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Applications of ^{211}At and ^{223}Ra in Targeted Alpha-Particle Radiotherapy

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Abstract

Targeted radiotherapy using agents tagged with α -emitting radionuclides is gaining traction with several clinical trials already undertaken or ongoing, and others in the advanced planning stage. The most commonly used α -emitting radionuclides are ^{213}Bi , ^{211}At , ^{223}Ra and ^{225}Ac . While each one of these has pros and cons, it can be argued that ^{211}At probably is the most versatile based on its half life, decay scheme and chemistry. On the other hand, for targeting bone metastases, ^{223}Ra is the ideal radionuclide because simple cationic radium can be used for this purpose. In this review, we will discuss the recent developments taken place in the application of ^{211}At -labeled radiopharmaceuticals and give an overview of the current status of ^{223}Ra for targeted α -particle radiotherapy.

Keywords

Alpha-particle radiotherapy; astatine-211; radium-223

INTRODUCTION

A potential advantage of systemic or targeted radiotherapy, compared to external beam radiation therapy, is the ability to eradicate not only the primary tumor but also metastatic lesions spread throughout the body. This reflects the ability of the radionuclide-carrying targeting agent to specifically sterilize cancer cells by binding to an epitope present on cancer cells rather than to exhibit effective killing in an anatomic region corresponding to the path of the radiation beam. Various combinations of targeting vehicles and radionuclides have been investigated in molecularly targeted radiotherapy. The vehicles can be small molecular weight compounds, intermediate oligopeptides, or high molecular weight antibodies. The most commonly used radionuclides in targeted radiotherapy are those that emit β -particles. Because the ranges in tissue of these ionizing radiations are rather large compared with the dimensions of a typical cancer cell, uniform uptake of the radiotherapeutic is not a *sine qua non* for its efficacy; adjacent cells not expressing the receptors/antigens and thus not capable of sequestering the radioactivity can be killed by the physical cross-fire effect. On the other hand, this has negative ramifications for the survival of nearby normal tissues. Another limitation of β -emitters is that the fraction of absorbed radiation dose that is deposited in tumor decreases with the decreasing size of the tumor. For example, it has been calculated that the ratio of fractional absorbed dose from an α -particle emitter (^{211}At) to that from ^{90}Y will be 9 and 33 for a 1000 μm and 200 μm diameter tumors, respectively [1]. On the other extreme, radiopharmaceuticals tagged with

radionuclides that emit Auger electrons need to be localized close to DNA in order to be therapeutically effective due to the very short range of these radiations [2]. Alpha particles have a range in tissue of about 50-90 μm —intermediate between that of β -particles and Auger electrons, but like Auger electrons, are radiations of high linear energy transfer (LET). A disadvantage of having a range that is equivalent to only a few cell diameters is that sterilization of adjacent cells that have not taken up the radiotherapeutic by physical cross-fire will be limited; however, recent studies have shown that this may be ameliorated by biological bystander effects [3]. It is worth noting that a case has been made for exploiting the $<0.1 \mu\text{m}$ range high LET properties of α -particle recoil nuclei by attempting to target them to the cell nucleus, for example, via incorporation into DNA [4]. In addition to their high LET, which is responsible for their high relative biological effectiveness [5], α -particle cytotoxic effectiveness is not dependent on the oxygen concentration, dose rate, and cell cycle status [6]. Targeted radiotherapy has the greatest chance of success in settings of minimal residual diseases such as micrometastatic lesions, residual tumor margins that remain after debulking the primary tumor by surgery, tumors in the circulation including lymphoma and leukemia, and malignancies that present as free-floating cells within body compartments and spread as thin sheets on compartmental walls [7]. The emission characteristics of α -particles are well-matched to the geometric features of these diseases and thus are ideal for their treatment.

Although there are about 100 radionuclides that are known to decay by the emission of α -particles, a number of factors must be taken into account in the choice of an α -particle emitter for a particular therapeutic application. It will be advantageous if the fraction of decays that yield α -particles is high and for certain applications, there should be minimal associated β -particles emission. Emission of gamma rays or x-rays, which are suitable for imaging, will be a plus because it permits determination of pharmacokinetics and dosimetry of the labeled therapeutic. Other considerations in selecting an appropriate α -emitter include physical half life of the radionuclide, which ideally should match the biological half life of the radiotracer or its active catabolites, ease of chemical synthesis and availability at a reasonable cost. A short half-life of the radionuclide demands that a high tumor-to-normal tissue absorbed dose ratio should be reached early. This often occurs if the plasma clearance rate is fast, but it also depends on the uptake rate and retention of radiotracer in tumors and other normal organs.

Astatine-211 [8], ^{213}Bi [9, 10], ^{212}Bi [11], ^{223}Ra [12], and ^{225}Ac [13] have been the most commonly investigated α -particle emitting radionuclides for targeted radiotherapy. Astatine-211 is probably the most promising among these for the α -particle therapy because of its relatively long half life, 100% of its decays results in α -emission and feasibility of imaging among its other favorable properties. During the past decade, an α -emitting isotope of the rare earth element radium, ^{223}Ra , has been used in the palliative treatment of bone metastases from breast and prostate cancers. In this article, various astatinated radiopharmaceuticals that have been developed and their potential applications will be described, especially those reported since our last review [8]. In addition, the therapeutic applications of ^{223}Ra will also be discussed.

^{211}At -LABELED RADIOPHARMACEUTICALS

Small Molecular Weight Compounds

[^{211}At]Astatide—Iodide accumulates in the thyroid and other organs such as the stomach facilitated by the sodium iodide symporter (NIS) and this phenomenon has been exploited for the therapy of thyroid carcinomas and non-thyroid tumors expressing NIS with [^{131}I]iodide after gene transfer [14]. Consistent with its halide nature, astatide has also been found to be a substrate for NIS [15]. One of the advantages of ^{211}At is that its half life is

better matched than that of ^{131}I with the rather rapid rate of washout of these halides from tumor cells. In preclinical studies, Willhauck *et al.* [16] have evaluated the potential utility of [^{211}At]astatide for the therapy of prostate cancer. LNCaP prostate cancer cells stably transfected with NIS under the control of a prostate-specific antigen promoter (NP-1 cells) accumulated [^{211}At]astatide copiously and the uptake was sensitive to treatment with perchlorate, a known NIS inhibitor. In comparison, the uptake of [^{211}At]astatide in P-1 cells transfected with a control vector was 15-fold lower and was similar to the uptake in NP-1 cells treated with perchlorate. From a clonogenic assay, it was demonstrated that only 1% of the NP-1 cells, compared to 95% of the P-1 cells, survived when treated with 100 kBq/ml of [^{211}At]astatide. Significant accumulation ($\sim 16\%$ ID/g) of [^{211}At]astatide in the positive but not the negative tumors was seen both by gamma scintigraphy and well counting in mice bearing both subcutaneous NP-1 and P-1 tumors after intraperitoneal injection of [^{211}At]astatide. From a therapeutic study it was shown that while P-1 tumors (mean tumor volume $109 \pm 53 \text{ mm}^3$) treated with ^{211}At or saline as well as NP-1 tumors treated with saline continued their growth throughout the observation period, NIS-expressing tumors treated with ^{211}At showed a significant reduction in average tumor volume. Furthermore, the therapeutic effect was somewhat more pronounced in smaller tumors (mean tumor volume $110 \pm 84 \text{ mm}^3$; $82 \pm 19\%$ reduction); in comparison, the average tumor volume was reduced by $71 \pm 14\%$ in larger tumors (mean tumor volume $516 \pm 132 \text{ mm}^3$) consistent with the anticipated advantage of α -particles for the treatment of smaller tumors. The utility of this approach for the treatment of extrathyroidal cancers will depend on the success of NIS transduction in these tumors *in vivo*, unless they naturally express NIS as has been reported in some cases [17, 18]. Induction of NIS expression has been shown to be feasible in preclinical models by intratumoral administration of viral vectors encoding NIS in subcutaneous and peritoneal tumors [19, 20]. Another potential issue with the NIS-targeted radiohalide therapy is the stunning of thyroid that has been observed with [^{131}I]iodide therapy. This is a phenomenon that results in reduced uptake of radioiodide by thyroid that has been pre-irradiated by an amount of radioactivity administered for imaging, presumably due to down-regulation of NIS. Lundh *et al.* [21] investigated whether ^{211}At as well as ^{123}I and $^{99\text{m}}\text{Tc}$ were able to induce similar stunning effects by determining whether NIS expression and iodide transport were affected differently by these radionuclides in comparison with ^{131}I . Down regulation of NIS and reduction in iodide transport were found to depend upon the relative biological effectiveness of the emitted radiation, with ^{211}At causing the highest degree of stunning; however, surprisingly, ^{211}At -irradiated cells regained iodide transport ability.

In a couple of recent publications, the determination of gene expression profiles after irradiating lymphocytes with [^{211}At]astatide (0.05 – 1.6 Gy over 30 min) has been reported [22, 23]. Although this work has no direct relevance to the effectiveness of α -particle therapy, it is germane to the understanding of potential deleterious effects of α -particles on normal tissues. Of the 338 early response genes that were modulated, 183 were up-regulated and the remaining down-regulated. Genes associated with cell death were found only in the up-regulated group while those involved with development were only found in the down-regulated group.

Particulates—Particulate materials, owing to their relatively large size, are less likely to leak out after direct administration into a cavity. Because α -particle therapy is suitable for tumors that grow as thin sheets on the surface of body cavities, microspheres, liposomes, nanoparticles and other particulate carriers labeled with ^{211}At have been investigated as potential therapeutic agents. However, no significant work investigating ^{211}At -labeled particulate agents has been reported within the last 2-3 years, which is not surprising, given the lack of molecular specificity of this type of delivery vehicle.

Meta-[²¹¹At]astatobenzylguanidine—Although structurally quite different, *meta*-iodobenzylguanidine (MIBG) is a functional analogue of the neurotransmitter norepinephrine and is sequestered into the cells of sympathomedullary tissues by the norepinephrine transporter (NET). NET is upregulated in a number of neuroendocrine tumors such as neuroblastoma and this fact has been exploited in targeting these cancers with radiolabeled MIBG both for diagnosis and therapy. While radioiodinated MIBG has excelled as an imaging agent, the therapeutic efficacy of [¹³¹I]MIBG, especially in the case of neuroblastoma, has not been that impressive. This lackluster performance is largely due to the suboptimal physical properties of the ¹³¹I for eradicating micro-metastatic lesions, which are often associated with neuroblastoma. Hypothesizing that an α -particle-emitting analogue of MIBG will be a better match to the geometrical characteristics of smaller tumors, we developed a method for the synthesis of *meta*-[²¹¹At]astatobenzylguanidine (MABG) at high specific activities (16,000 TBq/mmol) [24-26].

A number of preclinical studies have demonstrated MABG to be an excellent analogue of MIBG in terms of mechanism of uptake in NET-expressing cells and tissue distribution in human tumor xenograft models [26, 27]. In addition, MABG was found to be exquisitely cytotoxic especially for the treatment of smaller tumors [28, 29].

The utility of MABG for treating non NET-expressing cancers via a gene therapy approach also has been explored. Using human telomerase promoters, which are more specific for tumor than normal tissues, high levels of NET expression were induced in UVW glioma cells [30, 31]. The cytotoxicity of [¹³¹I]MIBG or MABG to wild type UVW glioma cells and those transfected with the NET gene under the control of RSV, and the telomerase promoters hTR or hTERT was determined in a novel multicellular mosaic spheroid model. Little reduction in survival fraction was observed in spheroids composed of wild-type UVW cells after treatment with either radiopharmaceutical suggesting that expression of functional NET is needed to achieve efficient cell kill of these radio-resistant glioma cells with these radiolabeled NET substrates. On the other hand, all NET expressing spheroids succumbed to [¹³¹I]MIBG-mediated cell kill in a radioactivity concentration-dependent manner regardless of the promoter driving NET expression. With MABG, the radioactivity amount required to reduce survival to 0.1% (log SF = 0.001) was roughly similar for all three transfectants, indicating that MABG resulted in an equivalent level of cell kill regardless of the strength of the promoter driving NET gene expression. The radioactivity levels required to achieve a given therapeutic effect with MABG were 400-1000-fold lower than those required when [¹³¹I]MIBG was used as the therapeutic, confirming the exquisite toxicity of MABG.

MIBG derivatives labeled with α -, β -, and Auger electron-emitting radionuclides including MABG have been evaluated for their ability to elicit a radiation induced biological bystander effect (RIBBE), which offers the exciting prospect of sterilizing cancer cells in which the NET gene is under expressed or not expressed and therefore not directly irradiated [3, 32-34]. RIBBE is a potentially important phenomenon for targeted radiotherapy because it could minimize the problem of heterogeneous MABG uptake and resultant dose deposition, which is one of the concerns with short range α -particles. When cells transfected to express NET were exposed to bystander toxin-containing medium from the treated cells, a bystander effect that was related to the amount of radioactivity taken up by donor cells was observed at all activity concentrations of [¹³¹I]MIBG. On the other hand, with high LET radiation-emitting [¹²³I]MIBG and [²¹¹At]MABG, an activity concentration-dependent cytotoxicity was observed at low activity concentrations but, with increasing activity, a survival nadir with respect to RIBBE was attained. At higher activity concentrations, the potency of bystander kill diminished suggesting that RIBBE is LET-dependent and that the RIBBE-generating mechanism is constrained for the high LET radiations above a threshold radioactivity amount. The combination of MABG and gene therapy approaches has potential

clinical application. About 92% of transitional cell carcinoma bladder tumors at all stages are telomerase-positive whereas only low levels telomerase expression have been reported in normal bladder mucosa under conditions of dysplasia and severe inflammation. This large differential in telomerase expression between normal bladder and tumor renders the telomerase promoters suitable candidates for bladder cancer specific expression of trans genes and thus making bladder cancer amenable to above approach of treatment [31].

Other low Molecular Weight Agents—Several other low molecular weight compounds labeled with ^{211}At have been synthesized and evaluated as targeted radiotherapeutics. These include agents undergoing DNA incorporation—astato-2'-deoxyuridine [4] and FIAU [35], naphthaquinone and methylene blue derivatives, steroids, melanoma-targeting benzamides, biotin derivatives, and bone-seeking bisphosphonates. Very little new work has been reported for low molecular weight agents since our last review [8] except for a study [36] wherein the bone-seeking agent 5- ^{211}At astatopyridine-3-amide-*N*-3-hydroxypropylidene-3,3-bisphosphonate was evaluated in an additional animal model. As reported earlier by Larsen *et al.*, [37] this work also showed high uptake and retention of this agent in bone. A number of cancer cells, but not normal ones, over-express certain amino acid transporters, which facilitates movement of various amino acids even across the blood-brain barrier. For this reason, amino acid transporters have been evaluated as targets for radioimaging and targeted radiotherapy of various cancers leading to the development of a number of radiolabeled amino acid derivatives. While attempts were made more than two decades ago to label tyrosine with ^{211}At , recently Meyer *et al.* [38] reported the synthesis of two ^{211}At -labeled phenylalanine derivatives, which were shown to undergo specific uptake in glioma cells *in vitro*. Finally, we have synthesized ^{211}At -labeled urea-based prostate-specific membrane antigen (PSMA) inhibitors that could potentially be useful in the targeted α -particle therapy of androgen-independent, advanced metastatic prostate cancers [39]. Preliminary results showed specific and high levels of uptake of these compounds in PSMA-expressing prostate cancer cells *in vitro* to a degree similar to that seen for their radioiodinated analogues.

In their continuing efforts to develop better prosthetic groups containing boron cage moieties for labeling biomolecules with ^{211}At , Wilbur *et al.* have compared *closo*-decaborate(2-) and *closo*-dodecaborate(2-) moieties [40]. They studied two sets of otherwise identical compounds that differed in the presence of a *closo*-decaborate(2-) or *closo*-dodecaborate(2-) moiety. With respect to inertness toward *in vivo* deastatination, both moieties seem to confer same degree of stability; however, compounds containing *closo*-dodecaborate(2-) substituents had a higher renal uptake and retention compared with the same compounds containing a *closo*-decaborate(2-) moiety. The counter ion also affected the biodistribution of these radiolabeled compounds. While tetrabutyl ammonium salts were excreted via the hepatobiliary route, triethylammonium did not influence the biodistribution as much.

Peptides and Non-Antibody Proteins—Radiolabeled peptides play a significant role in nuclear medicine due to their favorable pharmacokinetics, low antigenicity and ease of synthesis. The biological half lives of oligopeptides are ideally matched with the physical half life of ^{211}At and therefore astatinated peptides could be potentially useful in the targeted α -particle therapy of cancer. Other than the work cited in our earlier review no additional reports focused on astatinated peptides have appeared in the literature.

Recently, work on ^{211}At -labeling of a modular recombinant transporter (MRT) molecule and its evaluation for targeting EGFR-expressing tumors has been published [41]. The MRT molecule, is a multifunctional molecule, consisting of an internalizable ligand, a nuclear localization sequence (NLS), an endosomolytic module, and a carrier module. It was

designed and then genetically engineered to achieve both selective tumor targeting and nuclear delivery of the therapeutic. In this study, a MRT consisting of hEGF as the internalizable ligand, the optimized NLS peptide of SV40 large T-antigen, the translocation domain of diphtheria toxin as the endosomolytic module, and Escherichia coli hemoglobin-like protein (HMP) as the carrier module was constructed. This MRT was radioiodinated directly on its constituent tyrosine residues or using the residualizing label *N*-succinimidyl 4-guanidinomethyl-3-[*I]iodobenzoate ([*I]SGMIB) and labeled with ^{211}At using the corresponding astatinated prosthetic agent [^{211}At]SAGMB. When incubated with EGFR-expressing A431 human epidermoid carcinoma cells, the percent of radioactivity initially bound to the cells that was in the intracellular compartment was 3–4-fold higher for [^{125}I]SGMIB-MRT and [^{211}At]SAGMB-MRT compared to MRT radioiodinated by the direct method. Clonogenic survival of three different human EGFR-expressing cancer cell lines after exposure to both [^{211}At]SAGMB-MRT and as a control, free [^{211}At]astatide, was determined (data for A431 cell line shown in Fig. 1). The A_{37} values for [^{211}At]SAGMB-MRT for A431 carcinoma cells, U87MG. wtEGFR glioma cells, and D247 MG glioma cells were 19.7 kBq/mL (95% confidence interval, 24.4–15.9; ratio, 14.5), 11.9 kBq/mL (95% confidence interval, 10.6–13.4; ratio, 8.25), and 3.8 kBq/mL (95% confidence interval, 3.3–4.4; ratio, 18.3), respectively. When free [^{211}At]astatide was the therapeutic, these values were 285 kBq/mL (95% confidence interval, 257–314), 98.5 kBq/mL (95% confidence interval, 85.6–113.5), and 69 kBq/mL (95% confidence interval, 62.5–76.5), respectively. The enhanced cytotoxicity of the labeled MRT, compared to free [^{211}At]astatide, is most likely reflects a) a geometrically more favorable site of decay in close proximity of the cell nucleus and b) the action of $<0.1\ \mu\text{m}$ α -particle recoil nuclei facilitated by the nuclear delivery of ^{211}At by the MRT.

Monoclonal Antibodies and their Fragments

Immunoglobulins clear very slowly from plasma and thus a longer duration is needed to achieve a maximum tumor-to-normal tissue absorbed dose ratio with labeled mAbs. Due to the relatively short half life of ^{211}At , most of the activity from ^{211}At -labeled antibodies will decay before optimal tumor-to-normal tissue ratios can be achieved. Thus ^{211}At -labeled monoclonal antibodies (mAbs) may not be suitable for the targeted α -particle therapy if they have to be administered systemically. Nonetheless, the majority of research on the therapeutic applications of ^{211}At -labeled compounds has involved mAbs. Of course, one can resort to either a pretargeting approach or locoregional administration, and indeed these strategies have been investigated. Alternatively, small molecular weight fragments of conventional antibodies including $\text{F}(\text{ab}')_2$, Fab, scFv, minibody, diabody and nanobody (derived from camelid antibody) as well as affibody (antibody mimetic) could be utilized. These proteins have faster clearance rates than intact mAbs and could be used in targeted α -particle therapy after labeling them with ^{211}At . $\text{F}(\text{ab}')_2$ [42], Fab [43], scFv [44], diabody [45], and affibody [46] molecules all have been labeled with ^{211}At ; however, to date, ^{211}At -labeling of minibodies and nanobodies has not been reported.

With regard to the clinical potential of ^{211}At -labeled mAbs and fragments, the most promising setting for radio-immunotherapy (RIT) of colorectal cancer using an intact antibody is predicted to be as an adjuvant after the resection of the primary tumor, or for the therapy of small metastatic lesions. With this consideration in mind, a humanized antibody huA33 reactive to antigen A33 that is expressed homogeneously in more than 95% of all colorectal cancer was radiolabeled with ^{211}At using *N*-succinimidyl 4- ^{211}At astatobenzoate (*para*-SAB) and evaluated in athymic mice bearing subcutaneous SW1222 colon carcinoma xenografts [47]. After intravenous administration, the tumor uptake increased with time over the 21 h period studied. Uptake in tumor, especially at later time points, was considerably higher than in most other organs and was found to be specific to the presence of A33

antigen. The uptake in tumor at 8 and 21 h was about 15% and 23% ID/g, respectively, with a tumor-to-blood ratio of about 2.5 observed at 21 h. Dosimetric calculations indicated that, apart from blood, tumor received the maximum radiation absorbed dose. The authors concluded that this ^{211}At -labeled antibody is a good candidate for the treatment of metastatic colorectal carcinoma.

As pointed out above, diabodies have elimination half lives compatible with the physical half life of ^{211}At and thus could be more appropriate than intact mAbs for α -particle RIT when administered via the intravenous route. The potential of diabodies labeled with ^{211}At for the treatment of solid tumors has been investigated [45]. An anti-HER2 diabody C6.5, the GM17 diabody reactive to human Müllerian inhibiting substance type II receptor (MISIIR), and an anti-CEA diabody T84.66 were labeled with ^{211}At using *N*-succinimidyl *N*-[4- ^{211}At]astatophenethylsuccinamate and evaluated for the treatment of immunodeficient mice bearing HER2/-neu-positive MDA-MB-361/DYT2 tumors. A single i.v. injection of labeled C6.5 (45 μCi ; 1.7 MBq) resulted in a 57-d growth delay compared to controls. Three of the five mice that received the highest amount of radioactivity (45 μCi ; 1.7 MBq) exhibited a full remission and were tumor-free for 1 year following treatment; histopathologic examination revealed no signs of tumors. While delay in tumor growth was seen with ^{211}At -labeled T84.66 diabody, no complete responses were seen, possibly due to a relatively low expression of CEA compared with the specific activity of the labeled diabody. On the other hand, treatment with GM17 diabody was not effective at all, likely a result of a combination of low expression of this antigen and low affinity of the diabody for this molecular target.

Ovarian cancer is another potentially suitable setting for RIT using antibodies labeled with α -particle-emitting radionuclides. The potential usefulness of this strategy has been established in preclinical models, which formed the basis for a Phase I clinical study that was undertaken to determine the pharmacokinetics, dosimetry and toxicity of a ^{211}At -labeled mAb fragment [48]. The subjects in the study were a cohort of 9 women, who were initially successfully treated for ovarian carcinoma but later, relapsed and were treated long-term with salvage chemotherapy resulting in clinically and biochemically complete remission. The F(ab')_2 fragment of MX35, an antibody that recognizes the sodium-dependent phosphate transport protein 2b (NaPi2b) that is overexpressed in more than 90% of human ovarian epithelial cancers, was used for this study. It was labeled with ^{211}At using *N*-succinimidyl 3- ^{211}At]astatobenzoate (SAB). Patients received ^{211}At -MX35 F(ab')_2 (22.4–101 MBq/L) in Extraneal (1–2 L, 37 °C) by infusion via a peritoneal catheter over 30 min, together with 0.2 MBq of ^{125}I -human serum albumin (HSA) as a reference for *in vivo* stability; in some patients the thyroid was blocked. The decay-corrected ^{211}At and ^{125}I activity concentration in the peritoneal cavity decreased to 50% of the initial concentration (IC) at 24 h. In serum, the ^{211}At activity concentration increased to 6% of IC at 45 h compared with 10% for co-administered ^{125}I . For thyroid, these values for ^{211}At at 20 h were 127% when the thyroid was not blocked and 20% with blocking. Gamma imaging indicated abdominal distribution of ^{211}At -therapeutic similar to $^{99\text{m}}\text{Tc}$ -LyoMAA and no significant uptake in any organ other than unblocked thyroid. Bone marrow is generally the critical organ for RIT with β -emitters from a dosimetry perspective, even with intraperitoneal administration. From the radiation absorbed dose estimates obtained from this study, the peritoneum appeared to be the organ that may limit the administered activity. The highest activity concentration administered in this study (100 MBq/L) did not result in any adverse effects determined either subjectively or by evaluation of standard laboratory parameters. The authors predicted that, with their new labeling technique [49], they will be able to use higher amounts of activity in future studies. Taken together, these results are highly encouraging and suggest that it should be possible to deliver therapeutically effective

absorbed doses to microscopic ovarian carcinoma clusters within the peritoneal compartment by the intraperitoneal administration of $^{211}\text{At-MX35 F(ab')}_2$.

Theoretical considerations have indicated that the specific activity of an ^{211}At -labeled monoclonal antibody preparation may influence its therapeutic effectiveness. To investigate this possibility, mice inoculated with OVCAR-3 ovarian cancer cells were treated with equal amounts of ^{211}At -labeled MX35 F(ab')₂ with specific activities of 130, 32, 16 or 4 kBq/μg and 8 weeks later, were assessed for macro- and microscopic tumors and ascites [42]. The fraction of animals free of macro- and microscopic tumors and ascites (TFF) was 0.67, 0.73, 0.50, 0.50, and 0.17, respectively, for the groups treated with the $^{211}\text{At-MX35-F(ab')}_2$ of above specific activities; the TFF for the control group was zero. Only the difference in TFF of the group administered with specific activity 4 kBq/μg preparation was statistically significant compared to the 130 kBq/μg group. Although there was no significant difference in the therapeutic outcome for specific activities in the range of 130 to 16 kBq/μg, there was a trend of higher TFF with increasing specific activity suggesting that preparations with higher specific activities may be more beneficial. TFF was also determined in a different study from this group of investigators that was designed to evaluate the potential advantage of multi-cycle treatment with $^{211}\text{At-MX35-F(ab')}_2$ [50]. The TFF values were 0.17, 0.11, 0.39, 0.44, 0.44, and 0.67 when treated with 400 kBq of $^{211}\text{At-MX35 F(ab')}_2$ once or 2, 3, 4, 5, or 6 times, respectively. A significantly higher ($P < 0.05$) TFF was obtained when the treatment was repeated at least 3 times compared to a treatment regimen consisting of only 1 or 2 cycles. Treatment with unlabeled MX35 F(ab')₂ resulted in a TFF of zero. Moreover, ascites was present in 15 of 18 animals of the single cycle treatment group while none of the animals receiving 5 or 6 cycles had ascites. Taken together, repeated weekly intraperitoneal injections of up to 6 maximum tolerated amount of $^{211}\text{At-MX35 F(ab')}_2$ produced increased therapeutic efficacy without any observed toxicity, indicating a potential increase in the therapeutic index. In another theoretical study, this group of authors determined that tumor cure probability and metastatic cure probability was dependent on the radiation sensitivity of tumor cells [51].

Because kidneys are often the dose-limiting organs in targeted radiotherapy with molecules like F(ab')₂ that undergo rapid renal excretion, renal function was evaluated after intravenous administration of $^{211}\text{At-MX35-F(ab')}_2$ in athymic mice with and without subcutaneous OVCAR-3 ovarian cancer xenografts [52]. Mice were injected with 0.4, 0.8, or 1.2 MBq of $^{211}\text{At-MX35-F(ab')}_2$ in one, two or three fractions. The glomerular filtration rate (GFR) was utilized as an indicator of kidney function, and was determined using the plasma clearance of [^{51}Cr]EDTA. GFR was found to be dependent on the adsorbed doses to the kidneys. Absorbed doses of 16.4 ± 3.3 and 14.0 ± 4.1 Gy caused a 50% decrease in GFR 8-30 weeks after initiation of $^{211}\text{At-MX35-F(ab')}_2$ treatment in tumor- and non-tumor-bearing animals, respectively. Furthermore, the reduction in GFR progressed with time, suggesting that peak radiation effects on the kidney are manifested late. It should be noted that at activity levels close to the dose limit for severe myelotoxicity from this treatment regimen, the degradation in renal function was only minimal. The authors concluded that a mean absorbed dose of 10 Gy to the kidney was acceptable and that contrary to expectations, the kidneys would not be the dose-limiting organ when $^{211}\text{At-MX35-F(ab')}_2$ is administered systemically.

Carcinomatous meningitis (CM), a devastating disease characterized by the dissemination of malignant tumor cells into the subarachnoid space along the brain ventricle walls and spine, frequently occurs in HER2-positive breast carcinoma patients. The geometrical features of CM—free floating cancer cells and malignant coating of the surface of the intrathecal compartment—are such that treatment with a targeted α -particle therapeutic may offer the best chance of obtaining tumor control while minimizing deleterious side effects in

neighboring normal tissues of the central nervous system. With this goal in mind, the potential utility of ^{211}At -labeled anti-HER2 trastuzumab for treating CM was investigated in athymic rat model of CM [53]. Trastuzumab was labeled with ^{211}At using SAB and administered intrathecally to rats with CM derived from the MCF/HER2-18 cell line. The median survival of rats treated with 33 and 66 μCi (1.2 and 2.4 MBq) of labeled trastuzumab was 45 and 48 days, respectively, compared to 21 days for rats that received saline or unlabeled trastuzumab. Tumor growth along the neuroaxis was assessed by histopathological analysis and was in concordance with these results. In another experiment performed at a lower initial tumor burden, median survival was 68 and 92 days for the groups treated with 46 and 92 μCi (1.7 and 3.4 MBq), respectively, compared to 23 days for saline controls (Fig. 2). To determine the specificity of the therapeutic effect, an additional experiment was performed in which animals were administered saline, ^{211}At -trastuzumab (28 μCi ; 1 MBq) or a control antibody (TPS3.2) labeled with ^{211}At (30 μCi ; 1.1 MBq). Compared to 20 days for the saline group, the median survival was 36 and 29 days for animals treated with ^{211}At -trastuzumab and ^{211}At -TPS3.2, respectively (Fig. 3). Long term survivors were observed exclusively in the ^{211}At -trastuzumab cohort. These results are encouraging, suggesting that future studies in larger animals and in humans are warranted.

The potential of targeted radiotherapy using ^{211}At -labeled anti-CD45 antibody to replace total body irradiation as a conditioning regimen for hematopoietic cell transplantation has been investigated in normal mice [54]. An earlier study in a canine model using the same antibody labeled with ^{213}Bi showed sustained marrow engraftment; however, the limited availability, high cost, and short half-life of ^{213}Bi may preclude its clinical use, which prompted these investigators to evaluate ^{211}At -labeled mAb for this purpose. To accomplish ^{211}At -labeling, the antibody was derivatized with *N*-(15-(aminoacyl-*closo*-decaborate)-4,7,10-trioxatridecanyl)-3-maleimidopropionamide. From a prior study, this labeling approach was found to be superior to labeling using SAB [55]. Biodistribution studies indicated that the highest uptake of anti-CD45 antibody 30F11 occurred in the spleen, consistent with CD45 targeting and the spleen accumulation of ^{211}At -labeled 30F11 was considerably higher than that of ^{213}Bi -30F11. At equivalent radioactivity amounts of the two preparations, ^{211}At -30F11 was more effective in producing the desired myelosuppression effect than ^{213}Bi -30F11. All mice injected with 20 or 50 μCi (0.74 or 1.85 MBq) of ^{211}At -30F11, but none with the same activity amounts of ^{213}Bi -30F11, had lethal myeloablation. Severe reversible acute hepatic toxicity occurred with 50 μCi (1.85 MBq) of ^{213}Bi -30F11, but not with lower amounts of ^{213}Bi -30F11 or with any amount of ^{211}At -30F11. These results suggest that ^{211}At -labeled anti-CD45 antibody was far better than the ^{213}Bi -labeled mAb for delivering marrow-ablative absorbed doses without causing toxicities in other organs.

In an earlier study, Lindegren *et al.* have synthesized three ^{211}At -labeled, biotinylated, and charge-modified poly-L-lysine derivatives and evaluated these effector molecules for use in pre-targeted radioimmunotherapy. In a recent publication, the pretargeting approach was further evaluated using one of these effector molecules and an avidin-trastuzumab conjugate as the pre-targeting molecule [56]. After incubation for 1 h, $75.3 \pm 6.2\%$ of the added effector molecule had bound to avidin-trastuzumab pretargeted SKOV-3-cells *in vitro*. The non specific binding of the effector molecule was less than 1%. Based on these promising results, *in vivo* studies have been planned.

An anti-CD33 antibody labeled with ^{211}At was evaluated *in vitro* in HL-60 and K-562 myeloid cell lines to investigate the potential usefulness α -RIT for the treatment of acute myeloid leukemia [57]. The uptake of the labeled mAb in the low CD33-expressing K-562 cell line was considerably lower ($7.5 \pm 0.8\%$ of input radioactivity) than that in HL-60 cells ($27.6 \pm 1.2\%$ of input radioactivity), a high CD33-expressing cell line. Co-incubation with

excess unlabeled mAb reduced the uptake considerably in both cell lines. Treatment of HL-60 cells with the same concentration (4.29 $\mu\text{g/ml}$) of ^{211}At -labeled mAb (~1 molecule of 1000 molecules labeled, 500 kBq/ml) or the same mAb conjugated with the toxin calicheamicin (GO; 1:1 conjugation) resulted in the same degree of DNA double strand breaks (DDSBs). When GO was diluted with unlabeled mAb to create a molar ratio of ~1:1000 that is similar to that of ^{211}At -labeled mAb, or when the cells were treated with unlabeled mAb or free [^{211}At]astatide, no DDSBs were seen. Furthermore, DDSBs from ^{211}At -labeled mAb were dependent on the amount of the labeled antibody. A radioactivity-dependent apoptosis induction, as reflected by caspase activation, was seen with ^{211}At -labeled mAb or GO but not with unlabeled mAb. Cell killing, as assessed by cell viability assays, indicated that both ^{211}At -labeled mAb (~1:1000) and GO (1:1) were equally potent. These data suggest that ^{211}At -anti-CD33 may cause sufficient DDSBs to overcome a clinically relevant degree of anti-cytotoxic resistance such as that seen with calicheamicin-conjugated antibodies *in vivo*. Therefore these astatinated antibodies may be highly effective with regard to anti-leukemic therapeutic potential. However, ^{211}At -labeled mAb preparations with higher specific activity may be necessary for the therapy of tumors that express low levels of antigens.

^{223}Ra RADIUM AGENTS

The element radium was discovered in 1898 by Marie and Pierre Curie [58] and in early stages, was used in brachytherapy or external teletherapy [59, 60]. There are several naturally occurring radium nuclides but from a consideration of several factors including their half life, α -particle emission and the half life of radon daughters, ^{223}Ra has been generally considered to be the optimal radionuclide for internal radiotherapy. While the half life of ^{224}Ra is similar to that of ^{223}Ra , the relatively longer half life of its radon daughter (^{220}Rn , 56 s compared to 4 s for ^{219}Rn from ^{223}Ra) makes it less attractive due to the possibility of translocation of daughter products from the primary desired site of uptake to other normal organs, potentially increasing dose limiting toxic effects. However, ^{224}Ra has been used in the treatment of noncancerous bone diseases such as ankylosing spondylitis. Radium-223 has several favorable features for its application in α -particle therapy. It can be produced easily and inexpensively via a generator and its relatively long half life of 11.4 days makes the preparation and portability of its α -emitting radiopharmaceuticals very convenient. For bone targeting, this longer half life is advantageous because it allows greater incorporation of the radionuclide into bone surfaces before decay occurs. Radium-223 decays through a chain of radioactive daughters (Fig. 4) with the emission of approximately 28 MeV of total energy per decay with ~96% of the decay energy released as α -particles. Most of the work done to date on internal radiotherapy with ^{223}Ra has utilized the simple cationic form of radium but attempts also have been made to evaluate its use after incorporating into liposomes or mAbs.

Cationic Radium

Radium is chemically similar to barium and thus has a proclivity to bone, most likely by incorporating into bone mineral calcium hydroxy apatite. This fact has been exploited in the use of ^{223}Ra in the treatment of bone metastases especially those associated with breast and prostate cancer.

Preclinical Studies—A drawback of common bone targeting radiopharmaceuticals such as sodium [^{32}P]orthophosphate and [^{89}Sr]strontium chloride is that they cause significant irradiation of healthy bone marrow due to the longer range of β -particles. To determine whether ^{223}Ra could be utilized to circumvent this problem, the biodistribution of ^{223}Ra was compared with that of ^{89}Sr in normal mice to determine the relative uptake in soft tissues

and bone [61]. In addition, dosimetric analysis was performed to estimate the degree to which deposit of these two agents on the bone surfaces would irradiate healthy red marrow. After intravenous injection of [^{223}Ra]radium chloride, femur uptake of 41% ID/g was seen as early as 1 h. While the soft tissue uptake was considerably lower and decreased with time, bone radioactivity levels did not reduce significantly over 14 d (31% ID/g in femur). Although the biodistribution pattern of ^{89}Sr was similar, the femur uptake (18% and 21% ID/g at 1 h and 14 d, respectively) was considerably lower than that of ^{223}Ra . Only very little, if any, of the daughter radionuclides of ^{223}Ra redistributed from the site of radium decay in the bone to soft tissues. Dosimetric analysis indicated that, for a given absorbed dose to bone, ^{223}Ra delivered a greater absorbed dose to soft tissues such as the kidney and spleen than ^{89}Sr . However, because ^{223}Ra provides high, intensely localized radiation dose deposition to bone surfaces, the administered activity of ^{223}Ra can be adjusted in such a way as to deliver a therapeutically relevant absorbed dose to bone surfaces with acceptable radiation dose to the kidney and spleen. For example, in the case of ^{223}Ra , the estimated absorbed dose in a 250- μm sphere decreased steeply from approximately 65 Gy at 5 μm from the surface to 0 Gy at about 70 μm ; only small changes in absorbed dose with distance was seen in the case of ^{89}Sr . Moreover, the absorbed dose received by the marrow, the critical organ for limiting normal tissue toxicity, from ^{223}Ra would be considerably lower than that from ^{89}Sr .

In a companion study, these authors demonstrated a significant antitumor effect of [^{223}Ra]radium chloride in a bone metastases model [62]. First, biodistribution was determined in non tumor-bearing nude rats to confirm selective uptake in bone. Although significant uptake was also seen in bone marrow at 24 h ($1.0 \pm 0.3\%$ ID/g), unlike in bone matrix, it decreased considerably by 14 d ($<0.2\%$ ID/g). In the therapy experiments, control rats administered intravenously with a million of MT-1 breast cancer cells developed tumor-induced paralysis within 20-30 days. On the other hand, a significantly higher survival was seen when these tumor-bearing animals were administered 6 and 11 kBq of [^{223}Ra]radium chloride, with 36% of the animals treated with 11 kBq having symptom-free survival. In another treatment regimen, animals were administered with a bone resorption inhibitor, Aredia a day after injecting 0, 5, 10 or 30 kBq of [^{223}Ra]radium chloride. The group receiving Aredia alone has no survivors beyond 21 days, suggesting no therapeutic effect from this bone resorption inhibitor. Two out of 5 animals from the 10 and 30 kBq groups survived for more than 50 days. No bone marrow toxicity or body weight loss was seen in the treated group. These results suggested that bone metastases could be treated by the intravenous administration of [^{223}Ra]radium chloride without undue toxicity to bone marrow.

A short term acute toxicity study after intravenous administration of potentially toxic amounts of [^{223}Ra]radium chloride in Balb/c mice was then conducted [63]. Groups of 10 animals were treated with 0, 1250, 2500, or 3750 kBq/kg body weight of [^{223}Ra]radium chloride. Except for a few mice, most of the animals survived until the end of the 30 d observation period. Treatment with ^{223}Ra resulted in a radioactivity concentration-related minimal to moderate depletion of osteocytes and osteoblasts in the bones. A radioactivity concentration-related minimal to marked depletion of hematopoietic cells in the bone marrow, and a minimal to slight extramedullary hematopoiesis in the spleen and in the mandibular and mesenteric lymph nodes were also observed. With this range of radioactivity, the LD_{50} for acute toxicity, defined as death within 4 weeks of receiving the substance, was not reached. These results suggest that high amounts of ^{223}Ra did not completely inactivate the blood-producing cells. The relatively high tolerance to an α -particle emitter primarily localized in the skeleton was likely due to two factors—1) red bone marrow cells located distant from the bone surface were not affected due to the short

range of alpha particles and 2) any loss of blood-producing cells was compensated by the recruitment of peripheral stem cells.

A preliminary biodistribution of ^{223}Ra in canines has shown affinity for and stability within calcified tissues [64]. Elimination of radioactivity was mainly via the intestine and it resided in transit within the gut content with minimal activity in intestinal walls. The highest concentration of ^{223}Ra , as determined by α -track microautoradiography of canine specimens, was found on the surfaces of trabecular bone, in vertebra and in strongly osteoblastic bone matrix.

Clinical Studies—Encouraged by the above preclinical results, a Phase I study was initiated with the goal of determining the safety and tolerability of this radiotherapeutic in breast and prostate cancer patients with bone metastases and to evaluate pain palliation [65]. Twenty five patients—15 with prostate and 10 with breast carcinoma—were involved in this study. Groups of 5 patients received a single injection of 46, 93, 163, 213, or 250 kBq/kg of Alpharadin7 ($^{223}\text{RaCl}_2$ preparation from Algeta, Oslo, Norway) and were followed for 8 weeks. While no dose-limiting hemotoxicity was observed at any dosage level, some mild and reversible myelosuppression with a nadir at 2 to 4 weeks after administration occurred. While only grade 1 thrombocytopenia was seen, grade 3 neutropenia and leucopenia occurred in two and three patients, respectively. Ten out of 25 patients suffered from mild and transient diarrhea, and nausea and vomiting was a common occurrence in the high dosage cohort. It has been shown that reduction in serum alkaline phosphatase levels or prevention of increase in bone alkaline phosphatase levels could increase the time to progression in prostate cancer patients. While there was a decline in serum alkaline phosphatase in all patients, it was more prominent in patients with prostate carcinoma. The ^{223}Ra radioactivity distribution as determined by gamma scintigraphy indicated accumulation of radioactivity in skeletal lesions and was similar to that seen from $^{99\text{m}}\text{Tc}$ -MDP bone scans. More than half of the patients reported improved pain scores and a median survival of 20 months relative to the time of administration was seen. However, definite conclusions could not be drawn as a placebo control cohort was not included as a part of this study. In another phase I study involving 6 prostate cancer patients, the safety profile of repeated ^{223}Ra injections at two fixed dosage levels, administered with 3- or 6-week intervals between injections, was determined [64]. Patients received a total of up to 250 kBq/kg of ^{223}Ra in a fractionated regimen. Two patients received 2×125 kBq/kg of ^{223}Ra with a 6-week interval between administration and the other four patients received 5×50 kBq/kg of ^{223}Ra given with a 3-week interval between administrations. No significant adverse effects from repeated treatment were observed; the toxic effects experienced by patients given 5×50 kBq/kg were not significantly different from those experienced by patients from the previous study wherein the radioactivity was administered in a single lot.

A randomized, multicenter, double-blind, placebo-controlled, phase II study was conducted wherein 33 patients with hormone-refractory prostate cancer (HRPC) and bone pain, needing external beam therapy were treated with four 50 kBq/kg of ^{223}Ra (i.v.) at four week intervals; a cohort of 31 patients received just saline [66]. Only minimal hematological toxicity was observed. Thrombocytopenia was not seen in the treated group but was observed in one patient receiving placebo. On the other hand, grade 2+ neutropenia, which was reversible, was seen in 3 treated patients but not in the control group. In contrast, an earlier study involving the treatment of HRPC patients with the β -emitting radiotherapeutic ^{89}Sr reported that hematological toxicity was a significant problem. Grade 2+ thrombocytopenia was seen in 61% of ^{89}Sr -treated patients versus 10% in the placebo group. Notably, grade 4 thrombocytopenia was observed in 10% of patients in the ^{89}Sr group versus 2% in the placebo group. The authors attribute the beneficial effects of ^{223}Ra to the short range of its α -particles, which reduces bone marrow:bone dose ratios in

comparison to those associated with β -particle emitting ^{89}Sr . The low toxicity profile of ^{223}Ra should allow repeat treatment regimens that can be implemented without the need to wait for bone marrow recovery. A notable non-hematological toxic effect was constipation and this was unexpected because diarrhea was seen in the phase I study. The median relative change in bone alkaline phosphatase from baseline to 4 weeks after administration of the last ^{223}Ra activity was $-65 \cdot 6\%$ (95% CI $-69 \cdot 5$ to $-57 \cdot 7$) in the treated group and this value was $9 \cdot 3\%$ ($3 \cdot 8$ – $60 \cdot 9$) in the placebo group ($p < 0.0001$). The median relative change in PSA during the same time interval was -23.8% (range -98.6 to 545.6) in the ^{223}Ra group and 44.9% (-91.3 to 563.5) in the placebo group ($p = 0.003$). Although these results should be interpreted with caution due to the low number of subjects, the median overall survival in the ^{223}Ra group was 65.3 weeks compared with 46.4 weeks in the placebo group. It should be noted that treatment with ^{89}Sr was shown to significantly reduce alkaline phosphatase and PSA concentrations, but no effect was seen on overall survival. In short, ^{223}Ra was well tolerated with no myelotoxic effect and showed promise of efficacy warranting further evaluation in larger groups of patients. Preliminary reports describing additional Phase II studies (up to 225 patients) have appeared [12] and/or have been presented in conferences, and patients are currently being evaluated in a multi-center phase III study.

Chelating agents for Complexing ^{223}Ra

Radium-223 and ^{224}Ra have a relatively long half life compared to more widely investigated α -emitters such as ^{211}At and ^{213}Bi . Considering the slow plasma clearance of mAbs, ^{223}Ra may be a more appropriate nuclide for the applications of alpha radioimmunotherapy involving intravenous administration. A prosthetic group encompassing a chelating moiety is required for labeling mAbs with radiometals in a stable fashion. To facilitate labeling mAbs with ^{223}Ra , several chelating agents have been evaluated for its complexation [67]. Of the four chelating agents studied, calix[4]-tetraacetic acid appears to be the most promising. However, the authors predicted that this chelating agent may not be suitable for *in vivo* use due to the rapid dissociation of ^{223}Ra from the complex.

Liposome as a Carrier for ^{223}Ra

The preparation and serum stability of ^{223}Ra loaded onto sterically stabilized liposome has been reported [68]. A folate-derivatized F(ab')_2 fragment of a myeloma antibody was coated on to the above liposome and evaluated for its binding to folate receptors (FR) on OvCar-3 human ovarian carcinoma cells. More than 90% of ^{223}Ra was retained in the liposome after incubation in serum for 100 h, suggesting its potential usefulness for *in vivo* applications. Receptor-mediated binding of the ^{223}Ra -loaded liposome coated with the folate-derivatized F(ab')_2 was demonstrated. From an assay wherein its specific binding was determined as a function of concentration, there appeared to be two types of interaction between the ligand-coated liposome and receptor expressing cancer cells—a strong binding at lower concentrations and a weak one at higher concentrations. A potential advantage of this composite construct is that it can be used to target tumor cells expressing not only FR but the antigenic target of the myeloma antibody fragment. In another study these authors determined the biodistribution and radiation dosimetry of both ^{223}Ra encapsulated in pegylated liposomes with doxorubicin and free $[^{223}\text{Ra}]\text{RaCl}_2$ in normal mice [69]. The blood clearance half life for the liposomal radioactivity was more than 24 h compared to less than an hour for the cationic ^{223}Ra suggesting that, as seen *in vitro*, the liposome preparation is rather stable *in vivo* as well. On a % ID/gram basis, the spleen had the highest uptake among soft tissues, consistent with the tissue distribution expected for an intact liposome. On the other hand, on a per organ basis, liver has the highest uptake, again consistent with the disposition of a particulate in the body. Uptake of radioactive liposomes in soft tissues in general, and those of the reticuloendothelial system (RES) in particular, may be problematic

for cancer therapy. In this study, when mice were treated with unlabeled liposomes 4 d before administering the ^{223}Ra -loaded liposome, the uptake in the reticuloendothelial system was reduced, suggesting presaturation as a means to reduce uptake in RES. Dosimetry calculations indicated that the spleen was the organ that received the highest absorbed dose, even higher than femur and skull. Preliminary results from the evaluation of liposomal ^{223}Ra in osteosarcoma-bearing mice and dogs have been reported [59]. There were species-dependent differences in tissue distribution of ^{223}Ra activity. Tumor retention of radioactivity in mice relative to that in soft tissues was higher with maximum retention seen at 4-5 days after administration. In dogs, the uptake was considerably higher in both calcified and non-calcified tumor metastases of different organs compared to normal tissues. Spleen uptake was lower in dogs than in mice; on the other hand, uptake in most tumors was higher in dogs than in murine xenografts. Overall, liposome loaded with ^{223}Ra seems to be a promising agent for alpha radiotherapy of cancers especially for bone metastases.

SUMMARY AND CONCLUSIONS

Targeted α -particle radiotherapy remains a conceptually appealing approach for cancer treatment, particularly for malignancies that can best exploit the high LET and short range of this type of radiation. During the past few years, considerable progress has been made in translating this concept to the clinic with both ^{211}At - and ^{223}Ra -labeled radio-pharmaceuticals. With ^{211}At , the chemical nature of astatine and the physical properties of this radionuclide are such that a wide variety of approaches are possible and it is anticipated that new clinical trials will be initiated in the next few years with several of the molecules described in this review, notably ^{211}At -labeled trastuzumab and MABG. With ^{223}Ra , the vast majority of research has been focused on one compound – [^{223}Ra]radium chloride – exploiting the proclivity of cationic radium to localize in the bone. Given the poor *in vivo* stability of known radium complexes, the development of non-osseously targeted ^{223}Ra complexes will be difficult. On the other hand, given the clinical results obtained to date and involvement of a company in its development, we speculate that there is a good chance that [^{223}Ra]radium chloride will become the first α -particle emitting drug to receive approval for cancer treatment.

References

1. Humm JL. Dosimetric aspects of radiolabeled antibodies for tumor therapy. *J Nucl Med.* 1986; 27:1490–1497. [PubMed: 3528417]
2. Howell RW. Auger processes in the 21st century. *Int J Radiat Biol.* 2008; 84:959–975. [PubMed: 19061120]
3. Boyd M, Sorensen A, McCluskey AG, Mairs RJ. Radiation quality-dependent bystander effects elicited by targeted radionuclides. *J Pharm Pharmacol.* 2008; 60:951–958. [PubMed: 18644188]
4. Vaidyanathan G, Larsen RH, Zalutsky MR. 5- ^{211}At astato-2'-deoxyuridine, an alpha particle-emitting endoradiotherapeutic agent undergoing DNA incorporation. *Cancer Res.* 1996; 56:1204–1209. [PubMed: 8640798]
5. Claesson AK, Stenerlöv B, Jacobsson L, Elmroth K. Relative biological effectiveness of the alpha-particle emitter (^{211}At)astato-2'-deoxyuridine for double-strand break induction in human fibroblasts. *Radiat Res.* 2007; 167:312–318. [PubMed: 17316073]
6. Hall, EJ.; Giaccia, AJ. *Radiobiology for the Radiologist.* Lippincott Williams & Wilkins; Philadelphia: 2006.
7. Sautter-Bühl ML, Herbold G, Bühl H. Minimal residual disease: a target for radioimmunotherapy with ^{131}I -labeled monoclonal antibodies? Some dosimetric considerations. *Rec Res Cancer Res.* 1996; 141:67–75.
8. Vaidyanathan G, Zalutsky MR. Astatine radiopharmaceuticals: prospects and problems. *Curr Radiopharm.* 2008; 1:177. [PubMed: 20150978]

9. Cordier D, Forrer F, Bruchertseifer F, Morgenstern A, Apostolidis C, Good S, Muller-Brand J, Macke H, Reubi JC, Merlo A. Targeted alpha-radionuclide therapy of functionally critically located gliomas with ^{213}Bi -DOTA-[Thi8, Met(O2)11]-substance P: a pilot trial. *Eur J Nucl Med Mol Imaging*. 2010; 37:1335–1344. [PubMed: 20157707]
10. Lingappa M, Song H, Thompson S, Bruchertseifer F, Morgenstern A, Sgouros G. Immunoliposomal delivery of ^{213}Bi for alpha-emitter targeting of metastatic breast cancer. *Cancer Res*. 2010; 70:6815–6823. [PubMed: 20651254]
11. Wesley JN, McGee EC, Garmestani K, Brechbiel MW, Yordanov AT, Wu C, Gansow OA, Eckelman WC, Bacher JD, Flynn M, Goldman CK, MacLin M, Schwartz UP, Jackson-White T, Phillip CM, Decker J, Waldmann TA. Systemic radioimmunotherapy using a monoclonal antibody, anti-Tac directed toward the alpha subunit of the IL-2 receptor armed with the alpha-emitting radionuclides ^{212}Bi or ^{211}At . *Nucl Med Biol*. 2004; 31:357–364. [PubMed: 15028248]
12. Lieve K. Alpharadin, a ^{223}Ra -based alpha-particle-emitting pharmaceutical for the treatment of bone metastases in patients with cancer. *Curr Opin Investig Drugs*. 2009; 10:1346–1358.
13. Song H, Hobbs RF, Vajravelu R, Huso DL, Esaias C, Apostolidis C, Morgenstern A, Sgouros G. Radioimmunotherapy of breast cancer metastases with alpha-particle emitter ^{225}Ac : comparing efficacy with ^{213}Bi and ^{90}Y . *Cancer Res*. 2009; 69:8941–8948. [PubMed: 19920193]
14. Penheiter AR, Wegman TR, Classic KL, Dingli D, Bender CE, Russell SJ, Carlson SK. Sodium iodide symporter (NIS)-mediated radiovirotherapy for pancreatic cancer. *Am J Roentgenol*. 2010; 195:341–349. [PubMed: 20651188]
15. Carlin S, Akabani G, Zalutsky MR. *In vitro* cytotoxicity of ^{211}At -astatide and ^{131}I -iodide to glioma tumor cells expressing the sodium/iodide symporter. *J Nucl Med*. 2003; 44:1827–1838. [PubMed: 14602867]
16. Willhauck MJ, Samani BR, Wolf I, Senekowitsch-Schmidtke R, Stark HJ, Meyer GJ, Knapp WH, Goke B, Morris JC, Spitzweg C. The potential of ^{211}At for NIS-mediated radionuclide therapy in prostate cancer. *Eur J Nucl Med Mol Imaging*. 2008; 35:1272–1281. [PubMed: 18404268]
17. Moon DH, Lee SJ, Park KY, Park KK, Ahn SH, Pai MS, Chang H, Lee HK, Ahn IM. Correlation between $^{99\text{m}}\text{Tc}$ -pertechnetate uptakes and expressions of human sodium iodide symporter gene in breast tumor tissues. *Nucl Med Biol*. 2001; 28:829–834. [PubMed: 11578905]
18. Kilbane MT, Ajjan RA, Weetman AP, Dwyer R, McDermott EW, O'Higgins NJ, Smyth PP. Tissue iodine content and serum-mediated ^{125}I uptake-blocking activity in breast cancer. *J Clin Endocrinol Metab*. 2000; 85:1245–1250. [PubMed: 10720070]
19. Li H, Peng KW, Dingli D, Kratzke RA, Russell SJ. Oncolytic measles viruses encoding interferon beta and the thyroidal sodium iodide symporter gene for mesothelioma virotherapy. *Cancer Gene Ther*. 2010; 17:550. [PubMed: 20379224]
20. Trujillo MA, Oneal MJ, McDonough S, Qin R, Morris JC. A probasin promoter, conditionally replicating adenovirus that expresses the sodium iodide symporter (NIS) for radiovirotherapy of prostate cancer. *Gene Ther*. 2010; 17:1325–1332. [PubMed: 20428214]
21. Lundh C, Lindencrona U, Postgård P, Carlsson T, Nilsson M, Fossell-Aronsson E. Radiation-induced thyroid stunning: differential effects of ^{123}I , ^{131}I , $^{99\text{m}}\text{Tc}$, and ^{211}At on iodide transport and NIS mRNA expression in cultured thyroid cells. *J Nucl Med*. 2009; 50:1161–1167. [PubMed: 19525464]
22. Turtoi A, Schneeweiss FH. Effect of ^{211}At alpha-particle irradiation on expression of selected radiation responsive genes in human lymphocytes. *Int J Radiat Biol*. 2009; 85:403–412. [PubMed: 19382019]
23. Turtoi A, Sharan RN, Srivastava A, Schneeweiss FH. Proteomic and genomic modulations induced by γ -irradiation of human blood lymphocytes. *Int J Radiat Biol*. 2010; 86:888–904. [PubMed: 20653344]
24. Vaidyanathan G, Affleck DJ, Alston KL, Zalutsky MR. A tin precursor for the synthesis of no-carrier-added ^{211}At -MIBG and ^{211}At -MABG. *J Label Compd Radiopharm*. 2007; 50:177–182.
25. Vaidyanathan G, Affleck DJ, Alston KL, Zhao XG, Hens M, Hunter DH, Babich J, Zalutsky MR. A kit method for the high level synthesis of ^{211}At -MABG. *Bioorg Med Chem*. 2007; 15:3430–3426. [PubMed: 17387017]

26. Vaidyanathan G, Zalutsky MR. 1-(m-[²¹¹At]astatobenzyl)guanidine: synthesis via astato demetalation and preliminary *in vitro* and *in vivo* evaluation. *Bioconjug Chem*. 1992; 3:499–503. [PubMed: 1463779]
27. Vaidyanathan G, Friedman HS, Keir ST, Zalutsky MR. Evaluation of meta-[²¹¹At]astatobenzylguanidine in an athymic mouse human neuroblastoma xenograft model. *Nucl Med Biol*. 1996; 23:851–856. [PubMed: 8940730]
28. Cunningham SH, Mairs RJ, Wheldon TE, Welsh PC, Vaidyanathan G, Zalutsky MR. Toxicity to neuroblastoma cells and spheroids of benzylguanidine conjugated to radionuclides with short-range emissions. *Br J Cancer*. 1998; 77:2061–2068. [PubMed: 9649115]
29. Strickland DK, Vaidyanathan G, Zalutsky MR. Cytotoxicity of alpha-particle-emitting m-[²¹¹At]astatobenzylguanidine on human neuroblastoma cells. *Cancer Res*. 1994; 54:5414–5419. [PubMed: 7923174]
30. Boyd M, Mairs RJ, Keith WN, Ross SC, Welsh P, Akabani G, Owens J, Vaidyanathan G, Carruthers R, Dorrens J, Zalutsky MR. An efficient targeted radiotherapy/gene therapy strategy utilising human telomerase promoters and radioastatine and harnessing radiation-mediated bystander effects. *J Gene Med*. 2004; 6:937–947. [PubMed: 15293352]
31. Boyd M, McCluskey AG, Sorensen A, Mairs RJ. Experimental targeted therapy using [²¹¹At]meta-astatobenzyl-guanidine and transfection of the noradrenaline transporter gene. *Curr Radiopharm*. 2008; 1:197–202.
32. Boyd M, Ross SC, Dorrens J, Fullerton NE, Tan KW, Zalutsky MR, Mairs RJ. Radiation-induced biologic bystander effect elicited *in vitro* by targeted radiopharmaceuticals labeled with alpha-, beta-, and auger electron-emitting radionuclides. *J Nucl Med*. 2006; 47:1007–1015. [PubMed: 16741311]
33. Mairs RJ, Fullerton NE, Zalutsky MR, Boyd M. Targeted radiotherapy: microgray doses and the bystander effect. *Dose-Response*. 2007; 5:204–213. [PubMed: 18648605]
34. Mairs RJ, Boyd M. Optimizing MIBG therapy of neuroendocrine tumors: preclinical evidence of dose maximization and synergy. *Nucl Med Biol*. 2008; 35:9s–20s.
35. Vaidyanathan G, Zalutsky MR. Preparation of 5-[¹³¹I]iodo- and 5-[²¹¹At]astato-1-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl) uracil by a halodestannylation reaction. *Nucl Med Biol*. 1998; 25:487–496. [PubMed: 9720667]
36. Yang Y, Liu N, Liao J, Pu M, Liu Y, Wei M, Jin J. Preparation and preliminary evaluation of ²¹¹At-labeled amidobisphosphonates. *J Radioanal Nucl Chem*. 2010; 283:329–335.
37. Larsen RH, Murud KM, Akabani G, Hoff P, Bruland OS, Zalutsky MR. ²¹¹At- and ¹³¹I-labeled bisphosphonates with high *in vivo* stability and bone accumulation. *J Nucl Med*. 1999; 40:1197–1203. [PubMed: 10405142]
38. Meyer GJ, Walte A, Sriyapureddy SR, Grote M, Krull D, Korkmaz Z, Knapp WH. Synthesis and analysis of 2-[²¹¹At]-L-phenylalanine and 4-[²¹¹At]-L-phenylalanine and their uptake in human glioma cell cultures in-vitro. *Appl Radiat Isot*. 2010; 68:1060–1065. [PubMed: 20137958]
39. Vaidyanathan G, Mease RC, Affleck DJ, Chen Y, Welsh P, Hens M, Pomper MG, Zalutsky MR. An astatine-211 labeled PSMA inhibitor for targeted alpha-particle radiotherapy of prostate carcinoma. *J Nucl Med*. 2009; 50, 11P.
40. Wilbur DS, Chyan MK, Hamlin DK, Perry MA. Reagents for astatination of biomolecules. 3. Comparison of closo-decaborate(2-) and closo-dodecaborate(2-) moieties as reactive groups for labeling with astatine-211. *Bioconjug Chem*. 2009; 20:591–602. [PubMed: 19236022]
41. Rosenkranz AA, Vaidyanathan G, Pozzi OR, Lunin VG, Zalutsky MR, Sobolev AS. Engineered modular recombinant transporters: application of new platform for targeted radiotherapeutic agents to alpha-particle emitting ²¹¹At. *Int J Radiat Oncol Biol Phys*. 2008; 72:193–200. [PubMed: 18722270]
42. Elgqvist J, Andersson H, Haglund E, Jensen H, Kahu H, Lindegren S, Warnhammar E, Hultborn R. Intraperitoneal alpha-radioimmunotherapy in mice using different specific activities. *Cancer Biother Radiopharm*. 2009; 24:509–513. [PubMed: 19694586]
43. Wilbur DS, Chyan MK, Hamlin DK, Vessella RL, Wedge TJ, Hawthorne MF. Reagents for astatination of biomolecules. 2. Conjugation of anionic boron cage pendant groups to a protein

- provides a method for direct labeling that is stable to *in vivo* deastatination. *Bioconjug Chem.* 2007; 18:1226–1240. [PubMed: 17583925]
44. Demartis S, Tarli L, Borsi L, Zardi L, Neri D. Selective targeting of tumour neovasculature by a radiohalogenated human antibody fragment specific for the ED-B domain of fibronectin. *Eur J Nucl Med.* 2001; 28:534–539. [PubMed: 11357506]
 45. Robinson MK, Shaller C, Garmestani K, Plascjak PS, Hodge KM, Yuan QA, Marks JD, Waldmann TA, Brechbiel MW, Adams GP. Effective treatment of established human breast tumor xenografts in immunodeficient mice with a single dose of the alpha-emitting radioisotope astatine-211 conjugated to anti-HER2/neu diabodies. *Clin Cancer Res.* 2008; 14:875–882. [PubMed: 18245551]
 46. Steffen AC, Almqvist Y, Chyan MK, Lundqvist H, Tolmachev V, Wilbur DS, Carlsson J. Biodistribution of ^{211}At labeled HER-2 binding affibody molecules in mice. *Oncol Rep.* 2007; 17:1141–1147. [PubMed: 17390057]
 47. Almqvist Y, Steffen AC, Lundqvist H, Jensen H, Tolmachev V, Sundin A. Biodistribution of ^{211}At -labeled humanized monoclonal antibody A33. *Cancer Biother Radiopharm.* 2007; 22:480–487. [PubMed: 17803442]
 48. Andersson H, Cederkrantz E, Bäck T, Divgi C, Elgqvist J, Himmelman J, Horvath G, Jacobsson L, Jensen H, Lindegren S, Palm S, Hultborn R. Intraperitoneal alpha-particle radioimmunotherapy of ovarian cancer patients: pharmacokinetics and dosimetry of ^{211}At -MX35 F(ab')₂-a phase I study. *J Nucl Med.* 2009; 50:1153–1160. [PubMed: 19525452]
 49. Lindegren S, Frost S, Bäck T, Haglund E, Elgqvist J, Jensen H. Direct procedure for the production of ^{211}At -labeled antibodies with an epsilon-lysyl-3-(trimethylstannyl)benzamide immunoconjugate. *J Nucl Med.* 2008; 49:1537–1545. [PubMed: 18703591]
 50. Elgqvist J, Andersson H, Jensen H, Kahu H, Lindegren S, Warnhammar E, Hultborn R. Repeated intraperitoneal alpha-radioimmunotherapy of ovarian cancer in mice. *J Oncol.* 2010; 2010:394913. [PubMed: 19859581]
 51. Elgqvist J, Johansson BR, Partheen K, Danielsson A. Tumor cure probability during alpha-RIT of ovarian cancer with different radiation sensitivity. *Anticancer Res.* 2010; 30:2545–2551. [PubMed: 20682981]
 52. Bäck T, Haraldsson B, Hultborn R, Jensen H, Johansson ME, Lindegren S, Jacobsson L. Glomerular filtration rate after alpha-radioimmunotherapy with ^{211}At -MX35-F(ab')₂: a long-term study of renal function in nude mice. *Cancer Biother Radiopharm.* 2009; 24:649–658. [PubMed: 20025544]
 53. Boskovitz A, McLendon RE, Okamura T, Sampson JH, Bigner DD, Zalutsky MR. Treatment of HER2-positive breast carcinomatous meningitis with intrathecal administration of alpha-particle-emitting ^{211}At -labeled trastuzumab. *Nucl Med Biol.* 2009; 36:659–669. [PubMed: 19647172]
 54. Nakamae H, Wilbur DS, Hamlin DK, Thakar MS, Santos EB, Fisher DR, Kenoyer AL, Pagel JM, Press OW, Storb R, Sandmaier BM. Biodistributions, myelosuppression, and toxicities in mice treated with an anti-CD45 antibody labeled with the alpha-emitting radionuclides bismuth-213 or astatine-211. *Cancer Res.* 2009; 69:2408–2415. [PubMed: 19244101]
 55. Wilbur DS, Thakar MS, Hamlin DK, Santos EB, Chyan MK, Nakamae H, Pagel JM, Press OW, Sandmaier BM. Reagents for astatination of biomolecules. 4. Comparison of maleimido-closodecaborate(2-) and meta- ^{211}At astatobenzoate conjugates for labeling anti-CD45 antibodies with ^{211}At astatine. *Bioconjug Chem.* 2009; 20:1983–1991. [PubMed: 19731929]
 56. Frost S, Jensen H, Lindegren S. *In vitro* evaluation of avidin antibody pretargeting using ^{211}At -labeled and biotinylated poly-L-lysine as effector molecule. *Cancer.* 2010; 116:1101–1110. [PubMed: 20127953]
 57. Petrich T, Korkmaz Z, Krull D, Fromke C, Meyer GJ, Knapp WH. *In vitro* experimental ^{211}At -anti-CD33 antibody therapy of leukaemia cells overcomes cellular resistance seen *in vivo* against gemtuzumab ozogamicin. *Eur J Nucl Med Mol Imaging.* 2010; 37:851–861. [PubMed: 20107790]
 58. Mazon JJ, Gerbaulet A. The centenary of discovery of radium. *Radiother Oncol.* 1998; 49:205–216. [PubMed: 10075253]
 59. Bruland OS, Jonasdottir T, Fisher DR, Larsen RH. Radium-223: From radiochemical development to clinical applications in targeted cancer therapy. *Curr Radiopharm.* 2008; 1:203–208.

60. Dutreix J, Tubiana M, Pierquin B. The hazy dawn of brachytherapy. *Radiother Oncol.* 1998; 49:223–232. [PubMed: 10075255]
61. Henriksen G, Fisher DR, Roeske JC, Bruland OS, Larsen RH. Targeting of osseous sites with alpha-emitting ^{223}Ra : comparison with the beta-emitter ^{89}Sr in mice. *J Nucl Med.* 2003; 44:252–259. [PubMed: 12571218]
62. Henriksen G, Breistol K, Bruland OS, Fodstad O, Larsen RH. Significant antitumor effect from bone-seeking, alpha-particle-emitting ^{223}Ra demonstrated in an experimental skeletal metastases model. *Cancer Res.* 2002; 62:3120–3125. [PubMed: 12036923]
63. Larsen RH, Saxtorph H, Skydsgaard M, Borrebaek J, Jonasdottir TJ, Bruland OS, Klastrup S, Harling R, Ramdahl T. Radiotoxicity of the alpha-emitting bone-seeker ^{223}Ra injected intravenously into mice: histology, clinical chemistry and hematology. *In vivo.* 2006; 20:325–331. [PubMed: 16724665]
64. Bruland OS, Nilsson S, Fisher DR, Larsen RH. High-linear energy transfer irradiation targeted to skeletal metastases by the alpha-emitter ^{223}Ra : adjuvant or alternative to conventional modalities? *Clin Cancer Res.* 2006; 12:6250s–6257s. [PubMed: 17062709]
65. Nilsson S, Larsen RH, Fossa SD, Balteskard L, Borch KW, Westlin JE, Salberg G, Bruland OS. First clinical experience with alpha-emitting radium-223 in the treatment of skeletal metastases. *Clin Cancer Res.* 2005; 11:4451–4459. [PubMed: 15958630]
66. Nilsson S, Franzen L, Parker C, Tyrrell C, Blom R, Tennvall J, Lennernas B, Petersson U, Johannessen DC, Sokal M, Pigott K, Yachnin J, Garkavij M, Strang P, Harmenberg J, Bolstad B, Bruland OS. Bone-targeted radium-223 in symptomatic, hormone-refractory prostate cancer: a randomised, multicentre, placebo-controlled phase II study. *Lancet Oncol.* 2007; 8:587–594. [PubMed: 17544845]
67. Henriksen G, Hoff P, Larsen RH. Evaluation of potential chelating agents for radium. *Appl Radiat Isot.* 2002; 56:667–671. [PubMed: 11993940]
68. Henriksen G, Schoultz BW, Michaelsen TE, Bruland OS, Larsen RH. Sterically stabilized liposomes as a carrier for alpha-emitting radium and actinium radionuclides. *Nucl Med Biol.* 2004; 31:441–449. [PubMed: 15093814]
69. Jonasdottir TJ, Fisher DR, Borrebaek J, Bruland OS, Larsen RH. First *in vivo* evaluation of liposome-encapsulated ^{223}Ra as a potential alpha-particle-emitting cancer therapeutic agent. *Anticancer Res.* 2006; 26:2841–2848. [PubMed: 16886603]

Fig. (1). Clonogenic survival of A431 human epidermoid carcinoma cells after exposure for 4 h to varying activity concentrations of $[^{211}\text{At}]\text{SAGMB-MRT}$ (open diamonds) and $[^{211}\text{At}]\text{astatide}$ (closed squares). Confidence interval lines for $[^{211}\text{At}]\text{SAGMB-MRT}$ are not quite visible as they are very close to the regression line.

Fig. (2). Percentage of athymic rats with MCF-7/HER2-18 breast carcinoma carcinomatous meningitis surviving after intrathecal administration of 46 (dashed line) or 92 μCi (solid line) ^{211}At -labeled trastuzumab or saline (dotted line) 3 days after intrathecal injection of 5×10^6 tumor cells.

Fig. (3). Percentage of athymic rats with MCF-7/HER2-18 breast carcinoma carcinomatous meninges surviving after intrathecal administration of 28 µCi ²¹¹At-labeled trastuzumab (solid line), 30 µCi ²¹¹At-labeled TPS3.2 control mAb (dashed line) or saline 3 (dotted line) days after intrathecal injection of 5×10^6 tumor cells.

Fig. (4).
Decay scheme for Radium-223.