Radioimmunotherapy with alpha-emitting nuclides

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Abstract. This review discusses the application of alpha particle-emitting radionuclides in targeted radioimmunotherapy. It will outline the production and chemistry of astatine-211, bismuth-212, lead-212, actinium-225, bismuth-213, fermium-255, radium-223 and terbium-149, which at present are the most promising alpha-emitting isotopes available for human clinical use. The selective cytotoxicity offered by alpha particle-emitting radioimmunoconstructs is due to the high linear energy transfer and short particle path length of these radionuclides. Based upon the pharmacokinetics of alpha particle-emitting radioimmunoconstructs, both stochastic and conventional dosimetric methodology is discussed, as is the preclinical and initial clinical use of these radionuclides conjugated to monoclonal antibodies for the treatment of human neoplasia.

Key words: High linear energy transfer – Bismuth-213 – Astatine-211 – Bismuth-212 – Actinium-225 – Alpha particle-emitting radionuclides

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Introduction

Alpha particles are monoenergetic, high-energy helium nuclei (He-4) with high linear energy transfer (LET) in the range of 100 keV/µm. There are approximately 100 radionuclides [predominantly heavy elements ($Z \geq 82$)] that decay with alpha particle emission. Several review articles describing the applications of alpha-emitting radionuclides for the treatment of disease have recently appeared in the literature [1–4].

Based on practical dosimetric principles there are several advantages to using high LET radionuclides in radiotherapeutic applications [5]. For example, the mean LET value for the beta particle-emitting yttrium-90 is 0.2 keV/µm whereas that of astatine-211 is 97 keV/µm. Furthermore, the mean range in tissue of the ^{211}At alpha particle and the ^{90}Y beta particle is 70 µm and 3960 µm, respectively. Therefore, the cytotoxicity induced by alpha particles is far more selective.

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Apart from the naturally occurring radionuclides, the majority of alpha particle-emitting radionuclides are produced from reactor irradiations, incorporated into a generator (cow) and subsequently eluted. Currently, applications for specific alpha particle-emitting radionuclides produced from cyclotron irradiations are appearing.

Regardless of the source of the radionuclide, the radionuclide separation scheme must be capable of supplying a high-purity product, in terms of both chemical and radionuclidic purity. In the case of the relatively short-lived alpha particle-emitting radionuclides (such as bismuth-213, $t_{1/2}$ = 46-min) the processing time required to achieve elution of the radionuclide in the proper chemical form and to formulate the finished radiopharmaceutical must be short. Selection of appropriate nuclides must also take into account daughter nuclides that may be metabolized differently from the parent isotope. This is particularly important if the daughter is long-lived. Moreover, the materials utilized in the separation process, such as the generator resin support, must be judiciously selected to avoid detrimental radiation chemistry effects upon the formulated product, by either the parent or the daughters. At the current time most alpha-emitting isotopes are limited in availability and therefore are costly. In the following review, we will discuss the most promising alpha-emitting isotopes and their applications to human disease when conjugated to monoclonal antibodies.

Alpha particle-emitting radionuclides

Astatine-211

^{211}At decays with a 7.2-h half-life through a branched pathway (Fig. 1). One route is by direct alpha particle emission to bismuth-207 (42%) followed by electron capture to the ground state lead-207. The second is by electron capture to polonium-211 (58%) and prompt alpha particle emission to ^{207}Pb [6]. The ^{211}Po and ^{211}At average alpha particle energy is 6.8 MeV with a range in tissue of 55–80 µm, and a mean LET value of 99 keV/µm [4]. The ^{211}Po daughter emits K x-rays (77–92 keV) that are of sufficient energy to permit photon counting of samples and external imaging. The long 33.4 year half-life of ^{207}Bi is not a major safety concern, because the

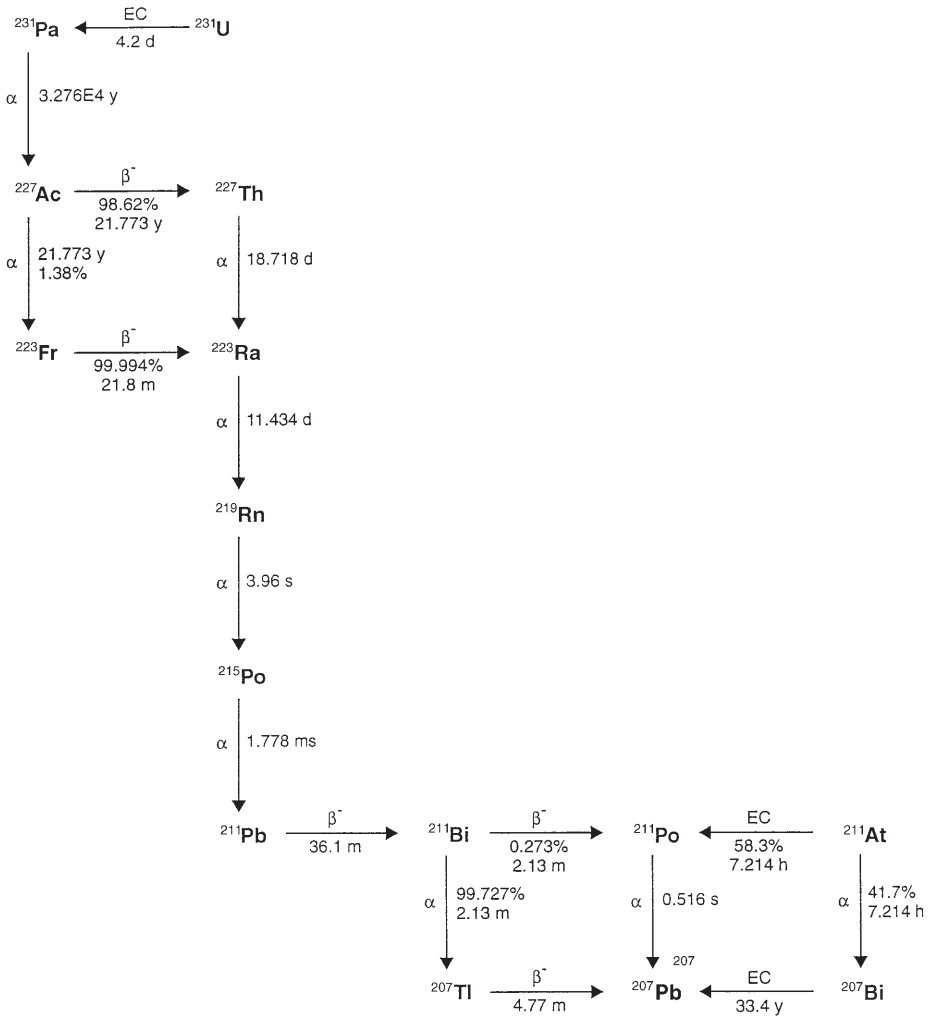

Fig. 1. The ^{231}U decay scheme

activity of ^{207}Bi generated is only 0.033 MBq (0.0009 mCi) from each 37 MBq (1 mCi) of ^{211}At [7].

Zalutsky et al. have described the cyclotron production of ^{211}At via the $^{207}Bi(\alpha,2n)^{211}At$ nuclear reaction on natural bismuth metallic targets. The ^{211}At species produced in this reaction is dry distilled from the target matrix and trapped in chloroform. It is important to maintain the alpha particle beam energy window within the target between 28.5 and 22.0 MeV to maximize the yield of ^{211}At and to minimize the production of ^{210}At. At-210 has an 8.1-h half-life, and decays to the alpha emitter ^{210}Po with a half-life of 138 days. As a bone-seeking radionuclide, ^{210}Po could lead to unacceptable bone marrow toxicity.

^{211}At has been successfully attached to monoclonal antibodies and antibody fragments using a two-step labeling method developed by Zalutsky et al. [8]. In the first step of this labeling procedure, *N*-succinimidyl 3-(trimethylstannyl)benzoate and *t*-butylhydroperoxide are added to the trap containing the ^{211}At/chloroform. Following a 15-min incubation period the product *N*-succinimidyl 3-[^{211}At]astato-benzoate (SAB) is purified by high-pressure liquid chromatography. The monoclonal antibody in borate buffer, pH 8.5, is added in the second step to the purified SAB and incubated on ice for 15 min. ^{211}At labeled antibody products can be prepared in this manner in 1.5-h at specific activities reported to 148 MBq/mg (4 mCi/mg).

The potential variablity in the radionuclidic purity of ^{211}At produced by the cyclotron irradiation and the fact that only a few research centers have the capability for production of ^{211}At remain potential drawbacks to the widespread use of this radionuclide. However, the 7.2-h half-life makes ^{211}At very attractive for radioimmunotherapy in which the targeting molecule does not gain immediate access to the tumor cells.

Bismuth-212

^{212}Bi is an alpha particle-emitting radionuclide that is obtained from the thorium-228 decay chain (Fig. 2) [6]. It has a half-life of 60.6-min and an average alpha particle energy of 7.8 MeV. ^{212}Bi decays via a branched pathway to thallium-208 and ^{212}Po and both in turn decay to stable ^{208}Pb. Each pathway results in the emission of an alpha particle. Radium-224 (3.6-day half-life) has been used to construct generators for the production of ^{212}Bi

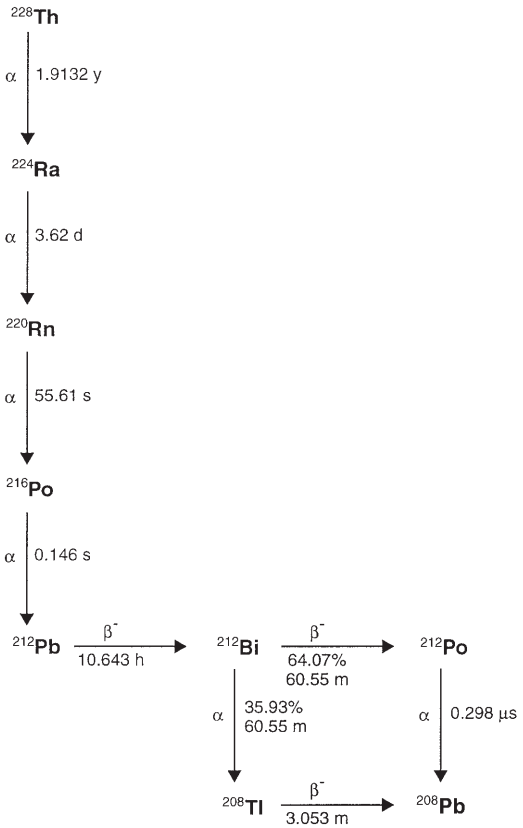

Fig. 2. The ^{228}Th decay scheme

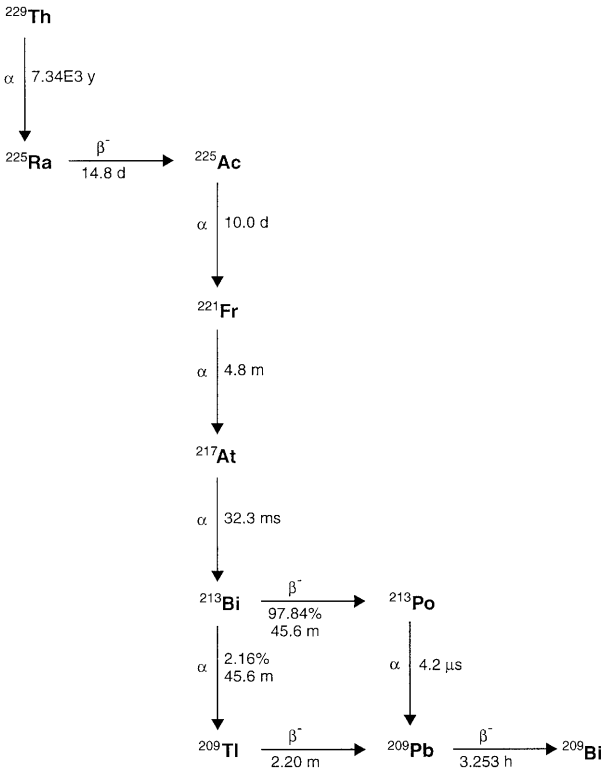

Fig. 3. The ^{229}Th decay scheme

and these generators have been used in preclinical studies by Atcher et al. [9]. This generator is constructed from ^{224}Ra that has been separated from its parent, ^{228}Th, by absorption of thorium nitrate in 9 *M* HNO_3 onto an anion exchange resin [10]. The ^{224}Ra eluated is evaporated to dryness and the residue dissolved in 0.1 *M* HCl. The acidic ^{224}Ra solution is then absorbed onto AGMP-50 cation exchange resin contained within a Teflon tube and fitted with barbed polypropylene fittings. By variation of the generator elution conditions (using either 0.5–2 *N* HCl or 0.2–2 *N* HI) the % yield of ^{212}Bi or its parent ^{212}Pb can be controlled.

The ^{208}Tl produced in the ^{212}Bi decay emits a 2.6 MeV gamma ray and along with the other medium- to high-energy gamma emissions in the decay scheme require that the ^{224}Ra cow be heavily shielded to minimize personnel radiation exposure. Additionally, there is a radon-220 daughter in the decay chain that requires that the ^{224}Ra cow be placed in either a gas-tight or trapped enclosure.

Bifunctional metal chelating moieties that can be readily attached covalently to protein molecules and stably bind bismuth radionuclides have been extensively investigated [11, 12]. The bifunctional metal chelating moieties are derivatives of functionalized diethylene triamine penta acetic acids and have been developed into stable radiometal chelating agents suitable for use in vivo.

Lead-212

^{212}Pb (10.6-h half-life) is also produced in the ^{228}Th decay scheme (Fig. 2) and can be produced using the ^{224}Ra generator system [9]. One strategy has been to label a monoclonal antibody with ^{212}Pb, which, when administered clinically would serve as an in situ ^{212}Bi generator [13]. Such an agent would actually produce a build-up of the dose rate in tissue and thereby result in some blood and bone marrow sparing. A number of lead and bismuth complexes have been prepared and evaluated for this unique in situ generator system [14, 15]. One difficulty of chelating ^{212}Pb results from the electron capture branch in the decay scheme, which through the concomitant charge build-up after Auger electron emission would destroy the chelating moiety.

Actinium-225

^{225}Ac can be obtained either from the natural decay of uranium-233 and its production of ^{229}Th [16] or from the neutron irradiation of ^{226}Ra by successive n,β capture decay reactions via ^{227}Ac, ^{228}Th to ^{229}Th [17, 18].

Geerlings et al. [19] have described a ^{225}Ac generator based on a design that adsorbs ^{229}Th oxide onto a titanium phosphate resin. Elution of this ^{229}Th cow with 0.5 *M* HNO_3 yields a mixture of radionuclides: ^{225}Ac,

^{225}Ra, and ^{224}Ra. A second column containing Dowex 50 W×8 is used to purify the ^{225}Ac by removing the ^{225}Ra, ^{224}Ra, and the ^{224}Ra decay products. The ^{225}Ac thus produced can be used directly as a reagent for radionuclide labeling or affixed onto a resin as parent for a ^{213}Bi generator product.

There are six daughters of ^{225}Ac which are produced in the cascade to stable ^{209}Bi (Fig. 3) [6]. For each ^{225}Ac decay there are subsequently five alpha and three beta disintegrations, most of high energy. As noted for ^{212}Pb above, the potential for using ^{225}Ac as a therapeutic radionuclide is limited by the availability of chelator moieties capable of binding the radionuclide as well as its daughters through alpha-decay [20]. Given the ^{225}Ac 10.0-day half-life, the high-energy alpha particle emission, and the favorable rapid decay chain to stable ^{209}Bi, this radionuclide would be an excellent candidate for use in cancer therapy utilizing the in vivo generator approach. However, the unlikely possibility of discovering suitable chelating agents able to withstand the immense alpha particle recoil energies for the actinium ion, limits the major application of ^{225}Ac as a cow for the generation of ^{213}Bi. (The alpha particle recoil energy is usually between 100 and 200 keV, which is slightly more than the binding energy of a macrocyclic metal-ion complex.)

Bismuth-213

^{213}Bi (45.6-min half-life) is a decay product of ^{225}Ac, as shown in Fig. 3. A clinically approved ^{213}Bi generator has been developed that provides up to 925 MBq (25 mCi) of pure, chemically reactive ^{213}Bi [21]. The generator is capable of providing a clinically useful radionuclide for 10 days with a minimum amount of shielding. At Memorial Sloan-Kettering Cancer Center "no carrier added" ^{225}Ac is obtained as the nitrate from the Institute for Transuranium Elements (Karlsruhe, Germany) or Oak Ridge National Laboratory (Tenn., USA) and dispersed onto an AGMP-50 resin. The ^{213}Bi reaches secular equilibrium with the ^{225}Ac after approximately 300 min. (6.5 ^{213}Bi $t_{1/2}$'s), however, after approximately 150 min. 90% of the maximum ^{213}Bi activity is available for elution from the generator. The ^{213}Bi is eluted as the $(BiI_5)^{2-}$ anion species with 0.1 *M* HI [22]. Labeling is accomplished by allowing the bismuth ion in a pH 4.5 acetate buffer to react with an antibody-chelate construct.

Antibody molecules appended [23] with the C-function alized *trans*-cyclohexyldiethylenetriamine pentaacetic acid moiety, CHX-A-DTPA [12], readily chelate the ^{213}Bi radionuclide. Reactions typically reach 90% completion in 10–20 min. The ^{213}Bi labeled antibodies are >90% immunoreactive and are purified via size exclusion chromatography. Specific activities can be achieved in the range of 370–925 MBq/mg (10–25 mCi/mg). Complete processing times are approximately 22–30 min [21, 24]. Clinical trials using a ^{213}Bi labeled anti-CD33 construct are in process [25]. Furthermore, in vivo patient imaging, taking advantage of the ^{213}Bi 440-keV photon emission, has been carried out [26] and detailed pharmacokinetics and dosimetry information in patients with leukemia has been obtained [27, 28]. (See more details below.)

One of the limitations to constructing alpha particle-emitting isotope generators is their rapid failure due to radiation damage. Generator failure is defined as exessive parent nuclide breakthrough, catastrophic radiolytic damage to the resin and to the body of the column (porosity and cracking), and sintering of the resin resulting in the production of fines which hinder the flow of solution through the generator.

The effects of radiation damage to ion exchange materials are well documented [29]. Radiation damage to the resin and the eluate solution is a concern given the large energy per transition values for the alpha-emitting radionuclides. The alpha energy per transition value is estimated to be approximately 1.0 E-12 Gy·kg/Bq·sec for the alpha-emitting radionuclides in the ^{225}Ac decay cascade based upon reference data [30]. The radiation dose due to ^{225}Ac and its daughters is particularly large, given the 10-day half-life, and could rapidly destroy the viability of the support resin and plastic components. For example, it is estimated that the daily dose to 1 or 100 mg of a polymeric cation exchange resin from 740 MBq (20 mCi) of ^{225}Ac is 2.0 E10 and 2.0 E08 cGy, respectively. It is known that cation exchange resins are considerably degraded when the total absorbed doses are greater than 1 E08 cGy [29]. Absorbed doses of this magnitude will be reached within the 1st day of loading 1 mg of resin with 740 MBq of ^{225}Ac.

An inorganic silica-based actinide resin has been employed to support ^{225}Ac in an effort to avoid generator failure [31]. Inorganic support resins should be more robust and experience less radiolytic damage than organic based support resins [32].

Fermium-255

^{255}Fm is an actinide element that has been proposed [33] as a good candidate for use in radioimmunotherapy based upon its 20.1-h half-life. ^{255}Fm has an alpha particle emission of 7.02 MeV (93.4%) in its decay to californium-251, which has a 900-year half-life. The ^{255}Fm could be produced from a generator constructed using einsteinium-255 (Fig. 4) [7], which has a half-life of 38.3 days. Millicurie quantities of ^{255}Fm have been produced at Oak Ridge National Laboratory by neutron activation of curium at the High Flux Isotope Reactor, producing a mixture of einsteinium isotopes from which the ^{255}Fm can be isolated [7].

Table 1. Candidate alpha particle-emitters for radioimmunotherapy

Radionuclide Daughters	Half-life	%[a]	Emissions	
				Particle Energy[b]
^{213}Bi	45.6 min	2	α	6 MeV
		98	β^-	444 keV
		17	γ	440 keV
^{213}Po	4.2 µs	98	α	8 MeV
^{209}Tl	2.2 min	2	β^-	659 keV
^{209}Pb	3.25 h	100	β^-	198 keV
^{209}Bi	Stable			
^{212}Bi	1.0 h	36	α	6 MeV
		64	β^-	492 keV
^{212}Po	298 ns	64	α	9 MeV
^{208}Tl	3.05 min	36	β^-	560 keV
		8	γ	510 keV
		31	γ	580 keV
		36	γ	2.6 MeV
^{208}Pb	Stable			
^{211}At	7.21 h	42	α	6 MeV
		19	γ	80 keV
^{211}Po	516 ms	58	α	7.5 MeV
^{207}Bi	32 years	24	γ	70 keV
		41	γ	570 keV
		31	γ	1 MeV
^{207}Pb	Stable			
^{225}Ac	10 days	100	α	6 MeV
^{221}Fr	4.9 min	100	α	6 MeV
		10	γ	218 keV
^{217}At	32.3 ms	100	α	7 MeV
^{213}Bi	See ^{213}Bi			
^{223}Ra	11.4 days	100	α	6 MeV
		40	γ	80 keV
		14	γ	270 keV
^{219}Rn	4 s	100	α	7 MeV
		10	γ	270 keV
^{215}Po	1.8 ms	100	α	7 MeV
^{211}Pb	36.1 min	100	β^-	447 keV
^{211}Bi	2.1 min	16	α	6 MeV
		84	α	7 MeV
		13	γ	350 keV
^{207}Tl	4.8 min	100	β^-	493 keV
^{207}Pb	Stable			
^{149}Tb	4.15 h	17	α	4 MeV
		61	γ	43 keV
		11	γ	48 keV
		28	γ	165 keV
		33	γ	352 keV
		20	γ	388 keV
		17	γ	652 keV
		12	γ	817 keV
		16	γ	853 keV
		30	e^-	7 keV
		20	e^-	8 keV
		10	e^-	115 keV
^{149}Gd	9.25 days	82	γ	41 keV
		15	γ	47 keV
		42	γ	150 keV
		23	γ	299 keV
		18	γ	347 keV
		53	e^-	7 keV
		40	e^-	8 keV
		17	e^-	30 keV
		21	e^-	101 keV
^{149}Eu	93 days	96	γ	40 keV
		12	γ	45 keV
		55	e^-	7 keV
^{145}Eu	5.9 days	17	γ	894 keV
^{145}Sm, ^{149}Sm	340 days, stable			

[a] Percent emitted per decay of parent radionuclide
[b] Mean β^- energy and approximate α, e^- and γ energies are listed

Radium-223

Figure 1 shows the ^{223}Ra decay scheme. The ^{223}Ra (11.4-day half-life) could be obtained from uranium mill tailings in large quantities [7] and a generator system has been developed [34] using a ^{227}Ac parent (21.8-year half-life). The advantage and disadvantage of this radionuclide in targeted radioimmunotherapy is the four alpha emissions. The radiotoxicity associated with four alpha particles would probably necessitate only one decay per tumor cell. However, the ^{223}Ra decays to radon-219, a gaseous product that would redistribute in vivo in an unknown way. Until the biodistribution of ^{223}Ra labeled antibodies and the daughter isotopes is investigated and understood, the clinical utility of this radionuclide remains questionable.

Terbium-149

^{149}Tb ([35] and references therein) is a lanthanide element that decays (4-h half-life) via alpha particle emission (17%), positron emission (4%), and electron capture (79%), and 1110 MBq (30 mCi) quantities have been prepared at the Isolde facility at the CERN spallation source. The production of this radionuclide required 4 microamps of 600 MeV protons from a synchrocyclotron incident upon a 122 g/cm^2 tantalum foil. The reaction products were ionized and accelerated from the source with a 60-kV potential. A magnetic field was used to separate the 149 mass component and deposit it onto an aluminum foil. It has also been produced in small [0.37 MBq (0.01 mCi)] quantities on a 10-mV tandem accelerator using the ^{141}Pr(^{12}C,4*n*)^{149}Tb reaction at 70 MeV. The separation and purification (removal of

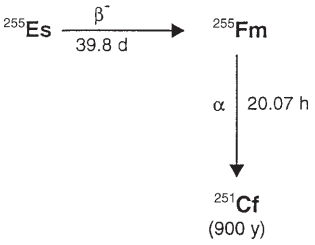

Fig. 4. The ^{255}Es decay scheme

metallic impurities) of the ^{149}Tb from the aluminum foil will be necessary prior to labeling antibody-chelate constructs. Additionally, the biodistribution and safety of the many ^{149}Tb decay products must be evaluated as these are lanthanides and presumably may deposit in mineral bone.

Dosimetry of Alpha Particle Emitter Radioimmunoconjugates

The dosimetry of alpha particle-emitting radionuclides may be distinguished from that of conventional beta particle-emitters by a number of features. Few alpha particle-emitters decay to stable or short-lived daughter products. Those that do, possess half-lives which are considerably shorter than the commonly used beta-emitters. The range of alpha particles are <100 μm and much shorter than beta particle emissions. The LET of alpha particles is 400 times greater than that of beta particles (80 keV/μm vs 0.2 keV/μm). Depending upon their emission characteristics, the relative biological effectiveness (RBE) of alpha particles for cell sterilization ranges from 3 to 7. Cell survival studies have shown that a single alpha particle track, originating from the cell′s surface and traversing the nucleus, is capable of resulting in cell death [36]. This is in contrast to the thousands to tens of thousands required of beta particles. Given the energy delivered along an alpha particle track and its potential cytotoxicity, the conventional methodologies for estimating mean absorbed dose may not always yield physically or biologically meaningful information. Instead, stochastic or microdosimetric methodologies may be required. Starting with quantitation of pharmacokinetics, these considerations are briefly examined below. In addition, decay of the parent alpha particle-emitting radionuclide yields a number of daughters whose dosimetry and pharamcokinetics must be considered separately. Table 1 lists alpha particle-emitting radionuclides that have been considered for radioimmunotherapy. Human studies have already been carried out with ^{213}Bi [25], and human studies with ^{212}Bi and ^{211}At are being planned [5, 37–41]. Studies with ^{225}Ac, ^{223}Ra, and ^{149}Tb are in early stages of development and analysis [19, 20, 35, 42–44].

Pharmacokinetics

Therapy using short-lived alpha particle-emitters requires an understanding of antibody pharmacokinetics that may not be extrapolated from experience obtained using iodine-131 or other longer-lived beta-emitters. With longer-lived radionuclides, the pharmacokinetics are dominated by biological clearance of the antibody. The distribution of antibody in the first several minutes to hours after administration yields residence times that are negligible in proportion to the overall residence times achieved for target and normal organs. This is in contrast to ^{213}Bi ($T_{1/2}$ = 45.6-min), for example, in which 20% of the total alpha particle emissions occur within the first 15-min after injection; 3-h post-injection only 6% of the total emissions remain.

For the shorter-lived alpha-emitters (^{213}Bi, ^{212}Bi, and to a lesser extent ^{211}At), therefore, relevant pharmacokinetic information will be obtained if imaging and/or sampling starts immediately after administration. The photon emission chosen for gamma camera imaging should possess an energy that is within the range of nuclear medicine imaging devices and that is unique to decay of the parent [26]. A further difference between the gamma camera images obtained from a short-lived radionuclide such as ^{213}Bi and those obtained from ^{131}I is that in representing a substantial fraction of the total decays they approximate images of dose distributions, rather than only radiolabeled antibody distributions.

Microdosimetry

A microdosimetric or stochastic analysis of energy absorbed in a target is required if a large fluctuation in the absorbed energy is expected. This may be quantitatively expressed as the variance in specific energy (energy absorbed per unit mass) relative to the mean specific energy. To understand the realm of microdosimetry versus conventional dosimetry it is worthwhile to consider the following example. If a population of tumor cells were to be exposed to a uniform distribution of alpha source with an average one alpha particle transversal or event per cell, then 37% of the tumor cells would receive no events. Therefore, whereas a radiation dose of 50 cGy from a uniform exposure of beta particles might result in all tumor cells receiving 1000±30 events, the same dose delivered by alpha particles would result in a very broad microdosimetric spectrum of energy deposition. The short-range local effects of alpha particles complete with the stochastic nature of high LET radiations presents a greater challenge to determine and understand the biological effects resulting from alpha particle-emitting radionuclides.

In radioimmunotherapy the geometric relationship between the sources and target tumor cells is not uniform and therefore the alpha particle hits cannot be assumed to be a Poisson distribution. Several distributions

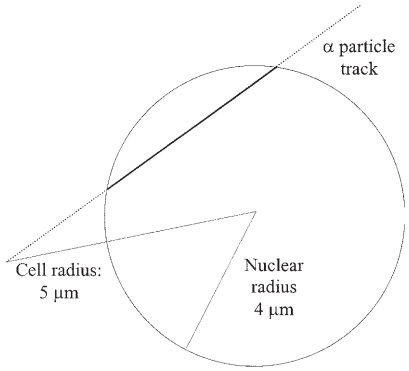

Fig. 5. Representation of the geometry used to derive Fig. 6. A cell is represented by two concentric spheres. The 4-μm-radius nucleus is the target

have been modeled and microdosimetric spectra calculated by either Monte Carlo [45, 46] or analytical [47, 48] methods. The results of microdosimetric calculations are specific energy probability densities [49]. Microdosimetric spectra have been calculated from measured autoradiographic data of radiolabeled antibody distribution in animal tumor models [50]. The transition from micro- to conventional dosimetry is dependent upon the number of alpha particle emissions and the magnitude of the target volume under consideration.

Microdosimetric analyses generally require direct sampling to estimate the activity in a given cellular geometry (e.g., per cell or per cluster). Bone marrow sampling, for example, may be used to obtain information regarding possible marrow toxicity. In such an analysis, the emissions must be placed relative to a particular cellular geometry. The initial result of such analyses is the single-hit probability density curve. Using the source-target geometry shown in Fig. 5, such a curve has been derived for ^{213}Bi (Fig. 6). The probability of delivering a particular range of specific energies may be obtained as the area under the curve that is bounded by the range of specific energies chosen. It is important to note that this is the probability density for specific energies, given that a single hit through the target volume has occurred. There will also be some probability that no hits have occurred; this cannot be depicted on the curve but may be thought of as a delta function at the origin (specific energy = 0). Since the geometry allows for only two possibilities – specific energy = 0, corresponding to no hit, and specific energy = alpha energy/spheroid volume, corresponding to complete absorption of alpha emission – the probability density curve is made up of two delta functions. To facilitate the application of microdosimetry to geometries that are clinically relevant, a formalism has been developed which makes it possible to perform basic microdosimetric assessments given the delta function (i.e., the probability that the specific energy is zero), the probability of no hits, and the mean specific energy due to a single hit. Once these parameters are determined for a particular radionuclide and geometry, it is possible to extend the analysis to multiple hits and thereby develop a simplified framework for estimating microdosimetric parameters [51, 52].

Conventional dosimetry

Radionuclide dosimetry that is based upon imaging-derived pharmacokinetics can be performed as specified in the MIRD pamphlets [53]. This methodology is also applicable to alpha particle-emitters for the determination of average radiation doses. However, it must be remembered that the range of alpha particles is much shorter than the limits of resolution of a gamma camera. In conventional dosimetry of alpha particle-emitting radionuclides it is assumed that all alpha and electron emissions arising from decay of the parent are locally deposited. The absorbed dose is then given by the cumulated activity concentration, $[\hat{A}]$, multiplied by the energy emitted per decay as electrons Δe, and alpha particles $\Delta\alpha$. To determine a radiation dose equivalent (in Sieverts), the alpha particle contribution to the dose should be adjusted for the relative biological effectiveness (RBE) of alpha particles [36]:

$$D = [\hat{A}]\times(\Delta e + \mathrm{RBE} \times \Delta\alpha). \quad (1)$$

In most cases, the photon contribution to the absorbed dose in an organ that concentrates an alpha particle-

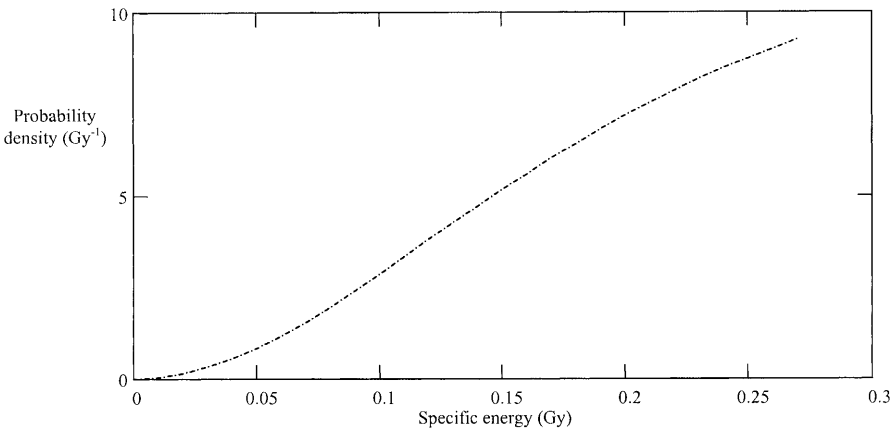

Fig. 6. The single-hit, specific energy probability density for an 8.4-MeV alpha particle (98% of ^{213}Bi alpha particle emissions)

emitter may be neglected as it is typically <1% of the total. All of the alpha particle-emitters under consideration for radioimmunotherapy yield radioactive daughters. The fate and biodistribution of radioactive daughters following alpha particle decay of the parent has not been examined. If the daughters remain in the vicinity of the parent then the parameters Δe and $\Delta\alpha$ should include the energy associated with their emissions, weighted by the yield of each daughter. If the half-life of the daughters is long in relation to the rate of diffusion of the daughter, and this may be dependent upon the chemical nature of the atom, then information regarding the redistribution must be explicitly incorporated into the dosimetric estimates.

Use of alpha-emitting radioconjugates in vivo in animals

One of the first studies demonstrating the feasibility of using alpha-emitting ^{212}Bi radioimmunoconjugates for therapy in vivo involved a T cell ascites tumor model in mice [39]. Both tumor and the IgM radioconjugates were injected intraperitoneally (i.p.). In this way, the rapid delivery of the short-lived isotope to targets was assured, without significant exchange of the radioconstruct from the peritoneal cavity to the systemic circulation affecting normal tissue toxicity. Injections of up to 14.8 MBq (0.400 mCi) of ^{212}Bi-labeled IgM were found to be safe. Unlabeled or radiolabeled control IgM were unable to cure any animals or prolong the survival beyond 30 days. In contrast, doses of 5.6–14.8 MBq (0.150–0.400 mCi) of ^{212}Bi-anti-thy 1.2 (specific) IgM were able to prolong survival in all animals for greater than 30 days, and at least half of treated animals were still alive at 2 months. Thus, these initial studies verified the specificity of the alpha-emitting constructs in a carefully circumscribed in vivo model.

Additional evaluation of the cytotoxic effects of the anti-thy 1.2 construct ex vivo led to the conclusion that apoptotic mechanisms were operative in lymphoma cell death after alpha particle irradiation [54]. Apoptosis was demonstrated by morphology with electron microscopy and the appearance of DNA ladders upon gel electrophoresis. As few as four alpha particles traversing the nucleus per cell were adequate to reduce cell numbers by 90%. More recently we have confirmed the presence of apoptotic cell death in another system (using human leukemia cell targets) by the use of flow cytometric methods (Lai, Weisburg, McDevitt, Scheinberg, unpublished results).

^{213}Bi labeled monoclonal antibodies targeting lung vasculature have been reported to be successful in therapy of lung tumor in a mouse model (see [16] and references listed therein).

Another murine peritoneal model involved human colon carcinoma zenografts of LS174T cells, treated at 1 week with single or multiple daily doses of ^{212}Bi-labeled B72.3, a murine Ig directed to the colorectal cell surface antigen TAG-72 [55]. Up to 7.0 MBq (0.190 mCi) for 3 days was administered with acceptable toxicity. Significant specific antitumor effects were seen and small changes in improved survival were observed as well.

Another murine model of radioimmunotherapy extensively studied is the Rauscher leukemia system, where a syngeneic leukemia is induced in the spleen and other organs [56]. Specific uptake of iodinated syngeneic antibody [56, 57], radiometal conjugated antibody [57, 58], and bismuth-labeled antibody [59] has been reported. A cyclohexylbenzyl DTPA modified Ig labeled with ^{212}Bi reduced tumor size at 3 weeks, when injected 8 days after tumor initiation, and doubled survival times [60]. ^{212}Bi-DOTA chelate constructs were also found to be pharmacologically stable in this murine erythroleukemia model [59] and in another T cell leukemia model [61]. The apparently good pharmacokinetics and therapeutic effects in these murine leukemia models have led to human trials (see below).

There has been intensive investigation of ^{211}At-labeled Ig and fragments in a human rhabdomyosarcoma meningitis model in athymic rats [62]. Single intrathecal doses resulted in significant prolongations of survival and possible cures [62]. This model has been used to develop a strategy for therapy of human carcinomatous meningitis that is expected to reach human trials in 1998. Similar work with a ^{211}At-labeled Ig reactive with melanomas and gliomas in xenograft mouse models also demonstrated specific uptake and adequate pharmacokinetics [4, 8].

Another strategy to deliver ^{211}At now being studied in animal models has relied on the labeling of small molecules, rather than the high molecular weight and purely diffusible Ig, that selectively accumulate in tumors, [2]. These have included ^{211}At-astato-benzylguanidine (MABG; in contrast to MIBG) for neuroblastoma [2] and ^{211}At labeled methylene blue for melanoma [63, 64]. Detailed discussion of these approaches is beyond the scope of this review.

Human clinical trials of alpha-emitting immunoglobulins

In 1996, the first human trials of an alpha-emitting radioimmunotherapeutic IgG construct, (^{213}Bi)CHX-A-DTPA-HuM195, began [25]. A genetically engineered, humanized IgG1 (HuM195), specific for a human myelogenous leukemia antigen (CD33) [65, 66], was used to investigate alpha particle therapy with ^{213}Bi. Conjugation of HuM195 to CHX-A-DTPA resulted in the attachment of up to ten ligand molecules per antibody, and the labeling efficiency with ^{213}Bi was typically more than 80% at specific activities of up to 740 MBq/mg (20 mCi/mg) [21, 67, 68]. The metal chelated HuM195 antibody construct was rapidly internalized into the cell in a time-dependent manner.

Preclinical testing of this radioimmunoconjugate in mice showed there was no uptake or loss of bismuth to mouse tissues, which do not express CD33, or to kidney, which has avidity for free, unbound bismuth. Mice injected i.p. with doses of [^{213}Bi]CHX-A-DTPA-HuM195 up to 740 MBq/kg (20 mCi/kg) showed no toxicity. Cumulative intravenous (i.v.) doses of [^{213}Bi]CHX-A-DTPA-HuM195 up to the 333–370 MBq/kg (9–10 mCi/kg) level showed very little toxicity, but 666 MBq/kg (18 mCi/kg) appears to be above the MTD for mice. Leukemia cell killing experiments with different specific activities of ^{212}Bi- or ^{213}Bi-labeled HuM195 showed dose- and specific activity-dependent killing of CD33+ HL60 cells. Both bismuth isotopes showed approximately 50% killing when two bismuth atoms were initially bound onto the target cell surface.

Based on the data in vitro, nine patients with relapsed (n = 8) or refractory (n = 1) acute myelogenous leukemia were treated with 10.4, 15.5, or 20.7 MBq/kg (0.28, 0.42, or 0.56 mCi/kg) [^{213}Bi]CHX-A-DTPA-HuM195 in three to six fractions over 2–4 days [25]. ^{213}Bi administered activities ranged from 555 to 1591 MBq (15–43 mCi) and HuM195 doses ranged from 1.6 to 4.4 mg. No acute toxicities and no extramedullary toxicity were seen, but myelosuppression lasting a median of 14 days was observed in six patients.

Uptake of ^{213}Bi in the bone marrow, liver, and spleen occurred within 10-min of administration and was maintained throughout the half-life of the isotope. No significant uptake was seen in any other organ [25, 27, 28]. Estimates of the absorbed doses delivered to the marrow ranged from 660 to 1220 cSv. Average doses to the spleen, liver, and blood ranged from 290 to 2200, from 240 to 1115, and from 110 to 530 cSv, respectively. These amounts were up to 4000 times higher than the estimated absorbed dose to the kidneys and 40000 times higher than that to the whole body. Five evaluable patients had transient reductions in peripheral blood leukemia cells and five patients also had decreases in the percentage of leukemia blasts in the bone marrow. This study is the first to show that targeted alpha particle therapy is feasible in humans.

Questions for the future

Despite significant advances in the theory and application of alpha-emitting radioconjugates, the path to their effective use in humans has been difficult. There remain several important unanswered questions:

1. Will widespread clinical application of either generator-derived or cyclotron-derived alpha-emitters be technically or economically feasible at therapeutic doses?
2. Will tumor control be achievable without significant second organ or marrow toxicity?
3. Will large, bulky tumors be successfully cleared by alpha-emitting radioimmunoconjugates, or will therapy be limited to minimal disease states?
4. Can alpha therapy be accomplished without long-term complications, such as renal damage or second neoplasms?

Based on the data available to date, we are persuaded that the next several years of clinical investigation will provide affirmative answers to each of these questions.

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