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Harnessing alpha-emitting radionuclides for therapy – radiolabeling method review

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ABSTRACT

Targeted alpha therapy (TAT) is an emerging powerful tool treating late-stage cancers for which therapeutic options are limited. At the core of TAT are targeted radiopharmaceuticals, where isotopes are paired with biological targeting vectors to enable tissue- or cell-specific delivery of alpha-emitters. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and diethylenetriamine pentaacetic acid (DTPA) are commonly used to chelate metallic radionuclides but have limitations. Significant efforts are underway to develop effective stable chelators for alpha-emitters and are at various stages of development and community adoption. Isotopes such as ^{149}Tb, $^{212/213}$Bi, ^{212}Pb (for ^{212}Bi), ^{225}Ac, $^{226/227}$Th have found suitable chelators, although further studies, especially *in vivo* studies, are required. For others including ^{223}Ra, ^{230}U and arguably ^{211}At, the ideal chemistry remains elusive. This review summarizes the methods reported to date for the incorporation of ^{149}Tb, ^{211}At, $^{212/213}$Bi, ^{212}Pb (for ^{212}Bi), ^{223}Ra, ^{225}Ac, $^{226/227}$Th and ^{230}U into radiopharmaceuticals, with a focus on new discoveries and remaining challenges.

KEYWORDS

Alpha-emitter, targeted alpha therapy, chelation, radiolabeling, review

DISCLOSURE

PS is a consultant for ARTMS, Inc. as Chief Technology Officer. JJW holds equity in Noria Therapeutics, which has licensed macropa for chelation of alpha emitters. No other potential conflicts of interest relevant to this article exist.

INTRODUCTION

Targeted Radionuclide Therapy has demonstrated significant therapeutic efficacy and survival benefit for some conditions, especially for late-stage disease with limited therapeutic alternatives (*1*,*2*). Several isotopes, such as lutetium-177, yttrium-90, strontium-89, samarium-153 and iodine-131 are in clinical use for treatment of neuroendocrine tumors (Lutathera™), hepatobiliary tumors (Therasphere™), bone metastases (Metastron™, Quadramet™), non-Hodgkins lymphoma (Bexxar™), respectively. Most isotopes used for targeted radionuclide therapy are beta emitters, with ^{223}Ra ([^{223}Ra]$RaCl_2$, Xofigo™) being the only FDA-approved alpha-emitter to date for the treatment of patients with metastatic castration-resistant prostate cancer (mCRPC) with symptomatic bone metastases and no known visceral metastatic disease. Alpha emitters have much higher linear energy transfer (LET, energy deposition per unit pathlength) than do beta emitters (~100 keV/µm vs. 1-2 keV/µm), and generate substantially more free radicals and lethal DNA double-strand breaks (*3*,*4*).

When delivered via tumor-specific targeting vectors, the short range of alpha particles (40-100 microns) enables highly selective targeting of cancers including micro-metastases, while potentially sparing surrounding healthy tissues. The cytotoxicity of alpha emitters is also independent of cell cycle or oxygen concentration (*5*,*6*), providing an advantage for treating hypoxic, often radiation-resistant tumors (*7*).

Targeted alpha therapy (TAT) has been fast-tracked by the FDA approval of [^{223}Ra]$RaCl_2$ for the treatment of bone metastases of mCRPC and the remarkable clinical effectiveness of ^{225}Ac-PSMA617, which is in pilot trials to treat mCRPC (*8*,*9*).

Few alpha-emitting radionuclides suitable for clinical use are available, considering half-life, decay mode and availability. Isotopes with potential medical applications and their properties are listed in Table 1. With the exception of [^{223}Ra]$RaCl_2$, TAT usually comprises an isotope, a targeting vector (e.g., small

molecule, peptide, antibody or engineered antibody), and a chelator that can form a stable complex and carry the isotope to deliver high LET radiation directly to cancer cells and the tumor microenvironment. An ideal chelator exhibits fast metal-complexation kinetics, selectivity for the radionuclide (because of inevitable metal impurities), high thermodynamic stability, high *in vivo* stability, and the ability to bind an imaging isotope for theranostics. Many of the isotopes in this review decay through multiple daughter products, each with their own unique coordination chemistry. This review intentionally focuses on chelates and biomolecule labeling as the issue of daughter recoil and coordination presents a unique set of physics and chemistry challenges that are beyond the scope of this review (*10*).

DOTA has been a standard chelator for radiometals and is still useful for coordinating Ac, Bi, Tb, Th and Pb, however issues remain with this chelate for alpha-emitters, including decreased thermodynamic stability towards large metal ions and slow chelation kinetics. High ligand concentration, which reduces the molar activity, and heating are required, which may compromise some targeting vectors. Sensitivity to metal impurities is another shortcoming. Thus, much work is required to develop suitable chelators for alpha emitters, particularly Ra, Th and U, plus arguably At. The need for novel, suitable labeling methods remains unmet for the rapidly expanding field of TAT. In this review, we will discuss the current state-of-art of labeling method development for alpha-emitters and their applications in TAT.

CHEMISTRY TO LABEL ALPHA EMITTERS

Terbium-149 (^{149}Tb)

Cyclen-based chelators, DTPA and dipicolinic acids (DPA) are among the many chelators reported to complex Tb^{3+} (*11*,*12*). Chelators that form stable complexes with Lu^{3+} may also do the same with Tb^{3+}, although binding may be weaker - Lu^{3+} has a larger atomic radius.

Medical applications of Tb isotopes are at an early stage - they bind DOTA effectively, and also (S,S)-cyclohexane-1,2-diamine-pentaacetic acid (CHX-A"-DTPA) preferentially for antibody labeling due to the fast kinetics and ambient temperature metal incorporation. PET imaging with ^{149}Tb-DOTANOC has shown excellent tumor visualization (*13*), while ^{149}Tb-DOTA-folate (cm09) was found to delay tumor growth in a dose dependent manner (*14*) and ^{149}Tb-CHX-A"-DTPA-rituximab (anti CD-20) demonstrated tumor-free survival in 89% of the mice treated two days after tumor inoculation (*15*).

To date, only DOTA and CHX-A"-DTPA have been studied with radio-Tb isotopes, and reports on their preclinical applications are limited (Figure 1); additional Tb chelates would yield more ideal candidates with improved kinetics or stability. The intense luminescent properties of various Tb complexes may be leveraged for use in optical/radio multi-modality imaging.

Astatine-211 (^{211}At)

Comprehensive reviews summarize organic and inorganic chemical methods for producing radioastatinated compounds (*16–18*). Astatine isotopes have short half-lives (longest is 8.1 h, none is stable) and radioiodine is commonly used as a surrogate when studying astatine chemistry, despite evidence of diverging in the reactivity with different labeling reagents and the *in vivo* stability of the products formed. Although At has metallic character, to date no chelating ligand evaluated provides an ^{211}At complex with enough *in vivo* stability for TAT applications (*16–18*). An exception may be a rhodium(III) astatide complex stabilized by a macrocyclic crown thioether (16aneS_4-diol) (*19*). Differences in the biodistribution of Rh[16aneS_4-diol]^{131}I and Rh[16aneS_4-diol]^{211}At suggest some deastatination of the latter, but the *in vivo* stability may be potentially useful (*19*). How stable the ^{211}At label must be before it can be useful for patient treatment has not been established.

A few astatine labeling methods utilize a non-activated aryl-astatine bonding approach (*16*–*18*), with the most widely used method involving organometallic compounds such as *N*-succinimidyl 3-(tri-alkylstannyl)benzoate **2** in electrophilic substitution reactions (Figure 2). This approach was employed in many preclinical studies, and more recently in two phase I clinical trials for treating brain or ovarian cancer (*20*,*21*). In these clinical trials, the ^{211}At-labeled radiopharmaceutical was prepared using a two-step labeling approach. A one-step labeling approach has been demonstrated by the Gothenburg team wherein the tri-alkylstannyl benzoate is conjugated prior to radiolabeling (*22*).

Non-activated arylboronic acid derivatives were shown to react rapidly with electrophilic astatine species. More recently nucleophilic substitution reactions using aryl boronic acids/esters have been shown to provide highly efficient ^{211}At-labeling. A series of ^{211}At-labeled compounds were prepared using boronic ester precursors and a Cu catalyst, including the ^{211}At-labeled PARP-1 inhibitor [^{211}At]MM4, **3a**, which exhibited a similar biodistribution profile as its ^{18}F and ^{125}I analogues and could potentially be useful for TAT applications (*23*,*24*). Copper-catalyzed astatination and radioiodination reactions using arylboronic acids can be conducted in aqueous solution at ambient temperature thus suitable for mAb labeling. Copper-catalyzed astatination and radioiodination of arylboronic acid-conjugated mAbs (compound 4, Figure 2), such as anti-CD138 antibody, 9E7.4 (*25*), have been reported.

Aryliodonium salts can also be used for mAb conjugate labeling via nucleophilic substitution. For example, asymmetric aryliodionium salts with one of the aryl rings bearing a *N*-hydroxysuccinimidyl (NHS) ester (compound 5a-c, Figure 2) were conjugated to anti-CD138 mAb for astatination and radioiodination studies (*25*). Striking differences were observed between radioiodination and astatination reactions in regioselectivity and in reaction conditions required to obtain optimal radiochemical yield; the astatination reaction using aryliodonium salts is more efficient than the radioiodination.

Another ^{211}At-labeling reagent that has been used in preclinical and clinical studies is the isothiocyanatophenyl-*closo*-decaborate(2-) (B10) boron cage molecule (compound 6, Figure 2) (*25*). The ^{211}At-labeled B10-conjugated anti-CD45 mAb BC8 is currently being evaluated in two phase I/II trials for hematopoietic cell transplantation in patients with leukemia, myelodysplastic syndrome and nonmalignant diseases. The aromatic B10 moiety provides high ^{211}At labeling efficiency (75-90% radiochemical yield, 1 min) with high *in vivo* stability. New boron cage ^{211}At-labeling reagents to potentially stabilize ^{211}At in a higher oxidation state, +3 or +5, are being evaluated for improved tissue distribution and more favorable pharmacokinetic properties (*25*).

^{211}At is a highly attractive radionuclide for TAT applications; to date, electrophilic substitution on unactivated aromatic rings is the most widely used astatination method for TAT preclinical and clinical research. Recent studies show the potential of nucleophilic substitution reactions for astatination of small molecules and mAbs (*23*,*24*). In addition to the aryl-astatine bonding approach, boron-astatine bonding provides an alternative astatination strategy. Other astatination strategies that involve the use of chelation chemistry, nanoparticles, etc. have been explored but have yet to show potential for use in humans (*26*).

Bismuth-212/213 ($^{212/213}$Bi)

DOTA and CHX-A”-DTPA are frequently used for Bi labeling, but neither is a good match for Bi^{3+}. Bi-DOTA or Bi-CHX-A”-DTPA complexes are not highly stable in human plasma (85% DOTA, 76% CHX-A”-DTPA, 2 hours) (*27*). Nonetheless, their efficacy was tested successfully *in vivo* for antibodies and peptides, with CHX-A”-DTPA primarily used for antibody conjugates and DOTA for peptides (Figure 3). Examples in clinical studies include ^{213}Bi-HuM195, ^{213}Bi-anti-CD20-mAb, ^{213}Bi-anti-EGFR-mAb, ^{213}Bi-anti-MCSP-mAb

(9.2.27), ^{213}Bi-DOTA-substance P and ^{213}Bi-DOTATOC. ^{213}Bi radiopharmaceuticals in clinical trials have been recently reviewed (*8*).

The nitrogen-rich cyclen-pyridine ligand L^{py} and analogs are reported to form highly stable complexes with $^{213/207}$Bi at RT (*28*). L^{py} showed selectivity towards Bi^{3+} over Ac^{3+}, and resistance to transmetalation when challenged with Cu^{2+}, Zn^{2+}, Fe^{2+} or Bi^{3+}, but *in vivo* studies are not yet reported. The phosphorus-containing cyclen chelates DOTP (also named DOTMP, Figure 3) and analogs form stable complexes with ^{213}Bi at RT as well, and ^{213}Bi-DOTP showed higher stability in human serum in 120 min compared to ^{213}Bi-DOTA or ^{213}Bi-CHX-A"-DTPA (*27*). ^{213}Bi-DOTP primarily accumulates in bone, different from free Bi^{3+} that accumulates in kidneys.

$^{205/206}$Bi has been used to evaluate novel chelates such as ({4-[2-(bis-carboxymethyl- amino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl}-acetic acid (NETA, a NOTA derivative) and 7-[2-(bis-carboxymethyl-amino)-ethyl]-4,10-bis-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl-acetic acid (DEPA, a DOTA derivative), synthesized specifically to provide a larger cavity to coordinate with Bi^{3+}. Both coordinate $^{205/206}$Bi at low or ambient temperature with high efficiency (*29*,*30*). Using a three-carbon linker in the bifunctional chelates, $^{205/206}$Bi-3p-*C*-NETA- and $^{205/206}$Bi-3p-*C*-DEPA-trastuzumab both showed effective tumor accumulation and low kidney uptake (*29*,*30*).

Overall, because of the short half-life of the Bi isotopes, development of novel chelators such as L^{py}, DOTP, NETA, and DEPA, capable of rapid, ambient temperature metal incorporation may help to improve radiochemical yields and molar activity of potential radiopharmaceuticals. Additional *in vivo* studies are required to evaluate stability and demonstrate suitability for TAT. There also remains potential for further development of S-donor chelates, informed by meso-2,3-dimercaptosuccinic and 2,3-dimercaptopropane-1-sulfonic acid that were used for chelation therapy to prevent Bi overdose in humans (*31*).

Lead-212 (^{212}Pb)

Although not an alpha-emitter itself, ^{212}Pb is used as an internal generator for ^{212}Bi. DOTA was one of the first chelators studied with ^{212}Pb (Figure 4). While data suggest favorable kinetic stability of Pb-DOTA complexes (*32*), release of Pb *in vivo* in the acidic tumor environment was reported in some cases and may prove a source of toxicity upon internalization and metabolic processing (*33*).

In a pre-targeting approach where ^{212}Pb-DOTA-biotin was injected into mice pre-inoculated with streptavidin-NR-LU-10 mAb. Despite the elevated dose to kidneys from free ^{212}Bi, the biodistribution relative to the ^{203}Pb counterpart was identical for all other organs, indicating little migration of ^{212}Bi once in tissue (*34*). ^{212}Pb-DOTA-Re(Arg^{11})CCMSH, a melanoma-targeting peptide was evaluated in B16/F1 xenografts (*35*), provided measurable therapeutic outcomes. ^{212}Pb-DOTA-103A mAb was studied for treating Rauscher leukemia virus (RVB3) (*36*). Despite success in eradicating the target, all animals succumbed to bone marrow toxicity, in contrast to the lack of marrow toxicity in a similar study using ^{212}Bi–103A–mAb. Given the observed susceptibility for some DOTA derivatives to be acid-labile at lower pH, it has been postulated that ^{212}Pb dissociation, followed by clearance from the target tissue and localization of the free P/Bi in bone marrow, leads to toxicity.

A more efficient and stable chelate for Pb, TCMC (1,4,7,10-tetraaza-1,4,7,10-tetra- (2-carbamoyl methyl)-cyclododecane), has since become the standard for Pb labeling (*37*). Bifunctional 4-nitrobenzylisothiocyanate derivatives were used to generate ^{203}Pb-TCMC-CC49 (*37*) and ^{212}Pb-TCMC-trastuzumab (*38*). TCMC-trastuzumab conjugates were determined to be more stable and more efficient at metal conjugation than their DOTA counterparts. Pb-TCMC-trastuzumab radioconjugate stability was analyzed in vitro, coupled with the demonstrated therapeutic effect and minimal toxicity of ^{212}Pb-trastuzumab in an orthotopic model of human prostate cancer cells (*38*). ^{212}Pb-TCMC-trastuzumab was used in a first in human dose escalation clinical trial and was well tolerated (*39*).

In 2020, a biodistribution study of several ^{203}Pb and ^{212}Pb-labeled TCMC-PSMA derivatives was also reported (*40*). One derivative demonstrated tumor growth delay, with the kidney as the dose-limiting organ. Another PSMA ligand, [^{212}Pb]Pb-NG001 showed ^{212}Pb and ^{212}Bi co-localized during the 24-hour study period, leading the authors to suggest that the rapid target internalization and non-target clearance of [^{212}Pb]Pb-NG001 prevented measurable amounts of ^{212}Bi from being released from the target tissue (*41*). Recently, ^{212}Pb-TCMC mAb376.96 was shown to bind the B7-H3 epitope found on ovarian cancer cells, with a 2-3-fold increased survival in tumor bearing mice over the control group (*42*).

A series of cyclen-based chelators (described as DOTA-1Py, -2Py, and -3Py) were compared to established chelators DOTA and TCMC. All chelates incorporated ^{212}Pb (and ^{203}Pb) efficiently, with radiochemical yields higher than DOTA and lower than TCMC (*43*). An intriguing report examining the complexation between Pb and calix[4]arene-1,3-crown-6 where the formation of a 1:1-Pb(II)-calix-complex was confirmed by ^{207}Pb NMR (*44*). Phosphonic acid chelates DOTP (DOTMP) and (tetra-methylenephosphonic acid (EDTMP) were labeled with Pb but found to have low stability *in vivo* (*32*).

Overall, ^{212}Pb coordination chemistry appears to have emerged from a multi-decade pre-clinical development phase and has advanced into a limited number of human clinical trials. The availability of the isotope and an imageable element-equivalent companion (^{203}Pb), coupled with extensive development of key chelators (i.e. TCMC) suggest an exciting future for this isotope.

Radium-223 (^{223}Ra)

So far, the only $^{223}Ra^{2+}$ chelator with demonstrated *in vivo* stability is macropa, characterized by low bone uptake of [^{223}Ra][Ra(macropa-β-alanine)] (*45*) (Figure 5). However, the bioconjugate of macropa to a PSMA ligand, DUPA, showed no difference to [^{223}Ra]$RaCl_2$ in subsequent biodistribution studies, highlighting the difficulty of developing stable chelates for Ra^{2+}. DOTA and Kryptofix 2.2.2 bind Ra^{2+} as

well, evidenced by competition extraction experiments against calix[4]arene tetraacetate (*46*), but the stability of these complexes has yet to be determined. Although calix[4]arene is effective in extracting Ra^{2+} in neutral pH solution, the stability of the complex is poor (*46*). Because of the challenges with Ra^{2+} chelation, there are reports on nanoparticles to immobilize ^{223}Ra, including liposomes, barium sulphate, lanthanum phosphate, and hydroxyapatite etc., and all of which have been recently reviewed (*26*).

There is no suitable chelator for *in vivo* delivery of ^{223}Ra has been identified to date, although macropa shows promise, with much work remaining to incorporate this useful isotope for TAT.

Actinium-225 (^{225}Ac)

Most ^{225}Ac chelates developed to date are macrocycles, however DOTA remains as the gold standard for Ac labeling for all clinical work. Examples include^{225}Ac-PSMA617, ^{225}Ac-DOTATOC, and ^{225}Ac-DOTA-HuM195 (*8*) (Figure 6). Labeling can be performed using 1- (*47*) or 2-step (*48*) methods, with the latter being used for heat-sensitive targeting biomolecules. On the clinical front, ^{225}Ac-DOTA bioconjugates of ^{225}Ac-PSMA617, ^{225}Ac-DOTATOC and ^{225}Ac-DOTA-HuM195 (*8*) have been evaluated. On the preclinical front, ^{225}Ac-DOTATOC (*49*,*50*), -F3 (*51*), -c(RDGyK) (*52*), -MC1RL (*53*), -HuM195 (*48*), -J591 (anti-PSMA) (*48*), -B4 (anti-CD19) (*48*), and -trastuzumab (*54*) are all reported. It is noteworthy that unexpected high liver or spleen uptake observed in some studies may not be related to the *in vivo* stability of ^{225}Ac-DOTA but rather to radiolysis (*55*), necessitating further study.

Large macrocyclic chelates have been used to ease the steric constraints for large metal ions and to increase the coordination number. Macrocycles with backbones of 18-members with 6 donors (N or O) have been extensively studied. Macropa showed high specificity towards Ac^{3+} (*56*). It has been used in a couple of PSMA targeting reagents (RPS070 (*56*), RPS074 (*57*)) and showed good *in vivo* stability, effective tumor uptake, and therapy efficacy. Crown, another emerging chelate with structural similarity

to DOTA, showed high labeling yields with Ac^{3+} at low concentrations (*55*,*58*). It was conjugated to an αMSH peptide targeting melanocortin 1 receptor and showed high tumor uptake and very low normal tissue/organ uptake (*55*). HEHA, one of the first chelates developed specifically for Ac^{3+}, showed promise in early studies (*59*,*60*) but a conjugate with mAb-B201B slowly released free Ac^{3+} from the targeting organ (lungs) (*61*). Other macrocyclic chelates such as PEPA (*59*), TETPA (*62*), TETA (*62*), DOTP (*63*), macropid (*64*) suffer either low labeling yield or poor *in vivo* stability, indicating increasing ring size or adding more donor atoms may not always work.

Acyclic chelates with picolinic acid moieties have shown significant promise as Ac^{3+} chelates as well. Py4pa demonstrated high Ac labeling yield at low chelate concentrations (*65*). The biodistribution of ^{225}Ac-py4pa-trastuzumab showed effective tumor accumulation and relatively low liver uptake. Bispa^2, CHXoctapa and noneunpa all showed excellent labeling yield at low chelate concentrations (*66–68*). Those chelates can coordinate ^{111}In as well, giving the advantage of potential theranostic pair.

Overall, new chelators such as macropa, crown and py4pa are highly promising because of the improved radiochemical yield, specific activity, and mild labeling conditions. One important area to address is whether the new chelators are capable of binding with an easily accessible imaging isotope, such as ^{68}Ga - imaging is essential for clinical translation of ^{225}Ac-DOTA radiopharmaceuticals. For the chelators with demonstrated ability to bind with an imaging isotope (Bispa^2, CHXoctapa, noneunpa), *in vivo* studies are necessary to assess the stability and applications.

Thorium-226/227 ($^{226/227}$Th)

Early studies of ^{227}Th chelation employed polyphosphonate-based ligands to target bone. Acyclic chelators ethylenediamine tetra(methylene phosphonic acid) (EDTMP) and diethylenetriamine penta(methylene phosphonic acid) (DTPMP) were evaluated in addition to the macrocyclic ligand (DOTMP)

for this purpose (*69*,*70*). In animal studies, all three complexes exhibited enhanced bone uptake compared to free [^{227}Th]Th^{4+}, indicating that the phosphonate groups can facilitate bone-targeting of this radionuclide and that these chelators form stable *in vivo* complexes with Th^{4+}.

Based on its success with many other radiometals, the bifunctional analogue of DOTA was studied to chelate ^{227}Th. Early coordination chemistry studies as well as more recent solid-state structural studies of Th-DOTA complexes (*71*–*73*) indicate they should be stable in aqueous solution (Figure 7)). The most common process involves a low-yielding two-step reaction; ^{227}Th is initially radiolabeled with bifunctional DOTA-NCS at elevated temperature and then conjugated to an antibody (*74*,*75*). A single-step radiolabeling reaction with a trastuzumab conjugated to DOTA required very long (2 d) reaction times (*76*). Despite the poor radiolabeling kinetics, the Th-DOTA complex retains good stability *in vivo* (*75*), enabling its use for various TAT applications.

Alternative chelators for DOTA have been investigated for ^{227}Th. In a recent study, the picolinic acid "pa" chelators octapa, neunpa-*p*-Bn-NO_2, pypa, and py4pa were investigated in this context (*77*). Among those four chelates, py4pa gave a high radiochemical yield (87% in 2.5 h) and the complex remained intact in PBS solution for over 2 weeks. Based on these promising results, py4pa was investigated for chelating the shorter lived ^{226}Th (*77*). Microwave irradiation afforded the radiolabeled complex in high yield, marking the first occasion of complexation of the ^{226}Th radionuclide.

Given the oxophilic nature of the Th^{4+} ion, the use of oxygen-rich chelating agents for these radionuclides is a promising approach. An octadentate hydroxypyridonate-based chelator bearing four bidentate 3-hydroxy-N-methyl-2-pyridinones (Me-3,2-HOPO) was validated to be an effective chelator for ^{227}Th (*78*). This ligand quantitatively incorporated ^{227}Th after 30 min at ambient temperature, marking a significant enhancement over the elevated temperatures required for DOTA; the resulting ^{227}Th complex was stable *in vivo*, as reflected by a lack of bone uptake. This chelator enabled several tumor-targeting

constructs to demonstrate the *in vivo* therapeutic potential of TAT with ^{227}Th (*79–81*). Detailed analytical studies on Th^{4+}-chelation with this ligand revealed that it possesses a large (> 20 orders of magnitude) thermodynamic selectivity for +4 over +3 ions (*82*). This large selectivity for +4 ions may explain the high stability of its Th^{4+} complex *in vivo*, as biologically common +3 ions such as Fe^{3+}, cannot effectively displace the actinide. The status of the Me-3,2-HOPO ligand as the current gold standard for ^{227}Th chelation has inspired additional studies on ligands of this type. A recently reported macrocyclic tetrapthalimide ligand is reported to have the highest thermodynamic affinity for Th^{4+} reported to date (log K = 10^{54}), representing a promising candidate for ^{227}Th TAT (*83*).

Overall, oxygen-rich chelators are effective for stabilizing the hard, oxophilic Th^{4+} ion *in vivo*. The HOPO-containing ligands are among the most promising candidates for use with Th-based TAT, but these chelators suffer from challenging chemical syntheses. The development of more readily accessible polydentate oxygen-rich chelators will meet an important need for advancing Th-based TAT.

Uranium-230 (^{230}U)

The chemically hard and oxophilic nature of the UO_2^{2+} ion dictates anionic oxygen-donor ligands. These conclusions are supported by many decades of $[UO_2]^{2+}$ coordination chemistry (*84*). Surprisingly, despite this, there have been few studies to develop chelators specifically for ^{230}U TAT. The lack of ^{230}U TAT studies is a consequence of the limited availability of the radionuclide and narrow knowledge of its potential importance in nuclear medicine. Researchers have investigated the interactions of the $[UO_2]^{2+}$ ion with abundant human serum proteins transferrin and albumin, in addition to other small molecules, like carbonate, that are present in blood in order to assess the needed efficacy of a potential ^{230}U chelator (*85*). An effective chelator for this radionuclide was predicted to have a stability constant in excess of 10^{19} (*85*); there are few chelators with such a high stability constant for $[UO_2]^{2+}$. For example, calixarene ligands

were found to form unstable complexes with $[^{238}UO_2]^{2+}$ that dissociated in serum (*86*). Given the expansive literature and efforts to develop uranium decorporation agents, however, it is likely that there are promising candidates for ^{230}U chelation extant. For example, the deferoxamine (DFO) complex of UO_2^{2+} has a stability constant within this range (*87*), as do a class of hexadentate equatorial-spanning terephthalamide(bis-hydroxypyridinone) ligands (*88*), suggesting that these chelators may be useful for ^{230}U TAT applications.

Although the $[UO_2]^{2+}$ ion predominates in aqueous solution, U^{4+} is also found. DOTA complexes of U^{4+} are well characterized and are stable to oxidation in aqueous solution (*72*,*73*,*89*). HOPO-based ligands can facilitate the reduction of $[UO_2]^{2+}$ to U^{4+} in water (*90*). HEHA, investigated for ^{225}Ac, also forms a stereochemically rigid and therefore potentially inert complex with U^{4+} (*91*). Thus, these results suggest that targeting the chelation of U^{4+}, perhaps by modulating the redox conditions of the radiochemical isolation and separation of ^{230}U, may provide an alternative effective means for delivering this radionuclide to tumor cells. Although ^{230}U has been proposed to be a promising candidate for TAT, there are currently no *in vivo* or *in vitro* studies that have demonstrated its efficacy for eliminating malignant cells. As such, chelation efforts for this radionuclide are somewhat underdeveloped; however, the principles of uranium chelation that have been established for other applications are likely pertinent to ^{230}U TAT applications.

CHALLENGES AND OPPORTUNITIES

Lutathera, as the first regulatory-approved (USA and EU) compound for peptide receptor radionuclide therapy (PRRT), has stimulated interest in targeted radiotherapy, particularly to treat patients with metastatic disease. Alpha-emitters are highly promising for radiotherapeutic agents, illustrating viability in the treatment of a wide range of malignancies. This surge of interest, particularly

in the actinides, however, has outpaced the understanding of the chemistry of each individual element; this remains poorly developed owing to the limited supply of the elements and the usually intense radioactivity of all isotopes. The chemistry depends on the reliable availability of alpha-emitting radionuclides with new approaches to produce and isolate them for therapy being critical. New production methods regularly coming on stream now mean supply is approaching levels capable of supporting pre-clinical as well as clinical studies on a routine basis, and further research into the coordination chemistry of these isotopes must catch up. With half-lives from around one hour (^{212,213}Bi) to weeks (^{227}Th), coupled with the radiological and biological considerations needed, the challenges to chelate these metals are considerable. What is evident, is that more specific chelation is required and specific isotope-chelate combinations will allow the field to move ahead apace.

An important step to advance the novel chelates is to find a readily available imaging isotope for those specific chelates for patient stratification and treatment monitoring, which can be challenging because the relevant alpha-emitters are much larger than currently available imaging isotopes. The challenge can be addressed by either designing and testing chelates that can accommodate both (*68*), or developing imaging radioisotopes, such as ^{134}Ce/^{134}La (*92*), that are more suitable to pair with the alpha-emitters.

One of the major impediments to using alpha-emitting radionuclides has been the need for improved chelators or labeling methods to ensure they remain stably complexed *in vivo* to deliver their payload to the target tissue and minimize the dose that can arise due to dissociation. More preclinical studies are required to evaluate or validate the emerging chelates.

Alpha recoil will cause the progenies to leave the chelates and free the daughters that may carry significant or even majority of the energy, like in the case of ^{225}Ac decay. Fast pharmacokinetics and

sufficient internalization are highly desirable to limit the off-target toxicity and maximize the tumor dose. Other methods to mitigate the risk include encapsulating in nanoparticles or localized administration (*10*).

The tumor microenvironment includes as 3 major cell types immune, stromal, and vascular cells and the potential to target each will become more and more exact using unique combinations of elements/isotopes with chelators and more specific targeting vectors. The incorporation of alpha-emitters into nanomaterials (with or without chelation) presents another front of interest to TAT.

Metal-ligand bonding in actinide complexes has been thought to be driven primarily by electrostatic interactions and steric constraints, with limited orbital interaction. Early efforts show that one can match isotopes to chelators but actinides show significantly more covalency that lanthanides (*68*). Further studies of fundamental actinide coordination and organometallic chemistry will enlighten more specific chelate designs.

In the near future, a focus on new tightly-binding, selective chelating ligands that yield high specific activity will be necessary. Reproducibly versatile and subsequently translatable chelation chemistry is required. Modular automation ("smart") technology will be essential for radiopharmaceutical synthesis that is adaptable to a variety of high-energy applications. The kit formulation, so important in SPECT - ^{99m}Tc being the archetype, will comprise varying combinations of metal ions, chelators, linkers and targeting vectors. The biology of alpha-emitting daughter isotopes must be explored as this could be a limiting factor in utility, based on their toxicity vs. therapeutic application.

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Table 1. Isotopes for targeted alpha therapy (TAT)

Isotope	Half-life	Common ion	Ionic radius, Å	Common coordination number	Hard/soft	pKa (aq.)
^{149}Tb	4.1 h	Tb^{3+}	1.04	8-9	Hard	7.9
^{211}At	7.2 h	At^{+}, AtO^{+}	-	-	-	-
^{212}Bi, ^{213}Bi	60.6 m, 45.6 m	Bi^{3+}	1.17	8	Intermediate	1.1
^{212}Pb (for ^{212}Bi)	10.6 h	Pb^{2+}	1.43	8	Intermediate	0.9
^{223}Ra	11.4 d	Ra^{2+}	1.48	8-12	Hard	3.1
^{225}Ac	9.9 d	Ac^{3+}	1.12	9-10	Hard	9.4
^{226}Th,^{227}Th	30.7 m, 18.7 d	Th^{4+}	1.05	>8	Hard	3.2
^{230}U	20.8 d	$[UO_2]^{2+}$	-	6	Hard	4.2

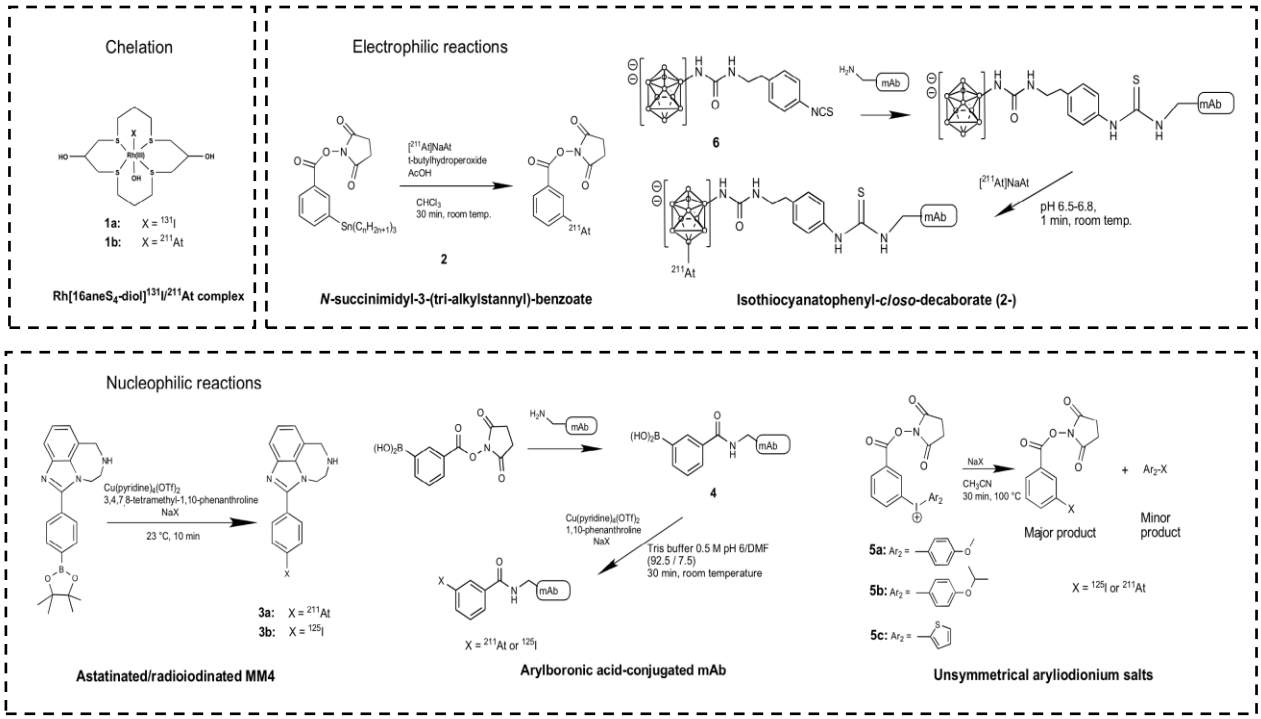
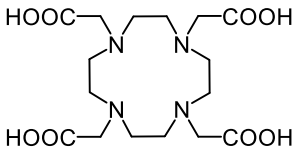
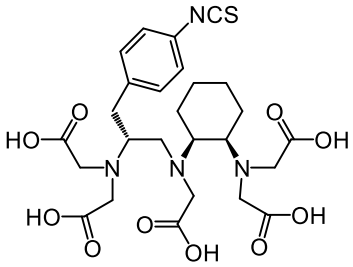


Figure 1. ^{211}At labeling methods. DMF: dimethylformamide; NaX: [^{125}I]NaI or [^{211}At]NaAt; mAb: monoclonal antibody.

Figure 2. Chelates for ^{149}Tb.

Radiometal	Ligand	Bioconjugates	Labeling conditions	*In vivo* stability	Ref.
^{149}Tb	DOTA 	DOTANOC DOTA-folate (cm09)	95˚C, 15 min, α-HIBA pH4.7	Stable	(*13*,*14*)
	p-SCN-Bn-CHX-A"-DTPA 	Rituximab	RT, 1 hr, α-HIBA	Stable	(*15*)

RT: room temperature.

Figure 3. $^{212/213}$Bi chelators.

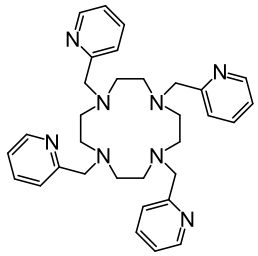
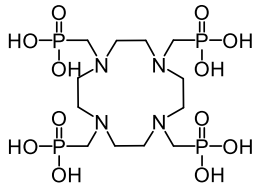
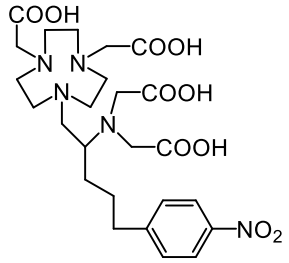
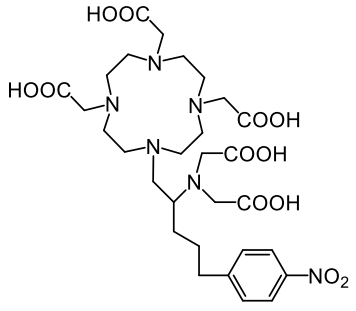
Radiometal	Ligand	Bioconjugates	Labeling conditions	*In vivo* stability	Ref.
^{213}Bi	DOTA (see Figure 2)	DOTATOC, Substance P	95˚C, 5 min, TRIS buffer	Stable	(*8*)
	CHX-A"-DTPA (see Figure 2)	lituzumab, anti-CD20-mAb, anti-EGFR-mAb, anti-MCSP-mAb (9.2.27),	RT or 80˚C, 5 min, NH_4OAc pH 5	Stable	(*8*)
	L^{Py} 	N/A	RT, 30 min, NH_4OAc pH 5	N/A	(*28*)
	DOTP (DOTMP) 	N/A	RT, 5 min, NaOAc pH 5	N/A	(*27*)
	3p-*C*-NETA 	Trastuzumab	37˚C 30 min, NaOAc pH 5.5	Stable	(*29*)
	3p-*C*-DEPA 	Trastuzumab	RT, 1 min, NaOAc pH 5.5	Stable	(*30*)

Figure 4. Chelators for ^{212}Pb.

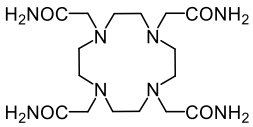
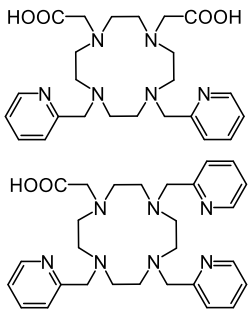
Radiometal	Ligand	Bioconjugates	Labeling conditions	*In vivo* stability	Ref.
^{212}Pb, ^{203}Pb	DOTA (see Figure 2)	mAb: AE1; 103A; CC49; B72.3 peptide/small molecule: biotin; CCMSH;	mAb: 35°C, 45 m, NH_4OAc pH 4 peptide/small molecule: 80˚C, 40 m, pH 4-9 (various buffers);	Acid-labile, lysosomal degradation	(*32*)
	TCMC 	CC49, trastuzumab, PSMA, NG001; PSMA; 376.96; VCAM-1	37˚C, 30 min. NH_4OAc in 0.1 M HCl	Stable	(*37*–*39*,*42*)
	DOTA-2Py and DOTA 3Py 	N/A	RT, <1 hr, NH_4OAc, pH 7	N/A	(*43*)

Figure 5. Chelators for ^{223}Ra.

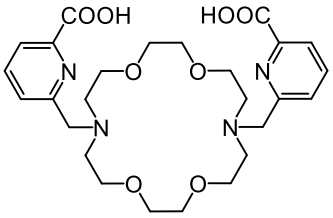
Radiometal	Ligand	Bioconjugates	Labeling conditions	*In vivo* stability	Ref.
^{223}Ra	 macropa	DUPA (PSMA ligand)	RT, 5 m, NH_4OAc pH 6	Stable with β-alanine, unstable with DUPA	(*45*)

Figure 6. Chelators for ^{225}Ac.

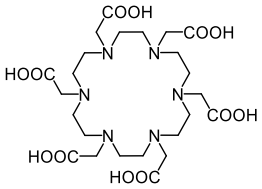
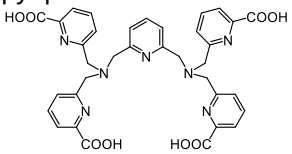
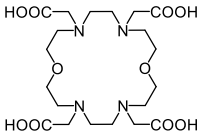
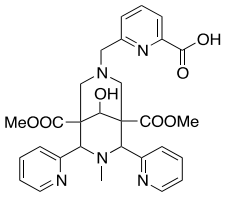
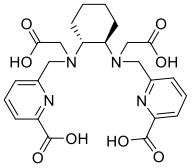
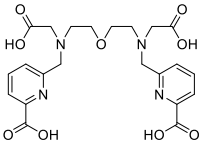
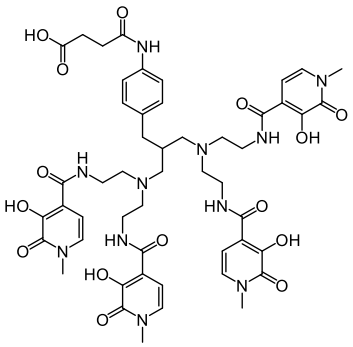
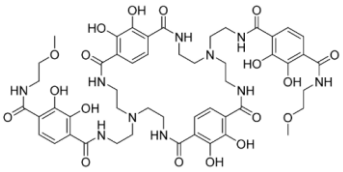
Radiometal	Ligand	Bioconjugates	Labeling conditions	*In vivo* stability	Ref.
^{225}Ac	DOTA (see Figure 2)	PSMA617, TATE, TOC, substance P, RGD, lintuzumab, trastuzumab, αMSH, PP-F11N	45-90˚C, 30-60 min, NaOAc, NH_4OAc, or TRIS, pH 4.5-9	Stable	(*48*–*51*,*53*,*54*,*67*)
	HEHA 	mAb 201B, mAb CC49, hu-ΔCH_2CC49	RT, or 40˚C, 30 min, NH_4OAc, pH 5 or 5.8	May slowly release Ac over time	(*59*–*61*)
	macropa (see Table 5)	RPS070, RPS074	RT, 5 min, NH_4OAc, pH 5.5-6	stable	(*56*,*57*)
	py4pa 	trastuzumab	RT, 30 min, NH_4OAc, pH 7	stable	(*65*)
	crown 	αMSH, TATE	RT, 15 min, NH_4OAc, pH 5-7	stable	(*55*,*58*)
	bispa^2 	N/A	RT, 15 min, NH_4OAc, pH 6	N/A	(*66*)
	CHXoctapa 	N/A	RT, 60 min, NH_4OAc, pH 5.5 or 7	N/A	(*67*)
	Noneunpa 	N/A	RT, 10 min, NH_4OAc, pH 7	N/A	(*68*)

Figure 7. Chelators for $^{226/227}$Th.

Radiometal	Ligand	Bioconjugates	Labeling conditions	*In vivo* stability	Ref.
$^{226/227}$Th	DOTA (see Figure 2)	Rituximab, trastuzumab	55-60°C, 30 m, NMe_4OAc, pH 5.5	Stable	(*71–76*)
	Me-3,2-HOPO 	Anti-CD33 mAb, anti-CD70 mAB, mesothelin targeted mAb.	RT, 30 m, citrate pH 5.5	Stable	(*78–82*)
	py4pa (see Table 6)	N/A	RT, 2.5 h, citrate pH 5-5.5	N/A	(*77*)
	macrocyclic tetrapthalimide 	N/A	Reflux in MeOH, 3 h	N/A	(*83*)