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The use of new radioisotopes for cancer treatment: Actinium-225

Elaborato in
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L'attinio-225 (^{225}Ac) è un radioisotopo promettente che racchiude un grande potenziale nel campo della cura del cancro. Essendo un radionuclide che emette particelle α, l'attinio-225 ha mostrato un'efficacia significativa nel colpire e distruggere le cellule tumorali riducendo al minimo i danni ai tessuti sani.

La radioterapia, che prevede l'uso di radiazioni per uccidere le cellule tumorali, è stata per lungo tempo una pietra miliare nel trattamento del cancro. L'attinio-225 offre un vantaggio unico rispetto ai metodi radioterapici tradizionali fornendo radiazioni intense e altamente localizzate direttamente ai siti tumorali. Questo radioisotopo viene utilizzato principalmente in combinazione con agenti di targeting specifici, come gli anticorpi monoclonali, per indirizzare direttamente le cellule tumorali. Questo approccio mirato aiuta a ridurre al minimo gli effetti collaterali e a migliorare i risultati per i pazienti.

Uno dei principali vantaggi dell'attinio-225 è la sua capacità di fornire particelle α ad alta energia con un corto raggio. Queste particelle hanno un forte effetto letale sulle cellule tumorali grazie al loro elevato trasferimento lineare di energia (LET), che provoca danni irreparabili al DNA all'interno delle cellule tumorali. Ciò rende l'attinio-225 particolarmente efficace contro i tumori resistenti ad altre forme di trattamento.

Tuttavia, esistono limitazioni associate all'uso dell'attinio-225 per il trattamento del cancro. Una delle sfide principali è la sua rarità e disponibilità limitata. L'attinio non è abbondante in natura e deve essere prodotto attraverso complesse reazioni nucleari che coinvolgono altri isotopi. Questa scarsità pone sfide in termini di costi, scala di produzione e accessibilità per un uso clinico diffuso.

Nonostante queste limitazioni, la ricerca in corso e i progressi nelle tecniche di produzione promettono di espandere la disponibilità dell'attinio-225 come strumento efficace nella terapia del cancro. Negli studi clinici si è dimostrato che è particolarmente promettente nel trattamento del cancro alla prostata, al polmone e al pancreas.

In conclusione, l'attinio-225 si dimostra molto promettente come approccio innovativo nel trattamento del cancro attraverso la radioterapia mirata. Anche se la sua rarità pone sfide per un'adozione diffusa, la ricerca in corso mira a superare queste limitazioni e a rendere questo potente strumento più accessibile ai pazienti che combattono varie forme di cancro.

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Introduction to radiation therapy with α radiations

1.1 Introduction to radiation therapy

Radiation therapy, also called radiotherapy, can be used to treat cancer. It is a treatment that uses high doses of radiation to kill cancer cells and shrink tumors. When it is used at low doses, radiation is used in x-rays to see inside the body. At high doses, radiation therapy kills cancer cells or slows their growth by damaging their DNA; cancer cells whose DNA is damaged beyond repair cease reproducing or die. When the damaged cells die, the body breaks them and then they are removed. Radiation therapy does not instantly kill cancer cells. Before cancer cells experience enough DNA damage to cause them to die, days or weeks of treatment are required. After radiation, cancer cells keep dying for several weeks or months.

Different outcomes can be achieved using radiotherapy:

- Curative radiotherapy tries to cure the cancer completely;
- Neo-adjuvant radiotherapy enhances the efficacy of other treatments (for instance it may be combined with chemotherapy or administered prior to surgery);
- Adjuvant radiotherapy reduces the risk of the cancer returning after surgery;
- Palliative radiotherapy helps to relieve symptoms if a cure is not achievable.

Radiotherapy can be administered in several ways. The most common types are:

- External radiotherapy, that involves using a machine to direct radiation beams at the cancer;
- Brachytherapy, where small pieces of radioactive implants are momentarily placed inside the body close to the cancer;
- Radioisotope therapy, that involves injecting a radioactive liquid into the blood or swallowing it in a drink;
- Intrabeam radiotherapy, where radiotherapy is delivered directly at the tumor during surgery.

During external radiotherapy, you lie on a table and a machine shoots radiation beams towards the cancer. It is a localized form of treatment. A person can go home without any issue once it is over.

In brachytherapy, seeds, ribbons, or capsules that contain a radiation source are implanted in or close to the tumor in your body. Similar to external beam radiation therapy, brachytherapy is a local treatment that targets only a specific part of your body. The implants are usually placed inside the body without surgery, but occasionally surgery is necessary to place them near the cancer. The period of time the implant remains in the body varies; it could be a few minutes or a few days. Tiny implants might occasionally be left inside the body permanently. Despite the fact that the radiation from the implants is painless, a patient may need to stay in the hospital for a few days until the implants is removed because it could be harmful to other people. Because permanent implants emit a very small amount of radiation that gradually decreases over time, they pose no risk to other people.

Radioisotope therapy is used for some types of cancers, for example thyroid cancer and prostate cancer. After receiving treatment, a person may be radioactive for a few days, necessitating a precautionary hospital stay until the amount of radiation has fallen to a safe level.

Intraoperative radiotherapy delivers a significant tumoricidal dose to a well specified target during surgery while simultaneously minimizing exposure to nearby normal structures. Compared to external beam radiotherapy, the advantages of this therapy include potential for dose escalation, reduced overall treatment time, and improved patient convenience.

Although all the benefits that come from radiotherapy, it can also cause some side effects, such as:

- Sore skin that might change color to red, lighter or darker than the usual skin tone;
- Feeling tired;
- Hair loss in the area receiving treatment;
- Feeling sick;
- Losing your appetite;
- Sore mouth;
- Diarrhea.

Most of these adverse effects can be treated or prevented and many will disappear after treatment stops.

1.1.1 How radiation treats cancer

Cancer is a disease characterized by uncontrolled growth, so there is a division beyond normal limits that brings to invasion of other organs (intrusion or destruction of adjacent tissue). It leads to

metastasis: the spread of the cancerous cells throughout the body via blood or lymphatics. There exist different types of cancer:

- Carcinoma: it forms along surfaces (epithelial cells), it usually develops in lung, breast, colon, and prostate;
- Sarcoma: it forms along supporting tissue, it is common in bones, fat, and muscles;
- Lymphoma: it forms in the lymph nodes;
- Leukemia: it forms in blood and bone marrow.

Radiation therapy roughly follows a law of halves: 50% of patient diagnosed with cancer will receive radiotherapy, 50% of those patients will be treated with curative intent, only 50% of curative intent patients will be cured (in the meaning of surviving more than 5 years disease free, that also highly depends on the site).

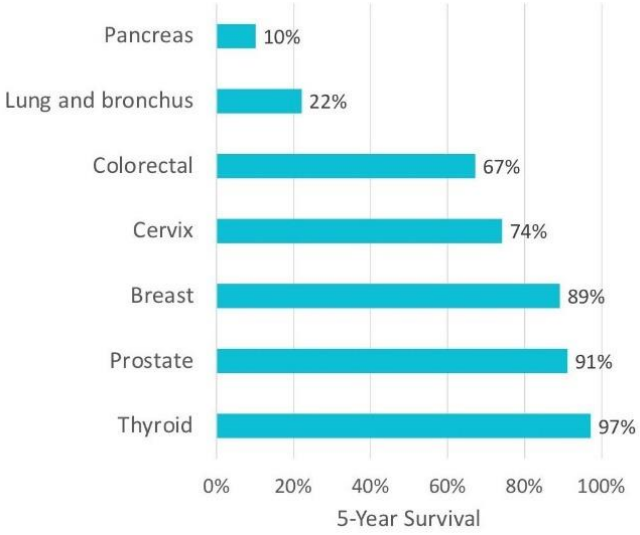

Figure 1: Canadian Cancer Statistic for 2022 of survival after 5 years, site dependent.

Radiation damages the architecture of a DNA strand by either direct action or indirect action following an ionization event:

- Direct Action, where the electron interacts with DNA molecule directly causing damage or a strand break;
- Indirect Action, where the radiation interacts with water nearby to create free radicals that bind to electrons in DNA molecule causing damage or a strand break.

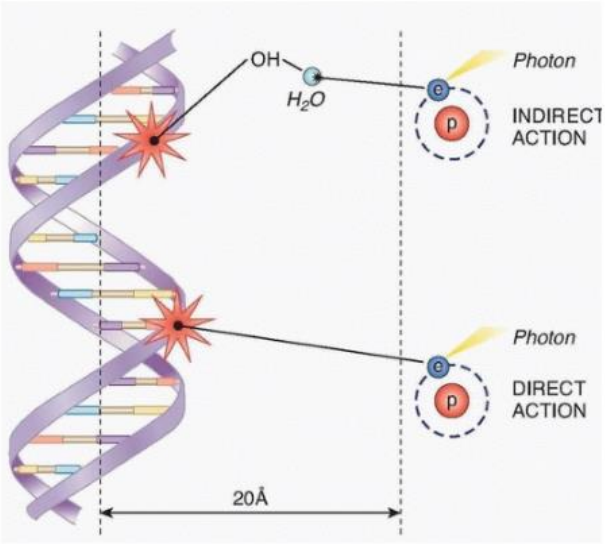

Figure 2: radiation damage to DNA.

Damaged DNA will either repair, die, or mis-repair (mutate). If cells cannot repair damage, cell death should occur at the next cell division. Abnormal cancer cells are more sensitive to radiation because they divide more quickly than normal cells. Over time, the damaged abnormal cells die off faster than healthy cells and the tumor shrinks. Normal cells can also be damaged by radiation, but they can repair themselves more effectively.

The amount and the type of radiation damage depends on several variables:

1. Type of Radiation (photons, electrons, protons, etc.);
2. Cell Cycle: late G_2 and mitosis are the most sensitive to radiation damage;
3. Oxygenation: oxygen fixation hypothesis supposes that readily available oxygen binds with free radicals, “fixing” the damaged molecule.

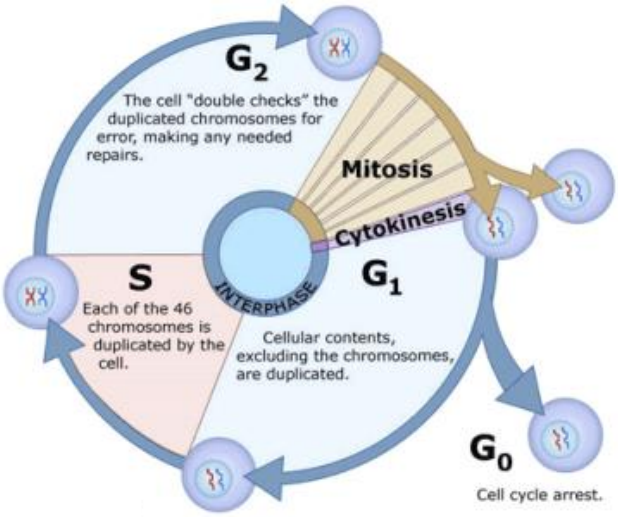

Figure 3: cell cycle.

The therapeutic dose is usually delivered in one day, but it can be also enhanced by delivering a therapeutic dose over multiple fractions, typically over several days. This enhancement is due to:

- Differences in radiosensitivity of cancer cells and healthy tissue;
- Healthy cells are better able to repair radiation damage than cancer cells;
- Healthy cells have a chance to repopulate;
- Cancer cells redistribute within the cell cycle and into more radiosensitive stages of the cell cycle, increasing cell kill over multiple treatments;
- As cells on the outside of the tumor die off, oxygen is better able to reach the necrotic core of some tumors (reoxygenation), increasing radiosensitivity due to oxygen fixation.

1.2 Introduction to radioisotope therapy

1.2.1 Radioisotopes

Radioisotopes are radioactive isotopes of a specific chemical element on the periodic table. Isotopes have a different number of neutrons in their nuclei, which contributes to their instability. Radioisotopes of a certain atom contain an unstable combination of neutrons and protons, which may result in excess energy (and instability).

There is at least one isotope for each chemical element listed on the periodic table, and radioisotopes are identifiable by that chemical and its atomic weight. For instance, when an isotope of iodine is expressed as iodine-131, it indicates it has an atomic weight of 131 neutrons. Iodine atoms typically weights 127 g.

Radioisotopes are used for both industrial and medical purposes. When radioisotopes are attached to a small molecule (e.g., a peptide), a radiopharmaceutical is created.

1.2.2 Radioisotope therapy

Radioisotope therapy, also known as targeted radionuclide therapy, uses radioisotopes drug to seeks out and kill cancer cells while causing the least amount of damage to nearby healthy cells. Although traditional radiation is the most popular radiation treatment option, radioisotope therapy enables treatment to target cancer cells throughout the body. Radioisotope therapy involves medication that

travels through the bloodstream, similar to chemotherapy. The primary distinction between this substance and chemotherapy is that it targets specific cells, minimizing the damage to healthy cells and limiting the possibility of side effects.

Depending on the type of cancer, different radioactive isotopes will be employed. For instance, thyroid cancer is treated with I-131, while prostate cancer may be treated with Ra-223 dichloride. Radioisotope therapy can be combined with other cancer treatments.

Since radiopharmaceuticals contain a radioisotope and a tracer connected to the pharmaceutical, the radioisotope can target a specific tissue or area of the body. Once the radioisotope begins to decay, it affects the targeted tissue or tumor because cancer cells absorb a greater amount of the radioisotope than non-cancerous cells do. The cancer cells are eventually destroyed by the increasing radiation exposure. The quantity of radiopharmaceutical administered is carefully determined based on the specific tumors, their location, and other factors.

1.2.3 Types of nuclear decay

The biological effect of targeted radionuclide therapy is obtained by energy absorption from the radiation that the radionuclide emits. The radionuclides used for targeted radionuclide therapy must release radiation with a relatively short path length, in contrast to the radionuclides used for nuclear medicine imaging which emit γ rays that can penetrate deeply into the body. For targeted radionuclide therapy there are three types of particles radiations that are important:

- β particles (β^- are electrons, β^+ are positrons);
- α particles (a sub-atomic matter - He atom - consisting of two protons and two neutrons);
- Auger electrons (the second electron that is ejected after the emission of an initial electron from an atom).

They can irradiate tissue volumes with multicellular, cellular and subcellular dimensions, respectively (Figure 4).

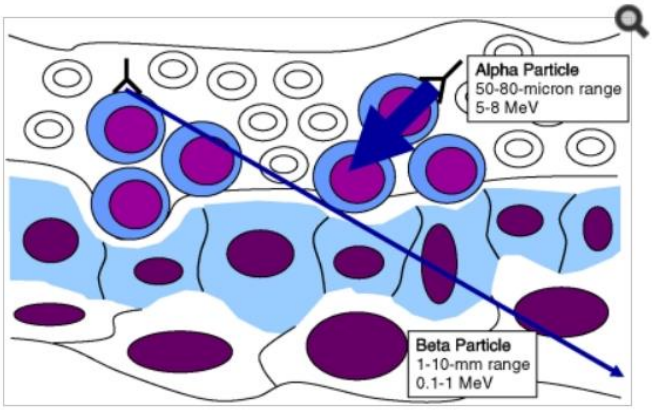

Figure 4: penetrating power of α and β particles.

In some cases, mixed emitters are used to allow both imaging and therapy with the same radionuclide (e.g., the mixed β/γ emitter iodine-131). Additionally, each of these categories contains a number of radionuclides with various tissue ranges, half-lives, and chemistries, opening the attractive option of tailor-making the properties of a targeted radionuclide therapeutic to suit the requirements of a specific patient.

β decay is the most common type of decay. It has been detected in isotopes of almost all elements. It is a type of radioactive decay in which an atomic nucleus emits a β particle (fast energetic electron or positron), transforming into an isobar of that nuclide. There are two types of β decay: β^- and β^+. In β^- decay a neutron is converted to a proton, and the process creates an electron and an electron antineutrino; while in β^+ decay, a proton is converted to a neutron and the process creates a positron and an electron neutrino. β+ decay is also known as positron emission.

β^- decay:

$$n \rightarrow p + \beta^- + \bar{\nu_e}$$

$$^{A}_{Z}X \rightarrow {}^{A}_{Z+1}Y$$

β^+ decay:

$$p^+ \rightarrow n + \beta^+ + \nu_e$$

$$^{A}_{Z}X \rightarrow {}^{A}_{Z-1}Y$$

α decay is a Coulomb repulsion effect. The nucleus reduces the Coulomb repulsion by getting rid of positive charges. It is a type of radioactive decay in which an atomic nucleus emits an α particle (helium nucleus) and thereby transforms or 'decays' into a different atomic nucleus, with a mass number that is reduced by four and an atomic number that is reduced by two. An α particle is identical to the nucleus of a helium-4 atom, which consists of two protons and two neutrons.

α decay:

$$^{A}_{Z}X_N \rightarrow {}^{A-4}_{Z-2}Y_{N-2} + {}^{4}_{2}He_2$$

The Auger effect is a physical phenomenon in which the filling of an inner-shell vacancy of an atom is accompanied by the emission of an electron from the same atom. When a core electron is removed, leaving a vacancy, an electron from a higher energy level may fall into the vacancy, resulting in a release of energy. This second ejected electron is called an Auger electron. For heavier atomic nuclei, it is more probable that the energy releases in the form of an emitted photon.

1.3 Radioisotope therapy with α particles

Radioisotope therapy with α particles is known as Targeted Alpha Therapy (TAT). Targeted alpha therapy relies on the coupling of α particle emitting radioisotopes to carrier molecules that target specific tumors, including monoclonal antibodies or peptides. Even though the tumor cells are spread through the body, these molecules have the capacity to selectively target them. They identify the targeted cancer cells through antigens that are expressed on the cell surface and can bind selectively to these cells, like a key fitting into a lock. In targeted alpha therapy, these carrier molecules act as vehicles to transport the radioisotopes to the cancer cells. It is called the "magic bullet" approach.

Radioisotopes that emit α particles appear especially promising to selectively destroy cancer cells. α particles have a high energy in the range of 5-9 MeV and at the same time a very short path length in human tissue below 0.1 mm, the equivalent to less than 10 cell diameters (in contrast to β radiation which can travels up to 100 times further in human tissue). As a result, the use of α emitters allows the precise targeting and destroying of single malignant cells, while reducing the toxicity to surrounding healthy tissue.

Even though there are many radionuclides that emit α particles when they decay, only a small number of them may be effectively used for therapeutic purposes. The broad distribution of radioisotopes can be limited by factors like the availability and the physical characteristics (e.g., half-life).

Actinium-225 is a highly promising α emitter for application in cancer therapy.

1.3.1 Characteristics of α radiation

α radiation consists of the emission of α particles, charged nuclear particles formed by two protons and two neutrons. It is characterized by high energy and shallow depth of penetration in human tissues. α radiation makes it possible to irradiate tumors selectively while sparing healthy tissues.

When the cohesive nuclear force is defeated by the electrostatic repulsion in nuclides with a lot of protons, a spontaneous nuclear transformation occurs resulting in the emission of α particles. As any other radioactive decay, α radiation is the result of a nuclear process and is not the property of a chemical element.

Many isotopes of radium, radon, polonium, actinium, uranium etc. exhibit pure α decay.

The radioisotope must be near cancer cells, or even better, inside the cancer cell, in order to reach its maximum potential. This can be achieved if the tumor cell has a distinctive biomolecule (such as a protein) on its surface that is missing from the healthy cell. These tumor-specific biomolecules can be targeted by biomarkers, that is by chemical species having a strong tendency to form stable chemical bonds. A radioligand is a biomarker that has been altered by the addition of a component that can capture the radionuclide and it can be used to transport the α emitter right to the tumor cell. A suitable radioligand must be chemically stable and very quick to reach the target (it is not ideal for the taxi to break down during the transport or for radiation decay to occur while traveling).

1.3.2 Differences with β particle and Auger electron emissions

β-emitting radioisotopes have the longest particle pathlength (≤12 mm) and lowest linear energy transfer (LET) (~0.2 keV/μm), supporting their effectiveness in medium to large tumors. Even though the long β-particle range is useful for evenly distributing radiation dose in heterogeneous cancers, it can also expose healthy tissue close to the tumor site to radiation.

Contrarily, Auger electrons have high LET (4–26 keV/μm) but a limited pathlength of 2–500 nm so they are effective only in single cells, thus necessitating the radionuclide to cross the cell membrane in order to reach the nucleus.

Finally, α-particles have a moderate pathlength (50–100 μm) and high LET (80 keV/μm) that make them particularly well suited for small neoplasms or micrometastases. Recent clinical studies highlighted the ability of α-radiotherapy to overcome treatment resistance to β-particle therapy, prompting a paradigm shift in the approach toward radionuclide therapy.

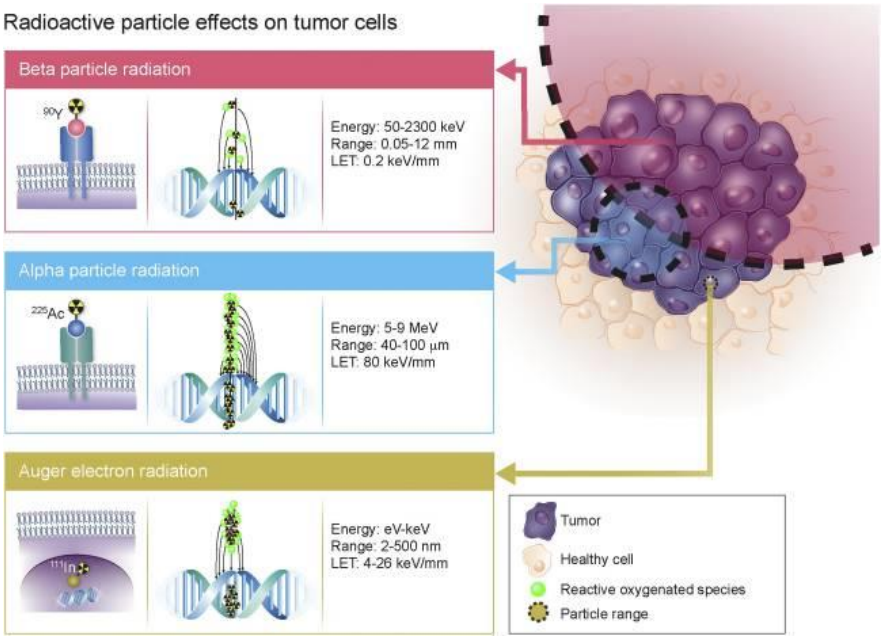

Figure 5: comparison of therapeutic energies, particle ranges, LET, and DNA damage potencies.

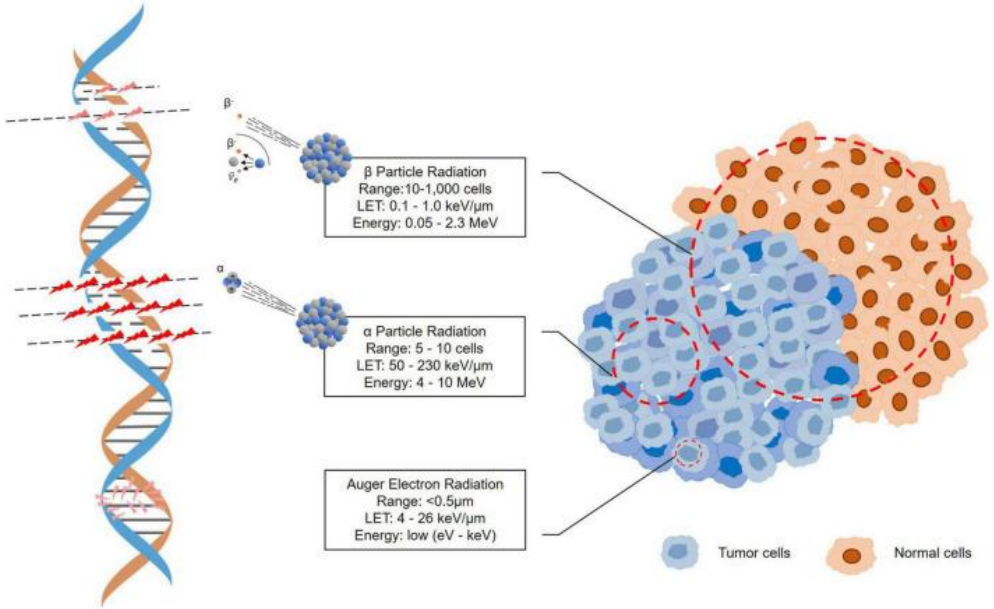

Figure 6: illustration of the tracks of α-particle, β-particle, auger electron radiation.

When α particles interact with biologic tissue, complex molecular pathways are started. The primary target of high-LET radiation is DNA, and a single α particle track can cause irreparable double-strand breaks. When α-tracks traverse the nucleus cytotoxicity results, whilst when they traverse the cytoplasm radiation-induced effects are more tolerable. In contrast, β-particle irradiation primarily causes single-strand breaks and has a cytotoxic potency that is about 500 times lower than α-particles.

The cross-fire effect is the ability of a particle to induce damage to multiple neighboring cells, offering an advantage in heterogeneous tumors. Due to the particle range, this cross-fire effect is believed to

be higher with β-emitters, but new studies demonstrating α-particles to have a significant therapeutic effect on large tumors question this concept.

It is significant that the ability of α particles to kill cells was demonstrated to be independent of cellular oxygenation, in contrast to β-particle radiotherapy, which primarily depends on the generation of reactive oxygen species.

1.3.3 Radioligands

Targeting components for targeted alpha therapy include antibodies, peptides, or small molecules; each has benefits and disadvantages. Antibodies exhibit favorable biodistribution when compared to small molecules, with high tumor uptake and low accumulation in healthy tissues. On the other hand, small molecules and peptides have greater tumor penetration and quicker clearance. Since targeting components have a wide range of pharmacokinetic characteristics, it is crucial to match the physical half-life of the therapeutic radionuclide to the biological half-life of the vector.

A radionuclide is conjugated to its vector using either a prosthetic group or a chelate. Actinium-225 radiolabeling of chelate necessitates harsh environmental conditions (high temperatures, pH extremes), which are not necessarily suitable for sensitive biomolecules like antibodies.

The slow kinetic clearance of antibodies produces blood toxicity and normal-tissue irradiation, which led to the development of a different delivery strategy called pretargeting, which separates the administration of the targeting vector from that of the radioisotope. First, an unlabeled antibody that binds both an antigen and the radioligand is administered, accumulates in the tumor, and then gradually leaves the blood and untargeted tissues. A low-molecular-weight radioligand is subsequently administered and diffuses into the tumor, binding to the antigen-associated pretargeting conjugate. The quick clearance of any extra radioligand results in improved tumor–to–normal-tissue ratios and lower radiation doses to healthy organs. The extraordinarily high affinity of avidin (or streptavidin) for biotin, bispecific antibodies, or bioorthogonal chemistry is used to interact between the radioligand and the pretargeted antibody. This method combines the advantages of antibodies (e.g., high targeting efficiency, penetration, long residence time) with those of small molecules (rapid clearance). Additionally, this approach enables the association of antibodies with short half-life radionuclides enhancing their therapeutic potential.

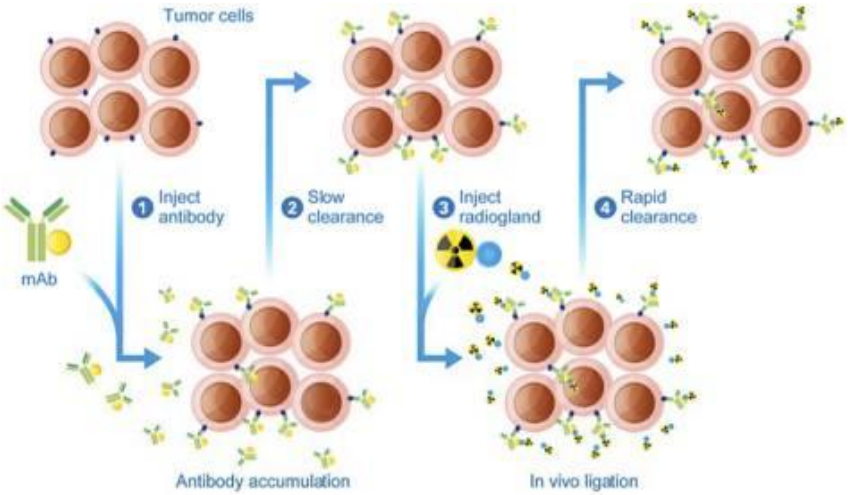

Figure 7: schematic representation of in vivo pretargeting (mAb= monoclonal antibody).

1.3.4 Hazards of α-emitters

Pure α-emitters do not constitute an external radiation hazard since an α-particle with a minimum energy of MeV is required to penetrate the protective skin layer (0.07 mm thick). The main concern is internalization and energy accumulation in healthy living tissues. Unfavorable radiation effects to humans from α-exposure include cancer induction, genetic diseases, teratogenesis, and degenerative alterations; the respiratory tract, bone, liver, and reticuloendothelial system are the most important target tissues. Furthermore, mutations and chromosomal abnormalities have been observed in the DNA of cells that have not been directly expose to α-particles, suggesting that the current genotoxic risks of α-emitters are underestimated.

Controlling the daughters' fate is equally crucial. On α-emission, recoil energy given to the daughter (100 keV) is about 1,000 times higher than the binding energy of any chemical bond, causing the daughter to be released. Redistribution depends on the distance traveled during the recoil process, diffusion processes, and active transport as well as the intrinsic affinity of the radionuclide for particular organs. The half-life of the daughter affects both the amount of time needed to reach the target and the toxicity to healthy organs. Redistribution of the recoil progeny is extremely difficult to measure and is performed mostly in postmortem ex vivo analysis of organs. One of the main drawbacks of ^{225}Ac radiotherapy is the redistribution of ^{213}Bi, a daughter of actinium-225, to the kidneys.

The problem of daughter redistribution can be effectively solved by using α-emitters with a short radioactive half-life and simple decay scheme. Nevertheless, the higher cytotoxicity of radioisotopes with longer half-lives and decay through numerous progenies motivated the creation of approaches

to regulate the fate of the daughters. A strategy is based on the development of a new form of brachytherapy, referred to as diffusing α-emitter radiation therapy. In this approach, wire sources impregnated with radionuclides are administered locally in or close to the solid tumor tissue. Encapsulation of α-emitting radionuclides into nanocarriers was evaluated to retain recoil daughters at the tumor site. ^{225}Ac-doped multishell nanoparticles were examined to encapsulate ^{225}Ac and its daughters.

The main warning of α-emitting radionuclides includes production and availability limitation.

Chapter 2

Actinium-225 production and technologies employed

2.1 The element actinium

Actinium was discovered in 1899 by Andrew Debierne, who extracted it from the uranium ore pitchblende. The name comes from the Greek word ‘actinos’, which means ‘ray’. This element is from the group of the Actinides and its atomic number is 89.

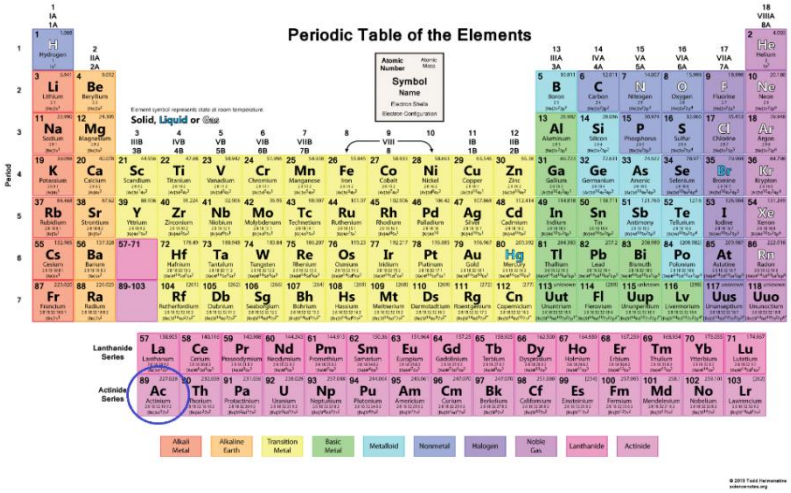

Figure 8: actinium in the periodic table of the elements.

Actinium is a soft, silvery-white radioactive metal. It glows blue in the dark due to its intense radioactivity that excites the air around it.

Figure 9: Actinium’s appearance.

This element has no known biological role; it is toxic due to its radioactivity. Actinium employed for research purposes is made by the neutron bombardment of radium-226. Actinium also occurs naturally in uranium ores. The one extracted from uranium ores is the isotope actinium-227 which has half-life of 21.7 years. It occurs naturally as one of the sequence of isotopes that originate with the radioactive decay of uranium-235. A ton of pitchblende contains around 150 mg of actinium.

2.1.1 Actinium's isotopes

Actinium has no stable isotopes and no characteristic terrestrial isotopic composition, so it does not have a standard atomic weight. There are 33 known isotopes, from ^{204}Ac to ^{236}Ac, and 7 isomers. Three isotopes can be found in nature, ^{225}Ac, ^{227}Ac and ^{228}Ac, as intermediate decay products of, respectively, ^{237}Np, ^{235}U, and ^{232}Th. Since ^{228}Ac and ^{225}Ac are extremely rare, ^{227}Ac makes up almost all the natural actinium.

The most stable isotopes are ^{227}Ac with a half-life of 21.772 years, ^{225}Ac with a half-life of 10.0 days, and ^{226}Ac with a half-life of 29.37 hours. All other isotopes have half-lives of less than 10 hours, with the majority falling under a minute. The shortest-lived known isotope is ^{217}Ac with a half-life of 69 ns.

2.2 The isotope actinium-225

Actinium-225 is an isotope of actinium. It undergoes α decay to francium-221 with a half-life of 10 days. ^{225}Ac is totally synthetic, with the exception of very small amounts found in nature that result from its decay chain.

It was discovered in 1947 as part of the previous unknown neptunium series, which ^{233}U was used to synthesize. A team of physicists from Argonne National Laboratory in the USA led by F. Hagemann first reported the discovery of ^{225}Ac and identified its 10-day half-life. The same decay scheme was independently discovered by a Canadian group under the direction of A. C. English.

Actinium-225 is part of the neptunium series, for it arises as a decay product of neptunium-237 and its daughters such as uranium-233 and thorium-229. It is the last nuclide in the chain with a half-life of more than a day until the penultimate product, bismuth-209 (half-life of 2.01×10^{19} years). The final decay product of ^{225}Ac is stable ^{205}Tl. As a member of the neptunium series, it does not occur in nature except as a product of trace quantities of ^{237}Np and its daughters formed by neutron capture reactions

on primordial ^{232}Th and ^{238}U. Compared to ^{227}Ac and ^{228}Ac, which respectively occur in the decay chains of uranium-235 and thorium-232, it is significantly rarer. Its abundance was estimated as less than 1.1×10^{-19} relative to ^{232}Th and around 9.9×10^{-16} relative to ^{230}Th in secular equilibrium (situation in which the quantity of a radioactive isotope remains constant because its production rate is equal to its decay rate).

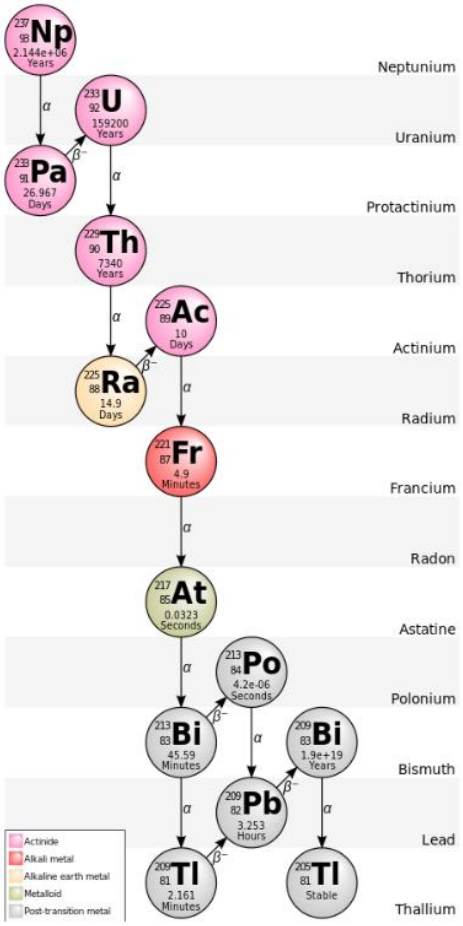

Figure 10: actinium-225 is part of the 4n+1 chain (the neptunium series).

2.2.1 Decay of actinium-225

^{225}Ac decays sequentially through 6 dominant radionuclide daughters to stable ^{209}Bi. A single ^{225}Ac decay yields net 4 α and 3 β disintegrations together with the emission of 2 useful γ-emissions; it is therefore classified as a nanogenerator. These daughters include ^{221}Fr (half-life of 4.8 m), ^{217}At (half-life of 32.3 ms), ^{213}Bi (half-life of 45.6 m), ^{213}Po (half-life of 4.2 μs), ^{209}Tl (half-life of 2.2 m), ^{209}Pb (half-life of 3.25 h) and ^{209}Bi (stable). The ^{213}Bi 440 keV γ emission has been used in imaging drug distribution. Due to the 10.0 d half-life of ^{225}Ac, the large α particle emission energies, and the favorable rapid decay chain to stable ^{209}Bi, this radionuclide was recognized as a potential candidate for use in cancer therapy.

The ^{225}Ac's daughter ^{213}Bi (with a half-life of 45.6 min) has received extensive research as a radionuclide for targeted alpha therapy in preclinical and clinical studies. ^{213}Bi forms stable complexes with nitrogen-rich chelators.

Free ^{225}Ac-acetate builds up mainly in the liver and bone. The ^{225}Ac's daughters ^{221}Fr and ^{213}Bi will preferentially accumulate in the kidneys and urine. Therefore, it is crucial to control the fate of the daughters of actinium-225 when injected into the body.

2.2.2 Decay law

Radioactive decay, also known as nuclear decay, is the process by which an unstable atomic nucleus loses energy by radiation. A material containing unstable nuclei is considered radioactive. Radioactive decay is a random event where unstable isotopes are under transformation in time.

We can assume that the number of unstable nuclei at time t is N(t) and that the probability of decay in an interval of time dt is λdt (constant for a given radioisotope). So, we can write the formula as:

$$dN = -\lambda N(t)dt$$

Dividing both sides by dt:

$$\frac{dN}{dt} = -\lambda N(t)$$

For t=0: $N(t) = N_0$

The solution shows the number of nuclei that remain after time t:

$$N(t) = N_0 e^{-\lambda t}$$

By the definition of activity, it is the rate at which a sample is decaying:

$$A = -\frac{dN}{dt} = \lambda N(t) = A_0 e^{-\lambda t}$$

When the activity reaches half of its initial value we reach the half-life of the element, defined as:

$$T_{\frac{1}{2}} = \frac{\ln(2)}{\lambda}$$

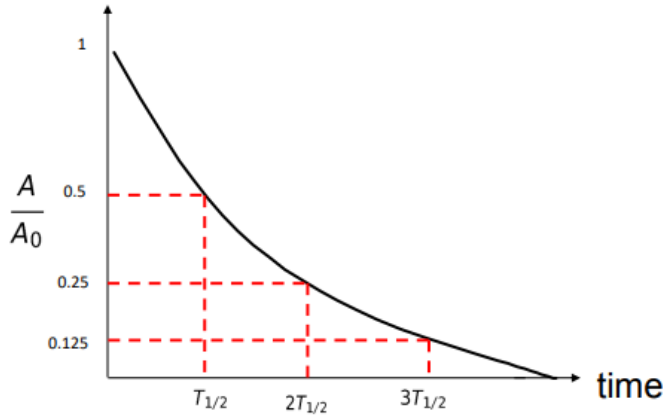

Figure 11: graph of the relative activity against time.

2.3 Radioisotope production

To produce a radioisotope there are many factors to consider:

- Clinical application;
- Chemical properties;
- Production method;
- Decay mode, energies;
- Half-life;
- Dose to the patient;
- Specific activity;
- Radionuclide purity;
- Costs.

To make radioisotopes is necessary to bombard a nucleus of a stable isotope with other particles such as neutrons, protons, α particles, etc. The two main methods are the nuclear reactor and the particle accelerator.

The nuclear reactor is mainly used to generate electricity, so the isotope production is a secondary application. However, it is still used to produce longer-life isotopes. In this method the neutrons are used to initiate more reactions when hitting a target, then the target can become radioactive. Three events can happen:

1. Neutron capture (n,γ): ${}^{A}_{Z}X + n \rightarrow {}^{A+1}_{Z}X$
2. Nucleon exchange (n,p): ${}^{A}_{Z}X + n \rightarrow {}^{A}_{Z-1}Y + p$
3. Another fission (n,f)

The particle accelerator can be small cyclotrons that can be installed in hospitals. It is useful for locally needed isotopes. A medical cyclotron has electric and magnetic fields that are used to accelerate charged ions, which are used to initiate reactions. Mainly, there can be two types of reaction:

1. (p,n) reaction ${}^{A}_{Z}X(p,n)_{Z+1}{}^{A}Y$, where an accelerated proton is captured by the target nucleus that releases a neutron;
2. (d, n) reaction ${}^{A}_{Z}X(d,n){}^{A+1}_{Z+1}Y$, where an accelerated deuteron is captured by the target nucleus that releases a neutron.

But there are more reactions that can occur: (p,2n), (d,3n), (d,p), (d,2n), and ^{109}Ag(α,2n)^{111}In.

During the production of radioisotopes, radionuclide generators are also used. A radionuclide generator is a device that contains a reasonably long-lived radionuclide (parent) that decays into a desired radionuclide (daughter). The radioactivity is inside a shielded container and can be transported and stored in the clinic. The parent is usually produced from nuclear reactions in the nuclear reactor. This is needed because the parent's half-life is longer than the daughter's one. Then the parent and the daughter should be easily separable using chemical methods. This makes easy the transportation from the production site to the hospital.

2.4 Actinium-225 production

Since ^{225}Ac does not occur in any appreciable quantities in nature, it must be synthesized in specialized nuclear reactors. The majority of ^{225}Ac is produced from the α decay of thorium-229 (^{229}Th), although this supply is limited due to the slow decay rate of ^{229Th} because of its relatively long half-life (7340 years). Stores of Th-229 were considered a waste product, leftover from nuclear programs in the 1940s and 1950s. For many years, Th-229 was kept in a secure facility until the discovery that Ac-225 and its decay daughter, Bi-213, had significant medical value. Then, scientists started "milking" the stores of Th-229, a process that involves separating Ac-225 from the other isotopes that result from thorium's decay.

It is also possible to breed ^{225}Ac from radium-226 in the ^{226}Ra(p,2n) reaction. First evidence of the ability to populate ^{225}Ac with a ^{226}Ra target came in 2005, but production and handling of ^{226}Ra are challenging due to the expense of extraction and risks posed by decay products such radon-222.

As an alternative, ^{225}Ac can be produced in spallation reactions on a ^{232}Th target irradiated with high-energy proton beams. The current techniques enable the production of millicurie quantities of ^{225}Ac,

but afterward it must be separated from other reaction products. This is done by allowing some of the shorter-lived nuclides to decay; actinium isotopes are then chemically purified in hot cells and ^{225}Ac is concentrated. Special care must be taken to avoid contamination with the longer-lived β-emitting actinium-227.

For many years, the majority of ^{225}Ac was produced in one facility - the Oak Ridge National Laboratory in Tennessee - further decreasing this isotope's availability even with minor contributions from other laboratories. Additional ^{225}Ac is now produced from ^{232}Th at Los Alamos National Laboratory and Brookhaven National Laboratory. The TRIUMF facility and the Canadian Nuclear Laboratories have formed a strategic partnership around the commercial production of actinium-225.

The low supply of ^{225}Ac limits its use in research and cancer treatment. The supply of ^{225}Ac that is now available is estimated to barely allow a thousand cancer treatments annually.

2.4.1 Producing actinium-225 by α decay of thorium-229

The primary source of ^{225}Ac is currently ^{229}Th generators, which can be milked over a three-week period and allow the separation of ^{225}Ra and ^{225}Ac. The Oak Ridge National Laboratory is a significant producer of ^{225}Ac, its ^{229}Th generator produces up to 33.3 GBq annually. However, due to the small number of generators in the world, there is a serious lack of this isotope for preclinical and clinical research. The production of ^{225}Ac/^{213}Bi generators is likewise hampered by the ^{225}Ac scarcity.

^{229}Th is itself an α-decay product from ^{233}U. Few thorium/actinium generators that can support clinical research have been documented due to the proliferation problems associated with ^{233}U. Thorium is recovered and purified by a combination of anion exchange and extraction chromatography from aged ^{233}U stockpiles. The chemical separation process for ^{225}Ra and ^{225}Ac consists of anion exchange separation using hydrochloric and nitric acids followed by cation exchange separation for the final purification.

However, the biggest issue with utilizing ^{225}Ac is the scarcity of ^{229}Th. Theoretically, there are many ways to produce ^{229}Th, the most common route being through the natural decay of ^{233}U. A secondary pathway of production of ^{229}Th can result through the neutron irradiation of ^{226}Ra via a primary which involves three neutron captures and two β decays, however, this route is limited due to unfavorable neutron cross sections for some intermediates and the co-production of substantial quantities of ^{228}Th.

2.4.2 Producing actinium-225 by proton irradiation of radium-226

An innovative technology for the industrialized production of actinium-225 is based on the physical reaction Ra-226(p,2n)Ac-225; this method involves bombarding radium-226 with protons generated by a medical cyclotron, in order to produce high quantities of Ac-225 at considerably lower prices.

In a target irradiation bunker, Ra-226 is mounted on a solid target that is then positioned in front of a beamline. A cyclotron will generate protons with an energy of up to 18 MeV, which exit through the beamline towards the Ra-226 target. The target is irradiated for an established period of time. Because of the massive impact of the proton bombardment, single protons enter the nucleus of Ra-226 atoms, which makes those atoms to become excited and unstable. In order to reach a more stable state, the excited Ra-226 atoms eject two neutrons from their nuclei, thereby transforming themselves into a different element: Ac-225. Ra-226 converts to Ac-225 at a very low rate, therefore the remaining Ra-226 is unaffected and can be reused endlessly, highly increasing the sustainability of the production.

2.4.3 Producing actinium-225 by photonuclear reaction with an electron accelerator

The most promising methods for producing ^{225}Ac on a large scale from ^{226}Ra targets involve the nuclear reaction ^{226}Ra(p,2n)^{225}Ac and possibly the photonuclear reaction ^{226}Ra(γ,n)^{225}Ra(β, 15d)^{225}Ac, both of which can be carried out using commercially available electron linear accelerator systems (linacs). The photonuclear reaction Ra-226(γ,n)Ra-225 can therefore produce industrial quantities of actinium-225 from radium-226 targets using electron beam accelerators. The Ra-225 decays via β-decay to Ac-225 with a half-life of 14.9 days.

The process starts with the acceleration of an electron that collides with a converter. The converter absorbs the energy from the electrons, converting it into a high-energy photon that collides with the target material, removing a neutron from the nucleus of the atoms resulting in radium-225.

Next all the actinium, including actinium-227, is completely removed by a chemical purification process, leaving a purified radium solution. The purified radium solution is allowed to decay and produce non-carrier-added Ac-225. Due to multiple radium solutions being on-line simultaneously, this approach significantly increases the production capacity and flexibility.

2.4.4 Producing actinium-225 by deuteron irradiation of radium-226

A method to produce Ac-225 is the bombardment of Ra-226 with deuterons accelerated in a cyclotron. Preferably, the incident energy of the deuterons ranges from 15 to 22 MeV. This process allows production of Ac-225 at high yields and purity levels.

This method is based on the transformation of instable Ra-226 - due to deuteron bombardment - into Ac-225 by emitting neutrons. This reaction is ultimately noted as Ra-226(d,3n)Ac-225 and it is more likely to occur than the (p,2n) reaction, as a result the production yield of Ac-225 is also higher.

During the process, other actinium radionuclides are produced (Ac-227, Ac-226, Ac-224) but in low concentrations compared to Ac-225. Since their half-life is very short, Ac-224 and Ac-226 are eliminated by natural decay and Ac-227 is produced in very low quantities. The Ac-225 yield is predicted to improve by 36% comparing it to the proton irradiation.

The process is preferably carried out in a cyclotron which normally enables deuterons to be accelerated to the desired energy range (between 18 and 20 MeV to provide the optimal Ac-225 production yields and purity levels).

To facilitate the handling of the highly toxic Ra-226 target material, the latter is advantageously placed in a sealed capsule of aluminum. Before the introduction into the capsule, the target material is preferably put in an envelope made of Ag, Ti or Nb, to prevent contamination of the target material with aluminum. These metals have a high conductivity and thus allow for a high deuteron current density during irradiation.

2.4.5 Producing actinium-225 by high-energy proton spallation of thorium-232

Possible pathways toward increasing ^{225}Ac production include high-energy proton spallation of ^{232}Th. A spallation is a violent reaction in which a target is bombarded by extremely high-energy particles. The incident particle, such as a proton, disintegrates the nucleus through inelastic nuclear reactions. The result is the emission of protons, neutrons, α-particles, and other particles.

A tri-institutional collaboration among Oak Ridge, Brookhaven, and Los Alamos National Laboratories recently produced millicurie quantities of ^{225}Ac by irradiating a natural thorium target at beam energies ranging from 78 to 192 MeV. Using this method, a 10-d irradiation campaign of a 5 g/cm^2 thorium target was able to produce curie levels of ^{225}Ac. The quality of the accelerator-

produced ^{225}Ac was equivalent to that of the ^{229}Th generated; however, the effect of coproduced ^{227}Ac has to be assessed.

Although this process can be scaled to increase production yields, it has some drawbacks, such as the requirement of high energy protons limiting the number of possible accelerators and the co-production of thousands of undesired isotopes often in much higher yields, raising waste issues. In addition, co-production of the long-lived and chemically inseparable actinium isomer, ^{227}Ac, introduces a number of health and regulatory challenges that will require additional mitigation efforts.

To reduce ^{227}Ac content, Ac-225 is separated from irradiated thorium and coproduced radioactive spallation and fission products using a thorium peroxide precipitation method followed by cation exchange and extraction chromatography. It offers a directly made Ac product with measured Ac-227 content of (0.15 ± 0.04)%. Repeating the final extraction chromatography step with the Ra-225 containing fraction results in the production of a second, indirectly manufactured Ac product with an Ac-227 content of $<7.5\times10^{-5}$%. The Ra-225 derived Ac-225 showed similar or improved quality compared to the initial, directly produced Ac-225 product in terms of chemical purity and radiolabeling capabilities, the latter of which was comparable with other Ac-225 sources.

Chapter 3

Application examples and clinical studies

3.1 Clinical application of actinium-225

Actinium-225 is one of the most effective radioisotopes for alpha therapy. Its decay chain provides four energetic α particles that have a short range in tissue (a few diameters) and have high energy, making them particularly effective in targeting and destroying the cancer cells. In particular, α particles are more effective at breaking DNA strands. While its half-life of about 10 days is perfect for completing the therapy cycle (from the preparation of the drug to patient's discharge from the hospital), it is long enough to facilitate treatment, but also short enough that little remains in the body months after treatment.

It is crucial for radionuclides employed in medical applications as radiation emitter is chemically pure (absence of other chemical elements) and isotopically pure (absence of other isotopes of the given element). For example, an actinium-225-based radiopharmaceutical should not contain actinium-227.

Despite its limited availability, ^{225}Ac has successfully completed various clinical trials that show its usefulness in targeted alpha therapy. Complexes including ^{225}Ac - such as antibodies labeled with ^{225}Ac - have been tested to target various types of cancer, including leukemia, prostate carcinoma, and breast carcinoma in humans. For instance, one experimental ^{225}Ac-based drug has demonstrated effectiveness against acute myeloid leukemia without harming the patient. More clinical trials of other drugs are now being conducted.

3.2 Historical use of actinium-225 in the medical field

The concept of applying ^{225}Actinium and its daughter nuclide ^{213}Bismuth for targeted alpha therapy of cancer was first proposed by Geerlings in 1993. Despite the nuclides' limited availability at the time, researchers recognized their potential for medical application, due to their favorable decay characteristics and chemical properties combined with the inherent advantages of α radiation of high energy and short-range in human tissue. The following two decades saw a significant amount of research, including the development of techniques for production of ^{225}Ac and ^{213}Bi, many preclinical studies and several clinical investigations. Early pioneering clinical work concentrated

on treatment of leukemia, non-Hodgkin's lymphoma (NHL), malignant melanoma, brain tumors, neuroendocrine tumors, and bladder cancer. In 2013, 20 years after Geerlings' original work, the compound ^{225}Ac-PSMA617 was first synthesized and studied at Joint Research Centre (JRC) Karlsruhe. While the JRC worked with hospitals in Heidelberg, Pretoria, and Munich to further improve the clinical use of ^{225}Ac-PSMA617, the amazing impact shown clinically in a growing number of patients sparked interest in using ^{225}Ac in TAT on a global scale. As a result, an increasing number of novel ^{225}Ac-labeled compounds are being developed right now, and production facilities have been established or are being built to keep up with the growing demand for the nuclide.

3.3 Clinical trials of actinium-225 by cancer type

Research has taken place on many different types of cancer, like leukemia, lymphoma, melanoma, brain-tumors, neuroendocrine-tumors, bladder carcinoma and most importantly castration resistant metastatic prostate cancer.

3.3.1 Leukemia

Leukemias are cancers of the white blood cells, which start in the bone marrow. They are divided in two categories: the type of white blood cell affected (lymphoid or myeloid), and how quickly the disease advances and worsens. There are four primary kinds of leukemia:

- Acute lymphoblastic leukemia (ALL);
- Chronic lymphocytic leukemia (CLL);
- Acute myeloid leukemia (AML);
- Chronic myeloid leukemia (CML).

Acute leukemia appears suddenly and grows quickly while chronic leukemia appears gradually and develops slowly over months to years. In 2023 it is estimated that more than 5,200 people were diagnosed with leukemia. The average age at diagnosis is 65 years old.

For the leukemia, ^{225}Ac-anti-CD33-mAb was used as a radioconjugate. 76 patients have been tested.

Early studies employing the anti-CD33 antibody lintuzumab labeled with β-emitters showed significant activity against acute myeloid leukemia (AML), although this resulted in persistent myelosuppression (decrease in bone marrow activity) that required hematopoietic cell transplantation (HCT). Targeted alpha therapy may destroy tumor more efficiently while sparing healthy cells. An

initial phase I trial of bismuth-213-lintuzumab in AML provided proof-of-principle for systemically administered α particle therapy. ^{213}Bi-lintuzumab showed quick targeting of disease sites without significant extramedullary toxicity. Target-to-whole body dose ratios were significantly improved compared to β-emitting immunoconjugates. All dosing levels of the drug produced anti-leukemic effects, and 78% of the patients experienced a reduction in the number of marrow blasts (blasts are stem cells that proliferate out of control in leukemia patients and impede the growth and development of healthy blood cells). In a subsequent phase I/II trial, ^{213}Bi-lintuzumab was administered following partial cytoreduction with cytarabine. Responses were observed in 24% of individuals who received the maximum tolerable dose of 37 MBq/kg or more. The 46-minute half-life of ^{213}Bi, however, continued to be a barrier to its broad use. Consequently, a more potent second-generation construct containing actinium-225 was developed.

A phase I study demonstrated that a single dose of ^{225}Ac-lintuzuamb was safe at doses of 111 kBq/kg or less and to reduce marrow blast in 67% of evaluable AML patients. Dose-limiting toxicity was prolonged myelosuppression (bone marrow suppression), and no evidence of radiation-induced nephrotoxicity was seen. Based on those results, ^{225}Ac-lintuzumab was investigated in a multicenter phase I/II trial in combination with low-dose cytarabine (a chemotherapy medication) for older patients with untreated AML. During the first cycle of therapy, two fractions of ^{225}Ac-lintuzumab (18.5-74 kBq/kg/fraction) were given one week apart after completion of chemotherapy. 5 of 18 patients (28%) had objective responses. All responses occurred after the first cycle. Responses were only observed in patients with peripheral blast counts <200/mL, indicating that the baseline peripheral blood blast count was a reliable indicator of the outcome. The most likely explanation for this is decreasing marrow targeting caused by preferential antibody binding to peripheral sites in patients with greater circulating blast counts. As a result of this observation, a multicenter phase II trial of ^{225}Ac-lintuzumab monotherapy was started in older AML patients, using hydroxyurea (a medication that treats leukemias) to lower peripheral blast counts if needed. Objective responses were seen in 9 of 13 patients (69%) after two doses of ^{225}Ac-lintuzumab (74 kBq/kg) administered one week apart. However, myelosuppression was longer than acceptable in this population, and additional patients were treated with two fractions of 55.5 kBq/kg each. ^{225}Ac-lintuzumab has demonstrated significant activity in AML both alone and in combination with chemotherapy. Future development of ^{225}Ac-lintuzumab includes combinations with standard chemotherapy and novel targeted agents for AML, treatment for measurable residual disease in AML, and conditioning before HCT in patients with high-risk myelodysplastic syndromes.

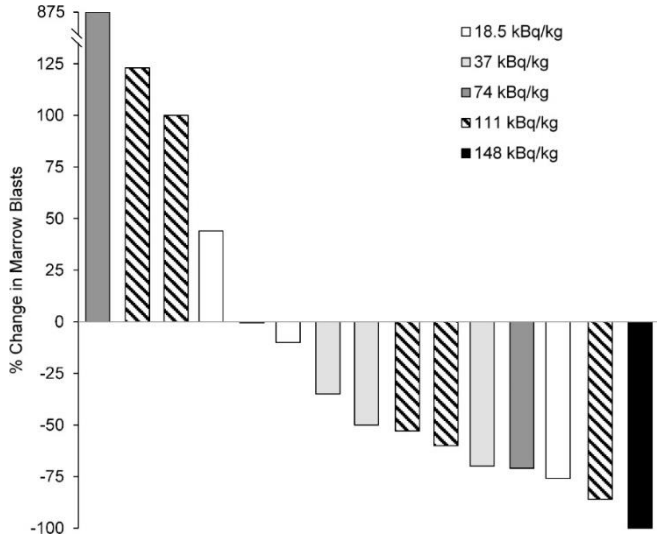

Figure 12: percentage change in bone marrow blasts after treatment with ^{225}Ac-lintuzuamb in 15 evaluable patients.

3.3.2 Glioma

Glioma is a common type of tumor originating in the brain. About 33% of all brain tumors are gliomas, which originate in the glial cells that surround and support neurons in the brain, including astrocytes, oligodendrocytes, and ependymal cells. Gliomas are called intra-axial brain tumors because they grow within the substance of the brain and often mix with normal brain tissue.

For the glioma, ^{225}Ac-Substance P was used as a radioconjugate. 21 patients have been tested.

Glioblastoma multiforme (GBM) is the most common, aggressive, and devastating malignant primary brain tumor in humans. There are not many alternatives for treating recurrent glioblastoma multiforme. No appreciable advancement in the management of malignancies was observed for many years. A therapy strategy that shows promise is local radiopharmaceutical treatment. GBM cells express high levels of the GPCR neurokinin type 1 receptor (NK-1R) and modified substance P can be used as its ligand for tumor cell targeting. Targeted alpha therapy with DOTA-Substance P (SP) labeled with the short-range α-emitter allows for selective irradiation and killing of tumor cells. In the initial stage, ^{213}Bi with a short half-time (45 min) was used for labeling of SP and local injection to the tumor and encouraging outcomes were reported. It seems that this radioisotope can be use in treatment of small tumors. The relatively short half-live of ^{213}Bi and a slow diffusion process constrains the ideal distribution of the tracer in the larger tumors. In this group of patients, a radioisotope with longer half-life might be preferred.

As a result, ^{225}Ac with a half-life of 9.9 days has been applied. 21 patients with histologically confirmed recurrence of the glia tumor grade II-IV were included in the study: grade II - 1 patient, grade III - 8 patients. grade IV -12 patients. All patients received standard treatment (surgery and radio-chemo-therapy). When a disease recurrence was discovered, resection of the tumor and implantation of the cat-cap system intratumoral or to the postsurgical cavity were performed. Few weeks later 20-40 MBq of ^{225}Ac-DOTA-SP was administered. ^{68}Ga-DOTA-Substance P was co-injected with ^{225}Ac-DOTA-SP to evaluate biodistribution using PET/CT. Therapeutic response was monitored with performance status and MRI imaging. In the group of patients with primary glioblastoma multiforme (grade IV) the progression-free survival (PFS) was 4-112 weeks; the overall survival (OS) from primary diagnosis was 32-128 weeks; OS from recurrence was 28-62 weeks; and OS from radioisotopic treatment was 8-48 weeks. Intracavitary/intratumoral injection of ^{225}Ac-substance P was well tolerated. Only minor, temporary side effects were observed (edema, epileptic seizures, aphasia). Patient recruitment and dose escalation are ongoing.

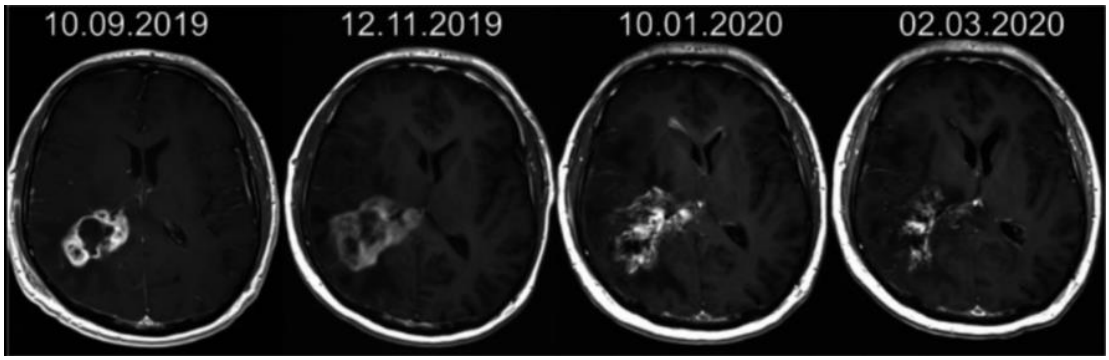

Figure 13: MRI images of a 42-year-old man diagnosed with recurrent glioblastoma NOS manifested 10 months after surgery and standard radiochemotherapy. The patient was treated with three cycles of 30-MBq ^{225}Ac-DOTA-SP with a total activity of 90 MBq. The T1-weighed enhanced MRI image before treatment and in follow-up presented shrinkage of the tumor.

3.3.3 Neuroendocrine tumors

Neuroendocrine tumors (NETs) are well-differentiated, low proliferating neuroendocrine neoplasms (NENs) that typically arises from gastroenteropancreatic structures and the lung, although NEN have been identified in nearly every tissue. NETs are considered uncommon diseases, making up only 0.5% of all malignancies, nonetheless, the incidence/prevalence has been increasing in many epidemiological studies over the past few decades.

For the neuroendocrine tumors, ^{225}Ac-DOTATOC was used as a radioconjugate. 34 patients have been tested.

As a heterogeneous disease with a wide range of symptomatology, NETs require multidisciplinary treatment and care, including medical control, surgery, chemotherapy, and internal or external radiation therapy. The foundation of therapy is still surgery with curative intent, whenever possible. However, in the case of metastatic disease, total excision is generally not an option due to the infiltration of other tissues and/or blood vessels or the number of metastatic sites. Systemic chemotherapy provides only small benefit in rapidly proliferating tumors. Therapeutic options such as somatostatin analogs (SSAs) or interferon-α may improve symptoms caused by hormonal excess or even lengthen the time to disease progression by offering hormonal and antiproliferative control over NETs, but rarely result in partial or complete tumor response. Unfortunately, external beam radiotherapy (EBRT) is ineffective in treating metastasized and secondary cancer sites that have spread outside the treatment area.

Theranostics, the idea of combining the inextricably linked disciplines of diagnostics and therapy, is a treatment option that has gained popularity over the past 25 years. Peptide receptor imaging and peptide receptor radionuclide therapy (PRRT) were the first successful examples of the theranostic concept, for imaging and treating cancer. PRRT has long been considered as a palliative treatment for NETs, but is currently receiving more and more attention as a very effective symptomatic and well-tolerated treatment that increases progression-free (and potentially overall) survival. As a conjunction with surgery, neoadjuvant therapy can make previously difficult-to-operate tumors operable by shrinking them, and as an adjuvant therapy, it may prevent tumor re-growth after surgical manipulation and growth of pre-existing micrometastases. In contrast to chemotherapy and EBRT, PRRT targets disease at the cellular level in the systemic treatment of non-resectable and metastasized NETs. About 80% of NETs have overexpressed somatostatin receptors (SSTRs) of different subtypes, offering a constantly developing method for NETs' diagnosis and treatment. The basic idea behind PRRT is to use a therapeutic radionuclide chelated to a SSTR binding peptide; as the compound binds to SSTR expressing tissue, DNA-damaging radiation is delivered nearly exclusively to tumor cells and its microenvironment while sparing the surrounding healthy tissue. Somatostatin, the native peptide, is a prime example of an SSTR-binding peptide. However, it is prone to rapid enzymatic degradation and consequently in vivo applications are not appropriate. Instead, synthetic peptides, such as those based on SSAs, have been developed with the aim of improving metabolic stability, tumor retention time, and affinity.

The first PRRT was performed in the early 1990s. The Rotterdam group successfully created ^{111}In-pentetreotide somatostatin scintigraphy (Octreoscan), and more than 1,000 patients were used to assess its imaging capabilities. Based on high uptake of ^{111}In-pentetreotide by tumors as demonstrated by imaging, Krenning's team successfully treated a patient with metastatic glucagonoma using a high dose of ^{111}In-pentetreotide, which led to a lowered level of circulating glucagon as well as decreased tumor size. This early work set the foundation for further development of this fascinating new area of radiomolecular precision medicine. For instance, the need for better radionuclides was discovered as a result of the experience with PRRT using ^{111}In-pentetreotide, as the properties of ^{111}In (decay by electron capture with a half-life of 2.8 days) do not provide good tissue penetration, which corresponds with a modest or nonexistent tumor shrinkage. Since then, novel peptides, chelators, and radionuclides in various combinations have been developed and made available, greatly improving PRRT. Stable radiolabeling with the high-energy β particle-emitter Yttrium-90 (^{90}Y-DOTATOC) was made possible by derivatizing Tyr3-octreotide and coupling it with a DOTA chelator, due to the Tyr3's higher binding affinity for SSTR2 than the native somatostatin analog (SSA) octreotide. Treatment with ^{90}Y-DOTATOC stopped rapid tumor progression, decreased the tumor marker neuron-specific enolase (NSE), and allowed disease stabilization. Since then, DOTATOC has gained popularity as a theranostic agent due to its greater diagnostic sensitivity when compared to Octreoscan and its potential therapeutic efficacy for treating SSTR-positive NETs when tagged with β-emitters, particularly Yttrium-90 (^{90}Y) and Lutetium-177 (^{177}Lu). Despite the success of ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE in the treatment of NETs in terms of progression-free survival, there were problems with ^{90}Y concerning renal toxicity and a low rate of complete remissions, suggesting an improvement in PRRT efficacy and a shift from using SSAs to somatostatin antagonists. So they developed α-PRRT using ^{225}Ac labeled ligands.

As of now, the majority of PRRTs rely on β-emitters, especially ^{90}Y and ^{177}Lu, because of their availability and known clinical effectiveness. However, the normal tissues nearby are also exposed to radiation due to the relatively large range of these radionuclides. Furthermore, hypoxic cancer tissue (with lack of oxygen) could be resistant to β-emitter treatment, causing radiotherapeutic failure of β-PRRT. Targeted alpha therapy offers a therapeutic option for patients who are resistant to β-irradiation treatments. ^{225}Ac has a lot of promise for use in TAT due to its relatively long half-life, many α-particle emissions in the decay chain, and quick decay to stable ^{209}Bi. Additionally, the theranostic potential of ^{225}Ac is made possible by the isomeric γ emissions with energy adequate for SPECT imaging.

The first clinical study of ^{225}Ac-PRRT in NET treatment was started in 2011 as a collaboration between the Joint Research Centre in Karlsruhe (Germany) and the University Hospital Heidelberg to treat patients with progressive NETs using ^{225}Ac-DOTATOC. Based on 46 treatment cycles in 34 patients, the maximum tolerable dose was determined to be 40 MBq. The treatment was found to be safe with doses of 18.5 MBq every 2 months or 25 MBq every 4 months, and a cumulative activity of 75 MBq regarding delayed toxicity. Despite the positive treatment outcomes seen in several patients, it was determined that additional research was required to improve patient selection and dosage regimens. Since then, there has been an increasing number of clinical trials with ^{225}Ac-PRRT in various NETs that examined whole-body SPECT/CT imaging potential, efficacy and safety, therapeutic effect, and comparison to β-PRRT. In the clinical trials no adverse effects were observed in patients with metastatic neuroendocrine tumors failing β-PRRT and this therapy showed improved overall survival.

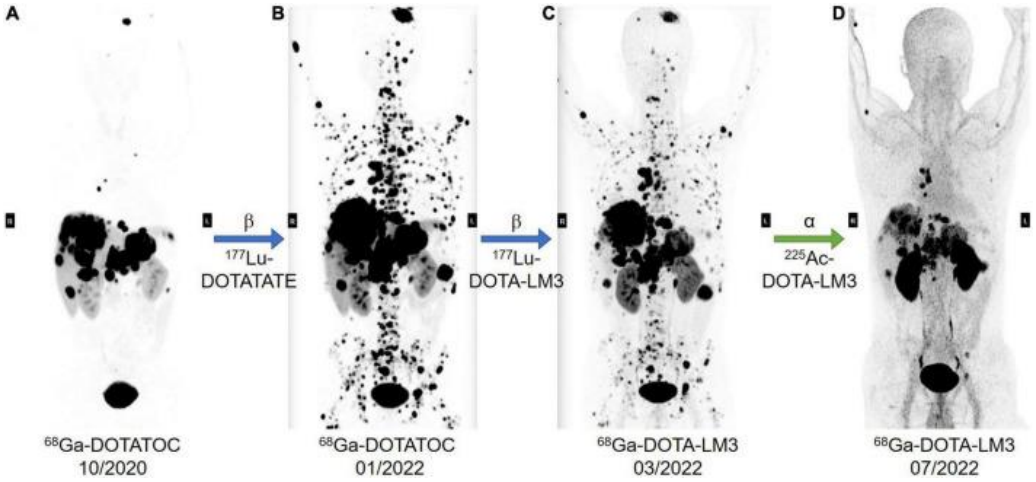

Figure 14: a 40-year-old patient was diagnosed with poorly differentiated non-functioning pancreatic-NET with bilobar liver and extensive bone metastases, Ki-67 index of 25% NEN-G3 without known mutations. The patients had previously undergone laparoscopic subtotal pancreatic resection and splenectomy, CAPTEM chemotherapy, transarterial chemoembolization (TACE) of right liver lobe and resection of abdominal lesion. In addition, the patient had previous ineffective treatment with Lanreotide, Everolimus, and Sunitinib. In total, the patient had received eight cycles of PRRT, and had a very poor prognosis, extensive bone metastases, even after multiple cycles treatment with ^{177}Lu-DOTATATE (**A,B**, ^{68}Ga-DOTATOC PET/CT before and after ^{177}Lu-DOTATATE treatment). The 9th PRRT cycle was performed with ^{177}Lu-DOTA-LM3 (**B,C**, PET/CT imaging before and after ^{177}Lu-DOTA-LM3 treatment), and the last two cycles with ^{225}Ac DOTA-LM3 (**C,D**, PET/CT imaging before and after ^{225}Ac-DOTA-LM3 treatment), 10 MBq in March and 15 MBq in May 2022, respectively. The cumulative administered

radio activities are 66.7 GBq of ^{177}Lu and 25 MBq ^{225}Ac. Restaging result in July 2022 showed excellent response to ^{225}Ac-DOTA-LM3 treatment with partial remission. As shown in the most recent PET/CT, there is dramatic improvement, especially concerning the previous innumerable bone metastases in the spine, ribs, and pelvis. The primary tumor in the pancreas, and the liver and bone metastases further decreased in size and number, and no new metastatic lesions were noted. The patient felt dramatically better in comparison to the previous treatments. After the last PRRT, he only experienced mild alopecia and mild pain in the upper right abdomen over 1 week, and has been physically active and gained 3 kg body weight over the past 2 months.

3.3.4 Prostate cancer

The breakthrough has been achieved with the development of an innovative compound for therapy of advanced prostate cancer, ^{225}Actinium-PSMA-617. The compound, which was first synthesized at JRC Karlsruhe in 2013, exhibits outstanding efficacy for treating advanced prostate cancer and provides a new therapeutic option for the second most common cancer in men worldwide. Additionally, it unequivocally indicates the great potential of targeted alpha therapy for the overall treatment of cancer.

For the prostate cancer, ^{225}Ac-PSMA617 was used as a radioconjugate. More than 400 patients have been tested.

The treatment uses the radioligand ^{225}ActiniumPSMA-617, which combines actinium-225 to a peptide that has a strong chemical affinity for the protein PSMA, also known as “Prostate Specific Membrane Antigen”. PSMA is on the surface of prostate cancer cells, but the healthy ones do not present it. Clinical tests allowed the scientists to determine the ideal radiation doses to deliver to the patient. The efficacy of the treatment has been monitored by PET and by measuring the concentration of PSA (prostate-specific antigen) in the blood of the patient at various times after the administration of the treatment.

In the evolution of TAT, the development of ^{225}Ac-PSMA617 for therapy of prostate cancer can be viewed as a turning point. The pharmacokinetics of PSMA617, in particular its quick tumor absorption within a few hours, its prolonged tumor retention as well as its rapid renal clearance of unbound compound, offer a great match to the decay characteristics of long-lived ^{225}Ac. The high level of internalization of PSMA617 in particular enables intracellular capture of the decay daughters of ^{225}Ac and utilizes their cytotoxicity for tumor cell destruction, while limiting toxicity of errant

daughters. After being created and in vitro characterized at JRC Karlsruhe in 2013/2014, ^{225}Ac-PSMA617 underwent clinical testing, which was initially conducted in partnership with University Hospital Heidelberg and later expanded to include Steve Biko Academic Hospital Pretoria and Technical University Munich.

For initial clinical application of ^{225}Ac-PSMA617, a standardized protocol was developed based on a dosimetry estimate and retrospective analysis of the effectiveness and tolerability of salvage therapies administered in 14 advanced stage metastatic castration-resistant prostate cancer patients, consisting of a treatment activity of 100 kBq/kg of ^{225}Ac-PSMA617 per cycle repeated twice every 8 weeks. In a group of 40 advanced stage patients the protocol showed significant antitumor efficacy, however xerostomia (dry mouth syndrome) was observed as predominant side effect that cause 10% of patients to stop receiving treatment. In subsequent clinical investigations, ^{225}Ac-PSMA617 was delivered in fixed activities of 8 MBq in the first cycle (equivalent to 100 kBq/kg body weight in a typical patient of 80 kg) for purposes of standardization and simplification of procedures. Additionally, given activities in the following cycles were de-escalated to 7-4 MBq as function of patient response in order to minimize harm to the salivary glands. An analysis of 73 patients treated with ^{225}Ac-PSMA617 at Steve Biko Academic Hospital in Pretoria showed therapeutic efficacy and increased tolerability of this de-escalation approach, demonstrating a Prostate specific antigen (PSA) decline of at least 50% in 70% of patients, while 83% of patients had PSA decline and 0% of patients discontinued treatment. Another interesting strategy for maintaining high therapeutic efficacy while minimizing toxicity to salivary glands is the combination of α and β emitter labelled PSMA617. A preliminary examination of a first group of 17 patients with advanced metastatic castration-resistant prostate cancer treated with a combination of 4 MBq ^{225}Ac-PSMA617 and 4 GBq ^{177}Lu-PSMA617 demonstrated improved tolerability with a response rate of 76% (PSA drop >50%). Notably, therapy with ^{225}Ac-PSMA617 was also discovered to be successful for brain metastases in individuals with advanced prostate cancer.

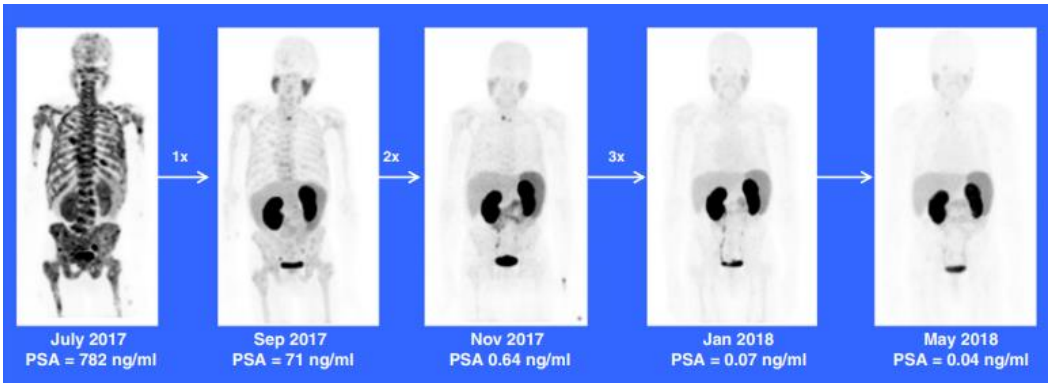

Figure 15: a treatment-naïve patient who presented with extensive bone metastasis at primary diagnosis achieved a complete remission after three cycles of ^{225}Ac-PSMA-617

with de-escalating activities of 8/7/ 6 MBq. He also remained symptom-free on 11-month follow-up with his serum PSA remaining below detectable level and the follow-up ^{68}Ga-PSMA-11 PET/CT scan remaining negative for disease recurrence.

3.4 Certification

3.4.1 FDA approval

Radiopharmaceuticals are being more broadly used in medical imaging and targeted therapies. These pharmaceutical agents include radioactive isotopes that emit radiation that can be detected by specialized imaging devices. Numerous radiopharmaceuticals have received FDA approval for a range of uses, including therapy, treatment evaluation, and diagnosis.

On February 2020 the U.S. Food and Drug Administration (FDA) has accepted its Type II Drug Master File (DMF) submission for Actinium-225 Nitrate (Accelerator-Produced). The material is produced through the proton bombardment of natural thorium targets at the high-energy accelerator facilities of the Department of Energy Isotope Program (DOE IP) at Los Alamos and Brookhaven National Laboratories. It is then separated and purified at Oak Ridge National Laboratory, which has the DMF for the product.

A DMF is a confidential, detailed document that is submitted to the FDA that contains details about the establishments, procedures, or equipment used in the manufacturing, processing, packaging, and storage of human drug products. If the DMF is active, clinical researchers and pharmaceutical firms can cite the filing in their regulatory submissions.

On April 2023, the FDA has granted approval to Fusion Pharmaceuticals for their radiopharmaceuticals actinium-225-FPI-2068 (FPI-2068) and related imaging analog indium-111-FPI-2107 (FPI-2107). According to the business, FPI-2068 was developed with AstraZeneca as part of a multi-asset collaboration agreement. FPI-2068 is a targeted alpha therapy designed to deliver actinium-225 to various solid tumors that express the proteins EGFR and cMET, cancer types include head and neck squamous cell, non-small cell lung, colorectal, and pancreatic ductal adenocarcinoma. According to the company, FPI-2068 will be the first initiative under Fusion's previously disclosed collaborative agreement with AstraZeneca to undergo clinical development. The deal includes joint discovery, development, and the option to co-market alpha therapies. Fusion will operationally

manage for a phase I study, while the following clinical development will be handled by AstraZeneca. Cost-sharing between the businesses will be equal throughout.

3.4.2 CE approval

Although many clinical studies were conducted in Europe, it seems aciìtinium-225 still needs to receive CE certification.

Chapter 4

Conclusion

Significant global interest in using ^{225}Ac as a therapeutic nuclide in TAT of cancer has been sparked by reports on the exceptional therapeutic efficacy of ^{225}Ac-PSMA617 for therapy of prostate cancer. It is projected that in order to meet the rising future demand for supply of the α-emitter a number of production routes will be utilized, including the extraction of additional ^{229}Th stocks from US legacy wastes and the implementation of various accelerator-based routes. In this respect common criteria for quality of the ^{225}Ac product need to be established in order to promote safe clinical use of the radionuclide regardless of its production pathway.

On the other hand, the limited availability of ^{225}Ac restricts its application in research and cancer treatment. It is estimated that the current supply of ^{225}Ac only allows about a thousand cancer treatments per year, reason why it is knows as ‘the rarest drug’. Only a few locations globally have thorium generators that can produce this material in research-scale quantities. In general, the production of ^{225}Ac is less than 2 Ci per year (like few grains of sand). The price for 1 mCi is US$ 800.

Despite its limited availability, several clinical trials have been completed, demonstrating the effectiveness of ^{225}Ac in targeted alpha therapy. Further clinical trials of other drugs are underway.

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