The Promise of Targeted α-Particle Therapy

Deborah A. Mulford, MD[1]; David A. Scheinberg, MD, PhD[1,2]; and Joseph G. Jurcic, MD[1]

[1]Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York; and [2]Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, New York

The use of monoclonal antibodies to deliver radioisotopes directly to tumor cells has become a promising strategy to enhance the antitumor effects of native antibodies. Since the α- and β-particles emitted during the decay of radioisotopes differ in significant ways, proper selection of isotope and antibody combinations is crucial to making radioimmunotherapy a standard therapeutic modality. Because of the short pathlength (50–80 μm) and high linear energy transfer (~100 keV/μm) of α-emitting radioisotopes, targeted α-particle therapy offers the potential for more specific tumor cell killing with less damage to surrounding normal tissues than β-emitters. These properties make targeted α-particle therapy ideal for the elimination of minimal residual or micrometastatic disease. Radioimmunotherapy using α-emitters such as ^{213}Bi, ^{211}At, and ^{225}Ac has shown activity in several in vitro and in vivo experimental models. Clinical trials have demonstrated the safety, feasibility, and activity of targeted α-particle therapy in the treatment of small-volume and cytoreduced disease. Further advances will require investigation of more potent isotopes, new sources and methods of isotope production, improved chelation techniques, better methods for pharmacokinetic and dosimetric modeling, and new methods of isotope delivery such as pretargeting. Treatment of patients with less-advanced disease and, ultimately, randomized trials comparing targeted α-particle therapy with standard approaches will be required to determine the clinical utility of this approach.

Key Words: targeted α-particle therapy; radiolabeled monoclonal antibodies; radioimmunotherapy

By targeting therapy to specific cell types and disease sites, monoclonal antibodies offer the possibility of decreased toxicity compared with conventional chemotherapy. Although some unlabeled antibodies are capable of inducing major clinical responses, monoclonal antibodies have also been used as delivery vehicles for cytotoxic agents—such as chemotherapy, toxins, and radioisotopes—in an effort to increase their antitumor effects. Despite the predominant use of β-emitters in radioimmunotherapy trials, investigators have long recognized potential advantages of

α-particle emitters. α-Particles are positively charged helium nuclei with a shorter range (50–80 μm) and higher energy (5,000–8,000 keV) than β-particles. We review the characteristics of various α-emitting isotopes for the targeted therapy of malignancies, issues in dosimetry, and recent preclinical and clinical studies. The reader is also referred to other published reviews on this subject (*1,2*).

RADIOBIOLOGY AND RATIONALE FOR α-PARTICLE THERAPY

Although the mechanisms by which radiation induces cell death are not completely understood, several processes have been implicated. Radiation induces single- and double-stranded DNA breaks (*3*), causes apoptosis (*4*), and initiates overexpression of p53, leading to delays in the G_1 phase of the cell cycle (*5*). Death of cells exposed to α-particles occurs only when the particles traverse the nucleus; high concentrations of α-particles directed at the cytoplasm have no effect on cell proliferation (*6*).

Linear energy transfer (LET) and relative biologic effectiveness (RBE) are essential radiobiologic concepts. LET refers to the number of ionizations caused by that radiation per unit of distance traveled. α-Particles have a high LET (approximately 100 keV/μm), whereas, β-particles have a far lower LET (0.2 keV/μm). The RBE for a type of radiation refers to the dose of a reference radiation, usually x-rays, that produces the same biologic effect as the type of radiation in question. The RBE of a type of radiation is in part related to its LET. The RBE of α-particles for cell sterilization ranges from 3 to 7, depending on emission characteristics.

The dependency of RBE on LET can be explained by several differences in the type and extent of cellular damage caused by low- and high-LET radiations. First, high-LET radiation generally causes more irreparable clustered and double-stranded DNA breaks than low-LET radiation (*7*). The maximum rate of double-stranded DNA breaks occurs at LETs of 100–200 keV/μm, since the distance between ionizations caused by the radiation at these LETs approximates the diameter of double-stranded DNA (2 nm). Second, high-LET radiation causes more severe chromosomal damage, including shattered chromosomes at mitosis and complex chromosomal rearrangements, than low-LET radiation (*7*). Third, high-LET α-irradiation causes more pronounced G_2-phase delays than low-LET γ-irradiation (*8*). The mechanisms behind these differences in cell cycle

effects have not been fully elucidated but may be related to differences in gene expression induced by low- and high-LET radiations (9).

The different physical properties of α- and β-particles confer theoretic advantages and disadvantages to each, depending on the clinical situation. Since the range of β-emissions extends for several millimeters, therapy with isotopes such as ^{131}I, ^{90}Y, and [188]Re can create a "crossfire effect," destroying tumor cells to which the radioimmunoconjugate is not directly bound. In this way, β-emitters can potentially overcome resistance due to antigen-negative tumor cells. Conversely, longer-range β-emissions may also produce nonspecific cytotoxic effects by destroying surrounding normal cells. These characteristics make β-particle therapy better suited for bulky tumors or large-volume disease.

In contrast, α-particles may be better suited to the treatment of microscopic or small-volume disease since their short range and high energies potentially offer more efficient and specific killing of tumor cells. In a microdosimetric model using single-cell conditions, 1 cell-surface decay of the α-emitter [211]At resulted in the same degree of cell killing as approximately 1,000 cell-surface decays of the β-emitter [90]Y (10). Based on these considerations, α-particle therapy has been investigated in a variety of settings, including leukemias, lymphomas, gliomas, melanoma, and peritoneal carcinomatosis.

SELECTED α-PARTICLE–EMITTING RADIOISOTOPES

Because >100 radioisotopes emit α-particles, and most decay too quickly to be of therapeutic use, we will confine our discussion of α-emitters to those that have therapeutic potential and have been investigated in animal models or humans (Table 1).

[225]Ac

[225]Ac (half-life, 10.0 d) decays by α-emission through 3 atoms, [221]Fr (half-life, 4.8 min), [217]At (half-life, 32.3 ms), and [213]Bi (half-life, 45.6 min), each of which also emits an α-particle (Fig. 1). [225]Ac can be produced by the natural decay of [233]U (2) or by accelerator-based methods (11). Because [225]Ac radioimmunoconjugates act as atomic nanogenerators, delivering an α-particle cascade to a cancer cell, they are approximately 1,000 times more potent than [213]Bi-

FIGURE 1. [229]Th decay scheme. [225]Ac is isolated from [229]Th sources and decays by α-emission through [221]Fr, [217]At, and [213]Bi, each of which also emits an α-particle.

containing analogs (12). Although this increased potency could make [225]Ac more effective than other α-emitters, the possibility of free daughter radioisotopes in circulation after decay of [225]Ac raises concerns about the potential toxicity of this radioisotope.

[213]Bi

[213]Bi decays to stable [209]Bi by emitting an α-particle and 2 β-particles (Fig. 1). Additionally, a 440-keV photon emission allows detailed biodistribution, pharmacokinetic, and dosimetry studies to be performed. Preparation of [213]Bi for clinical use requires a generator consisting of its parent isotope [225]Ac dispersed onto a cation-exchange resin from which [213]Bi can be eluted (13). After [213]Bi is eluted from the generator, the isotope is readily conjugated to antibody molecules appended with the C-functionalized *trans*-cyclohexyldiethylenetriamine pentaacetic acid moiety, CHX-A-DTPA (14).

[212]Bi

[212]Bi (half-life, 60.6 min) emits an α-particle with a mean energy of 7.8 MeV from the decay of [228]Th to stable [208]Pb. A generator that uses [224]Ra as the parent radionuclide facilitates the production of [212]Bi for labeling to antibodies (15). [208]Tl, produced by the decay of [212]Bi, emits a 2.6-MeV γ-ray along with other medium-to-high-energy γ-particles

TABLE 1
Characteristics of Selected α-Emitting Radioisotopes

Isotope	Particle(s) emitted	Half-life	Energy of α-particle (MeV)
[211]At	1 α	7.2 h	6
[225]Ac	4 α, 2 β	10 d	6–8
[212]Bi	1 α, 1 β	60.6 min	6
[213]Bi	1 α, 2 β	46 min	6
[223]Ra	4 α, 2 β	11.4 d	6–7
[212]Pb	1 α, 2 β	10.6 h	7.8
[149]Tb	1 α	4.2 h	4

that require heavy shielding to minimize radiation exposure to personnel, thereby limiting the clinical utility of this radioisotope.

^{223}Ra

^{223}Ra (half-life, 11.4 d) can be obtained from uranium mill tailings in large quantities and a generator system has been developed using an ^{227}Ac parent. Like ^{225}Ac, ^{223}Ra emits 4 α-particles over its decay scheme. Because of its bone-seeking properties, unconjugated cationic ^{223}Ra is a promising candidate for delivery of high-LET radiation to cancer cells on bone surfaces. Preliminary results of a clinical phase I study demonstrated pain relief and reduction in tumor marker levels in the treatment of skeletal metastases in patients with prostate and breast cancer (16).

^{211}At

^{211}At (half-life, 7.2 h) decays through a branched pathway with each branch resulting in the production of an α-particle in its decay to stable ^{207}Pb (Fig. 2). The α-particle produced by the decay of ^{211}At has a mean energy of 6.8 MeV and a mean LET of 97–99 keV/μm. Because of its long half-life, ^{211}At-labeled constructs can be used even when the targeting molecule does not gain immediate access to tumor cells. Additionally, its daughter, ^{211}Po, emits K x-rays that allow photon counting of samples and external imaging for biodistribution studies. ^{211}At is produced by the bombardment of bismuth with α-particles in a cyclotron via the ^{207}Bi (α, 2n) ^{211}At nuclear reaction (17) and is isolated from the cyclotron target using a dry distillation procedure (18). Few institutions, however, have a cyclotron capable of producing ^{211}At. Moreover, this radioisotope is retained less well than other α-emitting radiometals after internalization of the antigen–antibody complex (19).

RADIOLABELING

The integrity of a radioimmunoconjugates can be susceptible to catabolism after internalization into a target cell or to the direct effects of radioactive decay. Therefore, in vivo stability of a radioconjugate is required to maximize delivery of isotope to tumor and to prevent toxicity. A variety of methods are used to conjugate radioisotopes to antibodies,
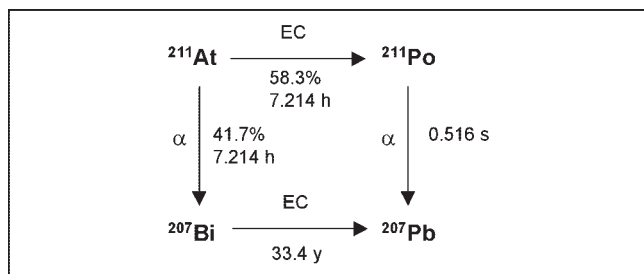
depending primarily on the nature of the radioisotope. ^{211}At is a halogen, like ^{131}I, and is usually labeled directly to antibodies by incorporation of an aryl carbon-astatine bond into the antibody (17). Methods used to create the aryl carbon-astatine bond usually involve an astatodemetallation reaction using a tin, silicon, or mercury precursor (18,20). Other radioisotopes require bifunctional chelators for linkage to antibodies. Chelators derived from DTPA include the cyclic dianhydride derivative (21) and the cyclohexylbenzyl derivative (CHX-A-DTPA) (14,22). CHX-A-DTPA is effective at chelating bismuth to antibodies, resulting in stable constructs that have been used effectively in clinical trials (14). The macrocyclic ligand 1,4,7,10-tetraazacyclododecane tetraacetic acid (DOTA) and its derivatives have been used effectively for labeling of antibodies with ^{225}Ac. A 2-step procedure was developed in which ^{225}Ac is first conjugated to DOTA-SCN followed by labeling of this construct to antibody (12).

DOSIMETRY

The generally shorter half-lives, shorter range, and higher LET of α-particle emitters distinguishes the dosimetry of these radionuclides from that of β-emitters. For longer-lived isotopes, pharmacokinetics are determined predominantly by the biologic clearance of the antibody. The distribution of the antibody within hours after administration yields residence times that are negligible in proportion to the overall residence times achieved in target and normal organs. In contrast, for ^{213}Bi, 20% of the total α-emissions occur within the first 15 min after injection, and only 6% of the total emissions remain after 3 h, due to its 46-min half-life.

Given the high energy of α-particles delivered over a short range, conventional MIRD methodologies that estimate mean absorbed dose over a specific organ volume may not always yield biologically meaningful information. Because of the physical properties of α-emissions, targeted cells may receive high absorbed-radiation doses, whereas adjacent cells may receive no radiation at all. Therefore, microdosimetric or stochastic analyses that account for the spatial distribution of various cell types and the distribution of α-decays within an organ or region of interest are necessary to estimate the absorbed dose to tumor cells and normal tissues more accurately. Since the geometric relationship between the radionuclide and the target cell is not uniform, α-particle hits cannot be assumed to be a Poisson distribution. Several distributions have been modeled, and microdosimetric spectra expressed as specific energy probability densities have been calculated. Based on this work, methods have been developed to perform basic microdosimetric assessments that account for the probability of the number of hits and the mean specific energy from a single hit (23).

FIGURE 2. ^{211}At decay scheme. ^{211}At decays through a branched pathway with each arm producing an α-particle in its decay to stable ^{207}Pb. EC = electron capture.

PRECLINICAL AND CLINICAL STUDIES

Mouse Models

The antitumor potential of α-particle radioimmunotherapy was noted in several early studies using xenograft models. Among the first studies, [212]Bi-containing radioimmunoconjugates were studied in lymphoma-bearing mice (21) and colon carcinoma-bearing mice (24). Injection of tumor-specific antibodies labeled with [212]Bi using cyclic DTPA dianhydride and glycyltyrosyl-lysyl-N-ε-DTPA, respectively, prolonged survival compared with controls. Much of the injected dose of [212]Bi, however, was taken up by the kidney, indicating instability of these radioimmunoconjugates (25). To overcome this, a different chelating agent, CHX-A-DTPA, was used to conjugate [212]Bi to the anti-gp70 antibody 103A (22). BALB/c mice were inoculated with the Rauscher leukemia virus, resulting in erythroleukemia that expresses glycoprotein gp70. Treatment of the mice with [212]Bi-103A resulted in decreased splenic tumor growth and prolonged median survival.

The results of many studies support the hypothesis that α-particle immunotherapy may be more effective in the treatment of small-volume disease than in the treatment of bulky tumors. Administration of [212]Bi-anti-Tac (anti-CD25) after inoculation of nude mice with a CD25-expressing plasmacytoma cell line, but before the development of overt disease, led to prolonged tumor-free survival and prevented development of tumors in some animals. In mice with established tumors, however, treatment with [212]Bi-anti-Tac failed to produce responses (26). Additionally, in several spheroid models of micrometastatic disease, α-particle therapy has been more effective in reducing the volume of smaller spheroids compared with larger ones (27,28).

In the few studies that have directly compared equivalent doses of α-emitters and β-emitters in animal models, α-emitters have been more effective in inhibiting tumor growth and improving survival rates. In mice with murine ovarian cancer that were treated with [211]At-tellurium colloid or with the β-emitters [165]Dy, ^{32}P, or ^{90}Y, [211]At-tellurium was the most effective and was often curative (29). Similarly, in a human colon cancer xenograft model in nude mice, a [213]Bi-labeled Fab′ fragment targeting a glycoprotein found on gastrointestinal cells (CO17-1A) prevented tumor growth and increased survival compared with the controls that received ^{90}Y-labeled Fab′ (30).

[213]Bi-Labeled Anti-CD33 for Myeloid Leukemia

Based on preclinical data that demonstrated specific tumor killing, safety, and favorable biodistribution in mice (31), a [213]Bi-labeled humanized anti-CD33 monoclonal antibody, HuM195, has been studied in clinical trials at Memorial Sloan-Kettering Cancer Center. Eighteen patients with advanced myeloid leukemias were treated in a phase I dose-escalation trial (32). Although myelosuppression occurred in all patients and transient minor liver function abnormalities were common, doses of up to 37 MBq/kg (1 mCi/kg) were safely administered to patients. Within 10 min after injection, γ-camera images demonstrated uptake of [213]Bi in the bone marrow, liver, and spleen, without significant uptake in other organs, including the kidney. The absorbed dose ratios between marrow, liver, and spleen and the whole body were 1,000 times greater with [213]Bi-HuM195 than with β-emitting HuM195 constructs used in similar patients in previous trials. Fourteen of the 18 patients had a reduction in the percentage of bone marrow blasts after therapy; however, there were no complete remissions, demonstrating the difficulty of targeting 1 or 2 [213]Bi atoms to each leukemic blast at the specific activities used in this trial (32).

Since α-particle immunotherapy appears to be more useful in the treatment of small-volume disease, a subsequent phase I/II study was undertaken in which patients were first treated with chemotherapy to achieve partial cytoreduction of the leukemic burden followed by [213]Bi-HuM195 (33). To date, >20 patients with acute myeloid leukemia were treated with cytarabine (200 mg/m²/d for 5 d) followed by [213]Bi-HuM195 at 4 dose levels (18.5–46.25 MBq/kg [0.5–1.25 mCi/kg]). Prolonged myelosuppression was dose limiting at the highest dose level. Complete responses, complete responses with incomplete platelet recovery, and partial responses were seen at the 2 highest dose levels. These preliminary results indicate that sequential administration of cytarabine and [213]Bi-HuM195 is safe and can lead to complete remissions in patients with acute myeloid leukemia.

[213]Bi-Labeled Antibodies Before Nonmyeloablative Transplantation

Although potentially curative for several malignancies, allogeneic marrow transplantation using myeloablative preparative regimens causes significant toxicity. To decrease the toxicity of treatment, nonmyeloablative strategies that rely on a graft-versus-tumor effect have been developed. In an effort to further reduce the toxicity associated with these nonmyeloablative regimens, [213]Bi-labeled antibodies to the pan-leukocyte antigen CD45 (34) and the T-cell receptor (TCRαβ) (35) have been used for immunosuppression before marrow transplantation in a canine model. Administration of both radiolabeled constructs before transplantation in conjunction with additional immunosuppressive agents resulted in prompt engraftment of transplanted marrow and resulted in stable mixed chimerism. Toxicities included transient myelosuppression and liver enzyme abnormalities. The use of [213]Bi-radioimmunoconjugates for this application in humans may be limited by the high activities (at least 74 MBq/kg [2 mCi/kg]) required to attain engraftment.

[225]Ac Atomic Nanogenerators

The marked increase in potency of [225]Ac constructs over [213]Bi-containing analogs in vitro led to studies in nude mice bearing human prostate carcinoma and lymphoma xenografts. Single becquerel (nanocurie) doses of [225]Ac-labeled tumor-specific antibodies significantly improved survival over controls and cured a substantial fraction of animals

(*12*). The increased potency of these ^{225}Ac constructs compared with ^{213}Bi can be explained by the longer (10 d) half-life of ^{225}Ac and by the ability of ^{225}Ac conjugates to act as atomic nanogenerators, emitting 4 α-particles within an individual tumor cell as it decays. Based on these promising preclinical data, a phase I trial investigating the use of ^{225}Ac-HuM195 for advanced myeloid leukemias is planned.

^{211}At-Labeled Antibodies

Investigators at Duke University have extensively studied ^{211}At-81C6, a chimeric antibody that targets tenascin, a glycoprotein overexpressed on gliomas relative to normal brain tissue. In a model of neoplastic meningitis, treatment with ^{211}At-81C6 after intrathecal injection of human rhabdomyosarcoma cells prolonged survival compared with controls (*36*). Based on these data, a phase I dose-escalation trial of ^{211}At-81C6 was initiated in patients with malignant gliomas after surgical resection of the tumor (*37*). Twelve patients have been treated to date. γ-Camera imaging showed that 99% of the ^{211}At decays occurred within the tumor cavity, indicating high in vivo stability of the radioimmunoconjugate. Early results have suggested that adjuvant therapy with ^{211}At-81C6 prolongs survival in these patients compared with historical controls.

Pretargeting Studies

In an effort to reduce radiation doses to normal organs and improve tumor-to-normal organ dose ratios, pretargeted methods of radioimmunotherapy have been developed. One pretargeting method involves administration of a monoclonal antibody or engineered targeting molecule conjugated to streptavidin, followed by administration of a biotinylated *N*-acetylgalactosamine-containing "clearing agent" to remove excess circulating antibody. Then, therapeutically radiolabeled biotin is infused. The radiolabeled biotin can bind specifically to "pretargeted" streptavidin at the tumor, whereas unbound radiolabeled biotin is rapidly excreted in the urine (*38*).

This approach has been applied to a mouse model of adult T-cell leukemia that expresses the cell-surface marker CD25 (*39*). After treatment with a streptavidin-labeled humanized anti-CD25 antibody and the clearing agent, immunodeficient mice with human adult T-cell leukemia received DOTA-biotin labeled with either ^{213}Bi or ^{90}Y. Treatment with ^{213}Bi reduced the levels of the surrogate tumor markers human β_2-microglobulin and soluble CD25 and improved survival compared with controls. Treatment with ^{90}Y, however, did not improve survival compared with controls. Mice treated with ^{213}Bi using the pretargeting approach survived longer than those treated with ^{213}Bi labeled directly to anti-Tac. This approach was also studied using an anti-CD25 single-chain Fv-streptavidin fusion protein followed by radiolabeled biotin (*40*). Significant antitumor effects were seen after administration of ^{213}Bi-DOTA-biotin to leukemic mice and, when combined with unconjugated anti-Tac, 7 of 10 mice were cured.

CONCLUSION

The role of radiolabeled monoclonal antibodies in the treatment of cancer is increasing. To date, most radioimmunotherapy trials have been performed with β-emitting isotopes. In contrast to β-emitters, the shorter range and higher LET of α-particles allow for more efficient and selective killing of individual tumor cells. Although some experimental models indicate that α-particle immunotherapy may eradicate large tumor burdens, most preclinical and clinical trials suggest that radioimmunotherapy with α-emitters may be best suited for the treatment of small-volume disease. Although results of early studies appear promising, there are several obstacles to the widespread use of α-particle immunotherapy. To address these difficulties, new sources and methods of production of α-emitters, improved chelation chemistry, better pharmacokinetic and dosimetry modeling techniques, and novel delivery methods must be explored. Additional preclinical and clinical investigations are necessary to define optimal radioisotopes, dosing regimens, and therapeutic strategies.

REFERENCES

1. Zalutsky MR, Bigner DD. Radioimmunotherapy with alpha-particle emitting radioimmunoconjugates. *Acta Oncol.* 1996;35:373–379.
2. McDevitt MR, Sgouros G, Finn RD. Radioimmunotherapy with alpha-emitting nuclides. *Eur J Nucl Med.* 1998;25:1341–1351.
3. Nunez MI, Villalobos M, Olea N, et al. Radiation-induced DNA double-strand break rejoining in human tumour cells. *Br J Cancer.* 1995;71:311–316.
4. Kolesnick RN, Haimovitz-Friedman A, Fuks Z. The sphingomyelin signal transduction pathway mediates apoptosis for tumor necrosis factor, Fas, and ionizing radiation. *Biochem Cell Biol.* 1994;72:471–474.
5. Pandita TK, Lieberman HB, Lim DS, et al. Ionizing radiation activates the ATM kinase throughout the cell cycle. *Oncogene.* 2000;19:1386–1391.
6. Munro TR. The relative radiosensitivity of the nucleus and cytoplasm of Chinese hamster fibroblasts. *Radiat Res.* 1970;42:451–470.
7. Kampf G. Induction of DNA double-strand breaks by ionizing radiation of different quality and their relevance for cell inactivation. *Radiobiol Radiother (Berl).* 1988;29:631–658.
8. Gadbois DM, Crissman HA, Nastasi A, et al. Alterations in the progression of cells through the cell cycle after exposure to alpha particles or gamma rays. *Radiat Res.* 1996;146:414–424.
9. Woloschak GE, Chang-Liu CM. Differential modulation of specific gene expression following high- and low-LET radiations. *Radiat Res.* 1990;124:183–187.
10. Humm JL. A microdosimetric model of astatine-211 labeled antibodies for radioimmunotherapy. *Int J Radiat Oncol Biol Phys.* 1987;13:1767–1773.
11. Koch L, Apostolidis C, Molinet R, et al. Production of Bi-213 and Ac-225, α-Immuno '97 Symposium Proceedings. Joint Research Centre, Institute for Transuranium Elements (ITU). Karlsruhe, Germany: 1998:20–25.
12. McDevitt MR, Ma D, Lai LT, et al. Tumor therapy with targeted atomic nanogenerators. *Science.* 2001;294:1537–1540.
13. McDevitt MR, Finn RD, Sgouros G, et al. An ^{225}Ac/^{213}Bi generator system for therapeutic clinical applications: construction and operation. *Appl Radiat Isot.* 1999;50:895–904.
14. Ma D, McDevitt MR, Finn RD, Scheinberg DA. Rapid preparation of short-lived alpha particle emitting radioimmunopharmaceuticals. *Appl Radiat Isot.* 2001;55:463–470.
15. Atcher RW, Friedman AM, Hines JJ. An improved generator for the production of ^{212}Pb and ^{212}Bi from ^{224}Ra. *Int J Rad Appl Instrum [A].* 1988;39:283–286.
16. Larsen RH. Biomedical application of alpha-particle-emitting radium-223. 225th American Chemical Society National Meeting. March, 23–27, 2003:40; New Orleans, LA. Abstract 11.
17. Zalutsky MR, Vaidyanathan G. Astatine-211-labeled radiotherapeutics: an emerging approach to targeted alpha-particle radiotherapy. *Curr Pharm Res.* 2000;6:1433–1455.
18. Zalutsky MR, Narula AS. Astatination of proteins using an N-succinimidyl

tri-n-butylstannyl benzoate intermediate. *Int J Rad Appl Instrum [A].* 1988;39: 227–232.

19. Yao Z, Garmestani K, Wong KJ, et al. Comparative cellular catabolism and retention of astatine-, bismuth-, and lead-radiolabeled internalizing monoclonal antibody. *J Nucl Med.* 2001;42:1538–1544.

20. Zalutsky MR, Garg PK, Friedman HS, Bigner DD. Labeling monoclonal antibodies and F(ab′)₂ fragments with the alpha-particle-emitting nuclide astatine-211: preservation of immunoreactivity and in vivo localizing capacity. *Proc Natl Acad Sci USA.* 1989;86:7149–7153.

21. Macklis RM, Kinsey BM, Kassis AI, et al. Radioimmunotherapy with alpha-particle-emitting immunoconjugates. *Science.* 1988;240:1024–1026.

22. Huneke RB, Pippin CG, Squire RA, et al. Effective alpha-particle-mediated radioimmunotherapy of murine leukemia. *Cancer Res.* 1992;52:5818–5820.

23. Humm JL, Roeske JC, Fisher DR, Chen GT. Microdosimetric concepts in radioimmunotherapy. *Med Phys.* 1993;20:535–541.

24. Simonson RB, Ultee ME, Hauler JA, Alvarez VL. Radioimmunotherapy of peritoneal human colon cancer xenografts with site-specifically modified ²¹²Bi-labeled antibody. *Cancer Res.* 1990;50(suppl):985s–988s.

25. Russ GA, Bigler RE, Tilbury RS, et al. Metabolic studies with radiobismuth. I. Retention and distribution of ²⁰⁶Bi in the normal rat. *Radiat Res.* 1975;63:443–454.

26. Hartmann F, Horak EM, Garmestani K, et al. Radioimmunotherapy of nude mice bearing a human interleukin 2 receptor alpha-expressing lymphoma utilizing the alpha-emitting radionuclide-conjugated monoclonal antibody ²¹²Bi-anti-Tac. *Cancer Res.* 1994;54:4362–4370.

27. Kennel SJ, Stabin M, Roeske JC, et al. Radiotoxicity of bismuth-213 bound to membranes of monolayer and spheroid cultures of tumor cells. *Radiat Res.* 1999;151:244–256.

28. Ballangrud AM, Yang WH, Charlton DE, et al. Response of LNCaP spheroids after treatment with an alpha-particle emitter (²¹³Bi)-labeled anti-prostate-specific membrane antigen antibody (J591). *Cancer Res.* 2001;61:2008–2014.

29. Bloomer WD, McLaughlin WH, Lambrecht RM, et al. ²¹¹At radiocolloid therapy: further observations and comparison with radiocolloids of ³²P, ¹⁶⁵Dy, and ⁹⁰Y. *Int J Radiat Oncol Biol Phys.* 1984;10:341–348.

30. Behr TM, Behe M, Stabin MG, et al. High-linear energy transfer (LET) alpha versus low-LET beta emitters in radioimmunotherapy of solid tumors: therapeutic efficacy and dose-limiting toxicity of ²¹³Bi- versus ⁹⁰Y-labeled CO17–1A Fab′ fragments in a human colonic cancer model. *Cancer Res.* 1999;59:2635–2643.

31. Nikula TK, McDevitt MR, Finn RD, et al. Alpha-emitting bismuth cyclohexyl-benzyl DTPA constructs of recombinant humanized anti-CD33 antibodies: pharmacokinetics, bioactivity, toxicity and chemistry. *J Nucl Med.* 1999;40:166–176.

32. Jurcic JG, Larson SM, Sgouros G, et al. Targeted alpha particle immunotherapy for myeloid leukemia. *Blood.* 2002;100:1233–1239.

33. Burke JM, Jurcic JG, Divgi CR, et al. Sequential cytarabine and alpha-particle immunotherapy with bismuth-213-labeled anti-CD33 monoclonal antibody HuM195 in acute myeloid leukemia (AML) [abstract]. *Blood.* 2002;100:339.

34. Sandmaier BM, Bethge WA, Wilbur DS, et al. Bismuth-213-labeled anti-CD45 radioimmunoconjugate to condition dots for nonmyeloablative allogeneic marrow grafts. *Blood.* 2002;100:318–326.

35. Bethge WA, Wilbur DS, Storb R, et al. Selective T-cell ablation with bismuth-213-labeled anti-TCRαβ as nonmyeloablative conditioning for allogeneic canine marrow transplantation. *Blood.* 2003;101:5068–5075.

36. Zalutsky MR, McLendon RE, Garg PK, et al. Radioimmunotherapy of neoplastic meningitis in rats using an alpha-particle-emitting immunoconjugate. *Cancer Res.* 1994;54:4719–4725.

37. Zalutsky MR, Cokgor I, Akabani G, et al. Phase I trial of alpha-particle-emitting astatine-211 labeled chimeric anti-tenascin antibody in recurrent malignant glioma patients [abstract]. *Proc Am Assoc Cancer Res.* 2000;41:544.

38. Axworthy DB, Reno JM, Hylarides MD, et al. Cure of human carcinoma xenografts by a single dose of pretargeted yttrium-90 with negligible toxicity. *Proc Natl Acad Sci USA.* 2000;97:1802–1807.

39. Zhang M, Yao Z, Garmestani K, et al. Pretargeting radioimmunotherapy of a murine model of adult T-cell leukemia with the alpha-emitting radionuclide, bismuth 213. *Blood.* 2002;100:208–216.

40. Zhang M, Zhang Z, Garmestani K, et al. Pretarget radiotherapy with an anti-CD25 antibody-stretavidin fusion protein was effective in therapy of leukemia/lymphoma xenografts. *Proc Natl Acad Sci USA.* 2003;100:1891–1895.