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Towards Translation of ^{212}Pb as a Clinical Therapeutic; Getting the Lead In!

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Abstract

Targeted α-particle therapy offers the potential for more specific tumor cell killing with less damage to surrounding normal tissue than β-emitters because of the combination of short path length (50–80 μm) with the high linear energy transfer (100 KeV μm^{-1}) of this emission. These physical properties offer the real possibility of targeted (pre-targeted) α-therapy suitable for the elimination of minimal residual or micrometastatic disease. Targeted and pre-targeted radioimmunotherapy (RIT) using α–emitters such as ^{212}Bi ($T_{1/2}$ = 1.01 h) and ^{212}Pb ($T_{1/2}$ =10.6 h) has demonstrated significant utility in both *in vitro* and *in vivo* model systems. ^{212}Pb, a promising α-particle emitting source, is the longer-lived parent nuclide of ^{212}Bi, and serves as an *in vivo* generator of ^{212}Bi. The radionuclide has been successfully used in RIT and pre-targeted RIT and demonstrated the enhanced therapeutic efficacy in combination with chemotherapeutics, such as gemcitabine and paclitaxel. The following perspective addresses the modes of radionuclide production, radiolabeling and chelation chemistry, as well as the application of ^{212}Pb to targeted and pre-targeted radiation therapy.

Introduction

The better understanding of the molecular differences between cancer and normal cells has led to the development of therapies that directly target cancer cells, including the use of monoclonal antibodies (mAbs) directed at tumor-associated antigen. Cancer therapy represents one major area in which mAbs have been successful. Although such antibody therapies have shown significant successes in cancer treatment, strategies to increase their efficacy are urgently needed. One such strategy is to link antibodies against tumor-associated antigens to highly toxic radionuclides, which brings to bear the killing power of these radionuclides directly onto tumor cells. Specially, targeted, cytocidal radionuclides (β- and α-particle emitters) can be localized in malignant tissue for therapeutic applications via the use of appropriate targeting vectors. These vectors include tumor antigen binding mAbs and their variants, or cell surface receptor binding peptides. Towards this end, FDA approval for two radiolabeled anti-CD20 mAbs, ^{90}Y-labeled ibritumomab tiuxetan (Zevalin) in 2002, and ^{131}I-labeled Bexxar in 2003 were landmark events in the developmental history of therapeutic radiolabeled mAbs.[1] The radiolabeled antibody is also recognized for its potential efficacy as both a monotherapy and for its enhanced efficacy when used in combination therapy.

New radioimmunotherapy (RIT) approaches incorporating α-particle emitters have been considered and have led to the development of both chelating agents and execution of pre-clinical studies. The α-particle has a very short path length (<100 μm), but a very high linear

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energy transfer (LET),[2] with typical energy deposition of ~100 keV/μm compared to 0.2 keV/μm typically for a β-particle. The relative biological effectiveness of high LET radiation exhibits no dose rate dependence and is effective even under hypoxic conditions.[3] The α-particle, a He nucleus, is quite relatively large compared to a β-particle, and the emission is associated with discrete high energies and a dense ionization track that is also associated with a high probability of inflicting irreparable and cytocidal DNA double strand breaks.[4–8] An individual cancer cell can in theory be killed by interaction with only a single α-particle traversing the nucleus of a cell.[9–11] The fundamental physics and radiobiology of a β-particle emitter provides a poor tumor to normal tissue dose ratio for treatment of single cell disease. On the other hand, delivery of an α-emitting radio nuclide to the cell membrane is sufficient to kill malignant cells, requiring only a few α-particle decays at the cell membrane due to 3 dimensional emission geometry considerations, to effect a 99.99% level of cell kill with correspondingly low normal tissue toxicity.[12] Consequently, α-emitters are well suited for hematologic disease, micrometastatic disease, and tumor cells near the surface of cavities. High homogeneity of antigenic expression is required for the complete destruction of micrometastases with high LET. Conversely, with radiations of low LET with a longer path length, the cross-fire of the β-emitters may make up for the non-homogeneity of antigen expression. A number of pre-clinical studies have concluded that α-emitters may be more effective than β-emitters administered at comparable doses in RIT.[13,14] However, high cost and/or limited or unresolved availability are major obstacles that have limited the clinical evaluation of mAbs radiolabeled with α-emitters. With the elimination of many obstacles and a better understanding of inherent imitations of mAbs, the active targeting and delivery vector of the radiation, many radiolabeled mAbs have been, or currently are being evaluated.

Although there are more than 100 α-particle emitting radionuclides, the majority of these radionuclides have half-lives that are either too short or too long for any meaningful or realistic therapeutic use, their production is not economically viable, or no viable chemistry for their use presently exists. The most widely studied α-particle candidates for therapy (Table 1) are ^{211}At ($T_{1/2}$ = 7.2 h), ^{212}Bi ($T_{1/2}$ = 61 min), ^{212}Pb ($T_{1/2}$ = 10.2 h), ^{213}Bi ($T_{1/2}$ = 46 min), and ^{225}Ac ($T_{1/2}$ = 10 days). Clearly, opportunities continue to exist in select areas of both coordination chemistry and conjugation chemistry. ^{212}Pb and ^{212}Bi are both promising α-particle emitting sources that have well-described radiochemistry for antibody linkage and are readily obtained from a ^{224}Ra generator.[15] ^{212}Pb is actually a β-emitter and is the immediate parental radionuclide of ^{212}Bi. Therefore, one strategy that has been devised has been to label a mAb with ^{212}Pb to serve then as an *in vivo* generator for the production of ^{212}Bi, thereby effectively extending the short half-life of ^{212}Bi. The major advantage of targeting ^{212}Pb to the tumor instead of ^{212}Bi is that ^{212}Pb delivers greater than 10 times the dose per unit of administered activity compared to ^{212}Bi alone or the α-emitter ^{213}Bi.[16] Therefore, the 10.6 h half-life of ^{212}Pb also makes dose preparation and administration easier, and permits all operations to be executed more efficiently than with the short half-life ^{212}Bi. This review describes the uses and strategies for ^{212}Pb as a potential radiotherapeutic, the use of ^{212}Pb radiolabeled mAbs directed approaches, the requisite and current chemistry, and discusses pre-clinical trials, with an emphasis on the development of ^{212}Pb towards clinical translation.

Production of ^{212}Pb -- Generators

Radionuclide generators are systems wherein a longer-lived parent radionuclide is used to continuously generate, by radioactive decay, a shorter-lived daughter radionuclide of interest and whereby that desired radionuclide can be selectively separated and obtained by chemical means. ^{212}Pb\$^{212}$Bi and ^{213}Bi are members of decay chains of the long-lived parents ^{232}Th and ^{233}U, respectively, and can therefore be produced by generators. ^{212}Pb is produced from

the decay chain of ^{228}Th and can be available from a ^{224}Ra generator that facilitates the on-site production of ^{212}Bi or ^{212}Pb, which may be selectively eluted by controlling the acid strength of HCl or HI eluant from that same ion-exchange based generator system and then used for radiolabeling mAbs, peptides, or other vectors conjugated with suitable bifunctional chelating agents.[17]

The original generator used ^{228}Th, which has a 1.9 y half-life, deposited on Na_2TiO_3 on which the ^{228}Th and its immediate daughter radionuclide ^{224}Ra are absorbed.[18] The ^{212}Pb and the other decay daughters (Figure 1) were separated by maintaining a flow of water over the parent to wash away the ^{220}Rn daughter, which has a half-life of 55s. The generator was operated by eluting ^{220}Rn with water into a reservoir, waiting a few minutes for the radon gas to decay to ^{212}Pb, followed by passing that solution through an organic cation-exchanger to absorb the ^{212}Pb. A theoretical maximum yield of ^{212}Pb (ca. 80 %) with a breakthrough level of ~2×10^{-4} Bq of ^{228}Th or ^{224}Ra per Bq of ^{212}Pb was reported. At radioactivity levels greater than 37 MBq (1 mCi), radiolytic breakdown of the ion-exchanger support caused increasing back pressure and decreasing yields.[15] All decays of the ^{212}Pb and ^{212}Bi lead to α-emissions, either directly, or through their daughter, ^{212}Po ($T_{½}$ = 0.3 μs). This generator based on ^{228}Th experienced problems with radiolytic damage in the resin with consequent diminished yield and was also a serious radiation safety problem.

Evaporation (emanation) based generator systems were also developed to overcome radiolytic effect limitations. This type of generator also based on the principle of collecting ^{220}Rn, however as a gas emanating from [^{229}Th] barium stearate, and accommodates a low radioactivity to mass ratio to avoid the destructive effects of radiolysis.[19] A 50 MBq (1.4 mCi) generator was evaluated where such a source could be moved into, or out from a collection chamber. When the source was inside the chamber, the decay product of ^{220}Rn, ^{212}Pb, was then deposited on the walls of a polyethylene bottle. The ^{212}Pb could be washed off the plastic surface with aqueous solutions without detection of any ^{228}Th ($<10^{-9}$ Bq of ^{228}Th per Bq of ^{212}Pb) breakthrough or other long-lived parent nuclides. The emanation yield was only 50 % initially and decreased gradually due to radiolytic damage of the barium stearate support.

An improved generator construct based on the same principle, but with a different method for collecting the ^{220}Rn and decay products has also been suggested.[20] In this construct, the amount of ^{228}Th doped barium stearate has been increased to lessen the destructive effects of radiolytic damage to the emanation ability. The collected yields of ^{212}Pb concomitantly increased to approximately 70 %. So far, characterization of the properties of emanation generators has only been possible with tracer levels of radioactivity due to limited availability of ^{228}Th.

To avoid problems originating from ^{228}Th-based generators, another generator based on ^{224}Ra ($T_{½}$ = 3.7 d) was designed.[15,21] ^{224}Ra is separated from ^{228}Th by absorbing ^{228}Th as the nitrate complex onto an anion-exchanger, while ^{224}Ra elutes through the column. The ^{224}Ra is then absorbed on to the macroporous organic cation ion-exchange resin (AG-MP-50) which then serves as the source for either a ^{212}Bi or ^{212}Pb. ^{212}Bi can selectively be eluted from the generator with low acid concentrations of HI (0.05–0.2 M). At higher acid concentrations of either HCl or HI (1–6 M), a mixture of both ^{212}Pb and ^{212}Bi can be eluted. In our preparation, ^{212}Pb was first eluted from the ^{224}Ra/^{212}Pb with 2 M HCl. The ^{212}Pb eluate was then diluted to 0.1 M HCl and loaded onto a small AG-50 × 4 resin and the ^{212}Bi eluted from the resin with 0.2 M HI. These generators have been available at source strength of ~0.7 GBq (0.02 Ci). Breakthrough of ^{224}Ra and ^{228}Th from these generators has been found to be 4×10^{-4} Bq of ^{224}Ra per Bq of ^{212}Pb and 10^{-6} Bq of ^{228}Th per Bq of ^{212}Pb.

This generator has given good yields of ^{212}Bi and its parent nuclide ^{212}Pb, but it must be regenerated after 1–2 weeks because of the short half-life of ^{224}Ra.

Chemistry of radiolabeling

The stable sequestration of the radionuclide *in vivo* is considered as one of the major aspects of targeted radiation therapy. It allows the delivery of radiation to tumor to be maximized while minimizing toxicity.[22] Continued interest exists in the area of synthesis of bifunctional chelating agents, their conjugation to mAbs and peptides, and their subsequent use for sequestering radioactive metal ions for use in RIT and radioimmunoimaging (RII) applications. A variety of technologies are used to conjugate radioisotopes to antibodies, dependent on the chemical nature of the radionuclide. One of the fundamental requirements is that the conjugation of a radionuclide to a mAb or the conditions imposed by the radiolabeling protocols must maintain the affinity/avidity of the mAb for its target antigen.

Choosing an appropriate bifunctional chelating agent that forms an adequately stable complex of the metallic radionuclide of choice and within the context of the application is a critical factor. Acylic diethylenetriaminepentaacetic acid (DTPA) and macrocyclic 1,4,7,10-tetraazacyclododecane tetraacetic acid (DOTA)-based chelators represent the most commonly utilized classes of agents used in RIT. Generally, DTPA and other acyclic chelates exhibit fast complex association rates, whereas DOTA derivatives and other macrocyclic chelates have slower complex dissociation rates. A sampling of bifunctional chelating agents derived from DTPA include the cyclic dianhydride derivative,[23] 1B4M-DTPA[24] and a family of *trans*-cyclohexyl derivatives that include the specific stereoisomer, CHX-A″DTPA. CHX-A″ DTPA is an effective chelator for ^{111}In, ^{90}Y, and ^{177}Lu.[25–27]

Numerous bifunctional analogs of the macrocycle DOTA (Figure 2) have been used effectively for labeling antibodies with ^{111}In, ^{90}Y, ^{177}Lu, ^{212}Pb, and ^{212}Bi.[22,28–32] DOTA complexes for lanthanides and other metal ions tend to yield eight coordinate square anti-prisms that exists in an equilibrium between isomeric arrangements for carboxylate arms and ring twists forms that saturate the coordination spheres about Bi(III) and Pb(II). A number of lead and bismuth complexes has been prepared and evaluated for unique *in situ* generator system.[33,34] Their favorable nuclear properties and availability make them well suited for *in vivo* assessment of new antitumor immunotherapeutic techniques. A problem with the clinical use of ^{212}Bi or ^{212}Pb radioimmunoconjugates (RICs) is the potential for radiotoxicity as a consequence of either premature release of the metal by the chelating agent or metabolic catabolism of the RIT releasing the radiometal.

Studies to evaluate the potential usefulness of a *C*-functionalized DOTA have led to conflicting results. Fundamental studies of the stability of the complex indicated that both the DOTA[Bi(III)] and DOTA[Pb(II)] complex were exceedingly stable and that a suitably stable complexes for *in vivo* applications formed.[34] Indeed, the complex thus formed with a *C*-functionalized DOTA was confirmed as stable *in vivo*; however, significant slow complex formation rates are a hindrance to the use of DOTA.[35] Functionalized DOTA ligands have also been reported to be highly sensitive to the presence of M(II) ion contamination of the radionuclide.[36] Thus, despite forming a kinetically inert complex with Bi(III), DOTA was not found suitable due to slow complex formation rates versus the constraints of the half-lives of ^{212}Bi, and, even more so, for the shorter half-life ^{213}Bi.

However, DOTA was also shown to be an adequately stable *in vivo* chelator for Pb(II),[34] which allows for conjugating and delivery of ^{212}Pb, the precursor of ^{212}Bi in targeted therapy. The ability to exploit the longer lived ^{212}Pb effectively allows time for tumor targeting of the α-emitter, ^{212}Bi. Key to its use, however, is the ability to form a stable chelate of ^{212}Pb that controls the *in vivo* localization through the decay event formation of

the ^{212}Bi. In theory, the decay of ^{212}Pb should not present a problem in retention of the ^{212}Bi. Conservation principles dictate that the recoil energy of the Bi nucleus is only about 0.5 eV. This is not sufficient to break a chemical bond, which requires ~10 eV. However, the γ-ray emitted when ^{212}Pb decays is internally converted over 30 % of the time.[37] The resulting cascade of conversion electrons makes that percentage of the metal–chelate complex unstable. Because the free highly energetic radio-bismuth that is ejected during this process poorly re-associates with DOTA, toxicity occurs as ^{212}Bi accumulates in or is formed within organ binding sites.

One of the challenges to the use of the Pb(II) radionuclides is the afore noted issue of acid catalyzed dissociation of the radiometal from DOTA, posing an additional obstacle to maintaining the ^{212}Pb-DOTA complex after ^{212}Pb-mAb internalization. Loss of ^{212}Pb post-internalization of mAb delivery to cells has been reported as a source of marrow toxicity when using a DOTA conjugate wherein the dissociated ^{212}Pb was free to be transported to the bone and subsequently then decay to ^{212}Bi.[35] Chappell *et al.* demonstrated that the Pb(II)[4-NCS-Bz-TCMC] complex was more stable compared to Pb(II)[-DOTA] complex, with increased resistance to complex dissociation under lower pH conditions.[38] The TCMC ligand (Figure 2) also showed many other advantages over the DOTA ligand including more efficient conjugation reaction to mAb CC49 resulting in a conjugation product with higher numbers of chelate conjugated while retaining antigen binding and immunoreactivity as well as greater radiolabeling yields. Thus, these combined advantages promote use of TCMC over use of DOTA mAb immunoconjugates for immunotherapeutic and imaging studies when using Pb(II) radionuclides.

Maumela *et al.* reported the remarkable stability of the Pb(II) complex formed with the *N*,*N*,*N*,*N*-tetraamide analog of DOTA, as this complex retained its integrity even at significantly high acidic conditions, *e.g.*, 0.5 M HCl.[39] Additionally, the binding affinity of this ligand for Pb(II) strongly differs from that observed for the 1,4,8,11-tetraazacyclotetradecane tetraacetic acid (TETA) analog wherein that Pb(II) complex is less stable by > 10 log units.[40] Hancock *et al.* reported a crystal structure of the Pb(II) complex of the *N*,*N*,*N*,*N*-tetraamide analog of DOTA that showed a water molecule bound to the metal center in such a way that a weak interaction between one water hydrogen atom and the Pb(II) was possible.[41]

The impact of choosing the correct macrocyclic platform for assembling a bifunctional chelating agents is well demonstrated by the differences in the succeptibility to acidic conditions demonstrated by these macrocyclic complexes. The macrocyclic bifunctional ligand, TCMC, which has been very efficiently radiolabeled with Pb(II) radionuclides has been found to be less labile at pH 3.5 than the corresponding DOTA complex, conferring enhanced resistance to acid-catalyzed dissociation within the cell.[38] Hence, TCMC continues to be used for sequestration of ^{212}Pb as opposed to DOTA.

More recently, Cuenot *et al.* have reported their studies of the Pb(II) complex of 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraaza-cyclododecane (TCMC), also known as DOTAM.[40] They related that the reaction between Pb(II) and TCMC produced a mononuclear complex, even under mild acidic conditions, which corroborates the radiolabeling experience of this ligand with ^{212}Pb as being quite rapid and efficient under similar conditions. Their crystal structure of the Pb(II)-TCMC complex revealed that the Pb(II) was fully encapsulated inside the TCMC with the eight-coordinate sphere saturated by the four ring nitrogens and the four amide oxygen atoms. Lastly, and most importantly, helical geometry of the four amide arms leaves no gap in the coordination sphere of the metal. This arrangement is indicative of a stereochemically inactive lone pair for Pb(II). This result is also highly supportive of this complex being extremely stable for *in vivo*

sequestration of Pb(II) and supports the suitability of TCMC for the potential clinical applications being reviewed here.

Dosimetry

The simple definition of absorbed dose is the energy absorbed in a particular volume divided by the mass of the volume; tumor response and normal tissue damage are a function of dose.[42] Generally, short half-lives, short range, high LET, and the complicated decay pathways of α-particle emitters differentiates their dosimetry from that of β-emitters. In targeted radionuclide therapy, dosimetry is complicated by several factors: heterogeneous radionuclide distribution, short-range particulate radiation, and few actual radioactive incidents per cell. A number of biological and chemical variables,[43,44] *e.g.*, heterogeneous radionuclide conjugation, heterogeneous target expression, antibody avidity, tumor vascularity, and interstitial pressure in the tumor have to be accounted for through model systems to estimate *in vivo* dose. Physical characteristics and the amount of the radionuclide are also factors.

The Medical standard approach to dosimetry calculations has been described by the Medical Internal Radionuclide Dose (MIRD)[45] and have been applied for *in vivo* experiments for dose determination after α-particle radiation therapy. Given the high energy of α-particles delivered over their short range, conventional MIRD calculations and models may not always yield biologically meaningful information. To estimate the absorbed dose coming from α-particles decaying through multiple unstable daughters, with their own intrinsic biodistribution, prediction of absorbed dose and potential toxicity, Hamacher and Sgouros reported on three models to estimate normal organ absorbed doses for the following parent radionuclides: ^{225}Ac, ^{212}Pb, ^{211}At, ^{223}Ra, and ^{213}Bi. Comparing doses in the case of a 0.1 g rapidly accessible tumor to those of a 10 g solid tumor, parent radionuclides with a short half-life yielded a higher dose burden to normal organs than longer lived radionuclides. At 20 Gy, the corresponding absorbed dose to a rapidly accessible 0.1 g tumor would be 1.7 and 0.9 Gy in liver and kidney from ^{225}Ac, 0.4 and 0.3 Gy to bone and small intestines from ^{223}Ra, and 2.2 and 3.0 Gy in small intestines from ^{212}Pb and ^{213}Bi, respectively.[46]

Thus, microdosimetry is typically required for α-emitter dosimetry for the analysis of cell culture experiments involving low concentrations of α-emitting nuclides. Such computations may be difficult to perform due to unknown microdistribution of the radionuclide.[47–49] Average cell survival probabilities derived from macroscopic dosimetry experiments also may not reflect the true cell survival probabilities. Evidence of bystander effects and delayed cell death has demonstrated that cells not hit or traversed by α-particles may express or communicate a decreased survival.[50–52] The impact of bystander effects related to the dose of ^{212}Pb has yet to be reported, however, papers relating bystander effects originating from ^{211}At studies are available and one would predict that similar effects would be generated with ^{212}Pb.[53,54] Likewise, RBE values reported from *in vitro* and *in vivo* experiments should be interpreted with caution. More accurate cell survival probabilities might be better obtained through *in vitro* experiments with absolute determination of the number of α-particle traversals of subcellular compartments.[9]

Surprisingly as yet, there are no dosimetry data results associated with actual ^{212}Pb radiolabeled mAbs thus far in the literature. This deficiency provides an area of opportunity for study that will have to be addressed as this radionuclide moves closer to translation to clinical trial evaluation.

Pre-clinical studies of Non-Targeted ^{212}Pb

The use of ^{212}Pb without a targeting vehicle as a therapeutic agent has been reported. In the study, ^{212}Pb in the form of a sulfur colloid was used to treat ovarian carcinoma after i.p. injection. A dose dependent survival was demonstrated. At doses of 70 μCi, death occurred as a result of gastrointestinal injury. Histologically, ^{212}Pb in the sulfur colloid caused extensive tumor necrosis compared to colloid alone. However, the use of the sulfur colloid may be limited as a carrier for ^{212}Pb because the colloid resulted in uneven peritoneal distribution of radionuclide to the bowel surface resulting in gastrointestinal toxicity at the higher doses.[55]

Rotmensch *et al.* used ferrous hydroxide as a colloidal carrier for ^{212}Pb because of higher retention time in the peritoneal cavity than ferric sulfide or hydroxide.[56] In this application, ^{212}Pb prolonged the mean survival time in a dose dependent manner after i.p. injection in the treatment of an ascites producing tumor. The radiosensitivity and chromosomal aberrations of cells increased with ^{212}Pb. However, no major side effects or toxicity was found with administration of up to 2.6 mCi of ferrous hydroxide-^{212}Pb.

Pre-clinical studies of Targeted ^{212}Pb

As noted previously, the limitations of the short half-life α-particle emitter, ^{212}Bi, may be effectively extended by using its *in vivo* generator parental radionuclide, ^{212}Pb, sequestered in a chelating agent conjugated to the carrier molecule. This increases uptake ratios between tumor and normal organs. However, as also noted, 36% of the ^{212}Bi formed could be lost from the DOTA complex on the *α*-decay of ^{212}Pb due to the electronic effects of the one of the decay pathways (Figure 1), potentially causing toxicity. A study from Ruble *et al.* demonstrated the efficacy of ^{212}Pb-labeled mAb 103A in treating the Rauscher leukemia virus (RVB3) resulting in histological cure in all animals. The animals showed no evidence of splenic tumor foci with a dose of just 0.74 MBq (20 μCi), but all of the animals died of bone marrow toxicity.[57] No other organ showed any sign of radiotoxicity. The radioactivity in the bone (3.2 % ID/g) was twice the level of that in the tissue of control animals treated with ^{212}Pb-B3 (1.6 % ID/g). The high bone marrow toxicity observed in this study sharply contrasted with the lack of marrow toxicity in the animals treated with ^{212}Bi-103A-mAb. These results show that ^{212}Bi lost from ^{212}Pb-mAb increased marrow toxicity as compared to ^{212}Bi-103A-mAb. Additionally, the study utilized bifunctional DOTA, whose complex begins to exhibit some measurable lability at the pH that would be encountered in the lyzosomes post-internalization of the radioimmunoconjugate. As a result of that acidic environment, ^{212}Pb itself could also have dissociated from the complex and then exited the tumor cells. Pb(II) at these very low concentrations is known to traffic in the blood and become transported to bone where it can bind to the bone, decay to ^{212}Bi, and be a source of toxicity. Attempts at limiting hematological and marrow toxicities by administration of heavy metal chelators such as 2,3-dimercapto-1-propanesulfonic acid (DMPS) and *meso*-2,3-dimercaptosuccinic acid (DMSA) have been explored albeit not in any great depth for ^{212}Pb.[58] While both were found to be effective in improving whole body clearance of both ^{212}Bi and ^{212}Pb as well as bone deposition levels, only DMPS was noted to have any impact on reducing renal accumulation.

HER2, expressed in a variety of epithelial cancers, is proving to be an ideal target for radioimmunotherapy.[59,60] Horak *et al.* evaluated the efficacy of ^{212}Pb-AE1-mAb for targeting HER2 on ovarian tumors in nude mice.[61] Transient bone marrow toxicity and lengthy renal toxicity were observed after i.v. injection of 0.93 MBq (25 μCi); doses of 1.48 MBq (40 μCi) resulted in a cellular bone marrow toxicity and subsequent death of all animals. However, treatment of three days post-inoculation s.c. tumors with 0.37–0.74 MBq

(10–20 μCi) of ^{212}Pb-AE1 resulted in 100 % tumor free survival for 180 days, with all control animals developing tumors by day 20. In additional studies, the growth of small, more established tumors (15 mm^3) was modestly inhibited, while the growth of larger tumors (146 mm^3) was unaffected following administration of ^{212}Pb-DOTA-AE1. The poor therapeutic efficacy of the larger tumors could be explained by long blood residence time, slow tumor targeting, and perhaps poor tumor penetration, resulting in low tumor/blood ratios for the ^{212}Pb-AE1-mAb.

Targeted α-radiation therapy with mAb, which binds to tumor-associated antigen, may be efficacious and more appropriately employed in a coordinated strategy for the treatment and management of disseminated peritoneal disease. There are, however, inherent limitations associated with their use for targeting and treatment of solid tumors.[62] ^{212}Pb-labeled trastuzumab for the treatment disseminated peritoneal disease has been suggested.[63] A pilot radioimmunotherapy experiment tested mice bearing LS-174T intraperitoneal (i.p.) xenografts as a model. The study established a maximum tolerated dose (MTD) of 0.74 – 1.48 MBq (20–40 μCi) for the mice. The median survival of animals receiving 0.37 MBq (10 μCi) increased from 19 to 56 days ($p = 0.008$). A multi-dosing regimen of ^{212}Pb-TCMC-trastuzumab administered at monthly intervals (up to 3 monthly doses of ^{212}Pb-TCMC-trastuzumab) increased median survival of mice bearing 3 d LS-174T i.p. xenografts to 110 days.

Approaches to increase the therapeutic efficacy of targeted radiation therapy have been explored. These include harnessing the potential of synergistic cytotoxicity of targeted α-particle radiation therapy with chemotherapeutics and radiosensitizers. The combination of gemcitabine (GEM) with RIT using β-emitters has been extensively studied.[64,65] For targeted α-particle therapy using ^{212}Pb-TCMC-trastuzumab has been evaluated in combination with gemcitabine (GEM) for treating disseminated peritoneal disease.[66] Treatment using mice bearing i.p. LS-174T xenografts with gemcitabine (GEM) followed 24–30 hr later by either 0.19 or 0.37 MBq (5 or 10 μCi) of ^{212}Pb-TCMC-trastuzumab resulted in improvement of median survival; from 31 to 51 days in the absence or presence of GEM with 0.19 MBq (5 μCi) of ^{212}Pb-TCMC-trastuzumab, respectively, and from 45 up to 70 days at the 0.37 MBq (10 μCi) dose in the absence or presence of GEM, respectively, compared to 16 days for untreated animals. Three weekly doses of gemcitabine in conjunction with ^{212}Pb resulted in a median survival of 90 days *vs* 21 days for the untreated group of animals. Treatment with two cycles of 10 μCi ^{212}Pb-TCMC-trastuzumab with two doses of GEM resulted in the greatest therapeutic efficacy with a median survival of 196.5 days. This therapeutic regimen combining chemotherapeutics and high LET radioimmunotherapy may have tremendous potential in cancer treatment, particularly in the context of microscopic and residual disease post-surgical resection.

Radioimmunotherapy using β-emitters combined with paclitaxel has been well studied for treatment of lymphoma in pre-clinical studies and found to be synergistic. The regimen is also sensitive to administration order and timing.[67,68] Milenic *et al.* have recently evaluated the ability of paclitaxel to potentiate the therapeutic efficacy of HER-2 targeting α-emitting high LET ^{213}Bi-CHX-A″-trastuzumab and ^{212}Pb-TCMC-trastuzumab in a multimodality regimen for the management of disseminated i.p. disease.[69] Combination treatment of paclitaxel administered 24 hr before, concurrently with, or 24 hr after ^{212}Pb-TCMC-trastuzumab (10 μCi) was studied to elucidate the optimal administration order of the two therapeutics. An enhanced therapeutic efficacy of ^{212}Pb-TCMC-trastuzumab was observed in the group that received 600 μg of paclitaxel 24 hr before the RIT. Nearly a 4-fold increase in the median survival, from 44 to 171 days, in this group was observed as compared with the group that received ^{212}Pb-TCMC-trastuzumab alone. The response appears to be quite

specific to the ^{212}Pb-TCMC-trastuzumab compared to ^{212}Pb-TCMC-HuIgG, a labeled non-specific human immunoglobulin.

Other Applications of Targeted ^{212}Pb

Liposomes carrying isotopes might also act as mediators of *in vivo* targeted radiotherapy at low total doses to the organism. Rosenow *et al.* described the properties of liposomes containing ^{212}Pb by encapsulation.[70] Liposomes incorporating ^{212}Pb remained at least partially intact *in vivo*. The potential of this tool for *in vivo* radioimmunotherapy lies in the possibility of maintaining cytotoxic activity in the circulation and in various organs for perfusion therapy of neoplasms or immune suppression.

A study with ^{212}Pb/^{212}Bi-ethylenediamine tetra-methylenephosphonic acid (EDTMP) demonstrated the intriguing possibility for therapy of osteosarcoma and bone metastases. EDTMP is a chelating agent with a high affinity for bone and has also been used to direct other radionuclides to bone for palliation therapy of bone lesions. However, EDTMP was found to be an unsatisfactory chelator for ^{212}Bi and ^{212}Pb due to the instability, resulting in very high kidney uptake values and lower bone uptake values compared to ^{212}Pb/^{212}Bi-DOTAMP.[71,72]

Hassfjell *et al.* proposed the use of ^{212}Pb and ^{212}Bi chelated to the bone-seeking ligand, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraphosphonic acid (DOTMP) for therapy of osteosarcomas, or bone metastases from breast, prostate, and lung cancers.[72] Both radiometal tetraphosphonate complexes localized rapidly in the bone matrix of mice, especially in regions with high bone turnover, a condition frequently observed in osteosarcomas and bone metastases. After 30 minutes, femur uptake of 16 % ID/g of ^{212}Bi-DOTMP and 10 % ID/g ^{212}Pb-DOTMP were reached. At this time point, the radioactivity in the blood was 0.6 % ID/g for both agents, and decreased to 0.06 % ID/g for ^{212}Bi and 0.02 % ID/g for ^{212}Pb, respectively, at later time points. Approximately one-third of the *in vivo* generated ^{212}Bi was lost from ^{212}Pb-DOTMP, similar to that reported for the ^{212}Pb-DOTA complex.

Diener *et al.* proposed a unique potential role for ^{212}Pb in radionuclide therapy by more stably encapsulating radionuclides inside of fullerenes, especially where conventional chelation chemistry is inadequate due to physical and/or chemical properties of radionuclide.[73] ^{212}Pb@C_{60} and its malonic ester derivatives allowed the ^{212}Pb detivative to be generated *in situ* from the decay of the parental ^{224}Ra. The ^{212}Pb appeared to recoil into C60 following α-decay from its parent. A preliminary biodistribution study in mice demonstrated that ^{212}Pb did not accumulate in bone after being administrated as an endohedral fullerene, in contrast to results with polyhydroxylated radiofullerenes and conventional polyaminocarboxylate chelators for ^{212}Pb, but showed rather slow clearance. Only ~2 % ID/g accumulated in the bone using the ^{212}Pb@C_{60} malonate. Studies to actively target this intriguing construct have surprisingly not appeared as yet.

Peptides, as opposed to mAb targeted α-therapy, have also been recently investigated to take advantage of both rapid targeting with cellular internalization combined with rapid clearance pharmacokinetics. Miao et al evaluated the therapeutic efficacy in the B16/F1 mouse melanoma animal model of a unique melanoma-targeting peptide radiolabeled with ^{212}Pb.[74] Treatment of melanoma-bearing mice with 50, 100, and 200 μCi of ^{212}Pb-DOTA-Re(Arg^{11})CCMSH extended their mean survival to 22, 28, and 49.8 days, respectively, when compared with 14.6-day mean survival of the untreated control group. Forty-five percent of the animals receiving 200 μCi doses of a ^{212}Pb-DOTA-Re(Arg^{11})CCMSH survived the study disease-free. The advantage of administering ^{212}Pb-DOTA-Re(Arg^{11})CCMSH is that the radiolabeled peptide will circulate, target melanoma tumor

cells and be cleared from the body as the ^{212}Pb-labeled peptide within the time frame of the radionuclide half-life. Only minimal amounts of the α-emitting ^{212}Bi compound will exist thereby minimizing normal tissue exposures from any "free" ^{212}Bi. Peptide-targeted ^{212}Pb, rapidly internalized and then retained by tumor cells decays to the α-particle emitting ^{212}Bi, localizing the highly toxic short-ranged α-radiation within the tumor cells. Once internalized and retained, the close proximity to the nucleus would facilitate a greater opportunity of traversing the nucleus and concomitantly increase the odds of cell death. Finally, the 10.6 h half-life of ^{212}Pb makes dose preparation and administration easier and more convenient than the short half-life ($T_{1/2}$ = 60.6 min) ^{212}Bi. No difference was detected in the biodistribution of ^{212}Pb and ^{212}Bi during the 48 hr study period, demonstrating that no significant amounts of ^{212}Bi were escaping the ^{212}Pb-DOTA-Re(Arg^{11})CCMSH molecule and redistributing *in vivo*. The rapid clearance combined with the rapid targeting and internalization of ^{212}Pb-DOTA-Re(Arg^{11})CCMSH likely prevented measurable amounts of ^{212}Bi from being released.

Pre-clinical studies of Pre-Targeted ^{212}Pb

Antibody pre-targeting is a process that addresses optimal delivery of radiation to tumors.[75] Su *et al.* evaluated an antibody pre-targeting system with mAb-steptavidin, clearing agent and DOTA-biotin for solid tumor radiotherapy using the *in vivo* ^{212}Pb/^{212}Bi generator.[76] Compared to its γ-emitting analogues, ^{212}Pb-DOTA-biotin was not stable and as with previous studies more than 30 % of the ^{212}Bi formed was released from ^{212}Pb-DOTA. However, the pre-targeting of ^{212}Pb/^{212}Bi provided good tumor uptake, tumor-to-blood ratios and normal non-target tissue/blood ratios with the exception of kidney, the primary biological deposition site for Bi(III). In addition, the dosimetry calculation of ^{212}Pb in the mouse xenograft model showed that the system provided a tumor dose of 93 rad/μCi and that the ratio of tumor to marrow and tumor to kidney was 386:1 and 12:1, respectively.

Applications of ^{203}Pb

One challenge associated with performing pre-clinical experiments with ^{212}Pb is the execution of accurate biodistribution and targeting assays of a ^{212}Pb-radiolabeled mAb. One viable option is to employ ^{203}Pb as a surrogate nuclide. ^{203}Pb has a favorable half-life ($T_{1/2}$ = 52 h) and decays with 80.1 % emission of γ-rays at 279 keV that is compatible with single photon emission computerized tomography (SPECT). This makes the radionuclide ideally suited as a matched radionuclide tracer ^{212}Pb targeted radionuclide therapy. The nuclide is potentially useful for imaging, tissue distribution studies, dosimetry data acquisition, as well as chemical exchange studies. ^{203}Pb can be easily produced via the ^{203}Tl(d, 2n)^{203}Pb reaction by irradiating natural Tl_2O_3 or an enriched Tl_2O_3 (^{203}Tl) target with 13.7 MeV deuterons from a cyclotron. Purified ^{203}Pb has been used to label trastuzumab, shown to be immunoreactive and demonstrated favorable biodistribution properties *in vivo*, indicating the suitability and feasibility of ^{203}Pb-labeled biomolecules to target cellular antigens.[77] Imaging and biodistribution studies performed with ^{203}Pb-DOTA-B72.3 in nude mouse bearing LS-174T tumors, showed no major accumulation of lead in the bone and other organs. Clear and distinct γ-camera images of LS-174T tumors were obtained by injection of ^{203}Pb-DOTA-B72.3 (Figure 3).[78] Miao *et al.* also evaluated DOTA-Re(Arg11)CCMSH radiolabeled with ^{203}Pb as a matched pair imaging agent for ^{212}Pb- DOTA-Re(Arg11)CCMSH.[79] ^{203}Pb- DOTA-Re(Arg11)CCMSH exhibited high melanoma uptake and a biodistribution pattern similar to that of ^{212}Pb-DOTA-Re(Arg11)CCMSH, highlighting its potential as a matched pair imaging probe for ^{212}Pb-DOTA-Re(Arg11)CCMSH (Figure 4).

Potential Prospects and Conclusion

The high LET of α-particle radiation and short path length, although not ideal for large burden disease, has been proposed as ideal for the treatment of smaller tumor burdens, micrometastatic disease, and disseminated disease. Furthermore, far greater cancer killing probabilities are often achievable with α-particle radiation than with alternative strategies. Despite the favorable properties of α-particle radiation, the development of α-particle RIT has been limited by the poor availability, as a result of limited amounts or economic limitations, or by the actual physical characteristics of α-emitting radionuclides.

Early RIT studies using α-emitting radioisotopes were performed with ^{212}Bi, in large part because of the availability of ^{224}Ra. The short half-life of ^{212}Bi creates the same limitations associated with even the shorter half-life of ^{213}Bi. ^{212}Pb, which decays to ^{212}Bi, offers a means to utilize the α–particles from ^{212}Bi decay for targeted therapy. Towards this end, a mAb radiolabeled with ^{212}Pb serves as an *in vivo* generator of ^{212}Bi thereby extending the delivery time for the ^{212}Bi daughter for actually arrive and impact target tumor tissues. The process also has the effect of reducing the dose that is needed to effect therapy. As noted above, a dose of 10 μCi of ^{212}Pb was equi-effective as a 500 μCi injected dose of ^{213}Bi in the identical model system.[69] Conservation principles dictate that the recoil energy of the Bi nucleus is only about 0.5 eV, which is not adequate by itself to break a chemical bond; however, the internal conversion process of one of the decay pathways of ^{212}Pb does provide a mechanism for loss of the ^{212}Bi daughter. Appropriate therapy strategies for ^{212}Pb exist that deal with this loss of ^{212}Bi within the context of the environment and disease presentation, *e.g.*, the targeting of intracavitary metastatic or micrometastatic malignancies are considered reasonable.

While both pre-clinical and early clinical studies appear promising, several obstacles obstruct the pathway to widespread acceptance and use of targeted α-therapy. Enhanced therapeutic efficacy can be attained through selective dose delivery to radiosensitive areas of tumors. To improve current dosimetry models, more accurate determination of the radionuclide microdistribution should be provided. Along with improvement of dosimetry for the clinical situation, cell survival probabilities after given numbers of α-particle traversals require a more accurate determination.

Chelation and linking chemistry remains a challenge for the multiple decay pathway radionuclides. Earlier studies have noted the inadequacy of DOTA in maintaining a stable complex during decay from ^{212}Pb to ^{212}Bi. Additionally, the DOTA-Pb(II) complex is modestly acid labile, which may be a source of toxicity when internalized and metabolized as a radioimmunoconjugate. Use of the TCMC-Pb(II) complex obviates the pH lability associated with DOTA, but the loss from the decay process remains unsolved and limits the choices of appropriate therapeutic applications. Recently, a possible application for α-particle RIT using coordination with Bi(III) and mAbs Pb(II) has been suggested.[80] A single-strapped analogue to porphyrin 5 coordinated with both Bi(III) and Pb(II) is stable leading to a possible application of a ^{212}Pb/^{212}Bi *in vivo* generator for medical applications. Despite having traversed many chelation challenges, more efficient conjugation and radiolabeling protocols remain to be developed to produce more consistent products with higher specific activities to optimize therapeutic potentials.

The clinical advantages and increases in efficacy obtained using combination therapies are becoming more evident. Chemotherapy in conjunction with α-particle RIT using the appropriate targeting vehicle would lead to efficient therapy following procedures such as cytoreductive surgery or peritoneal external beam radiation therapy. ^{212}Pb is a promising α-particle emitting source, providing alternative options in the treatment and management of

cancer. The recent advances from our laboratory and others demonstrate the tremendous potential of combination therapy studies using high linear energy transfer RIT with ^{212}Pb and chemotherapeutics such as gemcitabine and paclitaxel to treat disseminated peritoneal disease as well as other appropriately scaled disease.

Furthermore, the utilization of the matched pair approach using ^{203}Pb, which shows favorable pharmacokinetic and imaging properties, highlight new potential in therapy and imaging with mAbs, or peptides radiolabeled with ^{212}Pb. However, mAb based molecular imaging and RIT have yet to reach their full potentials in both the pre-clinical and clinical domains. Continued efforts to refine and optimize all of the components to improve efficacy and minimize toxicity along with carefully planned pre-clinical investigation and improved targeting strategies will facilitate translation into clinical evaluation to move the field forward.

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Biographies

Kwon Joong Yong received his B.A. in 1992 and M.S. in 1994 from the Kangwon National University, Republic of Korea. Under the supervision of Professor Okot-Kotber, he obtained his Ph.D. in 2004 from Kansas State University. He worked to develop methods for the purification and enhanced production of phytase from plant source. He was a Post-doctoral fellow at Georgetown University Medical Center in 2004–2009. He studied characterization of HDAC isoforms in breast cancer and developed novel HDAC inhibitors. He is presently in the research group of Dr. M. W. Brechbiel at the National Cancer Institute.

Martin W. Brechbiel received a B.A. in 1979 from Gettysburg College and a M.S. in 1982 from the U. of Delaware under the guidance of Professor Harold Kwart. After working for FMC Corp, he joined the National Cancer Institute in 1983. Thereafter, he worked to develop novel bifunctional chelating agents for sequestering radionuclides and their conjugation to immunoproteins under the direction of Dr. Otto A. Gansow while simultaneously obtaining a Ph.D from American U. in 1988 with Professor Thomas Cantrell. He remained with the NCI and in 2001 was appointed as the Section Chief of the Radioimmune & Inorganic Chemistry Section. His research group's activities span the range of continuing development of novel chelating agents for radionuclides, the development of contrast media for MRI, EPR, and CT imaging, SPECT and PET imaging agents, and the development of rationally designed targeted α-therapy regimens.

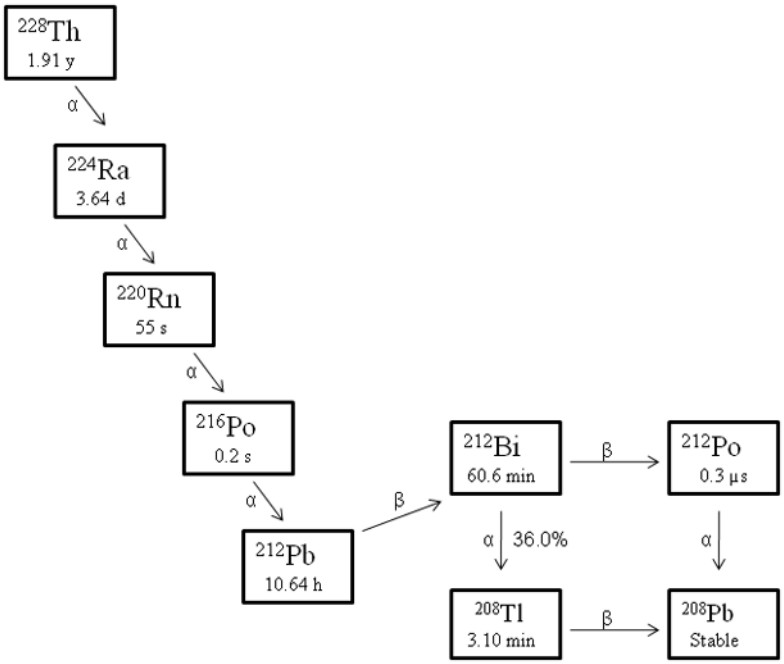

Figure 1.
Decay Schemes for the Production of ^{212}Pb

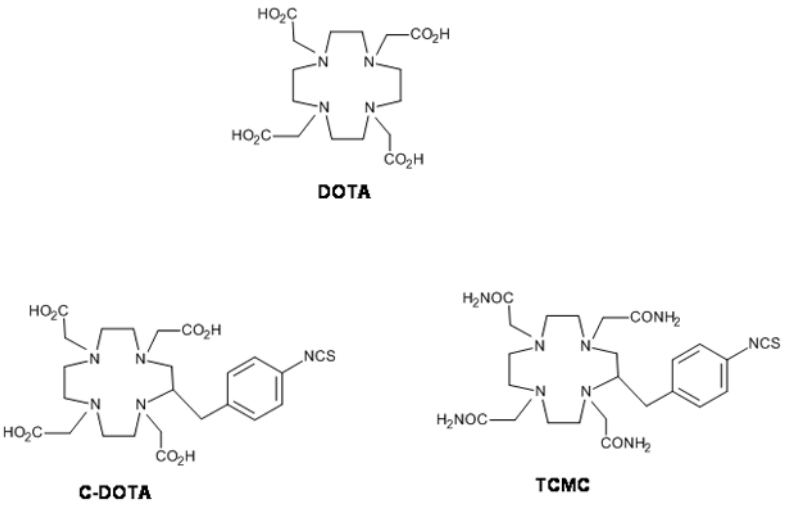

Figure 2.
Selected Structure of DOTA and its analogues in RIT

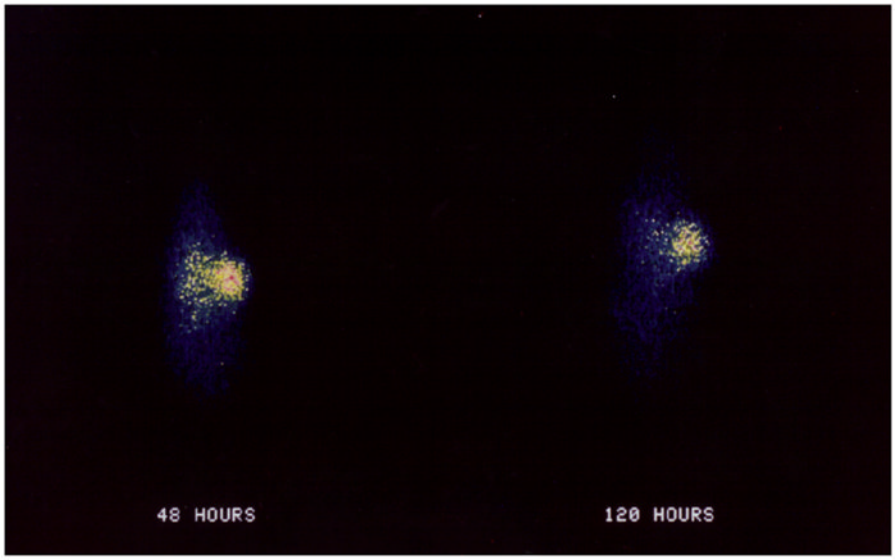

Figure 3.
Image with ^{203}Pb-B72.3-DOTA in athymic mice bearing LS-174T tumors.

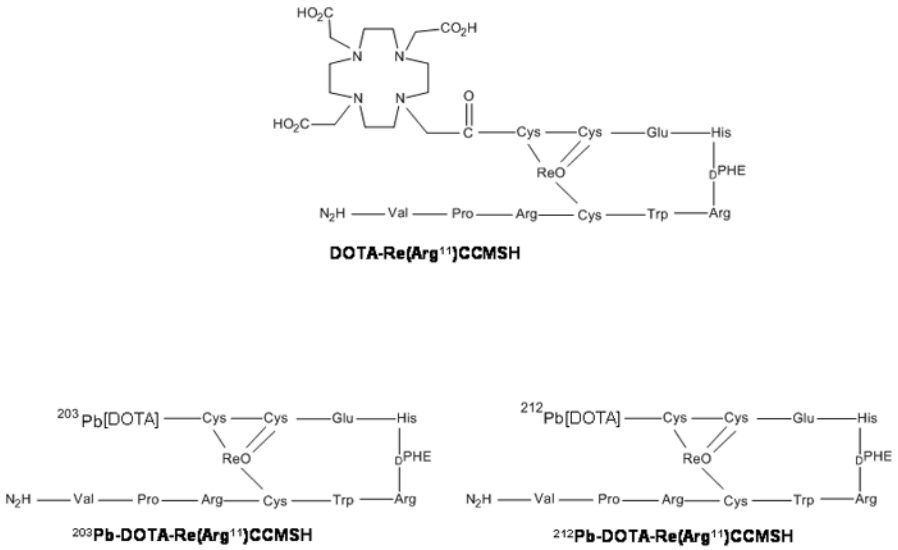

Figure 4.
Structure of ^{212}Pb-DOTA-Re(Arg^{11})CCMSH and ^{203}Pb-DOTA-Re(Arg^{11})CCMSH

Table 1

Selected Therapeutic Radionuclides

Radionuclide	Half-life (h)	Imaging decay	RIT decay	E_{max} (MeV)	Production
^{212}Pb	10.64	γ	β^- EC	0.57	^{224}Ra/^{212}Pb generator
^{212}Bi	1.01	γ	α β^- EC	6.09 6.05	^{228}Pb/^{212}Pb generator
^{213}Bi	0.76	γ	α β^- EC	5.87 5.55	^{228}Th/^{212}Pb generator
^{225}Ac	240.00	γ	α EC	5.83 5.79	n-capture of ^{232}Th→^{225}Ac or ^{226}Ra(p,2n)^{225}Ac
^{211}At	7.21	γ	α EC	5.87	^{209}Bi (α, 2n)^{211}At