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REVIEW

Radiopharmaceutical: An Update and Comparison of Preclinical Investigation Result of Alpha and Beta Emitter Radioisotope

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Abstract: Radiopharmaceuticals are radioactive compounds used for diagnostic imaging and therapeutic purposes. In diagnostic, gamma emitters are commonly utilized. Conversely, radioisotopes employed for therapy are beta and alpha emitters, which generally have contrasting Linear Energy Transfer (LET) and tissue penetration profiles. These distinguishable characteristics allow for the complementary or improvement of radiopharmaceuticals. For instance, noticeable breakthroughs have been made with the approval of targeted beta-emitting radiopharmaceuticals using agents such as Lutathera ([^{177}Lu]Lu-DOTA-TATE) or Pluvicto ([^{177}Lu]Lu-PSMA-617). However, with the increase in isotope production and purification technology, new radiolabeling variations in multiple α-emitting particles have emerged. Preclinical investigation is a critical multi-step process to evaluate the safety and effectiveness of radiopharmaceuticals before being tested in humans with the purpose of translating these innovations into clinical practice. Accordingly, a narrative review was conducted on the preclinical investigation of radiopharmaceuticals to ensure positive direction for radiopharmaceuticals study. From this narrative review, notable results were obtained for actinium-225 and lead-212 based radiopharmaceuticals. From the perspective of targeted beta therapy, limited studies on terbium-161 have revealed that it is more potent than lutetium-177 with the same targeting molecules in the same animal models. It has been concluded that targeted alpha therapy is generally better than targeted beta therapy in many preclinical settings.

Keywords: radiopharmaceuticals, targeted alpha therapy, targeted beta therapy, preclinical investigation

Introduction

Radiopharmaceuticals represent a transformative class of drugs that harness the unique properties of radioisotopes for both diagnosing and treating diseases, with a particular emphasis on oncology.[1] These agents offer distinct advantages over conventional therapies by enabling the selective delivery of radiation to malignant cells, thereby minimizing collateral damage to healthy tissues.[2] Radiopharmaceuticals are composed of two main components which are radioisotopes and vectors. Chelators and linkers are added to aid radioisotopes stability on the process of delivery to the targeted tumor. Chelators, including DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), bind to the radioisotope and form a stable complex, preventing its release into normal tissue, while the linker connects the complex to the vector.[3] Radioisotope therapy is designed to selectively target specific tumors.[4] Vectors, including small molecules, peptides, and antibodies, were labeled with radioisotopes to grant specificity, radiolabeling.[5] The goal is to deliver a precise radiation dose to the tumor while minimizing its impact on healthy tissues.[6] The scheme usually involves the diagnosis of a certain cancer using positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging. For instance, fluorine-18 fluorodeoxyglucose (PET/CT) has a capability to diagnose gall bladder, thyroid, or even lymphoma.[7–9] After being diagnosed, a specific radiopharmaceutical is administered, enabling precise and noninvasive therapy.[10]

The historical trajectory of radiopharmaceutical therapy has been marked by pioneering scientific achievements, notably the introduction of Iodine-131 (^{131}I) in 1942 for the treatment of Graves' disease and later differentiated thyroid cancer. Starting with ^{131}I for cancer treatment, since then, more than 60 radiopharmaceuticals have been approved for various types of

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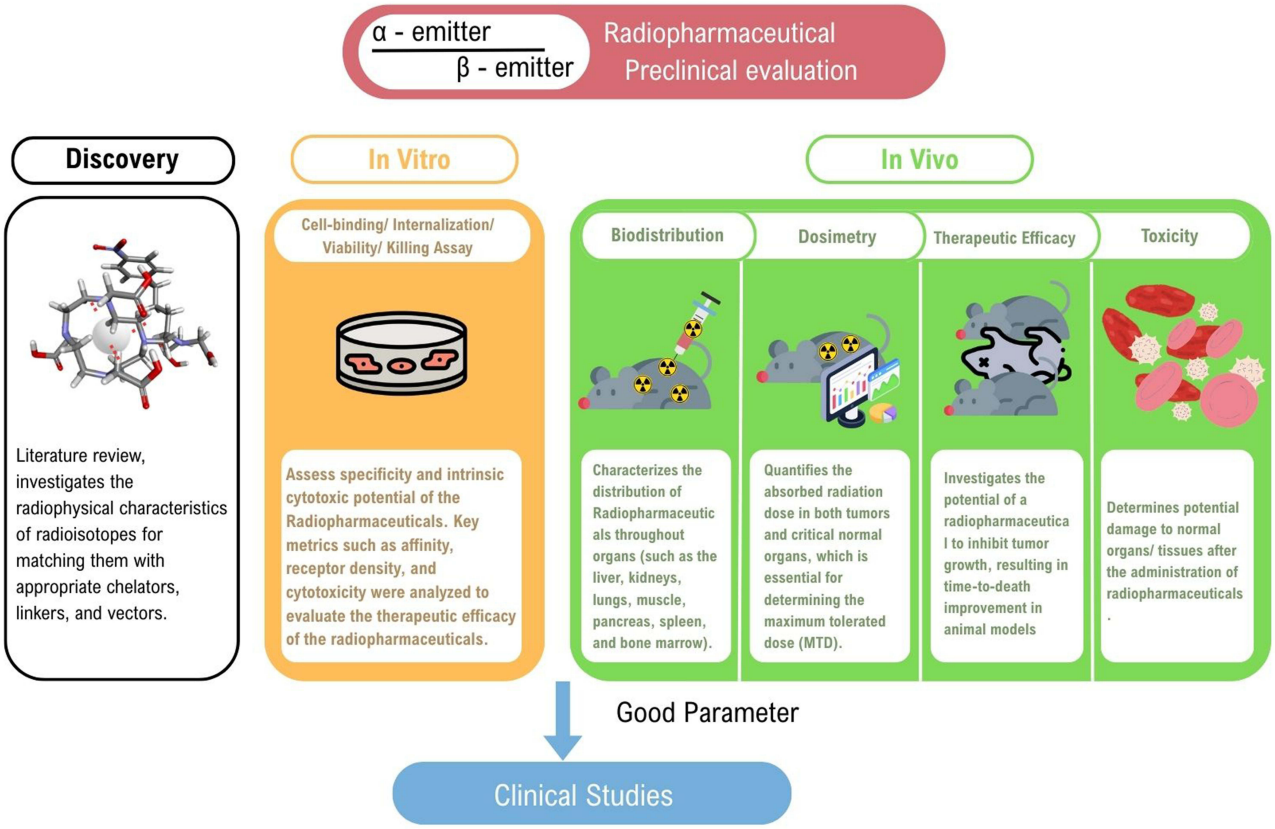
Graphical Abstract



cancers. The combination of precise diagnosis with a selective, high cell-killing capability agent is defined as radiotheranostic.[11] The arrival of agents such as Lutathera ([^{177}Lu]Lu-DOTA-TATE), Pluvicto ([^{177}Lu]Lu-PSMA-617), and their complementary diagnostic imaging agents, Netspot ([^{68}Ga]Ga-DOTA-TATE), and Locametz ([^{68}Ga]GaPSMA-11) have gained radiopharmaceutical recognition as potential subject to be extensively studied because of their capability to eliminate cancer, especially metastatic and refractory cancers, which poses a challenge for chemotherapy or other therapeutic agents.[12]

Another type of particle that has distinct characteristics compared to beta particles is alpha particles. This particle has high linear energy transfer and low tissue penetration compared to beta particles.[13] In 2013, Bayer's [^{223}Ra]$RaCl_2$, the first α-particle radiopharmaceutical, markedly improved metastatic cancer treatment. Marcu et al, in 2018, have made a global comparison of alpha- and beta-emitting particles based on their preclinical and clinical trials.[14] However, with the increase in isotope production and purification technology and the emergence of new radiolabeling variations in multiple α-emitting particles such as astatine-211 (^{211}At), actinium-225 (^{225}Ac), bismuth-213 (^{213}Bi), and lead-212 (^{212}Pb) for various cancers, an update is needed to ensure a positive direction for the development of therapeutic agents for cancers, not only α-emitting particles but also β-emitting particles, judging from the early stage of the test: preclinical trials.

Radiophysical and Radiobiological Properties of Alpha and Beta Emitters

Alpha Emitters: Energy, LET, Tissue Penetration, Mechanism of Action

Alpha particles have been an interesting subject to discuss as alpha particles exhibit strong biological effects owing to their limited penetration in tissues, typically only 50–80 μm, and their high linear energy transfer (LET) along this short path. These particles generally have energies between 5 MeV and 9 MeV and vary in half-life. For instances, ^{225}Ac has a long half-life at the value of 10 days, yet ^{213}Bi has a short half-life only at the count of 45.6 minutes.[15,16] However, they

still share the same profile in producing LET values in the range of approximately 80–100 keV per micrometer.[13,17] This highly localized energy deposition is a critical advantage, as it minimizes damage to surrounding healthy tissues, making alpha emitters ideal for targeting small tumor clusters or individual cancer cells.[18]

This is illustrated in Figure 1, which shows that radiation from alpha particles primarily targets the cell nucleus.[19] High LET radiation delivers intense radiotoxicity to each alpha particle, producing a cytotoxic response that does not rely entirely on reactive oxygen species.[20] This characteristic is especially beneficial when treating tumors with low oxygen levels.[21] The DNA damage caused by alpha particles frequently results in complex double-strand breaks (DSBs).[22]

Beta Emitters: Energy, LET, Tissue Penetration, Mechanism of Action

Beta particles are relatively low-mass particle, electrons or positrons, which possess significantly greater penetration capabilities than alpha particles due to their smaller size. This longer range allows beta emitters to irradiate larger or more diffuse tumors, or those with heterogeneous expression patterns, ensuring a more uniform dose distribution across a broader area. Most β-particles have a LET of 0.1–1.0 keV/μm and an energy of 50–2300 keV which is smaller than alpha particles.[23] They have roughly the same half-life, such as Lutetium-177 (^{177}Lu) and ^{131}I which have half-life of approximately 6.6 and 8.0 days, respectively.[24,25]

Ionizing radiation from these radionuclides induces considerable DNA damage within target cells, primarily through the generation of reactive oxygen species (ROS) as a result of water radiolysis. This oxidative stress can trigger the upregulation of specific proteins associated with the apoptotic pathways.[26] The design of irradiation protocols is tailored to the characteristics of the tumor, such as type, size, and heterogeneity, as well as pharmacokinetics and biodistribution of the administered radionuclides.[27]

Key Differences and Therapeutic Implications

The fundamental radiophysical differences between the alpha and beta emitters lead to distinct therapeutic implications. The characteristics of the alpha and beta emitters are listed in Table 1. At the bottom of this section, alpha emitters offer unparalleled precision for microscopic disease or small lesions due to their high LET and short range, delivering a highly potent, localized dose.[28,29] In contrast, beta emitters provide a broader kill zone, making them suitable for larger tumor burdens or more diffuse disease.[30] The ROS-independent mechanism of alpha emitters offers a distinct advantage in hypoxic tumor microenvironments, which often presents a challenge for beta emitters.[21,31]

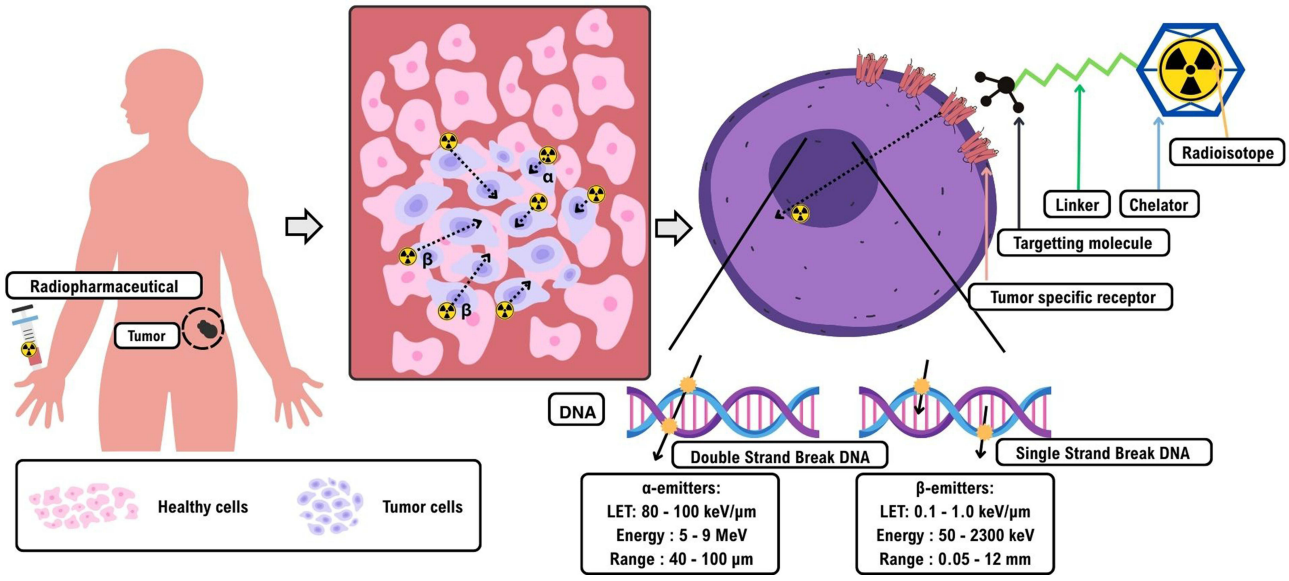


Figure 1 Targeted Radionuclide Therapy Mechanism of Action.

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Table 1 Summary of Alpha and Beta Particle LET, Energy, Half-Life, and Tissue Penetration

Particle Type	Radioisotope [Ref]	LET (keV/μm)	Particle Energy (MeV)	Physical Half-Life (hour)	Tissue Penetration (mm)
Alpha	Radium-223[32]	80 - 100	5.87	273.6	0.1
	Actinium-225[15]		5.8	237.8	0.047–0.085
	Bismuth-213[16]		6 – 8.4	0.76	0.04–0.08
	Lead-212[33]		6.0	10.6	0.04–0.08
	Astatine-211[34]		7.5	7.2	0.08
Beta	Lutetium-177[25]	0.1–1.0	0.133	158.4	0.673
	Iodine-131[24]		0.606	192.0	2
	Terbium-161[35]		0.154	165.6	0.29

The logistical considerations also vary significantly between the two types. Radioisotopes with very short half-lives, such as ^{213}Bi (46 minutes), pose substantial challenges for manufacturing, transport, and clinical scheduling, necessitating advanced infrastructure and rapid deployment for optimal dose administration.[36] Conversely, longer-lived isotopes such as ^{177}Lu (7 days) and ^{225}Ac (10 days) offer greater logistical flexibility but require careful consideration of prolonged patient radiation exposure.[15,25]

At the preparation phase, radioisotope is chelated with chelator that has been paired with antibody. Radioisotope ionic radius plays crucial part in stability of radiopharmaceutical. For instances, ^{212}Pb has an ionic radius of 1.19 Å and 6 coordination number.[37] ^{212}Pb is chelated with chelator with large cavity, macrocyclic chelator such as DOTA, and high coordination number so ^{212}Pb would remain in a complex form throughout its circulation in the body. Release of any free radioisotope would increase the risk of radiotoxicity, as illustrated in Figure 2 below, such as Pb^{2+} that mimic Ca^{2+} and has a high likelihood to retain in bones.[38]

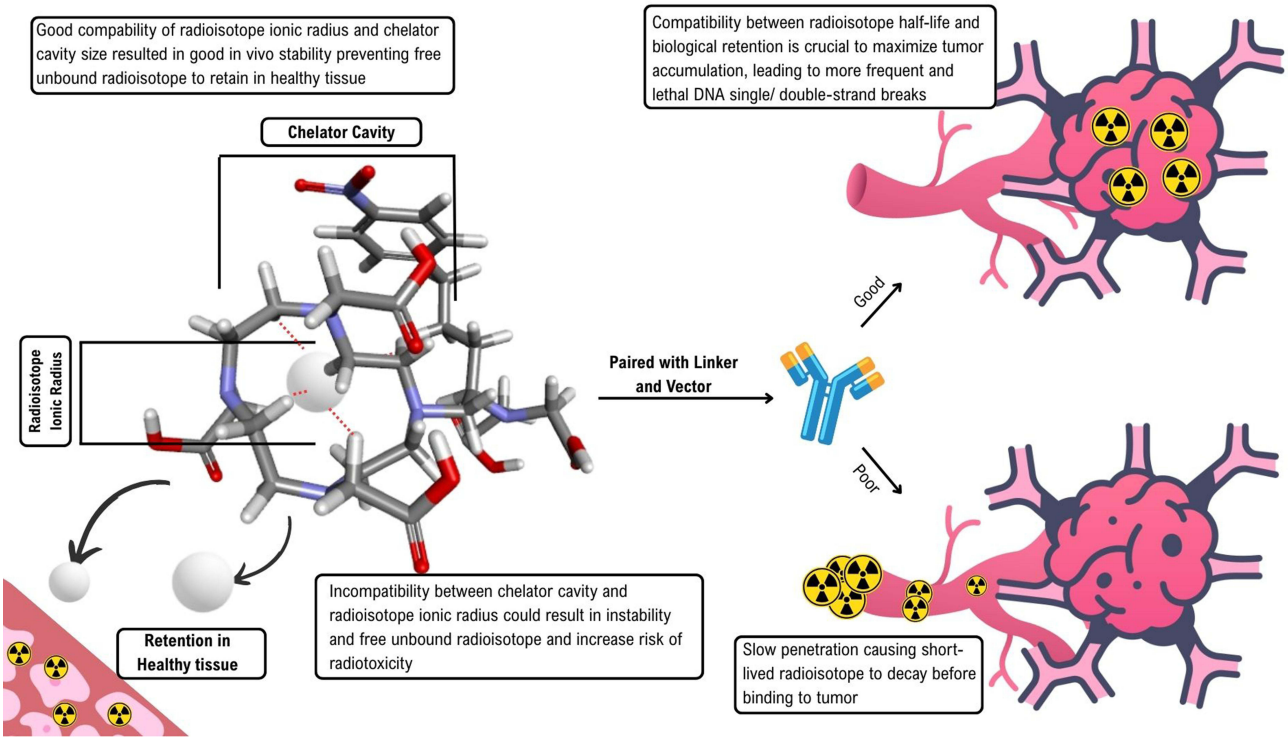

Figure 2 Radioisotope, Chelator, Linker, and Vector Characteristics Affected it Therapeutic Potential.

Each of these radioisotopes has their distinctive characteristic which will affect their compatibility with their conjugated vector. Physical half-life of radioisotope should match the biological half-life of vector. Antibody vectors typically have 2 to 5 days of biological half-life are therefore deemed to be compatible with long half-life radioisotope such as ^{225}Ac, ^{177}Lu, ^{131}I, and ^{161}Tb.[39] On the other hand, peptide and small-molecule vectors have shorter half-life and faster clearance, yet higher penetration to the tumor tissues, making them compatible with short half-life radioisotope such as ^{213}Bi, ^{212}Pb, and ^{211}At. Specifically, their rapid tumor accumulation ensures the radioactive payload is delivered before the isotope decays away.[40,41]

Differences of released energy per decay event when radiopharmaceutical interacted with tumor receptor would contribute to the outcome of therapeutic. For instances, ^{177}Lu and ^{161}Tb exhibit only subtle differences in their mean beta energies $E\beta^{-}_{Average}$, which are approximately 133 keV and 154 keV, respectively.[42,43] However, ^{161}Tb has additional advantages of emitting conversion electrons (3–50 keV) and 2 Auger electrons per decay, which could potentially result in a higher cellular dose on a shorter range.[44] In comparison to alpha and beta particles, alpha emitters such as ^{225}Ac emits 4 alpha particles per decay event (5.87 MeV), significantly higher compared to ^{177}Lu. For alpha-emitters, simple surface binding is inadequate, high percentage of vector internalization, defined as the radiopharmaceutical penetrates the membrane and enters the cell, is a prerequisite for ^{225}Ac therapy to ensure the cytotoxic energy is deposited in range to the tumor DNA.[45,46]

Key Preclinical Investigation Parameters in Radiopharmaceutical Development

Cell-Binding/Internalization/Viability/Killing Assays

These in vitro assays assess the specificity and intrinsic cytotoxic potential of the radiopharmaceuticals. Key metrics included K_d values (dissociation constant), Bmax (maximum number of binding sites), percentage of binding, percentage of internalization, IC_{50} (half maximal inhibitory concentration), and the observed reductions in cell viability.[47,48] Lower K_d values indicate higher affinity, and a high Bmax value indicates that the target cell has many binding sites/receptors. The percentage of binding in radiopharmaceuticals refers to how much of a radiopharmaceutical attaches to its target; for instance, 10.45%±0.45% of 99mTc-labeling attached to LNCaP cells after 4-hour incubation.[49] Upon binding to the membrane, the radiopharmaceuticals that are taken inside of the cells were measured. Internalization assays provide insights into the ability of tracers to enter the intracellular space by passive diffusion or active mechanisms. High internalization upon cell binding indicates that radiopharmaceuticals are taken into the cell rather than only binding to the cell.[50] Lower IC_{50} indicates higher potency of agents to inhibit 50% of biological processes in tumors.[51]

Finally, cell viability measured the percentage of cells survivability, potential radiopharmaceutical significantly reduced the percentage of surviving cells. For instance, administration of 10, 20, and 60 Gy radiation dose of ^{131}I reduced cell viability up to 61% by causing apoptosis to the TFK-1 cell lines and it has positive correlation with the doses of radiation.[52] Another example is 100 kBq/mL [^{225}Ac]Ac-DOTA-SP that reduce cell viability to 80% after 72 hours of incubation, and it can continue reduce cell viability up to 50% after 5 and 6 days of treatment. Cell viability differences in time are caused by alpha particles severe double-strand breaks. It is explained in the flow cytometry analysis that late apoptosis is the main pathway. This is caused by the cells' inability to repair DNA damage during G2/M (preparation) phase thus cannot continue to the mitosis phase.[53]

Biodistribution

Biodistribution characterizes the distribution of radiopharmaceuticals throughout organs (such as the liver, kidneys, lungs, muscle, pancreas, spleen, and bone marrow). The designs of these studies reflect the planned indications of the agent. For instance, in preclinical settings, the PC-3 PIP cell line for prostate cancer may be used to observe the accumulation and retention of the agent.[54] The key metric is the percentage of injected dose per gram (%ID/g) in tumors and organs (eg, the kidneys, liver, spleen, blood, and salivary glands), interpreted as tumor uptake and organs uptake.[55] High tumor uptake indicates high accumulation of the agent in the tumor, and if tumor uptake remains high through several points of time, it indicates agent retention in the tumor, both of which are indicative of potential efficacy. Conversely, high and retained off-target uptake indicates potential toxicity.[56]

Biodistribution profiles are critical predictors of a radiopharmaceutical's clinical potential, where the balance between tumor accumulation and off-target clearance contributed to the efficacy and safety. An antibody hTAB004 conjugated by

DOTA is radiolabeled with ^{225}Ac resulting in high tumor uptake of 65±15%ID/g while maintaining off-target uptake in critical organs below 10%ID/g. This agent achieved 100% survival in animal models until the end of study (52 days).[57] This could be resulted by ^{225}Ac long half-life at the value of 9.9 days paired with hTAB004 antibody that typically has a long circulating time, biological half-life, thus maximizing the cytotoxic potential of ^{225}Ac before being excreted outside the body.[15] Modifying biodistribution of agents could also be a potential strategy, [^{225}Ac]Ac-crown-TATE study highlights that the tumor-to-kidney ratio improved significantly over time, rising from 1.3:1 at 4 hours to 3.9:1 by 120 hours. This indicates that while the kidneys receive an initial dose during clearance, long half-life of ^{225}Ac would prevent damage to the kidney at the early hours of injection but, by the long-term, exposure is heavily weighted toward the tumor, which is desirable for safety.[58]

Dosimetry and Dose-Limiting Organs

Dosimetry quantifies the absorbed radiation dose in both tumors and critical normal organs, which is essential for determining the maximum tolerated dose (MTD) and identifying the dose-limiting organs. Key metrics include the mean absorbed dose (in Gy/MBq or mGy/kBq) to the tumor, kidneys, spleen, lungs, and red marrow. A high mean absorbed dose in a certain organ indicates a dose-limiting organ. Thus, the dose (Bq) that is administered must not exceed the threshold radiation (Gy) for that organ, this dose represents the agent's MTD.[27] For instance, based on published threshold doses from external beam irradiation data, an absorbed dose that ranges from 18–23 Gy in whole kidney volume would increase risk of kidney injury by 5% over 5 years.[59] With this information, if doses that are administered exceed the MTD, the risk of kidney injury could be mitigated by the co-administration of amino acids (lysine and arginine) and polygelines (eg, gelofusine [Braun]).[60]

Therapeutic Efficacy Assessment

Therapeutic efficacy assessment investigates the potential of a radiopharmaceutical to inhibit tumor growth, resulting in time-to-death improvement in animal models.[61] This in-vivo investigation involved the implantation of human cancer cells (xenografts) into immunodeficient animals. The tested agents were administered and observed over time to evaluate their clinical potency of the tested agents.[62] Therapeutic efficacy is typically measured by parameters such as tumor growth delay, median survival time, tumor volume decrease, complete remission (CR) rates, and partial response (PR) rates. Delay in tumor growth and median survival were measured in units of time. Tumor growth delay involves comparing the differences in time it takes for a tumor to grow to a predetermined size between the treatment and control groups, while median survival is time at which 50% of the animal subjects were still alive. The decrease in tumor volume quantifies the reduction in the size of a tumor following treatment in a unit of percentage between the difference in volume after a certain point of time per initial volume. Complete remission, also known as complete response, indicates the disappearance of all signs of tumor in response to treatment, while partial response or partial remission means that the tumor has responded to treatment, but has not been eliminated.[63] Improved survival and tumor regression in animal models are indicators of potential radiopharmaceuticals and are likely to proceed to the next stage of the study.[55]

Toxicity and Safety Profile Evaluation

Toxicity determines potential damage to normal organs/tissues after the administration of radiopharmaceuticals. When treated with radioligand therapy, the kidneys and bone marrow were the two most significant dose-limiting tissues.[64–66] The investigation involved administration of the tested agents to xenografted animals for a predetermined time. Blood samples were taken at several points in time to observe hematological changes, such as white blood cells (WBC), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), red blood cells (RBC), hemoglobin (HGB), and platelets (PLT). Significant hematological changes could indicate bone marrow suppression.[67] At the end of the predetermined time, the animals were euthanized and organs such as the thyroid, salivary glands, stomach, small intestine, spleen, kidney, and bone were extracted. Histological analysis allows visualization of the damages induced by the tested agents. For instance, 1.00 and 1.93 MBq of [^{211}At]At-NpG-PSMA induced kidney damage, as observed by shrinking glomeruli in the stained kidney sections.[68] In conclusion, abnormalities in organ tissues indicate the potential toxicity of the agents.[69]

DNA Double-Strand Break (DSB) Analysis

This study provides direct mechanistic evidence for the impact of radiation at the cellular level by assessing DNA damage, specifically double-strand breaks (DSBs), which are a critical mechanism of action in radiation therapy.[70] The metric is the number of foci observed in γH2AX-positive cells. For instance, [^{225}Ac]Ac-DOTA-YS5 treatment resulted in 4.90 foci observed at 14 days post-injection suggesting the effective induction of DNA double-strand breaks in tumor cells. This analysis confirmed that the radiopharmaceutical induced cytotoxic DNA damage and could be used to compare the relative potency of different radiopharmaceuticals or isotopes in inducing this damage. Changes in DSB repair pathways can also contribute to resistance, making this a valuable parameter for understanding therapeutic limitations.[70,71] Table 2 below summarizes the key preclinical investigation parameters in radiopharmaceutical development.

Ideally, preclinical investigations of radiopharmaceuticals must include radiolabeling data, cell binding-internalization, stability tests in PBS and HS, cell viability/toxicity, clonogenic tests, DNA DSB analysis, biodistribution data, tumor-bearing mice, and renal retention.[47] However, through literature review, we concluded that preclinical investigations mostly include dosimetry, biodistribution, and therapeutic efficacy. The significance of these tests is presented in Table 2. Dosimetry is responsible for the determination of MTD, biodistribution to predict therapeutic efficacy or toxicity, and therapeutic efficacy as the goal outcome of a radiopharmaceutical that is to eliminate the tumor.

Updates in Preclinical Investigation of Targeted Alpha Therapy (TAT) Radiopharmaceuticals

TAT radiopharmaceuticals are gaining significant attention in cancer therapy due to their high linear energy transfer and short range, offering potent and localized cell killing. Recent preclinical investigations have explored various alpha emitters conjugated with targeting molecules for the treatment of specific cancers. Table 3 below elaborates on targeted alpha-emitting radiopharmaceuticals that have been investigated and their cell lines corresponding to their planned disease.

Table 2 Key Preclinical Investigation Parameters in Radiopharmaceutical Development

Parameter [Ref]	Description	Key Metrics	Significance
Cell-binding/ Internalization/ Viability/ Killing Assays[47,49,50]	In vitro evaluation of radiopharmaceutical's affinity to target cells, internalization, and ability to induce cell death.	K_d, Bmax, % binding, % internalization, EC_{50}, IC_{50}, cell viability reduction.	Early validation of targeting specificity and intrinsic cytotoxic potential; guides initial dose selection.
Biodistribution[55,56]	In vivo evaluation and visualization of radiopharmaceutical distribution in animal models over time.	%ID/g in tumor and organs, tumor-to-organ uptake ratios.	Predicts therapeutic efficacy and potential off-target toxicity; informs dosing strategy; basis for human dosimetry.
Dosimetry and Dose-Limiting Organs[27]	In vivo evaluation of radiopharmaceuticals absorbed dose in organs of animal models.	Mean absorbed dose (Gy/MBq or mGy/kBq) to tumor and organs; identification of dose-limiting organs.	The basis of dose selection for the therapeutic efficacy assessment and clinical study.
Therapeutic Efficacy Assessment[63]	In vivo evaluation of radiopharmaceutical's treatment effectiveness.	Median survival days, % tumor growth inhibition, complete/partial responses, tumor volume changes.	Direct indicator of potential clinical benefit; establishes optimal therapeutic doses.
Toxicity and Safety Profile Evaluation[68,72]	In vivo evaluation of physiological change that is related to organ injuries of animal models.	Changes in blood counts (WBC, RBC, platelets), elevated liver/kidney enzymes, histological evaluations, body weight changes.	Identifies maximum tolerated dose (MTA).
DNA Double-Strand Break (DSB) Analysis[70,71]	Assessment of DNA damage, specifically double-strand breaks.	Number of foci (eg, γH2AX-positive cells).	Confirms mechanism of action; compares potency; helps understand resistance.

Table 3 Updates in Preclinical Investigation of Targeted Alpha Emitting Radiopharmaceuticals

Cancer Type	Radiopharmaceuticals [Ref]	Experimental Type (Cell Lines)
Prostate cancer	[^{225}Ac]Ac-PSMA-617[50]	PC-3 PIP cells
	[^{225}Ac]Ac-SibuDAB[50]	PC-3 PIP cells
	[^{225}Ac]Ac-L1[73]	PC3 PIP cells
	[^{225}Ac]Ac-DOTA-YS5[71]	22Rv1 cells
	[^{225}Ac]Ac-Macropa-Pelgifatamab[63]	C4-2, LNCaP CDX, KUCaP-1 PDX cells
	[^{213}Bi]Bi-L1[73]	PC3 PIP cells
	[^{212}Pb]Pb-L2[74]	PC3 PIP cells
	[^{212}Pb]Pb-TCMC-YS5[75]	PC3, PC3-luc cells
	[^{212}Pb]Pb-DOTAM-GRPR1[76]	PC-3 cells
	[^{211}At]At-PSMA1[77]	LNCaP cells
	[^{211}At]At-PSMA5[77]	LNCaP cells
	[^{211}At]At-PSMA6[77]	LNCaP cells
	[^{211}At]At-NpG-PSMA[68]	LNCaP cells
NETs[a]	[^{225}Ac]Ac-MACROPATATE[78]	H69 cells
	[^{225}Ac]Ac-crown-TATE[58]	AR42J cells
	[^{212}Pb]Pb-DOTAMTATE[51]	AR42J cells
	[^{225}Ac]Ac-DOTA-JR11[79]	H69 cells
	[^{212}Pb]Pb-eSOMA-01[80]	NCI-H69
	[^{212}Pb]Pb-PSC-PEG-T[61]	AR42J cells
Thyroid cancer	[^{225}Ac]Ac-DOTA-CCK-66[72]	AR42J cells
Breast cancer	[^{225}Ac]Ac-DOTA-hTAB004[57]	HCC70 cells
Glioblastoma multiforme	[^{225}Ac]-DOTA-SP[53]	T98G, U87MG, U138 MG cells
Multiple myeloma	[^{213}Bi]Bi-9e7.4[81]	5T33 MM cells
Non-hodgkin's lymphoma	[^{213}Bi]Bi-rituximab[82]	Raji green fluorescent protein luciferase lymphoma cells
	[^{212}Pb]Pb-rituximab[83]	EL4-hCD20-luc cells
Malignant peritoneal mesothelioma	[^{212}Pb]Pb-TCMC-hIgG1[84]	MSTO-211H, NCI-H226 cells
	[^{212}Pb]Pb-TCMC-chOI-3[84]	MSTO-211H, NCI-H226 cells

Comparing radiopharmaceutical's therapeutic efficacy is not a straightforward process.[85] From the same type of emitters, the amounts of alpha particles emitted per decay event could indicate which of these radioisotopes is the most potent. For example, a single atom of ^{225}Ac undergoes a cascade of decays, releasing a total of four high-energy alpha particles.[86] While ^{212}Pb undergoes much more complex route of emission, it first decays into bismuth-212 (^{212}Bi) through beta decays then 36% of ^{212}Bi would decay into thallium-208 by producing alpha particles. At the same isolated cancer cells, ^{225}Ac far exceeded ^{212}Pb in terms of therapy outcome. However, ^{212}Pb shorter half-life benefitted in terms of toxicity and potential issues in storing radioactive waste from patients.[87] Thus, it is not ideal to conclude that a radiopharmaceutical can proceed to the clinical stage based only by its therapeutic outcomes in animal models.

^{225}Ac-Based Radiopharmaceuticals

Recent preclinical evaluations of actinium have resulted in novel radiolabeling of many target cancers. In the case of prostate cancer, subcellular dosimetry of [^{225}Ac]Ac-PSMA-617 was performed by Lee and showed that [^{225}Ac]Ac-PSMA-617 deposited 0.129 Gy in the nucleus, resulting in a 464-fold higher absorbed dose compared to [^{177}Lu]Lu-PSMA-617, which deposited 2.78×10^{-4} Gy.[50] Radiopharmaceuticals that are studied by Buslinger et al and Banerjee et al, respectively, [^{225}Ac]Ac-SibuDAB and [^{225}Ac]Ac-L1 (small peptides) have shown promising biodistribution profiles with good tumor uptake, 80±8%ID/g at 24 hours and 45.8% to 49.0%ID/g at 2–8 hours post-injection, respectively.[50,73] SibuDAB is a new class of PSMA ligands comprising (s)-ibuprofen as an albumin binding entity. (S)-ibuprofen binds reversibly to serum albumin, which is the most abundant protein in the blood plasma which extend the radiopharmaceutical's circulation in the body. It optimized the cytotoxic potential of ^{225}Ac as it has long half-life.[50] L1, Low Molecular Weight Compound, is built on 4-bromobenzyl Lys-urea-Glu-targeting moiety, short linker, and DOTAMA as a chelator. The targeting moiety is critical for tight binding, p-bromo-benzyl moiety achieves high tumor retention primarily by increasing the binding affinity of the molecule to the PSMA receptor, indicated by tumor uptake, compared to other Low Molecular Weight Compound that are experimented.[88]

The application of actinium, which has a long half-life, is beneficial and achieves good results when combined with monoclonal antibodies. Another study by Bidkar et al, an in-vitro study revealed [^{225}Ac]Ac-DOTA-YS5 good binding of 19.25% and viability of IC_{50}=10.09 nCi/mL. It effectively induced DNA double-strand breaks, with 4.90 foci observed at 14 days post-injection. [^{225}Ac]Ac-DOTA-YS5 showed an extended biodistribution profile with tumor uptake increased significantly over time from 11.64%ID/g at 24 hours to 31.78%ID/g at 408 hours. However, significant renal injury was confirmed at 0.5 mCi of dose.[61] A study by Schatz et al revealed that [^{225}Ac]Ac-Macropa-Pelgifatamab showed promising therapeutic efficacy, including partial responses and stable disease in C4-2 xenograft models, total tumor eradication and complete remission in LNCaP CDX models, and partial responses in KUCaP-1 PDX models.[63]

Another type of cancer that has been extensively studied is neuroendocrine tumor. Study by Ingham et al demonstrated that [^{225}Ac]Ac-crown-TATE exhibited higher tumor uptake, peaking at 11.1±1.5%ID/g at 4 hours and retaining 6.92±2.03%ID/g at 120 hours, compared to [^{225}Ac]Ac-MACROPATATE, a study conducted by King et al, with 9%ID/g at 2 hours and 4%ID/g at 24 hours post-injection. However, [^{225}Ac]Ac-MACROPATATE demonstrated superior outcome of median survival days with less dose compared to [^{225}Ac]Ac-crown-TATE (46.3 kBq of administration resulted in median survival of 55 days and 55 kBq of administration resulted in median survival of 26 days, respectively).[58] Even though [^{225}Ac] Ac-MACROPATATE achieved better survival outcome of animal models, this could be due to the cell lines that are used, H69, grew relatively slow than AR42J cell lines that are used on [^{225}Ac]Ac-crown-TATE. When normalizing with the control groups, [^{225}Ac]Ac-crown-TATE achieved a 5–6-fold extension in median survival, significantly outperforming [^{225}Ac]Ac-MACROPATATE, which only achieved a 2-fold extension. This could be due to Crown chelator having more coordination number, large cavity, which are preferred with a large radioisotope like ^{225}Ac, this increased in vivo stability and optimize the delivery of cytotoxic ^{225}Ac to the tumor.[58,78] Another compound with long biodistribution profile was [^{225}Ac]Ac-DOTA-JR11, this compound showed high tumor uptake, increased at 4 hours post-injection (19.3±2.6%ID/g) and decreased over time (8.1±0.3%ID/g at 72 hours post-injection).[79]

Recent studies of ^{225}Ac have targeted medullary thyroid carcinoma, triple-negative breast cancer, and glioblastoma multiforme. For medullary thyroid cancer, [^{225}Ac]Ac-DOTA-CCK-66 at 37 kBq demonstrated a mean survival of 54±6 days in animal models.[72] In another subject, triple negative breast cancer, Kelly et al found that [^{225}Ac]Ac-DOTA-hTAB004 exhibited a high tumor uptake, reaching 65±15%ID/g, and low off-target uptake (blood, bone, kidneys, liver, lungs, muscle, pancreas, spleen) reaching < 10% ID/g) at 120 hours post-injection. [^{225}Ac]Ac-DOTA-hTAB004 demonstrated potential therapeutic efficacy. Administration with 18.5 kBq of this agent resulted in all mice surviving until endpoint (48 d).[57] For glioblastoma multiforme, a study of [^{225}Ac]Ac-DOTA-SP by Majkowska-Pilip et al, in-vitro evaluation of [^{225}Ac]Ac-DOTA-SP, a neuropeptide vector, resulted in cytotoxic effect that reduced cell viability to 80% at 72 hours post-injection at high dose (100 kBq/mL).[53] Table 4 below summarizes the most significant results of ^{225}Ac based radiopharmaceutical studies.

Table 4 Observation Summary of ^{225Ac} Based Radiopharmaceuticals

Radiopharmaceutical [Ref]	**Observation**
[^{225}Ac]Ac-PSMA-617[50]	At subcellular dosimetry, Actinium deposited 0.129 Gy per decay event.
[^{225}Ac]Ac-SibuDAB[50]	Good tumor uptake, 80±8%ID/g at 24 hours.
[^{225}Ac]Ac-L1[73]	37 and 74 kBq of administration results in median survival days for 56 and 79 days in PSMA-positive PC-3 PIP tumors.
[^{225}Ac]Ac-DOTA-YS5[75]	Demonstrated good binding of 19.25% and viability of IC50=10.09 nCi/mL.
	Effectively induced DNA double-strand break with 4.90 foci observed at 14 days post-injection.
	Extended biodistribution profile of tumor uptake 11.64% ID/g at 24 hours, increasing to 31.78% ID/g at 408 hours post-injection.
[^{225}Ac]Ac-Macropa-Pelgifatamab[63]	In C4-2 xenograft model: treatment with a single dose of 300 kBq/kg resulted in partial responses (PR) in 7/10 and stable disease (SD) in 3/10 mice.
	In LNCaP CDX model: treatment with 70, 125, or 250 kBq/kg, led to total tumor eradication and complete remission.
	In KUCaP-1 PDX model: treatment with 150 kBq/kg resulted in 2/9 CR, 1/9 PR, and 4/9 SD and 300 kBq/kg resulted in 8/8 PR.
[^{225}Ac]Ac-crown-TATE[58]	High tumor uptake of 11.1±1.5%ID/g at 4 hours and retaining 6.92±2.03%ID/g at 120 hours.
	55 kBq of administration resulted in median survival of 26 days.
[^{225}Ac]Ac-MACROPATATE[78]	Exhibited tumor uptake reaching 9%ID/g at 2 hours and 4%ID/g at 24 hours.
	46.3 kBq of administration resulted in median survival of 55 days.
[^{225}Ac]Ac-DOTA-JR11[79]	High tumor uptake reached 19.3±2.6%ID/g at 4 hours and 8.1±0.3%ID/g extended till 72 hours.
[^{225}Ac]Ac-DOTA-CCK-66[72]	37 kBq of agents resulted in 54±6 days mean survival.
[^{225}Ac]Ac-DOTA-hTAB004[57]	High tumor uptake reaching 65±15%ID/g and low off-target uptake (blood, bone, kidneys, liver, lungs, muscle, pancreas, spleen) reaching less than 10% ID/g at 120 hours.
[^{225}Ac]Ac-DOTA-SP[53]	Reduced cell-viability to 80% at 72 hours with 100 kBq/ mL of agent.

^{213}Bi-Based Radiopharmaceuticals

The extremely short half-life of ^{213}Bi means that it delivers its dose very rapidly, which is advantageous for fast-growing tumors, but necessitates efficient targeting and rapid administration logistics. Recent preclinical findings for ^{213}Bi by Banerjee et al included labeling with low-molecular-weight protein and monoclonal antibody (mAb). For prostate cancer, labeling with low molecular weight (L1) exhibited rapid tumor uptake (18.9±3.1%ID/g at 10 minutes post-injection, increasing to 29.4 ±8.0%ID/g at 2 hours). Initial renal uptake was high (49.0±21.2%ID/g at 10 minutes) but declined rapidly. Treatment with as much as 3.7 MBq resulted in 35 days in tumor growth.[73] Conjugating 9e7.4 mAb, as demonstrated by Fichou et al, for multiple myeloma demonstrated potential therapeutic efficacy, as observed by 3.7 MBq of [^{213}Bi]Bi-9e7.4, resulting in a median survival of 80 days.[81] A study conducted by Havlena et al revealed that [^{213}Bi]Bi-rituximab (non-Hodgkin's lymphoma) demonstrated dose-dependent effects. Treatment with a single dose of either 1.295 MBq or 3.7 MBq led in mice remaining alive after 28 days (end of study), with a 3.7 MBq dose resulting in a 75% potential cure rate.[82]

The key findings for each ^{213}Bi based radiopharmaceutical are presented in Table 5 below. Despite logistical challenges, the high alpha energy and observed therapeutic efficacy highlight its potent cytotoxic potential, especially in hematological malignancies where rapid systemic distribution might be beneficial.

Table 5 Observation Summary of ^{213}Bi Based Radiopharmaceuticals

Radiopharmaceutical [Ref]	Observation
[^{213}Bi]Bi-L1[73]	Rapid tumor uptake of 18.9±3.1%ID/g at 10 minutes and 29.4±8.0%ID/g at 2 hours. At 3.7 MBq, 35 days of tumor growth delay was observed.
[^{213}Bi]Bi-9e7.4[81]	At 3.7 MBq, median survival of 80 days was observed.
[^{213}Bi]Bi-rituximab[82]	At 1.295 MBq or 3.7 MBq, mice remaining alive after 28 days. At 3.7 MBq, 75% potential cure rate.

^{212}Pb-Based Radiopharmaceuticals

Many preclinical studies have demonstrated the potential of lead-based radiopharmaceuticals in prostate cancer. Banerjee et al labeled Low-Molecular Weights Compound with lead. L2 is built on 4-iodoobenzyl Lys-urea-Glu-targeting moiety, short linker, and DOTAMA as a chelator. It allows ^{212}Pb achieved a low off-target absorbed dose, with a tumor absorbed dose of 8.0 mGy/kBq, a kidney absorbed dose of 4.4 mGy/kBq, and a blood absorbed dose of 0.1 mGy/kBq. The distinguishing feature of the compound is its fast clearance kinetics, resulting in tumor uptake values of less than 10% ID/g. This behavior is advantageous given the short half-life of ^{212}Pb, as it facilitates immediate cytotoxic efficacy while minimizing prolonged renal exposure, thereby addressing the dose-limiting toxicity common to Pb-based agents.[74] J. Li et al reported that [^{212}Pb]Pb-TCMC-YS5 had high survival rates and extended median survival days in various models (55–100.5 days). The tolerated dose range for this agent was 0.185–0.74 MBq and no significant hematological toxicity was observed compared with the control group.[61] Preclinical evaluation of a novel compound, a GRPR-targeting antagonist, was conducted by Saidi et al [^{212}Pb]Pb-DOTAM-GRPR1. Tumor uptake was 5%ID/g at 24 hours after injection. The kidney was identified as the dose-limiting organ, but the absorbed dose to the kidney was 7.5-fold lower than the 23-Gy threshold for kidney injury, and treatment up to 1665 kBq was well tolerated, with no signs of radiation- or lead-induced damage in the kidneys, gastrointestinal mucosa, bone marrow, or other organs. Administration of 370 kBq × 4 at 3-week intervals resulted in median survival of 19 weeks.[76]

Another type of cancer that has been extensively studied is neuroendocrine tumor. The somatostatin agonists, octreotate (TATE) and octreotide (TOC), were examined and labeled with lead. A study by Stallons et al, [^{212}Pb]Pb-DOTAMTATE, was observed to 20%ID/g at 1 hour post-injection and remained constant for 4 hours until 24 hours post-injection. Dose-dependent effect was observed, 0.37 MBq administration of this agent resulted in median survival of 8.5 weeks.[51] A new series of octreotate derivatives, dubbed eSOMA, was labeled by Chapeau et al, containing either the DO3AM or p-Bn-SCN-TCMC chelator, two functionalized DOTAM derivatives, and an Amcha or Pip linker was tested with ^{212}Pb. [^{212}Pb]Pb-eSOMA-01 is built on DOTAM chelator, Amcha (trans-4-(aminomethyl)cyclohexanecarboxylic acid) linker, and TATE as vector. DOTAM (1,4,7,10-Tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane), also known as TCMC, forms highly stable complex with ^{212}Pb due to its four ring nitrogens and four amide oxygen atoms fully saturate the metal's coordination sphere. This structural arrangement encapsulates the ion, making the complex exceptionally inert in vivo and resistant to dissociation.[89] [^{212}Pb]Pb-eSOMA-01 demonstrated high tumor uptake, approximately 16% ID/g at 1h, decreasing to ~9% at 4h and ~6% at 24h. In-vitro evaluation showed IC_{50} (nM) of unlabeled complexes is inferior compared to DOTAMTATE, at the value of 2.54±0.20 vs 0.91±0.18. However, the Lead-complexed versions showed the opposite, the structure changes because the chelator physically closes around the metal resulting in higher binding affinity to the tumor receptor. [^{212}Pb]Pb-eSOMA-01 demonstrated superior affinity (lower IC_{50}) compared to [^{212}Pb]Pb-DOTAM-TATE (5.29 nM vs 7.60 nM).[80] Preclinical evaluation of a lead specific chelator (PSC) conjugated to radiopeptide by Li et al, [^{212}Pb]Pb-PSC-PEG-T, revealed good biodistribution profile with specific uptake in tumors, peaking at 1 hour post-injection (5.2%ID/g at 1 hour; 2.0%ID/g at 5 hour), accompanied by fast clearance were observed in 3 hour post-injection (tumor: 1.2%ID; kidneys: 6.6%ID), however minimal retention in the kidney was observed (0.9%ID in tumor; 1%ID in kidneys).[61]

In some cancers, monoclonal antibody labeling with ^{212}Pb yields good results. For malignant peritoneal mesothelioma, preclinical findings of [^{212}Pb]Pb-TCMC-hIgG1 (human IgG1 isotype control) and [^{212}Pb]Pb-TCMC-chOI-3 (IgG1 chimeric

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variant) by Lindland et al showed good internalization of 36.0±10.3% and 55.2±8.1% at 24 hours post-treatment, respectively. As an antibody with specific binding, chOI-3 demonstrates a high affinity for the CD146 antigen, with a dissociation constant (K_d) of approximately 1.8 nM. This high affinity facilitates stable binding and subsequent internalization. At lower doses, [^{212}Pb]Pb-TCMC-chOI-3 demonstrated superior therapeutic efficacy compared to [^{212}Pb]Pb-TCMC-hIgG1, as measured by median survival times of 55 and 42 days, respectively.[84] For non-Hodgkin lymphoma, promising results were observed by Durand-Panteix et al biodistribution profile of [^{212}Pb]Pb-rituximab, tumor uptake reached 13.4–18.4%ID/g at 6–48 hours post-injection, with low off-target uptake (<5%ID/g). It was effective in early-stage lymphoma models, resulting in a high survival rate of approximately 75% at over three months post-treatment with a single injection. However, it is less effective in advanced-stage lymphomas. Dose-dependent bone marrow suppression was observed, leading to significant decreases in leukocyte, platelet, and hemoglobin counts, although these results were reversible, with hemoglobin remaining low after three months.[83] While the kidneys are often dose-limiting, several studies have reported good tolerability and absorbed doses below injury thresholds, suggesting that with careful dosimetry and targeting, ^{212}Pb can achieve good therapeutic outcomes. The main outcomes of the various ^{212}Pb radiopharmaceuticals are presented in Table 6 below.

^{211}At-Based Radiopharmaceuticals

Several studies have been conducted to utilize ^{211}At for prostate cancer. Watabe et al investigated a series of PSMA molecules radiolabeled with ^{211}At. The three PSMA analogs have different amino acid residues in their side chains, Gly-Lys, ©-G©(R)-Glu, and (S)-Glu-(S)-Glu in PSMA1, PSMA5, and PSMA6, respectively. [^{211}At]At-PSMA-5 exhibited

Table 6 Observation Summary of ^{212}Pb-Based Radiopharmaceuticals

Radiopharmaceutical [Ref]	**Observation**
[^{212}Pb]Pb-L2[74]	Tumor absorbed dose of 8.0 mGy/kBq, kidney absorbed dose of 4.4 mGy/kBq, and a blood absorbed dose of 0.1 mGy/kBq were observed.
[^{212}Pb]Pb-TCMC-YS5 [75]	At 0.74 MBq, in PC3 subcu-CDX model, administration of treatment resulted in 80% mice surviving for 55 days after treatment. At 0.74 MBq, in mCRPC CDX model, administration of treatment resulted in 100.5 median survival days. At 0.37 MBq and 0.74 MBq, in the PDX model, administration of treatment resulted in 56 and 99 median survival days, respectively.
[^{212}Pb]Pb-DOTAM-GRPR1[76]	Tumor uptake of 5%ID/g at 24 hours. At 370 kBq x 4, median survival of 19 weeks was observed.
[^{212}Pb]Pb-DOTAMTATE[51]	Tumor uptake of 20%ID/g at 1, 4, and 24 hours. At 0.37 MBq, median survival of 8.5 weeks was observed. IC_{50} = 0.91±0.18 nM.
[^{212}Pb]Pb-eSOMA-01[80]	Tumor uptake of 16%ID/g at 1 hour and 9%ID/g at 4 hours and 6% ID/g at 24 hours. IC_{50} = 5.29 nM.
[^{212}Pb]Pb-PSC-PEG-T[61]	Good biodistribution profile with tumor uptake of 5.2%ID/g at 1 hour and 2.0%ID/g at 5 hours. Minimal retention in the kidney was observed at 24 hours (0.9%ID in tumor; 1%ID in kidneys).
[^{212}Pb]Pb-TCMC-hIgG1[84]	Internalization = 36.0±10.3% At 371 kBq, median survival of 55 days was observed.
[^{212}Pb]Pb-TCMC-chOI-3[84]	Internalization = 55.2±8.1% At 348 kBq, median survival of 42 days was observed.
[^{212}Pb]Pb-rituximab[82]	Tumor uptake of 13.4–18.4%ID/g at 6–48 hours. At 277.5 kBq, 75% cure rate at early stage and median survival of 28 days at advance stage of the disease were observed. Dose-dependent bone marrow suppression was observed.

excellent tumor growth suppression in a xenograft model. The result is [^{211}At]PSMA5 exhibited higher tumor retention compared to [^{211}At]PSMA1 and [^{211}At]PSMA6 (30.6±17.8, 12.4±4.8, and 19.1±4.5%ID/g at 3 h versus 40.7±2.6, 8.7 ±3.5, and 18.1±2.2%ID/g at 24 h, respectively). Not only that, [^{211}At]PSMA5 was subjected to slow deastatination in mice, resulting in not more than 1.0% of the injected doses of the metabolites, including astatide ions. It may have connection with PSMA5 side chain being ©-G©(R)-Glu, (R) is an unnatural configuration of molecule which are harder to decompose and make it more stable Further evaluation revealed a high mean absorbed thyroid dose; however, no pathological abnormalities related to the administration of [^{211}At]PSMA-5 were observed in the brain, salivary glands, thyroid, heart, liver, gallbladder, pancreas, rectum, kidney, bladder, adrenal glands, or prostate.[77]

A more recent development by Yaginuma et al, [^{211}At]At-NpG-PSMA, featuring a neopentyl-glycol structure, showed significant in vivo stability against deastatination. It exhibited high tumor uptake (42.0±13.1%ID/g at 3 hours), with minimal uptake in non-target tissues like the thyroid (0.28±0.20%ID/g), stomach (0.71±0.12%ID/g), and salivary glands (0.88±0.10% ID/g) at 3 hours. This low off-target accumulation suggests successful mitigation of deastatination, which is a common challenge for ^{211}At-labeled compounds. This agent demonstrated dose-dependent anti-tumor effect which with 1.00 MBq treatment of this agent resulted in −76.4±19.2% change and the 1.93 MBq group a −59.5±41.6% change in tumor volume, compared to the control group that experience tumor volume increased by 796.0±437.6% at 15 days post-treatment. However, the kidney was identified as a dose-limiting organ, with mild renal tubule regeneration observed at 1.00 MBq and moderate changes were observed by 1.93 MBq group at 35 days post-treatment.[68] Table 7 below summarizes the results for each ^{211}At based radiopharmaceuticals.

Updates in Preclinical Investigation of Targeted Beta Therapy (TBT) Radiopharmaceuticals

Beta-emitting radionuclides, with their longer tissue penetration compared to alpha emitters, are well-suited for treating larger or more diffuse tumors. Recent preclinical studies have continued to refine their application, focusing on optimizing their targeting, efficacy, and safety. Table 8 below elaborates on targeted beta-emitting radiopharmaceuticals that have been investigated and their cell lines corresponding to their planned disease.

At the subject of TBT, ^{177}Lu is the most studied radioisotope following its success as an approved agent. Emerging radioisotopes, such as terbium-161 (^{161}Tb), can be attached to the same cancer-targeting molecules to treat tumors, such as neuroendocrine tumors.[91] The unique characteristic of ^{161}Tb is that ^{161}Tb also emits a significant shower of low-energy Auger and electron conversion. This emission releases an energy at the value of 46.5 keV per decay. The high LET (~4–26 keV/μm) and short tissue range (~2–500 nm), by hypothesis, this would benefit ^{161}Tb in an isolated cancer cell.[42] Because of this short tissue range, a vector that is needed not only specific but also has a good internalization, this data are essential for ^{161}Tb to proceed to the clinical stage.[96]

Table 7 Observation Summary of ^{211}At Based Radiopharmaceuticals

Radiopharmaceutical [Ref]	**Observation**
[^{211}At]At-PSMA1[77]	Tumor uptake of 12.4±4.8%ID/g at 3 hours and 8.7±3.5%ID/g at 24 hours.
[^{211}At]At-PSMA5[77]	Tumor uptake of 30.6±17.8%ID/g at 3 hours and 40.7±2.6%ID/g at 24 hours.
[^{211}At]At-PSMA6[77]	Tumor uptake of 19.1±4.5%ID/g at 3 hours and 18.1±2.2% ID/g at 24 hours.
[^{211}At]At-NpG-PSMA[68]	High tumor uptake of 42.0±13.1%ID/g at 3 hours. Minimal retention in non-target tissues. Dose-dependent tumor volume reduction was observed with 1.00 and 1.93 MBq resulting in −76.4±19.2% and −59.5 ±41.6% change in tumor volume. At 1.93 MBq, moderate changes in kidney were observed.

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Table 8 Updates in Preclinical Investigation of Targeted Beta Emitting Radiopharmaceuticals

Cancer Type	Radiopharmaceuticals [Ref]	Experimental Type (Cell Lines)
Prostate tumor	[^{177}Lu]Lu-L1[88]	PC3 PIP cells
	[^{177}Lu]Lu-Alb-L2[54]	PC3 PIP cells
	[^{177}Lu]Lu-Alb-L3[54]	PC3 PIP cells
	[^{177}Lu]Lu-Alb-L4[54]	PC3 PIP cells
	[^{177}Lu]Lu-Alb-L5[54]	PC3 PIP cells
	[^{177}Lu]Lu-Alb-L6[54]	PC3 PIP cells
	[^{177}Lu]Lu-Ibu-DAB-PSMA[90]	PC3 PIP cells
	[^{177}Lu]Lu-rhPSMA-10.1[55]	LNCaP, 22Rv1 cells
NETs[a]	[^{177}Lu]Lu-DOTATOC[91]	AR42J cells
	[^{177}Lu]Lu-DOTA-LM3[91]	AR42J cells
	[^{177}Lu]Lu-DOTA-JR11[92]	H69 cells
	[^{161}Tb]Tb-DOTA-LM3[91]	AR42J cells
	[^{161}Tb]Tb-DOTATOC[91]	AR42J cells
	[^{161}Tb]Tb-Crown-TATE[93]	AR42J cells
Cholangiocarcinoma	Unconjugated ^{131}I[52]	TFK-1, HuCCT1
Human solid tumors	[^{177}Lu]Lu-OncoFAP-23[94]	SK-RC-52.hFAP, CT-26.hFAP
Non-hodgkin's lymphoma	[^{177}Lu]Lu-CHX-A′-DTPA-Rituximab[95]	Raji cells

^{177}Lu-Based Radiopharmaceuticals

A preclinical evaluation of ^{177}Lu-Labeled PSMA-based Low-Molecular-Weight was conducted by Banerjee et al revealed that injection of 111 MBq [^{177}Lu]Lu-L1 resulted in a potential therapeutic efficacy outcome with 60% of animal survival until 190 days after administration.[88] Boinapally et al study on a series of novel albumin-binding ^{177}Lu-labeled PSMA-based low-molecular-weight to the low-molecular-weight results in, initially, alb-L4 exhibited the highest tumor uptake at 2 hours post-injection, reaching 40.89±4.73%ID/g, followed by alb-L6, alb-L5, alb-L2, and alb-L3 ^{177}Lu-labeled compounds. Shockingly, at 48 hours post-injection, alb-L5 reached peak tumor uptake with a value of 127.44±22.85%ID/g, followed by alb-L4, alb-L2, alb-L3, and alb-L6. These findings suggest that alb-L4 and alb-L5 have superior tumor-targeting and retention abilities. These two new series of albumin binding ligand is built on DOTA-monoamide chelator, long linker, and albumin binding moiety ibuprofen (IBU) for alb-L4 and 4-(para-iodophenyl) butyricacid (IPBA) for alb-L5. While the IBU moiety facilitated more rapid initial tumor accumulation, the IPBA moiety demonstrated superior long-term retention, maintaining significantly higher radioactivity levels within the tumor over extended periods.[54]

Another study on PSMA molecules by Tschan et al discovered that ibuprofen as an albumin-binding entity conjugated via a linker composed of a diaminobutyric acid (DAB) and a lysine residue resulted in [^{177}Lu]Lu-Ibu-DAB-PSMA. As previously explained, the incorporation of an ibuprofen moiety allows the radioligand to bind reversibly to serum albumin in the blood and extend the blood circulation time thus [^{177}Lu]Lu-Ibu-DAB-PSMA (prostate cancer), with 5 MBq of treatment, demonstrated a remarkable result which is that more than 50% of mice survived until the end of study, 84 days.[90] A novel preclinical investigation of PSMA-based conjugation was conducted by Foxton et al. However, [^{177}Lu]Lu-rhPSMA-10.1 showed moderate tumor uptake of 4.9%ID/g, 15 hours post-injection.[55]

^{177}Lu-labeled somatostatin receptor (SSTR) analogues have been extensively studied in preclinical settings for neuroendocrine tumors with overexpression of SSTR. Preclinical studies of this non-internalizing SST analogues/antagonist (DOTA-LM3, DOTA-JR11) resulted in much higher tumor accumulation compared to SSTR agonists (DOTATATE, DOTATOC). SSTR antagonists have an ability to bind to more binding sites on the receptor than the agonist. A study by Borgna et al revealed that in-vitro evaluation of [^{177}Lu]Lu-DOTATOC, which localizes in the cytoplasm, resulted in EC_{50} value of 8.2 MBq/mL and 10 MBq/mL resulted in 5% γH2AX-positive cells, indicating DNA double-strand breaks. Treatment with 2×10 MBq of this agent resulted in a median survival of 19.5 days. SSTR antagonists are also a favorable choice for SSTR2-mediated peptide receptor radionuclide therapy (PRRT); for instance, 2×10 MBq of DOTA-LM3, which localizes in cell membranes, resulted in superior tumor growth delay of 35±7 days and a median survival of 48.5 days, compared to [^{177}Lu]Lu-DOTATOC.[91] Another type of SSTR antagonist is JR-11. Albrecht et al revealed a higher tumor uptake because of its higher ability to bind into more binding sites. [^{177}Lu]Lu-DOTA-JR11 has a high mean absorbed dose to tumor at a value of 464.4 mGy/MBq, and mean absorbed dose to kidney was 406.9 mGy/MBq. Treatment with 2×20 MBq of this agent resulted in a remarkable median survival of 207 days. However, severe haematological toxicity was observed at 30 MBq.[92]

A study on human solid tumors was also conducted using ^{177}Lu. As mentioned by Galbiati et al, [^{177}Lu]Lu-OncoFAP-23 is built through the trimerization of the original OncoFAP ligand which is a small organic ligand that binds with high affinity to Fibroblast Activation Protein (FAP). This high selectivity results in high tumor uptake (42%ID/g at 24 hours and 16%ID/g at 96 hours post-injection) with favorable off-target ratios (tumor-to-kidney of 30, tumor-to-liver of 62, and tumor-to-spleen of 108). It also showed dose-dependent anticancer effects, including complete remissions.[94] A study of [^{177}Lu]Lu-CHX-A′-DTPA-Rituximab (non-Hodgkin's lymphoma), rivaling [^{212}Pb]Pb-rituximab, revealed long biodistribution profile, reaching 9.1±1.5%ID/g at 24 hours, 17.2±1.8%ID/g at 48 hours, and 23.3±4.8%ID/g at 72 hours post-injection.[95] However, antibody vector typically has long biological half-life causing accumulation in organs known to catabolize antibodies, including the blood, liver, kidneys, and spleen, which suggests a low specificity of tumor targeting. All the highlighted results for ^{177}Lu based radiopharmaceuticals are presented in Table 9 below for review.

Table 9 Observation Summary of ^{177}Lu-Based Radiopharmaceuticals

Radiopharmaceutical [Ref]	Observation
[^{177}Lu]Lu-L1[88]	At 111 MBq, 60% of animal survive until 190 days after administration.
[^{177}Lu]Lu-Alb-L2[54]	Highest tumor uptake of 26.41±6.73%ID/g at 24 hours and 3.39±1.03%ID/g at 192 hours.
[^{177}Lu]Lu-Alb-L3[54]	Highest tumor uptake of 30.55±7.44%ID/g at 24 hours and 5.94±1.38%ID/g at 192 hours.
[^{177}Lu]Lu-Alb-L4[54]	Highest tumor uptake of 40.89±4.73%ID/g at 2 hours and 42.22±14.05%ID/g at 192 hours.
[^{177}Lu]Lu-Alb-L5[54]	Highest tumor uptake of 127.44±22.85%ID/g at 48 hours and 70.96±2.34%ID/g at 192 hours.
[^{177}Lu]Lu-Alb-L6[54]	Highest tumor uptake of 38.73±1.26%ID/g at 2 hours 2.22±0.37%ID/g at 192 hours.
[^{177}Lu]Lu-Ibu-DAB-PSMA[90]	At 5 MBq, 50% of mice survive until the end of study (84 days).
[^{177}Lu]Lu-rhPSMA-10.1[55]	Tumor uptake of 4.9%ID/g at 15 hours.
[^{177}Lu]Lu-DOTATOC[91]	EC_{50} = 8.2 MBq/mL.
[^{177}Lu]Lu-DOTA-LM3[91]	At 10 MBq/mL, DNA double-strand breaks were observed, indicated by 5% γH2AX-positive cells.
[^{177}Lu]Lu-DOTA-JR11[92]	At 2×20 MBq, median survival of 207 days was observed.
[^{177}Lu]Lu-OncoFAP-23[94]	At 2×10 MBq, tumor growth delay of 35±7 days and a median survival of 48.5 days were observed.
[^{177}Lu]Lu-CHX-A′-DTPA-Rituximab[95]	Extended tumor uptake with 9.1±1.5%ID/g at 24 hours, 17.2±1.8%ID/g at 48 hours, and 23.3±4.8%ID/g at 72 hours. Accumulation in normal tissue such as blood, liver, kidneys, and spleen.

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^{131}I-Based Radiopharmaceuticals

Iodine-131 (^{131}I) has a half-life of eight days and emits beta particles (0.606 MeV) and gamma particles (0.364 MeV). A study by Brito et al revealed that in other preclinical settings, such as cholangiocarcinoma, the in vitro evaluation of ^{131}I significantly decreased cell survival in a dose-dependent manner. HuCCT1 cells were more sensitive to ^{131}I irradiation than TFK-1 cells were. The irradiated HuCCT1, undergoing cell death with irradiation of 60 Gy ^{131}I, decreased significantly to a value of 7.17±2.80% and initial apoptosis of 6.00±0.82% cells. Cell viability decreased with increasing ^{131}I dose, primarily because of the increased percentage of cells undergoing initial apoptosis. For TFK-1 cells, viability decreased significantly from 89.17% (control) to 61.00% at 60 Gy, with initial apoptosis increasing from 4.83% to 31.17%.[52] The results are summarized in Table 10 below.

^{161}Tb-Based Radiopharmaceuticals

It was found that SSTR agonists and antagonists can be labeled with ^{161}Tb. A study by Borgna et al comparing terbium-based and lutetium-based agents in neuroendocrine tumor preclinical settings reported that [^{161}Tb]Tb-DOTA-LM3 (SSTR antagonist) was superior than [^{161}Tb]Tb-DOTATOC (SSTR agonist). In biodistribution profile, [^{161}Tb]Tb-DOTA-LM3 tumor uptake reached 35±7%ID/g at 4 h and 21±4%ID/g at 48 hours post-injection, whereas [^{161}Tb]Tb-DOTATOC displayed fast clearance with tumor uptake at the value of 15±1%ID/g, 6.3±0.6%ID/g, 3.7±0.7%ID/g at 0.5 hours, 24 hours, and 48 hours post-injection, respectively. Furthermore, at the same dose (2 × 10 MBq), [^{161}Tb]Tb-DOTA-LM3 demonstrated a median survival of 49 days, whereas [^{161}Tb]Tb-DOTATOC demonstrated a median survival of 21 days.[91] These differences could be due to SSTR antagonists has more binding affinity on overexpressed SSTR tumor. A new chelating ligand has been developed that contains large molecules and is suitable for combination with the SSTR agonist (TATE). As observed by Wharton et al, a fast accumulation of activity, [^{161}Tb]Tb-Crown-TATE, was obtained with tumor uptake reaching 38.5±3.5%ID/g at 2 hours post-injection. This study proven Crown to be a versatile chelator, as mentioned before, it could hold actinium stably. It labeled efficiently under mild conditions (room temperature, 10 minutes, pH 6.0) with high purity (>99%).[93] Table 11 below presents the results of the study.

Comparative Analysis: Alpha vs Beta Emitters in Preclinical Settings

This section focuses on preclinical studies, in which both types of emitters were conjugated to the same targeting molecule and applied to the same cancer type. This approach is crucial because it minimizes confounding variables related to targeting specificity or tumor biology. The following Table 12 analyzed key preclinical parameters such as biodistribution, dosimetry, therapeutic efficacy, toxicity, and DNA Double-Strand Break (DSB).

Table 10 Observation Summary of ^{131}I Based Radiopharmaceuticals

Radiopharmaceutical [Ref]	Observation
Unconjugated ^{131}I[52]	At 60 Gy, cell viability decreased to 61.00%.

Table 11 Observation Summary of 161Tb-Based Radiopharmaceuticals

Radiopharmaceutical[Ref]	Observation
[^{161}Tb]Tb-DOTA-LM3[91]	Tumor uptake of 35±7%ID/g at 4 hours and 21±4%ID/g at 48 hours.
[^{161}Tb]Tb-DOTATOC[91]	At 2×10 MBq, median survival of 49 days was observed.
[^{161}Tb]Tb-Crown-TATE[93]	Tumor uptake of 15±1%ID/g, 6.3±0.6%ID/g, 3.7±0.7%ID/g at 0.5 hours, 24 hours, and 48 hours, respectively.

Table 12 Comparative Preclinical Findings of Radioisotopes Shared Targeting Vectors

Cancer Type	Targeting Vector	Alpha Emitter [Ref]	Beta Emitter [Ref]	Key Findings and Analysis
Prostate tumor	PSMA-617	^{225}Ac[97]	^{177}Lu[97]	The treatment outcome was determined with therapeutic efficacy assessment which observed the survival time of tumor xenograft animal models after being administered with the tested compound. Administration of actinium resulted in higher time to death for half of the animal models, median survival days, 70 days vs 30 days for lutetium. In conclusion, the therapeutic evaluation indicates that actinium offers a significant survival advantage over lutetium, proven its compatibility with chelator/ vector in this diseases.
	L1	^{225}Ac[73]	^{177}Lu[88]	Biodistribution evaluation which evaluate the distribution of compound in the body revealed that actinium exhibited the highest tumor uptake, at 24 hours post-injection, (49.0±17.9%ID/g), compared to lutetium (< 20%ID/g). However, comparing the therapeutic efficacy, lutetium with higher dose than actinium (111 MBq x 1 and 9.3 kBq × 6, respectively) demonstrated the highest survival time. While Actinium is a more potent and specific agent, the study indicates that Lutetium can still achieve better survival outcomes through high-dose administration, suggesting that maximum tolerated dose (MTD) and total injected activity are just as critical to survival as tumor affinity
	L2	^{212}Pb[74]	^{177}Lu[54]	Lead could specifically target tumors better from its organ-absorbed doses profile, which could be observed by the compound absorbed at the value of 8.0 mGy/kBq to the tumor—nearly twice the dose absorbed by the kidneys (4.4 mGy/kBq). On the other hand, lutetium exhibited an unfavorable biodistribution profile. Although lutetium achieved a high tumor uptake of 26.41 ± 6.73%ID/g at 24 hours, its overall distribution in healthy organs was considered inferior to lead. This could increase the risk of radiotoxicity.
	YS5	^{225}Ac[71] ^{212}Pb[75]	–	Therapeutic efficacy which evaluates the outcome of the treatment revealed that administration of actinium (0,0185 MBq) resulted in 50% of the animal models surviving until 131 days compared to lead (0,74 MBq) with 80% of the animal models having already been euthanized at 55 days post-injection. From its therapeutic efficacy evaluation, those studies indicate that actinium is more compatible in this vector and model due to its 4 alpha-particle emissions compared to Lead's single alpha emission.
Neuroendocrine tumor	Crown-TATE	^{225}Ac[58]	^{161}Tb[93]	Biodistribution evaluation resulted in terbium exhibited higher tumor uptake, reaching 38.5 ± 3.5%ID/g at 2 hours post-injection, while the highest tumor uptake for actinium was 11.1±1.5%ID/g at 4 hours post-injection. Both terbium and actinium exhibited low kidney uptake (7.71±2.11%ID/g at 2 hours and 8.4 ±1.4%ID/g at 4 hours post-injection, respectively). Both agents successfully target tumors with low kidney retention, terbium demonstrate significantly higher tumor uptake efficiency. However, the therapeutic efficacy of actinium may still be potent due to the high LET of alpha particles. Further therapeutic efficacy assessment is needed and Internalization study is suggested on actinium as it is more important for alpha particles than beta particles that have high penetration ranges.
	DOTATOC	–	^{177}Lu[91] ^{161}Tb[91]	In vitro tumor cell viability revealed that terbium EC_{50}, activity concentration necessary to reduce cells activity by 50%, was 5-fold lower than lutetium, indicating that, terbium is 5-fold more potent than lutetium. This profile contributed to the therapeutic efficacy results. Tumor growth delay, the time it takes to reach a predetermined size of tumor, for terbium was 9.0 ± 5.5 days, not significantly higher ($p > 0.05$), compared to lutetium, only 6.0 ± 4.4 days thus resulting in comparable median survival days of 21 for terbium and 19.5 days for lutetium.
	LM3	–	^{177}Lu[91] ^{161}Tb[91]	In vitro tumor cell viability, MTT assays revealed that terbium EC_{50} was 102-fold lower than lutetium, indicating that, terbium is 102-fold more potent than lutetium. This profile contributed to the therapeutic efficacy results. Tumor growth delay for terbium was 44 ± 5 days, significantly higher ($p < 0.05$), compared to lutetium, only 35 ± 7 days thus resulting in 100% animal models that are treated with terbium surviving in the end of study (49 days) compared to lutetium with its median survival days of 48.5 days.
	DOTA-JR11	^{225}Ac[79]	^{177}Lu[79]	The evaluation focused on the biodistribution and off-target uptake, examining accumulation of compound in healthy tissues. It was observed that the absorbed dose of actinium in dose-limiting organs was higher, kidney (952.6 mGy/kBq vs 406.9 mGy/MBq), liver (271.4 mGy/kBq vs 38.5 mGy/MBq), compared to lutetium. These actinium accumulations could result in greater risk of radiotoxicity thus limiting its dosage or co-administration with amino acid cocktails could be a viable strategy for the treatment.

(*Continued*)

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Table 12 (Continued).

Cancer Type	Targeting Vector	Alpha Emitter [Ref]	Beta Emitter [Ref]	Key Findings and Analysis
Non-hodgkin's lymphoma	Rituximab	^{212}Pb[83] ^{213}Bi[82]	^{177}Lu[95]	Biodistribution evaluation resulted in tumor uptake of lead is higher than lutetium only at 24 hours post-injection (13.4–18.4%ID/g vs 9.1±1.5%ID/g). At 48 hours post-injection, while lead tumor uptake remained, lutetium exhibited an increase thus resulting in a higher tumor uptake (17.2±1.8%ID/g). Although biodistribution data for bismuth and therapeutic efficacy data for lutetium were not available for direct comparison, the therapeutic efficacy assessment highlighted the potency of lead. Treatment with 277.5 kBq of lead resulted in a 75% survival rate at 3 months, demonstrating superior efficacy compared to Bismuth-213, even when Bismuth was administered at higher doses.

Conclusion

Based on preclinical investigations of ^{225}Ac, ^{213}Bi, ^{212}Pb, ^{211}At, ^{177}Lu, ^{161}Tb, and ^{131}I-based radiopharmaceuticals, actinium is the most studied TAT, followed by ^{212}Pb, ^{213}Bi, and ^{211}At. On the side of TBT, ^{177}Lu is predominant in TBT study as it is also used as a comparator radiopharmaceutical for emerging radiopharmaceuticals. The comparison of radiopharmaceuticals is not a straightforward process because many variables can interfere with the results. However, in TRT subjects, the following results were obtained:

1. ^{225}Ac is a potent radiopharmaceutical, and some studies have shown that ^{225}Ac has superior efficacy compared to ^{177}Lu. However, due to its high physical half-life, toxicity investigation is required for its clinical translation.
2. In addition to actinium, ^{212}Pb has also demonstrated potential therapeutic efficacy. However, some studies have stated that ^{212}Pb is associated with safety concerns in the kidneys. Future studies on ^{212}Pb should focus on mitigating kidney damage by amino acid co-administration or optimal dosing of radiopharmaceuticals.
3. For TBT, a limited number of studies have revealed that ^{161}Tb has potential because of its good biodistribution profile and better therapeutic efficacy than ^{177}Lu.
4. ^{177}Lu remains an option as the base for radiopharmaceuticals. Studies have shown that high tumor uptake results in good therapeutic efficacy. However, for beta-emitting particles, a higher dose is required to have a relatively similar effect, and a high tissue penetration range causes damage to nearby organs. Therefore, dosimetry and toxicity investigations are required for clinical translation.

While the number of preclinical investigations that are reviewed were limited, with the emergence of bifunctional chelators and vectors that enhance the specificity and safety of radiopharmaceuticals. TATs have generally demonstrated better therapeutic efficacy than TBTs in tumors due to its compatibility with emerging chelator/vector mentioned in this article.

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Disclosure

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