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Liberal, F. D. C. G., O'Sullivan, J. M., McMahon, S. J., & Prise, K. M. (2020). Targeted Alpha Therapy: Current Clinical Applications. *Cancer biotherapy & radiopharmaceuticals*. <https://doi.org/10.1089/cbr.2020.3576>

Published in:
Cancer biotherapy & radiopharmaceuticals

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

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Targeted Alpha Therapy: Current Clinical Applications

Francisco D. C. Guerra Liberal^{1,2}, Joe M. O'Sullivan^{1,3}, Stephen J. McMahon¹ and

Kevin M. Prise¹

1. The Patrick G Johnston Centre for Cancer Research, Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7AE, United Kingdom
2. Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
3. Clinical Oncology, Northern Ireland Cancer Centre, Belfast Health and Social Care Trust, Belfast, Northern Ireland, United Kingdom

(Corresponding author) – f.liberal@qub.ac.uk

Keywords

Alpha-Particles, Radium-223, Actinium-225, Thorium-227, Radiopharmaceutical

CRedit author statement

Francisco Guerra Liberal: Investigation, Writing- Original draft preparation, Writing - Review & Editing; Stephen J. McMahon: Writing - Review & Editing, Conceptualization; Joe O'Sullivan: Writing - Review & Editing; Kevin M Prise: Writing - Review & Editing, Supervision, Funding acquisition

All authors of this manuscript have reviewed and approved it before submission.

Conflict of Interest

Francisco Guerra Liberal and Stephen J McMahon declare no conflict of interests regarding the publication of this paper. Joe O'Sullivan has received honoraria for Speakers bureau and Advisory Board from Bayer. Joe O'Sullivan has received institutional research funding from Bayer. Kevin M Prise has received speaker honoraria from Bayer.

Funding

The authors gratefully acknowledge the support of Fundação para a Ciência e Tecnologia (FCT-MCTES), Radiation Biology and Biophysics Doctoral Training Programme (RaBBIT, PD/00193/2012); Applied Molecular Bioscience Unit - UCIBIO (UIDB/04378/2020) and CEFITEC Unit (UIDB/00068/2020); and scholarship grant number SFRH/BD/114448/2016 to (FL). The work was funded by the Movember Prostate Cancer UK Centre of Excellence (CEO13_2-004) and the Research and Development Division of the Public Health Agency of Northern Ireland (COM/4965/14).

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Abstract

Alpha emitting radionuclides have been approved for cancer treatment since 2013, with increasing degrees of success. Despite this clinical utility, little is known regarding the mechanisms of action of α -particles in this setting, and accurate assessments of the dosimetry underpinning their effectiveness are lacking. However, Targeted Alpha Therapy (TAT) is gaining more attention as new targets, synthetic chemistry approaches, and α -particle emitters are identified, constructed, developed and realised.

From a radiobiological perspective, α -particles are more effective at killing cells compared to low linear energy transfer (LET) radiation. As well as these direct effects it is now evident from preclinical and clinical data that α -emitters are capable of both producing effects in non-irradiated bystander cells and stimulating the immune system, extending the biological effects of TAT beyond the range of α -particles.

The short range of α -particles makes them a potent tool to irradiate single cell lesions or treat solid tumors by minimizing unwanted irradiation of normal tissue surrounding the cancer cells, assuming a high specificity of the radiopharmaceutical and good stability of its chemical bonds. Clinical approval of $^{223}\text{RaCl}_2$ in 2013 was a major milestone to the widespread application of TAT as a safe and effective strategy for cancer treatment. Additionally, ^{225}Ac -PSMA treatment benefit in metastatic Castrate Resistant Prostate Cancer refractory to standard therapies patients is another game-changing piece in the TAT clinical application short history. Investigations on TAT are growing with active clinical studies with different radionuclides, combination therapies or in different clinical settings.

Despite the remarkable advances in TAT dosimetry and imaging, it has not yet been used to its full potential. Labelled ^{227}Th and ^{225}Ac appear to be promising candidates and could represent the next generation of agents able to extend patient survival in several clinical scenarios.

Introduction

Targeted Alpha Therapy (TAT) is an attractive therapeutic option in patients with multiple micrometastases. It offers many advantages including easy administration, the ability to treat multiple lesions simultaneously, and the possibility of combination with other therapeutic approaches for improved efficacy.¹ TAT utilizes an α -particle emitting radionuclide, primarily for treatment of cancer but also, much less commonly, in some other diseases, such as HIV, bacterial and fungal infections.^{2,3}

TAT is typically delivered by attaching an α -emitting radionuclide to a biological molecule with targeting capabilities, such as a monoclonal antibody, with the help of a bi-specific chelating agent. This selectively delivers a high radiation dose directly to the target, with generally limited toxicity to the surrounding normal tissues.⁴ Advances in the understanding of tumor biology together with progress in monoclonal antibody technology, chemical labeling techniques and other related disciplines are providing significant advances in the development of new clinical applications of α -emitting radionuclides in novel therapeutic agents.

The concept of clinical targeted therapy is not new and has been discussed since the “magic-bullet” concept was first presented by Paul Erlich at the beginning of the 20th century⁵. As a key example, hyperthyroidism and thyroid cancer have been successfully treated using ^{131}I for over 80 years.⁶ Other β -emitting radionuclides, including samarium-153 (^{153}Sm) and strontium-89 (^{89}Sr), have been used for palliative treatment of bone metastases since the middle of 20th century.¹ Remarkably, Radium-223 (^{223}Ra) is the first radiopharmaceutical agent to demonstrate improved overall survival among castration resistant prostate cancer patients with symptomatic bone metastases, with mild side effects owing to its localized dose deposition.⁷ Its clinical use was approved by

the FDA in 2013, as Xofigo® ($^{223}\text{RaCl}_2$, formerly Alpharadin) from Bayer Healthcare after the publication of the ALSYMPCA trial. Recently, a new class of therapeutic radiopharmaceuticals for TAT have been proposed. They consist of an α -emitting radionuclide (^{225}Ac or ^{227}Th) complexed to a chelator conjugated to a monoclonal antibody.

Despite the expanding interest in TAT, in many preclinical and clinical studies, dosimetry and biological information at the microscopic level is sparse or not accurately defined.⁸ A better understanding of these factors will provide better tools to maximize the benefits of TAT, avoid undesired toxicity and determine the optimal quantity of an α -emitting radiopharmaceutical to be injected to a given patient. Similarly, routine clinical use of TAT will require new approaches with respect to dosimetry and imaging to monitor treatment efficacy.

TAT therapy has been shown to be a potent weapon in the treatment of some cancers, and many novel refinements are now being published, expanding the number of potential applications. Therefore, it is timely to provide an overview of recent progress in the field of TAT research.

Radiobiological Properties and Dosimetry of α -Therapy

An α -particle is an energetic (4 to 9 MeV) positively charged Helium-4 (^4He) nucleus, with a +2 electrical charge.⁹ They have a short path length, typically less than 100 μm , that is characterized by high LET (60-230 keV/ μm), depositing 1500 times more energy per unit path length than a β -particle.¹⁰ Simulations and experiments have shown that a few α -particles crossing a cell nucleus are enough to kill a cell whereas more than 10000 β -particles are needed to achieve the same biological effect.¹¹ This combination

of short range and highly cytotoxic interactions means alpha particles primarily kill target cells by self-irradiation, offering true selectivity with only minor collateral damage to nearby healthy tissues.¹²

This biological effectiveness of high LET radiation, in particular α -particles, is understood in terms of their propensity to induce complex DNA double-strand breaks (DSBs) and multiple clusters of DSBs in target cells, making cellular repair mechanisms ineffective.¹³ Also, the greater efficacy of α -particles has been demonstrated to be independent of the oxygenation status of the cell. Conversely, low LET radiotherapy is less effective in hypoxic cells and at low dose rates. Values for relative biological effectiveness (RBE) for *in vitro* and *in vivo* cell survival have been reported ranging from 3 to 8 for α -particles, indicating that, for an equal amount of energy deposited in a cell, the probability of cellular death is much greater than for low LET radiation.¹⁴ Radiobiological characterization of targeted radionuclide therapy has been reviewed by Pouget et al.^{8,15} However, the relevant radiobiology of α -particle radionuclides is far from being entirely understood and new insights are needed to maximize the effectiveness of this new modality.

Moreover, given the complex and stochastic nature of α -particle interactions, their dosimetry presents a number of challenges. Although most of α -emitting radionuclides can be imaged in γ -cameras, at present neither treatment planning nor post-therapeutic dosimetry are performed in clinical practice, due to the technical limitations of these techniques. But it has been an active area of research with several advances in clinical imaging and dosimetry in recent years.¹⁶

As stated above, following the *in vivo* biodistribution and pharmacokinetics of ^{223}Ra is feasible by γ -camera imaging, but poor spatial resolution makes it impossible to produce

accurate quantitative information at a cellular scale.¹⁶ Nevertheless, this can still produce useful clinical information. Hindorf et al. defined the imaging characteristics of ²²³Ra and its feasibility¹⁷, which enables the study of biodistribution and pharmacokinetics in human patients.^{18,19} Also, Abou et al. confirmed in animal models that ²²³Ra is deposited at the bone surface surrounding the tumor and skeletal accumulation was dependent on the local blood vessel density.²⁰ A correlation has been demonstrated between uptake of ²²³RaCl₂ with that of ^{99m}Tc and ¹⁸F, which was also associated with the absorbed dose in the lesion.^{21,22} Recently, studies have also been conducted to explore the usefulness of SPECT/CT ²²³Ra.^{23,24}

With regards to dosimetry, most conventional approaches are limited to average parameters such as absorbed dose to a macroscopic volume. Current dosimetry calculations predict a much higher level of hematotoxicity than experienced clinically. For example, a phase I clinical trial where patients received up to 925 kBq of ²¹³Bi reported α -particles doses of 1 Gy to the blood system. However, from microdosimetric calculations the actual dose to important cell targets was 2 cGy for endothelial cells and 10 cGy for lymphocytes, less than 10% of the macroscopic dose to the blood.⁹ This highlights that conventional dosimetric approaches are not always valid, highlighting the need to understand the distribution of dose at the cellular level to accurately predict biological effects.²⁵ Meaningful dosimetry studies with TAT require detailed information on the geometry of the target, as well as pharmacokinetic data of the α -emitting radionuclide and possible fate of daughters at a cellular and subcellular scale.¹⁴ Usually, this is achieved with geometrical approximations and mathematical modelling to describe the uptake, clearance and concentration of the radiopharmaceutical.

Palm et al. constructed a model to optimize radionuclide therapy, using radionuclide pharmacokinetics to estimate the absorbed dose in different tissues.²⁶ Hobbs et al. developed a bone marrow toxicity model for $^{223}\text{RaCl}_2$ and concluded that cellular scale dosimetry is necessary to explain the good toxicity profile of ^{223}Ra clinically observed.²⁷ Recently, Moreira et al. modelled different tumour growth models and three possible ^{223}Ra uptake scenarios to determine the most realistic scenario based on clinical data from the ALSYMPCA trial.²⁸ The authors concluded that only a sub-population of target cells are directly affected by ^{223}Ra with strong evidence of saturation in these hot-spots. This data confirms that TAT has not yet been used to its full potential, and using body weight to calculate the administered activity in a treatment fraction may potentially lead to many patients being overtreated (high toxicity) or undertreated (no clinical effect). Furthermore, Taprogge et al. showed by mathematical models that retention of ^{223}Ra in the human skeleton requires 2 different compartments, with an initial location to the bone surface followed by incorporation into the bone matrix.²⁹

Off-Target Effects of α -Particles

It is clear from the physical characteristics of TAT that due the short path length of α -particles relative to target dimensions and incomplete targeting, some cell nuclei receive multiple hits while others receive no direct irradiation, giving rise to an inhomogeneous irradiation profile.³⁰ Those cells which are not directly irradiated may diminish the overall therapeutic benefit. Because of this, early studies had predicted some advantage of β -emitting radionuclides in macrolesions due the crossfire effect (dose delivered outside the targeted cell due to long particle ranges) reducing problems associated with heterogeneous uptake in the target.³¹

However, biological off-target effects have been observed in response to ionizing radiation, including high LET irradiation. These are frequently termed “the bystander effect” and are most commonly seen as cell killing occurring in cells which see no direct irradiation.³² These effects may particularly important in heterogeneous regimens, where indirect killing of non-targeted cells is required to ensure maximal therapeutic benefit. In addition to local bystander effects, longer-range adaptive effects, mostly inflammatory responses in distant tissues, have also been reported. In some pre-clinical studies, bystander effects have been as effective as the direct effect after low-dose exposure, suggesting that under these conditions and in a low-dose rate scenario, a bystander response could have a large impact on overall effectiveness.³³ These effects are not currently considered in conventional treatment planning.

A series of elegant studies have clarified the role of bystander effects after irradiation with several radionuclides. In 2002 Xue et al. showed contradictory bystander responses in the same *in vivo* model after treatment with ¹²⁵I and ¹²³I, short range Auger emitters with substantial differences in half-life (13.3 hours and 60.5 days respectively).³⁴

In a different study, Boyd and colleagues compared the bystander effect induced by different radiation qualities.³⁵ In particular, they compared the effectiveness of indirect effects following external beam γ -radiation exposure with that following exposure to a β -emitter (¹³¹I), an Auger emitter (¹²³I), or an α -emitter (²¹¹At) in two different tumour lines. The standard bystander response mediated by external beam irradiation is an increase at low doses followed by a plateau. In contrast, treatment of cells with an α -emitting radionuclide led to a U-shaped response curve, with an increased cell kill in the recipient cells at low activities, but a decreased effect at increasing activities. This U-shape bystander curve response was also reported by Karthik et al. in human

lymphocytes exposed to external α -particles.³⁶ Finally, Ladjohounlou et al. reported that bystander effects contributed up to 36% of cell killing when α -emitters were used in *in vivo* and *in vitro* experiments³⁷

Understanding the mechanism of bystander effects may help TAT achieve its full potential and have profound implications on the clinical applications of radionuclides. Bystander effects in *in vivo* and *in vitro* radionuclide therapy have been reviewed by Brady et al.³⁸ In addition, there is now evidence that TAT can stimulate the immune system to deliver a better and more robust antitumor response.^{39,40}

Another mechanism by which α -emitting radionuclides may overcome heterogeneous uptake is through tumor anti-vascular α -therapy (TAVAT) as shown by Allen et al.⁴¹ TAVAT assumes that α -emitting radionuclides deposit energy in multiple hot-spots near capillaries in the target volume, causing closure of capillaries and subsequent reduction of oxygen and nutrients, leading to regression in solid tumors.

New TAT Radionuclides

The approval of $^{223}\text{RaCl}_2$ for clinical use has stimulated clinical interest in TAT, with an increasing number of clinical trials around the world. α -emitting radionuclides which have been proposed for therapeutic application and are undergoing clinical investigation include Astatine-211 (^{211}At), Bismuth-212 (^{212}Bi), Bismuth-213 (^{213}Bi), Actinium-225 (^{225}Ac) and Thorium-227 (^{227}Th), summarized in Table 1. Production of these radionuclides ranges from nuclear reactors to cyclotrons and generator systems. There is a continuous effort to develop simpler and more efficient production methods to make α -emitting radionuclides more widely available. Another key aspect of TAT is

the need to establish efficient chemical procedures to bind the α -radionuclide to a suitable carrier. The merits of these different α -emitters are summarized below.

Astatine-211

Astatine-211 is a radiohalogen with a half-life of 7.2 hours and offers many potential advantages for TAT due to its average range in soft tissues of 57 μm , average LET of 97 keV/ μm and single α -particle emission per decay. However, its use is constrained by its limited current availability.⁴² ^{211}At decays through two different paths, via electron capture (58%) with emission of 77-92 keV X-rays or via emission of an α -particle (42%) with energy of 5.87 MeV (Figure 1). The decay product of the electron capture, Polonium-211 (^{211}Po $T_{1/2}$ = 0.52 s) then decays by α -emission (100%) with energy of 7.45 MeV and a range in soft tissues of 71 μm .⁴³ Therefore, each ^{211}At decay results in one α -particle with mean energy of 6.78 MeV, leaving stable ^{207}Pb .⁴³

Although ^{211}At can be produced by α -bombardment of enriched bismuth via the $^{209}\text{Bi}(\alpha, 2n)^{211}\text{At}$ nuclear reaction, the availability of cyclotrons offering α -particle beams of sufficient energy (20-30 MeV) is limited. Typically, isolation of ^{211}At from bismuth is performed either by dry distillation or wet chemical approaches. Currently, ^{211}At is routinely produced via cyclotron nuclear reaction in the United States (Seattle, Bethesda, Durham) and Denmark (Copenhagen). Even with his short half-life it can be distributed to centers distant from production sites.

Alternative methods for producing ^{211}At involve production of Radon-211 (^{211}Rn), which can be obtained by proton spallation of uranium or thorium targets or by irradiation of natural bismuth with lithium-7 (^7Li) atoms. $^{211}\text{Rn}/^{211}\text{At}$ generators may facilitate easier distribution due to the longer half-life of ^{211}Rn ($T_{1/2}$ = 14.6 h).⁴⁴

The inherent cost for production of ^{211}At is relatively inexpensive and comparable to that of commercially available β -emitters.

The 7.2 hours half-life of ^{211}At is acceptable for chemical labelling and clinical pharmacokinetics even with slower target tissue uptake. Another advantage is that its second decay branch to ^{211}Po emits X-rays with energy of 77-92 keV, enabling biodistribution and dosimetry studies. Currently, its radiolabeling chemistry with proteins and antibodies is the subject of extensive studies in pre-clinical trials. Nevertheless, *in vivo* instability of the labeled compound and scarce availability of the radionuclide remains major challenges with ^{211}At .⁴²

Elgqvist et al. investigated the therapeutic efficacy of ^{211}At labeled with a fragment of a monoclonal antibody (mAb) in a pre-clinical model of ovarian cancer.^{45,46} The results of these studies showed that dosimetry to the cell nuclei is strongly dependent on the tumor size - while in small tumors (radius < 30 μm) all cells are eradicated, in tumors with radii greater than 75 μm , there is a small fraction of unirradiated cells in the core that explain the low tumor-free fraction despite the high mean absorbed dose in the tumor (>22 Gy for an injected activity of 400 kBq of $^{211}\text{A-MX35 F(ab')}_2$).⁴⁶ Previously, Charlton reported similar results using Monte Carlo simulation.⁴⁷

Moreover, promising results were also obtained in two Phase I trials of ^{211}At conjugated for treatment of malignant gliomas or ovarian cancer, which confirmed its safety and failed to identify a dose-limiting toxicity.^{48,49} Several radiolabeled complex antibodies with ^{211}At have been shown to be selective in different preclinical models, and highly cytotoxic for several types of cancer.⁵⁰⁻⁵⁴

Recently, a novel α -emitting radiopharmaceutical was developed by incorporating ^{211}At into the chemical structure of a small-molecule PARPi ($^{211}\text{A-MM4}$). This novel

radiopharmaceutical showed promising efficacy both *in vitro* and *in vivo*.⁵⁵ Additionally, it was shown that low-dose-fractionated therapy improved its toxicity profile in animal models and gave a more durable antitumor response. Different cell lines also demonstrated differential sensitivity to α -particle irradiation *in vitro*, suggesting sensitivity to this agent depended on underlying biology. Fractionated treatment with ²¹¹At labeled to the anti-prostate stem cell antigen (PSCA) A11 minibody also showed strong tumor growth inhibition on both macro- and micro-lesions.⁵² There is also an ongoing phase I/II clinical study using ²¹¹At labeled to anti-CD45 antibody for the treatment of acute myeloid leukemia (AML) and acute lymphoblastic leukemia, before bone stem cell transplant.⁴²

Bismuth-212 and Bismuth-213

Bismuth-212 with a half-life of 60.6 min is a hybrid α/β -emitter with an α -emission with mean energy of 7.8 MeV available from the decay of ²²⁸Th to stable ²⁰⁸Pb.⁵⁶ Since its half-life is too short for realistic transportation between sites a generator that uses ²²⁴Ra as parent radionuclide provides on-site production of ²¹²Bi for radiolabeling of target vectors. As its half-life is very short making radiopharmaceutical preparation challenging, the idea of an *in vivo* ²¹²Pb/²¹²Bi generator came, as ²¹²Pb is a longer-lived parent ($T_{1/2}$ = 10.6 h) and decays into ²¹²Bi after emission of an electron.⁵⁷ (Figure 2 a). The 10.6 h half-life of ²¹²Pb allows time for preparation of the radiopharmaceutical as well as tissue targeting before much of the ²¹²Bi is produced and decays. ²¹²Pb has demonstrated significant utility in both *in vitro* and *in vivo* models.⁵⁷ The major disadvantage of ²¹²Bi is the high-energy gamma (γ) emission of ²⁰⁸Tl (E_{γ} = 2.6 MeV), that

requires heavy shielding to minimize radiation exposure, thereby limiting the clinical utility of this radionuclide.

There are some studies of ^{212}Bi radiolabeled with tumor-specific antibodies reporting prolonged survival compared with controls.^{58,59} So far, no clinical trials have been reported for ^{212}Bi compounds. However, a Phase I clinical trial which investigated the use of ^{212}Pb -trastuzumab for treatment of HER2+ ovarian, colon and pancreatic tumors has been completed, showing minimal toxicity.⁶⁰

Bismuth-213 is also a hybrid α/β -emitter with a half-life of 45.6 min, derived from the α -decay of ^{225}Ac .⁵⁶ The major disadvantage is the cost, as large amounts are needed to attain an effective treatment due to the short half-life.⁶¹ ^{213}Bi predominantly decays via β -emission to the ultra-short lived ^{213}Po ($T_{1/2} = 4.2 \mu\text{s}$), which in turn decays by pure α -emission with energy of 8.37 MeV. The remaining 2.2% decays to Thallium-209 (^{209}Tl) via α -particle emission ($E_{\alpha} = 5.55 \text{ MeV}$, 0.16%, $E_{\alpha} = 5.9 \text{ MeV}$, 2.01%).⁶² Finally, ^{209}Tl decay via β -emitter ^{209}Pb ($T_{1/2} = 3.25 \text{ h}$) into stable ^{209}Bi (Figure 2 b). Most of the total particle energy emitted in each ^{213}Bi disintegration originates from α -decay (92.7%), which drives its cytotoxic effects, while only 7.3% of decay energy is contributed by β -emission. Additionally, the decay of ^{213}Bi is accompanied by a 440 keV photon emission (probability of 26.1%) that allows ^{213}Bi biodistribution to be monitored for dosimetry and pharmacokinetic studies. The major impediment for its clinical use has been concern regarding the natural accumulation of free bismuth in the kidneys.⁶³

The therapeutic potential of ^{213}Bi labeled with antibodies and peptides has been demonstrated in patients with leukemia.⁵⁹ Additionally, ^{213}Bi labeled with plasminogen activator inhibitor type 2 (PAI2) has been reported to inhibit tumor growth in prostate and breast cancer models, resulting in a dose-dependent tumor growth inhibition with

minimal toxicity up to 3.7 MBq of systemically administered ^{213}Bi -PAI2 in mouse models.^{64,65} Derrien et al. showed a survival benefit of labeled ^{213}Bi in mouse models of peritoneal carcinomatosis of ovarian origin.⁶⁶ A Phase I trial for AML treatment showed feasibility and safety with **activities** up to 37 MBq/kg being well tolerated.⁶⁷ A subsequent Phase I/II trial with sequential administration of chemotherapy (cytarabine) and ^{213}Bi -lintuzumab has concluded it is well-tolerated and capable of inducing remission in patients with AML.⁶⁸

Additionally, TAT with ^{213}Bi -labeled DOTATOC in 25 patients with challenging neuroendocrine tumors refractory to therapy with β -emitters resulted in a long-lasting antitumor response.⁶⁹ Thus, in a similar concept ^{213}Bi -PSMA-617 was administered in two cycles in a patient with metastatic Castrate Resistant Prostate Cancer (mCRCP), that was progressive following conventional therapy. With a total delivered **activity** of 592 MBq, these patients also demonstrated biochemical response and remarkable tumor regression.⁷⁰ Furthermore, ^{213}Bi -labeled Substance P analog have been tested by locoregional administration in 67 patients with grade II to IV glioma.^{71–73} Treatment was fractionated in up to 8 cycles at 2 month intervals, with remarkable tumor regression. Recently, a pilot study used intravesical administration of ^{213}Bi -labeled anti-EGFR in 12 bladder cancer patients who were resistant to standard therapy, and showed remarkable tumor remission with a safe toxicity profile.⁷⁴

Radium-223

Most current research activity in TAT is focused on ^{223}Ra . As a calcium mimetic, ^{223}Ra has an intrinsic affinity to bone, forming complexes with the bone hydroxyapatite crystals at sites of high bone turnover, with almost no redistribution of daughter nuclides.⁷⁵

^{223}Ra has a physical half-life of 11.4 days with a six-stage decay chain, emitting 4 α -particles with mean energy of 5.87 MeV and tissue penetration of 54 μm prior to reaching the stable ^{207}Pb . ^{223}Ra releases 95.3% of its energy as α -particles, 3.6% as β -particles with very little (1.1%) γ -emission (Figure 3).⁷ ^{223}Ra is generally artificially produced, via a generator from the ^{227}Ac ($T_{1/2} = 21.8$ years) parent.⁷⁶

The long physical half-life of ^{223}Ra provides appropriate time for its preparation, long distance distribution and administration to patients. Its low γ -emission is favorable from the point of view of handling, radiation protection and treatment on an outpatient basis. Nonetheless, due to its poor chemistry the availability of appropriate chelating agents with sufficient *in vivo* stability is an issue for applications other than treatment of bone metastases. The maintenance and biological follow-up of the decay daughter products continues to be a continuing challenge.⁶¹

A major milestone was reached in the ALSYMPCA study which demonstrated a survival benefit of ^{223}Ra admission together with a favorable safety profile.⁷ $^{223}\text{RaCl}_2$ (Xofigo®) was approved for clinical use by the FDA and European Medicines Agency (EMA) in 2013 and has been widely used for men with symptomatic mCRPC. Its peak skeletal uptake occurs within 1 h of injection, with no subsequent redistribution. Its blood clearance is rapid after intravenous administration and the skeletal uptake is estimated to range between 40% to 60% of the administered activity.⁷⁷ Additionally, non-negligible levels of $^{223}\text{RaCl}_2$ can be found in the spleen, stomach and intestinal organs that rapidly transits to the gastrointestinal tract for excretion.⁷⁸

In clinical reports $^{223}\text{RaCl}_2$ had a pain response in up to 71% of patients, and had significant positive effects in bone alkaline phosphatase (ALP), prostate-specific antigen (PSA) levels and overall survival.^{79,80} Data from these studies showed that there was no

evidence of long-term toxicity during the 2-year follow-up and the most common forms of adverse events were transient diarrhea, fatigue, nausea and vomiting.⁷⁵

The improvement in overall survival and low toxicity of ^{223}Ra opens the possibility for it to be combined with other modalities, such as docetaxel or external beam radiotherapy, and the extension of its application to skeletal metastases from other primary tumors. However, questions remain regarding its treatment schedule and dosing for optimized patient benefit, as the dosing and scheduling used in the ALSYMPCA trial was not necessarily optimal.⁸¹

Thus, a new phase II trial was performed to compare standard regimen (55 kBq/kg every 4 weeks for 6 cycles) against higher activity (88 kBq/kg every 4 weeks for 6 cycles) or for an extended schedule (55 kBq/kg every 4 weeks for 12 cycles). But neither of these alternative regimens were more effective than the standard $^{223}\text{RaCl}_2$ schedule.⁸¹

Suominen et al. provided insights into the mechanisms of action of $^{223}\text{RaCl}_2$ showing a dose-dependent inhibition of osteoclast differentiation, data that supports the dual effect of $^{223}\text{RaCl}_2$ on the cancer cycle by targeting both osteoclast and tumor cells.⁸²

Malamas et al. showed $^{223}\text{RaCl}_2$ treatment stimulated the immune system by inducing T cell-mediated cell death in different cancers.³⁹ Subsequently, a phase II trial of patients with advanced breast cancer and progressive bone-dominant disease demonstrated that ^{223}Ra has selective uptake in areas of increased bone metabolism in these patients, and one-third of target lesions showed significant metabolic decrease.⁸³ A phase I/II clinical trial testing the feasibility and safety of the anti-metabolite chemotherapy agent Capecitabine plus ^{223}Ra is ongoing in patients with advanced breast cancer.⁸⁴ Subbiah et al. also extended the application of $^{223}\text{RaCl}_2$ to osteosarcoma with a phase I escalation

trial finding minimal hematologic toxicity and recommending a follow-up phase II study with a monthly activity of 100 kBq/kg.⁸⁵

Despite the apparent additional benefits of $^{223}\text{RaCl}_2$ plus hormonal therapy, the ERA-223 trial showed that $^{223}\text{RaCl}_2$ should not be administered in combination with abiraterone plus prednisone or prednisolone as it significantly increases the risk of osteoporotic fractures.^{86,87} It is hypothesized that increased fracture risk is the result of multifactorial insults to bone microenvironment from androgen deprivation, ionizing radiation and prednisone.⁸⁸ However, concomitant use of bone protecting agents dramatically decreased the risk of osteoporotic fractures in the ERA-223 trial, and are now required in future combination studies with ^{223}Ra .

The ERA-223 trial highlighted the importance of controlled trials to assess the safety and benefit of combination therapy. Many $^{223}\text{RaCl}_2$ clinical trials are currently active and recruiting (Supplementary Table 1). The main focus is on long-term follow-up, new scheduling, expansion of clinical settings, and combination of $^{223}\text{RaCl}_2$ with other agents. These combination studies mainly focus on five combination strategies: abiraterone, enzalutamide, docetaxel, immunotherapy, and PARP inhibitors.⁸⁹

$^{223}\text{RaCl}_2$ in combination with paclitaxel was tested in a phase I trial with 15 patients with acceptable toxicity when combined with weekly administration of paclitaxel.⁹⁰ A similar phase I/II trial combining $^{223}\text{RaCl}_2$ with docetaxel is ongoing.⁹¹

In an elegant study, Leung et al. demonstrated the potential impact of bystander effects of $^{223}\text{RaCl}_2$ therapy showing different cellular sensitivities *in vivo*.⁹² However, *in vitro* studies have shown significant variation in cellular sensitivity across different tumor cell models after exposure to ^{223}Ra , suggesting further work is needed before applying it to different cancers.^{93,94}

Actinium-225

Actinium-225 is a pure α -emitter with a half-life of 10 days. It decays sequentially by α -emission through three relatively short-lived daughter radionuclides, ^{221}Fr ($T_{1/2} = 4.8$ min), ^{217}At ($T_{1/2} = 32.3$ ms) and ^{213}Bi ($T_{1/2} = 45.6$ min), each of which then also emits an α -particle with a large cumulative energy of 28 MeV.⁹⁵ Then ^{213}Bi decays via β -disintegrations and α -emission into stable ^{209}Bi . Thus the predominant decay chain of ^{225}Ac yields 4 alpha particles, along with 2 β -disintegrations of 1.6 and 0.6 MeV maximum energy, (Figure 4).⁹⁶ Additionally, useful γ -emission for *in vivo* imaging is generated in the ^{225}Ac decay scheme from decay of ^{221}Fr (218 keV, 11.6% emission probability) and ^{213}Bi (440 keV, 26.1% emission probability).⁹⁵ Its long half-life of 10 days and the multiple α -particles generated in the decay chain render ^{225}Ac a highly cytotoxic radionuclide.⁹⁶

^{225}Ac is mainly produced by isolation from ^{229}Th , which is a decay product of ^{233}U , yet the quantity available through this route is not sufficient for commercial applications as there is no large-scale production of ^{233}U .⁹⁵ At present viable sources of ^{229}Th are available at the Directorate for Nuclear Safety and Security of the Joint Research Center (JRC), formerly known as Institute for Transuranium Elements (Germany), Oak Ridge National Laboratory (ORNL) (USA) and the Institute of Physics and Power Engineering (IPPE) (Russia). Overall, the average global production is 63 GBq of ^{229}Th .⁹⁷ Alternatively, ^{225}Ac can also be produced in cyclotrons delivering 18 MeV proton beams by bombardment of radioactive radium through the $^{226}\text{Ra}(p,2n)$ reaction.⁹⁵

In theory, ^{225}Ac could be as much as ~100 times more potent than ^{213}Bi -conjugated analogs for the same activity, by virtue of the α -cascade to the target cell and the longer half-life of ^{225}Ac , which is 313-fold greater than the ^{213}Bi half-life.⁹⁸ An ^{225}Ac decay on

the nucleus deposits the same dose as 4 ^{213}Bi decays. This increased potency provides a clinically relevant dose, even when activities as small as 37 kBq/Kg of radionuclide are administered.⁹⁹

But the major impediment for its clinical use has been concern regarding the biological fate of its daughter radionuclides, particularly ^{213}Bi which accumulates in the kidneys. Issues relating to suitable chelating agents and the chelation chemistry to sequester this element *in vivo* remain to be solved, as none of the radiolabeling methods investigated so far have proven to efficiently form the required complex.⁹⁸

TAT using ^{225}Ac conjugated to antibodies or other carriers are promising treatments for many malignant diseases such as myeloid leukemia, ovarian cancer, bladder cancer, melanoma and non-Hodgkin's lymphoma. A successful phase I clinical trial with ^{225}Ac linked to an anti-CD33 antibody demonstrated anti-tumor activity against AML and a safe toxicity profile at activities below 111 kBq/kg.⁹⁹ A phase I/II trial demonstrated the feasibility of fractionated-dose ^{225}Ac -lintuzumab combined with the cytosine arabinoside chemotherapy agent Cytarabine, inducing remission with activity of 74 kBq/kg per fraction.¹⁰⁰

After the encouraging results of ^{213}Bi - DOTATOC, a follow-up investigation using ^{225}Ac -DOTATOC was tested in 39 patients with progressive neuroendocrine tumors. This showed promising efficacy and the maximum tolerable activity was considered to be 40 MBq for a single cycle, or 25 MBq every 4 months and 18.5 MBq every 2 months for multiple fractions.¹⁰¹

^{225}Ac -PSMA-617 was studied in patients with mCRPC.¹⁰² In Kratochwil et al., 40 heavily pre-treated patients with advanced mCRPC were treated with 3 cycles of 100 kBq/kg of ^{225}Ac -PSMA-617 with promising anti-tumor activity, supported by positive PSA decline

(>50%) in 63% of patients, where severe xerostomia was the main side effect of treatment.¹⁰³ Despite the long physical half-life of ^{225}Ac , this suggests the peptide targeting was sufficiently stable to provide the desired pharmacokinetic properties and prevent accumulation of free ^{225}Ac in undesired organs.¹⁰⁴ Sathekge extended the ^{225}Ac -PSMA-617 treatment schedule to chemotherapy-naïve patients, with good antitumor activity assessed by PSA level and imaging in 80% of patients, with mild and reversible xerostomia in all patients. This new data can potentially move TAT treatments into earlier-stage cancers.¹⁰⁵

In a comparative modeling study, ^{225}Ac -PSMA produces doses to the target cells nine hundred-fold greater than ^{177}Lu -PSMA and 14 times more than $^{223}\text{RaCl}_2$ per unit of activity retained in the target volume.¹⁰⁶ This study highlights the importance of subcellular localization of the α -emitting radionuclide as ^{225}Ac and ^{223}Ra have similar decay chains, however ^{225}Ac -PSMA is found in the cytoplasm and membrane of cancer cells, while $^{223}\text{RaCl}_2$ is located in the bone compartment surrounding the tumor.

A clinical trial in older patients with advanced myeloid leukemias treated with 2 activities of 74 kBq/Kg ^{225}Ac -labeled lintuzumab, anti-CD33 monoclonal antibody, has demonstrated a 56% response rate with moderate side effects.¹⁰⁷ Another phase I/II trial is investigating treatment ^{225}Ac -labeled lintuzumab after chemotherapy to reduce leukemia burden in patients with AML (Supplementary Table 2).

Thorium-227

Thorium-227 has a physical half-life of 18.72 days, and is a α -emitter with an average energy of 6.02 MeV, along with frequent γ -emission ($E_\gamma = 236$ keV, 11.2%, $E_\gamma = 50$ keV, 8.5%).⁵⁶ ^{227}Th decays via radioactive ^{223}Ra and the other short-lived radionuclides in its

decay chain to stable ^{207}Pb , emitting a total of 5 α -particles (Figure 5). ^{227}Th can be produced by natural decay of ^{227}Ac ($T_{1/2} = 21.8$ years). ^{227}Ac can be produced in a reactor by neutron irradiation of ^{226}Ra that produces ^{227}Ra ($T_{1/2} = 42$ min), which in turn decays by β -disintegration to provide the desired ^{227}Th . The long half-life of ^{227}Th allows for transportation and preparation of the radiopharmaceutical.¹⁰⁸ As well as its daughter ^{223}Ra , ^{227}Th is of interest for development of therapeutic bone-seeking radiopharmaceuticals. Furthermore, Algeta (Norway) (a subsidiary of Bayer (Germany), that developed Xofigo®), is also developing ^{227}Th -labelled antibodies for therapeutic use. Additional sources of ^{227}Ac will be needed for worldwide availability of ^{227}Th -labelled pharmaceuticals.

Due to its chemical properties, ^{227}Th can be complexed to a variety of antibodies and proteins. Current research using ^{227}Th conjugates centers on developing and testing new radiopharmaceuticals for the treatment of breast, lymphoma, renal, AML, ovarian and prostate cancers (Supplementary Table 3).^{40,109}

Therapy using the anti-CD20 monoclonal antibody rituximab labeled with ^{227}Th was demonstrated to kill lymphoma cells in nude mice bearing human B-lymphoma xenografts, without any serious toxicity.¹⁰⁷ Additionally, Dahle et al. demonstrated that ^{227}Th -rituximab has a better therapeutic effect per unit of absorbed dose than $^{99\text{Tc}}$ -tiuxetan-ibritumomab (Zevalin) or external beam irradiation.¹¹⁰ The authors also suggested treatment fractionation though multiple injections can maximize the therapeutic effect per dose unit while minimizing toxicity.

Preclinical studies of ^{227}Th -trastuzumab for treatment of human epidermal growth factor receptor 2 (HER2) positive ovarian and breast cancer showed encouraging antitumor effect without serious toxicity.^{111,112} ^{227}Th -PSMA was shown to be selective

and has a potent antitumor activity *in vitro* and *in vivo*.¹¹³ This preclinical data encouraged the further investigation of this agent in an ongoing phase I trial in mCRPC. Mesothelin (MSLN) targeting ²²⁷Th conjugate demonstrated a significant antitumor effect *in vivo* with good tolerability, showing reduced cellular viability with an upregulation of immune response.¹¹⁴ These encouraging results led to a phase I clinical study on the use of ²²⁷Th-MSLN in patients with solid tumors known to express mesothelin. Furthermore, MSLN targeting ²²⁷Th conjugates have demonstrated synergistic antitumor effect when combined with DNA damage response inhibitors, particularly ATRi and PARPi in preclinical cancer models.¹¹⁵

α -Emitter Selection

The clinical use of ²²³RaCl₂ showed clear benefits in overall survival in mCRPC, supporting the proposed theoretical therapeutic advantages of α -particles over the more widely used β^- . Other α -emitting radionuclides which are the subject of on-going research for therapeutic application include ²¹¹At, ²¹²Bi, ²¹³Bi, ²²⁵Ac, and ²²⁷Th.

The selection of an α -emitting radionuclide for radiopharmaceutical development requires careful consideration. It has been shown that is possible to clinically use radionuclides with a broad range of physical half-lives, from 45 min to almost 19 days, as long as the physical half-life matches the pharmacokinetic parameters of the radiopharmaceutical and are appropriate for the desired application. As all α -particles will completely lose their energy in less than 100 μ m, corresponding to only a few cell diameters, the precise kinetic energy of the generated α -particles is less critical for normal tissue sparing.

Other characteristics, in addition to the physical properties, should also be taken into consideration during the selection of the ideal radionuclide. These include both the feasibility and cost of radionuclide production, as well as the capability to easily manipulate the radionuclide to produce a radiopharmaceutical that is stable *in vivo*.

Half-life has a major impact in the logistics of production and distribution. An ideal radionuclide should permit widespread distribution from the production sites to all over the world. A longer half-life will ensure minimal waste due to decay during radiochemical processing and distribution of the radiopharmaceutical. However, radionuclides produced via a generator represent a convenient way to produce short-lived radionuclides at user sites through the decay of a long-lived parent, if suitable parents are available.

Radiopharmaceutical design remains a key challenge to optimizing therapeutic benefit. Many TATs are characterized by inhomogeneous distributions of the radiopharmaceutical, with some cells in the target potentially receiving no radiation. This implies that the radionuclide should be able to be produced with high specific activity for radiolabeling targeting molecules to ensure that sufficient dose is delivered to the target without saturating the limited number of receptors available at the target sites.⁴ However, lower specific activity may be suitable for bone pain palliation and treatment of joint inflammation by radiation synovectomy.

Another important aspect that should be taken into account is the detachment of daughters from the targeting carrier due to the intense recoil energy upon α -decay (>100 keV) that exceeds the energy of chemical bonds. The resulting free daughter nuclides can then diffuse from the target, leading to energy deposition in healthy tissue.¹⁰⁹ Palm et al. demonstrated, using microdosimetric calculations, that the energy

deposited in the target could be reduced by 50% if diffusion of daughters of ^{211}At was taken into account.¹¹⁶ Furthermore, a study evaluating the anti-CD33 mAb lintuzumab ^{225}Ac -labeled for the treatment of leukemia in mice found that most of the radiation dose in the kidneys was due to renal uptake of free ^{213}Bi released following α -particle decay of ^{225}Ac .⁶³ Therefore, it is of importance to study the fate of all radionuclides involved in the decay chain.

Despite the encouraging progress in TAT, treatments usually do not have optimal pharmacokinetics. Ideally, radiopharmaceuticals should have a rapid clearance from the blood stream with a robust tumour uptake. This led to the suggestion of a pre-targeting approach, a concept proposed by Goodwin et al. in 1988.¹¹⁷ This is a two steps procedure: in the first step a pre-targeting non-radioactive molecule is administrated and adequate time is given for its localization to the specific antigen in the target volume. Then, after clearance of the pre-targeted molecule, the radiolabeled molecule is injected, and will rapidly diffuse in the target and specifically bind to the pre-targeted molecule. This approach improves the target-to-normal tissue ratios of absorbed dose. The pre-targeting approach is very promising for short half-life radionuclides such as ^{211}At and ^{213}Bi .

Development of a Novel α Emitting Radiopharmaceutical

The development of novel radiopharmaceutical is a long and costly process, estimated to cost \$800 – 1,700 million over 10 or more years.¹¹⁸ The development process starts with target identification. After target identification, it is crucial to find the lead molecule to be radiolabeled. Following the lead molecule selection, biological properties of the radiopharmaceutical must be evaluated *in vitro* and *in vivo*, to evaluate affinity,

saturability, stability, pharmacokinetics, and biodistribution. Further toxicology studies in animals are done to assure safety prior to translation into humans. Usually, phase I clinical trials include safety studies in healthy humans, although this is frequently unfeasible for radiopharmaceuticals. Phase II and III will define the settings and efficacy of the radiopharmaceutical.¹¹⁹

In the particular case of development of novel radiopharmaceuticals for the treatment of mCRPC, molecular targets have been recently identified. PSMA (prostate specific membrane antigen) is a transmembrane protein highly and specifically expressed at all tumor stages on the surface of cancer cells and its levels are correlated with the aggressiveness of the disease, making it an attractive target.¹²⁰

This antibody was conjugated with DTPA and radiolabeled with ¹¹¹In for imaging and commercialized under the name ProstaScint® (Cytogen Corporation).¹²¹ A huJ591 mAb was radiolabeled with ¹⁷⁷Lu and evaluated in clinical trials in subjects with mCRCP.¹²² Recently, PSMA-617, a ligand with optimal pharmacokinetics, was conjugated with a DOTA chelator for PSMA-targeted radioligand therapy.¹²³

Nevertheless, some patients fail to respond to ¹⁷⁷Lu-labeled PSMA ligands, and despite the good tolerability, the long path length of β -particles continues to be a risk factor.¹²⁴ This opens an opportunity for α -radiation therapy labeled to PSMA ligands. Kratochwil et al. took this opportunity to evaluate PSMA-targeted α -radiation therapy with ²²⁵Ac-PSMA-617 in a first-in-human trial, in one case of contraindication for treatment with β -emitters and another where the patient failed to response to ¹⁷⁷Lu-PSMA-617 therapy. These patients experienced a remarkable benefit from TAT.¹⁰⁴

Conclusions

TAT allows selective delivery of highly toxic radiation to target cells with reduced harm to surrounding cells. Because of their high LET and short path length, α -emitting radionuclides offer a number of competitive advantages over other therapeutic radionuclides, including: higher RBE; reduced dependence of radioresistance mechanisms due to increased direct DNA damage; and reduced toxicity due to their limited range. However, the widespread acceptance of α -emitting radionuclides for clinical applications has been obstructed by low availability of radionuclides and concerns about potential toxicity due to free daughter radionuclides.

Although nonuniform dose distribution among target cells is still a significant limiting factor, there is now evidence that TAT can promote tumor regression and stimulate the immune response. Building on this concept, the complexity of therapeutic radiopharmaceuticals is changing from a simple radioactive element such as ^{223}Ra to a conjugated carrier molecule (i.e. ^{225}Ac -PSMA-617). This opens an opportunity to extend the application of TAT to other malignant diseases. Obviously, there are many challenges still ahead at this stage.

Current research into α -emitting radiopharmaceuticals is mainly focused on the development of more stable and selective carriers. The urgent need for more specific binding was highlighted by some challenges with the use of $^{223}\text{RaCl}_2$, whose non-specific attraction to actively growing bone leads to uptake on healthy bone tissue.

Consequently, TAT-specific radiobiology also needs to be studied. It is essential to clarify the contribution of indirect effects to the lethality of α -emitting radiopharmaceuticals, taking into account their unique microdosimetry. It is starting to be widely accepted that biological effects of TAT extend beyond the range of α -particles, due the bystander

effects, stimulation of immune system and TAVAT, which could fundamentally change how TAT is planned.

Also, there is a need to conduct further controlled randomized trials with sufficient patient numbers to be able to properly compare and evaluate different radionuclides and conjugating agents. It is clear that TAT is far from its full potential and using body weight to calculate the administered activity may not be the optimal parameter.

A greater understanding of the radiobiology of high LET α -particles can lead to the design of safer and more efficient approaches for the delivery of α -particles radiation. Advances in TAT imaging and dosimetry will provide the fundamental knowledge for optimization and treatment planning. With regards to TAT, labelled ^{227}Th and ^{225}Ac seem to be promising and could represent the next approaches to extend survival in the future for several clinical settings, alone or in combination with other treatments. However, the current supply of ^{227}Th and ^{225}Ac is certainly insufficient for routine applications in hospitals worldwide and developing new supply chains will be critical.

Conflict of Interest

FGL, and SJM declare no conflict of interests regarding the publication of this paper. JOS has received honoraria for Speakers bureau and Advisory Board from Bayer. JOS has received institutional research funding from Bayer. KMP has received speaker honoraria from Bayer.

Acknowledgements

The authors gratefully acknowledge the support of Fundação para a Ciência e Tecnologia (FCT-MCTES), Radiation Biology and Biophysics Doctoral Training Programme (RaBBIT, PD/00193/2012); Applied Molecular Bioscience Unit - UCIBIO (UIDB/04378/2020) and

CEFITEC Unit (UIDB/00068/2020); and scholarship grant number SFRH/BD/114448/2016 to (FL). The work was funded by the Movember Prostate Cancer UK Centre of Excellence (CEO13_2-004) and the Research and Development Division of the Public Health Agency of Northern Ireland (COM/4965/14).

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Figure Legend

Figure 1: Decay scheme of Astatine-211. The $T_{1/2}$ is shown inside of each box, and between boxes the energy of α transition (MeV) and the percentage of the branch when necessary. The chemistry of Astatine allows radiolabeling with a molecule of interest.

Figure 2: a) Decay scheme of Bismuth-212. b) Decay scheme of Bismuth-213. The $T_{1/2}$ is shown inside of each box, and between boxes the energy of α transition (MeV) and the percentage of branch when necessary. The chemistry of Lead allows radiolabeling with a molecule of interest

Figure 3: Decay scheme of Radium-223. The $T_{1/2}$ is shown inside of each box, and between boxes the energy of α transition (MeV) and the percentage of branch when necessary. Due to its poor chemistry ^{223}Ra has not yet been successfully combined with targeting molecules.

Figure 4: Decay scheme of Actinium-225. The $T_{1/2}$ is shown inside of each box, and between boxes the energy of α transition (MeV) and the percentage of branch when necessary. The good chemistry of Actinium allows radiolabeling with a molecule of interest.

Figure 5: Decay scheme of Thorium-227. The $T_{1/2}$ is shown inside of each box, and between boxes the energy of α transition (MeV) and the percentage of branch when necessary. The chemistry of Thorium allows radiolabeling with a molecule of interest.

Tables

Table 1: Main characteristics of the potential α emitting radionuclides for clinical applications.

Radionuclide	$T_{1/2}$	Primary E_{α} (MeV) (%)	Mean E_{α} (MeV)	Average Range in soft tissue (μm)	Main Method of producing	Particle Emitted per Decay	Emissions Useful Imaging	References
^{211}At	7.2 h	5.87 (42)	6.78	57	Cyclotron	1 α , 1EC	77-92 KeV X-rays	42, 43, 44
^{212}Bi	60.6 min	6.05 (36)	7.80	71	Generator $^{224}\text{Ra} \rightarrow ^{212}\text{Bi}$	1 α , 1 β^-	238 keV γ -ray	56, 57
^{213}Bi	45.6 min	5.90 (2.2)	8.32	78	Generator $^{225}\text{Ac} \rightarrow ^{213}\text{Bi}$	1 α , 2 β^-	440 keV γ -ray	56, 62
^{223}Ra	11.4 days	5.87 (100)	6.59	54	Generator $^{227}\text{Ac} \rightarrow ^{223}\text{Ra}$	4 α , 2 β^-	84, 95 and 270 keV γ -ray	56, 75
^{225}Ac	10 days	5.83 (100)	6.87	58	Generator $^{229}\text{Th} \rightarrow ^{225}\text{Ac}$	4 α , 2 β^-	218 and 440 keV γ -ray	94, 95, 96
^{227}Th	18.7 days	6.02 (100)	6.46	53	Generator $^{227}\text{Ac} \rightarrow ^{227}\text{Th}$	5 α , 2 β^-	84, 95, 236 and 270 keV γ -ray	56, 107

Fig. 1

Fig. 2 a)

Fig. 2 b)

Fig. 3

Fig. 4

Fig. 5