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Targeted α -Therapy:

Past, Present, Future?

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Abstract

Monoclonal antibodies have become a viable strategy for the delivery of therapeutic, particle emitting radionuclides specifically to tumor cells to either augment anti-tumor action of the native antibodies or to solely take advantage of their action as targeting vectors. Proper and rational selection of radionuclide and antibody combinations is critical to making radioimmunotherapy (RIT) a standard therapeutic modality due to the fundamental and significant differences in the emission of either α - and β -particles. The α -particle has a short path length (50–80 μm) that is characterized by high linear energy transfer ($\sim 100 \text{ keV}/\mu\text{m}$). Actively targeted α -therapy potentially offers a more specific tumor cell killing action with less collateral damage to the surrounding normal tissues than β -emitters. These properties make targeted α -therapy appropriate therapies to eliminate or minimal residual or micrometastatic disease. RIT using α -emitters such as ^{213}Bi , ^{211}At , ^{225}Ac , and others has demonstrated significant activity in both *in vitro* and *in vivo* model systems. Limited numbers of clinical trials have progressed to demonstrate safety, feasibility, and therapeutic activity of targeted α -therapy, despite having to traverse complex obstacles. Further advances may require more potent isotopes, additional sources and more efficient means of isotope production. Refinements in chelation and/or radiolabeling chemistry combined with rational improvements of isotope delivery, targeting vectors, molecular targets, and identification of appropriate clinical applications remains as active areas of research. Ultimately, randomized trials comparing targeted α -therapy combined with integration into existing standard of care treatment regimens will determine the clinical utility of this modality.

Introduction

Kohler and Milstein's hybridoma / monoclonal antibody (mAb) technology resurrected the concept that antibodies might serve as magic bullets as proposed by Ehrlich.¹ Their seminal publication provided a clear opening towards the development of antibody targeted radiation.²

In the 1980's, murine mAbs against tumor-associated antigens (TAA) generated multitudes of pre-clinical studies that provided proof-of-concept of the potential of cancer treatment using radiolabeled mAbs. These studies also demonstrated discordance in predictability of their therapeutic efficacy. Foremost was a seemingly inevitable patient production of human anti-

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murine immunoglobulin antibodies (HAMA) after one to three treatments.³ Other factors limiting treatment included (1) inadequate therapeutic dose delivered to tumor lesions; (2) insufficient activation of effector function(s); (3) slow blood compartment clearance; (4) low mAb affinity and avidity; (5) trafficking to or targeting of normal organs; (6) heterogeneous antigen distribution on tumor cells; and (7) insufficient tumor penetration.³ In part, these limitations were addressed by chemical modification of the mAb, but many of these challenges have been addressed with genetic engineering applied to eliminating HAMA by either production of chimeric mAbs, CDR grafting, or complete humanization of the protein.⁴

Investigators are now able to fully explore the real therapeutic potential of radiolabeled mAbs. With the elimination of many obstacles and a better understanding of the inherent limitations of mAbs, the active targeting and delivery vector of the radiation, many radiolabeled mAbs have been, or currently are being evaluated in Phase III trials. The FDA approved two radiolabeled mAbs for the treatment of non-Hodgkin's lymphoma (NHL), Zevalin and Bexxar, making the approval of additional targeted radiation therapy products probable.⁵ However, both agents are radiolabeled with β -emitters, ^{90}Y ($t_{1/2} = 2.67$ d) and ^{131}I ($t_{1/2} = 8.07$ d), respectively.

Radionuclides that decay by emission of β -particles emit electrons with maximum kinetic energies of 0.3-2.3 MeV with corresponding ranges of ~ 0.5 -12 mm in tissue. This lengthy range reduces the need for cellular internalization and so targeting close to or at the cell membrane is sufficient. The range of β -particles, as compared to the diameter of cells, permits β -particles to traverse several cells (10-1000), an effect that has been termed "crossfire". Crossfire is critical to β -particle emitter therapy to improve tumor dose homogeneity and to ensure sufficient dose to each cell.⁶ Single cell disease such as leukemia, micrometastases, post-surgical residual disease, and other disseminated types of cancer may not be curable with targeted β -particle therapy. Humm and Cobb reported that to attain a cell kill probability of 99.99% for single cells, several hundreds of thousands of β -decays at the cell membrane are required.⁷ Concomitantly, a very large portion of the dose would also be deposited in the surrounding normal tissue by virtue of this same lengthy range. Therefore, the fundamental physics and radiobiology of β -particle radiation provides a poor tumor-to-normal-tissue dose ratio for treatment of single cell disease. Selection of an α -particle emitter over a β -particle emitter creates a scenario wherein such diseases may be treatable with targeted radionuclide therapy. Energy deposition per unit path length in tissue for α -particles is far higher than for β -particles, due to the greater mass and charge of the α -particle, which is a mono-energetic, high-energy helium nucleus (^4He). The average energy imparted per unit path length, termed linear energy transfer (LET), is 60-230 keV/ μm for α -particles and is therefore classified as high LET radiation.⁸ For comparison purposes, the LET values for β -particles are typically between 0.1 and 1 keV/ μm ; low-LET radiation. A single α -particle traversal of the cell nucleus has a 20-40% probability of killing the cell.⁹⁻¹¹ A typical α -particle kinetic energy of 5-9 MeV results in a 50-90 μm range in tissue, or to ~ 2 -10 cell diameters. Delivery of an α -emitting radionuclide close to, or at the cell membrane, remains sufficient for therapy with α -particles to kill targeted malignant cells. In a single cell disease scenario, taking into account omnidirectional decay and geometry, only a few hundred α -particle decays at the cell membrane are required for a 99.99% level of cell kill with a correspondingly significant decrease in collateral toxicity to normal tissue.⁷ Thus, given a good targeting vehicle suitable for a α -particle emitter, a highly localized and cytotoxic radiation dose can be delivered to cancer cells with minimal damage to surrounding normal tissue.

Additionally, high LET radiation is well established as being far more lethal to cells than low LET radiation.¹²⁻¹⁶ Differences between high and low LET radiation is often described through their relative biological effectiveness (RBE). RBE is defined as the ratio between a given test radiation dose and a reference radiation dose (originally 250 kV X-rays) wherein the

test and reference radiation doses result in equal biological effect. RBE values for *in vitro* and *in vivo* cell survival of 3-8 have been reported for α -particles.^{18,23,24} The primary cause for higher cell toxicity has been hypothesized as originating from the increased frequency of clustered DNA double-strand breaks (DSBs) observed with high LET radiation.^{13,17-19} The cytotoxicity of α -particles has also been shown to be independent of both dose rate and oxygenation status of the irradiated cells.¹² Low LET radiotherapy is less effective on hypoxic cells and at low dose rates.¹²

Radionuclides

There are greater than >100 radionuclides that emit α -particles. However, the overwhelming majority either decay too quickly or too long to be of meaningful therapeutic use, or no viable chemistry exists for their use, or there is no viable supply. Therefore, this discussion will be limited to those α -emitters that fall within the boundaries of reasonable use and have been investigated in animal models or humans.

¹⁴⁹Tb

¹⁴⁹Tb ($t_{1/2} = 4$ h) is a lanthanide that decays via a complicated set of mechanisms: α -decay (17%), β^+ -decay (4%), and electron capture (79%) (Figure 1). Production has been at the CERN spallation facility and small amounts have also been produced using a 10-mV tandem accelerator using the ¹⁴¹Pr(¹²C,4n)¹⁴⁹Tb reaction at 70 MeV. Separation and purification from the target materials has proven challenging when faced with the levels of purity required for RIT applications.^{20,21} Parts per million contamination can completely compromise radiolabeling protocols.

²¹¹At

²¹¹At is a cyclotron produced radionuclide by virtue of bombardment of a bismuth target with α -particles in a cyclotron via the ²⁰⁷Bi(α , 2n)²¹¹At nuclear reaction.²² Isolation from the cyclotron target is routinely performed by means of dry distillation procedures.^{23,24} Few institutions, however, possess a cyclotron of adequate energy range that is capable of producing ²¹¹At.

²¹¹At ($t_{1/2} = 7.2$ h) decays through a branched pathway with each branch resulting in the production of an α -particle in its decay to stable ²⁰⁷Pb (Figure 2). The α -particles from ²¹¹At have a mean energy of 6.8 MeV with a mean LET of 97-99 keV/ μ m. Because of its relatively long half-life, ²¹¹At-labeled constructs can be used even when the targeting molecule does not gain immediate access to tumor cells. Additionally, its daughter, ²¹¹Po, emits K x-rays that allow photon counting of samples and external imaging for biodistribution studies. This radionuclide, by virtue of behaving analogously iodine halogen chemistry, is also not retained as well as other α -emitting radiometals post-internalization into cells, which is a factor to be considered.²⁵

²¹²Bi

²¹²Bi ($t_{1/2} = 60.6$ min) emits an α -particle with a mean energy of 7.8 MeV from the decay of ²²⁸Th to stable ²⁰⁸Pb (Figure 3). A generator that uses ²²⁴Ra as the parent radionuclide provides for on-site production of ²¹²Bi for radiolabeling targeting vectors such as mAbs since the half-life is too short for realistic transportation between sites.²⁶ The ²²⁴Ra actually originates from weapons development and is extracted from ²²⁹Th, currently at Pacific Northwest Laboratories with the ²²⁸Th originally being purified from ²³²U.²⁶ One daughter from the decay of ²¹²Bi, ²⁰⁸Tl, emits a 2.6-MeV γ -ray that requires heavy shielding to minimize radiation exposure to personnel, thereby limiting the clinical utility of this radioisotope. However, it is unclear what level of shielding is really necessary in a clinical setting due to the

combination of both actual dosing schedules and short half-life. After ^{212}Bi has been selectively eluted from the ion-exchange resin of the ^{224}Ra generator either in the form of chloride or the tetraiodide complex, the isotope can be used after pH adjustment to radiolabel mAbs, peptides, or other vectors conjugated with a suitable bifunctional chelating agent such as C-functionalized *trans*-cyclohexyldiethylenetriamine pentaacetic acid derivative, CHX-A" DTPA (Figure 4).²⁷⁻³⁰

^{212}Pb

^{212}Pb ($t_{1/2} = 10.2$ h) is actually a β^- -emitter and is the immediate parental radionuclide of ^{212}Bi . Its inclusion here is justified since ^{212}Pb has been evaluated as an *in vivo* generator for the production of ^{212}Bi thereby effectively extending the half-life of ^{212}Bi to be ~ 11 h (Figure 3). However, during the decay processes, $\sim 30\%$ of the formed ^{212}Bi is released from the chelation environment.³¹ Nonetheless, the combination of greater efficacy as compared to ^{212}Bi on the basis of μCi vs. mCi lowered administered dose, and issues then of availability vs. cost, all combined with appropriate usage continue to promote the use of this radionuclide as a viable therapeutic within specific limitations. ^{212}Pb is available from the same ^{224}Ra generator that facilitates production of ^{212}Bi , and may be selectively eluted by controlling pH of HCl eluant from that same ion-exchange based generator system vs. ^{212}Bi for labeling mAbs.²⁶ Concerns regarding the a 2.6-MeV γ -ray from the ^{208}Tl daughter are greatly diminished due to decreased dose levels combined with half-life.

^{213}Bi

^{213}Bi is also available from a very similar generator based technology from its parent radionuclide ^{225}Ac dispersed onto a cation-exchange resin to prevent charring and decomposition of resin due to the confined radiation flux.^{32,33} The source of ^{225}Ac in the United States is currently limited to Oak Ridge National Laboratories where the source materials extend back to ^{225}Ra extracted from ^{229}Th which again has its origin in weapons development from ^{233}U .^{8,34} ^{213}Bi decays to stable ^{209}Bi by emitting an α -particle and 2 β^- -particles (Figure 4). Additionally, a 440-keV photon emission allows biodistribution, pharmacokinetic, and dosimetry studies to be performed. Similarly to ^{212}Bi , after elution from the ^{225}Ac generator, ^{213}Bi is readily conjugated to mAbs, peptides, or other vectors that have been modified with a suitable bifunctional chelating agent, such as CHX-A" DTPA.²⁷⁻³⁰

^{223}Ra

^{223}Ra ($t_{1/2} = 11.4$ d) can be provided in a generator form from the ^{227}Ac ($t_{1/2} = 21.8$ y) parent and is also available from uranium mill tailings in large quantities. Similar to ^{225}Ac (*vide infra*), ^{223}Ra ultimately provides for the emission of 4 α -particles through its decay scheme and daughters (Figure 6).³⁵ Because of inherent bone-seeking properties, cationic ^{223}Ra may be a promising candidate for delivery of high-LET radiation to cancer cells on bone surfaces. A Phase I clinical study demonstrated pain relief and reduction in tumor marker levels in the treatment of skeletal metastases in patients with prostate and breast cancer.³³ Development of chelation chemistry actively targeted ^{223}Ra continues to be pursued, however, the retention and biological trafficking of the decay process daughters remain a problematic challenge. The first daughter in the ^{223}Ra decay pathway is ^{219}Rn , a gaseous product that would pose a serious challenge to control *in vivo*. Thus, the biodistribution and targeting as well as those issues pertaining to control and trafficking of the decay daughters remains under investigation.

^{225}Ac

^{225}Ac ($t_{1/2} = 10.0$ d) decays sequentially by α -emission through three daughter radionuclides, ^{221}Fr ($t_{1/2} = 4.8$ min), ^{217}At ($t_{1/2} = 32.3$ ms), and ^{213}Bi ($t_{1/2} = 45.6$ min), each of which then also emits an α -particle (Figure 5). ^{225}Ac can be produced by the natural decay

of ^{233}U or by accelerator-based methods.^{34,35,37} Targeted ^{225}Ac as a therapeutic, in theory may be as much as ~1,000 times more potent than ^{213}Bi -containing analogs by virtue of this α -particle cascade to a cancer cell.³⁸ While this increased potency might render ^{225}Ac more effective than other α -emitters, the biological fate of the free daughter radioisotopes in circulation after decay of ^{225}Ac is unresolved; the qualities of the chelation chemistry used to sequester this element *in vivo* are equally unresolved.³⁸⁻⁴⁰

Radiolabeling -- Chemistry

One of the fundamental key aspects of targeted radiation therapy is the stable sequestration of the radionuclide *in vivo*.⁶ *In vivo* stability of a radioconjugate is paramount to maximizing the delivery of radiation to tumor while minimizing toxicity. All of the above radionuclides have specific biological sites of deposition which will then pose issues of unacceptable toxicity to normal tissue; $^{211}\text{At} \rightarrow$ thyroid, gut and lungs, $^{212}\text{Pb} \rightarrow$ red blood cells and bone, $^{212}\text{Bi}/^{213}\text{Bi} \rightarrow$ kidney, $^{223}\text{Ra} \rightarrow$ bone, $^{225}\text{Ac} \rightarrow$ bone and liver.^{8,22,38} Radioconjugates can also be susceptible to catabolism post-internalization into target cell or to the direct effects of radioactive decay. A variety of methods are used to conjugate radioisotopes to antibodies, dependent on the chemical nature of the radionuclide.

^{211}At is generally treated as a halogen and like ^{131}I can be used to directly radiolabel mAbs by incorporation of an aryl carbon-astatine bond into the antibody using tyrosine residues.²² However, such chemistry results in labile products resulting in loss of the ^{211}At . Methods have been developed in multiple laboratories based on small "linker" molecules that create an aryl carbon-astatine bond involving an astatodemetalation reaction using a tin, silicon, or mercury precursor.^{22,23,41} These small molecules have a reactive site for astatination while then also generally possessing an active ester for protein or peptide modification and radiolabeling. Regardless of these developments, most of these agents still suffer from some small measure of instability leading to unacceptable loss of the ^{211}At . Recent results indicate that this deficiency may be resolved although additional evaluation remains to confirm these results.^{42,43}

The other radionuclides discussed above are metallic in nature and thus require chelation chemistry or bifunctional chelators for linkage to antibodies. A sampling of bifunctional chelating agents derived from DTPA include the cyclic dianhydride derivative,⁴⁴ 1B4M-DTPA (MX-DTPA, tiuxetan),⁴⁵ and a family of *trans*-cyclohexyl derivatives that includes the specific stereoisomer, CHX-A" DTPA (Figure 4).²⁸⁻³⁰ The CHX-A" DTPA is effective chelating, ^{111}In , ^{90}Y , ^{177}Lu , and to date is the only reported DTPA derivative found to form suitably stable complexes with either of the above noted bismuth radionuclides conjugated to mAbs or peptides *in vivo*.²⁸⁻³⁰ resulting in radioimmunoconjugates that have been used effectively in clinical trials.²⁷ While studies of the physical characteristics and coordination chemistry have not been performed on this specific enantiomeric form, CHX-A", studies on the parental ligand CyDTPA and DTPA and their Bi(III) complexes have been reported.⁴⁶ Eight coordinate structures, both square anti-prisms, for each complex were reported. However, the impact of the *trans*-cyclohexyl ring were clearly noted by shorter Bi-ligand bond distances, but more importantly by all eight elements of the coordination sphere originating from CyDTPA while one carboxylate donor in the DTPA complex originates from a neighboring molecule. This characteristic very easily translates into the significant differential in *in vivo* complex stability found for the CHX-A" DTPA.

Numerous bifunctional analogs of the macrocyclic ligand 1,4,7,10-tetraazacyclododecane tetraacetic acid (DOTA) (Figure 7) have been used effectively for labeling of antibodies with ^{111}In , ^{90}Y , ^{177}Lu , ^{212}Pb , and ^{212}Bi .^{6,8,47-50} Structurally, DOTA complexes for lanthanides and other metal ions tend to be eight-coordinate square anti-prisms that exist in an

equilibrium between isomeric arrangements of the carboxylate arms and ring twists forms that saturate the coordination spheres about Bi(III) and Pb(II). However, the complex formation mechanism as reported for lanthanides can doubtlessly be extended to Bi(III) which correlates with compromising the use of DOTA with ^{212}Bi or ^{213}Bi .^{51,52} One simply does not have the luxury of time when using short half-life radionuclides except in select cases where the formation rate might be accelerated by heating; this option is not available when using proteins as the targeting vector and may be limited to small molecules and peptides.⁵³

Unfortunately, despite also forming highly stable complexes with ^{212}Pb ,⁵⁴ the Pb(II) DOTA complex has been shown to be more susceptible to acid catalyzed dissociation of Pb(II) than a corresponding tetra-primary amide derivative, TCMC (Figure 7).⁵⁵ Loss of ^{212}Pb post-internalization of mAb delivery to cells has been reported as a source of marrow toxicity. Hence, TCMC continues to be used for sequestration of ^{212}Pb as opposed to DOTA. DOTA derivatives have also been evaluated for sequestration of ^{225}Ac , however, and despite reported stability,³⁸ this result conflicts with the reported use of a bifunctional derivative of the 12-coordination site chelating ligand, 1,4,7,10,13,16-hexaazacyclohexadecane- N,N',N'',N''',N''',N'''' -hexaacetic acid (HEHA) (Figure 8).^{39,40} The development of Ac(III) chelation chemistry is considerably hampered by the simple fact that there are no stable isotopes of this element. As such, very little is actually known about the coordination chemistry of Ac(III). One can infer or extrapolate from La(III), however the ionic radius is known to be ~10% greater for Ac(III) (112 pm),⁵⁶ and while a coordination number of eight might be arrived at by this process, saturating the coordination sphere may in fact require a greater number of donors more effectively distributed about the metal ion. Unfortunately, it is completely unclear that any research group is currently engaged in those fundamental studies required to define the coordination chemistry of Ac(III).

Chelation chemistry for ^{223}Ra as noted above remains unresolved for adequate *in vivo* sequestration of this element with controlled targeting via a biological vector, *e.g.* a mAb.⁵⁷ Again, development of Ra(III) chelation chemistry for these applications has been considerably hampered by the simple fact that there are no stable isotopes of this element. Various chelating agents have been evaluated, but as yet none meet the minimal criteria for such applications.

A limited number of reports exist that evaluate chelation chemistry for ^{149}Tb *in vivo*.⁵⁸⁻⁶⁰ Use of CHX-A DTPA has been reported, yet no definitive *in vivo* stability and biodistribution studies are available at this time using this chemistry. Evaluations of *in vivo* pre-clinical efficacy of this radionuclide have been equally limited.⁶⁰ Contrary to several of the above listed radionuclides, Tb(III) is available in a stable form and as such a wealth of knowledge is available in the literature on lanthanide complexation chemistry.⁶¹ Despite the abundant literature relating to lanthanide coordination chemistry, both derivatives of DOTA and CHX-A DTPA seem to be bifunctional chelating agents of choice which may also then indicate that little more development is required in that regard.

The vast majority of these bifunctional chelating agents have been functionalized with an aryl isothiocyanate group for conjugation to amine moieties resident on proteins, peptides, or other vectors.⁶ Other reactive functional groups such as reactive halogens, maleimides, and active esters have seen far less study and seem to be limited to specific arenas of use. Almost all when used to form conjugates with proteins form random distributions reacting with available functionalities. Additionally, in the case of conjugating chelating agents to proteins, *e.g.* mAbs, the number of chelates possible per protein molecule has to be determined empirically to retain the biological activity of the protein. In the case of peptides, the chelating agent is generally at the *N*-terminus in a 1:1 form arranged synthetically. In general, bifunctional chelating agents are reacted with the targeting vector first and after optimization of the conditions to achieve acceptable conjugation conditions, radiolabeling of the conjugate is executed. Pre-

radiolabeling of the bifunctional chelating agent prior to conjugation, which has been investigated with ^{225}Ac ,³⁸ tends to not be performed due to issues of low conjugation yields versus radiolysis issues. Additionally, for ^{225}Ac both low complexation yields despite heating and then short conjugation reaction times severely impact the specific activity of the ^{225}Ac labeled protein product. Clearly, opportunities exist in select areas of both coordination chemistry and conjugation chemistry. Both areas seems to be settled in regards to Bi(III) radionuclides as well as for ^{212}Pb . The loss of formed ^{212}Bi from the β^- -decay of ^{212}Pb seems an insurmountable problem,³¹ but one that simply limits the viable applications of this radionuclide with chemistry that is equally adequate. One must seriously question the need for further derivatives of DOTA and DTPA for that matter since no significant gains arising from either basic structure seem forthcoming. No greater stability was needed nor has been achieved by the continued production of DOTA derivatives in this field and it is unlikely that any further improvements will achieved through the creation of additional DTPA derivatives. However, refinements in conjugation chemistry strategies continue to be needed to optimize yields and efficiency.

Development of novel ligands suitable for *in vivo* sequestration of the both Ac(III) and Ra(II) remains an area of opportunity since those ligands in use now were simply adopted from other applications or for chelating other radionuclides. Clearly, only with absolute unequivocal complex stability of both ^{225}Ac and ^{223}Ra will researchers be able to deconvolute and address issues surrounding the fate and trafficking of the decay product daughters and then be able to determine if these radionuclides have a real place in the cancer therapy armamentarium. Other than just stability, the inability to produce final radiolabeled products in high specific activity continues to challenge use of the metallic α -emitters.

Considerable progress has been made in the development of linkers for forming ^{211}At conjugates although it remains clear that this effort still is reaching asymptotically towards completely achieving suitable stability for intravenous administration. Clinical trials remain treating cavitory disease scenarios and pre-clinical studies continue to demonstrate small, but meaningful levels of instability *in vivo* of the At-C bond.

Pre-clinical Studies

^{149}Tb

There are limited *in vivo* evaluation studies reported using ^{149}Tb no doubt due to limitations associated with its production (see above). One study demonstrates high-efficiency elimination of leukemia in a SCID mouse model using the FDA approved anti-CD20 mAb, rituximab, labeled with ^{149}Tb .⁶⁰ RIT using 150 μCi (5.5 MBq) of ^{149}Tb -rituximab 2 days after intravenous administration of $5 \cdot 10^6$ Daudi cells resulted in >120 days of tumor free survival in 89% of treated animals. In contrast, all control mice (no treatment or treated with 5 or 300 μg unlabeled rituximab) developed lymphoma disease dying within 43 and 118 days, respectively. Interestingly, one can see that the high dose of rituximab had significant response, but no cures. This result very clearly defines the potential impact and opportunities of combination therapy, in this case integrating the effector functions of the targeting vector with targeted α -therapy.

When the study was terminated, $28.4\% \pm 4\%$ of the long-lived daughter activity from ^{149}Tb decay remained *in vivo*; 91.1% was located in bone tissue with 6.3% in the liver. Significant daughter radionuclide activity was also determined to be in the spleen and interpreted to be indicative of elimination of killed cancer cells through the spleen. Alternately, the spleen may have been a focal point of disease leaving those daughters retained in that organ. While encouraging, one has to remain concerned about the impact of retained daughter radionuclide and whether any later effect toxicity might be generated by these radionuclides. This aspect is

a recurring area of concern for many α -emitters that have multiple daughter and/or branching decay pathways.

^{211}At

Studies using ^{211}At date back greater than 50 years, however, recent history defines the leading center for targeted therapy using this radionuclide to be Duke University. Investigators there have extensively studied both ^{211}At production and radiolabeling chemistry while executing an array of pre-clinical studies developing numerous small molecule linkers for astatination of proteins and peptides.^{22,41} Studies with ^{131}I -81C6, a chimeric antibody targeting tenascin, a glycoprotein over-expressed on gliomas, were extended to ^{211}At -81C6. Treatment of a neoplastic meningitis model with ^{211}At -81C6 prolonged survival compared with controls.⁶² These studies were subsequently extended and extrapolated into one of the landmark clinical trials using targeted α -therapy (*vide infra*).

^{212}Bi

Early reports on ^{212}Bi -containing radioimmunoconjugates included studies by Macklis et al,⁴⁴ and Simonson et al.⁶³ Tumor-specific antibodies radiolabeled with ^{212}Bi using either the cyclic DTPA dianhydride or glycytyrosyl-lysyl-*N*- ϵ -DTPA, respectively, reported prolonged survival of colon carcinoma-bearing mice as compared with controls. However, both of these studies noted significant renal deposition of ^{212}Bi .

To overcome this deficiency, the value of the bifunctional chelating agent, CHX-A DTPA,²⁸ was defined by evaluation of ^{212}Bi labeled anti-gp70 mAb 103A.⁶⁴ Therapy of murine Rauscher leukemia virus (RVL) resulted in decreased splenic tumor growth and prolonged median survival. Additionally, ^{212}Bi -anti-Tac (anti-CD25) was used to treat CD25+ tumor bearing mice which led to enhanced tumor-free survival and prevented development of tumors in some animals. However, in mice with established tumors, this same therapy failed to provide any responses.⁶⁵

^{212}Pb

Ruble et al. studied the efficacy of ^{212}Pb radiolabeled mAb 103A treating RVL resulting in histological cure in all animals with a dose of 0.74 MBq (20 μCi). However, marrow toxicity originating from loss of the decay daughter, ^{212}Bi , could not be obviated by administration of a heavy metal chelator.⁶⁶

Horak et al. investigated ^{212}Pb -AE1-mAb targeting HER2 on ovarian tumors in nude mice.⁶⁷ 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 acellular bone marrow and subsequent death of all animals. However, 0.37-0.74 MBq (10-20 μCi) of ^{212}Pb -AE1 resulted in 100% tumor free survival for 180 days when treating animals bearing a 3-day s.c. tumor; all controls developed tumors by day 20. Treatment of larger tumors (15 and 146 mm^3) resulted in no complete remissions.

Studies demonstrating feasibility of targeted α -therapy using ^{212}Pb -labeled trastuzumab to treat disseminated peritoneal disease have been reported.⁶⁸ A pilot RIT experiment treated mice bearing 5 d LS-174T (i.p.) xenografts determined a maximum tolerated dose (MTD) of 0.74 - 1.48 MBq (20-40 μCi). 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 -trastuzumab increased median survival of mice bearing 3 d LS-174T i.p. xenografts to 110 days using up to 3 monthly doses of ^{212}Pb -trastuzumab.

Combination of gemcitabine as a radiosensitizing agent with ^{212}Pb -trastuzumab has been recently evaluated for treating disseminated peritoneal disease.⁶⁹ A pilot study treated 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 -trastuzumab. Improved survival resulted with both doses of ^{212}Pb -trastuzumab; 31 to 51 d in the absence or presence of GEM with 0.19 MBq (5 μCi) of ^{212}Pb -trastuzumab, and 45 to 70 d at the 0.37 MBq (10 μCi) dose vs. 16 d for untreated animals. A second study examined multiple doses of GEM combined with a single ^{212}Pb -trastuzumab dose. One group of animals received a total of three doses of GEM, one before and two weekly doses following ^{212}Pb -trastuzumab. Median survival was 63 d whereas those receiving the single GEM dose before RIT experienced a median survival of 54 d. This finding was further extended to one vs. two treatment cycles wherein a cycle consisted of sequential treatment with GEM, ^{212}Pb -RIT, followed by one or two additional weekly doses of GEM. The greatest benefit, a median survival of 196.5 d, was achieved by treating with two cycles of ^{212}Pb -trastuzumab with two doses of GEM.

^{213}Bi

Kennel et al. investigated targeting blood vessels in lung tumors as a therapeutic approach with ^{213}Bi labeled mAb's 201B and 34A in a murine model with lung tumors of EMT-6 mammary carcinoma and IC-12 tracheal carcinoma.⁷⁰ Animals with tumor cross-sections of 5-10 cell diameters (50-400 cells/tumor) achieved 100% cure at a dose of 5.6-7.4 MBq (150-200 μCi), but the cure rate dropped markedly with larger tumors. Cured animals sacrificed after 73-75 days due to respiratory distress revealed significant lung damage, including fibrosis and edema.

Engineered mAb Hu-CC49 ΔCH2 radiolabeled with ^{213}Bi was evaluated for efficacy treating mice with s.c. LS174T tumors ($83.8 \pm 31.5 \text{ cm}^3$).⁷¹ Doses as high as 37 MBq (1.0 mCi) per animal were administered i.p. with all of the animals exhibiting a tumor growth arrest and ~50% of the animals being cured. Lower doses of 18.5-27.8 MBq (500-750 μCi) provided positive responses with ~33% being cured, 33% responding with delayed tumor growth and ~33% not responding. An MTD was not reached, however the results achieved treating a solid tumor were very encouraging and may also indicate that contrary to accepted thought that solid tumors may in fact be successfully treated with α -emitters, and that the limitations to using ^{213}Bi may in part lie within the properties of delivery vector.

The Allen group in Australia has executed several pre-clinical studies.⁷²⁻⁷⁵ Single and multiple dose toxicity and efficacy of ^{213}Bi labeled plasminogen activator inhibitor type 2 (PAI2) in regressing prostate cancer model has been reported. Tumor growth was inhibited with single doses of 947 or 1421 MBq kg^{-1} (25.6 - 38.4 mCi kg^{-1}), complete tumor growth inhibition was achieved at a total dose of 947 MBq kg^{-1} (25.6 mCi kg^{-1}) given on five successive days.⁷² In a related study a single local or i. p. injection of ^{213}Bi -PAI2 completely regressed tumor growth and lymph node metastases. Control animals and one of five mice treated with 111 MBq kg^{-1} (3 mCi kg^{-1}) ^{213}Bi -PAI2 developed metastases in the lymph nodes while no lymphatic spread of cancer was found in the 222 MBq kg^{-1} (6 mCi kg^{-1}) treated groups at 2 days and 2 weeks post-cell inoculation.⁷⁴

Anti-colorectal cancer mAb c30.6 radiolabeled with ^{213}Bi was found to exhibit high tumor uptake and retention of the radiolabel, potentially offering a new approach for control of colorectal cancer.⁷⁴

The ^{213}Bi -PAI2 has also been evaluated for control of micrometastatic breast cancer treating a 2-day post-inoculation of MDA-MB-231 breast cancer cells. A single local injection of ^{213}Bi -PAI2 completely inhibited tumor growth while a single systemic (i.p.) administration

resulted in dose-dependent tumor growth inhibition with up to 3.7 MBq (100 μ Ci) of ^{213}Bi -PAI2 being well tolerated.⁷⁵

Locoregional RIT of dissemination gastric cancer using ^{213}Bi provided good therapeutic results with a single administration that was dependent on the time interval between tumor inoculation and therapy.⁷⁶ Single versus double i.p. injections of mAb d9MAb labeled with ^{213}Bi were evaluated for therapeutic efficacy and toxicity. Two applications of 0.37 MBq (10 μ Ci) of ^{213}Bi -d9MAb at days 1 and 8 after tumor inoculation significantly prolonged median survival vs. a single injection.

The mAb, J591 radiolabeled with ^{213}Bi was evaluated for efficacy in a murine model using LNCaP tumors.⁷⁷ Cytotoxicity experiments achieved significantly improved median tumor-free survival (54 days) relative to the control (33 days), or no treatment (31 days). Reduction of PSA levels also correlated to tumor response.

Milenic et al evaluated ^{213}Bi -trastuzumab for treatment of disseminated peritoneal disease.⁷⁸ A dose of 500 μ Ci was determined to be the effective operating dose for future experiments based on changes in animal weight treating 3 day LS-174T i.p. xenografts. Median survival increased from 19 days to 43 days and 59 days with 18.5 and 27.8 MBq (500 and 750 μ Ci), respectively. RIT targeting HER2 was proposed to be potentially beneficial even for those patients currently ineligible for immunotherapy due to low antigen expression.

Allogeneic marrow transplantation using myeloablative radiation preparative regimens causes significant toxicity despite being potentially curative for several malignancies. To reduce toxicity associated with non-myeloablative regimens, ^{213}Bi -labeled mAbs targeting CD45 and TCR $\alpha\beta$ were evaluated for immunosuppression prior to marrow transplantation in a canine model.^{79,80} Administration of either radiolabeled mAb prior to transplantation combined with immunosuppressive agents resulted in engraftment of transplanted marrow and stable mixed chimerism. Toxicities included transient myelosuppression and liver enzyme abnormalities. Use of ^{213}Bi radiolabeled mAbs for this application in humans however may be limited by estimated requirements of $> 74 \text{ MBq kg}^{-1}$ [2 mCi kg^{-1}] doses.

^{225}Ac

^{225}Ac has been investigated for targeted α -therapy in attempts to harness the cascade of 4 α -emissions in the decay chain (Figure 5) to provide a much greater radiation dose while also administering far less radionuclide. Increased potency, and hence impetus for investigation, lies within the long half-life and that ^{225}Ac conjugates may act as *in vivo* generators. Both direct and indirect radiolabeling methods have been investigated and neither has actually proven to be optimal for efficiently forming the requisite complex.^{38,40} Issues surrounding stability of the ^{225}Ac complex remain debatable and, of far greater concern, is the control and fate of the decay product daughter radionuclides as the chelation chemistry cannot be expected to survive the decay event or even remain suitable for the daughter elements.

The bifunctional derivative HEHA was evaluated for sequestering ^{225}Ac for targeted α -therapy (Figure 8).⁴⁰ HEHA conjugated to mAb 201B determined that ^{225}Ac was delivered to lung efficiently, was slowly released with an initial $t_{1/2} = 49 \text{ h}$, and accumulated in the liver. The decay daughters of ^{225}Ac were also released from the lung; levels of ^{213}Bi , the third alpha-decay daughter were deficient in the lungs and in excess in the kidney. Injected doses of ^{225}Ac -201B of 0.037 MBq (1.0 μ Ci), delivering a minimum calculated absorbed dose of $\sim 6 \text{ Gy}$ to the lungs, was effective in killing lung tumors, but also proved acutely radiotoxic. Animals treated with $> 0.037 \text{ MBq}$ (1.0 μ Ci) of ^{225}Ac -201B died dose dependently of a wasting syndrome within days. These studies concluded that ^{225}Ac RIT was compromised not only by

unstable chelation chemistry, but critically so by radiotoxicity associated with trafficking of the decay daughter radioisotopes.

The Sloan-Kettering group found that DOTA derivatives could be used forming the ^{225}Ac complex first followed by conjugation to mAbs.³⁸ The *in vivo* efficacy of ^{225}Ac -J591 was evaluated in an intramuscular LNCaP tumor model. A single administration of ^{225}Ac -J591 12 days post-inoculation with tumor caused tumor regression and significantly improved ($P < 0.0001$) median survival to 158 days compared to the mice treated on day 15 (63 days) compared to controls (33 days). The mice survived at least 10 months without measurable PSA levels or evidence of tumor at the time of death (293 days). Mice bearing a disseminated human Daudi lymphoma were treated using ^{225}Ac -B4 1 day post-tumor dissemination showed a dose dependent response in median survival times: 165 days (6.3 kBq), 137 days (4.3 kBq), and 99 days (2.1 kBq) with the latter dose being significant versus controls ($p = 0.05$). Approximately, 40% of mice treated with the highest dose were tumor-free at 300 days.

An interesting target for targeted α -therapy is neovascular endothelium. E4G10, a mAb that targets vascular endothelial cadherin, was radiolabeled with ^{225}Ac and evaluated in a model of prostatic carcinoma.⁸¹ Treatment with ^{225}Ac -E4G10 resulted in tumor growth inhibition, decreased serum prostate specific antigen level, and markedly prolonged survival, which was enhanced by administration of paclitaxel. Immunohistochemistry revealed decreased vessel density and enhanced apoptosis. Additionally, residual tumor vasculature appeared normalized without toxicity being observed in vascularized normal organs.

A major impediment to clinical use of ^{225}Ac has been concern regarding radiotoxicity of systemically released daughter radionuclides, particularly ^{213}Bi which accumulates in the kidneys.⁸² Oral metal chelation with 2,3-dimercapto-1-propanesulfonic acid or *meso*-2,3-dimercaptosuccinic acid significantly reduced renal ^{213}Bi uptake; however, DMPS was more effective than DMSA. These results were also confirmed in a non-human primate model. Renal ^{213}Bi and ^{221}Fr activities were significantly reduced by diuretics, furosemide and chlorothiazide. Impact on renal ^{213}Bi activity was further enhanced by combining DMPS with either chlorothiazide or furosemide ($P < 0.0001$). Lastly, liposomes have been investigated for retention of both ^{225}Ac along with formed daughter radionuclides.⁸³ While ^{225}Ac could be sequestered in liposomes, retention of the daughter radionuclides was size dependent phenomenon and was the basis for assembly of liposomes of suitable size and complexity to perform targeted α -therapy via immunoliposomes. Based on this set of pre-clinical data, a Phase I trial investigating the use of ^{225}Ac -HuM195 for advanced myeloid leukemias has been initiated at Sloan-Kettering.

Targeted α -therapy versus targeted β -therapy

Few studies directly compare the efficacy of α -emitters and β -emitters *in vivo*. Greater efficacy in inhibiting tumor growth and improved survival rates have been demonstrated using α -emitters. A ^{213}Bi -labeled Fab' fragment of mAb CO17-1A was prevented growth of a human colon cancer xenograft while increasing survival compared to the ^{90}Y -labeled Fab'.⁸⁴

Pre-targeted RIT was developed to reduce radiation dose to normal organs while improving tumor-to-normal organ ratios. One pre-targeting strategy involves administering a mAb or targeting vector conjugated to streptavidin, followed by administration of a biotinylated *N*-acetylgalactosamine-containing "clearing agent" to remove excess circulating antibody. Thereafter, radiolabeled biotin is infused to bind to the "pre-targeted" streptavidin at the tumor, leaving the unbound radiolabeled biotin to be rapidly excreted.⁸⁵ This strategy was used to treat disseminated CD25+ adult T-cell leukemia DOTA-biotin radiolabeled with either ^{213}Bi or ^{90}Y .⁸⁶ Treatment with ^{213}Bi reduced the levels of the surrogate tumor markers $\beta_2\text{M}$ and soluble CD25, and improved survival while treatment with ^{90}Y failed to improve survival and

resulted in significant toxicity. This same strategy was extended to study of the anti-CD25 single-chain Fv-streptavidin fusion protein followed by radiolabeled biotin achieving similar results with 7 of 10 mice cured.⁸⁷

Clinical Studies

²¹¹At

Based on pre-clinical results at Duke University, a Phase I dose-escalation trial of ²¹¹At-81C6 was initiated in patients with malignant gliomas post-surgical resection of their tumor.⁸⁸ Twelve patients have been treated to date. Imaging (γ -camera) showed 99% of the ²¹¹At decays occurred within the tumor cavity, indicating high *in vivo* stability of the radioimmunoconjugate in this intracavitary administration approach. Early results suggest that ²¹¹At-81C6 as an adjuvant therapy prolongs survival in these patients.

²¹³Bi

The ²¹³Bi-labeled humanized anti-CD33 monoclonal antibody, HuM195, was translated to a landmark clinical trial at Memorial Sloan-Kettering Cancer Center.⁸⁹ Eighteen patients with advanced myeloid leukemia were treated in a Phase I dose-escalation trial and with myelosuppression in all patients along with transient minor liver function abnormalities. Doses of up to 37 MBq kg⁻¹ (1 mCi kg⁻¹) were safely administered. Uptake of ²¹³Bi was demonstrated by γ -camera imaging to be in the bone marrow, liver, and spleen, without significant uptake in other organs, and most importantly, absent from the kidney. Absorbed dose ratios between marrow, liver, and spleen and the whole body were 1,000 times greater with ²¹³Bi-HuM195 than with previously evaluated HuM195 radiolabeled with β^- -emitters. Fourteen of 18 patients had a reduction in the percentage of bone marrow blasts after therapy. There were no complete remissions thereby demonstrating the difficulty of targeting an adequate number of ²¹³Bi atoms to each leukemic blast at the specific activities used in this trial.

A Phase I/II study followed wherein patients were first treated with chemotherapy to achieve partial cytorreduction of the leukemic burden followed by ²¹³Bi-HuM195.⁹⁰ Greater than 20 patients with acute myeloid leukemia were treated with cytarabine (200 mg/m²/d for 5 d) followed by ²¹³Bi-HuM195 at 4 dose levels (18.5-46.25 MBq kg⁻¹ [0.5-1.25 mCi kg⁻¹]). Prolonged myelosuppression was dose limiting at the highest dose level. Complete responses, complete responses with incomplete platelet recovery, and partial responses were achieved at the 2 highest dose levels. These preliminary results indicate that sequential administration of cytarabine and ²¹³Bi-HuM195 can lead to complete remissions in patients with acute myeloid leukemia. These studies have recently been extended to a Phase 1 study using ²²⁵Ac.

Alternate delivery methods and uses

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.

A melanoma-targeting peptide, (DOTA)-Re(Arg¹¹)CCMSH, was radiolabeled with ²¹²Pb for biodistribution and therapy studies executed in a B16/F1 melanoma-bearing murine tumor model. Treatment with 1.85, 3.7 and 7.4 MBq (50, 100, and 200 μ Ci) of ²¹²Pb[DOTA]-Re(Arg¹¹)CCMSH extended mean survival to 22, 28, and 49.8 days, respectively, as compared with 14.6-day mean survival of the controls; 45% that received 7.4 MBq (200 μ Ci) surviving disease-free.⁹¹

The somatostatin analogue [DOTA⁰, Tyr³]octreotide (DOTATOC) was labeled with ²¹³Bi. Significant decreases in tumor growth rate were observed in rats treated with >11 MBq (300

μCi) of ^{213}Bi -DOTATOC 10 days post-inoculation with tumor compared with controls ($P < 0.025$). Treatment with $>20 \text{ MBq}$ ($540 \mu\text{Ci}$) resulted in greater tumor reduction.⁵³

While RIT applications have overwhelmingly been oncologic in nature, Dadachova and co-workers opened up a novel arena to study the use of microbe-specific mAb 18B7 which binds to capsular polysaccharides of the human pathogenic fungus *Cryptococcus neoformans*.^{92, 93} When radiolabeled with ^{213}Bi , biofilm metabolic activity was reduced 50% while unlabeled 18B7, ^{213}Bi -labeled non-specific MABs, and γ - and β -radiation failed to have an effect. Their results indicate targeted α -therapy to be a novel option for the prevention or treatment of microbial biofilms on indwelling medical devices. These researchers have also investigated targeted α -therapy for infectious diseases for several fungal and bacterial infections.

Prospects & Conclusions

The role of radiolabeled mAbs in the treatment of cancer remains a burgeoning enterprise. The majority of RIT trials have been performed with β^- -emitting isotopes and this currently remains true. Contrasting to β^- -emitters, the shorter range and higher LET α -particles allow for more efficient and selective killing of individual tumor cells. And, while some experimental pre-clinical models have demonstrated that targeted α -therapy may have a significant impact on large tumor burdens, the majority of pre-clinical and clinical trials thereafter clearly pursue the accepted paradigm that RIT with α -emitters are best suited for the treatment of small-volume disease. While both pre-clinical and early clinical studies appear promising, several obstacles obstruct the path to widespread acceptance and use of targeted α -therapy. To traverse these obstacles, issues pertaining to supply and economics need to be resolved by the creation of new sources and methods for production of these medically valuable radionuclides. Current supplies of those radionuclides associated with the ^{225}Ac and ^{224}Ra decay pathways remain limited to those sources of naturally isolated by-products from weapons development within the United States. Additional sources of both reside within Russia and other locations and within recovery of nuclear fuel materials during their reprocessing. Both reactor and accelerator routes to ^{225}Ac have been proposed although meaning product remains to be delivered. Beyond just production of these radionuclides, assembly and transport of generators of sufficient activity amounts to support clinical trials have yet to really be produced; current clinical use requires multiple generators linked together. Lastly, the costs of these radionuclides has more than tripled in the past decade for a variety of reasons seriously challenging the ability of many researchers from both remaining in or entering the field. Production of ^{211}At is also hampered by production limits both in amounts and feasible locations due to cyclotron energy constraints.

Chelation and linking chemistry, while partially resolved for some radionuclides as noted earlier, remains a challenge for the multiple decay pathway radionuclides. The practical chemical challenges of studying the coordination chemistry of Ac(III) and Ra(II) directed towards the requirements for these applications place such studies well beyond all but a few facilities where is equally unclear that such studies might ever be performed. Opportunities remain within the area of conjugation chemistry. Despite having traversed many chelation challenges, more efficient conjugation and Radiolabeling protocols remain to be developed that result in more consistent products with higher specific activities to optimize therapeutic potentials. Better pharmacokinetic and dosimetry modeling techniques are also required that actually address the cellular micro-dosimetry aspect of targeted α -emitters since their targets exist at the individual cell by cell therapy..

Novel delivery methods must be developed and carefully studied and in part this approach has been initiated (*vide infra*). Careful and comparable pre-clinical (and clinical investigations) will be required to define optimal radioisotopes, dosing regimens, and therapeutic strategies. This latter aspect is of particular importance as it is obvious that while single dose

administration protocols dominate clinical investigation, the clinical reality remains that RIT and targeted α -therapy will have to become integrated into clinical standards of care, become combined with both external beam radiation therapy and chemotherapies to become real, accepted therapeutic modality.

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Figure 1.
Decay scheme for ^{149}Tb

Figure 2.
Decay scheme for ^{211}At

Figure 3.
Decay scheme for ^{212}Pb and ^{212}Bi

Figure 4. Structures of DTPA, cyclic dianhydride of DTPA, CyDTPA, 1B4M-DTPA, and CHX-A'' DTPA

Figure 5.
Decay scheme for ^{225}Ac and ^{213}Bi

Figure 6.
Decay scheme for ^{223}Ra

Figure 7. Structures of DOTA, and the bifunctional analogs C-DOTA, PA-DOTA, CHX-DOTA, *lys*-DOTA, and TCMC

BF-HEHA

Figure 8.
Structures of the bifunctional chelating agent BF-HEHA