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An overview of targeted alpha therapy

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Abstract

The effectiveness of targeted α-therapy (TAT) can be explained by the properties of α-particles. Alpha particles are helium nuclei and are ~8,000 times larger than β^--particles (electrons). When emitted from radionuclides that decay via an α-decay pathway, they release enormous amounts of energy over a very short distance. Typically, the range of α-particles in tissue is 50–100 μm and they have high linear energy transfer (LET) with a mean energy deposition of 100 keV/μm, providing a more specific tumor cell killing ability without damage to the surrounding normal tissues than β^--emitters. Due to these properties, the majority of pre-clinical and clinical trials have demonstrated that α-emitters such as ^{225}Ac, ^{211}At, ^{212}Bi, ^{213}Bi, ^{212}Pb, ^{223}Ra, and ^{227}Th are ideal for the treatment of smaller tumor burdens, micrometastatic disease, and disseminated disease. Even though these α-emitters have favorable properties, the development of TAT has been limited by high costs, unresolved chemistry, and limited availability of the radionuclides. To overcome these limitations, more potent isotopes, additional sources, and more efficient isotope production methods should be addressed. Furthermore, better chelation and labeling methods with the improvements of isotope delivery, targeting vehicles, molecular targets, and identification of appropriate clinical applications are still required.

Keywords

Radioimmunotherapy; Alpha particles; Antibody; Actinium; Astatine; Bismuth; Lead; Radium; Thorium; Alpha targeted therapy; Alpha emitters

Introduction

Alpha and β^--particles, two kinds of particle radiation, were discovered by Rutherford in 1898 and were characterized as helium nuclei and electrons, respectively. Due to their ability to destroy tissue, these radiation particles were quickly applied to therapeutic applications such as radiation cancer therapy. One proposed approach was to deliver the killing power of these highly toxic radionuclides directly to tumor tissue via the appropriate targeting vectors, for instance, tumor antigen binding monoclonal antibodies (mAbs) and cell surface receptor binding peptides. This approach has continued to be a popular strategy and has been actively pursued for several years. Finally, as a result of these efforts, two radiolabeled antibodies

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Conflicts of interest None

targeting CD20 were approved by the FDA for treatment of non-Hodgkin's lymphoma (NHL). One was ^{90}Y-labeled ibritumomab tiuxeran (Zevalin) in 2002 and the other is ^{131}I-labeled tositumomab (Bexxar) in 2003.

Even though the clinical utility of α-particle has been traditionally perceived as being limited to clinical applications that permit rapid targeting and cellular uptake to address half-life limitations, interest in α-particle has continued to be a burgeoning area of interest. Treatment of accessible disease, intratumoral administration strategies, and direct targeting of tumor vasculature have all been promoted as means to counter both half-life limitations as well as to better match physical half-life with biological half-life. As a consequence, new radioimmunotherapy (RIT) approaches with α-particle emitters have been considered and developed by numerous researchers. While pre-clinical studies date back in time for decades, targeted α-therapy (TAT) first appeared in the clinical research literature in 1999 with the first patient being treated in 1995 [1]. The effectiveness of TAT can be explained by the properties of α-particles. Alpha particles are helium atoms that are about 8,000 times larger than β^--particles (electrons). When emitted from radionuclides that decay via an α-decay pathway, they release enormous amounts of energy over a very short distance. Typically, the range of α-particles in tissue is 50–100 μm. They have high linear energy transfer (LET) with a mean energy deposition of 100 keV/μm, resulting in a lower number of particle emissions required for cell kill linked, in part, to an inability of DNA double-strand break repair potential and lack of oxygen effects on cytotoxicity [2, 3]. Thus, the cytotoxicity of α-particles may be extremely effective and may also be more dose independent than β^--emissions with cell death occurring from a single or a few α-particle traversals of the cell nucleus [4, 5].

The growing interest of TAT has led to the development of new chelating agents because the stable sequestration of the radionuclide in vivo is a critical component of targeted radiation therapy. In vivo stability of radioimmunoconjugates makes therapy possible by the achievement of maximum delivery of radiation to tumor while minimizing toxicity. Radioimmunoconjugates can also be subject to the direct effects of daughter radionuclides and to catabolism in targeted cells after internalization. There are various chelating agents available dependent on the chemical nature and the coordination chemistry of the radionuclides (Figs. 1 and 2).

The clinical evaluation of RIT with α-emitters generally has been limited due to high costs of the radionuclide, unresolved chemistry, and limited availability of the radionuclide. With the elimination or reduction of many of these obstructions combined with a better understanding and new generation of monoclonal antibodies, numerous research groups have successfully evaluated many radiolabeled monoclonal antibodies for TAT.

There have been a number of α-emitting radionuclides considered for targeted therapy application. However, only a very limited number of α-emitting radionuclides on the list are realistically available for such applications due to either a too short or too long half-life, a lack of realistically viable chemistry, complicated decay pathways, or actual production/availability issues. For instance, ^{213}Bi, an α-emitting nuclide with a half-life of 46 min, has been introduced into the therapeutic field and has been evaluated in the clinic. However, the

short half-life of ^{213}Bi remains a major concern regarding its widespread use, limiting its delivery ability to only the readily accessible cancer cells, e.g., to leukemias, to actual tumor vasculature, or through intratumoral administrations. One resolution to this constraint is to deliver the α-emitter in the form of a generator to the target cell, allowing production of radionuclides in situ, such as ^{225}Ac (parent of ^{213}Bi) and ^{212}Pb (parent of ^{212}Bi), which will generate in vivo effective α-particles to eradicate tumor cells. This concept, “in vivo generators”, was later termed, “nanogenerators”. The major advantage of this approach is that the longer half-life parental isotopes may facilitate targeting to and extravasation into tumor tissue. The longer half-life of the parental radionuclides also permit all operations to be executed more efficiently than with the short half-life isotopes as well as than the potential delivery of the accumulated range of emissions associated with the decay chain at the tumor site for greater efficacy.

This review will focus on those α-emitters suitable for pre-clinical and clinical uses such as actinium-225 (^{225}Ac), astatine-211 (^{211}At), bismuth-212 (^{212}Bi), bismuth-213 (^{213}Bi), lead-212 (^{212}Pb), radium-223 (^{223}Ra), and thorium-227 (^{227}Th) (Table 1). New concepts, approaches, and techniques will be described as well. As yet, numerous review articles related to the development of TAT have been published and are readily available [6-21], and this discussion will again be principally limited to providing an update those reviews.

^{225}Ac

Properties, chemistry, and radiolabeling

Actinium was discovered by Andre Louis Debierne in 1899 and Friedrich Oskar Giesel in 1902 [22]. Actinium occurs naturally in association with uranium radionuclides and can be obtained from either the decay of ^{233}U or by the neutron transmutation of ^{226}Ra (Fig. 3) [23]. Actinium-225 is a radiometal with a half-life of 10 days and there are six radionuclide daughters in the decay path to a final stable state ^{209}Bi. For each decay event of ^{225}Ac, there are four α and two β^--emissions with high energy (8.38 MeV for α-decay). Given the 10-day half-life, the high energy α-particle emission, and the favorable rapid decay chain to stable ^{209}Bi, ^{225}Ac has been considered a potential candidate for use in cancer therapy, particularly in regards for use as an in vivo generator. However, the coordination chemistry of Ac (III) is not well defined primarily due to there being no stable isotopes to promote routine chemical studies. Therefore, use of ^{225}Ac as a therapeutic has been limited by the availability of suitable chelating agents stably binding the radionuclide as well as the daughter radionuclides generated by its decay [24, 25]. In addition, the chelating agent will be challenged by the recoil energy of α-particle emission (100–200 keV), which is higher than the binding energy than that of the environment defined by the coordination complex.

Chappell et al. developed a chelating agent, 2-(4-isothiocyanatobenzyl)-1,4,7,10,13,16-hexaazacyclohexadecane-1,4,7,10,13,16-hexaacetic acid (HEHA-NCS, Fig. 2), and radiolabeled three mAbs with ^{225}Ac [26]. HEHA is a macrocyclic chelating agent with six nitrogens and six carboxylic acids available as donors to its coordination sphere as well as a large macrocyclic cavity to match the large ionic radius of Ac(III). Due to its properties, HEHA was proposed as potentially useful for forming a complex with ^{225}Ac, but the study concluded that RIT with ^{225}Ac-HEHA was compromised by unstable coordination

chemistry and release of the daughter radionuclides in vivo. Another strategy was proposed by the group from the Sloan-Kettering Cancer Center, which was the use of ^{225}Ac envisioned as a “nanogenerator” approach [27]. In this study, McDevitt et al. used 2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (C-DOTA, Fig. 2) to form an ^{225}Ac complex which, after transport into the target cells, decayed and daughter isotopes were retained. This system was efficacious in vitro and in vivo for the targeted cells and limited the toxicity to non-target cells.

Pre-clinical studies

Neovascular endothelium is an interesting target for TAT. Tumor vasculature targeting is the rapid, efficient, and selective method of endoradiotherapy delivery to tumor endothelium by peptides, antibodies, antibody fragments, and nanoparticles with α-emitters [28, 29]. This endoradiotherapy directly at tumor vasculature may prove extremely proficient since the endothelium is immediately adjacent to blood which carries the radiolabeled compound so that radiolabeled compounds have no need to be highly diffusible through the tumor interstitium. E4G10, a mAb that targets vascular endothelial cadherin, was radiolabeled with ^{225}Ac and evaluated in mice bearing LNCaP prostate tumor cell xenografts [30]. Treatment with ^{225}Ac-E4G10 resulted in tumor growth inhibition, decreased serum prostate-specific antigen levels, and prolonged survival. This therapeutic benefit was, in turn, enhanced by administration of paclitaxel. Immunohisto-chemistry revealed decreased vessel density and enhanced apoptosis of the tumor. In addition, residual tumor vasculature appeared normalized without toxicity being observed in vascularized normal organs. E4G10 was also evaluated as a single wall carbon nanotube (SWCNT) conjugate constructs with ^{225}Ac-DOTA in a murine xenograft model of human colon adenocarcinoma (LS174T). Therapy using this “nano” assembly resulted in reduced tumor volumes and improved animal survival rates as compared to the experimental controls [31].

Retention of the daughter radionuclides generated by the decay of ^{225}Ac at the desired tumor site would be expected to increase efficacy, while conversely the release and redistribution of those same daughter radionuclides would be predicted to increase toxicity. Sofou et al. [32] proposed liposomal encapsulation of ^{225}Ac to retain the daughters at the target site, but only 14% of daughter radionuclides were retained at the target. In their following study involving multivesicular liposomes (MUVELs) evaluated to increase daughter retention [33], these researchers found that PEGy-lated MUVELs stably retained 98% of the encapsulated ^{225}Ac, as well as 31% of the ^{213}Bi daughter. MUVELs were also conjugated to an anti-HER2/*neu* antibody and evaluated in vitro with SKOV3-NMP2 ovarian cancer cells, showing significant cellular internalization (83%).

Sgouros’ group compared the efficacy of ^{225}Ac with ^{213}Bi and ^{90}Y in a mouse model bearing NT2.5 xenografts, a rat HER-2/*neu*-expressing mouse mammary tumor cell line [34]. In this study, anti-rat HER-2/*neu* monoclonal antibody (7.16.4) was conjugated to SCN-CHX-A″-DTPA (for ^{213}Bi and ^{90}Y) and *p*-SCN-Bn-DOTA (for ^{225}Ac). Treatment with ^{225}Ac-7.16.4 was found to eradicate the breast tumor, leading to long-term survival (1 year) of the mice. Use of ^{225}Ac was much more effective than either ^{213}Bi-7.16.4 (61 days) or ^{90}Y-7.16.4 (50 days). Dosimetry calculations indicated that the ^{225}Ac-treated tumor (9.6

Gy) received significantly higher dose than either ^{213}Bi (2.0 Gy) or ^{90}Y (2.4 Gy). Biodistribution studies suggested that only a small portion of the ^{225}Ac daughter radionuclides, ^{221}Fr and ^{213}Bi, were released from the target site.

Recently, there has been increasing interest in nanomaterials for use in the diagnostic and therapeutic fields. Woodward et al. [35] synthesized La(^{225}Ac)PO_4 nanoparticles conjugated to monoclonal antibody mAb 201B, which targets normal mouse lung endothelium. Based on biodistribution and imaging studies, ^{225}Ac and the associated daughter radionuclides rapidly accumulated in mouse lung after i.v. injection with 30% ID/lung (>200% ID/g) of La(^{225}Ac)PO_4 nanoparticles at 1 h p.i. in the lung. In addition, an in vivo release study of ^{213}Bi was performed and the data indicated a deficiency of ^{213}Bi activity relative to the equilibrium ^{213}Bi/^{225}Ac ratio in the lungs. At 24 h p.i., 80% of the ^{213}Bi, one daughter of ^{225}Ac decay, was retained in the target area, and the retention of ^{213}Bi increased to 87% at 120 h p.i. In vitro analysis demonstrated that 50% of the daughter radionuclides were sequestered in the La(^{225}Ac)PO_4 nanoparticles. One should also note that with this example of a targeted nanoparticle that access to and penetration into tumor was traversed by the targeting vehicle and target and that extravasation of nanoparticles into tumor remains a challenge to their use regardless of any attractions associated with nanotechnology. If the size of intact an IgG is considered an obstacle to delivery and penetration of solid tumors, then the use of nanoparticles, generally larger than antibodies, remains challenged by these same size limitations.

Clinical studies

There has been only one clinical trial using ^{225}Ac to date. HuM195, a humanized antibody, targets CD33 on acute myeloid leukemia cells and has been extensively investigated in clinical trials with β^--emitters and α-emitters [91, 92]. Studies with HuM195 were extended to ^{225}Ac due to its favorable properties for RIT, and based on pharmacokinetics, dosimetry, and toxicity studies of ^{225}Ac-HuM195 conducted with cynomolgus monkeys [36]. These studies resulted in a phase I clinical trial was started by the Scheinberg group at Memorial Sloan-Kettering Cancer Center and which is still recruiting patients [37]. The main goal of this trial was to define both safety and the maximum tolerated dose of ^{225}Ac-HuM195 for patients with advanced myeloid leukemia. The starting dose was 0.5 μCi/kg of ^{225}Ac-HuM195 with three to six patients being treated at each dose level, with dose escalation proceeding if less than 33% of patients in a cohort experienced dose limiting toxicity. Thereafter, six patients were planned to be treated at the maximum tolerated dose. Blood, urine, and bone marrow were to be measured after treatment to determine the toxicity, pharmacokinetics, immunogenicity, and anti-cancer effects.

^{211}At

Properties, chemistry, and radiolabeling

Astatine-211 is one of the most promising radionuclides for α-particle therapy, and notation of its potential medical utility dates back as far as 50 years [38]. Astatine is the heaviest halogen without a stable isotope and ^{211}At has a half-life of 7.2 h which provides enough time for distillation and radiolabeling. Also, the half-life is reasonably well matched to the

pharmacokinetics of a variety of molecular entities such as peptides, mAbs (intact and fragments), and small molecules. Astatine-211 emits two α-particles through a split decay pathway with energies of 5.87 and 7.45 MeV, a range in soft tissue of 55–80 μm, and an LET of 99 KeV/μm. One decay pathway is by direct α-particle emission to ^{207}Bi (42% of decay) followed by electron capture to the stable ^{207}Pb (Fig. 4). The other pathway is by electron capture to ^{211}Po (58% of decay) followed by α-particle emission to the ground state ^{207}Pb. In addition, the ^{211}Po daughter emits 77–92 KeV polonium K X-rays that enable external imaging, including SPECT, and γ counting for biodistribution studies, all additional advantages. Even though ^{211}At has very attractive properties for RIT, its widespread use as a clinical therapeutic has been seriously limited by availability and radiochemical purity. Astatine-211 is cyclotron produced by bombarding a bismuth target with 22–28.5 MeV α-particles via the ^{209}Bi(α, 2n)^{211}At nuclear reaction followed by the dry distillation to isolate ^{211}At in solution, e.g., chloroform or methanol [39, 40]. In order to minimize the production of ^{210}At, which decays to ^{210}Po with a half-life of 138 days and could lead to safety and toxicity issues, the beam energy window should be kept below 28.5 MeV. However, only a few institutions in the USA have cyclotrons available with an appropriate α-particle beam for production of ^{211}At and possess facilities for target processing.

Although astatine is generally treated as a halogen, it also has significant metallic characteristics in certain environments [41, 42]. The aryl carbon–halogen bond strength for astatine is significantly lower than that of iodine [8] which precludes using standard direct radio-iodination methods for labeling mAbs with ^{211}At. Such methods lead to labile products resulting in rapid in vivo loss of ^{211}At. To resolve this problem, approaches have been developed in several research groups based on small linker molecules that create an aryl carbon–astatine bond involving an astatodemetallation reaction using tin, silicon, or mercury precursors. Zalutsky et al. [39, 40] developed the two-step method for radiolabeling intact and mAb fragments. In the first step, *N*-succinimidyl 3-(trimethylstannyl)benzoate and the oxidant *tert*-butyl hydroperoxide were added into ^{211}At/chloroform followed by 15-min incubation and HPLC purification. The second step was addition of the protein to the purified product followed by 15-min incubation in an ice bath. The whole process takes 1.5 h making this approach practical for providing ^{211}At-labeled mAb with good specific activity, high immunoreactivity as well as with reasonable in vivo stability.

While generally dry distillation is used for ^{211}At purification, wet extraction technique has also been developed due to the demand for a high yield of ^{211}At [43]. In this technique, the irradiated bismuth target was dissolved in nitric acid, and the ^{211}At extracted by di-isopropylether (DIPE) instead of being distilled into a solution. The *N*-hydroxysuccinimidyl-*meta*-trimethylstannylbenzoate ester (MeSTB) has been used for radiolabeling with ^{211}At obtained by wet extraction methods [43, 59].

Pre-clinical studies

One leading center for TAT using ^{211}At has been Duke University. The Zalutsky group there has extensively studied production, radiolabeling chemistry and conducted pre-clinical studies with ^{211}At [2, 8, 40, 44, 45]. Recently, Zalutsky and co-workers evaluated the therapeutic effect of intrathecal administration of ^{211}At-labeled trastuzumab in rats bearing

MCF-7/HER2-18 breast carcinoma xenograft as a model for breast carcinoma carcinomatous meningitis (CM) [46]. CM is a form of metastatic cancer spreading to the subarachnoid space of the brain and spinal cord; most therapies are ineffective against CM. In this study, they found that median survival increased significantly versus the control group. For instance, the animals that received 33 and 66 μCi of ^{211}At-trastuzumab the median survival was 45 and 48 days, respectively, while for the saline and cold trastuzumab groups for the median survival was 21 days.

Investigators at the Göteborg University at Sweden are also actively involved in TAT using ^{211}At. Elgqvist et al. investigated the efficacy of targeted α-therapy of ovarian cancer in mice using ^{211}At-labeled mAb MX35 and MX35 F (ab′)$_2$ [47-49]. Animals inoculated intraperitoneally (i.p.) with OVCAR-3 cells received TAT treatment by i.p. injection. Two months after treatment, the mice were euthanized and the presence of macroscopic/microscopic tumors and ascites was determined. When tumor radius was ≤30 μm, the tumor-free fraction (TFF) of animals treated with ^{211}At-labeled-MX35 F(ab′)$_2$ was 95%. Compared to those treated with the non-specific antibody [Rituximab F(ab′)$_2$], the specific antibody, ^{211}At-labeled-MX35 F(ab′)$_2$ gave a significantly higher TFF when the tumor radius exceeded the range of the α-particles. This result was explained by a high mean absorbed dose from the activity bound to the tumor surface combined with the ability of the F(ab′)$_2$ to penetrate farther into the tumor as compared to the intact IgG [49]. Another efficacy study of an ^{211}At-labeled antibody was performed with a HER-2 positive ovarian carcinoma by Palm et al. [50], and the responses to single dose and fractionated treatment with unlabeled and ^{211}At-labeled trastuzumab were evaluated. Tumor-bearing mice were injected with ^{211}At-trastuzumab (400 kBq) followed by 5, 50, or 500 μg of trastuzumab 7 days later. Compared to 5 μg group, tumors were reduced by 87% with 50 μg of trastuzumab and completely eradicated in mice given 500 μg of trastuzumab.

The Wilbur group has investigated the application of α-particle emitting radionuclides as a replacement of total body irradiation (TBI) for hematopoietic cell transplantation (HCT) [51, 52]. Allogeneic HCT is an effective method for treating patients with hematopoietic disease; mAb labeled with β$^-$-emitting radionuclides had been previously studied for increasing specificity and efficacy with less toxicity from TBI. However, due to the long path length of their emissions and low dose rates, β$^-$-emitting radionuclides were not suitable for targeting hematopoietic cells as compared to α-emitting radionuclides. Biodistribution and toxicity studies were performed with radioimmunoconjugates of the antimurine CD45 antibody 30F11 labeled with ^{211}At and ^{213}Bi. The results indicated that lower doses of ^{211}At-30F11 were sufficient to achieve myelosuppression and myeloablation with less toxicities compared to ^{213}Bi-30F11 [52].

There are other research groups who have studied the efficacy of TAT with ^{211}At. Almqvist and co-workers targeted the A33 antigen, expressed on 95% of colorectal carcinomas, using ^{211}At-labeled humanized monoclonal antibody A33 (huA33) [53, 54]. At 8 h p.i., tumor had the highest uptake (~15% ID/g) than any other organ except blood. The tumor/blood ratio of ^{211}At-huA33 increased with time and reached 2.5 at 21 h p.i. In a dosimetry study, blood had the highest absorbed dose (8.9 Gy/MBq), and tumor received 6.2 Gy/MBq, which is higher than the normal organs besides the thyroid [54]. Another group from Sweden has

focused on targeted α-therapy with ^{211}At using chimeric mAb U36 in head and neck squamous cell carcinoma [55, 56]. Tumor uptake (10% ID/g at 21 h p.i.) increased with time while ^{211}At-U36 activity in other organs decreased excluding thyroid. In the therapy study, 18 of 20 mice responded to therapy, indicated by stabilized or decreased tumor volume [56].

The therapeutic efficacy of ^{211}At-labeled anti-CD25 [interleukin-2 receptor α (IL-2Rα)] monoclonal antibody, 7G7/B6 has also been evaluated as a potential therapeutic agent for CD25 expressing leukemias and lymphomas by the Waldmann group [57]. Their study demonstrated that the combining ^{211}At-7G7/B6 with daclizumab (unmodified anti-Tac) significantly improved therapeutic efficacy [58]. Mice were treated with 0.44 MBq of ^{211}At-7G7/B6 and 100 μg of daclizumab given weekly (0, 7, 14, and 21 days). The combination treatment resulted in 91% of mice surviving through 94 days, whereas only 36% and 50% of mice from the ^{211}At-7G7/B6 and daclizumab groups, respectively, were alive at 94 days.

Clinical studies

A limited number of clinical trials have evaluated the therapeutic potential of ^{211}At. The study with ^{211}At-ch81C6, a chimeric antibody targeting tenascin, a glycoprotein over-expressed in gliomas, was extended to clinical trials. Zalutsky et al. investigated the feasibility and safety of this therapy in patients with recurrent malignant brain tumors [60, 61]. Eighteen patients were treated with ^{211}At-ch81C6 administered into a surgically created resection cavity (SCRC). No patient presented with dose-limiting toxicity and 96.7% of ^{211}At decays occurred inside the SCRC. No toxicities of grade 3 or higher were derivable from the treatment and no patient required repeat surgery for radionecrosis. Eight of 14 patients with recurrent glioblastoma multiforme survived for a year and two patients survived for 3 years after treatment with 144 MBq of ^{211}At-ch81C6. This study clearly demonstrated that the regional administration of ^{211}At-ch81C6 was attainable, safe, and associated with a possible therapeutic benefit for patients with recurrent central nerve system tumors.

Another clinical trial has been initiated at the University of Gothenburg, Sweden. Andersson and co-workers investigated the pharmacokinetics and dosimetry of ^{211}At-MX35 F(ab′)$_2$ [62]. Women with recurrent ovarian carcinoma were enrolled in this phase I study. Nine patients were infused with ^{211}At-MX35 F(ab′)$_2$ via peritoneal catheter, and the result has demonstrated that ^{211}At-MX35 F(ab′)$_2$ by intraperitoneal administration is feasible to achieve therapeutic doses in microscopic tumor clusters without significant toxicity.

^{212}Bi

Properties, chemistry, and labeling

Bismuth-212 can be obtained from the decay chain of ^{228}Th. It has a half-life of 60.6 min and decays to stable ^{208}Pb via either ^{208}Tl (36%) or ^{212}Po (64%) (Fig. 5). Both pathways emit β$^-$-particles and α-particles with an average energy of 7.8 MeV and path length in tissues of 40–100 μm, localizing cellular toxicity in a small region. The combination of α- and β$^-$-particles may help to increase the therapeutic efficacy for tumor heterogeneity.

However, the short half-life of ^{212}Bi has in part been a major limitation for radiolabeling processes and RIT applications. This problem was partly resolved by the availability of a ^{224}Ra generator which makes local production of ^{212}Bi feasible [63, 64]. Still, applications including i.v. injection of mAb remain challenging with the short physical half-life of ^{212}Bi. Another serious perceived challenge to the clinical evaluation of ^{212}Bi has been the 2.6 MeV γ-ray originating from the ^{208}Tl daughter produced in ^{212}Bi decay chain, which requires significant shielding to minimize radiation exposure to health care workers, making it unclear what level of shielding is needed for a clinical environment due to the combination of actual dosing logistics, short half-life, and safety concerns.

Bismuth-212 can be reasonably bound by bifunctional metal chelating agents such as derivatives of functionalized diethylenetriamine pentaacetic acid (DTPA, Fig. 1) conjugated to mAbs, their fragments, and peptides including the cyclic DTPA anhydride derivative, 2-(*p*-isothiocyanatobenzyl)-DTPA (SCN-Bz-DTPA), isothiocyanatobenzyl derivative of C-functionalized *trans*-cyclohexyl DTPA (CHX-A″-DTPA), and 2-(*p*-isothiocyanatobenzyl)-6-methyl-DTPA (Mx-DTPA, 1B4M-DTPA). However, only the CHX-A″-DTPA forms suitably stable complexes with bismuth radionuclides in vivo [65, 66]. DOTA has also been successfully used for labeling mAbs with ^{212}Bi and demonstrated in vivo stability in an animal model [67, 68]. However, the slow formation kinetics of radiolabeling DOTA with ^{212}Bi seriously compromises the use of this chelation technology. As such, there have been no recent pre-clinical studies reported in the literature using ^{212}Bi with this Bi(III) radionuclide effectively being supplanted by ^{213}Bi.

^{213}Bi

Properties, chemistry, and labeling

Bismuth-213 is a decay product of ^{225}Ac making it generator available and ^{213}Bi decays via a branched pathway by α-particle emission with an energy of 8.37 MeV to ^{209}Bi (Fig. 3). Bismuth-213 has a half-life of 45.6 min, shorter yet than ^{212}Bi; however, ^{213}Bi additionally emits a 440-keV γ-ray (26.5%) allowing imaging, biodistribution, pharmacokinetics, and dosimetry studies to be performed and, more importantly, also lacks the high energy γ-ray of ^{212}Bi. Bismuth-213 for clinical use requires a generator due to the short half-life, and currently, the Oak Ridge National Laboratory produces the only ^{225}Ac/^{213}Bi generator in the USA. Primarily, methods to separate ^{213}Bi from ^{225}Ac were introduced by Boll et al. [69, 70]. The ^{225}Ac/^{213}Bi generator has since been further developed by Memorial Sloan-Kettering Cancer Center in order to supply carrier-free ^{213}Bi [71-74]; however, the initial generator design dates to Pippin et al. [75]. After elution from the generator, ^{213}Bi is readily used for radiolabeling chelate conjugated mAbs, peptides, or other vectors without significant safety measures or shielding required other than gloves and monitoring devices. Similarly to ^{212}Bi, the bifunctional chelating agent, CHX-A″-DTPA (Fig. 1), can readily attach covalently to protein molecules and has been proven to be the agent of choice for sequestering radio-bismuth stably in vivo [65, 66, 76].

Pre-clinical studies

The Allen group in Australia has actively studied the efficacy of ^{213}Bi-labeled mAb as TAT agents [77-81]. The mAb 9.2.27 has a high specificity for human melanoma cell surfaces. The biodistribution of ^{213}Bi-labeled mAb 9.2.27 showed no evidence of retention of the α-radioimmunoconjugate in non-targeted organs. Tumor growth was completely inhibited at 2 days post-inoculation. In conclusion, the targeted α-therapy was effective for the treatment of micrometastatic melanoma when the tumor was in a pre-angiogenic stage. In addition, a multiple dose treatment regimen was more effective than a single dose [77].

The toxicity and efficacy of ^{213}Bi-labeled plasminogen activator inhibitor type 2 (PAI2) in a prostate cancer model has been examined [79]. In this particular study, tumor growth was inhibited following the administration of single doses of either 947 or 1,421 MBq/kg at 3 days post-tumor inoculation. Complete tumor growth inhibition was also observed when a total dose of 947 MBq/kg was given on five successive days. Treatments given at 6, 12, and 18 days post-tumor inoculation showed significantly slower tumor growth than the controls. Additionally, all treatments were tolerated in mice and rabbits as monitored by biochemical and hematological studies; multiple doses were no more toxic than the single dose regime [79].

This group also evaluated ^{213}Bi-labeled C595 anti-MUC1 mAb for control of ascites in an ovarian cancer ascites mouse model; MUC1 is over-expressed in ovarian cancer tissues [80]. A single dose of 355 MBq/kg of ^{213}Bi-labeled C595 increased survival by 25 days when administered 9 days after tumor implantation. The maximum tolerated dose was more than 1,180 MBq/kg with monitoring up to 21 weeks. This study demonstrated that ^{213}Bi-C595 can effectively target and kill ovarian cancer cells with acceptable levels of toxicity.

The Sgouros group at Johns Hopkins has focused on the targeting of metastatic breast cancer [82, 83]. They have investigated the efficacy of ^{213}Bi-labeled anti-rat HER-2/*neu* mAb 7.16.4 in a rat/*neu* transgenic mouse model for metastatic mammary carcinoma. The maximum tolerated dose was 120 μCi; treatment at this dose prolonged median survival to 41 days as compared to 28 days for treatment with the control antibody. Overall, ^{213}Bi-7.16.4 was effective for treating early stage HER-2/*neu* metastases [82].

Liposomes have been studied for several decades as vehicles for drug delivery, and incorporating polyethylene glycol (PEG) chains has made it possible to effectively increase their circulatory half-life. Lingappa and co-workers radiolabeled a liposome-CHX-A″-DTPA conjugate with ^{213}Bi and compared the efficacy of that product to a liposome-CHX-A″-DTPA-^{213}Bi-7.16.4 and ^{213}Bi-7.16.4 in rat HER2/*neu* transgenic mice. Based on the survival times, the ^{213}Bi-labeled liposome was as effective as antibody targeted ^{213}Bi in this animal model [83].

Park et al. were interested in evaluating a pre-targeting system using antibody–streptavidin (Ab-SA) constructs and radiolabeled biotin [84]. In this study, mice with Ramos lymphoma cells were given anti-CD20 1F5(scFv)$_4$SA fusion protein. A dendrimeric clearing agent and ^{213}Bi-DOTA-biotin were then injected as per protocol. Tumor uptake was 16.5±7.0% ID/g 90 min p.i. compared to 2.3±0.9% ID/g for the control. Tumor growth was significantly

delayed by treatment with 1F5(scFv)$_4$SA and ^{213}Bi-DOTA-biotin (600 μCi). The median survival time was 90 days compared to 23 days for the control. In addition, the treatment was well tolerated with no significant toxicity reported.

Locoregional RIT of disseminated gastric cancer in mice using ^{213}Bi showed good therapeutic results with a single dose depending on the time interval between tumor inoculation and therapy. Bloechl et al. compared single versus double i.p. injections of mAb d9MAb labeled with ^{213}Bi for efficacy and toxicity [85]. Single and double applications of 0.37, 0.74, or 1.48 MBq of ^{213}Bi-d9MAb were injected into mice at 1 and 8 days after tumor inoculation. Double doses of 0.37 MBq significantly prolonged the median survival as compared with a single dose. In an advanced stage of the disease, double injection of 0.74 MBq was much more effective than a single dose of 1.48 MBq without any toxicity.

Milenic et al. has investigated the dual targeting efficacy of targeted α-therapy using trastuzumab, which binds to HER-2 receptor and humanized CC49 mAb (HuCC49ΔCH2), which binds to tumor-associated glycoprotein 72 (TAG-72) [86]. A therapy study was performed in which 500 μCi of ^{213}Bi-trastuzumab and ^{213}Bi-HuCC49ΔCH2 was injected (i.p.) alone and concurrently to mice with 3-day tumor burden. Sequential administration, ^{213}Bi-trastuzumab followed by ^{213}Bi-HuCC49ΔCH2 or vice versa, resulted in greater therapeutic efficacy than single dose and produced a median survival of 36 days and 40 days, respectively. The highest therapeutic efficacy was achieved with the concurrent administration of ^{213}Bi-trastuzumab and ^{213}Bi-HuCC49ΔCH2. The median survival was 147 days, which translates to a therapeutic index of 9.8.

The short half-life of ^{213}Bi (45.6 min) is well matched with the peptide-receptor radionuclide α-therapy. Wild and co-workers recently prepared two ^{213}Bi-labeled peptides, DOTA-PEG4-bombesin (DOTA-PESIN) and DO3A-CH_2CO-8-aminooctanoyl-QWAVGHLM-NH_2 (AMBA), and compared them with ^{177}Lu-DOTA-PESIN in a human prostate carcinoma xenograft model (PC-3 tumor) [87]. The maximum tolerated dose of ^{213}Bi-DOTA-PESIN and AMBA was 25 MBq (5×5 MBq) while ^{177}Lu-DOTA-PESIN was 112 MBq (4×28 MBq). With this dose level, ^{213}Bi-labeled peptides were clearly more efficient than ^{177}Lu-DOTA-PESIN. For example, an additional injection of ^{213}Bi-labeled peptides reduced the risk of recurrent disease by 57%, whereas ^{177}Lu-DOTA-PESIN treatment showed no effect on abatement of recurrent disease. Moreover, the median survival time with ^{213}Bi-labeled peptides was extended by 15 weeks, five times greater than that obtained from ^{177}Lu-DOTA-PESIN treatment.

Clinical studies

With the replacement of ^{212}Bi with ^{213}Bi, a number of clinical trials have been initiated and executed. The therapeutic efficacy of α-emitting ^{213}Bi on B-cell-lineage lymphoma has been investigated with various antibodies. In pre-clinical experiments, ^{213}Bi-labeled anti-CD20-CHX-A″-DTPA showed a strong cytotoxic potency for targeting lymphoma cells. This radioimmunoconjugate also demonstrated a favorable in vivo stability and biodistribution pattern. No other toxic effects occurred beside the induced myelosuppression [88]. Consequently, Heeger and co-worker at German Cancer Research Center started a phase I dose escalation trial to determine toxicity and feasibility as well as dosimetry and

pharmacokinetics [89]. Nine patients with B-cell malignancy were enrolled and received doses of ^{213}Bi-anti-CD20 from 385 to 1,640 MBq. No acute or extramedullary toxicity were observed except a mild leukopenia (two patients). Two patients responded to radioimmunotherapy. Currently, this trial has been continued at the University Hospital Düsseldorf, Germany.

An open-label phase I dose escalation study of ^{213}Bi-labeled mAb 9.2.27 for metastatic melanoma was initiated by the Allen group [90]. Twenty-two patients with stage IV/in-transit metastasis were treated with 55–947 MBq of ^{213}Bi-9.2.27. Overall, 6% demonstrated almost complete response, 14% showed partial response, 50% stable disease and 30% progressive disease. Most of the patients showed reductions of disease in 8 weeks based on the tumor marker melanoma inhibitory activity protein. No toxicity was detected during the dose escalation study.

A humanized anti-CD33 mAb, HuM195, targeting myeloid leukemia cells, radiolabeled with ^{213}Bi was translated to a phase I dose-escalation trial at Memorial Sloan-Kettering Cancer Center [91]. Eighteen patients with advanced myeloid leukemia were treated with 10.36 to 37.0 MBq/kg of ^{213}Bi-HuM195. No significant toxicity was detected. Uptake of ^{213}Bi, determined by γ-camera imaging, was found to be mainly in the bone marrow, liver, and spleen without significant uptake in other organs. Absorbed dose ratios between bone marrow, liver, and spleen and the whole body were 1,000 times higher with ^{213}Bi-HuM195 than with β^--emitters labeled HuM195. Fourteen of 18 patients had reductions in the percentage of bone marrow blasts, and 14 patients had reductions in circulating blasts after therapy. This study is the first proof-of-concept for systemic targeted α-particle immunotherapy in humans.

A phase I/II trial followed to determine the maximum tolerated dose and effects of ^{213}Bi-lintuzumab (HuM195) by Jurcic et al. at Memorial Sloan-Kettering Cancer Center [92]. Thirteen patients newly diagnosed and 18 patients with acute myeloid leukemia were enrolled and treated with cytarabine continuous infusion for 5 days. Doses of ^{213}Bi-lintuzumab (18.5–46.25 MBq/kg) were then injected over 1 to 2 days. Myelosuppression was the primary toxicity, and the maximum tolerated dose was 37 MBq/kg. Two of 21 patients treated with the maximum tolerated dose died. At all dose levels, marrow blasts reductions were observed. Based on biodistribution and pharmacokinetic studies, CD33 sites were saturated by ^{213}Bi-lintuzumab after partial cytoreduction by cytarabine.

Substance P is tachykinin peptide neurotransmitter which targets the neurokinin type-1 receptor (NK-1), and NK-1 is consistently over-expressed in World Health Organization (WHO) grade II–IV gliomas. The Merlo group conjugated 1,4,7,10-tetraazacyclododecane-1-glutaric acid-4,7,10-triacetic acid (DOTAGA) to substance P and was then performed a pilot trial of TAT using ^{213}Bi [93, 94]. In this pilot study, five patients with WHO grades II–IV were enrolled and the treatments were given via an implanted catheter system (intratumoural injection). Four patients received 1.07–2.0 GBq of ^{213}Bi-DOTA-substance P within one therapeutic cycle, and one patient received 7.36 GBq with four therapeutic cycles. Lengthy retention of ^{213}Bi-DOTA-substance P was observed at the target site, and no local or systemic toxicity was detected according to the NCI Common

Toxicity Criteria (CTC) scale. The pre-therapeutic functional score (the Barthel Index) was 75 and 80 with the two glioblastoma multiforme (GBM) which means mild restrictions in activities of daily living. After treatment, both patients improved to 90 out of 100. MR imaging demonstrated that there were radiation-induced necrosis and demarcation of the tumors [94].

^{212}Pb

Properties, chemistry, and labeling

Lead-212 is a product of the ^{228}Th decay chain and also can be generated by the previously mentioned ^{224}Ra generator that again facilitates on-site production [63]. Pb-212 (10.6 h half-life) is a β^--emitter and is the parent of ^{212}Bi (Fig. 5). One strategy which has been proposed and investigated has been to label a mAb with ^{212}Pb and use this conjugate again as an in vivo generator for ^{212}Bi to effectively traverse the problem of short half-life ^{212}Bi [95]. Several lead and bismuth complexes have been studied for use in this in vivo generator system [96-98].

There are distinct advantages of targeting tumor with ^{212}Pb over ^{212}Bi and ^{213}Bi. Pb-212 can deliver more than 10 times the dose per unit of administered activity as compared to either ^{212}Bi or ^{213}Bi. In addition, the 10.6 h of half-life is very reasonable for dose preparation and administration. However, there is major complication associated with radiolabeling a mAb with ^{212}Pb due to the electron capture and Auger electrons which may compromise the chelating moiety. Many bifunctional DOTA derivatives have been used for labeling antibodies with ^{212}Pb, but the Pb(II)–DOTA complex has been shown to permit dissociation of Pb(II) at a pH comparable to that in lysozymes post-internalization into cells as compared with the tetra amide derivative, 1,4,7,10-tetraaza-1,4,7,10-tetra-(2-carbamoyl methyl)-cyclododecane (TCMC, Fig. 2) [99]. The subsequently free ^{212}Pb has been reported as a source of bone marrow toxicity [100]. Therefore, TCMC has become the chelation chemistry of choice for sequestrating ^{212}Pb.

Pre-clinical studies

Milenic and co-workers have proposed and demonstrated the effective use of ^{212}Pb-labeled trastuzumab for the treatment of disseminated peritoneal disease [101]. In studies using mice bearing LS-174T i.p. xenografts, the maximum tolerated dose was 0.74–1.48 MBq, and the median survival of animals with 0.37 MBq was prolonged from 19 to 56 days. A multi-dose regime of ^{212}Pb–TCMC–trastuzumab administered monthly (up to 3 months) increased the median survival of mice bearing 3-day LS-174T i.p. xenografts to 110 days.

This group also performed a combination study of ^{212}Pb–TCMC–trastuzumab and gemcitabine (GEM) for treating disseminated peritoneal disease [102]. Treatment with GEM followed 24–30 h later by 0.19 or 0.37 MBq showed an improvement of median survival from 31 (without GEM) to 51 (with GEM) days at the 0.19 MBq, and from 45 (without GEM) to 70 (with GEM) days at the 0.37 MBq compared with 16 days for the untreated group. Three weekly treatments of GEM with ^{212}Pb prolonged the median survival to 90 days as compared with 21 days for the untreated mice. Treatment with two cycles of 0.37

MBq of ^{212}Pb–TCMC–trastuzumab with two doses of GEM achieved the greatest therapeutic efficacy with a median survival of 196.5 days.

Milenic et al. have also evaluated the efficacy of the combination treatment of paclitaxel with ^{212}Pb–TCMC–trastuzumab [103]. Paclitaxel was given 24 h prior to, concurrent with, or 24 h post-administration of ^{212}Pb–TCMC–trastuzumab to study the optimal order for administration. Enhanced therapeutic efficacy was found in the group receiving 600 μg of paclitaxel administered 24 h before the RIT. The median survival increased from 44 to 171 days compared with the group that received ^{212}Pb–TCMC–trastuzumab alone.

The strategy of antibody pre-targeting has been developed to address the limitations of antibodies in the delivery of radiation to tumor [104, 105]. Su et al. evaluated the potential of antibody pretargeting therapy with ^{212}Pb as an in vivo generator of ^{212}Bi for α-particle radiotherapy [106]. In this study, NR-LU-10 antibody–streptavidin conjugate was administrated first followed 20–24 h later by the *N*-acetyl-galatos-amine-biotin clearing agent. Thereafter, ^{212}Pb/^{212}Bi-DOTA-biotin was injected 6 h after. Their results demonstrated that the tumor uptake of DOTA-biotin labeled with ^{212}Pb and ^{212}Bi was higher than 25% ID/g and remained at that level for up to 24 h p.i while the non-targeted radioactivity was rapidly cleared via the renal excretion route. Consequently, biodistribution data showed the high kidney uptake at an early time point (14.5% ID/g at 2 h p.i.).

Radiolabeled peptides have also been evaluated as potential therapeutic agents for TAT during the last decade. The advantages of using radiolabeled peptides over antibodies are simple chemical synthesis, easy labeling, fast uptake, rapid clearance, uniform distribution into tissues, and a lower potential of immunogenicity; however, that same rapid clearance can also be a source of renal toxicity as well as an economic drain of an expensive radionuclide. Due to their properties, some peptides, RGD, octreotide, α-melanocyte-stimulating hormone analogues, and bombesin derivatives, are feasible for targeted α-therapy [107]. Miao et al. investigated the therapeutic efficacy of the melanoma targeting rhenium-cyclized peptide [Re(Arg^{11})CCMSH] radiolabeled with the ^{212}Pb/^{212}Bi in vivo generator on B16/F1 murine melanoma bearing C57 mice [108]. In this study, treatment with 50, 100, and 200 μCi of ^{212}Pb[DOTA]-Re(Arg^{11})CCMSH significantly decreased tumor growth rates and extended the mean survival times to 22, 28, and 49.8 days, respectively, as compared with 14.6 days of the untreated control group. ^{212}Pb[DOTA]-Re(Arg^{11})CCMSH exhibited a rapid tumor uptake and prolonged retention with rapid clearance. Consequently, only minimal amounts of measurable ^{212}Bi were released.

Clinical studies

Due to the Brechbiel group's pre-clinical data of ^{212}Pb–TCMC–trastuzumab [101-103], the first phase I clinical trial employing ^{212}Pb has been sponsored by Areva Med LLC. This phase I trial, opened at the University of Alabama, Birmingham in 2011, is designed to determine the toxicity profile of ^{212}Pb–TCMC–trastuzumab, its dose-limiting toxicities, and its anti-tumor ability in patients with HER-2 positive intraperitoneal cancers, specifically in the area of ovarian cancer. The number of participants with adverse events after treatment of ^{212}Pb–TCMC–trastuzumab will be measured as an indicator of dose-limiting toxicity. The human immune response against ^{212}Pb–TCMC–trastuzumab via i.p. infusion will also be

characterized. Anti-tumor effects will be monitored by physical examination, radiographic imaging, and tumor marker studies. Furthermore, the plasma pharmacokinetics and excretion mechanism of radioactivity from the peritoneal cavity will be determined by γ-camera imaging.

^{223}Ra and ^{227}Th

Properties, chemistry, and labeling

Radium-223 can be produced from a ^{227}Ac generator [109] and is also available from uranium mill tailings in large quantities [110]. Radium-223 has a half-life of 11.4 days and emits four α-particles and two β^--particles in its decay pathway to stable ^{207}Pb (Fig. 6). There are advantages and disadvantages with these four α-particles. From a therapeutic point of view, having four α-particles available to provide a therapy dose is an advantage. On the other hand, they may be a disadvantage from the radiolabeling and toxicity point of view For example, ^{219}Rn, the gaseous decay product daughter of ^{223}Ra, can redistribute in the body and damage the non-target tissues, particularly from the subsequent decays of that radionuclide. Thus, development of a suitable ligand for in vivo sequestration of radium remains an area of opportunity since those ligands currently in use have simply been adopted from other applications or for chelating other radionuclides. Even though various chelating agents have been evaluated, development of Ra(II) chelation chemistry for in vivo therapy applications has been held back considerably due to the lack of stable isotopes with which the chemistry can be evaluated.

^{223}Ra has been known for a potential candidate for delivery of radiation to cancer cell on bone surface due to its propensity for bone and has been extensively investigated in pre-clinical studies. As a consequence, Alpharadin (^{223}Ra chloride) was developed by the Norwegian company Algeta ASA partnered with Bayer Schering Pharma AG and has a natural affinity for bone metastases with the potent and localized tumor cell killing ability of α-particle emission, which leads to minimal damage of non-targeted tissues. Currently, Alpharadin is in several phase II and III clinical trials as a potential α-radiation treatment for patients with bone metastases associated with cancers such as prostate and breast [111-113].

Thorium-227 (Fig. 6) has a half-life of 18.7 days and can be obtained continuously from ^{227}Ac, which in turn can be generated by thermal neutron irradiation of ^{226}Ra [114]. Due to a long half-life, ^{227}Th may be used to radiolabel, administrate, and target the tumor before a significant amount of ^{223}Ra is generated. Even though a considerable amount of ^{223}Ra is accumulated in the bone, this may not cause bone marrow toxicity due to the short range of the α-particle [115]. Therefore, there may be an opportunity for use of this radionuclide as a therapeutic with reasonable toxicity incurred. Furthermore, the low γ-components greatly reduce the necessity for patient shielding.

The therapeutic candidate, ^{227}Th, was successfully conjugated to two mAbs, rituximab and trastuzumab using the bifunctional chelator, *p*-SCN-Bn-DOTA by Dahle and co-workers [116, 117]. The immunoreactivity, serum and in vivo stability, antigen-binding ability, and the effect on in vitro cell growth as well as in vivo biodistribution were then explored. Overall, ^{227}Th-labeled radioimmunoconjugates demonstrated a relevant stability in serum

and in vivo, with a significant antigen-dependent inhibition of cell growth. The overall radiolabeling yield was 17% and the immunoreactive fraction of ^{227}Th–DOTA–rituximab was 56–65%.

Pre-clinical studies

Norwegian Radium Hospital in Norway is the leading center for targeted therapy using ^{227}Th. As mentioned above, the Dahle group has developed the novel low-dose rate α-emitting radionuclide ^{227}Th on rituximab conjugated to DOTA, and tested the efficacy in mice bearing Raji lymphoma xenografts [118]. Treatment with 200 to 1,000 kBq/kg demonstrated delay in tumor growth and prolonged mean survival as compared with the control without any serious toxicity. A significant increase in growth delay with a dose of 1,000 kBq/kg was achieved compared to 200 kBq/kg (from 40 days to 17 days), whereas the median survival showed an inverse result (from 75 days to 119 days). Overall, the low-dose rate strategy using ^{227}Th seems to be feasible against macroscopic tumor and single tumor cells.

The relative biologic effects (RBE) of α-radiation from ^{227}Th–rituximab have been also investigated by Dahle et al. [119]. In this study, Zevalin and external beam radiation were used for comparison purposes. Tumor growth was measured two to three times a week after injection of ^{227}Th–rixuximab, Zevalin, or external beam radiation. For the first week, the absorbed radiation dose rate in tumor reached 0.1 Gy/day and then gradually decreased to 0.03 Gy/day at 21 days. With Zevalin, the maximum dose rate (0.2 Gy/day) was obtained 6 h p.i. which then decreased to 0.01 Gy/day after 7 days. In terms of RBE, treatment with ^{227}Th–rituximab was 2.5 to 7.2 times more effective in inhibiting tumor growth than external beam radiation while the Zevalin treatment was 1 to 1.3 times more effective external beam radiation.

^{227}Th–rituximab has been investigated for long-term radiotoxicity to evaluate possible side effects [120]. Various doses, saline, cold rituximab, or 50, 200, and 1,000 kBq/kg ^{227}Th–rituximab, were injected into tumor-bearing animals and observed for 1 year. Mice with Raji lymphoma xenografts were included in this study as well. Mice treated with 1,000 kBq/kg experienced weight loss as well as a decrease in WBC and platelet count. Therefore, the no-observed-adverse-effect level (NOAEL) was 200 kBq/kg. The maximum tolerated activity was 600 to 1,000 kBq/kg, and the maximum tolerated dose for bone marrow was 2.1 to 3.5 Gy. Based on these results, the dose-limiting toxicity was determined to be bone marrow suppression.

Heyerdahl et al. evaluated the cytotoxicity of low-dose rate ^{227}Th–DOTA–trastuzumab for treating HER-2 positive breast and ovarian cancer [121]. In this study, three HER-2 positive cell lines were investigated, the breast cancer cell lines BT-474 and SKBR-3 and the ovarian cancer cell line SKOV-3. MCF-7 (HER-2 negative breast cancer cell line) was included as a control. SKBR-3 cells received 50% of the mean absorbed radiation dose from internalized activity and 50% from cell surface bound activity, whereas BT-474 and SKOV-3 cells got 75% of the radiation dose from internalized activity and 25% from cell surface bound activity. BT-474 cells treated with 2.5 kBq/ml ^{227}Th–trastuzumab showed a mean absorbed radiation dose of 2 to 2.5 Gy. Cell growth inhibition and apoptosis were induced in all cell

lines at the clinically relevant activity concentration. The cytotoxic effect was higher than that of external beam radiation (relative biological effectiveness=1.2).

Clinical studies

As noted above, Alpharadin (^{223}Ra chloride) is currently in several phase II and III clinical trials as a potential α-radiation treatment agent and has also been evaluated in two open-label phase I trials and three double-blind phase II trials for castration-resistant prostate cancer (CRPC) and bone metastases. Based on trial data (37 patients for phase I and 255 patients for phase II), Parker et al. released the hematologic and safety profile [122]. Out of 292 patients treated with ^{233}Ra, less than 1% experienced common toxicity criteria (CTC) grade 4 hematologic toxicity, 4% showed grade 3 anemia, and less than 3% had grade 3 toxicity for platelets, neutrophils, or WBC. Mild reversible neutropenia was found after repeated ^{223}Ra treatments (25, 50, 80 kBq/kg given every 4 or 6 weeks). There was no sign of renal or hepatic toxicity. More adverse events and serious adverse events were observed in the placebo group than in the ^{223}Ra-treated group in phase II trials. In the phase I trials, there was no dose-limiting toxicity. Consistent improvement in disease-related biomarkers and pain, and a highly favorable safety profile was observed. In one trial, the median overall survival increased by 4.5 months with ^{223}Ra treatment as compared with placebo group.

Prospects and conclusions

Compared with β^--emitters, α-particle emitters have a shorter range and higher LET. Due to these properties, the majority of pre-clinical and clinical trials have demonstrated that α-emitters are ideal for the treatment of smaller tumor burdens, micrometastatic disease, and disseminated disease where in fact the α-emitters could be efficiently delivered. As such, the rational matching of disease presentation with realistic accessibility and delivery is a key criterion to their successful use in a clinical setting. Even though α-particle emitters have favorable properties, the development of TAT has been limited by high costs, unresolved chemistry, and limited availability of the radionuclides. To overcome these limitations, new sources and better production methods of medical grade radionuclides are issues that continue to remain unresolved. Current supplies for some α-emitters, especially for those radionuclides associated with the ^{225}Ac and ^{224}Ra decay pathways, remain limited by naturally isolated by-products from weapons development within the USA with the parental feedstock being slated for disposal. Currently, only two sources are available for “clinical grade” ^{225}Ac, Oak Ridge National Laboratory in Oak Ridge, TN, USA, and the Institute for Transuranium Elements in Karlsruhe, Germany. Production of ^{211}At is also hampered by production limits both in amounts and feasible locations due to cyclotron energy constraints. Besides the immediate production limits, assembly and transport of generators of sufficient activity amounts to support multiple clinical trials really have yet to be produced at adequate levels. Furthermore, the costs of these radionuclides have continued to increase over the past decade, which poses a serious obstacle that challenges both those researchers who currently work on or are entering this area of research.

There are still remaining issues associated with “nanogenerators” even though the strategy of the long half-life parental radionuclides serving as in vivo generators for the delivery and

production of desired therapeutic daughter radionuclides effectively extends their respective short half-life radionuclides. Attempts to sequester daughter radionuclides have led to the creation of larger and larger constructs. As a consequence of such strategies, this comes at a cost associated with limits or compromises to the use of efficient targeting systems combined with then limitations imposed on both penetration and extravasation into tumor. In addition, the in vivo stability and metabolism of these constructs is not altogether well defined, and radiologic side effects are observed which are likely to be mediated by release of daughter radionuclides from the chelate and construct.

From a chemistry point of view, there are still challenges remaining regarding the coordination and conjugation chemistry for the multiple decay pathway radionuclides. More efficient conjugation and radiolabeling methods need to be developed that lead to stable products with higher specific activities to maximize therapeutic efficacy. Better pharmacokinetics and dosimetry studies are also required that actually address the cellular micro-dosimetry aspect of targeted α-emitters since their targets exist at the individual cell level for therapy.

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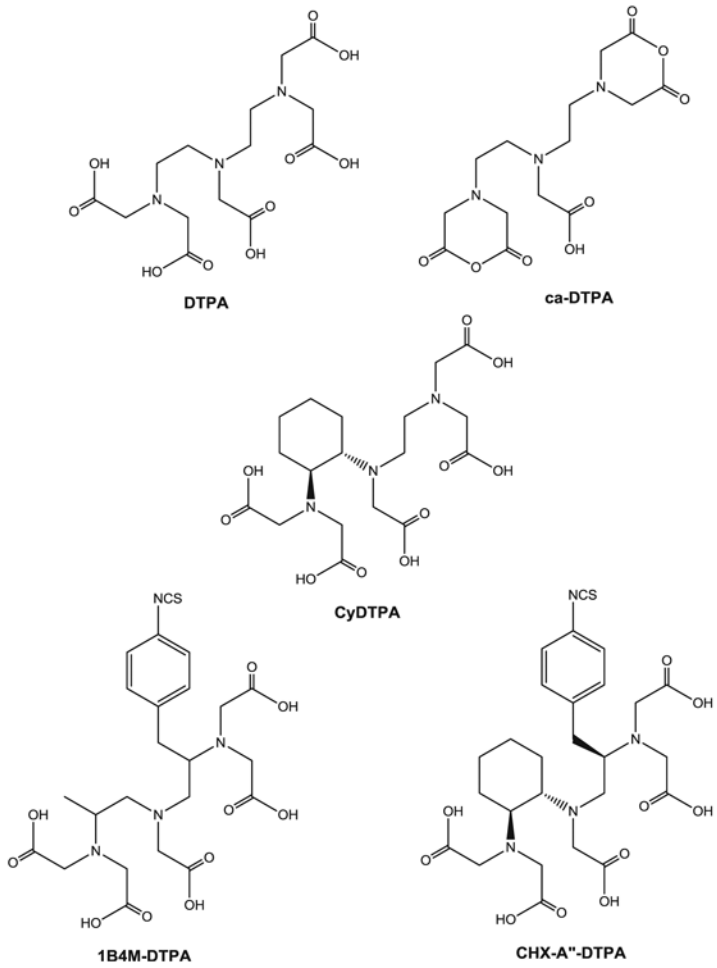

Fig. 1.
Structures of DTPA derivatives

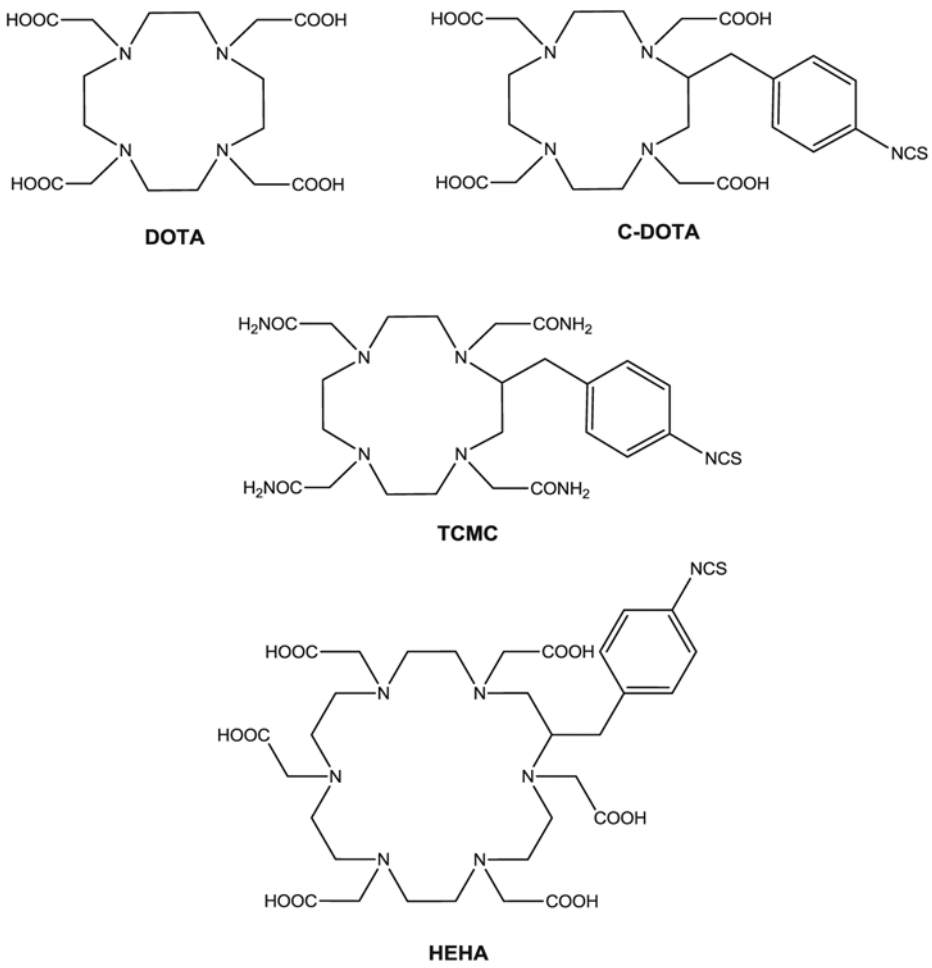

Fig. 2.
Structures of DOTA, TCMC, and HEHA

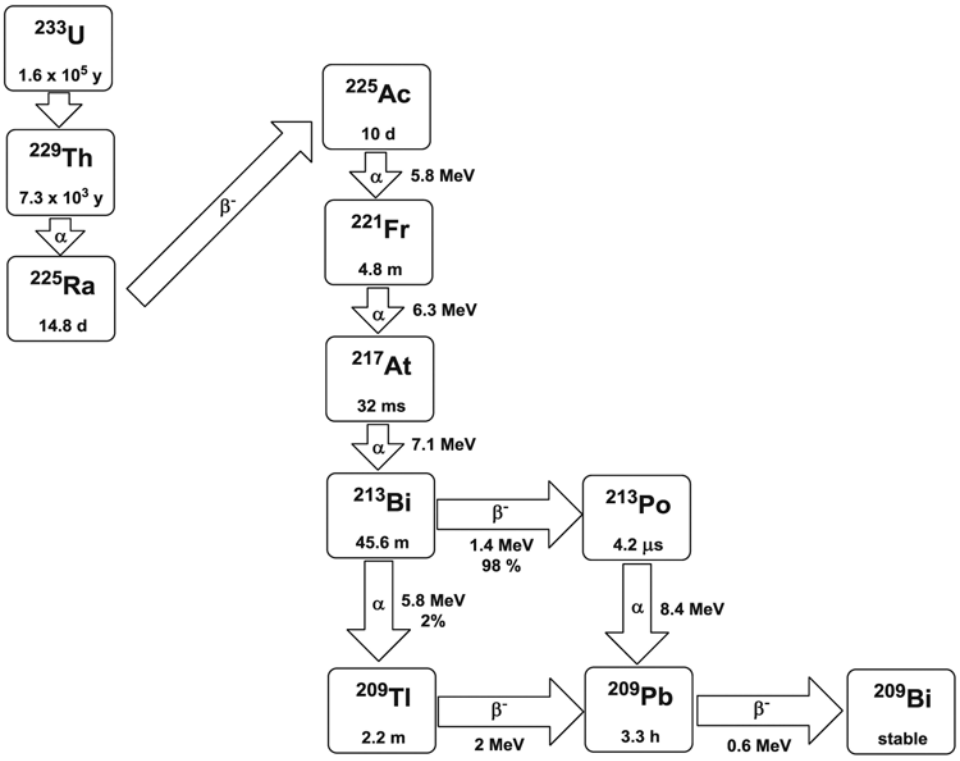

Fig. 3.
Decay scheme of ^{225}Ac and ^{213}Bi

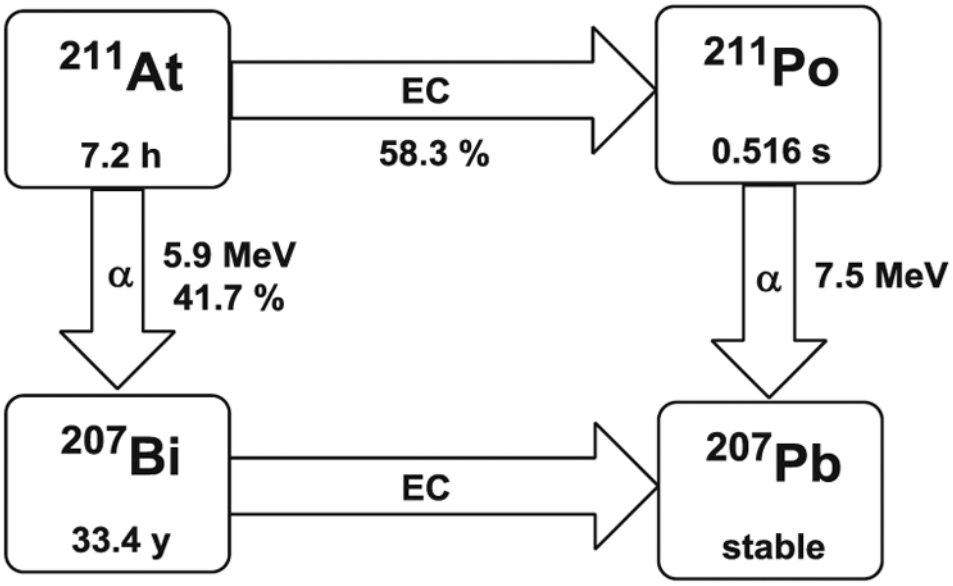

Fig. 4.
Decay scheme of ^{211}At

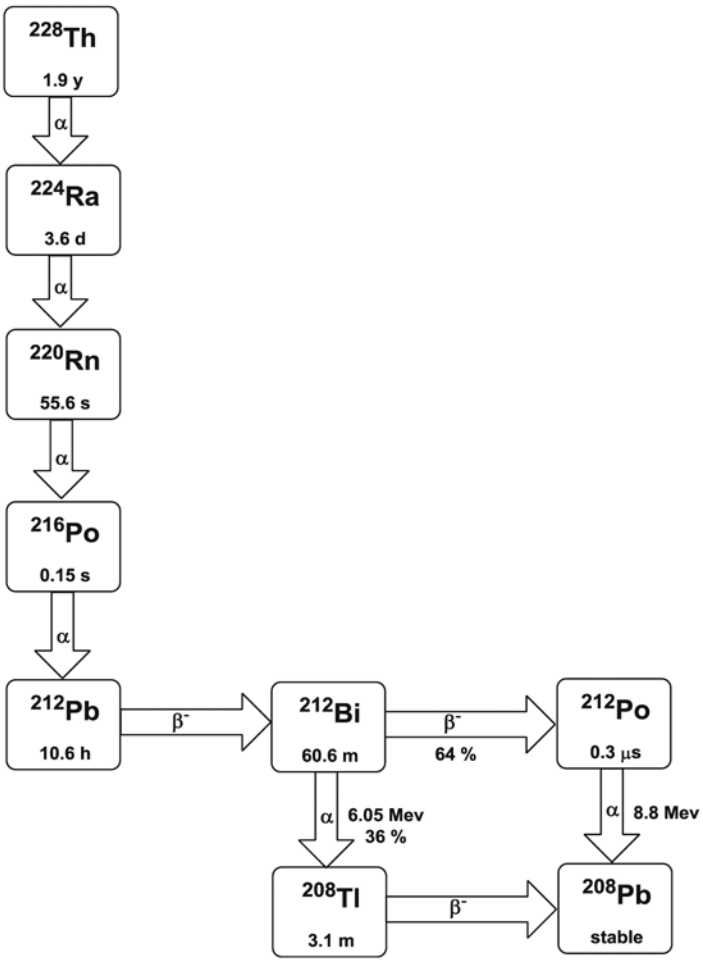

Fig. 5.
Decay scheme of ^{212}Bi and ^{212}Pb

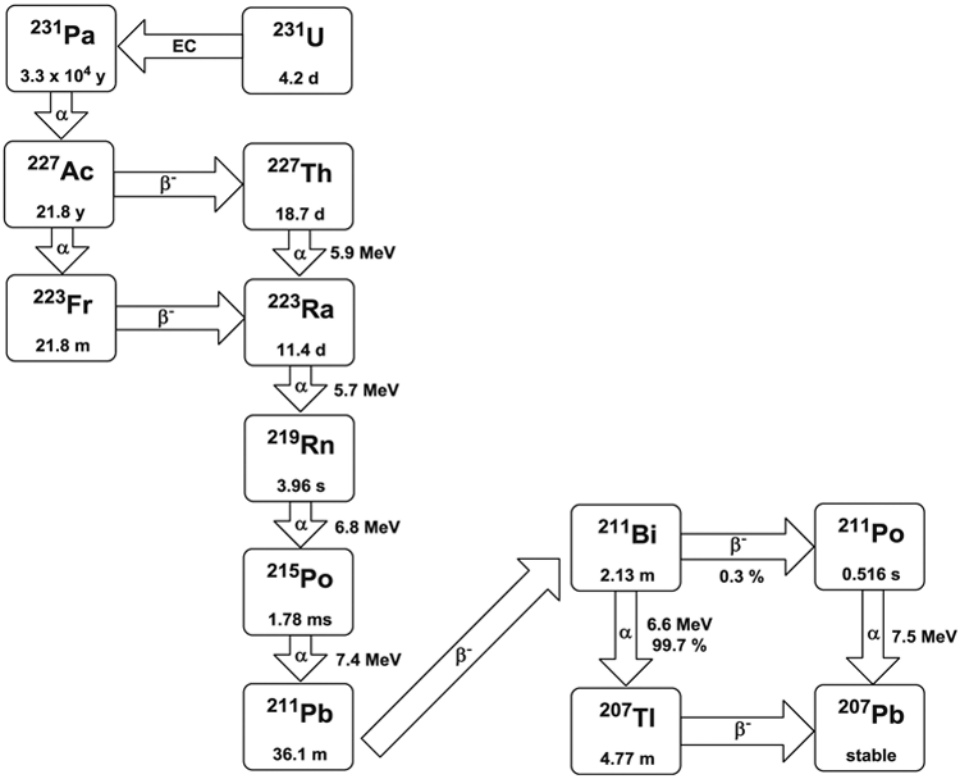

Fig. 6.
Decay scheme of ^{223}Ra and ^{227}Th

Table 1

Candidates for targeted α-therapy

Radionuclide	Daughters	Half-life	Emission	Energy	Production	Chelating agents
^{225}Ac	^{221}Fr, ^{217}At, ^{213}Bi, ^{213}Po, ^{209}Tl, ^{209}Pb	10 days	5 α, 3 β^-	5.8 MeV	^{233}U natural decay Cyclotron	HEHA, DOTA
^{211}At	^{211}Po, ^{207}Bi	7.2 h	2 α, 2 EC	5.9 MeV	Cyclotron	
^{212}Bi	^{212}Po, ^{208}Tl	60.6 min	2 α, 2 β^-	6.05 MeV	^{228}Th natural decay ^{224}Ra generator	CHX-A″-DTPA
^{213}Bi	^{213}Po, ^{209}Tl, ^{209}Pb	45.6 min	2 α, 3 β^-	5.8 MeV	^{225}Ac generator	CHX-A″-DTPA
^{212}Pb	^{212}Bi, ^{212}Po, ^{208}Tl	10.6 h	2 α, 3 β^-	6.05 MeV	^{224}Ra generator	DOTA, TCMC
^{223}Ra	^{219}Rn, ^{215}Po, ^{211}Pb, ^{211}Bi, ^{211}Po, ^{207}Tl	11.4 days	5 α, 3 β^-	5.7 MeV	^{227}Ac generator	
^{227}Th	^{223}Ra, ^{219}Rn, ^{215}Po, ^{211}Pb, ^{211}Bi, ^{211}Po, ^{207}Tl	18.7 days	6 α, 3 β^-	5.9 MeV	^{227}Ac generator	DOTA