To be published in "Current Radiopharmaceuticals"

November 2022

Collider Accelerator Department

Brookhaven National Laboratory

U.S. Department of Energy

USDOE Office of Science (SC), Nuclear Physics (NP) (SC-26)

DISCLAIMER

Current State of $^{44}Ti/^{44}Sc$ Radionuclide Generator Systems and Separation Chemistry

Christine E. Schmidt[1,2]*, Leah Gajecki[3]*, Melissa A. Deri[1,2], Vanessa A. Sanders[3]

1. *Ph.D. Program in Chemistry, Graduate Center of the City University of New York, New York, NY 10016*

2. *Department of Chemistry, Lehman College of the City University of New York, New York, New York 10468*

3. *Collider Accelerator Department, Brookhaven National Laboratory, Upton, NY 11973 USA*

**These authors contributed equally to this work.*

Corresponding Author: Vanessa A. Sanders, P.O. Box 5000, Building #801, Upton, New York, 11973, Telephone (631)344-7230, Fax (631)344-5962, vsanders@bnl.gov

Abstract

In recent years, there has been an increased interest in $^{44}Ti/^{44}Sc$ generators as an onsite source of ^{44}Sc for medical applications without the need of a proximal cyclotron. The relatively short half-life (3.97 hour) and high positron branching ratio (94.3%) of ^{44}Sc makes it a viable candidate for positron emission tomography (PET) imaging. In this review, current $^{44}Ti/^{44}Sc$ generator designs are discussed, focusing on their chemistry, drawbacks, post-elution processing, and relevant preclinical studies of the ^{44}Sc for potential PET radiopharmaceuticals.

Keywords: Scandium, titanium, generators, PET, radioisotope production, diagnostics, radiolabeling, separations

1. Introduction

Scandium-44 (^{44}Sc) has been identified as a promising radionuclide for positron emission tomography (PET) imaging. ^{44}Sc possesses advantageous decay characteristics suitable for PET imaging that complement other known PET imaging radionuclides (***Table 1***). The 3.97 hour half-life makes it a suitable candidate for labeling small to medium biomolecules (peptides, antibody fragments, etc.). ^{44}Sc has shown improved image resolution compared to the analogous ^{68}Ga systems (full-width at half-maximum of 2.30 ± 0.12 mm vs. 2.48 ± 0.22 mm, respectively) as would be expected with the lower average positron energy of ^{44}Sc compared to ^{68}Ga (632 keV versus 830 keV, respectively).[1-3] Additionally, a benefit to using ^{44}Sc is the capability for longer imaging times due to its slightly longer half-life compared to the 1.13 hour half-life of ^{68}Ga.

Table 1. *Decay properties of ^{44}Sc compared to select clinically relevant PET imaging isotopes.[3]*

Radionuclide	Half-life	$E_{\beta+}$ Average (keV)	$I_{\beta+}$(%)	Eγ (keV)	Production Route
^{11}C	20 min	386	99.8	-	^{14}N(p,α)^{11}C
^{18}F	110 min	250	96.7	-	^{18}O(p,n)^{18}F
^{44}Sc	3.97 h	632	94.3	1157, 1499	^{44}Ca(p,n)^{44}Sc ^{44}Ca(d,2n)^{44}Sc ^{44}Ti/^{44}Sc generator
^{64}Cu	12.7 h	278	17.6	1346	^{64}Ni(p,n)^{64}Cu
^{68}Ga	68 min	830	88.9	1077	^{68}Ge/^{68}Ga generator
^{89}Zr	78.4 h	396	22.7	909, 1713	^{89}Y(p,n)^{89}Zr

The use of scandium isotopes in radiopharmaceuticals is also favorable due to the similar coordination chemistry of Sc(III) to that of the lanthanides and Ga(III), making labeling studies more straightforward as labeling protocols using DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and DOTA derivatives have already been established.[1, 4-7] Additionally, another isotope of scandium, ^{47}Sc, is of interest for therapeutic applications due to its beta particle emissions with energies of 143 keV (64.8%) and 204 keV (31.6%).[8] Thus, these chemically identical radioisotopes of scandium have the potential to be used as a theranostic matched pair by being chelated using the same bifunctional chelator.[8, 9]

What makes ^{44}Sc even more attractive as a PET imaging radionuclide is its potential to be produced and isolated from a generator system. Radionuclide generators provide a convenient and quick local source of a short-lived daughter nuclide from the decay of a long-lived radionuclide parent in a small self-contained system, making it a widely available source for hospitals and research institutions regardless of location. The cost of a cyclotron used for PET isotope production is around 1.6-2.1 million dollars, so a generator may also be a more financially viable option for facilities without an existing cyclotron.[10] In addition, cyclotron production of radionuclides involves the co-production of other isotopes that then require exhaustive post irradiation purification to isolate the isotope of interest in a pure and usable form. The potential of radionuclide generators to supply medical radionuclides in an efficient and easy manner has been driving research in this area over the past few decades.

PET imaging has become an effective method for diagnostics in the medical field, and while there are many PET imaging facilities available worldwide, there are far fewer cyclotrons to provide adequate imaging isotopes at these locations. The exact number of PET and PET/CT scanners available worldwide in 2022 is not established, but some values from the early 2000s are available. In 2008, there were about 700 cyclotrons installed worldwide.[11, 12] In 2015, Goethals et. al reported an increase to 1218 cyclotrons with around 1050 of them being small medical cyclotrons (<20 MeV) present in hospitals, universities, and small-scale commercial radionuclide production plants.[13] A 2009 study estimated that approximately 2000 PET/CT scanners were installed in the United States alone, and 350 were installed in Europe.[14] This discrepancy in the number of PET imaging facilities versus cyclotrons demonstrates the need for onsite radionuclide generators to produce necessary imaging isotopes.[15-17]

Isotope procurement through generator production has many benefits over irradiation production. Optimal generator systems allow for readily available and isolated isotopes at repeated time intervals, depending on the half-lives of the parent/daughter pair, in a pure and radiolabeling practical form. On the other hand, irradiation products need extensive post-irradiation processing to isolate the desired isotope, which often requires several time-consuming chromatographic separations. For radionuclides that have extremely short half-lives (e.g ^{82}Rb, $t_{1/2}$ = 75 s; ^{62}Cu, $t_{1/2}$ = 9 min), generators are the *only* onsite option available for production as shipping of the daughter isotope is not conceivable.

The success of radionuclide generators depends largely on the selection of parent/daughter pairs with appropriate half-lives, decay characteristics, and viable radiochemical separations. Ideally, the lifetime of the parent radionuclide is sufficiently longer than that of the daughter ($t_{1/2,p} \gg t_{1/2,d}$), which allows for the quantity of the daughter isotope to remain constant because its production rate (from the decay of the parent isotope) is equal to its decay rate; a condition known as secular equilibrium. Using parent-daughter pairs that are in secular equilibrium allows for frequent elutions of the generator yielding near quantitative activities of the daughter radionuclide without depletion of the parent, potentially creating a source of daughter radionuclide for the lifetime of the parent. In the case of transient equilibrium, the half-life of the daughter is not negligible compared to the half-life of the parent, and the quantity of daughter being produced slowly decreases over time. It is still possible to prepare generators with isotope pairs which reach transient equilibrium, but the yield of the generator noticeably diminishes over time.

There are several radionuclide generators currently in use for both research and commercial purposes to supply radionuclides for PET imaging. Examples of these generators include ^{68}Ge/^{68}Ga, ^{72}Se/^{72}As, ^{52}Fe/^{52m}Mn, ^{62}Zn/^{62}Cu, and ^{82}Sr/^{82}Rb (***Table 2*** and ***Table 3***). Shared traits of these generators include parent isotopes with much longer half-lives than the daughter isotopes, chemically viable separation of the isotopes using chromatographic methods, and straightforward succinct decay schemes. In order for a generator system to be ideal in a clinical setting, there are further requirements that need to be met: (1) there should be minimal to no parent isotope breakthrough observed, (2) the generator lifetime should be as long as the half-life of the parent, (3) the volume of the eluent required should be small (a few mL) to obtain the maximum activity in the smallest volume, and (4) the eluent should not require substantial post-elution purification (i.e. should be free of any competing organic binders and/or extreme pH levels not conducive to biological processes). A ^{44}Ti/^{44}Sc generator is an obvious candidate for addition to the existing

pool of PET imaging radionuclide generators listed, due to the promising PET characteristics displayed by ^{44}Sc and the generator conducive characteristics of the ^{44}Ti/^{44}Sc parent daughter pair.

Table 2. *Decay properties of select generator produced isotopes with PET imaging potential.[15, 18]*

Radionuclide	Half-life	$E\beta^+_{average}$ (MeV)	$I\beta^+$(%)	Generator Production
^{44}Sc	3.97 h	0.597	94.3	^{44}Ti/^{44}Sc
^{52m}Mn	21 min	1.13	97.0	^{52}Fe/^{52m}Mn
^{62}Cu	9.74 min	1.28	97.0	^{62}Zn/^{62}Cu
^{68}Ga	68 min	0.74	88.9	^{68}Ge/^{68}Ga
^{72}As	26 h	1.02	88.0	^{72}Se/^{72}As
^{82}Rb	1.27 min	1.41	95.0	^{82}Sr/^{82}Rb

Table 3. *Characteristics of a selection of radionuclide generators with potential for PET imaging.[15]*

Generator	Solid Support	Eluent	Parent $t_{1/2}$	Daughter $t_{1/2}$	Equilibrium
^{68}Ge/^{68}Ga	TiO_2	Dilute HCl	270 days	68 min	Secular
^{72}Se/^{72}As	Anion exchange resin	Dilute HCl	8.4 days	26 h	Transient
^{52}Fe/^{52m}Mn	Anion exchange resin	Concentrated HCl	8.3 days	21 min	Secular
^{62}Zn/^{62}Cu	Anion exchange resin	HCl/NaCl	9.19 h	9.74 min	Transient
^{82}Sr/^{82}Rb	SnO_2	Dilute NaCl	25.6 days	1.27 min	Secular

As seen in ***Figure 1***, ^{44}Ti decays by electron capture to ^{44}Sc, providing cyclotron-independent production of ^{44}Sc, which then beta decays with a high positron branching ratio (94.3%) to stable ^{44}Ca. The long half-life of the parent isotope ^{44}Ti (58.9 years) compared to that of ^{44}Sc (3.97 hours) allows for secular equilibrium to be reached, and potential daily elutions of ^{44}Sc from a long-lasting source of ^{44}Ti.

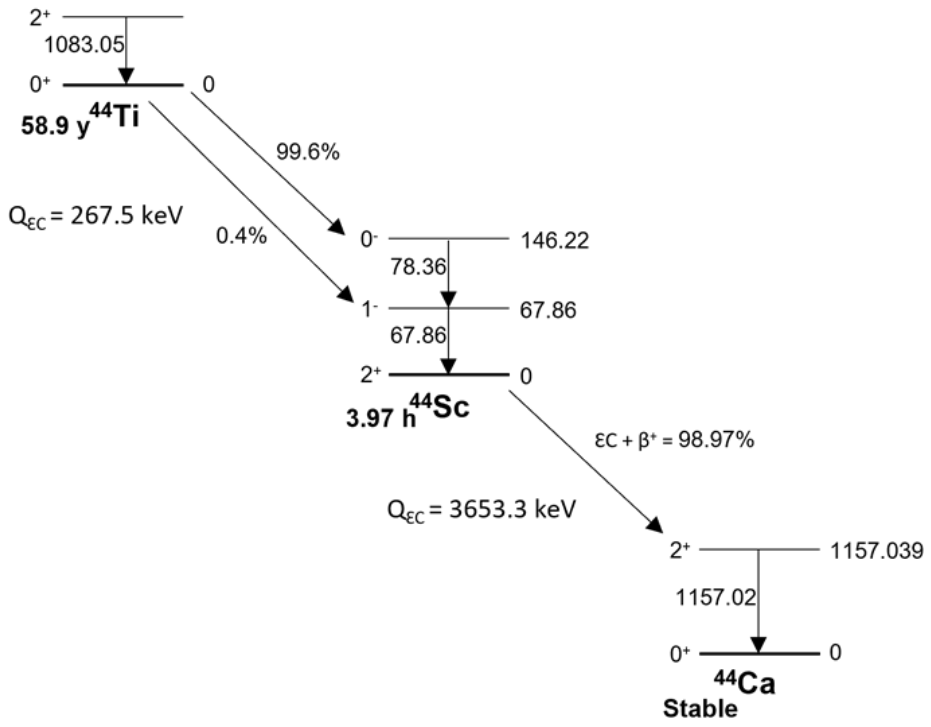

Figure 1. *Decay Scheme of ^{44}Ti and ^{44}Sc.*

Current literature suggests separation of ^{44}Ti from the natural ^{45}Sc target material is well developed, and isolation of appreciable amounts of pure ^{44}Ti is possible to construct ^{44}Ti/^{44}Sc generator systems. However, the use of commercial resins to radiochemically isolate ^{44}Sc from ^{44}Ti decay exhibit ^{44}Ti breakthrough during ^{44}Sc elution, leading to short generator lifetimes, potentially due to inadequate binding to the resin or deterioration of the resin over time. Due to the long half-life of ^{44}Ti these generators could theoretically be used for multiple years at a time. Additionally, the purity and chemical form of the eluted ^{44}Sc from these generators is also a concern. Many of the eluents used are not conducive to subsequent radiolabeling experiments which then requires additional purification of the ^{44}Sc through further chromatography. The current literature also lacks detail on the specific chemistry and mechanisms involved in the chromatographic separation involved in these generator systems which we hope to explain or postulate when no literature explanation is given.

The aim of this review is to briefly discuss the topics of ^{44}Ti and ^{44}Sc production methods and provide a comprehensive analysis of the existing literature on the development of ^{44}Ti/^{44}Sc generators, and the challenges still present in translating these systems to widespread use. This is a comprehensive review from reported literature and an attempt to normalize the results for comparison. However, sometimes this is not possible due to differences in the level of detail published.

2. Accelerator Production

2.1 Direct Production of ^{44}Sc

The direct production of ^{44}Sc using particle accelerators has been covered in great depth by Chernysheva et al.,[19] but a summary of direct production has nonetheless been included below to provide context and comparison for the generator systems at the center of this review.

The requirements of ^{44}Sc direct production are to maximize the ^{44}Sc yield and to minimize co-produced radionuclidic impurities. Accelerator production routes co-produce ^{44}Sc with its excited isomer ^{44m}Sc. ^{44m}Sc ($t_{1/2}$ = 58.61 h) mainly decays by internal transition (IT, 98.8%) to its ground state ^{44g}Sc by emitting an intense gamma-ray at 271.24 keV (86.7%). It can also decay by electron capture (EC, 1.2%) to stable ^{44}Ca. The longer -half-life of ^{44m}Sc may lead to unfavorable additional dosage for shorter clinical studies, and its presence is largely redundant for diagnostic imaging.[20] Because of these considerations, an additional requirement of direct ^{44}Sc production is to minimize this ^{44m}Sc/^{44g}Sc ratio. ***Table 4*** highlights several ^{44}Sc production methods, although this review does not include an exhaustive list of all possible production methods.

Due to the availability of medical cyclotrons, proton- and deuteron-induced reactions are common for medical isotope production.[21-27] Production of ^{44}Sc with a proton beam can be achieved with either an enriched ^{44}Ca target [21, 22] via the ^{44}Ca(p,n)^{44}Sc reaction or a natural calcium target via ^{nat}Ca(p,n)^{44}Sc.[23, 26] Irradiation of enriched target materials led to production of ^{44}Sc in high radionuclidic purity (>99%) as well as higher yields based on lower incident beam energy compared to irradiation of natural target materials.[21-23, 27] When ^{nat}Ca is irradiated with protons, multiple scandium isotopes are produced including ^{43}Sc ($t_{1/2}$ = 58.6 h), ^{47}Sc ($t_{1/2}$ = 3.35 d), and ^{48}Sc ($t_{1/2}$ = 43.67 h). This is due to the fact that natural calcium contains many isotopes including ^{40}Ca (96.94%), ^{42}Ca (0.647%), ^{43}Ca (0.135%), ^{44}Ca (2.09%), ^{46}Ca (0.004%), and ^{48}Ca (0.187%). Although contaminants are present, this reaction route can provide a cost-effective approach for pre-clinical trials. However, enriched calcium targets are preferred due to the higher yields and purity of ^{44}Sc.

Table 4. *Various production reactions for ^{44}Sc including beam energy and corresponding EOB yields (calculated to normalize results). *Estimated from values obtained 9 h after EOB. ** Calculated theoretical yields at 24 MeV incident beam energy.*

Reaction	Target Form	Target Purity	Incident Beam Energy (MeV)	EOB Yield (MBq/(μA·h)	Reference
^{44}Ca(p,n)^{44}Sc	$^{44}CaCO_3$	Enriched ^{44}Ca (94.5%)	10.0	50.4	[21]
	$^{44}CaCO_3$	Enriched ^{44}Ca (97.0%)	11.0	26.7	[27]
	^{44}CaO	Enriched ^{44}Ca (97.0%)	18.0	141.8*	[22]
^{nat}Ca(p,n)^{44}Sc	$^{nat}Ca(NO_3)_2$, $4H_2O$	Natural ^{40}Ca (96.4%)	16.0	32.5	[23]
^{44}Ca(d,2n)^{44}Sc	$^{44}CaCO_3$	Enriched ^{44}Ca (99.0%)	16.0	220	[28]

^{47}Ti(p,α)^{44}Sc	$^{47}TiO_2$	Enriched ^{47}Ti (99.0%)	24.0	130**	[25]

^{44}Sc can also be produced via the reaction ^{44}Ca(d,2n)^{44}Sc using an enriched $^{44}CaCO_3$ target with incident deuteron energies over 15 MeV.[24, 28, 29]. These studies demonstrated that ^{44m}Sc/^{44}Sc thick-target production yield ratios are higher with deuterons than with protons regardless of the incident beam energy.[24, 28] Based on the lower ^{44m}Sc/^{44}Sc production ratio and greater abundance of facilities capable of proton irradiations, proton-based production routes are advantageous compared to deuteron routes.[30]

An unconventional reaction route using titanium targets has also been studied to produce ^{44}Sc.[25] The ^{47}Ti(p,α)^{44}Sc production route utilizes an enriched $^{47}TiO_2$ target. While natural Ti (7.44% ^{47}Ti) can be used as a more economical route, co-production of ^{43}Sc and ^{47}Sc can be problematic. Exploration of this production route is in the early stages and optimization of production conditions require further investigation.

Where accelerators are available, the direct production of ^{44}Sc is possible and well researched using a variety of target materials and production routes. However, each of these routes present challenges with radionuclidic purity, target material cost, and/or processing time. Alternatively, it is possible that generator production of ^{44}Sc can overcome these limitations and increase accessibility of ^{44}Sc for research and clinical applications.

2.2 Production of Parent ^{44}Ti

Critical to the success of any ^{44}Ti/^{44}Sc generator system is the availability of the parent isotope, ^{44}Ti. Two different accelerator production methods for ^{44}Ti are shown in ***Table 5*** both of which use an inexpensive, monoisotopic natural Sc target. The main route is via the ^{45}Sc(p,2n)^{44}Ti reaction due to the availability of high intensity proton beams. Daraban et al. measured the maximum cross section to be 40 mb between 23-25 MeV.[31] The irradiation time required to produce the maximum yield of a radionuclide is proportional to its half-life, so ^{44}Ti would need an irradiation of upwards of 60 years in order to obtain the maximum yield. The second ^{44}Ti production route, ^{45}Sc(d,3n)^{44}Ti, has an even lower cross section than the proton induced reaction (21 mb) and also requires higher beam energies (21-50 MeV).[32] While these production routes for ^{44}Ti have relatively low cross sections, production reactions only have to be done once to provide a persistent source of ^{44}Sc for the lifetime of the generator system. The amount of ^{44}Ti produced from these irradiations is sufficient to then produce clinically relevant amounts of ^{44}Sc as the parent nuclide decays. The primary advantage of the use of a ^{44}Ti/^{44}Sc generator compared to direct accelerator production of ^{44}Sc is the longer half-life of ^{44}Ti allowing for production and transportation from centralized accelerator facilities followed by assembly and use of generators at other locations without local access to medical cyclotrons.

Table 5. *Production reactions for ^{44}Ti with beam energy and corresponding cross section measurements.*

Reaction	Target Form	Target Purity	Incident Beam Energy (MeV)	EOB Yield (MBq/(μA·h))·10^{-6}	Reference
^{45}Sc(p,2n)^{44}Ti	$^{45}Sc_2O_3$	99.5%	up to 38	5.04	[31]
^{45}Sc(d,3n)^{44}Ti	^{45}Sc Foil	99.5%	50	2.50	[32]

3. Current $^{44}Ti/^{44}Sc$ Generator Systems

Beginning in the 1960s, several $^{44}Ti/^{44}Sc$ generator designs were investigated for potential medical application. Current generators in the literature make use of commercial anion exchange (AG 1-X8 and TEVA) or hydroxamate-based chelating (ZR) resins. The anion exchange generators boast longer lifetimes eluting with dilute mixtures of oxalic and hydrochloric acid, but in general use very large volumes of eluent, and the oxalates can compete with ligands in the ^{44}Sc eluent inhibiting direct radiolabeling. The ZR resin generator can elute ^{44}Sc in smaller volumes of dilute hydrochloric acid without the use of oxalic acid, but ^{44}Ti breakthrough occurs much sooner in these systems than the anion exchange resin generators. An in-depth discussion and comparison of current generators in the literature will be explored in this section, as well as potential improvements of the current methods towards a more clinically relevant $^{44}Ti/^{44}Sc$ generator system.

3.1 Dowex/AG 1-X8

The very first $^{44}Ti/^{44}Sc$ generator was conceived in 1967 at Brookhaven National Laboratory by Margaret Greene.[33] The simple system made use of anionic exchange resin, Dowex 1-X8 (50-100 mesh), eluting ^{44}Sc with 0.1 M $H_2C_2O_4$ (oxalic acid)/0.2 M HCl mixtures. In a generator constructed in a 2 cm tube with 10-15 mL of resin, 60-70% yield was obtained by eluting the generator with 50 mL of the aforementioned eluent mixture of dilute acids. This design was based on previous anion exchange studies conducted that showed Sc(III) is adsorbed on anion exchange resins as oxalate or citrate complexes, and these complexes can be destroyed and therefore Sc(III) eluted using dilute HCl.[34, 35] Ti(IV) was found to be strongly adsorbed on anion exchange resins in the presence of oxalates, suggesting that adsorption and separation of the two metals was possible with the proper ratio of $H_2C_2O_4$ and HCl. In Greene's generator, the ^{44}Ti was loaded onto the generator in 0.1 M $H_2C_2O_4$ and likely strongly adsorbed on the resin as $[Ti(C_2O_4)_3]^{2-}$. The generator was then eluted by the addition of 0.2 M HCl to the 0.1 M $H_2C_2O_4$ which selectively destroys the anionic $[Sc(C_2O_4)_2]^-$ species allowing for elution of the neutral Sc(III) species. Increasing the concentration of HCl in the mixture will eventually protonate the dianionic Ti(IV) species and the neutral Ti oxalate complex will be eluted. Greene reports that the oxalate ions can be removed from the ^{44}Sc eluent by first evaporating to dryness, followed by washing with peroxide and finally evaporating to dryness again. Unfortunately, the reported results from this study did not go into any further detail on column size/bed volume, fraction elution profile data, the amount of ^{44}Ti activity loaded, resin capacity, or any post-elution processing/radiolabeling studies. This first publication did however prove the potential for a viable $^{44}Ti/^{44}Sc$ generator using commercially available anion exchange resin and dilute acids.

This work was then further studied by Rösch more recently using AG 1-X8 resin, where a more in-depth study of K_d values of Sc and Ti for different ratios of $C_2H_2O_4$ and HCl was reported.[36] Distribution coefficient values for several different molarities and ratios of HCl and $H_2C_2O_4$ were conducted, and it was determined that the best

separation can be obtained with 0.2 M HCl/0.1 M $H_2C_2O_4$ (K_d: Ti > 1000, Sc = 1.7), 0.125 M HCl/0.025 M $H_2C_2O_4$ (K_d: Ti = 1050, Sc = 2.68) or 0.065 M HCl/0.005 M $H_2C_2O_4$ (K_d: Ti > 1000, Sc = 4.0). It was thought that the most desirable choice would be to use the most dilute mixture in terms of generator lifetime and potential radiolabeling studies. Distribution coefficient studies were conducted using the same acid mixtures with cation exchange resin AG 50-X8, but at all concentrations and ratios the K_d values for ^{44}Ti were low.

To monitor the potential of these mixtures in a generator system, two pilot generators were made with AG 1-X8 200-400 mesh Br^- resin in PEEK columns (40 mm x 3 mm), the bed volumes of these generators or mass of resin used were not given. Both generators were loaded with 300 kBq (8 μCi) of ^{44}Ti in 0.1 M $H_2C_2O_4$, and both were eluted three times a week with 10 mL of 0.2 M HCl/0.1 M $H_2C_2O_4$. One generator was eluted in the traditional "direct" method where solvent is eluted in one direction, where the second generator was additionally regenerated by eluting in alternate directions also known as "reverse" elutions. With the direct elution generator, the yield of ^{44}Sc eluted was initially high (250 kBq/6.8 μCi), but then decreased steadily after the first 15 elutions to less than 50 kBq (1.4 μCi), this decrease was accompanied by a consistent increase in ^{44}Ti breakthrough. The authors state that after 30 elutions of the direct generator 50% of the ^{44}Ti had been eluted, and almost all of the ^{44}Ti was eluted after 50 elutions. The reverse generator however resulted in consistent quantities of ^{44}Sc eluted over the 50 elutions, with negligible ^{44}Ti breakthrough in the first 10 elutions, and a slight increase to 0.2% for the following 40 elutions. These generators were eluted a total of 50 times over the span of one year, giving roughly weekly elutions.

A 185 MBq (5 mCi) generator was also constructed using the same resin in a 150 mm x 3 mm PEEK column with a bed volume of 0.55 mL. The ^{44}Ti was loaded onto the column in 0.1 M $H_2C_2O_4$ and then the generator eluted with 0.07 M HCl/0.005 M $H_2C_2O_4$. This generator was eluted roughly once weekly for a total of 54 times over the span of a year. This generator was eluted with 20 mL of eluent in 2 mL fractions, and it was determined that 85% of the total ^{44}Sc activity was eluted in fractions 4-7 (8 mL total) with total elution activity of 97% in the entire 20 mL elution. Much less elution information is given on this generator in the publication, only that initial ^{44}Ti breakthrough in the third elution of 900 Bq (0.02 μCi; 0.0004%) decreased to 150 Bq (0.004 μCi; 0.00008%) by the seventh elution and for the 54th elution. No information is given for elutions between 7 and 54.

While the AG 1-X8 ^{44}Ti/^{44}Sc generator system is an impressive first iteration of this technology, there are many drawbacks to this method, and it needs further improvement before this generator can be a viable clinical option. The 20 mL of eluent needed to obtain reasonable amounts of ^{44}Sc is far too large of a volume for use in radiolabeling and will at the very least need to be concentrated/reduced to a smaller volume. The use of $H_2C_2O_4$ (even in dilute concentrations) to elute the generator is also an issue as oxalates are competitive binding ligands that will inhibit radiolabeling reactions. A discussion of the implication of the presence of oxalates can be found *vide infra*. These hindrances force the need of several subsequent purification steps and columns before the ^{44}Sc is in a suitable form for radiopharmaceutical labeling reactions which then decreases the final activity of ^{44}Sc available.

3.2 ZR

The only non-ion exchange mechanism/resin reported in the literature for a $^{44}Ti/^{44}Sc$ generator is the use of commercially available ZR resin from Triskem Intl.© reported by Fassbender et al. [37] Previously, Dirks et al. at Triskem were the first to report the high retention of Ti(IV) on the ZR resin with respect to Sc(III) in dilute acids suggesting that a generator could be plausible.[38] The manufacturers describe the ZR resin as a "hydroxamate extractant" containing resin and give a partial structure of the main functional group used (***Figure 2***). No further data on the composition or structure of the resin is disclosed, but presumably the hydroxamate group acts as a chelate for the metal ions to form a chelating resin opposed to an ion exchange resin. The metal is likely covalently bound through the oxygens of the hydroxamate in a bidentate manner, and unsurprisingly this resin shows high affinity for several metals. Hydroxamate based resins have been used for Zr/Y separation in the literature,[39] so the use of a similar resin for Ti/Sc separation is a reasonable follow up endeavor due to the similarities in the Zr(IV)/Y(III) and Ti(IV)/Sc(III) pairs.

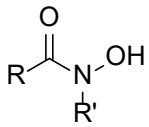

Figure 2. "*Hydroxamate extractant" group as provided by Triskem/Eichrom [40]*

For chelating resins, in general, the main mechanism and driving force of selectivity is dependent on the metal ligand bonds, and metals are removed from the resin by use of high concentrations of acid whereby the chelating heteroatoms in the resin structure are protonated and the metal displaced.[41] Thus, it is possible to elute certain metals by use of simple dilute acids, such as HCl, and there is no need for chelators, such as oxalates, to form ionic structures with the metal as was seen in the AG 1-X8 case. In a generator system this may be a huge benefit as direct radiolabeling is possible if the eluent is free of coordinating ions.

The ZR resin specifically is also an extraction chromatographic resin which means it uses aspects of both liquid-liquid extraction as well as chromatography. These resins are made of microporous matrix materials such as polymers, with the extractant impregnated on the porous support.[42, 43] Since the ZR resin uses hydroxamate groups as the extractant, the mechanism of chromatographic separation is a hybrid between liquid-liquid extraction and chelating resins. As is seen in liquid-liquid extraction, this method is prone to leeching and sorption deterioration over time; this may lead to the organic extractant being eluted off the column with the metal as well as gradual breakthrough of the parent radionuclide over time. Due to this, extraction chromatographic resins may not be conducive to repetitive elutions over an extended period of time as is required in generator systems, while they are still successful in radionuclide separations where the column/resin is not used long term.[37]

Fassbender et al. reported on two different generators using the ZR resin: one using a direct elution method and one using a reverse elution method as previously described.[44] In the direct elution generator, 1 mL of ZR resin

is loaded in a 4 x 0.8 cm, 5 mL Bio-Rad column that had been pre-conditioned in 6 M HCl. 9.8 kBq (0.26 μCi) of ^{44}Ti was loaded in 6 M HCl and the generator eluted with 12 mL of 0.05 M HCl in 1 mL fractions followed by 85 mL of 0.05 M HCl in 5 mL fractions. The time frame of the elutions was not given so it is unclear if this was all in one day or eluted over several days however after 40 bed volumes of eluent 0.23% ^{44}Ti breakthrough was observed. Similar to the AG 1-X8 system the authors also evaluated a reverse generator. Here, a 150 x 4 mm PEEK column was loaded with 2 mL of resin (pre-conditioned in 0.05 M HCl) on one side, then 3.7 MBq of ^{44}Ti (100 μCi) loaded followed by an additional 1 mL of resin so that the ^{44}Ti fraction is "centered" between the resin plugs. This generator was eluted using 5 mL of 0.05 M HCl in alternating directions, no more than once daily, in attempts to minimize ^{44}Ti breakthrough. No data on the ^{44}Sc elution behavior is given, but the ^{44}Ti breakthrough did decrease to 4.1×10^{-4}% and was only observed in the reverse direction elutions (every second elution). This breakthrough remained consistent over 65 bed volumes and did not increase over time as was seen in the direct elution generator.

The increased lifetime of the reverse generator may largely be due to the alternating elution method but may also be strongly impacted by the type/size of column used in that experiment versus the columns used in the direct elution experiment. The length of the column used in the reverse generator experiment is much longer than the column used in the direct generator experiment (150 x 4 mm vs. 4 x 0.8 cm respectively), and the increased length increases the distance the ^{44}Ti must travel before breakthrough. The fact that the authors saw ^{44}Ti breakthrough on every second elution (the side that had only 1 mL of resin compared to 2 mL on the other side) suggests that path length is a contributing factor. While the reverse elution method is still a valid and successful way to increase the generator lifetime, in order to compare between direct and reverse elutions directly the size of the column should be consistent between the two experiments, as was conducted in the AG 1-X8 publication.[36]

3.3 ZrO_2

In 1973, a ^{44}Ti/^{44}Sc generator with a zirconium oxide resin support was reported by Seidl and Lieser.[45] This is the only inorganic-based ^{44}Ti/^{44}Sc generator currently in the literature to the authors' knowledge. The generator system utilized zirconium oxide hydrate (BioRad) with mesh sizes of 100-200 and 50-100. The most favorable pH range for eluting ^{44}Sc was determined to be between 1.7 and 3.7. Further experimentation was completed in this range showing that decreasing pH resulted in increased ^{44}Sc yields. However, after reaching a pH of 1.7, ^{44}Sc yields decreased drastically. The generator was therefore eluted with 30 mL of 0.01 M HCl for up to 6 months. A 42-46% radiochemical yield and 0.02% ^{44}Ti breakthrough were reported.

The mechanism of elution for this generator system is also ion exchange, specifically anion exchange. Metal hydrous oxides are known to be amphoteric ion exchangers, where under alkaline conditions they act as cation exchangers and in acidic conditions as anion exchangers.[46] In acidic conditions the zirconium dioxide lattice becomes protonated and mono–cationic, which is compensated by anionic counterions which can then be exchanged.[47] Seidl and Lieser suggest that in the hydrochloric acid solutions of this generator system that the titanium is in a dianionic form of $[TiCl_6]^{2-}$ where the scandium is monoanionic ($[ScCl_4]^-$) which allows for selective

elution of the scandium in dilute acid eluents. While this mechanism is similar to that of the AG 1-X8 resin, the ZrO_2 generator has the advantage of only needing HCl to elute and no $H_2C_2O_4$ which should ideally allow for direct radiolabeling of the generator eluent. Unfortunately, no labeling studies were conducted in this publication to verify this hypothesis.

3.4 TEVA

The most recent $^{44}Ti/^{44}Sc$ generator reported in the literature utilizes commercially available TEVA (TEtraValent Actinides) resin.[48] Similar to the ZR resin and also produced by Triskem, TEVA resin is an extraction chromatographic resin that contains an aliphatic quaternary amine as the functional group which facilitates an anion exchange mechanism. This resin has similar properties to strong base anion exchange resins that have very similar quaternary amine groups, but since the functional groups are not fixed to the polymer matrix and are fluid, this provides more flexibility to coordinate around the ^{44}Ti ion.[49] The manufacturer of the resin claims these properties allow for higher uptake of anions on the TEVA resin and thus lower acid concentrations may be used to achieve separations, however this leads to the same potential issues with extractant leaching over repeated elutions as noted with ZR resin. The speciation and mechanism of separation is likely the same as was discussed in the anion exchange resin section (AG 1-X8) as the chemical structure of the resin functional groups is similar and the same eluents are used.

Larenkov et al. go into great depth studying and optimizing the generator conditions and eluents used. Distribution coefficients were determined over a range of varying $H_2C_2O_4$ and HCl concentrations and the best results obtained were for 0.1 M $H_2C_2O_4$/ 0.05-0.2 M HCl (D_g in mL/g of $^{44}Ti = 4.7 \times 10^4 – 2.2 \times 10^4$, $^{44}Sc = 9.3 – 2.7$) and 0.025 M $H_2C_2O_4$/ 0.125 M HCl (D_g in mL/g of $^{44}Ti = 1.8 \times 10^4$, $^{44}Sc = 6.8$). These results differ slightly from what was found in the anion exchange resins *vide supra*. The authors also tested varying $H_2C_2O_4$/HCl concentrations on a 100 mm × 2.1 mm column loaded with 150 mg of TEVA resin and 1.5 MBq (40.5 μCi) of ^{44}Ti to see which mixture gives the highest recovery of ^{44}Sc in 5 mL of eluent. It was found that the use of more dilute $H_2C_2O_4$ (0.005-0.025 M) was not effective in eluting enough ^{44}Sc, and that a minimum concentration of 0.1 M $H_2C_2O_4$ is needed to obtain yields > 90%. This is inconsistent with results found with the AG 1-X8 resin, although the volume of eluent, column size, and amount of resin are different between the two studies so a direct comparison is not available.[36] There was a slight increase in ^{44}Sc yield with increasing HCl concentration from 0.05-0.2 M, although the concentration of HCl does not seem to impact the ^{44}Sc elution yield as much as the $C_2H_2O_4$.

A 5 MBq (135 μCi) generator was set up for long term evaluation of the elution behavior and characteristics. This generator was prepared in the same 100 mm × 2.1 mm column as previously reported. This generator was eluted regularly over the period of a year (at least 3 times a week, no more than once a day, for a total of 120 elutions). This generator was initially eluted manually with the use of a syringe and cannula, but then transitioned to the use of a vacuum pump for more consistent results. Throughout this period the ^{44}Sc yield was 91 ± 6% and the ^{44}Ti breakthrough did not exceed 1.5×10^{-5}%. It was also determined that 95% of the eluted ^{44}Sc was obtained in the first 0.5 mL, and the ^{44}Ti breakthrough mainly occurred in the 1.5-2.0 mL fraction.

Through use of autoradiography images, the authors also monitored the movement of the ^{44}Ti on the column over time. It was found that after 120 elutions that the bulk activity of ^{44}Ti had shifted down the column 7 mm, and that the width at half peak height of the ^{44}Ti zone increased from 13 to 19 mm. This generator was eluted in direct mode (one direction), so this shift of ^{44}Ti down the resin bed can be expected and is likely caused by the leeching/deterioration of adsorption seen in extraction chromatographic resins mentioned earlier. It is also of note that the length to inner diameter ratio of this column is larger than any of the other generators in this review. (***Table 6***). This variable clearly has drastic impacts on generator lifetime through observed ^{44}Ti breakthrough. An interesting comparison study would include the ZR, TEVA and anion exchange resin in generators prepared in the same size column to see how the length and or inner diameter directly impacts the time until breakthrough is observed. It seems possible that decreasing the column inner diameter and increasing the length (thereby increasing the path length Ti would have to travel before breakthrough) of the column used for the generator may be an easy and quick way to increase the lifetime of the generator without changing any other variables.

Table 6. *Summary of generator properties discussed in this review.*

Sorbent	Elution mode	^{44}Ti Activity loaded (µCi)	Eluent	Eluent Volume (mL)	^{44}Sc Yield (%)	Column size	Amount of resin used	^{44}Ti Breakthrough	Frequency of elution	Ref.
Dowex 1-X8	Direct	-	0.1 M $C_2H_2O_4$/ 0.2 M HCl	30-50	60-70	2 cm tube	10-15 mL	0.1% after 40 elutions	-	[33]
AG 1-X8	Direct	8	0.1 M $C_2H_2O_4$/ 0.2 M HCl	10	≈ 17-83	40 × 3 mm	-	50% after 30 elutions	3× /week	[36]
	Reverse				≈ 93			0.2% after 50 elutions		
	Reverse	5000	0.005 M $C_2H_2O_4$/ 0.07 M HCl	20	97	150 × 3 mm	0.55 mL	4.0×10^{-4}% after 3 elutions 8.0×10^{-5}% after 54 elutions	Weekly	
ZR resin	Direct	0.26	0.05 M HCl	-	-	4 × 0.8 cm	1 mL	0.23% after 40 BV	-	[37]
	Reverse	100		5		150 × 4 mm	3 mL	4.1×10^{-4}% after 65 BV	-	
ZrO_2	Direct	1	0.01 M HCl	30	42-46	-	2-5 g	0.02%	-	[45]
TEVA resin	Direct	135	0.1 M $C_2H_2O_4$/ 0.2 M HCl	1	91	100 × 2.1 mm	150 mg	1.5×10^{-5}% after 120 elutions	120 elutions over a year (at least 3×/week)	[48]

4. Post-elution processing, ^{44}Sc radiolabeling and imaging

4.1 AG 1-X8

The same authors who published the AG 1-X8 generator work also published another paper discussing post-elution processing of ^{44}Sc from their AG 1-X8 generator system.[44] Due to the large volumes of eluent along with the presence of competing oxalates and very low pH of the dilute acids, direct radiolabeling is not possible. This publication explored the use of several different cation exchange resins to concentrate and isolate the ^{44}Sc eluent. The authors tested AG 50W-X4 (200-400 mesh, H^+), AG 50W-X8 (200-400 mesh, H^+) and Chelex 100 (200-400 mesh, Na^+) resin. Various amounts of cation exchange resin were prepared in "small plastic syringes" to mimic miniature columns and 2.55-3.0 mL of ^{44}Sc eluent (in 0.005 M $C_2H_2O_4$/0.07 M HCl) was passed through the columns at a rate of 1 mL/min. It was determined that by using similar amounts of resin, the higher crosslinked AG 50W-X8 had the highest retention of ^{44}Sc (50 mg, 88.7%), followed by AG 50W-X4 (50 mg, 50.5%) and Chelex 100 displaying the lowest retention (51 mg, 42.2%). It was observed that as the mass of the resin was increased, the amount of ^{44}Sc retained on the resin increased as well; the highest retention was seen with 200 mg of AG 50W-X8 (99.9%), followed by 160 mg of AG 50W-X4 (98.0%) and the lowest retention was obtained with 51 mg of Chelex 100 (42.2%).

To test the elution of the ^{44}Sc from the cation exchange resin, miniature columns filled with 80 mg of AG 50W-X8 were loaded with 2.55-3.0 mL of ^{44}Sc generator eluent and small volumes of varying buffers and solvents were passed through and the recovery of ^{44}Sc was determined. Results showed that increasing the concentration of acid (HCl) or base (NaOH) also increased the recovery yield, but the extreme pH values of the resulting eluent is unsuitable for subsequent radiolabeling, thus other eluents containing organic complexing agents were tested. While EDTA and diammonium oxalate both gave good recovery of ^{44}Sc (87.6% and 94.7% respectively), both are strongly competitive ligands and therefore need to be destroyed by heating prior to radiolabeling experiments. From this study, it was concluded that the most appropriate conditions for ^{44}Sc recovery was the use of 3.0 mL of 0.25 M ammonium acetate buffer at a pH of 4, which resulted in a 90.4% recovery yield. This was due to the stability constant of acetate ions with Sc(III) being low (log K = 3.48), allowing the acetate ions to not interfere in radiolabeling reactions.[50] The use of this post-elution cation exchange column acts to isolate the ^{44}Sc in a form sufficient for radiolabeling.

Radiolabeling with AG 1-X8 generator produced ^{44}Sc was conducted by Pruszynski et al. on post-elution purified ^{44}Sc using the AG 50W-X8 column described earlier.[7, 44] ^{44}Sc was eluted from an AG 1-X8 generator using 20 mL of 0.005 M $H_2C_2O_4$/0.07 M HCl and the eluent was directly post-processed on a column filled with 53 mg of AG 50W-X8 resin, the ^{44}Sc was adsorbed on the resin and then eluted using 2-3 mL of 0.25 M ammonium acetate buffer (pH 4). The authors conducted several radiolabeling tests varying concentration of DOTATOC, reaction time, temperature, and pH of the reaction mixture. Authors report radiochemical yields greater than 98% when 21 nmol of DOTATOC was reacted with ^{44}Sc for 25 minutes at 95°C in a buffer solution at pH of 4. Unfortunately, the authors do not disclose the amount/activity of ^{44}Sc that was obtained from the generator or amount used in the

radiolabeling reactions, so it is impossible to directly compare the radiochemical yield to other ^{44}Sc radiolabeling studies conducted under similar reaction conditions. The high radiochemical yield obtained does suggest that the use of generator-produced ^{44}Sc from an AG 1-X8 column does not hinder radiolabeling when used in conjunction with the AG 50W-X8 post processing.

Another paper by Eigner et al. used generator-produced ^{44}Sc in radiolabeling studies with DOTA-Puromycin.[51] The ^{44}Sc was obtained from the generator and processed in the exact same manner as the Pruszynski publication.[7, 44] In this study, 150 MBq (4.05 mCi) of ^{44}Sc was mixed with 55.5 nmol of DOTA-Puromycin in 0.25 M ammonium acetate buffer pH 4 at 95°C for 20 minutes while shaking. This reaction gives radiochemical yields of 55% to 65% using a ligand:Sc ratio of 10,000:1, which is similar to results obtained in non-generator produced ^{44}Sc radiolabeling experiments.[6, 26, 52]

A study conducted by Kerdjoudj et al. compared the radiolabeling efficiency of both generator produced and accelerator produced ^{44}Sc with DOTA and monophosphorus acid DOTA derivatives.[5] The generator-produced ^{44}Sc was obtained in the same method as the two previously described papers in this section, and the cyclotron produced ^{44}Sc was produced by irradiation of a natural $CaCO_3$ target and processed on a DGA resin column as described elsewhere.[28] Authors varied the radiolabeling solution pH, temperature and ligand molar ratio and compared both radioscandium. For both the accelerator and generator produced ^{44}Sc there was a general trend of increasing yield with increasing temperature for all four ligands tested (DOTA, DO3AP, DO3AP^{ABn}, and DO3AP^{PrA}) to 70°C after which the yield remained consistent (≈95-100%) until 100°C. Similar trends were also found between the two sources of ^{44}Sc in varying the pH of the reaction solution from 2 to 6, although slightly higher yields were found in the case of the generator derived ^{44}Sc. Another study compared the effect that different metal:ligand ratios have on the radiolabeling yields, in these reactions the experimental conditions were kept constant (30 minutes, pH 4 at 70°C). Overall trends of increasing yield with increasing amount of ligand used was seen in radioscandium from both production routes, but the amount of ligand needed to obtain a minimum yield of 95% was lower for the accelerator derived ^{44}Sc (0.2 nmol) than the generator derived ^{44}Sc (5 nmol). The authors attribute this to the amount of competing cold metal impurities in the two samples. The authors also state that the radioscandium from the two sources differ in specific activity, the calculated specific activity of the cyclotron ^{44m}Sc/^{44}Sc being higher than the generator ^{44}Sc (10 MBq nmol^{-1} and 2 MBq nmol^{-1}, respectively). Ligand specific trends are also evident in the data but are beyond the scope of this review.

As reported above, AG 1-X8 generator produced ^{44}Sc has been evaluated a few times in the literature for binding to DOTA-conjugates. However, there are only two reported in-human studies, one used a ^{44}Ti/^{44}Sc generator to obtain ^{44}Sc.[53, 54] Eppard et al. obtained ^{44}Sc eluent from the 185 MBq (5 mCi) AG 1-X8 generator reported by Filosofov et al., and utilized the post-elution processing procedure reported by Pruszynski et al.[7, 36, 44] Following these literature procedures, authors obtained 142 MBq (3.8 mCi) of oxalate free ^{44}Sc in 3 mL of solution after the elution and processing: this correlates to a 23% loss of ^{44}Sc in the elution and processing described for this system (assuming a quantitative amount of ^{44}Sc eluted from the generator which is unlikely). The entirety of this ^{44}Sc (3 mL in 0.25 M NH_4OAc) was then used in subsequent radiolabeling with 40 μL (38 nmol) of PSMA-617 and 160 μL of

EtOH, this gives a relative ligand:Sc ratio of 7,800:1. This solution was heated at 95°C for 20 minutes, after which the radiolabeled product was purified on a C-18 column with 1.5 mL of 50% EtOH. The final product was formulated with 8.5 mL of 0.9% NaCl followed by sterile filtration. Using this purified solution, patients were intravenously injected with 50.45 MBq (1.36 mCi) of ^{44}Sc in a total volume of 10 mL. PET imaging studies conducted on the patients showed comparable results, in terms of image quality and noise level, between ^{44}Sc-PSMA-617 and ^{68}Ga-PSMA-11. The ^{44}Sc-PSMA-617 did, however, allow for acquisition of later time point imaging at 19 h which is not possible with ^{68}Ga-PSMA-11.

Furthermore, another study conducted showed that ^{44}Sc-PSMA-617 more closely resembled overall tissue distribution with that of ^{177}Lu-PSMA-617 compared to ^{68}Ga-PSMA-11 and ^{68}Ga-PSMA-617 further demonstrating the clinical potential of this radioisotope in theranostic applications.[55] These studies demonstrate the clinical viability of a ^{44}Ti/^{44}Sc generator to provide radionuclide ^{44}Sc as a PET imagining agent for prostate cancer, although authors point out the limited availability of ^{44}Sc currently is a huge limitation.

4.2 ZR

Radchenko et al. conducted a labeling study with ^{44}Sc obtained from the ZR resin-based generator.[37] DOTA was used as a chelating agent. For a preliminary study, ~300 kBq (8 µCi, 110 µL) of ^{44}Sc was eluted from a ZR resin column using 0.05 M HCl. The solution was added to 0.25 M ammonium acetate buffer (pH 4) and 5 µL of DOTA (30 nM), achieving a 1:15 reported ratio of Sc:DOTA. This preliminary study resulted in >94% of ^{44}Sc chelated with DOTA after 15 minutes. Varying concentrations of DOTA (0.01 - 10 nM), Sc:DOTA ratios, time intervals, and temperature were then tested. After 40 minutes, 1 nM DOTA (1:1.67; reported ratio) showed labeling yields >60%.

4.3 TEVA

Similar to the AG 1-X8 generator, the TEVA eluent contained oxalates and was too acidic for use in direct radiolabeling, so the authors studied different methods of post-elution purification to obtain the radioscandium in a labeling appropriate form. One method to remove the oxalates was decarboxylation of the $C_2H_2O_4$ with hydrogen peroxide followed by evaporation, this method had previously been suggested by Greene.[33] The TEVA generator eluent was evaporated dry and washed with 500 µL of 30% H_2O_2 twice before being reconstituted in 1.0 M HCl and diluted to 1.0 mL with water to give a final concentration on 0.1 M HCl. This method gives 87% ^{44}Sc recovery with final residual $C_2H_2O_4$ in concentrations of 0.0001-0.0003 M. While this post-elution purification technique proved successful, authors state that it would not be conducive to automation so alternate methods were explored, although proof of concept to the validity of this purification was shown in radiolabeling studies using the ^{44}Sc obtained from this post-elution procedure. Radiolabeling reactions were conducted at 95°C in 1.0 mL of 4.5 pH ammonium acetate buffer with 1.0-4.0 MBq (27-108 µCi) of ^{44}Sc, varying the reaction times and ligand amounts (2-10 nmol of PSMA-

617). A radiolabeling yield of >95% can be achieved within 15 minutes using 2, 5 and 10 nmol of PSMA-617 under these conditions. It was found that after 5 minutes, 2 nmol of ligand gives 95% yield, 5 nmol gives 97% and 10 nmol gives 99%. After 10 minutes both 5 and 10 nmol ligand reactions give 99% and 2 nmol gives 98%. While these yields are high, a very large excess of ligand is also being used (between 14,000:1 and 290,000:1 depending on the amount of ^{44}Sc, and ligand used).

Larenkov et al. also examined the maximum amount of $C_2H_2O_4$ residue that can be present in the ^{44}Sc sample before it is detrimental to the radiolabeling yields. In these studies, reactions were conducted at 95°C for 30 minutes in ammonium acetate buffer at pH 4.5 with 20 nmol of PSMA-617. The concentration of $C_2H_2O_4$ present in the labeling reactions was varied from 0.1-0.0001 M and it was found that sufficiently high yields (>95%) can be obtained at concentrations of 0.001 M and lower, and that the yield decreases drastically starting at 0.05 mol/L dropping all the way to 0% at 0.1 M. The ligand:Sc ratio in these reactions is extremely large, ranging from 146,000:1 to 580,000:1 depending on the amount of ^{44}Sc used. The drastic effect that the concentration of $C_2H_2O_4$ has in the labeling reaction, even under large ligand excess, demonstrates the harmful impact that using $C_2H_2O_4$ as an eluent has on radiolabeling and the need for adequate post-elution processing.

Similar to the anion exchange column generator purification, the use of an additional column chromatography to concentrate and purify the ^{44}Sc eluent was employed. Several different cation exchange, solid phase extraction (SPE), and chelating resins were tested for the sorption of ^{44}Sc eluent from the TEVA resin. These sorption results showed that the Presep PolyChelate resin demonstrated the highest sorption rate of ≥99%, where all other resins tested showed low sorption rates ranging from 8-57%. Desorption of ^{44}Sc was performed in 0.1-3 M HCl and it was shown that an increase in HCl concentration results in a higher ^{44}Sc yield with less eluate volume. Although a higher amount of ^{44}Sc was obtained by using 3 M HCl (95% desorption), high amounts of $C_2H_2O_4$ (0.008-0.010 M) were present in the resulting elutions. The amount of $C_2H_2O_4$ present surpassed the maximum permitted concentration for sufficient radiolabeling. Additional washing of the resin with water and ethanol did not significantly improve the amount of $C_2H_2O_4$ present. The authors chose to use 0.1 M HCl (93% desorption) for further experiments due to it having the lowest exhibited residual $C_2H_2O_4$ concentration (0.001-0.005 M).

^{44}Sc eluted from the Presep PolyChelate column was reacted with 20 nmol of PSMA-617 for 30-minutes at 95°C and a pH of 4.5. The highest reported yield of ^{44}Sc-PSMA-617 synthesis was 92%, although an average radiolabeling reaction yield of only 75 ± 10% was achieved. It was noted that microwave heating significantly improved reaction yields for ^{44}Sc-PSMA-617 to >95%. Unlike the results from reactions conducted in a block heater of ^{44}Sc-PSMA-617, labeling reactions preformed with PSMA-I&T under the same conditions give consistently high yields (>95%) with reactions conducted in a block heater and >99% yields with microwave heating. This suggests that the DOTAGA chelating agent is less sensitive to the presence of chemical impurities compared to DOTA, and more readily forms ^{44}Sc complexes. It can be concluded that microwave heating improved radiolabeling yields and helps to shorten the reaction time. The sensitivity of the PSMA-617 to chemical impurities in the labeling solution led the authors to investigate further purification of the Presep PolyChelate eluent in attempts to remove the impurities hindering the radiolabeling yields. The use of TK221 resin was investigated as distribution coefficients of ^{44}Sc in HCl

media showed quantitative trapping of the ^{44}Sc on the resin. This work investigated a range of desorption eluents with reduced acidity that would be appropriate for subsequent radiolabeling. Results concluded that washing the column with water (0.2 mL) then eluting the column with 0.5 mL of 1 M NH_4OAc (pH 4.5) to elute ^{44}Sc from TK221 resin resulted in the highest ^{44}Sc recovery of 97%. The use of these eluents made the resulting ^{44}Sc solution suitable for direct radiolabeling. Using the same reaction conditions mentioned above (95 °C, 30 min, V = 1.0 mL, pH = 4.5, 20 nmol of PSMA-617), high ^{44}Sc radiolabeling yields were achieved at ≥95%. Increasing the amount of PSMA-617 also increased ^{44}Sc labeling yields. The authors noted that no ^{44}Ti was detected in samples after these combined post-elution processes demonstrating promising results for clinical applications.

5. Conclusion

The development of ^{44}Ti/^{44}Sc generators has been investigated over the past several decades, with recent advancements showing the potential for a clinically relevant system. Use of the anion exchange resin AG 1-X8 has been studied the most and shows promising results of obtaining high yields (97%) of ^{44}Sc in moderate volumes of dilute $C_2H_2O_4$/HCl mixtures. Even more promising results were obtained using an extraction chromatographic anion exchange resin (TEVA). High yields (91%) of ^{44}Sc were likewise eluted in mixtures of dilute $C_2H_2O_4$/HCl, but the volume was reduced drastically to just 1 mL of solution, and the lifetime of the generator before ^{44}Ti breakthrough was increased through the use of the TEVA resin. Both improvements may potentially stem from the use of a longer and narrower column for the generator which increases the path length and decreases the volume or may be due to the different binding characteristics of the resin used. While the direct generator eluent from these systems is not suitable for radiolabeling, extensive research of sufficient post-elution processing was conducted demonstrating a viable process to both concentrate the ^{44}Sc into a smaller volume of solution and remove the competing oxalates. These post-elution protocols allowed for sufficient radiolabeling of generator produced ^{44}Sc with DOTA and DOTA derivative chelators.

Two non-anion exchange resin generators have also been reported, one using ZrO_2 as a matrix and the other using extraction chromatographic ZR resin. The ZrO_2 publication stated that 42-46% ^{44}Sc could be obtained in 30 mL of 0.01 M HCl with very little ^{44}Ti breakthrough, but unfortunately no further data or radiolabeling experiments were reported and no other literature reports of this generator matrix have been published. The ZR resin publication improved the elution solution composition compared to the AG 1-X8 generator by only using dilute HCl without the use of $C_2H_2O_4$. This generator displayed very little ^{44}Ti breakthrough when a reverse elution using 0.05 M HCl method was used, although no data was given on the recovery of ^{44}Sc obtained in the elution. The eluted ^{44}Sc was, however, successfully used in a direct radiolabeling experiment with DOTA without the need or use of any post-elution processing.

Across all of the generators presented in this review, the use of reverse method elution versus direct method elution, as well as the use of longer and more narrow columns increased the lifetime of the generator before ^{44}Ti breakthrough was observed. The anion exchange resins, specifically the TEVA generator, display longer lifetimes,

but the eluent requires post-elution processing before radiolabeling can be achieved. The ZR resin generator produces ^{44}Sc in a form conducive for direct radiolabeling, but the lifetime of the generator is short and the efficiency of ^{44}Sc recovery has not been examined or published. While the generators presented here all show great promise and potential for use in clinical settings, more improvement and further research is needed to obtain an ideal system. The AG 1-X8 generator demonstrates the viability of a ^{44}Ti/^{44}Sc generator in a scale appropriate for clinical use, the TEVA generator displays the most impressive lifetime and smallest volumes of eluent, while the ZR generator is most beneficial for direct radiolabeling experiments. Finding these three impressive accomplishments in a single generator system would give the best chance of obtaining a successful and clinically feasible generator, and the research presented so far is very promising and suggests that this may be achieved.

List of Abbreviations

PET = positron emission tomography

DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid

CT = computed tomography

EC = electron capture

EOB = end of bombardment

TEVA = TEtraValent Actinides

SPE = solid phase extraction

Consent for publication

Not applicable.

Conflict of Interest

The authors have no conflicts of interest financial or otherwise.

Funding

The authors would like to thank the Department of Energy Core R&D Funding (ST5001030) for their generous support. Additionally, this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number SC2GM130464. This work was also supported in part by the Department of Energy Isotope Program's Grant DE- SC0022550, the Horizon-broadening Isotope Production Pipeline Opportunities (HIPPO) program.

Acknowledgments

The authors would like to thank Drs. Cathy S. Cutler, Jennifer Shusterman, and Lynn Francesconi for their helpful discussions. The authors would also like to thank the Department of Energy Core R&D Funding (ST5001030) for their generous support. Additionally, this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number SC2GM130464. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This work was also supported in part by the Department of Energy Isotope Program's Grant DE- SC0022550, the Horizon-broadening Isotope Production Pipeline Opportunities (HIPPO) program. Finally, the authors would like to thank all the researchers for without their work this review could have not been compiled.

References

[1] Domnanich, K.A.; Muller, C.; Farkas, R.; Schmid, R.M.; Ponsard, B.; Schibli, R.; Turler, A.; van der Meulen, N.P., (44)Sc for labeling of DOTA- and NODAGA-functionalized peptides: preclinical in vitro and in vivo investigations. *EJNMMI Radiopharm Chem,* **2017**, *1*, (1), 8.

[2] Rosar, F.; Buchholz, H.G.; Michels, S.; Roesch, F.; Piel, M.; Miederer, M.; Reuss, S.; Schreckenberger, M., A performance comparison of Sc-44 to Ga-68 using preclinical PET scanners. *Journal of Nuclear Medicine,* **2018**, *59*, (supplement 1), 1776-1776.

[3] Bunka, M.; Muller, C.; Vermeulen, C.; Haller, S.; Turler, A.; Schibli, R.; van der Meulen, N.P., Imaging quality of (44)Sc in comparison with five other PET radionuclides using Derenzo phantoms and preclinical PET. *Appl Radiat Isot,* **2016**, *110*, 129-133.

[4] Koumarianou, E.; Loktionova, N.S.; Fellner, M.; Roesch, F.; Thews, O.; Pawlak, D.; Archimandritis, S.C.; Mikolajczak, R., ^{44}Sc-DOTA-BN[2-14]NH_2 in comparison to ^{68}Ga-DOTA-BN[2-14]NH_2 in pre-clinical investigation. Is ^{44}Sc a potential radionuclide for PET? *Appl Radiat Isot,* **2012**, *70*, (12), 2669-2676.

[5] Kerdjoudj, R.; Pniok, M.; Alliot, C.; Kubicek, V.; Havlickova, J.; Rosch, F.; Hermann, P.; Huclier-Markai, S., Scandium(III) complexes of monophosphorus acid DOTA analogues: a thermodynamic and radiolabelling study with (44)Sc from cyclotron and from a (44)Ti/(44)Sc generator. *Dalton Trans,* **2016**, *45*, (4), 1398-1409.

[6] Huclier-Markai, S.; Kerdjoudj, R.; Alliot, C.; Bonraisin, A.C.; Michel, N.; Haddad, F.; Barbet, J., Optimization of reaction conditions for the radiolabeling of DOTA and DOTA-peptide with (44m/44)Sc and experimental evidence of the feasibility of an in vivo PET generator. *Nucl Med Biol,* **2014**, *41 Suppl*, e36-43.

[7] Pruszynski, M.; Majkowska-Pilip, A.; Loktionova, N.S.; Eppard, E.; Roesch, F., Radiolabeling of DOTATOC with the long-lived positron emitter ^{44}Sc. *Appl Radiat Isot,* **2012**, *70*, (6), 974-979.

[8] Muller, C.; Bunka, M.; Haller, S.; Koster, U.; Groehn, V.; Bernhardt, P.; van der Meulen, N.; Turler, A.; Schibli, R., Promising prospects for ^{44}Sc-/^{47}Sc-based theragnostics: application of ^{47}Sc for radionuclide tumor therapy in mice. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine,* **2014**, *55*, (10), 1658-1664.

[9] Domnanich, K.A.; Muller, C.; Benesova, M.; Dressler, R.; Haller, S.; Koster, U.; Ponsard, B.; Schibli, R.; Turler, A.; van der Meulen, N.P., (47)Sc as useful beta(-)-emitter for the radiotheragnostic paradigm: a comparative study of feasible production routes. *EJNMMI Radiopharm Chem,* **2017**, *2*, (1), 5.

[10] Volkovitsky, P.; Gilliam, D.M., Possible PET isotope production using linear deuteron accelerators. *Nucl Instrum Meth A,* **2005**, *548*, (3), 571-573.

[11] Synowiecki, M.A.; Perk, L.R.; Nijsen, J.F.W., Production of novel diagnostic radionuclides in small medical cyclotrons. *EJNMMI Radiopharm Chem,* **2018**, *3*, (1), 3.

[12] *Cyclotron Produced Radionuclides: Principles and Practice*. INTERNATIONAL ATOMIC ENERGY AGENCY: Vienna, **2009**.

[13] Goethals, P.; Zimmermann, R.; Online, **2015**.

[14] Buck, A.K.; Herrmann, K.; Stargardt, T.; Dechow, T.; Krause, B.J.; Schreyogg, J., Economic evaluation of PET and PET/CT in oncology: evidence and methodologic approaches. *J Nucl Med Technol,* **2010**, *38*, (1), 6-17.

[15] Dash, A.; Chakravarty, R., Radionuclide generators: the prospect of availing PET radiotracers to meet current clinical needs and future research demands. *American journal of nuclear medicine and molecular imaging,* **2019**, *9*, (1), 30-66.

[16] Schmor, P., Review of Cyclotrons for the Production of Radioactive Isotopes for Medical and Industrial Applications. *Reviews of Accelerator Science and Technology,* **2012**, *04*, (01), 103-116.

[17] Braccini, S.; Alves, F., Special Issue "Instruments and Methods for Cyclotron Produced Radioisotopes". *Instruments,* **2019**, *3*, (4), 60.

[18] Rosch, F.; Baum, R.P., Generator-based PET radiopharmaceuticals for molecular imaging of tumours: on the way to THERANOSTICS. *Dalton Trans,* **2011**, *40*, (23), 6104-6111.

[19] Chernysheva, M.; Loveless, S.C.; Brossard, T.; Becker, K.; Cingoranelli, S.; Aluicio-Sarduy, E.; Song, J.; Ellison, P.; Nolen, J.; Rotsch, D.A.; Lapi, S.E.; Engle, J.W., Accelerator Production of Scandium Radioisotopes: Sc-43, Sc-44, and Sc-47. *Current radiopharmaceuticals,* **2021**, *14*, (4), 359-373.

[20] Chaple, I.F.; Lapi, S.E., Production and Use of the First-Row Transition Metal PET Radionuclides (43,44)Sc, (52)Mn, and (45)Ti. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine,* **2018**, *59*, (11), 1655-1659.

[21] Krajewski, S.; Cydzik, I.; Abbas, K.; Bulgheroni, A.; Simonelli, F.; Holzwarth, U.; Bilewicz, A., Cyclotron production of ^{44}Sc for clinical application. *Radiochimica Acta,* **2013**, *101*, (5), 333-338.
[22] van der Meulen, N.P.; Hasler, R.; Talip, Z.; Grundler, P.V.; Favaretto, C.; Umbricht, C.A.; Müller, C.; Dellepiane, G.; Carzaniga, T.S.; Braccini, S., Developments toward the Implementation of ^{44}Sc Production at a Medical Cyclotron. *Molecules,* **2020**, *25*, (20), 4706.
[23] Severin, G.W.; Engle, J.W.; Valdovinos, H.F.; Barnhart, T.E.; Nickles, R.J., Cyclotron produced (44)gSc from natural calcium. *Appl Radiat Isot,* **2012**, *70*, (8), 1526-1530.
[24] Duchemin, C.; Guertin, A.; Haddad, F.; Michel, N.; Metivier, V., Production of scandium-44 m and scandium-44 g with deuterons on calcium-44: cross section measurements and production yield calculations. *Phys Med Biol,* **2015**, *60*, (17), 6847-6864.
[25] Loveless, C.S.; Blanco, J.R.; Diehl, G.L., 3rd; Elbahrawi, R.T.; Carzaniga, T.S.; Braccini, S.; Lapi, S.E., Cyclotron Production and Separation of Scandium Radionuclides from Natural Titanium Metal and Titanium Dioxide Targets. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine,* **2021**, *62*, (1), 131-136.
[26] Hernandez, R.; Valdovinos, H.F.; Yang, Y.; Chakravarty, R.; Hong, H.; Barnhart, T.E.; Cai, W., (44)Sc: an attractive isotope for peptide-based PET imaging. *Mol Pharm,* **2014**, *11*, (8), 2954-2961.
[27] van der Meulen, N.P.; Bunka, M.; Domnanich, K.A.; Muller, C.; Haller, S.; Vermeulen, C.; Turler, A.; Schibli, R., Cyclotron production of (44)Sc: From bench to bedside. *Nucl Med Biol,* **2015**, *42*, (9), 745-751.
[28] Alliot, C.; Kerdjoudj, R.; Michel, N.; Haddad, F.; Huclier-Markai, S., Cyclotron production of high purity (44m,44)Sc with deuterons from (44)CaCO3 targets. *Nucl Med Biol,* **2015**, *42*, (6), 524-529.
[29] Alliot, C.; Audouin, N.; Barbet, J.; Bonraisin, A.C.; Bosse, V.; Bourdeau, C.; Bourgeois, M.; Duchemin, C.; Guertin, A.; Haddad, F.; Huclier-Markai, S.; Kerdjoudj, R.; Laize, J.; Metivier, V.; Michel, N.; Mokili, M.; Pageau, M.; Vidal, A., Is there an interest to use deuteron beams to produce non-conventional radionuclides? *Front Med (Lausanne),* **2015**, *2*, 31.
[30] Talip, Z.; Favaretto, C.; Geistlich, S.; Meulen, N.P.V., A Step-by-Step Guide for the Novel Radiometal Production for Medical Applications: Case Studies with (68)Ga, (44)Sc, (177)Lu and (161)Tb. *Molecules,* **2020**, *25*, (4), 966.
[31] Daraban, L.; Adam Rebeles, R.; Hermanne, A.; Tarkanyi, F.; Takacs, S., Study of the excitation functions for ^{43}K, ^{43,44,44m}Sc and ^{44}Ti by proton irradiation on ^{45}Sc up to 37MeV. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms,* **2009**, *267*, (5), 755-759.
[32] Hermanne, A.; Adam Rebeles, R.; Tarkanyi, F.; Takacs, S.; Takacs, M.P.; Csikai, J.; Ignatyuk, A., Cross sections of deuteron induced reactions on ^{45}Sc up to 50MeV: Experiments and comparison with theoretical codes. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms,* **2012**, *270*, 106-115.
[33] Greene, M.W.; Hillman, M., A Scandium Generator. *Int J Appl Radiat Is,* **1967**, *18*, (7), 540-&.
[34] Vickery, R.C., The Extraction and Purification of Scandium. *J Chem Soc,* **1955**, (0), 245-251.
[35] Walter, R.I., Anion Exchange Studies of Sc(III) and V(IV) - Separation of Scandium, Titanium and Vanadium. *J Inorg Nucl Chem,* **1958**, *6*, (1), 58-62.
[36] Filosofov, D.V.; Loktionova, N.S.; Rösch, F., A ^{44}Ti/^{44}Sc radionuclide generator for potential application of ^{44}Sc-based PET-radiopharmaceuticals. *Radiochimica Acta,* **2010**, *98*, (3), 149-156.
[37] Radchenko, V.; Meyer, C.A.L.; Engle, J.W.; Naranjo, C.M.; Unc, G.A.; Mastren, T.; Brugh, M.; Birnbaum, E.R.; John, K.D.; Nortier, F.M.; Fassbender, M.E., Separation of (44)Ti from proton irradiated scandium by using solid-phase extraction chromatography and design of (44)Ti/(44)Sc generator system. *J Chromatogr A,* **2016**, *1477*, 39-46.
[38] Dirks C., B.A., Maudoux N. In *61st Radiobioassay and Radiochemistry Measurements Conference*: Iowa City, IA, USA, **2015**.
[39] Holland, J.P.; Sheh, Y.; Lewis, J.S., Standardized methods for the production of high specific-activity zirconium-89. *Nucl Med Biol,* **2009**, *36*, (7), 729-739.
[40] ZR Resin product sheet. https://www.triskem-international.com/scripts/files/61eef0f5e9fa69.45400285/PS_ZR-Resin_EN_210908.pdf (3/2/2022),
[41] MEYERS, P., How chelating resins behave. *Plating and surface finishing,* **1998**, *85*, (10), 22-29.
[42] Triskem Extraction Chromatography Technical Document. https://www.triskem-international.com/scripts/files/5fbc1413c82157.37153904/technical-doc-worldwide-en-web.pdf (3/15/2022),

[43] Vyas, C.; Park, J.; Yang, S., Application of Extraction Chromatographic Techniques for Separation and Purification of Radiometals Sc-44,47 and Cu-64, 67. *Journal of Radiopharmaceuticals and Molecular Probes,* **2016**, *2*, 84-95.
[44] Pruszynski, M.; Loktionova, N.S.; Filosofov, D.V.; Rosch, F., Post-elution processing of (44)Ti/(44)Sc generator-derived (44)Sc for clinical application. *Appl Radiat Isot,* **2010**, *68*, (9), 1636-1641.
[45] Seidl, E.; Lieser, K.H., Die Radionuklidgeneratoren ,^{113}Sn/^{113m}In, ^{68}Ge/^{68}Ga und ^{44}Ti/^{44}Sc. *ract,* **1973**, *19*, (4), 196-198.
[46] Qureshi, M.; Varshney, K.G., First edition. ed; CRC Press: Boca Raton, **2019**.
[47] Altshuler, G.N.; Malyshenko, N.V.; Popova, A.N., The Ion-Exchange Properties of Polymeric Zirconium Phosphate and Zirconium Dioxide. *Inorganic Materials: Applied Research,* **2018**, *9*, (4), 746-750.
[48] Larenkov, A.A.; Makichyan, A.G.; Iatsenko, V.N., Separation of (44)Sc from (44)Ti in the Context of A Generator System for Radiopharmaceutical Purposes with the Example of [(44)Sc]Sc-PSMA-617 and [(44)Sc]Sc-PSMA-I&T Synthesis. *Molecules,* **2021**, *26*, (21), 6371.
[49] TEVA Resin Product Page. https://www.eichrom.com/eichrom/products/teva-resin/ (3/16/2022),
[50] Itoh, H.; Ikegami, Y.; Suzuki, Y., Stability Constants of Scandium Complexes. II. Dicarboxylate Complexes Species. *Bulletin of the Chemical Society of Japan,* **1984**, *57*, (12), 3426-3429.
[51] Eigner, S.; Vera, D.R.; Fellner, M.; Loktionova, N.S.; Piel, M.; Lebeda, O.; Rosch, F.; Ross, T.L.; Henke, K.E., Imaging of protein synthesis: in vitro and in vivo evaluation of (44)Sc-DOTA-puromycin. *Mol Imaging Biol,* **2013**, *15*, (1), 79-86.
[52] Muller, C.; Bunka, M.; Reber, J.; Fischer, C.; Zhernosekov, K.; Turler, A.; Schibli, R., Promises of cyclotron-produced ^{44}Sc as a diagnostic match for trivalent beta--emitters: in vitro and in vivo study of a ^{44}Sc-DOTA-folate conjugate. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine,* **2013**, *54*, (12), 2168-2174.
[53] Eppard, E.; de la Fuente, A.; Benesova, M.; Khawar, A.; Bundschuh, R.A.; Gartner, F.C.; Kreppel, B.; Kopka, K.; Essler, M.; Rosch, F., Clinical Translation and First In-Human Use of [(44)Sc]Sc-PSMA-617 for PET Imaging of Metastasized Castrate-Resistant Prostate Cancer. *Theranostics,* **2017**, *7*, (18), 4359-4369.
[54] Singh, A.; van der Meulen, N.P.; Muller, C.; Klette, I.; Kulkarni, H.R.; Turler, A.; Schibli, R.; Baum, R.P., First-in-Human PET/CT Imaging of Metastatic Neuroendocrine Neoplasms with Cyclotron-Produced (44)Sc-DOTATOC: A Proof-of-Concept Study. *Cancer biotherapy & radiopharmaceuticals,* **2017**, *32*, (4), 124-132.
[55] Umbricht, C.A.; Benesova, M.; Schmid, R.M.; Turler, A.; Schibli, R.; van der Meulen, N.P.; Muller, C., (44)Sc-PSMA-617 for radiotheragnostics in tandem with (177)Lu-PSMA-617-preclinical investigations in comparison with (68)Ga-PSMA-11 and (68)Ga-PSMA-617. *EJNMMI research,* **2017**, *7*, (1), 9.