The Development of the α-Particle Emitting Radionuclides ^{212}Bi and ^{213}Bi, and Their Decay Chain Related Radionuclides, for Therapeutic Applications

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Received November 8, 2000

Contents

I. Introduction

The three main weapons in the arsenal for cancer treatment continue to be surgery, external beam radiotherapy, and chemotherapy. Nevertheless, inoperable tumors, tumors situated close to radiation sensitive organs, metastatic disease, and single cell diseases such as leukemia and lymphoma are often difficult to eradicate with these traditional modalities. Interest in the use of targeted radionuclide therapy has increased to address some of these obstacles. Targeted radionuclide therapy involves specific localization of a radionuclide emitting ionizing radiation to cancer cells to deliver a cytotoxic radiation dose to the cancerous tissue while sparing surrounding healthy tissue.

Radionuclides alone rarely possess properties that specifically target cancer cells. Therefore, carrier molecules that specifically target cancer cells are required. The radionuclide chosen, regardless of emission character, should also reflect the carrier's tumor targeting and clearance properties and its tumor residence time. Applicable classes of radionuclides are Auger-electron, β-particle, and α-particle emitters. Auger-electron emitters are highly cytotoxic primarily within a micron of the decay site, due to the emission of a cascade of low energy electrons per decay.[1,2] Thus, a carrier molecule must transport the Auger-electron emitter into the cell nucleus to be highly cytotoxic.[3,4] Despite this limitation, significant responses have been observed with high doses of a ^{111}In labeled peptide.[5] This success is likely due to the longer range conversion electrons emitted by ^{111}In. The β-particle emitters are more suitable for targeted radionuclide therapy and emit electrons with maximum kinetic energies of 0.3−2.3 MeV with corresponding ranges of ∼0.5−12 mm in tissue. Thus, cellular internalization is unnecessary and targeting close to or at the cell membrane is sufficient. The potentially long 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 important in therapy with β-particle emitters to improve tumor dose homogeneity and to ensure sufficient dose to each cell. Monoclonal antibodies (mAb's) radiolabeled with β-particle emitters have shown promising results in treatment of several cancers.[6−13] Regardless of positive results, single cell disease such as leukemia, micrometastases, and other dispersed cancer types 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 would be needed.[14] Additionally, due to the range of the β-particles, a very large portion of the dose would be deposited in surrounding normal tissue. Therefore, β-particles give a poor tumor-to-normal-tissue dose ratio for treatment of single cell disease. By selecting an α-particle emitter over β-particle emitters, such diseases may still be treatable with targeted radionuclide therapy.

The energy deposited per unit path length in tissue is far higher for α-particles than for β-particles, due to the greater mass and charge of the α-particle. 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

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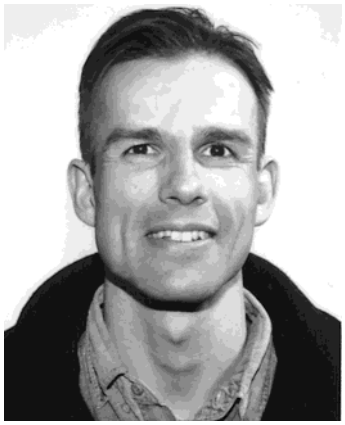
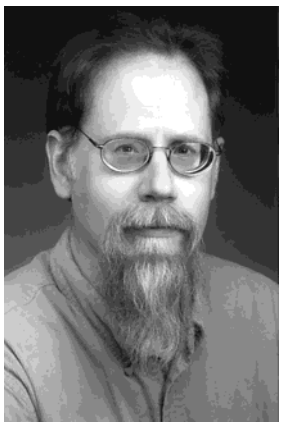
10.1021/cr000118y CCC: $36.00 © 2001 American Chemical Society
Published on Web 06/07/2001



Sindre Hassfjell is a native of Oslo, Norway. He received his B.Sc. in 1989, M.Sc. in 1992, and Ph.D. in 1997, all from the University of Oslo. Under the supervision of Professor P. Hoff, he explored the possibilities of targeted radionuclide therapy and diagnostic of sclerotic bone cancer and bone metastases with the α-particle emitters ^{211}At and ^{212}Bi, and the γ- and X-ray emitters ^{125}I and ^{165}Er, respectively. At the same time, he developed a ^{212}Pb/^{212}Bi generator. In 1992, he received Hafslund Nycomed's Best Young Scientist Award. He was a research assistant, teaching laboratory courses in radiochemistry and inorganic chemistry at the University of Oslo in 1992–1997. In 1997–1998, he worked as a researcher for Axis Biochemicals ASA. He was a Postdoctoral Fellow at the University of Oslo in 1998–1999, and at the National Institutes of Health, Bethesda, in the research group of Dr. M. W. Brechbiel in 1999–2000. He is presently employed at the Norwegian Defense Research Establishment.



Martin W. Brechbiel received his B.A. in 1979 from Gettysburg College and a M.S. in 1982 from the University of Delaware under the guidance of Professor Harold Kwart. After working for FMC Corp, he joined the National Cancer Institute in 1984. 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 University in 1988 with Professor Thomas Cantrell. He remained with the NCI and in 1997 was appointed Acting 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, and the medicinal chemistry of novel metal complexes.

radiation. In comparison, β-particles have LET values typically between 0.1 and 1 keV/μm and therefore are classified as low-LET radiation. A single α-particle traversal of the cell nucleus has a 20−40% probability of killing the cell.[15−17] A typical α-particle kinetic energy of 5−9 MeV, results in a 50−90 μm range in tissue, corresponding to approximately 2−10 cell diameters. Targeting close to, or at the cell membrane, is therefore sufficient for therapy with α-particle emitters. In the single cell disease scenario, a few hundred α-particle decays at the cell membrane would be needed to achieve the 99.99% level of cell kill, and the short range of α-particles would result in significantly decreased collateral toxicity to normal tissue.[14] Thus, given a good targeting mechanism for a suitable α-particle emitter, a highly localized and cytotoxic radiation dose could be delivered to cancer cells with a minimum of damage to normal tissue.

Numerous experiments have established that high LET radiation is far more lethal to cells than low LET radiation.[18−22] The difference between high and low LET radiation is often described through the relative biological effectiveness (RBE). The RBE is defined as the ratio between a given test radiation dose and a reference radiation dose (originally 250 kV X-rays) where the test and reference radiation doses are resulting 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] It has been hypothesized that a primary cause for higher cell toxicity is the increased frequency of clustered DNA double-strand breaks (DSBs) observed for high versus low LET radiation.[19,25] Furthermore, the destructiveness of α-particles has been shown to be independent of both dose rate and the oxygenation status of the irradiated cells.[18] Low LET radiotherapy conversely is less effective on hypoxic cells and at low dose rates.[18]

The most widely studied α-particle emitter candidates for therapy are ^{212}Bi ($t_{1/2}$ = 61 min), ^{213}Bi ($t_{1/2}$ = 46 min),^{225}Ac ($t_{1/2}$ = 10 days), and^{211}At ($t_{1/2}$ = 7.2 h). Like ^{99m}Tc, both ^{212}Bi and ^{213}Bi are conveniently produced by generators. Generators, also often referred to as cows, are systems wherein a longer-lived parent radionuclide is used to continuously generate, by radioactive decay, a shorter-lived daughter radionuclide of interest and where the desired radionuclide can be selectively obtained by chemical means. Both ^{212}Bi and ^{213}Bi have branched decay series that result in the emission of α-particles, β-particles, and γ-rays (Figures 1 and 2). A disadvantage of ^{212}Bi is the emission of high-energy γ-rays, specifically 2.6 MeV in 36% abundance. This necessitates extensive shielding of personnel involved in production, delivery, and patient treatment. ^{213}Bi has the advantage of emitting lower energy γ-rays suitable for γ-camera imaging. Unfortunately, both bismuth radioisotopes have relatively short half-lives, which require rapid targeting as well as efficient production of radiolabeled carrier molecules to minimize loss of radioactivity. This review will focus on the chemistry and related experimental results relevant to the development of the therapeutic use of ^{212}Bi and ^{213}Bi through the year 2000. The relevant work on the development of related parental radionuclides, ^{212}Pb and ^{225}Ac, respectively, as therapeutics, will also be addressed. The use and development of the α-emitter ^{211}At as applied to radiopharmaceutical development and other α-particle emitters have been reviewed elsewhere.[26−28]

II. Chemistry of Bismuth (III)

Comprehensive reviews of the general medicinal chemistry of bismuth compounds have recently been

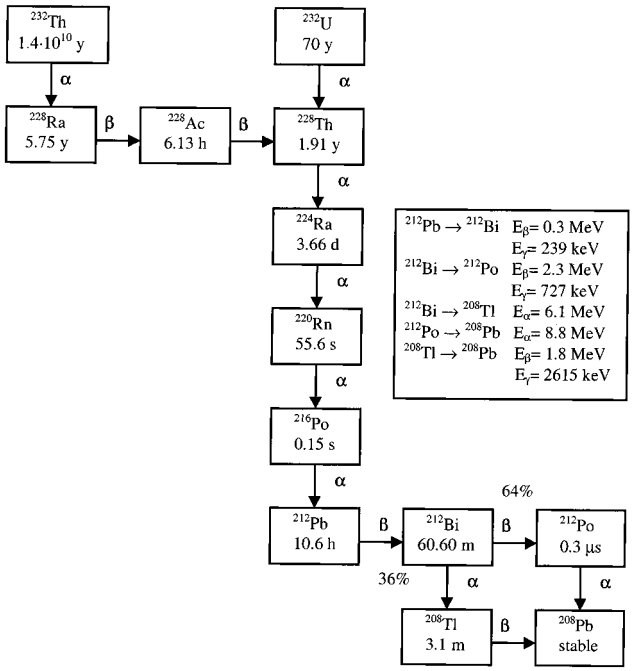


Figure 1. Decay schemes for the production of ^{212}Bi and ^{212}Pb.

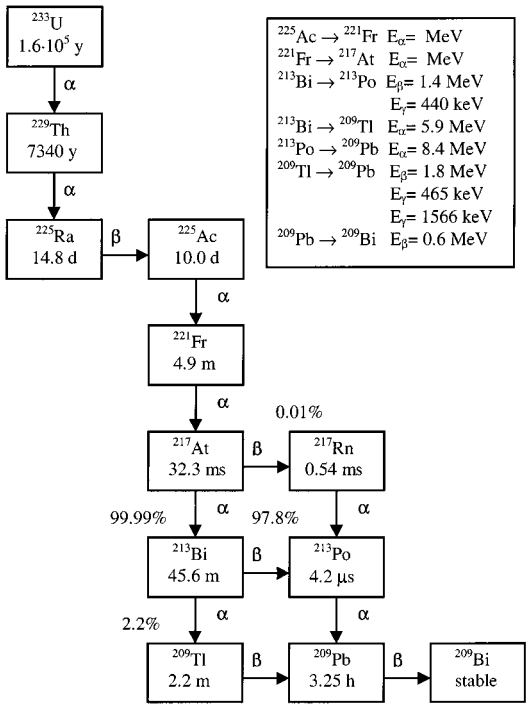


Figure 2. Decay schemes for the production of ^{213}Bi and ^{225}Ac.

published.[29,30] In brief, bismuth is the heaviest stable element in the periodic table with an ionic radius of 103 pm, with a normal oxidation state of 3+, although 5+ species are well-known as oxidative reagents.[31] Stable, nonradioactive, bismuth is widely used in antiulcer and antibacterial drugs. Besides having a strong affinity for oxygen and nitrogen donors, Bi forms very stable complexes with sulfur and halogens, especially iodide. Chelating agents with oxygen and nitrogen donors or compounds with thiolate donors form very stable complexes with Bi(III) and the coordination number varies from 3 to 9. Bi(III) is known to bind to Zn(II) sites (e.g., metallothionein) and Fe(III) sites (e.g., transferrin) in proteins. In its unchelated form, Bi(III) is excreted relatively quickly through the renal clearance system, with the kidneys being a temporary deposit organ.

III. Sources and Generators for ^{212}Bi

Production modes for ^{212}Bi have been reviewed by both Mirzadeh and Lambrecht et al.[27,32] The parent radionuclides for ^{212}Bi generators are ^{228}Th and ^{224}Ra, which are members of the natural decay chain of ^{232}Th ($t_{1/2} = 1.4 \times 10^{10}$ years) (Figure 1). ^{228}Th can be produced in two practical ways. The first is extraction and purification of ^{228}Ra from ^{232}Th and then separation of ^{228}Th from ^{228}Ra in ca. 1 year intervals. Since there are 4.1 GBq (0.11 Ci) of ^{228}Th per ton of ^{232}Th (>35 years old), several tons of ^{232}Th containing minerals would need to be processed for production of reasonable amounts of generator material. The second method for production of ^{228}Th is by double neutron capture and successive β decays of ^{226}Ra. ^{226}Ra targets can be made by separation from ^{238}U and is available both in Europe and the USA. At the Oak Ridge National Laboratory (ORNL) high flux isotope reactor (HFIR), 1.4 TBq (37 Ci) can be produced per gram of ^{226}Ra in 24 days at a neutron flux of 1×10^{15} ns^{-1} cm^{-2} (th/epi = 10).[27] The process of successive neutron captures will also produce ^{227}Ac and ^{229}Th. The ^{227}Ac and its radioactive decay products could be removed by first separating ^{227}Ac from the Th isotopes and then allowing time for the decay of ^{227}Th ($t_{1/2} = 18.7$ days) and its radioactive daughters to reach an acceptably low level. The ^{229}Th ($t_{1/2} = 7340$ years) cannot be removed this way, but it is only present at the level of 4×10^{-5} Bq of ^{229}Th per Bq of ^{228}Th.

The only potentially available generator system for ^{212}Bi is the ^{224}Ra/^{212}Bi generator produced at the University of Chicago, recently relocated from the Argonne National Laboratory. ^{224}Ra ($t_{1/2} = 3.6$ days) is separated from ^{228}Th by adsorbing ^{228}Th as nitrate complexes on an anion-exchanger, while ^{224}Ra elutes through the column. The ^{224}Ra is then adsorbed on the macroporous organic cation ion-exchange resin (AG-MP-50) which then serves as the ^{212}Bi (or ^{212}Pb) generator.[33−35] Selective elution of ^{212}Bi (ca. 50%) is achievable with 1−0.25 M HCl or 0.05−0.2 M HI with little ^{212}Pb breakthrough (~0.1%). At greater acid concentrations, 1−6 M HCl or 0.2−1 M HI, a mixture of ^{212}Pb and ^{212}Bi will elute (ca. 90%).[35] These generators have been available at a 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. Generator systems have also been made that are based on ^{228}Th, but none of these have proven able to produce the radioactivity levels required by therapeutic applications. Zucchini and Friedman adsorbed ^{228}Th and ^{224}Ra on a column of Na_2TiO_3 from a very dilute HCl solution (pH = 6).[36] 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 the 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 $\sim 2 \times 10^{-4}$ Bq of ^{228}Th or ^{224}Ra per Bq of ^{212}Pb was reported. To attain reasonable yields close to the theoretical maximum, this procedure had to be repeated continuously for a day or more. At radioactivity levels greater than 37 MBq (1 mCi), radiolytic breakdown of the ion-exchanger support caused increasing back pressure and decreasing yields.[35,37]

Other ^{228}Th based generators have utilized the property of ^{220}Rn to emanate from ^{228}Th doped barium stearate.[38−40] Hassfjell and Hoff described a 50 MBq (1.4 mCi) generator where such a source could be moved into, or out from, a collection chamber.[40] When the source was inside the chamber,^{212}Pb generated from the ^{220}Rn, which then was deposited on the walls. The ^{212}Pb could be washed off with aqueous solutions (70−99%) without detection of any ^{228}Th ($<10^{-9}$ Bq of ^{228}Th per Bq of ^{212}Pb) breakthrough. The emanation yield was only 50% initially and decreased gradually due to radiolytic damage of the barium stearate. Recently, Hassfjell has suggested an improved generator construct based on the same principle, but with a different method for collecting the ^{220}Rn and decay products.[41] 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 have been increased to approximately 70%. So far, characterization of generator properties has only been possible with tracer levels of radioactivity due to limited availability of ^{228}Th.

IV. Sources and Generators for ^{213}Bi

Production modes for ^{213}Bi have been reviewed by both Mirzadeh and Lambrecht et al.[27,32] The parent radionuclide for making ^{213}Bi generators is ^{225}Ac, which is a decay product of ^{229}Th (Figure 2). They are all members of the decay chain of the now extinct ^{237}Np ($t_{1/2} = 2.1 \times 10^6$ years). A possible source of ^{229}Th is the ^{233}U stockpile at the National Repository at ORNL, produced in the 1960−70s for a molten salt breeder reactor program. It has been estimated that ~12 g or 93 GBq (2.5 Ci) of ^{229}Th might be extracted from this stockpile.[27] However, the ^{233}U stock also contains ^{232}U which produces a mixture of ^{228}Th and ^{229}Th. As mentioned previously, ^{229}Th may also be produced by successive neutron capture of ^{226}Ra in high flux reactors such as at HFIR at ORNL. Under comparable conditions to those for production of ^{228}Th (1.4 TBq), the yield of ^{229}Th is only 55 MBq (1.5 mCi). Thus, large amounts of ^{226}Ra and long irradiation times would be needed to produce enough generator material for therapeutic applications. While this production route for ^{229}Th has advantages of few separation steps and coproduction of ^{228}Th, the high-energy γ-rays from the ^{228}Th decay chain, coupled with the ^{222}Rn ($t_{1/2} = 3.8$ days) produced from ^{226}Ra, put high demands on shielding and the production logistics. To the best of our knowledge, this production route can only be conducted at the transuranium facility at ORNL. Pure ^{225}Ac is obtainable from ^{229}Th by separating Ra(II) from Th(IV), letting the ^{225}Ac grow in, and then separating the ^{225}Ac from the Ra-(II).[27] This separation could be accomplished by several steps of ion-exchange and extraction chromatography.

A third source for ^{229}Th is extraction of ^{229}Th from processing waste of ^{233}U, which has been explored at ORNL.[42,43] An extensive purification with several steps of precipitation, dissolution, anion-exchange (Reillex HPQ), and extraction chromatography (bis-(2-ethylhexyl)phosphoric acid (HDEHP) impregnated Amberlite XAD-7 beads (Ln-Spec)) was required due to bulk metal and organic compound impurities. By processing waste ^{233}U, ORNL was able to recover 2.4 GBq (65 mCi) of ^{229}Th of 0.4% specific activity. Limited quantities of ^{225}Ac is available from the European Institute for Transuranium Elements (ITU, Karlsruhe, Germany)[44] where Geerlings et al. reported use of a titanium phosphate column with adsorbed ^{229}Th to obtain ^{225}Ac.[45] ^{225}Ac may also be available from Pacific Northwest National Laboratory (Richland, WA). The ITU also pursues an alternative production route by irradiating ^{226}Ra targets with a proton beam, making ^{225}Ac in a (p,2n) reaction. Yet another production method of ^{225}Ac from ^{229}Th was proposed by Tsoupko-Sitnikov et al.,[46] wherein the different complex strengths of Ac, Ra, and Th with citric acid were exploited. ^{229}Th and its decay products were loaded onto a cation-exchanger column (Aminex-A5) from a solution of 0.25 M ammonium citrate (pH < 1), and ^{229}Th and ^{225}Ac were selectively eluted at pH = 1.8−2.5 and at pH > 3.5, respectively. The ^{225}Ra could be eluted with 4 M nitric acid. With 2.2 MBq (60 μCi) of radioactivity, the ^{225}Ac separation yield was reported to be 100% with no breakthrough of parent radionuclides.

Several ^{213}Bi/^{225}Ac generators have been described in recent years. Geerlings et al. reported use of two organic cation-exchanger columns (Dowex 50W-X8), the first for separating ^{225}Ac from $^{224/225}$Ra with the second acting as the ^{225}Ac/^{213}Bi generator and eluting with 2 M HCl to obtain the ^{213}Bi.[45] The source strength of these generators was ca. 37 MBq (1 mCi), and neither the purity of the ^{225}Ac, the yield of ^{213}Bi, nor the breakthrough level, was reported. Pippin et al. evaluated this generator using lower acid molarity for improved compatibility with antibody labeling, which resulted in decreased ^{213}Bi yield.[47] El Samad et al. also reported use of a similar ^{213}Bi/^{225}Ac generator,[48] but with the ^{225}Ac initially extracted from ^{233}U by two strong anion-exchange columns (Dowex 1X8). Since their objective was a study of ^{209}Tl, only low-level generators were described. Boll et al. chose an organic strong cation-exchange resin (AG 50W-X4) with a lower degree of cross-linking for the ^{213}Bi/^{225}Ac generator. The purified ^{225}Ac in a 1 M HNO_3 solution was loaded onto a 2 × 15 mm column containing ~20 mg of resin, utilizing 0.15 M HI as eluant for the ^{213}Bi.[43] At a source strength of 31 MBq (0.83 mCi), the column performance was stable for 18 days, a reasonable lifetime for a generator, and the yields of ^{213}Bi were 91−98% with breakthrough levels of 10^{-5}−10^{-4} Bq of ^{225}Ac per Bq of ^{213}Bi and of 10^{-3} Bq of ^{224}Ra per Bq of ^{213}Bi. However, higher ^{225}Ac loads produced radiolytic damage to the resin limiting the usefulness of this generator construct. In

these generator systems, ^{225}Ac was adsorbed by simply passing the ^{225}Ac solution through the column. The radioactivity was then effectively concentrated on a small portion of the upper layer of the resin in the column resulting in a large radiation dose to this layer and radiolytic damage at this location. Several researchers have described ways of making functional generators with higher ^{225}Ac loads. Wu et al. described a generator employing a potentially greater radiation resistant inorganic cation ion-exchange resin (Ac-Resin).[49] Furthermore, the column volume was increased to 1 mL and the ^{225}Ac was distributed more homogeneously throughout the upper two-thirds of the resin. The lower third of the resin was to eliminate ^{225}Ac breakthrough. In a continuous operation cycle of ca. 6 min, the ^{213}Bi was eluted from the generator with 6 mL of 1.0 M HCl, the eluate diluted to 0.2 M HCl, and the ^{213}Bi loaded onto a smaller organic cation ion-exchange (AG MP-50) column. Finally, ^{213}Bi was eluted with 0.5 mL of 0.1 M HI, ready for antibody labeling. The overall yield was greater than 85% and without ^{225}Ac observed in the eluate from the second column. This generator has been used with up to 1.0 GBq (27 mCi) of ^{225}Ac, without radiolytic damage with over 2 weeks of use. McDevitt et al. have described a similar approach for distributing the ^{225}Ac more homogeneously over the resin.[50] The ^{225}Ac was mixed with 200 mg of AG MP-50 resin in 3 mL of 1.5 M HCl for 30 min and then loaded onto a 2 × 55 mm column. To remove breakthrough of ^{225}Ac, a second column with 100 mg of AG MP-50 was applied in addition to a 10 mg plug of unloaded resin in the generator. ^{213}Bi was eluted quantitatively with 3 mL of 0.1 M HCl/NaI, with breakthrough of 10^{-11} and 10^{-13} Bq of ^{225}Ac per Bq of ^{213}Bi without or with the second catch column, respectively. Approximately 1.0 GBq (27 mCi) generators were prepared for a Phase I clinical trial.[51] Another proposed method for making stable, high activity ^{213}Bi/^{225}Ac generators was disclosed by Bray and co-workers.[52] A 4 mL 0.25−1.0 M HCl solution of ^{225}Ac in radioactive equilibrium with its decay products was drawn through a disk containing a thin film strong anion-exchanger (Anex, 3M). ^{213}Bi was adsorbed onto the anion-exchanger as chloride complexes and a wash solution of 4 mL of 5 mM HCl that removed traces of ^{225}Ac and reduced the HCl concentration. Finally, the ^{213}Bi was eluted from the disk with a stripping solution, preferably 0.05 M NaOAc (pH 5.5). The results reported a 92% yield of ^{213}Bi in 4 mL of stripping solution after a total elapsed time of 6 min, without any other radionuclides being present. The destructive radiation dose to a resin may be eliminated by separating the ^{213}Bi from a ^{225}Ac feed solution, rather than by elution of a ^{225}Ac cow.

V. Chelation Chemistry

For in vivo stable attachment of radio-bismuth to carrier molecules, a bifunctional chelating agent is needed for forming a radiometal complex that must be exceedingly stable in vivo. Covalent chelator linkages to mAb's have been accomplished through reaction with the ϵ-amines of lysine residues,[53−55] a chemically introduced thiol,[56] or oxidative introduction of aldehyde groups to introduce chelating agents by reductive amination.[57]

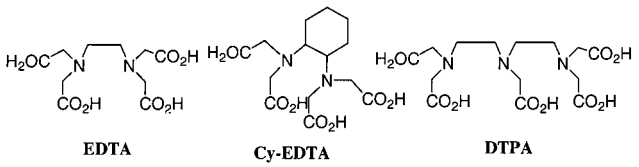


Figure 3. Acyclic polyaminocarboxylate chelating agents.

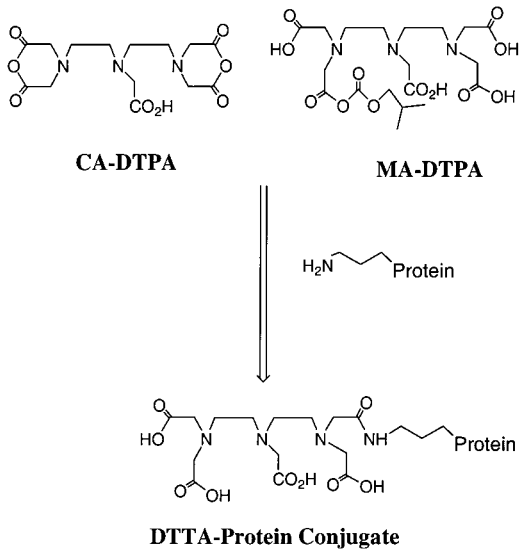


Figure 4. Reaction product of either the cyclic dianhydride of DTPA or the mixed anhydride of DTPA with protein.

The development of suitable bifunctional chelating agents for conjugating Bi(III) radionuclides to proteins began by abstraction of existing chelating reagents from the chemistry literature. These reagents were then modified to produce an active species derivative for protein conjugation. Acyclic DTPA (Figure 3) was recognized very early to be an effective chelating agent with high thermodynamic stability constants for a variety of metal ions.[58] The Bi(III) complex of this ligand was thought to be possibly adequate to meet the requirements of forming kinetically inert complexes in vivo while retaining reasonable complex formation rates, a critical consideration for the short half-life Bi isotopes. Thus, one finds the stability constant for the DTPA complexes of Bi(III) to be $10^{35.6}$, providing the impetus for the evaluation of two of the first derivatives for linking DTPA to proteins.[58] DTPA dianhydride[54] and DTPA carbonic anhydride[53] both utilized one of the carboxylate arms to link the ligand to protein via an amide group (Figure 4), therefore decreasing the denticity to heptadentate or to converting one of the donor groups to an amide carbonyl oxygen.

This compromise in denticity had been initially overlooked as contributing to complex instability because of the extreme ease of use associated with these reagents. An early demonstration of the potential of targeting ^{212}Bi to specific cells was accomplished by using the isobutylcarbonic anhydride of DTPA reacted with mAb anti-Tac.[59] While this chelate conjugate was highly successful in vitro, and later when employed in the treatment of malignant cells in a compartmentalized animal model,[60] the lack

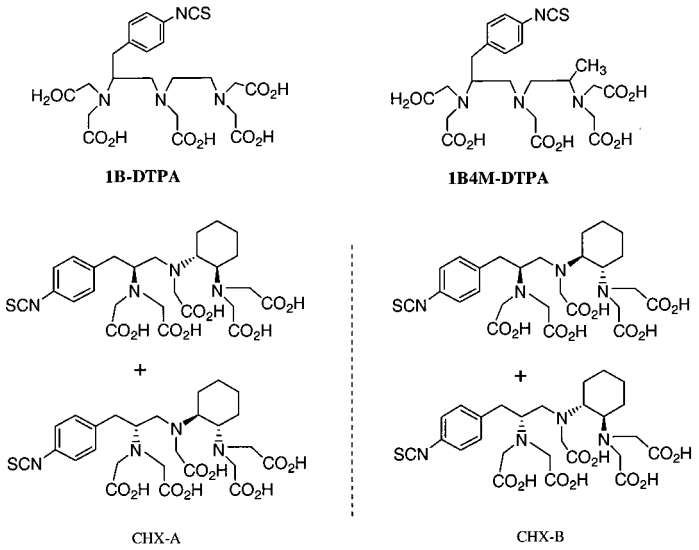


Figure 5. C-Functionalized bifunctional DTPA derivatives evaluated for forming stable radio-Bismuth complexes in504 vivo.

of acceptable stability was also readily apparent with this chelating agent.

This instability was initially attributed to inadequate denticity providing an unstable complex in vivo,[61] and hence evaluation of bifunctional octadentate DTPA derivatives was initiated. A C-functionalized DTPA (1B-DTPA, Figure 5) with an aryl isothiocyanate group on the carbon backbone structure for protein linkage substantially increased the in vivo stability of Bi(III) complexes.[62] Additional C-functionalization of the DTPA ligand with a methyl group (1B4M-DTPA, Figure 5) was shown to further increase in vivo stability with Bi(III), a result that was clearly indicated from the chemical literature from a combination of both inductive and steric effects.[63]

However, despite the initial very high stability constant of the DTPA[Bi(III)] complex, none of these bifunctional DTPA derivatives demonstrated acceptable in vivo stability when conjugated to mAb and radiolabeled with $^{205/206}$Bi. Further review of the chemical literature revealed that the *trans*-cyclohexyl-EDTA (Cy-EDTA) (Figure 3) Bi(III) complex possessed a stability constant close to that of the DTPA complex.[58] Merging the *trans*-cyclohexyl substructure with the previously reported bifunctional DTPA provided the family of CHX-DTPA ligands (Figure 5).[64,65] These ligands were substantial improvements over the prior bifunctional DTPA reagents because the preorganization geometry conferred by the *trans*-cyclohexyl unit[66] provided not only exceptional stability for the Bi(III) isotopes, but also retained the rapid complex formation kinetics. The stepwise development of a suitably stable Bi(III) chelate is depicted in Figure 6. The effects of adding an alkyl group or inclusion of the *trans*-cyclohexyl ring to a bifunctional DTPA are clearly indicated by the decreased accretion of $^{205/206}$Bi in the kidneys and statistical congruency with the biodistribution of radio-iodinated monoclonal antibody. These ligands exist in several stereochemical configurations, and while very significant differences have been reported for the in vivo stability with ^{88}Y radiolabeled immunoconjugates with these ligands,[67] those differences are irrelevant to use with ^{212,213}Bi(III) due to half-life considerations.

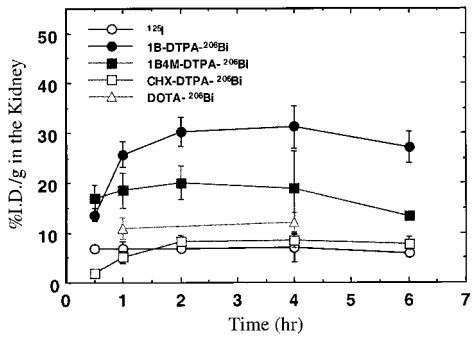


Figure 6. Evaluation of stability C-functionalized bifunctional DTPA derivatives for forming stable radio-Bismuth complexes in vivo conjugated to mAb B72.3.

The solid-state structure of the cyclohexyl DTPA provided some insight into the increased in vivo stability of the corresponding Bi(III) complex.[68] The coordination number was eight and in general, the Bi−ligand bond distances were shorter than those observed for the corresponding DTPA complex. Also, several of the DTPA complexes exist with the last site of the complex being filled with an oxygen of a neighboring ligand, thus leading to polymeric systems that could be a source of instability in vivo.[68] Clearly, the contribution of the *trans*-cyclohexyl ring provides significant preorganization of the geometry of the ligand that favors the formation of a stable Bi-(III) complex.[66]

Concurrent with development of an acyclic bifunctional chelating agent for Bi(III) radioisotopes, the inherent potential of macrocyclic polyaminocarboxylates ligands such as NOTA, DOTA, and TETA (Figure 7) were readily apparent. Their macrocyclic nature confers a high degree of preorganization and limits conformational disorder while providing a range of both denticity and cavity size. Thus, the use of numerous bifunctional C- and N-functionalized macrocylic ligands for biological applications have been reported.[69−73]

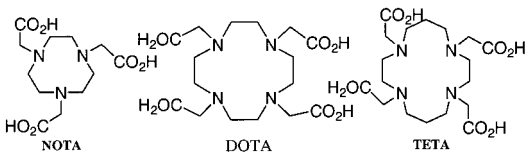


Figure 7. Macrocyclic polyaminocarboxylate chelating agents.

Studies to evaluate the potential usefulness of a C-functionalized DOTA led to conflicting results. Fundamental studies of the stability of the complex indicated that the DOTA[Bi(III)] complex was exceedingly stable and that a suitably stable complex formed.[74] Indeed, the complex thus formed with a C-functionalized DOTA was found to be acceptably stable in vivo; however, this high kinetic stability was also a hindrance to the use of DOTA, due to significantly slow complex formation rates.[61] Functionalized DOTA ligands have also been reported to be highly sensitive to the presence of M(II) ion contamination of the radionuclide, principally Ca(II).[75] Thus, DOTA, despite forming a kinetically inert complex with Bi(III), was not found suitable due to slow complex formation rates.[61] However, DOTA was also shown to be an adequately stable in vivo chelator for Pb(II),[76] which allows for conjugating and delivery of ^{212}Pb, the precursor of ^{212}Bi (Figure 1).

Thus, while research continues on developing stable chelating agents for targeted radionuclide therapy, the CHX-DTPA ligands fulfill the requirements for rapid complex formation with $^{212/213}$Bi and for in vivo stability when conjugated to protein. This has been well documented from numerous preclinical results[63,77−80] and from subsequent clinical results with ^{213}Bi (vide infra).

VI. Dosimetry

The absorbed dose (D), defined as average absorbed energy per unit mass, is an important parameter in conventional radiotherapy. Both tumor response and normal tissue damage are reported as a function of dose. In targeted radionuclide therapy, dosimetry is complicated by several factors: (i) heterogeneous radionuclide distribution, (ii) short-range particulate radiation, and (iii) few radiative incidents per cell.[81] The first factor results from several biological and chemical variables, i.e., heterogeneous radionuclide conjugation, heterogeneous antigen (target molecule) expression, antibody avidity, poor tumor vascularization, and high interstitial pressure in the tumor.[82,83] Very often the targeting agent is not able to penetrate multiple layers of tumor cells.[84,85] Along with the radionuclide distribution, physical characteristics and the amount of the radionuclide determine the latter two factors.

Small numbers of α-particle traversals of cell nuclei result in a broad distribution, *f*(z), of specific energies (individual cell "doses"), with some cell nuclei receiving very few and even zero α-particle traversals.[86,87] To achieve curative cancer treatment, low survival probability ($<10^{-8}$) for all cancer cells should be obtained. Dosimetry for α-particle radionuclide therapy should therefore ensure not only a sufficient average dose, but also a low probability of cells with zero traversals.

The Medical Internal Radiation Dose (MIRD) committee produced principles for dose calculations in radionuclide therapy.[88] The mean absorbed dose from given radionuclides from a source to a target region was calculated, assuming homogeneous radionuclide distribution within a region. The concept of region extends to the subcellular level.[89] Some models for inhomogeneous radionuclide distribution in tumors have also been proposed.[90] The MIRD model and a Monte Carlo transport code for electrons and photons have been used in in vivo experiments for dose determination after α-particle irradiation.[91−93] In current clinical trials with α-particle emitters, use of the MIRD model with a RBE value of 5 has been reported.[94] None of the above models take into account the width of the specific energy distribution and hence cannot provide the probability of zero hit cells for a specific treatment plan.

In microdosimetry, the stochastic variations of energy deposited within small targets (e.g., cell nuclei) are considered.[95,96] Estimates of the average dose ($D = \bar{z}$) and the fraction of cells receiving zero (or any number of) α-particle traversals may be obtained from microdosimetric calculation of the specific energy distribution, *f*(z). Since such microdosimetric computations for actual clinical situations may be difficult to perform due to unknown microdistribution of the radionuclide, Roeske and Stinchcomb have proposed a dosimetric framework where microdosimetric moments are implemented in the MIRD formalism.[97]

To improve current dosimetry models, more accurate determination of the radionuclide microdistribution should be provided. Autoradiography and histological samples of tumor models and normal tissue might improve on this.[98−101] Along with improvement of dosimetry for the clinical situation, cell survival probabilities after given numbers of α-particle traversals require a more accurate determination. The average cell survival probabilities derived from macroscopic dosimetry experiments may not reflect the true cell survival probabilities from α-particle traversals.[102,103] Furthermore, evidence of bystander effects and delayed cell death demonstrate that cells hit by no α-particles may express decreased survival.[104−106] In experiments using no microdosimetric determination and measuring the average survival of cells instead of individual cell survival, such bystander effects will be disguised. Likewise, the RBE values reported from in vitro and in vivo experiments should be interpreted with caution. More accurate cell survival probabilities may be obtained through in vitro experiments with absolute determination of the number of α-particle traversals of subcellular compartments (cytoplasm, nucleus, etc.).[15] With this novel technique, differences in cell shape may also be accounted for making possible the calculation of traversed path length, energy, and specific energy deposited.

When the radionuclides in question are not pure α-particle emitters, such as ^{212}Bi and ^{213}Bi (and their radioactive daughters) the high energy β-particles

and γ-rays (Figures 1 and 2) also need to be taken into account, even if they contribute only a small fraction of the total patient dose. The γ-ray components are mainly of concern for radiation protection of personnel in a clinical setting, especially so for ^{212}Bi. For ^{212}Bi, the radioactive daughters will probably not relocate to any significant extent from the tumor site due to their short half-lives. ^{213}Bi, however, has a β-particle emitting daughter, ^{209}Pb, with a 3.25 h half-life. This is probably long enough for ^{209}Pb to relocate from the tumor and localize in other organs, blood cells, and in the bone structure. For calculations of such dose contributions, the MIRD formalism may be used.

VII. In Vitro and In Vivo Experiments with ^{212}Bi/^{213}Bi Conjugated to Immunoproteins

The most widely used targeting carrier vehicle has been monoclonal antibodies (mAb's) of the IgG type. The IgG mAb's are proteins of ~150 kDa, which recognize specific cell surface macromolecules (antigens) and bind to them with affinities ranging from $10^{-7}-10^{-11}$ M^{-1}. Numerous mAb's directed against antigens that are highly expressed on cancer cells, and weakly expressed or even absent on normal cells, have been produced and evaluated. Since mAb's were often originally generated in mice, patients frequently developed immune responses toward them. To obviate this situation, mAb's are now engineered into chimeric or humanized intact versions.[107] To improve therapeutic efficacy in radioimmunotherapy, mAb's have been enzymatically cleaved into smaller immunoreactive fragments. Thus, Fab′ (50 kDa) and $(Fab)'_2$ (100 kDa), and more recently, sFv's (25 kDa), diabodies (50 kDa), minibodies (80 kDa), and other variants have been developed through bioengineering.[83,108−113]

^{212}Bi and ^{213}Bi have been evaluated in in vitro and in vivo models with radiolabeled mAb conjugates to form highly specific and cytotoxic reagents. Kozak et al. reported approximately 20 times higher cytotoxicity of a human T-cell leukemia line targeted with ^{212}Bi labeled mAb as compared to an irrelevant ^{212}Bi labeled mAb, as assayed by protein synthesis inhibition and clonogenic death.[59] A RBE of 10 for nontargeted ^{212}Bi irradiation of cells as compared to ^{137}Cs γ-rays when assaying protein synthesis was also reported. In a similar study, Kurtzman et al. reported results of clonogenic assays on human pancreatic carcinoma cells from tumors.[114] Targeted and nontargeted ^{212}Bi were 20 and 5 times more efficient than 15 MeV X-rays, respectively.

The ability of the murine T-lymphoma cell line EL-4 to incorporate ^{3}H-thymidine was reduced by a factor of 40 when comparing targeted to nontargeted ^{212}Bi.[60] Mice injected i.p. with EL-4 cells and administered 5.5−8.5 MBq (150−230 μCi) i.p. of a ^{212}Bi antibody construct 1 day later, showed 70 days of tumor free survival in 80% of the animals versus the controls all dying within 33 days after treatment with an irrelevant ^{212}Bi-mAb.

A selective cytotoxic effect on B-cells in mice has also been demonstrated with a B-cell specific ^{212}Bi-mAb. Injections of 5.9 kBq (0.16 μCi) and 68 kBq (1.8 μCi) of ^{212}Bi-mAb reduced ^{3}H-thymidine incorporation in B-cells and T-cells in regional lymph nodes to 50%, respectively.[115] The possibility of preventing allograft rejection with ^{212}Bi coupled to anti-Tac mAb has also been suggested as potentially useful. The in vitro results indicated that the radiolabeled immunoconjugate was able to selectively eliminate alloresponsive cells generated during an allogenic mixed lymphocyte reaction.[116] In all these studies, nontargeted ^{212}Bi-mAb exhibited some therapeutic effect. These experiments clearly indicated the potential for efficient and highly specific therapy with α-particle emitters, although some caution must be exercised in interpretation of these results due to instability of the ^{212}Bi antibody conjugates. In an i.p. human colon cancer xenograft model in mice, targeted therapy with i.p. injections of ^{212}Bi-B72.3 (anti-TAG-72) mAb were investigated.[117] In dissected animals, 12 days posttreatment, a 56% reduction of tumor mass as compared to control tumors of ~3 g after receiving 16.7 MBq (450 μCi) of the immunoconjugate was observed. With smaller tumors (control 0.8 g), 3 of 4 mice had no observable tumor after receiving 4 × 6.7 MBq (4 × 180 μCi) on consecutive days. However, only 10% of the treated animals survived beyond 95 days. The targeted therapy approach was not optimal in this study due to secretion of the antigen and instability of the ^{212}Bi conjugate that used the GYK-DTPA[57] for sequestering the bismuth isotope.

Hartmann et al. studied an in vivo stable ^{212}Bi radiolabeled mAb anti-Tac that used the CHX-A DTPA (Figure 4) to evaluate therapy of murine myeloma tumors transfected with the human Tac antigen.[78] Animals receiving i.p. 5.5−7.4 MBq (150−200 μCi) of ^{212}Bi-anti-Tac mAb i.p. 3 days postinjection of tumor cells showed 75% tumor free survival (180 days). All animals receiving a nonspecific ^{212}Bi-Mab developed tumors within 35 days. In a similar experiment, but with subcutaneous (s.c.) tumors and intravenous (i.v.) injection of the radiopharmaceutical, efficacy was reduced to 30% tumor free survival (120 days) and treatment of larger s.c. tumors (936 mm^3) resulted in only modest tumor growth retardation. Biodistribution data indicated that targeting to these larger tumors was too slow in relationship to the half-life of ^{212}Bi. The animals receiving 7.4 MBq (200 μCi) ^{212}Bi-Mab showed reversible bone marrow suppression, less severe for i.p. versus i.v. injection, resulting from the large percentage and long circulation time of the mAb in the blood.

Few comparisons of therapeutic efficacy of the α-particle emitters ^{212}Bi and ^{213}Bi with a β-particle emitter in the same tumor model have been performed. In a Rauscher murine erythroleukemia, a stable ^{212}Bi-103A-mAb conjugate (CHX-A-DTPA) was used.[77] Localization of 40% ID/g to tumor-bearing spleens at 1 h postinjection was reported with an uptake ratio between leukemic cells and normal spleen cells of 14. When injected day 8 of the disease with 5.5 MBq (150 μCi), no histological evidence of erythroleukemia was found and the spleens had normal weight. However, animals given the same dose at day 13 of the disease were seen to still possess leukemic foci. With this 50-fold higher tumor burden,

complete remission was observed with 1.0 MBq (27 μCi) of ^{90}Y-103A-mAb; however, bone marrow toxicity was also noted.[118] Comparatively, no toxicity was observed in animals injected with doses as high as 7.4 MBq (200 μCi) of ^{212}Bi-103A-mAb. Interpretation of these results is complicated by the large differences in half-life ($t_{1/2}$ = 64 h for ^{90}Y), catabolism of antibody with subsequent release of radiometal, and organ cross-fire dose due to the longer range of the β-particle. Nevertheless, it is worth noting the lack of bone marrow toxicity coupled with the therapeutic efficacy of the targeted ^{212}Bi, even with a poor spleen-to-blood uptake ratio of approximately 1. With ^{90}Y, favored by the longer half-life, the spleen-to-nontarget organ ratios were at least 20.

The half-life of ^{212}Bi may be effectively extended by conjugating its longer-lived parent ^{212}Pb ($t_{1/2}$ = 10.6 h.) to the carrier molecule and thereby generating the α-particle emitter in vivo. Uptake ratios between tumor and organs may then be increased, and treatment of larger tumors might be possible. However, during the β-decay process of ^{212}Pb, 36% of the ^{212}Bi formed was lost from the DOTA complex which then might contribute to toxicity.[119] To test this hypothesis, ^{212}Pb was conjugated to the 103A-mAb and evaluated in the erythroleukemia model.[120] Animals at both day 8 and 13 of the disease showed no evidence of splenic tumor foci after injection with 0.74 MBq (20 μCi) of ^{212}Pb-103A-MAb, but all died of bone marrow toxicity. Histology showed acellular bone marrows devoid of erythroid and myeloid cells. No other organ showed any sign of radiotoxicity, despite high kidney levels of ^{212}Bi (up to 50% ID/g) due to the ^{212}Bi lost from the ^{212}Pb-mAb conjugates. Control animals receiving the same doses of ^{212}Pb-103A-mAb and leukemic animals receiving an irrelevant ^{212}Pb-mAb also suffered from marrow toxicity, although most survived. Interestingly, their blood levels of ^{212}Pb and ^{212}Bi (30−20% ID/g) were approximately twice that of leukemic animals receiving ^{212}Pb-103A-mAb while the bone uptake in ^{212}Pb-103A-mAb treated leukemic mice (3.2% ID/g) was twice the observed level in the control animals. Unchelated ^{212}Pb released from leukemic cells after mAb internalization and catabolism likely explains the increased bone uptake. The high bone marrow toxicity observed in this study contrasts with the lack of marrow toxicity in the animals treated with ^{212}Bi-103A-mAb. Thus, the results show that ^{212}Bi lost from ^{212}Pb-mAb increased marrow toxicity as compared to ^{212}Bi-103A-mAb. Clearly, the deposition of ^{212}Pb to the bone is highly toxic due to the increased solid angle for α-particle traversal of stem cells from the in vivo generated ^{212}Bi. In an attempt to counter this scenario, 2,3-dimercapto-1-propane-sulfonic acid (DMPS) was administered to intercept "free" ^{212}Pb prior to deposition at the bone, but with minimal success. This compound had previously been shown to efficiently reduce ^{206}Bi levels in mice.[121]

Horak et al. investigated the potential of ^{212}Pb-AE1-mAb targeting the HER2/neu oncoprotein on ovarian tumors in nude mice.[122] Transient bone marrow toxicity and long time renal toxicity was observed after i.v. injection of 0.93 MBq (25 μCi) and increasing to 1.5 MBq (40 μCi) resulted in acellular bone marrow and subsequent death of all animals. Three days post-inoculation s.c. with tumor cells, treatment 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. Therapy of larger tumors (15 and 146 mm^3) was less efficient with no complete remissions. Also shown in this study was that the AE1-mAb had a long blood residence time and slow tumor targeting for larger tumors. This resulted in poor tumor/blood ratios for the ^{212}Pb-AE1-mAb, which reasonably explains the poor therapeutic efficacy of larger tumors. Despite this, the potential for treatment of larger tumors with ^{212}Pb has been shown, and also noteworthy, the need for more rapid targeting vectors was illustrated. The importance of keeping cell incorporated ^{212}Pb entrapped, and reducing the loss of radiogenically formed ^{212}Bi, has also been shown. The acidic lysosomal environment inside cells was anticipated to potentially be a major source of instability of the ^{212}Pb-DOTA complex after ^{212}Pb-mAb internalization. Recent efforts to address the acid lability have resulted in the synthesis and in vitro evaluation of a C-functionalized DOTA tetraamide, that forms stable complexes with Pb(II), with increased resistance to complex dissociation at lower pH conditions, as compared to DOTA.[123]

Kennel et al. presented a therapeutic approach of targeting blood vessels in lung tumors.[91] The mAb's 201B and 34A that target thrombomodulin in normal lung endothelia radiolabeled with ^{213}Bi were evaluated in a murine model with lung tumors of EMT-6 mammary carcinoma and IC-12 tracheal carcinoma (>200/lung). The mAb conjugates target the lung rapidly (minutes) and in high yield (38% of ID), and 100% cure was achieved in animals with tumor cross-sections of 5−10 cell diameters (50−400 cells/tumor) receiving 5.6−7.4 MBq (150−200 μCi) of ^{213}Bi-201B-mAb. The cure rate dropped markedly with larger tumors, and the tumors killed all untreated animals within 20 days. All cured animals were sacrificed after 73−75 days due to respiratory distress. Histological examination revealed significant lung damage, including fibrosis and edema. Attempts to prevent pulmonary fibrosis by inhibiting the activity of tumor necrosis factor α (TNF-α) was unsuccessful with the nontumor bearing mice receiving ^{213}Bi-201B-mAb having their life-span being reduced.[124] A comparison between ^{90}Y-201B-mAb and ^{213}Bi-201B-mAb resulted in similar therapeutic efficacy and toxicity with therapy of larger tumors being marginally more effective with ^{90}Y than with ^{213}Bi.[125] Reduction of the severe lung toxicity should be possible if specific targeting of tumor blood vessels could be achieved. Further improvement in therapeutic efficacy with ^{213}Bi is likely if specific cancer cell targeting could be added to the protocol.

Behr et al. reported the results from targeting a human colon cancer (GW-39) xenograft in nude mice with ^{213}Bi and ^{90}Y conjugated to the CO17-1A-Fab′.[92] At equitoxic dosing (doses causing equal toxicity), ^{213}Bi was significantly more therapeutically effective than ^{90}Y, both in s.c. tumors and in liver micro-

tumors. Doses to tumor and organs were calculated according to the MIRD formalism aided by Monte Carlo simulations of particle histories.[126] Tumor volume multiplication times versus dose of s.c. tumors of 100−200 mg (approximately 6 mm) was measured. A linear relationship from 1 to 2 Gy and upward was found for ^{213}Bi. For ^{90}Y, a less steep curve was reported, with minimal tumor growth retardation for doses below 5 Gy, resulting in RBE's of 2−3. Therapy of mice with microscopic tumor colonies of 250−500 μm in the liver (>250/liver) was performed at the maximum tolerated dose (MTD), corresponding to 26 MBq (700 μCi) ^{213}Bi and 9.3 MBq (250 μCi) ^{90}Y. This resulted in 95% cure for the animals receiving ^{213}Bi as compared to 20% for the animals receiving ^{90}Y with the control animals dying within 6−9 weeks. Targeting was rapid and the Fab′ conjugates were internalized, favorable for efficient α-particle therapy with ^{213}Bi. Interestingly, myelotoxicity occurred at 5−8 Gy and kidney toxicity at 50−70 Gy, equally for both radionuclides.

Recently, specific targeting with ^{213}Bi-J591-mAb of bone metastases from prostate cancer has been suggested.[127] Similar to HuM195, this mAb is rapidly internalized into the targeted cells without release of the radiometal. Nude mice with a LNCaP xenograft treated after 2 days with 4.8 MBq (0.13 mCi) ^{213}Bi-J159-mAb showed 46 days of tumor free survival as compared to 30 days for control animals.

Recently, the engineered mAb Hu-CC49ΔCH2 has been evaluated for efficacy when labeled with ^{213}Bi and used to treat mice with LS174T tumors in the flank (83.8 ± 31.5 cm^3). 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 with ∼50% of the animals being cured. Lower doses of 18−28 mBq (500−750 μCi) also provided positive responses with ∼33% being cured, 33% responding with delayed tumor growth, but then with ∼33% not responding to the therapy at all. While a maximum tolerated dose was not found in this study, the results at targeting a solid tumor with such a short half-life radionuclide are very encouraging and point out that the limitation in the use of the bismuth isotope really lies in the delivery vector.[128]

Radionuclide therapy of multiple myeloma with ^{213}Bi-mAb has recently been suggested.[129] Specific versus nonspecific ^{213}Bi-mAb targeting in vitro of a human multiple myeloma cell line resulted in large cell survival differences, at most 10^{-10} versus 0.1, respectively, as assayed by ^{3}H-thymidine incorporation.

To assess the potential for eradication of micrometastases, an in vitro model of cells growing in spheroids has been utilized. Low cell kill efficiency was noted when 1 mm spheroids were targeted with a ^{212}Bi-mAb.[130] However, for spheroids of diameter 0.1−0.4 mm, cell killing efficiency of 90−99% with specific ^{213}Bi-mAb has been reported.[91,125] In another study, the volume of 0.12 mm spheroids targeted with a ^{213}Bi-mAb, decreased over a 2-month period. Microscopic analysis showed that the outer 2−3 cell layer portion had deteriorated, with the inner core of cells remaining static.[127] The antibody conjugates in these studies targeted the outer surface of the spheroids with negligible radial penetration. Thus, as the spheroid size increases, the fraction of the cells spared from α-particle irradiation also increased, with diminished therapeutic efficacy.

VIII. Other Applications of Targeted ^{212}Bi

To the best of the authors' knowledge, only two other methods for in vivo carriers of ^{212}Bi, besides antibodies have been reported. Rosenow et al. described the properties of liposomes containing ^{212}Pb and reported them to be at least partially intact in vivo.[131] The rationale for this study was the possibility of maintaining cytotoxic activity in the circulation and in various organs for perfusion therapy of neoplasms or immune suppression.

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.[132] 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. Maximum femur uptake of 26% ID/g of ^{212}Bi-DOTMP was reached within 15 min postinjection. At this time point, the radioactivity in the blood was 2% ID/g, and rapidly decreasing, similarly so from other organs. 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.[119] No further studies have been undertaken to clarify the therapeutic potential and the limits for bone marrow and renal toxicity.

IX. Nontargeted Radiotherapy with ^{212}Bi

Several papers have advocated possible use of ^{212}Bi without a targeting vehicle, i.e., in a colloidal form or as the oxychloride, as a therapeutic means for microscopic carcinoma of the ovary.[133−135] When ^{212}Bi was compared with ^{32}P, 250 keV X-ray, and *cis*-platinum, RBE's of 2−8 were reported for survival of human ovarian cancer cells, either in single cell suspensions or with cells grown as 0.1- and 0.8-mm spheroids. Autoradiography demonstrated that radioactivity was distributed throughout the cross-section of the spheroid. Toxicity and therapeutic studies with i.p. administration of ^{212}Pb and ^{212}Bi in mice with i.p. Ehrlich carcinoma were also reported. ^{212}Pb administered as ferrous hydroxide colloids cured 24% of the mice, but this approach was abandoned due to inhomogeneous distribution.[136] Administration of 3.3 MBq (90 μCi) of ^{212}Bi 2 days post-inoculation resulted in a 40% survival for 3 months, while controls died within 21 days without any radioactivity induced toxicity being reported.[135] Biodistribution and toxicity studies in rabbits (4.5 kg) showed that 70−90% of the injected ^{212}Bi remained in the peritoneal fluid. After administration of 2.2 GBq (60 mCi) ^{212}Bi transient thrombocytopenia and leukopenia was seen, and necroscopy revealed mild blunting of intestinal villi and doses above 3.0 GBq

(80 mCi) were fatal. The advantage of this nontargeted approach is the ability of the unconjugated ^{212}Bi to diffuse into spheroids. However, the lack of specific targeting seems likely to be an inefficient way of delivering an α-particle dose to tumor cells in the peritoneum, possibly resulting in a low tumor to healthy tissue dose ratio and a larger fraction of unhit cells.

X. ^{225}Ac, Potential Suitable Chelation Chemistry and Applications

The parent radionuclide of ^{213}Bi, ^{225}Ac ($t_{1/2}$ = 10 days), has been proposed for use as a therapeutic.[45,137] The decay process of ^{225}Ac includes four α-emissions and two β-particle emissions to a stable ^{209}Bi daughter (Figure 2) resulting in a total release of energy per ^{225}Ac decay of ~28 MeV. This large energy deposition would greatly enhance the statistical probability of cell kill per atom thus requiring much lower amounts of radioactivity to be administered. Additionally, the much greater half-life of ^{225}Ac versus either ^{213}Bi or ^{212}Bi would permit more convenient logistics in handling clinically both in production of the patient dose and its physical delivery. Complimentarily, targeting and delivery to disease in vivo with the increased half-life of ^{225}Ac might also permit α-particle treatment of cancers where rapid targeting was not easily achieved. Complete decay of 37 kBq of ^{225}Ac (1.0 μCi), including daughters, has been reported theoretically to provide a dose of 6 Gy (600 rad) of energy to 4 g of tumor, which would then lead to 99.99% cell kill.[45] Also, a therapeutic dose of 5 mg of radiolabeled mAb at a specific activity of 370 MBq/mg with a targeting efficiency of 0.01%/g while making an assumption of 5×10^4 available antigens on the surface of malignant cells has been proposed based upon results obtained from spheroid cytotoxicity studies.[137] In both of these cases, numerous assumptions were made, and it remains unknown whether such amounts of ^{225}Ac can actually be delivered and just how much of the energy of the daughters contributes to a tumoricidal dose.

The promise of this α-particle therapy alternative is encumbered with major obstacles. First, conjugating ^{225}Ac to protein using a bifunctional chelating agent is not easily accomplished. Second, the recoil energy after the α-particle (~0.1 MeV) is much larger than the chemical binding energy of an ^{225}Ac conjugate. Finally, the first formed daughter, e.g., ^{221}Fr, a 1+ ion, would require significantly different chelation chemistry as opposed to ^{225}Ac. Thus, maintaining an intact conjugate of the radioactive daughters after the first α-particle emission seems a dubious prospect. Release of the α-particle emitting decay products with subsequently trafficking in vivo, leading to unacceptable toxicity, results. However, the first two daughters, ^{221}Fr ($t_{1/2}$ = 4.8 min) and ^{217}At ($t_{1/2}$ = 0.032 s), may be adequately short-lived to ensure that they do not significantly travel from the tumor site. Furthermore, proponents of the use of ^{225}Ac for RIT have put forth that the daughters may be formed as highly energetic, short-lived species that will bind to endogenous structures in vivo and thus minimize the departure of the ^{221}Fr and ^{217}At. Some evidence for this possibility has been demonstrated in treating model spheroids, although in vivo evidence has yet to be obtained.[137] The chemical species of the formed ^{217}At may be irrelevant due to the short half-life, ^{213}Bi ($t_{1/2}$ = 46 min) clearly has an adequate half-life to traffic, localize in the kidney, and cause serious renal toxicity.[138] Regardless of these opposing positions, resolution of this issue will require performing the fundamental experimentation.

^{225}Ac is a 3+ metal ion. Thus, the natural choice is to link it to a mAb, or other carrier molecule, via a suitable bifunctional chelating agent meeting the same criteria as related for $^{212/213}$Bi(III), at least for the parent radionuclide. Contrary to the development of bifunctional chelating agents for Bi(III) isotopes for which there was a plethora of coordination chemistry literature available for guidance, there is virtually no comparable information available for Ac(III). The reported stability constant of the ^{225}Ac-EDTA complex is $10^{14.2}$, and the ionic radius of Ac(III) has been estimated to be 1.14 Å.[139,140] Finally, there are no stable isotopes of actinium available from which one can conveniently develop chemistry, although one might presume significant analogous chemistry to lanthanides, keeping in mind the greater ionic radius.

^{225}Ac has been shown to be highly toxic when administered intravenously, with much of the dose being deposited primarily in the liver as well as in the bone.[141] Thus, reductions in toxicity and liver and bone deposition have been used as markers for evaluating the in vivo stability of different ^{225}Ac chelates.[141] Studies with ^{225}Ac-citrate evaluated in vivo uptake and found better blood clearance, greater liver uptake, and lower bone uptake as compared to ^{169}Yb-citrate, but also poor whole body clearance.[142] Ethylenediaminetetramethylenephosphonic acid (EDTMP) was also evaluated as an ^{225}Ac chelator by Beyer et al., and again high liver uptake and poor excretion were noted indicating instability of the complex.[143] Conventional chelates such as EDTA and CHX-A″ DTPA (Figures 1 and 4) have also been evaluated in vivo.[141] These chelates effected a reduction of the liver dose somewhat with CHX-A″ DTPA being most efficient. However, the ^{225}Ac CHX-A″ DTPA complex still had a MTD of ca. 100 kBq (2.7 μCi) in mice. Higher doses of ^{225}Ac-DTPA resulted in 100% mouse mortality within 8 days.[141] Thus, derivatives of acyclic polyaminocarboxylate ligands, such as EDTA or DTPA which do form complexes with ^{225}Ac, have been demonstrated as far too labile in vivo.[139,141]

The use of calix[4]arene ligands have been proposed for sequestration of ^{225}Ac in vivo.[144,145] One report demonstrated selectivity and extraction characteristics of a calix[4]arene derivative for ^{225}Ac but contained no actual stability data.[144] A second report detailed results on conjugation of a bifunctional calix[4]arene to a mAb, immunoreactivity of the product, and studies on potential immunogenicity.[145] However, no data pertaining to actual stability, either in vitro or in vivo for the ^{225}Ac complex was presented, nor were any radiolabeling or complexation data included.

Concurrently, Deal et al. reported on the screening of chelating agents for complexing ^{225}Ac and the potential development of stable chelating agents for ^{225}Ac.[146] The macrocyclic ligand, 1,4,7,10,13,16-hexaazacyclohexadecane-1,4,7,10,13,16-hexaacetic acid (HEHA), which offers 12 coordination sites and a large binding cavity, formed an ^{225}Ac complex that was rapidly eliminated from the blood. The accumulation of ^{225}Ac in the accretion organs, i.e., liver and bone,[138] was negligible and a substantial reduction in observable toxicity was noted versus other screened ^{225}Ac complexes.[141,146] A bifunctional version of this ligand was synthesized and evaluated in vitro for stability and in vivo as a therapeutic.[147,148] The in vitro serum stability evaluation produced mixed results. Observations indicated that this was an unstable complex when conjugated to mAb's, although the results were equally interpretable as resulting from the high LET α-emissions actually destroying the protein during the course of study.[147] The in vivo stability and therapeutic potential of an ^{225}Ac-HEHA-mAb radioimmunoconjugate was investigated by Kennel et al.. employing a murine lung tumor model.[79] The results indicated that the in vivo stability of the complex was greatly improved over prior evaluable radioimmunoconjugates, yet still not completely stable. Initially, there was significant uptake of ^{225}Ac at the tumor with > 300% ID/g found in the lungs at 4 h postinjection. However, this deceased with a $t_{1/2}$ = 49 h while the level of ^{225}Ac increased in the liver.[148] Trafficking of the decay daughters was determined by measuring deviations from the equilibrium ratio of ^{213}Bi:^{225}Ac. The results indicated that the tumor and liver were deficient in ^{213}Bi while the inverse was measured in the kidneys, clearly originating from ^{213}Bi being formed, not being chelated, not being retained within the tumor matrix, and then localizing into the kidneys. The therapy experiments produced equally mixed results. Doses as low as 37 kBq (1.0 μCi) were efficacious and cured 8/10 mice, yet were equally radiotoxic for those same mice. Interestingly, the likely target organ for death appeared to involve the gastrointestinal tract, and the actual cause of death was not well defined. This might be supportive of a theory that the formed ^{221}Fr daughter is trafficking and mimicking K^{+1}, which could virtually eliminate any application of ^{225}Ac as a therapeutic due to a lack of viable chelation chemistry. Alternatives that have been proposed that might address this obstacle include the encapsulation of ^{225}Ac in a fullerene molecule.[148] This seems a logistical challenge at minimum without any clear indication that even this structure would survive the chain of decay events intact. Another possibility is to limit use of the ^{225}Ac−HEHA complex to be conjugated to a reagent that targets exceptionally rapidly and results in rapid internalization. This option also seems challenged if the formed ^{221}Fr daughter would mimic K^{+1} and be pumped out of the cell thereby causing toxicity throughout the body. In sum, despite significant advances in the chelation chemistry of Ac(III), it remains unsettled whether ^{225}Ac can be utilized as a therapeutic.

XI. Clinical Trials with ^{212}Bi/^{213}Bi

To date, only one clinical trial with ^{213}Bi has been initiated. This was done by the group at Memorial Sloan-Kettering Cancer Center, applying the humanized mAb HuM195 for therapy of myeloid leukemia.[51] The antibody binds to the CD33 antigen expressed on the myeloid and monocytic leukemia cells, myeloid progenitors, and monocytes. This mAb rapidly targets leukemic cells in vivo with subsequent internalization of the radioimmunoconjugate into the cells. The mAb conjugate can be efficiently produced at a specific activity of 1.1 GBq/mg (37 mCi/mg). Specific targeting of HL60 cells in vitro yields 50% lethality at 2−3 ^{213}Bi atoms per cell. In mice, bone marrow toxicity was dose-limiting beginning at 6.92 MBq (187 μCi), given as 4 i.v. injections over 2 days, and 14.8 MBq (400 μCi) for one i.p. injection.[80]

In the Phase I trial, 18 patients with relapsed acute myeloid leukemia received i.v. 0.6−1.6 GBq (16−43 mCi) of ^{213}Bi-CHX-A HuM195 in 3−6 fractions over 2−4 days.[51,94] The antibody conjugate was found to localize only in blood, bone marrow, liver, and spleen, measured directly by γ-camera and blood samples. Dosimetry calculations (MIRD) reported the dose to these organs to be in the range 2−20 Sv (a RBE of 5 was applied) with the rest of the body receiving only 2−6 mGy from the γ-rays. Of evaluable patients, 10 of 12 had reductions in peripheral blood leukemia cells, and 12 of 18 had decreases in bone marrow blasts. Transient myelosuppression was observed in some patients but no acute toxicity.

XII. Discussion and Potential Future

A. The in Vitro and in Vivo Experiments

The in vitro and in vivo data have clearly shown the potential of $^{212/213}$Bi in cancer therapy. Even a single α-particle traversal results in significant probability of cell death, a cytotoxity rarely achievable with other therapeutic agents. Furthermore, there is no residue of long-lived toxic compounds. After only half a day, a therapeutic dose of $^{212/213}$Bi has decayed to nanograms of stable ^{208}Pb or ^{209}Bi, respectively. Comparisons between $^{212/213}$Bi versus low-LET radiation and chemotherapeutic agents have clearly shown higher cytotoxicity from the α-particle irradiation. Furthermore, far greater cancer cell kill probabilities are often achievable with α-particle irradiation than with alternative strategies. These studies have also clearly shown the importance of cellular targeting for efficient therapy with $^{212/213}$Bi, and the potential for specific cancer cell killing with little normal tissue damage. For single cell diseases and dispersed cancers, incorporation of the α-particle emitter into the cell by endocytosis of immunoconjugates further increases therapeutic efficacy. As an illustration, Macklis et al. calculated the mean dose to a cell nucleus from ^{212}Bi decay at various locations in a cell with cellular and nuclear diameters of 18 and 11 μm, respectively.[149] For decay in the nucleus, the dose is approximately 35 cGy, decreasing to 5 cGy at the nuclear envelope, 1 cGy at the cell membrane, and 0.1 cGy for decay sites 26 μm away from the cell

membrane. For tumors where the intracellular distance is less than 90 μm, cross-fire dose becomes increasingly important with decreasing intracellular distance and increasing tumor size, and will at some point dominate the dose contribution to each cell.[90]

For evaluation of the applicability of a $^{212/213}$Bi radiopharmaceutical toward a tumor model, multicellular spheroids have already been of substantial value.[91,99,127,150] Such a model system could be employed for evaluation and optimization of carrier molecule tumor kinetics (e.g., influence of size, pI, and affinity),[83] for studying tumor sterilization/growth retardation after different radioactivity levels and radionuclide distributions, and investigating the effect of repeated $^{212/213}$Bi conjugate incubations.

In in vivo animal models, $^{212/213}$Bi immunoconjugates have been very efficient in therapy of small tumors, less so for larger tumors, a not unexpected result due to slow tumor penetration of the large immunoconjugates. Fractionation of the dose and injection over a few consecutive days has not resulted in increased therapeutic efficacy in rodents. However, larger intervals between fractions and repeated injections might still be interesting to evaluate the possibility of eradicating larger tumors. Increased therapeutic efficacy with $^{212/213}$Bi versus low-LET irradiation has been observed in relevant tumor models. Comparison between alternative relevant treatment strategies, also nonradioactive if applicable, in the same tumor model is a sound way of evaluating the therapeutic efficacy of $^{212/213}$Bi radiopharmaceuticals, and should be performed more often. The SCN-CHX-DTPA reagents meet the demand for rapid and in vivo stable chelation of radiobismuth to targeting carriers and further effort in synthesis of bifunctional bismuth chelators seems superfluous.

The in vivo results with ^{212}Pb labeled antibodies have been somewhat disappointing due to bone marrow toxicity from ^{212}Pb and ^{212}Bi released from the immunoconjugate. Preliminary results from NeoRx Inc., indicate better results with rapid tumor localization of a ^{212}Pb-DOTA-biotin conjugate (pretargeting), high tumor/blood and tumor/organ ratios, and entrapment of tumor-localized ^{212}Pb and ^{212}Bi.[151] Thus, ^{212}Pb-DOTA conjugates might be therapeutically effective against some tumors. Furthermore, the more acid stable chelator conjugate of Chappell et al. might improve the overall therapeutic effectiveness of ^{212}Pb.[123] A substantial further improvement would be the synthesis of a chelator also capable of retaining all the in situ generated ^{212}Bi. The probable reason for the 36% break up of the complex are the highly positive charged states of ^{212}Bi that result from conversion of the 238 keV γ-line with a subsequent Auger electron cascade.[119] Thus, a potential way of stabilization might be incorporation of electron rich donor groups into the chelator to rapidly quench the excited state of the electron deficient ^{212}Bi.

B. Generators

Production of ^{212}Bi and ^{213}Bi with high purity and in high yields are possible on a routine basis at radioactivity levels of approximately 1 GBq (27 mCi). Increased radioactivity levels on the generators might be needed for further implementation of $^{212/213}$Bi into routine clinical treatment of cancer. It is not entirely clear if the published generator constructs can accommodate higher radioactivity levels without radiolytic break down. If so, simple increases in size or multiple generators linked together might be viable solutions. Extensive shipment of ^{212}Bi/^{224}Ra generators, required by the short half-life of ^{224}Ra (3.6 days) could be troublesome and expensive due to the high-energy γ-emissions from ^{208}Tl. Restriction of ^{212}Bi therapy to centers capable of producing the generators from ^{228}Th, or construction of a stable ^{228}Th based generator, might then be necessary. The ^{213}Bi/^{225}Ac generators are more easily handled due to less high-energy γ-radiation. For future cancer therapy with $^{212/213}$Bi, a more reliable supply of ^{228}Th and ^{229}Th in larger quantities and increased generator strength would be needed. If the generator size must be increased accordingly to avoid resin damage, the increased elution volume of $^{212/213}$Bi might cause problems in carrier conjugation. To achieve reasonable yields, the conjugation of the radiobismuth to carriers and subsequent purification must be done in minutes, and the conjugation reaction is therefore performed directly in the elution volume. For carriers of larger size, such as mAbs, the concentration is preferably ~5.0 mg/mL to ensure good radiolabeling efficiencies, and only separation of uncomplexed radiobismuth from carrier is reasonably possible, separation of radiolabeled from nonradiolabeled carrier is not. The highest specific radioactivity for ^{213}Bi-mAb achievable for clinical use today is approximately one ^{213}Bi atom per 1000 mAb molecules. To achieve complete tumor sterilization when the cells are organized in clusters this might be sufficient due to cross-fire, but possibly not enough for adequate single cell kill probability, unless the antigen density on the cancer cell is high ($>5 \times 10^5$). As for the discussed leukemia trial, the observed antigen regeneration combined with repeated injections might be sufficient to raise the mean number of ^{213}Bi per cell to a level where complete tumor sterilization is possible.[152] Still, a significant therapeutic effect may be achieved at current levels. However, a higher specific activity would probably improve the therapeutic potential. Potential methods to increase the specific activity of the radiolabeled therapeutic may be increased generator strength, reduction of the $^{212/213}$Bi elution volume, or decreased mAb concentration.

C. Carrier Molecules

As pointed out, rapid targeting and homogeneous distribution of the radionuclide in adequate numbers on the cancerous site is crucial to derive full therapeutic efficacy from $^{212/213}$Bi radiopharmaceuticals. Although mAb's allow specific deposition of radionuclides in tumors, the targeting and blood clearance is often too slow to generate adequate tumor/nontumor dose ratios and a homogeneous radionuclide distribution. Thus, complementary targeting strategies are needed.

By exploiting pretargeting strategies, where the antibody and the radionuclide are injected separately,

increased targeting speed and more homogeneous radionuclide deposition might be achieved. Additionally, the specific activity could be increased. In one approach, a dual specific antibody conjugate (DSC) capable of binding both a cancer cell antigen and a small radionuclide conjugate (1−2 kDa) can be administered. When the DSC level on the cancer cells is optimal, and levels in blood and organs are low, the radionuclide conjugate is administered.[153−156] In a related approach, cancer cells are first targeted with a biotinylated antibody, followed by administration of avidin/streptavidin, proteins (~60 kDa) that can bind up to 4 molecules of biotin (10^{15} M^{-1}).[157] Finally, a biotin radionuclide conjugate is administered, for instance, biotin coupled to ^{111}In-DTPA or ^{90}Y-DOTA.[158−160] Alternatively, the antibody is conjugated with streptavidin/avidin before injection, the tumor targeted, and then a biotin radionuclide conjugate is administered.[161,162] When using DSCs, ^{111}In-DTPA,[153,154] or ^{90}Y-DOTA[156] could be employed as the hapten (antibody binding moiety) as well. In both approaches, an agent for clearing surplus mAb/avidin/streptavidin conjugate from the blood is often applied prior to the radionulide conjugate is administered. These pretargeting procedures result in tumor/blood ratios >1 only minutes after injection of the radionuclide conjugate. Both approaches could be applied with $^{212/213}$Bi using either biotin-CHX-DTPA or biotin-DOTA conjugates,[161,163] or simply the $^{212/213}$Bi-DOTA/$^{212/213}$Bi-CHX-DTPA conjugates both as haptens and radionuclide carriers. Furthermore, Axworthy et al.,[161] utilizing the biotin approach, reports of similar or increased tumor radioactivity concentration as compared to direct mAb conjugated radioactivity. With such small radionuclide carrier molecules, development of rapid separation procedures (e.g., HPLC),[164] producing carrier-free radionuclide conjugates could be possible. Thus, only metal-ion conjugates would be administered to the patients, thereby avoiding the competition for the target sites from all the unlabeled molecules. Yields of several thousands of $^{212/213}$Bi atoms per cancer cell could then be possible, theoretically producing a single cell survival probability of $<10^{-10}$. Furthermore, the rapid targeting and blood clearance of such small radionuclide conjugates permits the possibility of obtaining favorable tumor/blood and tumor/organ ratios compatible with the short half-life of $^{212/213}$Bi in larger tumors and less easily accessible cancers other than, for instance, leukemia. One will probably encounter challenges from immunogenicity in these pretargeting techniques, and with the biotin/avidin system, a biotin decreased diet before treatment may be necessary for the patient.[165] Furthermore, the pretargeting techniques also suffer from inefficiency of capturing the final low molecular weight radionuclide carrier, which might have a significant impact on the economics of this approach.[165] Another possible disadvantage resulting from this capturing inefficiency might be kidney and bladder toxicity, due to renal clearance of most of the administered radioactivity (~70−90%) in the first hours postinjection.[153,159−161] If a ^{212}Pb-conjugate were used in these pretargeting approaches, the appealing possibility of separating the ^{212}Pb-conjugate from the ^{212}Bi-conjugate, and if necessary free ^{212}Bi, exists.[164] By injecting essentially pure ^{212}Pb-conjugate, the levels of ^{212}Bi would be very low in the initial time point when most of the radioconjugate would be excreted. Thus, the initial large dose from the α-particles to the renal clearance system, and especially to the blood/bone marrow, would be substantially reduced. Then, if the ^{212}Pb and the generated ^{212}Bi localized in the cancerous cells remained entrapped there; this scenario would be close to being a perfect in vivo generator system. Of course, it remains to be seen if the assumed advantages of these pretargeting strategies will hold for the α-particle emitters.

Rapid specific tumor targeting with high affinity and avidity might also be achieved with small mAb fragments.[83,109] Single chain sFv's exhibit tumor targeting and blood clearance kinetics compatible with the short half-life of $^{212/213}$Bi.[166,167] Furthermore, autoradiography has shown that scFv's distribute more homogeneously in tumors than other mAb fragments and intact mAbs due to their smaller size. Yokota et al. found maximum uptake of scFv in 0.5−1 cm human colon carcinoma xenografts in mice 30 min after i.v. injection, as compared to 48 h for intact IgG.[167] Although the tumor uptake of scFv versus intact IgG was considerably reduced, the maximum levels of scFv and IgG in the tumor more than 10 μm away from blood vessels was similar, showing that considerable amounts of IgG were restricted to the intravascular space. A disadvantage of scFv, in addition to low tumor uptake, remains high kidney localization and retention.[166] Finally, production of carrier-free $^{212/213}$Bi labeled scFv would probably not be achievable.

Other small molecules, besides DOTMP,[132] which exhibits rapid pharmacokinetics, might also be useful as carriers for $^{212/213}$Bi to cancerous sites. Alternatives could be small peptides[168−170] or amino acid[171] and vitamin analogues.[172]

Combinations of different targeting strategies may give an even more specific and homogeneous radionuclide distribution at the cancerous site within the lifetimes of $^{212/213}$Bi. For instance, pretargeting or conventional targeting of several different antigen sites could increase the specificity due to an increased number of possible target sites. Such a strategy could also increase the dose homogeneity if the different antigens had independent frequency distributions. Involvement of untargeted cancer cells would then be less likely. By the same reasoning, combining pretargeting or conventional targeting strategies with small molecules that target other cellular functions (such as those mentioned above) could further increase specificity, tumor uptake, and radionuclide distribution homogeneity. Other approaches to increase the therapeutic efficacy might be synergistic cytotoxicity with chemotherapeutics[173] or increasing the target receptor expression with, e.g., interferon[174] or adenoviral vectors.[170] Also, the probability of killing the fraction of cancer cells with originally low antigen expression should be increased.

D. Normal Tissue Damage

Organs most at risk for receiving acute damage from $^{212/213}$Bi radiopharmaceuticals are the renal clearance system, the blood vessels, and the bone marrow. To date, the renal clearance system in mice has shown surprisingly high tolerance levels for clearance of $^{212/213}$Bi labeled radiopharmaceuticals, indicating an RBE value close to unity.[92] There are few studies where the processing of free bismuth or bismuth labeled radiopharmaceuticals in the kidneys has been investigated. Free bismuth is distributed very nonhomogeneously throughout the whole kidney, varying by a factor of 10, with the central region containing lesser amounts of radioactivity than the periphery.[175] Free bismuth is also incorporated into kidney cells to some extent.[138,176] Speidel et al. studied morphological changes in monolayers of bovine aortic endothelial cells in vitro after α-particle irradiation from ^{212}Bi-DTPA in the medium.[177] They found no massive cell damage for doses up to 73 Gy, indicating no particular toxicity to the vascular system after administration of $^{212/213}$Bi radiopharmaceuticals. Additionally, for bone marrow, the most radiosensitive and often dose-limiting organ, no excessive toxicity for α- versus β-particle irradiation has been found.[92,93] Furthermore, repeated injection of a MTD of a ^{213}Bi-immunoconjugate did not result in increased bone marrow toxicity.[93] These findings hold promise for cancer therapeutic applications of $^{212/213}$Bi, though further studies are warranted. Late effects, such as carcinogenesis has been observed following administration of α-particle emitters.[178−184] For therapy of nonterminal patients, the probability for induction of such late damage needs to be addressed.[185,186] More accurate knowledge of late damage induction probabilities may come from microbeams[187−189] or the retrospective absolute α-particle hit determination method.[190]

E. Concluding Remarks

The short half-life of $^{212/213}$Bi and the short range of the α-particles make these radionuclides suitable for therapy of cancers, which is expressed as disseminated small cell clusters and single cells, e.g., lymphoma, leukemia, and ovarian cancer. Although the short half-life is a disadvantage for therapy of larger, less easily accessible tumors, this property may work advantageously for loco-regional tumor injections and fast, easily accessible cancers. In the ^{213}Bi-mAb leukemia trial, organs other than blood, bone marrow, spleen, and liver received a negligible dose because the ^{213}Bi had decayed below toxicity inducing levels before catabolites or nontargeted $^{212/213}$Bi-mAbs had cleared out of the treatment region. Similarly, loco-regional treatment of glioma and intraperitoneal disseminated ovarian cancer with $^{212/213}$Bi radiopharmaceuticals should spare vital organs and whole body from any significant dose. Improvement in targeting strategies could also open for efficient α-particle therapy of metastasized cancers, for instance, from the large patients groups with breast, lung, and prostate cancer.

XIII. Acknowledgment

The authors thank Christina Soyland, Ekaterina Dadachova, and Barbara Keller for their careful readings and salient technical commentary. The views expressed in this article do not necessarily represent those of either the NIH or DHHS.

XIV. References

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CR000118Y