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Introduction to radiopharmaceuticals with a focus on their special characteristics and the choice of radionuclides for a clinical trial center

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Inhalt

Abstract Deutsch

Radiopharmaka nehmen innerhalb der Arzneimittel eine Sonderstellung ein. Dies liegt einerseits in ihrer radioaktiven Natur und den daraus folgenden Eigenheiten, welche sowohl die Messbarkeit im Sinne ihrer Herstellung und Qualitätskontrolle, aber auch ihre klinischen Eigenschaften beeinflussen. Dies spiegelt sich nicht zuletzt auch in diversen Sonderregularien wider. Radiopharmaka bestehen in den meisten Fällen aus nichtradioaktiven Vorläuferstoffen, welche die Bindung an die Zielstruktur als auch die Pharmakokinetik maßgeblich prägen. Diese werden mit Radionukliden markiert, welche entweder therapeutische, diagnostische Eigenschaften, oder eine Kombination von Beiden aufweisen. Dosimetrie ist die Evaluierung der Strahlenwirkung auf verschiedene Gewebe als auch auf den gesamten Körper und spielt eine wichtige Rolle in der klinischen Entwicklung von Radiopharmaka. Mit dieser Methode kann sowohl die Pharmakokinetik bestimmt werden, als auch die Abschätzung der Gefahr regulatorisch und klinisch relevanter Nebenwirkungen getroffen werden. Aufgrund der weit verbreiteten Anwendung von Radiopharmaka zur Diagnostik werden in der vorliegenden Arbeit auch wichtige Aspekte der Evaluierung von Radiodiagnostika in klinischen Prüfungen anhand des Beispiels von [^{89}Zr]Zr-Girentuximab erläutert. Im abschließenden Teil der Arbeit werden verschiedene Radionuklide, welche als Grundlage des Betriebs eines neuen Forschungszentrums mit GMP- konformer Herstellung an der Medizinischen Universität Wien dienen sollen, in ihren Eigenschaften erklärt und ihr zukünftiges Potenzial erläutert.

Abstract English

Radiopharmaceuticals have distinct features that distinguish themselves from conventional pharmaceuticals. This is primarily due to their radioactive nature, that influences both their pharmaceutical as well as their clinical properties. As a result, they are also regulated in a specific manner. In most cases, radiopharmaceuticals consist of non-radioactive precursor substances that largely determine their pharmacokinetics and receptor binding. Precursors are radiolabelled with radionuclides that carry either diagnostic or therapeutic functions, or both. Dosimetry is a method to evaluate radiation effects on different tissues as well as the whole body. It allows to determine the pharmacokinetics of a radiopharmaceutical as well as it allows to estimate the occurrence of future clinically and regulatory relevant side effects. Due to the widespread use of diagnostic radiopharmaceuticals in the detection and follow-up of disease, their evaluation in clinical trials will be explained by the example of [^{89}Zr]Zr-Girentuximab.

In the final part of the work, various radionuclides intended to form the basis for the operation of a new research center with GMP-compliant production at the Medical University of Vienna are described with respect to their properties and their future potential.

1. Introduction

1.1 General information

According to Directive 2001/83/EC, a medicinal product is "*Any substance or combination of substances presented as having properties for treating or preventing disease in human beings*; or (b) *Any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.*" A radiopharmaceutical is „Any medicinal product which, when ready for use, contains one or more radionuclides (radioactive isotopes) included for a medicinal purpose."(1)

Radiopharmaceuticals usually consist of a nonradioactive chemical (or biological) precursor, e.g. a peptide, small molecule or antibody(fragment). The chemical precursor is radiolabelled with a radionuclide. Only a very small number of radiopharmaceuticals merely consists of a formulated radionuclide without a nonradioactive precursor. The most prominent examples are Iodine radioisotopes, e.g. ^{125}I or ^{131}I, [^{223}Ra]$RaCl_3$ (Xofigo®) and [^{99m}Tc]$TcO4^-$ eluted from ^{99}Mo/^{99m}Tc generators for immediate use.

Formulation of the drug product after radiolabelling (the formation of the (radio)active substance) is inseparable from the radiolabelling process.

The principal design of a radiopharmaceutical is shown in figure 1.

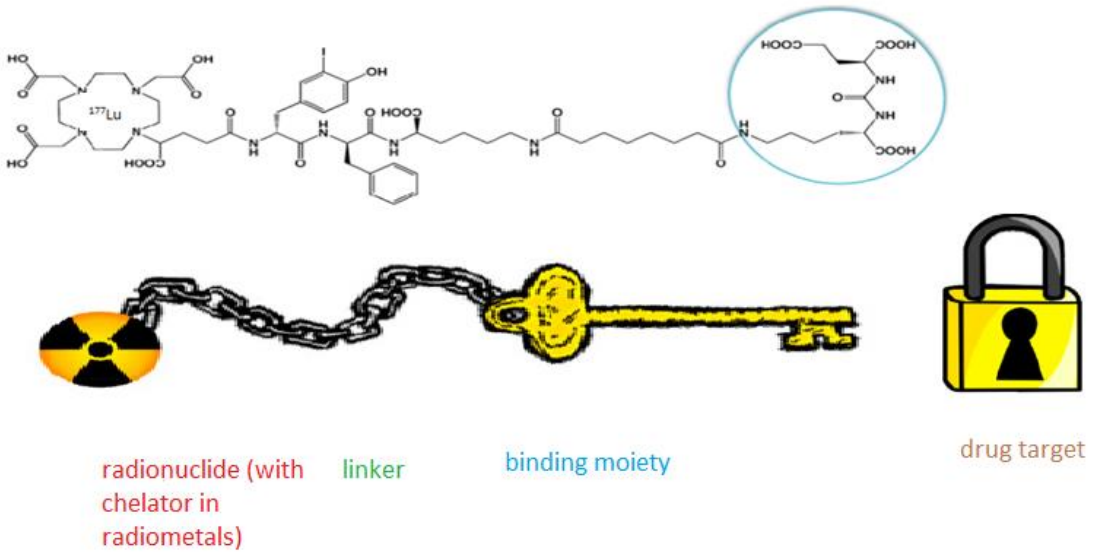


Figure 1 General design of radiopharmaceuticals and exemplary structure of [^{177}Lu]Lu-PSMA$^{I\&T}$,PSMA binding motif circled in blue, modified after(2, 3)

Radiopharmaceuticals are either used for therapeutic or diagnostic purposes, or both. Radionuclides used in therapeutic radiopharmaceuticals emit corpuscular radiation, whereas diagnostic radiopharmaceuticals emit high- energy electromagnetic waves. PET (positron emission tomography) radionuclides decay by positron emission that is followed by annihilation of the positron with an electron, e.g. from surrounding tissue and emission of two 511 kEv (kilo electron volt) gamma rays in a 180-degree angle (Figure 2). After detection by a PET scanner detector system, a line of response is calculated to define the origin of the annihilation event and therefore the site of interest, e.g. a tumor, in the body.

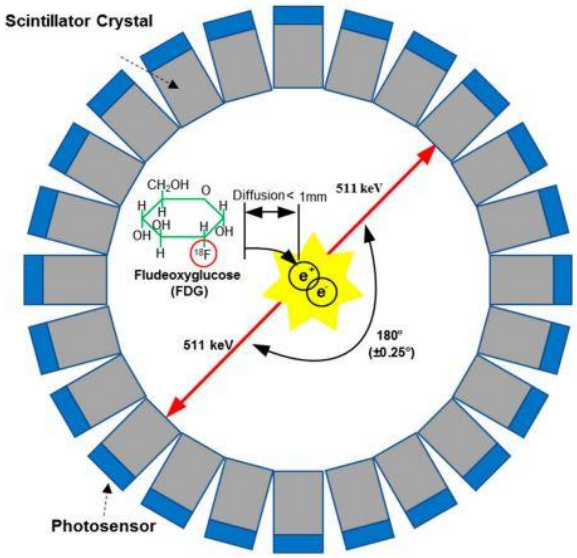


Figure 2 Emission of two 511 kEv gamma rays after decay of Fluorine-18 as part of [^{18}F]FDG (fluorodeoxyglucose), a commonly used PET tracer. Taken from(4); e^-: electron; e^+: positron

Although every PET radionuclide decays by this mechanism, there are differences in emission energies of the positron itself that determine the distance of the annihilation event of the actual uptake site (e.g. the tumor). The correlation of positron energy and positron range of commonly used radionuclides is displayed in Figure 3.

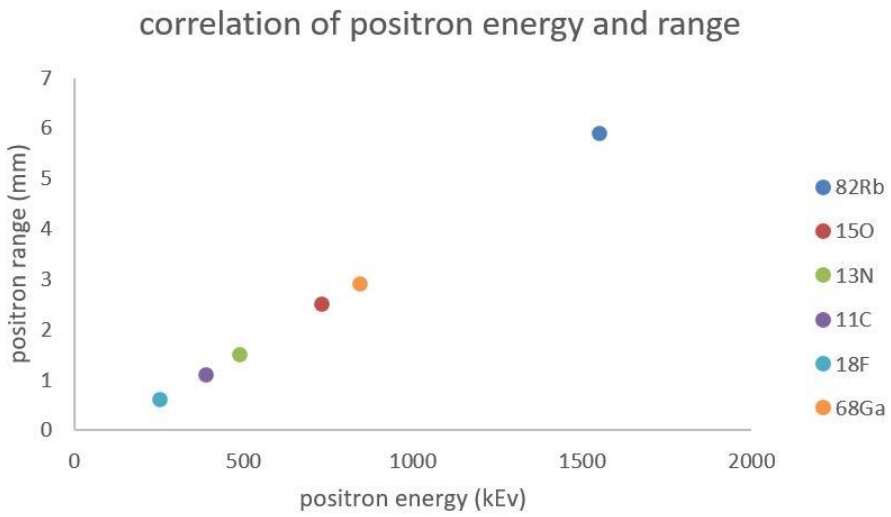


Figure 3 correlation of mean positron energy and mean range, data taken from ((5))

Many common radionuclides have diagnostic and therapeutic properties due to mixed emission of e.g. β^- and γ^- radiation (^{177}Lu, ^{131}I) or even α, β^- and γ radiation (^{225}Ac, ^{212}Pb). They are classified by their main- or situation dependent intended use and predominant effect. In the case of ^{131}I capsules for thyroid imaging and therapy, they are classified as diagnostic or therapeutic by the contained activity, since the therapeutic effect is dominant in higher doses or even absent in lower doses. In therapeutic radionuclides, concomitant gamma (e.g. ^{177}Lu, ^{225}Ac) or PET emission (e.g. ^{90}Y) allows posttherapeutic imaging. This can be exploited to assess the biodistribution and target region uptake of the therapeutic radiopharmaceutical at a single time point. Further, full dosimetry protocols can be applied to determine OADs (organ absorbed doses), ED (effective dose) and pharmacokinetics of the radiopharmaceuticals.(6-8)

1.2 Dosimetry

Dosimetry describes the assessment of distribution and excretion of a radiopharmaceutical and determination of the OADs (unit Gray (Gy)) as well as the whole-body ED (unit Sievert (Sv)).

In phase I clinical trials, but also in later phases of clinical development of therapeutic radiopharmaceuticals, dosimetry is performed to determine the organ at risk (OAR) based on its OAD and as a result the maximum cumulative activity for a certain treatment regimen.

Usually, a starting dose for first-in-human studies is based on preclinical pharmacology studies. Scaling from animal to human doses is often based on normalized body surface area but may also be based on body weight or pharmacokinetic parameters. Subsequent dose escalation/dose finding is usually based on the expected dose- response profile based on preclinical studies with risk adjusted increments.(9)

Radioligand therapy is a form of targeted therapy. Therefore, the goal of phase I dose finding often is not the maximum tolerated dose (MTD) but the optimal biological dose (OBD), which is the lowest activity yielding the highest efficacy. However, additionally to clinical safety and efficacy signals, the tolerance absorbed doses (TD) to individual organs must be considered. The so-called 5% normal tissue complication probability thresholds (NTCPs) are derived from external beam radiation therapy (EBRT) data. According to the definition, an OAD that meets the respective threshold, e.g. 23 Gy for the kidneys, leads to a 5% chance of causing an adverse event, e.g. renal impairment. As an example, the PSMA-directed radiopharmaceutical [^{177}Lu]Lu-PSMA-617 (Pluvicto®) was investigated in various dosimetry studies. A cumulative exposure of 44.4 GBq divided into 6 x 7.4 GBq cycles was determined to yield an OAD to the kidneys in the range of the 23 Gy NTCP threshold as well as the 2 Gy red marrow threshold (see Figure 4).

	Absorbed dose per unit activity (mGy/MBq)[a] (N=29)		**Calculated absorbed dose for 7 400 MBq administration (Gy)[a]**		**Calculated absorbed dose for 6 x 7 400 MBq (44 400 MBq cumulative activity) (Gy)[a]**	
Organ	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Adrenals	0.033	0.025	0.24	0.19	1.5	1.1
Brain	0.007	0.005	0.049	0.035	0.30	0.22
Eyes	0.022	0.024	0.16	0.18	0.99	1.1
Gallbladder wall	0.028	0.026	0.20	0.19	1.2	1.1
Heart wall	0.17	0.12	1.2	0.83	7.8	5.2
Kidneys	0.43	0.16	3.1	1.2	19	7.3
Lacrimal glands	2.1	0.47	15	3.4	92	21
Left colon	0.58	0.14	4.1	1.0	26	6.0
Liver	0.090	0.044	0.64	0.32	4.0	2.0
Lungs	0.11	0.11	0.76	0.81	4.7	4.9
Oesophagus	0.025	0.026	0.18	0.19	1.1	1.1
Osteogenic cells	0.036	0.028	0.26	0.21	1.6	1.3
Pancreas	0.027	0.026	0.19	0.19	1.2	1.1
Prostate	0.027	0.026	0.19	0.19	1.2	1.1
Red marrow	0.035	0.020	0.25	0.15	1.5	0.90
Rectum	0.56	0.14	4.0	1.1	25	6.2
Right colon	0.32	0.078	2.3	0.58	14	3.4
Salivary glands	0.63	0.36	4.5	2.6	28	16
Small intestine	0.071	0.031	0.50	0.23	3.1	1.4
Spleen	0.067	0.027	0.48	0.20	3.0	1.2
Stomach wall	0.025	0.026	0.18	0.19	1.1	1.1
Testes	0.023	0.025	0.16	0.18	1.0	1.1
Thymus	0.025	0.026	0.18	0.19	1.1	1.1
Thyroid	0.26	0.37	1.8	2.7	11	16
Total body	0.037	0.027	0.27	0.20	1.6	1.2
Urinary bladder wall	0.32	0.025	2.3	0.19	14	1.1
Effective dose[b]	0.120 mSv/MBq	0.043 mSv/MBq	0.886 Sv	0.315 Sv	5.319 Sv	1.892 Sv

Figure 4 Absorbed organ doses and effective dose resulting from Pluvicto® therapy, taken from and modified after the SmPC (summary of medicinal Product Characteristics)(10)

The effective dose, measured in Sv, has no threshold and therefore a single decay can cause secondary neoplasia. This should be considered in synopsis with the disease burden and life expectancy. As seen in Figure 6, six cycles of Pluvicto® cause an effective dose of 5.319±1.892 Sv. 1 Sv is assumed to increase the lifetime risk of cancer by 5 percentage points in comparison to the general population (25→30%). (10-13)

Dosimetry further allows head-to-head comparison with established radiopharmaceuticals targeting the same receptor and may provide early signals whether further clinical development is justified. In the case of radionuclides with one or more radioactive daughters, dosimetry imaging of their different gamma lines specific for the respective daughters allows investigation of daughter pharmacokinetics, that may differ from the pharmacokinetics of the intact molecule labelled with the mother radionuclide. A highly relevant example is the investigation of [^{225}Ac]Ac-PSMA-I&T pharmacokinetics. ^{225}Ac decays via a decay chain with multiple alpha decays, with Francium-221 (^{221}Fr) as first and Bismuth -213 (^{213}Bi) as third daughter. Due to high recoil energies of alpha decays, daughters are expelled from the radiopharmaceutical and therefore undergo different biodistribution than the intact compound. By collecting signals at 217 kEv (^{221}Fr gamma line) and 440 kEv (^{213}Bi gamma line), altered pharmacokinetics of recoiled ^{213}Bi were shown.(14-16)

1.3 The strength of a radiopharmaceutical drug substance

Here, the quality parameters specific activity and the carrier added/non carrier added or carrier free status shall be described. Molar activity describes the Bq/mol and is used for compounds with known molecular mass, e.g. in small molecules. Specific activity describes the Bq/g and is used in radiopharmaceuticals, where the molecular mass of the labelled compound is unknown, e.g. antibodies. Both units change over time due to radioactive decay and have to be stated in relation to a point of time, e.g. at end of production. (17)

$$A_m = \frac{Activity_{A^*}(GBq)}{n_A(\mu mol) + n_{A^*}(\mu mol)} \simeq \frac{Activity_{A^*}(GBq)}{n_A(\mu mol)}$$

Figure 5 Calculation of molar activity, taken from(18)

The therapeutic radiopharmaceutical [^{177}Lu]Lu-PSMA-I&T is not separated from its unlabelled precursor PSMA-I&T and therefore the apparent molar and specific activities are important. These terms also include the presence of the product, unlabelled precursor, nonradioactive labelled precursor (e.g. [^{176}Lu]Lu-PSMA-I&T) and radiolabelled and nonradioactive impurities. Since the

precursor and the nonradioactively labelled product are also highly affine to PSMA, potential competition reactions on the receptor may occur, especially in preparations with low apparent molar activity. The apparent A_m is primarily influenced by the precursor amount used in the start of the synthesis, which should be as low as possible. Currently, optimized synthesis conditions published in the literature are as low as 11.6 µg PSMA-I&T/GBq (Giga Bequerel) n.c.a. (non-carrier added) ^{177}Lu.(19)

To calculate the molar ratio between precursor and radionuclide, a fundamental equation linking radioactivity and the molar amount of a radionuclide is used:

$$\text{Amount (in mol)} = \text{activity (in Bq)} \times \text{half-life (in s)} \times 2.3957 \times 10^{-24}$$

$$\text{Amount (in mol)} = 1\text{x}10^{9} \text{ x } 576.288\text{x}10^{3}\text{ x}2.3957 \times 10^{-24} = 1.4 \text{ nmol}$$

According to this equation, 1 GBq of 177Lu corresponds to 1.4 nmol of the radionuclide.(20)

With a molecular mass of 1498.4 g/mol, 11.6µg of PSMA-I&T precursor correspond to 7.7 nmol.(21) This means a molar excess of 5:1 for PSMA-I&T. Therefore, in an optimized synthesis protocol with 100% radiolabelling yield, ~80% of the precursor mass is still present as unlabelled competitor in the drug product.

A radiopharmaceutical consists of three different legal and scientific entities: **the chemical or biological precursor, the radionuclide** and the resulting **drug product**. In the exceptions stated in chapter 1.1, the chemical or biological precursor is missing. In the following sections, the particularities of each entity will be explained.

1.4 The radionuclide

Radionuclides (syn. Radioisotopes) carry the diagnostic or therapeutic function of a radiopharmaceutical. They can be provided either by a radionuclide generator, a cyclotron or from a nuclear reactor. Currently approved radioisotopes are either authorised as generators (e.g. GalliAd® ^{68}Ge/^{68}Ga generator; UltratechneKow® ^{99}Mo/^{99m}Tc generator) or as radionuclide precursors, e.g. [^{177}Lu]$LuCl_3$, usually presented as low- volume, sterile solutions. Interestingly, radionuclide precursors as defined in Directive 2001/83 as “Any other radionuclide produced for the radio-labelling of another substance prior to administration”. “Any other” refers to the differentiation between radionuclide generator, radionuclide kit and radioactive precursor in the directive. Radionuclide kits don`t contain radionuclides but are merely nonradioactive substance mixtures, e.g.

chemical precursor combined with buffer and stabilizers. Every radionuclide that is not a radionuclide generator, is a radioactive precursor, except those that are not “for the radiolabelling of another substance prior to administration”. These radionuclides are active substances of authorised medicinal products with medical indications, as opposed to the “chemical” indication “for radiolabelling”.(1, 22)

1.4.1 Radionuclide Generators

By the definition of Directive 2001/83/EC, a radionuclide generator is “Any system incorporating a fixed parent radionuclide from which is produced a daughter radionuclide which is to be obtained by elution or by any other method and used in a radiopharmaceutical.” In such a generator, both, the mother and daughter nuclide are considered active substances. The generators themselves are authorised medicinal products with the aforementioned “chemical indication”. Interestingly, ^{99m}Tc generators contain “chemical” as well as medical indications, since ^{99m}Tc- generator eluate is also used for direct application. The general setup of a generator shall be visualized via the licensed GalliAd® generator (figure 6).

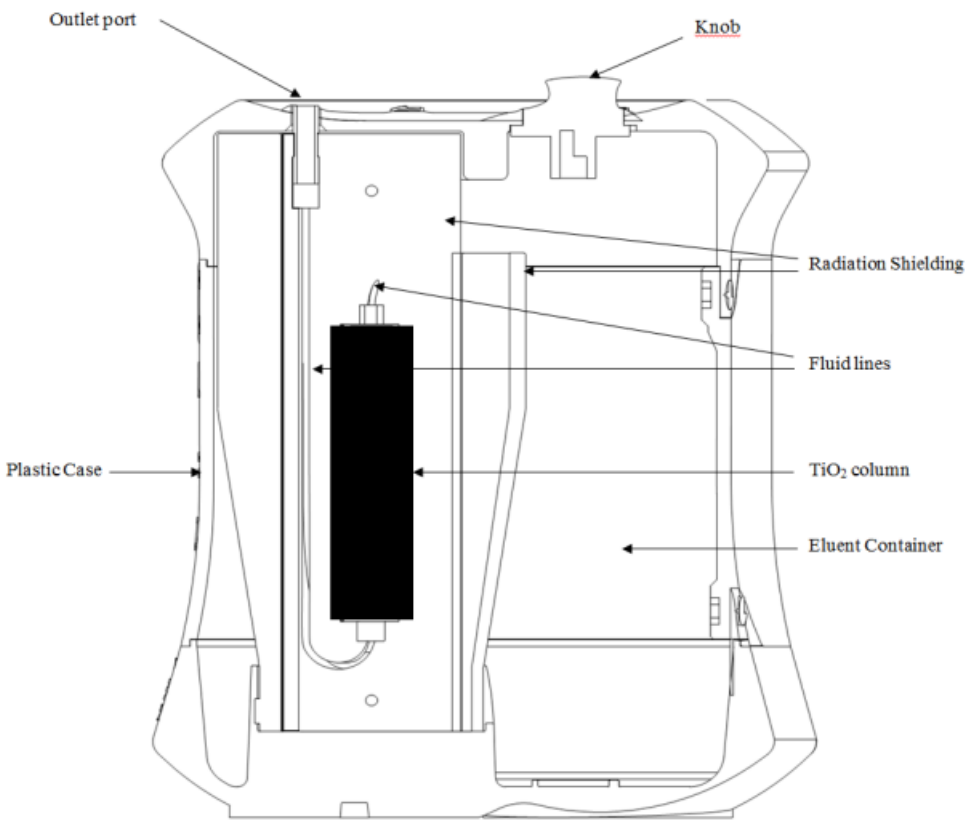


Figure 6 The mother nuclide is attached the TiO_2 column. Sterile hydrochloric acid solution is contained in the eluent container and after turning the knob and connection either of a pump or a vacuum vial, a fixed volume of the eluent flows over the column and selectively elutes the daughter nuclide. Scheme taken from (23)

1.4.2 Cyclotron based radionuclide production

In a cyclotron, particles (most often protons) are accelerated on a circular track and eventually collide with a target. For example, one of the main production routes of ^{18}F is the bombardment of a $H_2{}^{18}O$-water containing target with protons. The subsequent nuclear reaction yields $[^{18}F]F^-$ after uptake of a proton by ^{18}O and subsequent emission of a neutron.(24)

Currently, the most established radionuclides for human use based on cyclotron production are carbon-11, fluorine-18, nitrogen-14 and copper-64. Currently, Copper-64 (Cuprymina® radiopharmaceutical precursor solution) and Germanium-68/Gallium-68 generators (the mother nuclide ^{68}Ga) contain the only cyclotron- produced radionuclides authorised by the EMA (European Medicines Agency). They have been granted marketing authorisation for radiolabelling (=the indication) and not for direct human use. (23, 25)

1.4.3 Atomic in-vivo nanogenerators

Atomic in-vivo nanogenerators are radionuclides that 1) decay via decay chains to multiple daughter nuclides and 2) these daughter nuclides individually contribute to the therapeutic effect by α or β^- decay. The most advanced nanogenerator in terms of clinical development is ^{225}Ac (Figure 7). By December 2023, more than twenty clinical trials with ^{225}Ac- labelled IMPs (Investigational Medicinal Products) were registered at clinicaltrials.gov. (26, 27)

First and foremost, to discuss the particularities of Actinium-225, the nature of its decay must be understood (Figure 7).

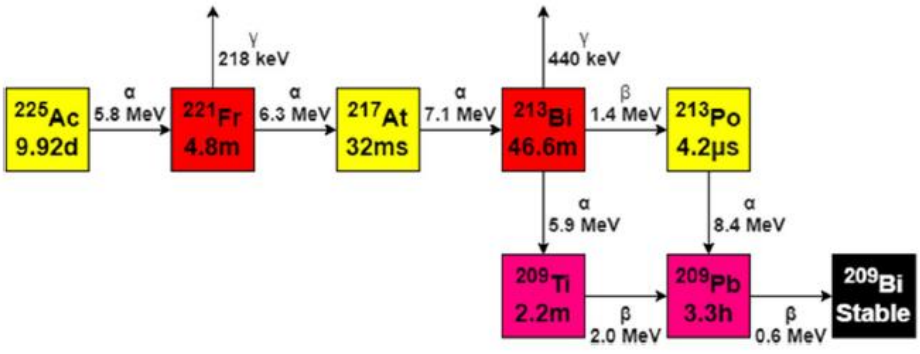
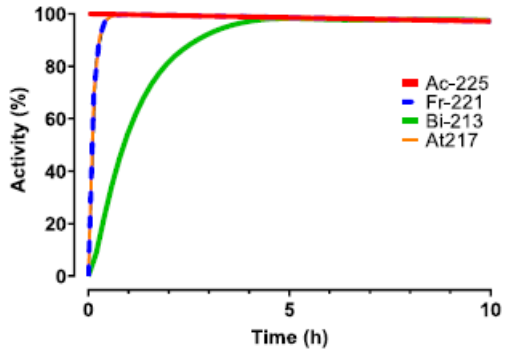


Figure 7 decay chain of ^{225}Ac; 4 net α particles, 2 β^- particles and 2 measurable gamma lines are generated; taken from(28)

As opposed to well- established radionuclides, the mother nuclide ^{225}Ac cannot be reliably measured, due to the net emission of 4 alpha, 2 Beta particles and two prevalent and detectable gamma rays with 218 keV (emitted by Francium-221) and 440 keV (emitted by Bismuth-213). By using wide energy windows, the detected signals would be of different origin, e.g. ^{213}Bi in a non- equilibrium state and ^{221}Fr in an equilibrium state and additional Bremsstrahlung signals created from alpha and

beta particles. In the currently available literature, the quality control parameters of synthesis yield, radiochemical yield and radiochemical purity are measured via detection of the ^{221}Fr gamma line in equilibrium with ^{225}Ac. A secular equilibrium occurs if the half- life of a mother nuclide is much longer than the half- life of the respective daughter. After six half- lives of the daughter, the measured activity of the daughter nuclide corresponds to approximately 98% of the activity of the mother nuclide and therefore a reliable assumption of the true activity of the mother can be made (Figure 8).(28)



$t_{1/2}$ ^{225}Ac (9.91 d)>>> $t_{1/2}$ ^{221}Fr (4.8 min) ;eq_{sec} after ~30 min

$t_{1/2}$ ^{225}Ac (9.91 d)>>> $t_{1/2}$ ^{213}Bi (45.6 min) ;eq_{sec} after ~274 min

Figure 8 Ingrowth of the different daughter nuclides over time; modified after(28)

Accordingly, because of its shorter half- life, measurements are most frequently based on the ^{221}Fr gamma line.

Starting from the delivery of $^{225}AcCl_3$ as raw material, it can be assumed in most cases that since the last separation process influencing the secular equilibrium between ^{225}Ac and its daughters, such as column-based purification of ^{229}Th/^{225}Ac generator, enough time has passed for the different secular equilibria to have formed. In this case, no waiting time is necessary to reliably measure the delivered radioactivity.

After radiolabelling, solid phase extraction with reversed-phase C18 columns is often performed to separate unbound ^{225}Ac (and unbound daughter nuclides) from the desired product. Unbound daughter nuclides are present due to the delivered solution being in secular equilibrium and are constantly generated by decay of ^{225}Ac and the recoil effect (alpha particles contain a manifold of the energy of any known chemical bond), that leads to release of its daughter nuclides from the radiopharmaceutical. However, this separation leads to disruption of the equilibria (Figure 9).

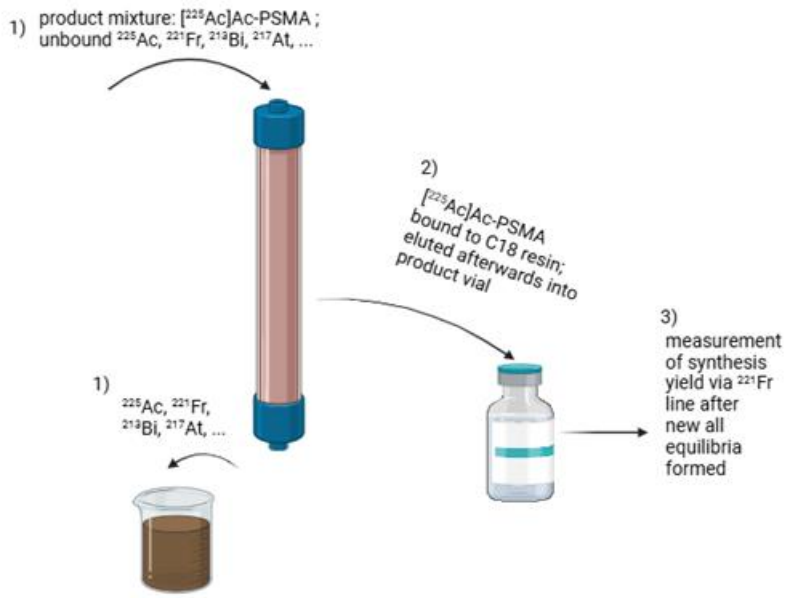


Figure 9 solid phase extraction as purification method, e.g. for [^{225}Ac]Ac-PSMA compounds

1.4.4 Determination of unbound ^{225}Ac via TLC

TLC (thin-layer chromatography) is commonly used for the determination of unbound radionuclide, most often by developing with sodium citrate as mobile phase. Unbound ^{225}Ac is a bone- seeker and therefore a critical quality attribute to prevent myelosuppression resulting from poor product quality.

In sodium citrate TLC, unbound ^{225}Ac migrates with the front while the product stays at the bottom. Due to the presence of recoiled daughters that also move with the solvent front, the signal at the top of the TLC strip is confounded and decay of the respective daughter nuclides must be waited for (Figure 10). Meanwhile, ^{225}Ac/^{221}Fr equilibrium forms, leading to an increasing signal at the product peak. A sufficient waiting time allows accurate determination of free ^{225}Ac. In batches with borderline high amounts of free ^{225}Ac, sufficient waiting time is important to prevent batch rejection due to premature measurement.

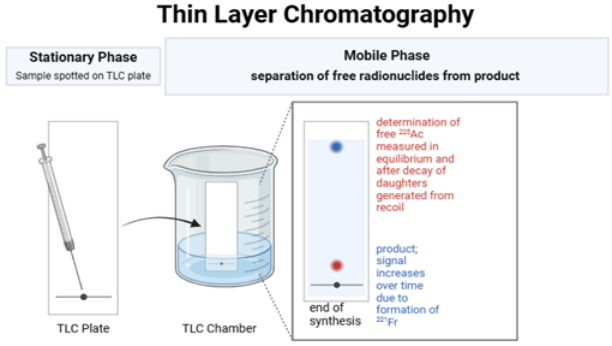


Figure 10 Scheme of TLC in quality control of ^{225}Ac- labelled compounds

1.4.5 [^{225}Ac]Ac-PSMA HPLC method validation

As a result of the reasons stated above, direct inline HPLC measurement is not reliable or suitable for ^{225}Ac-labelled radiopharmaceuticals. Therefore, fractions of the HPLC eluate are collected and measured in ^{225}Ac/^{221}Fr equilibrium. In reversed- phase HPLC columns, free radiometals elute with the solvent front and are therefore measured first. Since the presence of recoiled daughters is inevitable, their decay must be waited for until the remaining radioactivity can be attributed to ^{225}Ac, represented by ^{221}Fr in equilibrium. Later fractions contain the product and degradation/rearrangement products. Due to the time intensive procedures, reduction of the total number of collected fractions is a method to save time. However, especially in byproducts that are difficult to separate and require sophisticated methods with slow gradients and long columns, the collection of too much eluate in one fraction compromises the separation efficiency of the HPLC method. (29)

The glu- urea- lys PSMA binding motif is part of all established PSMA- directed precursors, such as PSMA-617, PSMA-11 and PSMA-I&T (Figure 13).

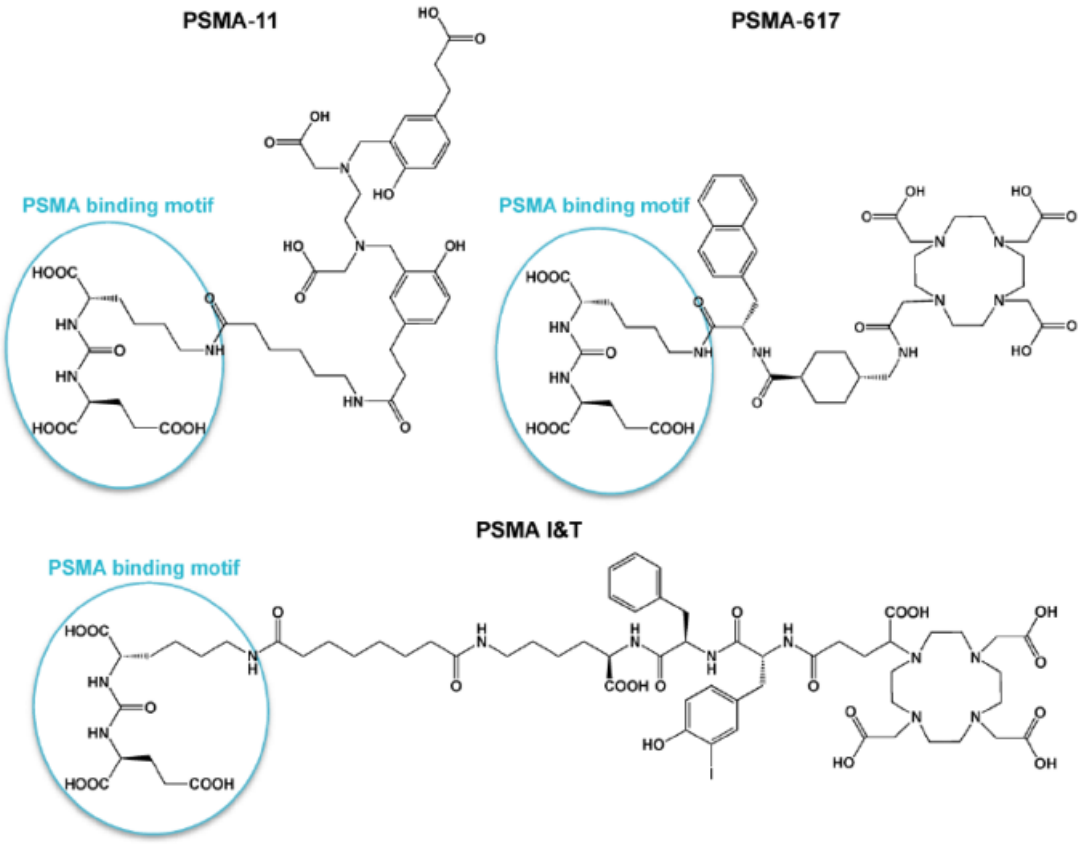


Figure 11 PSMA binding motif in established compounds; taken from(30)

Under the influence of heat, the binding motif undergoes cyclization to form three different hydantoin byproducts. Due to the high similarity to the intact product, HPLC baseline separation of these byproducts and the intact product is challenging (Figure 14).

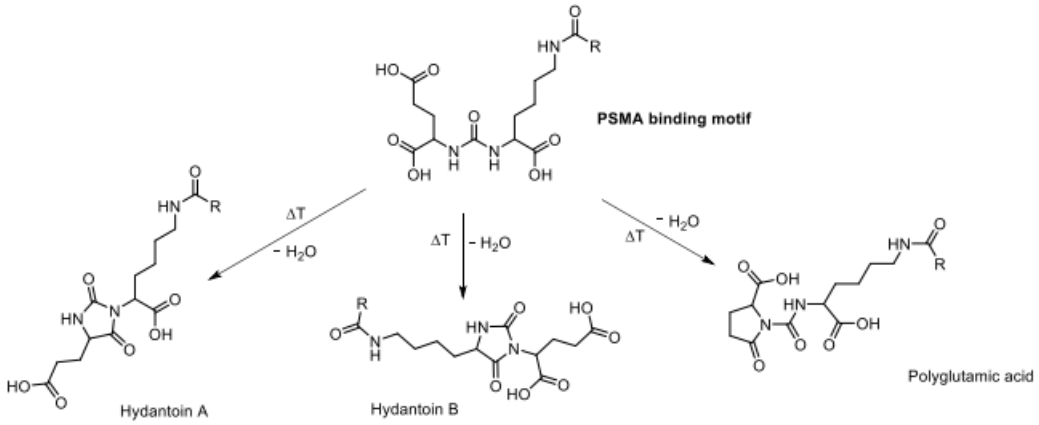


Figure 12 Heat- dependent rearrangement of the PSMA- binding motif; taken from(3)

In current literature regarding [^{225}Ac]Ac-PSMA quality control, fraction collection is performed with fractions comprising of 30-40 seconds of runtime each, e.g. a total of 30 fractions in a 20-minute run. Currently, none of the authors managed to measure hydantoine byproducts by HPLC fraction collection, representing a major part of radiochemical impurities. The hydantoine byproducts are hidden under peak tailing of the product. A comparison between fraction collection of [^{225}Ac]Ac-PSMA in the literature and validated methods for the detection of hydantoins in [^{177}Lu]Lu-PSMA, is displayed in Figure 14.

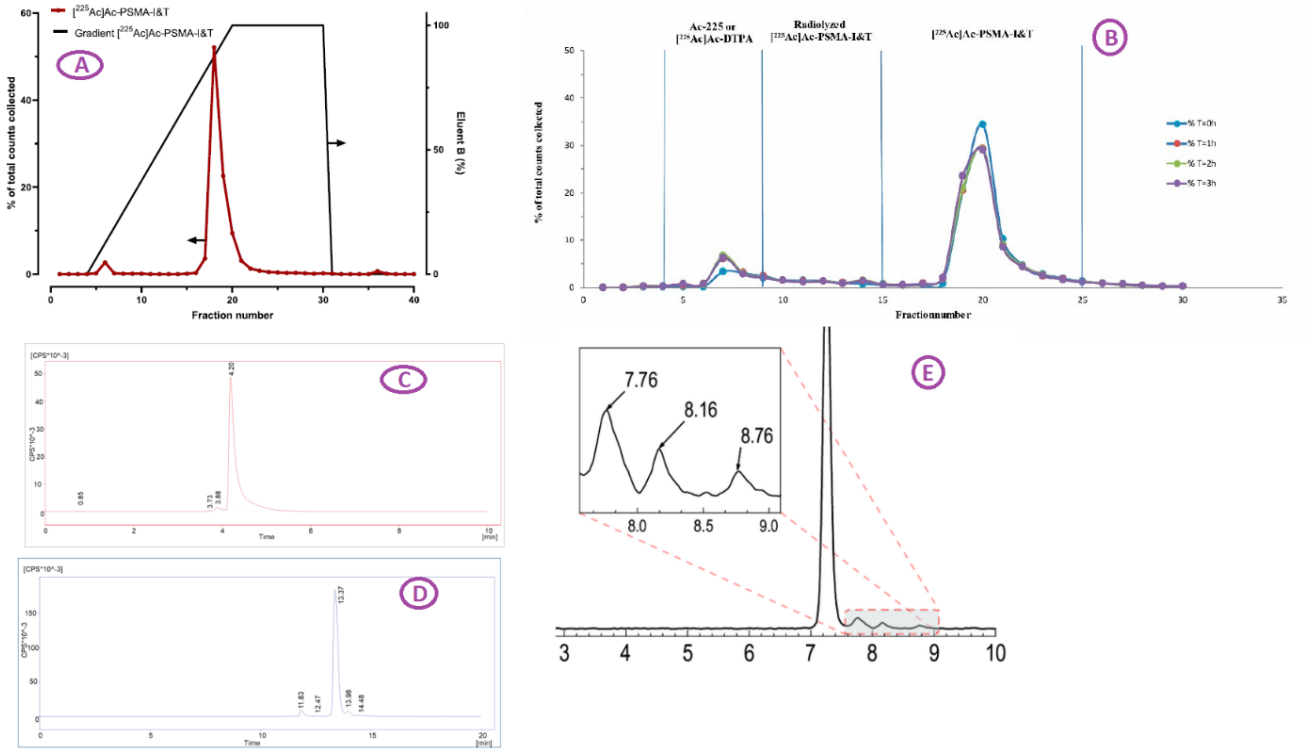


Figure 13; A, B: HPLC fraction collection of [^{225}Ac]Ac-PSMA-I&T with product peak tailing; C,D: [^{177}Lu]Lu-PSMA-I&T HPLC measurement with C) unresolved hydantoins and D) visible hydantoin peaks; E) resolution of hydantoin peaks in [^{177}Lu]Lu-PSMA-617; chromatograms modified after(3, 28, 31, 32)

According to the literature, hydantoins account for approximately 2-3% of the total radioactivity in the product and show no binding affinity to PSMA.(3, 32)

1.5 Chemical precursors

Chemical precursors for radiopharmaceutical preparations, or simply `chemical precursors`, are non-radioactive substances obtained by chemical synthesis for combination with a radionuclide.(22, 33)

In the EMA guideline on radiopharmaceuticals, the radionuclide and the chemical precursor are to be viewed as active substances.

It is stated that a chemical precursor should either comply with a respective substance monograph in the Ph. Eur, or data should be provided in an active substance master file (ASMF) or full details of manufacture according to EMA guideline 3AQ5A should be provided. For new precursors, the latter two options apply. The chemical precursor data in an IMPD is displayed in the substance (“S”) section, together with the description of the radionuclide. (34)

1.6 Biological precursors

Recently, significant progress was made in the development of novel antibody-based radiopharmaceuticals for imaging and therapy, e.g. in PSMA- targeted radioligand therapy of prostate carcinoma, which became the most prevalent field of research in nuclear medicine academia and industry over the last years. Currently, only Pluvicto® ([^{177}Lu]Lu-PSMA-617) is approved as PSMA- targeted radioligand therapy in prostate cancer, but similar candidates are in advanced clinical development (e.g. ([^{177}Lu]Lu-PSMA-I&T). One of the major drawbacks is that ^{177}Lu has a significantly longer physical half- life ($t_{1/2}$ ~160 hours) than the effective half- life of the radioconjugate [^{177}Lu]Lu-PSMA-617 (terminal $t_{1/2}$ of 41.6 hours). In metastases, the effective half- life is 61h. The resulting problem is that a major part of the drug is excreted without the majority of decay events having occurred at the target site and even after uptake into the target, the tumour residence time is much lower than the physical half- life of the radionuclide.(10, 35, 36)

There, antibodies and antibody fragments come into play, because they better match the half-lives of the currently established therapy isotopes. A major disadvantage of intact antibodies is their slow blood clearance. In imaging agents, this means lower target to background contrast agents, which could be overcome by imaging several days after tracer administration. In therapeutic radio antibodies, myelosuppression could be more likely. Antibody fragments, such as Fab fragments or ScFv were developed to reduce blood circulation time and solve these problems (Figure 14). (37, 38)

1.6.1 Therapeutic radioantibodies versus small molecules

Currently, the therapeutic antibody most advanced in clinical development is [^{177}Lu]Lu-Rosopatamab-Tetraxetan (syn. [^{177}Lu]Lu-J591). It was first described in 1997 and is currently investigated in a phase III trial in mCRPC patients. As opposed to the approved therapy algorithm of Pluvicto with 7.4 GBq every 6 weeks for up to 6 cycles, TLX591 uses a fractionated dose regimen with only 2x 2.8 GBq administered 14 weeks apart. If safety and efficacy data would prove to be at least non- inferior to Pluvicto®, this could be a potential breakthrough by reducing total treatment time to two weeks as opposed to 24-36 weeks when using Pluvicto® or similar small molecule- based substances. Also, total administered activity would be reduced from 30-44 GBq to 5.6 GBq. In a phase I dosimetry trial, [^{177}Lu]Lu-J591 showed a whole body effective half- life of approximately 10 days. In Pluvicto® and [^{177}Lu]Lu-PSMA-I&T, the respective values were only 35 and 42h. On the one hand, the higher whole-body residence time of [^{177}Lu]Lu-J591 enables higher tumor doses with less radioactivity, but at the potential cost of higher bone marrow dose and resulting myelosuppression.(10, 39-41)

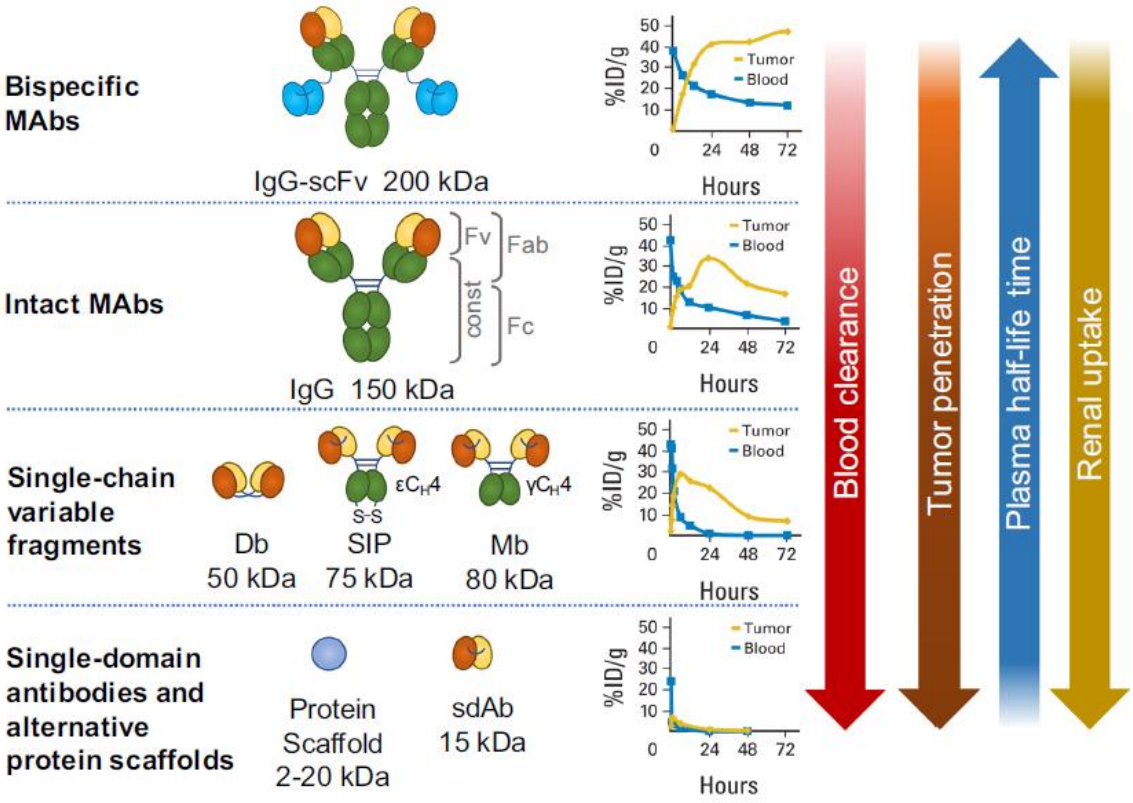


Figure 14 Antibody- based vectors, modified after(22)

1.7 (Early) clinical development in nuclear medicine

Well-equipped radiopharmacies, usually located in university clinics, are capable of providing early access to novel radiopharmaceuticals for diagnosis and therapy. For example, [^{177}Lu]Lu-PSMA-617 was used for therapy of metastatic, castration- resistant prostate cancer, at the University Clinic Vienna from 2015 on and continued until the precursor became unavailable for academic centers. Proper clinical development that eventually led to the approval of Pluvicto® only started in 2016 with a dose escalation trial. Legal options to use experimental radiopharmaceuticals in the clinic are displayed in figure 15.

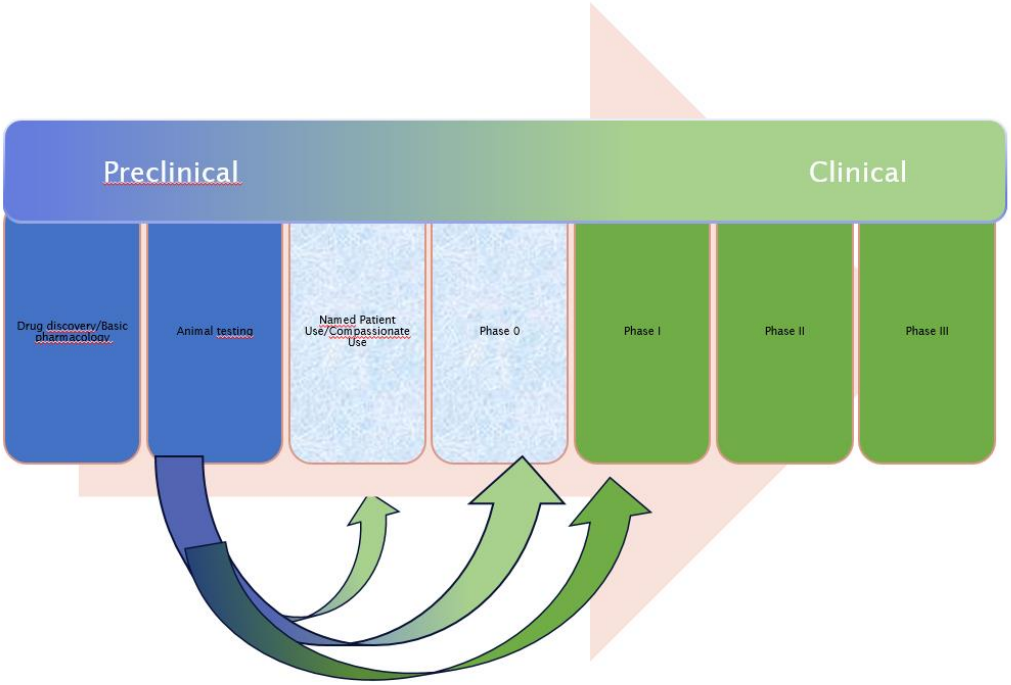


Figure 15 Experimental radiopharmaceuticals in the clinic; named patient use is regulated in §8 AMG (Arzneimittelgesetz), compassionate use is regulated in §8(a)AMG.(42)

Retrospective analyses of named patient use applications provide early safety and efficacy signals ahead of proper clinical development. For example, a variety of radiopharmaceuticals for [^{225}Ac]Ac-PSMA therapy is currently subject to clinical trials. On the other hand, a recent multicentric, retrospective study already included 488 patients, providing preliminary safety and efficacy signals within the limitations of retrospective studies.(43)

1.8 The role of radiopharmaceuticals in clinical trials

In clinical trials are either developed as genuine therapeutic or diagnostic agents or diagnostic agents function as auxiliary medicinal products (AxMPs) to assess study endpoints for development of a therapeutic pharmaceutical.(44)

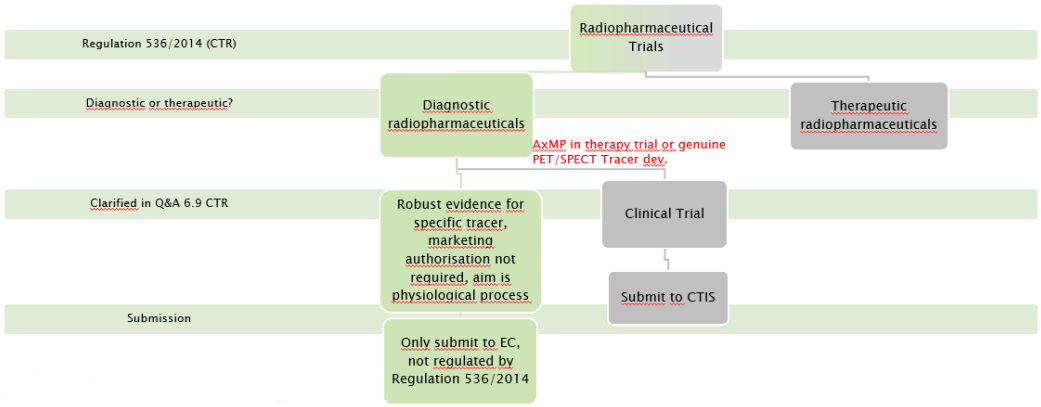


Figure 16 Radiopharmaceuticals in clinical trials: decision tree between clinical trials, other studies and regulatory implications

1.8.1 Clinical development of diagnostic radiopharmaceuticals

In comparison to regular pharmaceuticals, radiopharmaceuticals are used for diagnostic purposes in great measure. Due to low molar amounts of the injected substances, often onetime use and low pharmacological activity, side effects are rare and often associated with contrast agents injected during image acquisition or excipients and of allergic nature. Systematic reviews show a median frequency of adverse events during the use of diagnostic radiopharmaceuticals ranging from 0.0016-0.0019%. This is much lower than 1-2% of reported adverse events in conventional therapeutic drugs.(45)

Energy dose deposition to organs is usually below the threshold for deterministic radiation injuries and therefore of stochastic nature, increasing the chance to develop cancer with the cumulative effective dose. For subjects without an expected immediate benefit, e.g. healthy probands in a clinical trial, 10- year effective dose may not exceed 30 mSv in Austria. This corresponds to roughly 1-2 whole body [^{18}F]FDG-PET/CT scans (20.0 ± 5.6 mSv per scan), with the majority of radiation dose mediated by the CT component. For patients with an expected benefit, although not further clarified how this is determined, radiation exposure must be taken into consideration beforehand. However, this is a general requirement in medical application of ionizing radiation. (46, 47)

Clinical development of a diagnostic radiopharmaceutical shall now be explained at the example of [^{89}Zr]Zr-Girentuximab, a PET tracer. It is currently awaiting FDA approval decision and is used under named patient use at the University Clinic Vienna, Division of Nuclear Medicine.

The aim in phase 1 of diagnostic trials is to obtain pharmacokinetic data as well as initial safety data and testing of various mass doses of the agent. In the phase I [^{89}Zr]Zr-Girentuximab trial, patients were randomised to receive either 5mg or 10 mg of the tracer. Notably, the amount of radioactivity was kept constant between groups. In radiopharmaceuticals, increasing the dose of non-radiolabelled precursor can lead to an improved safety profile, due to blocking effects on off- target binding sites. A slight effect was seen in this trial.(48)

Among several efficacy parameters for diagnostic methods that are described, sensitivity and specificity are used most frequently. These key parameters require a standard of truth. If such is not available, efficacy might be measured according to the impact on patient management or improved clinical outcome. (49)

Positive predictive value (PPV) and negative predictive value (NPV) are also frequently used.

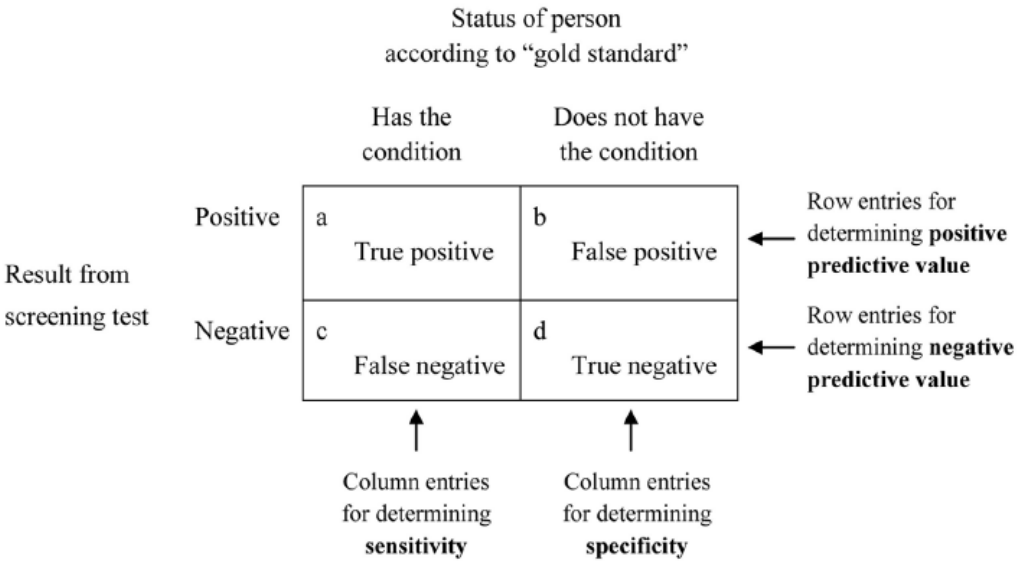


Figure 17 Key diagnostic performance parameters, taken from(50)

Sensitivity describes "*The proportion of correctly classified participants among those with the target condition*" calculated via the following formula:

$$\left[\frac{a}{a+c}\right] * 100$$

It is therefore the proportion of correctly detected events among all individuals experiencing the event, e.g. renal cancer is detected by a PET imaging method in a cohort of 15 patients. 5/15 patients were detected as positive by PET, whereas 10 patients were detected as positive by an established reference standard. Therefore sensitivity is 33%. In simpler words: The capability of detection of a present event. A low sensitivity means a higher rate of undetected events.

Specificity describes "*The proportion of correctly classified participants among those without the target condition*" calculated via the following formula:

$$\left[\frac{d}{b+d}\right] * 100$$

It is therefore the proportion of patients that were accurately determined as not experiencing an event among all patients who did not experience the event. If 5/15 patients were detected as negative by PET, whereas 10 patients were determined as negative by an established reference standard, specificity is 33%. In simpler words: The capability of correct nondetection of an absent event. A lower specificity means a higher rate of false positive measurement results.

PPV is the probability of people with a positive result to have the respective condition.

$$\left[\frac{a}{a+b}\right] * 100$$

NPV is the probability of absence of a condition in people with a negative result.

$$\left[\frac{d}{c+d}\right] * 100$$

To sum it up, sensitivity and specificity indicate a test`s performance with reference to a standard method, whereas NPV and PPV indicate the probability of an individual patient to have or have not the condition, based on his test result.(50)

1.9 ZIRCON Phase III trial

ZIRCON was a recently finished, multicenter prospective, randomized trial to evaluate [^{89}Zr]Zr-Girentuximab, a carboanhydrase IX (CAIX) directed antibody in comparison to histopathological diagnosis after partial or total nephrectomy in patients with indeterminate renal masses ≤7cm, suspicious for renal clear cell carcinoma.

Among three readers, mean sensitivity, specificity, PPV and NPV irrespective of tumor mass subgroups are displayed in table 1.

Table 1 ZIRCON trial results, data from(37)

Parameter	Mean (95%CI)
Sensitivity	85·5% (81·5–89·6)
Specificity	87·0% (81·0–93·1)
Positive Predictive Value	92·9% (90·2–95·7)
Negative Predictive Value	75·2% (71·2–79·3)

Notably, all PET positive lesions were malignant, with papillary carcinoma as the most prevalent histology subtype (8/11 false positives).

As discussed above, false positives influence specificity and PPV. In the real world setting, knowing that all PSMA PET positive patients had a form of kidney cancer, with a 92.9% probability of having clear cell renal cell carcinoma and 100% probability of having a malignant kidney lesion, the overall added value would probably increase depending on the specific management of the renal cancer subtypes leading to false positive results, in particular, papillary carcinoma. The NPV of 75.2% and sensitivity of 85.5% reflect the presence of false negative results. The authors suggested low CAIX expression as the probable cause.

1.10 Influence of performance parameters of radiopharmaceutical AxMPs on study designs:

Several factors influence statistical trial planning, in particular selected power, significance level, effect size, expected attrition and measurement error (Figure Y).

Changes in response between groups can be measured by PET imaging. The most practical and clinically used method is the measurement of the standard uptake value (SUV) based on a single time point PET image, while dynamic imaging including full kinetics analysis allows better differentiation between background and actual tumor uptake. For example, in a cohort of breast cancer patients undergoing neoadjuvant chemotherapy, which was divided into tertiles based on the Baseline SUV and subsequently correlated with measurements based on dynamic [^{18}F]FDG imaging, the correlation between SUV and dynamic [^{18}F]FDG flux as response parameters was lowest for the low SUV group and highest for the high SUV group (Figure 18). Therefore, sensitivity of static [^{18}F]FDG imaging is dependent on the initial SUV and must therefore be considered in clinical trial planning, to prevent misinterpretation of treatment efficacy.(51)

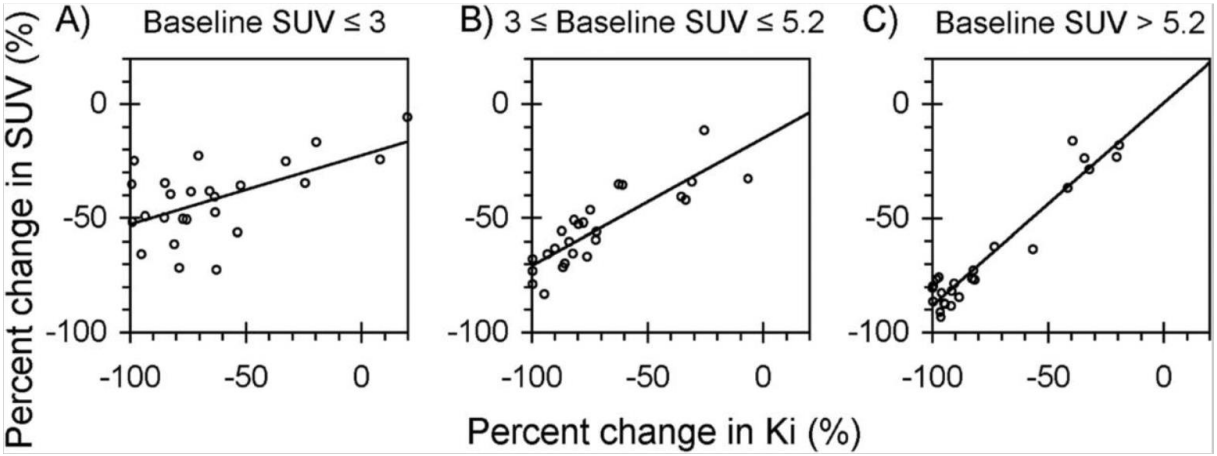


Figure 18 Increasing correlation between static imaging (SUV) results and [18F]FDG flux (Ki) results from dynamic imaging dependent on Baseline SUV; in Baseline SUV ≤3, a -100% change in FDG flux corresponded to a -52% SUV change, in 3 ≤ baseline SUV ≤ 5.2 -100% change in FDG flux corresponded to -71% SUV change and in the Baseline SUV >5.2 cohort -100% FDG flux corresponded to -88% change in SUV.(51)

Another important parameter to consider is measurement error, which reflects inter- observer variability, usage of different scanners and scanning protocols and other parameters, which influence the power of PET imaging as a surrogate marker. For example, in the 3≤SUVbaseline≤5.2 group, a measurement error of 20% (e.g. multi-center study) and a sample size of 100 yields a power of 94%, whereas the same cohort and a measurement error of 40% due to poor standardization of image protocols and other factors would require the triple amount of patients to achieve a power of 86% (Figure 19).(51)

Trial Scenario	N	Power			
		1st tertile $SUV_{baseline}$≤3 50% sens.*	2nd tertile 3≤$SUV_{baseline}$≤5.2 70% sens.*	3rd tertile $SUV_{baseline}$>5.2 90% sens.*	kinetic modeling 100% sens.*
Single site, $\sigma^{\pm}$ =10%	20	61%	88%	98%	99%
	30	78%	97%	99%	99%
Multi-center, $\sigma^{\pm}$ =20%	50	42%	70%	89%	94%
	100	71%	94%	99%	99%
Muiti-center, $\sigma^{\pm}$ =40%	100	24%	42%	61%	71%
	300	58%	86%	97%	99%

Figure 19 Influence of SUV baseline and measurement error on power; based on 20% true percentage point change in [^{18}F]FDG uptake(51)

1.11 Planning of a radiopharmaceutical clinical research center

A radiopharmaceutical production site is limited to the radionuclides permitted for use in the respective area. As opposed to an industrial radio pharmacy site, the overall radiation burden in an academic site will rather be shared between a big variety of radionuclides to attract as many cooperation opportunities throughout industry partners as possible, instead of focusing on a small number of products with large batch sizes for large scale production. Further, radiation protection law requires the definition of maximum activity per room or location at a single time point and the weekly activity-time-product expressed to check the validity of the proposed radiation protection concept. Amendments to the approved list of radionuclides are possible but time- consuming. Therefore, an important aspect of site planning is the definition of a set of radionuclides that reflects most of current and future industry demands as well as academic research interests.

A variety of physical and chemical properties are critical for the choice of radionuclides: half- life, decay type, decay energy, radiochemistry, feasibility and route of radionuclide production, commercial and academic awareness and availability of true theranostic pairs (Table 2).

Table 2 Considerations for the choice of radionuclides(9, 52, 53)

Parameter	Implications
Half- life	Therapeutic: must match biokinetics of the vector (small molecule, antibody fragment, …) to optimise energy dose deposition in target tissue; Diagnostic: Matching kinetics allow high imaging contrast; logistics: longer half- life allows farther distribution after central production and less time pressure while handling, regardless of production
Decay type	Imaging and/or therapy
Decay energy	Image resolution, therapeutic efficacy, off target dose eliciting side effects
Radiochemistry	Easy and robust radiolabelling, preparation of easy-to-use radiopharmaceutical kits
Route of radionuclide production	Commercial availability, supply stability, decentralized production possibility
Commercial and academic awareness	Generates high request, opportunity for cooperations
Availability of true theranostic pairs	Predictability of biodistribution and therapeutic effects via imaging ; “you see what you treat”

2. Methods

Thorough literature research on PubMed, Web of Science and Scopus. Further, EANM and IAEA (International Atomic Energy Agency) documents were taken into account where useful to identify radionuclides of importance for future clinical development efforts. Further, roundtables with experts from various professions were held to discuss feasibility and prioritization of the choice of radionuclides.

3. Results and Discussion

The following consensus list of radionuclides was defined as basis for our new radiopharmaceutical trial center. In the following section, use cases will be discussed and explained according to the interplay between physical/chemical properties, production routes, regulatory and scientific status and nuclide supply. An overview of the current industry landscape regarding the development of radiopharmaceuticals based on each radionuclide will be given.

Table 3 List of radionuclides, data obtained from various sources (1-8)(54, 55)

Radionuclide	Production in clinical cyclotron (y/n)	Symbol	T1//2 (h)	Primary intended use	Particle relevant for primary intended use	Energy of relevant emission (kEv(particle nature))	Radioactive Progeny	Stable progeny	EU-approved radionuclide available (as generator or precursor)
Carbon-11	y	^{11}C	20.4	PET	β^+	960	none	^{11}B	no
Fluorine-18	y	^{18}F	109.8	PET	β^+	690	none	^{18}O	no
Copper-64	?	^{64}Cu	12.7	PET	β^+	653	none	^{64}Ni/^{64}Zn	yes
Gallium-68	y	^{68}Ga	68.0	PET	β^+	1900 (β^+)	none	^{68}Zn	yes
Zirconium-89	y	^{89}Zr	78.4	PET	β^+	396(β^+); 909(γ)	^{89m}Y ($t_{1/2}$= 15s)	^{89}Y	no
Lutetium-177	n	^{177}Lu	6.7	Therapy	β^-	497 keV (β^-) 384 keV (β^-) 176 keV (β^-) 113 keV (γ) 208 keV (γ)	none	^{177}Hf	yes
Yttrium-90	n	^{90}Y	64.1	Therapy	β^-	2281 (β^-)	none	^{90}Zr	yes
Copper-67	?	^{67}Cu	61.9	Therapy	β^-	182-576 (β^-)	none	^{67}Zn	no

Copper-61	y	^{61}Cu	3.33	PET	β^+	560-1216 (β^+)	none	^{61}Ni	no
Actinium-225	n	^{225}Ac	237.6	Therapy	α	5800-8400 (α)	^{221}Fr, ^{217}At, ^{213}Bi, ^{213}Po, ^{209}Pb	^{209}Bi	no
Lead-212	n	^{212}Pb	10.64	Therapy	α	6200/8900(α)	^{212}Bi, ^{208}Tl, ^{212}Po	^{208}Pb	no
Lead-203									
Terbium-161		^{161}Tb	165	Therapy	β^-/Auger	154 (β^-)/3-50 (Auger/conversion)	none	^{161}Dy	no
Terbium-149	?	^{149}Tb	4.1	Therapy	α / β^+	3967 (α)/730(β^+)	^{145}Eu, ^{149}Gd,^{145}Sm,^{14} ^{9}Eu, ^{145}Pm	^{149}Sm, ^{145}Nd,^{1} ^{141}Pr	no
Terbium-152	?	^{152}Tb	17.5	PET	β^+	1140 (β^+)	none	^{152}Gd	no

3.1 Rationale for the choice of PET radionuclides

3.1.1 ^{11}C

^{11}C is a short- lived radionuclide for on- site radiolabelling after cyclotron production. It is one of the main PET radionuclides in academic centres with an on- site cyclotron, but of no use for industrial development due to its very short half- life. At the Medical University of Vienna, ^{11}C-labelled radiotracers were primarily used for psychiatric studies throughout the last years. Besides its use for PET imaging as a diagnostic tool, ^{11}C labelled tracers are also frequently used for exploratory clinical (Phase 0) trials in conventional drug development, e.g. early pharmacokinetic studies, since substituting a ^{12}C atom with its ^{11}C isotope allows radiolabeling without alteration of the investigated substance`s structure. Radiosynthesis of ^{11}C-labelled conventional pharmaceuticals under GMP could be a company requirement in Phase 0 trials and therefore is included in the approval request.

3.1.2 ^{18}F

With a half-life of 110 minutes, ^{18}F-labelled tracers are suitable for wider distribution. Further, a positron released after ^{18}F decay travels a relatively short path length, due to low positron emission energy and therefore allow high resolutions of PET images due to the annihilation radiation generated closely to the site of origin (e.g. a tumor). Currently, no ^{18}F radionuclide precursor is approved and therefore, the use in clinical trials is limited to either approved, industry produced ^{18}F-labelled tracers or to facilities equipped with a cyclotron for in- house labelling.

3.1.3 ^{68}Ga

Due to its widespread availability as approved ^{68}Ge/^{68}Ga generator or by on-site cyclotron production and well established radiolabelling chemistry, ^{68}Ga is one of the main radionuclides used for companion diagnostics of established radioligand therapies, especially with ^{177}Lu- or ^{225}Ac-labelled radiopharmaceuticals, sometimes using the same precursor for PET imaging and therapy (e.g. [^{68}Ga]Ga-PSMA$^{I\&T}$ and [^{177}Lu]Lu-PSMA$^{I\&T}$).

3.2 Radionuclides for ImmunoPET

3.2.1 ^{89}Zr

One of the main principles in effective PET tracer design is the matching of the physical half-life of the radionuclide with the pharmacokinetic half- life of the precursor. In antibody- based imaging (ImmunoPET), antibodies or fragments thereof are radiolabelled. ^{89}Zr is a main radionuclide for antibody- based PET imaging, due to its relatively long half- life of 3 days which matches the long biological half- life and slow distribution kinetics of many antibodies. One practical example would be ^{89}Zr-DFO-Girentuximab, a CAIX targeting antibody. After application of ^{89}Zr-DFO-Girentuximab, a waiting time from injection until image acquisition of 5±2 days is necessary due to slow distribution kinetics. Many other established PET nuclides would have decayed by that time. Advantageous decay characteristics of ^{89}Zr versus its competitor ^{124}I are displayed in Table X.(37, 56, 57)

Table 4 Comparison between radionuclide properties of ^{89}Zr and ^{124}I

Parameter	Advantage vs ^{124}I
Positron energy	396 kEv positron (^{89}Zr) versus 687 kEv and 974 kEv positrons (^{124}I) allows higher image resolution
Gamma energies	^{124}I gamma lines (100-150 kEv) interfere with 511 kEv annihilation radiation detection

Currently, no ^{89}Zr solution for radiolabelling is approved in the EU and the current strategy for future supply is the acquisition of cyclotron facilities and immediate radiolabelling after production. (58)

^{89}Zr is produced via proton irradiation of ^{89}Y (=natural Y, 100% abundance) solid phase targets via the ^{89}Y(p,n)^{89}Zr reaction. Targets either consist of Yttrium foil or Niobium coins coated with Yttrium. Possible irradiation byproducts ^{88}Zr (via the ^{89}Y(p,2n)^{88}Zr reaction) and ^{88}Y (via the ^{89}Y(p,pn)^{88}Y) may form above their energy threshold of 13.3 MeV. Therefore, it is important to keep the proton beam energy under 13 MeV (Figure 21). (59)

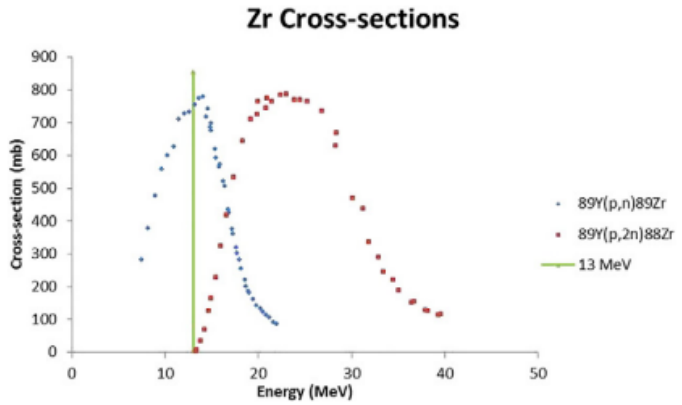


Figure 20 Cross- section values of ^{89}Zr and ^{88}Zr formation dependent on proton beam energy(60)

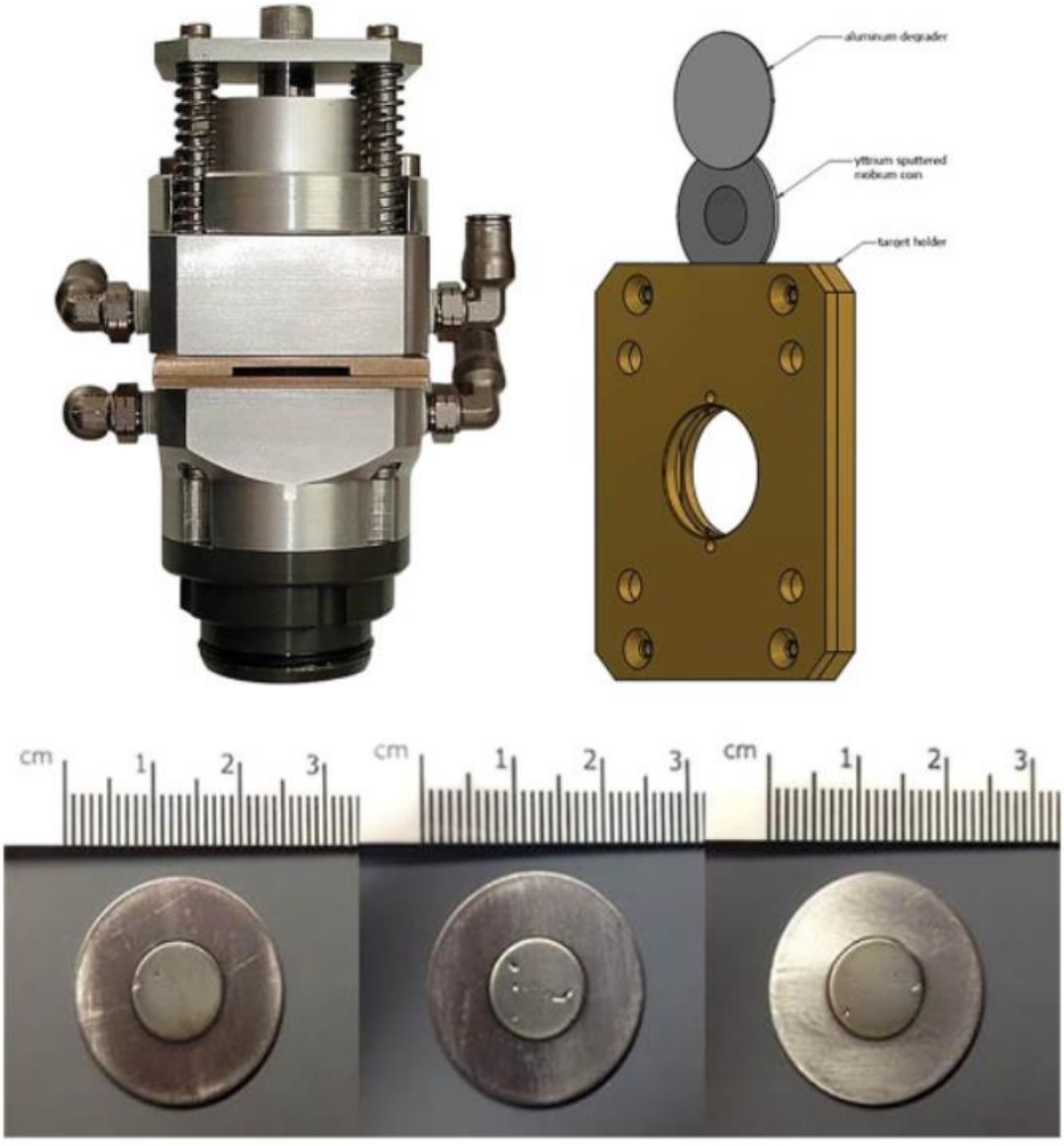


Figure 21 Coin target holder (top left), target assembly (top right, aluminum degrader used for beam energy reduction) and ^{89}Y sputtered target coins (bottom), taken from(60)

After irradiation, at first ^{89}Zr is dissolved from the solid target with (concentrated) HCl and applied on a hydroxamate resin. This resin preferably traps Zr(IV), Ti(IV) and Nb(V) over Y(III), Sc(III) and Fe(III). Rinsing the hydroxamate resin with 2 M HCl and water further decreases the amount of 89Y and Fe(III). This allows effective separation of ^{89}Zr from ^{89}Y and coproduced radioisotopes during irradiation, e.g. ^{52}Mn, ^{54}Mn, ^{56}Co, ^{65}Zn, ^{67}Ga, ^{96}Tc and ^{48}V. Subsequently, ^{89}Zr is eluted with oxalic acid over a sterile filter into the product/labelling vial.

Control of radionuclidic identity and purity is performed by high- purity germanium detectors, by determining the absence of characteristic gamma signals of potential impurities (Figure 23). Nonradioactive metal contaminants are measured by ICP-MS after decay of ^{89}Zr and therefore is only possible due to parametric release. (59, 61, 62)

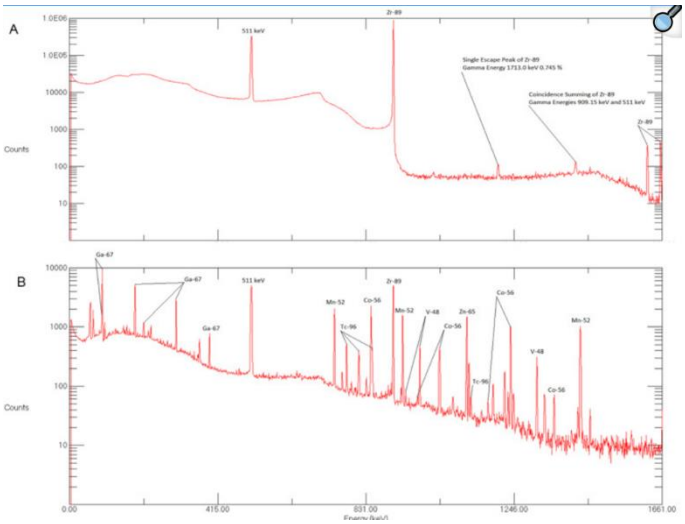


Figure 22 A: HPGe spectrum of hydroxamate- resin- purified ^{89}Zr 8 hours after EOB (short- lived contaminants decayed); B: HPGe spectrum of contaminants separated by hydroxamate resin, found in the waste. Taken from(59)

3.2.2 ^{61}Cu

^{61}Cu has advantageous decay characteristics in comparison to the more established ^{68}Ga and ^{18}F (Table 5). A variety of production paths have been described, either via the ^{nat}Ni(d,x)^{61}Cu, ^{60}Ni(d,n)^{61}Cu, ^{61}Ni(p,n)^{61}Cu, ^{64}Zn(p,α)^{61}Cu or ^{64}Zn(p, α)^{61}Cu reaction have been developed.(55, 63)

Table 5 Advantageous characteristics of ^{61}Cu

Parameter	Advantage vs ^{18}F and ^{68}Ga
Half- life	Longer $t_{1/2}$ enables distribution to remote sites; later time- point imaging after injection possible, increases contrast
True theranostic pair	Yes, with therapeutic ^{67}Cu; Ga or F isotopes have no true theranostic pairs
Chemistry	Advantage of straight forward chelator chemistry versus multi- step ^{18}F radiosyntheses

3.2.3 ^{64}Cu

^{64}Cu has a relatively long half- life of 12.7h. With its mixed β^+/ β^- emissions, it is theoretically suited for therapy and PET imaging but mostly investigated as a PET radionuclide or as a diagnostic companion in ^{67}Cu-based therapy. Potential applications include the radiolabelling of antibody fragments due to their relatively faster kinetics in comparison with intact antibodies, which would rather be labelled with longer- lived ^{89}Zr. Besides ^{68}Ga, it is the only commercially available, approved radionuclide for radiolabelling of PET tracers. Cyclotron production is possible with similar methods as ^{61}Cu (25, 64)

3.2.4 ^{152}Tb

Due to its long half-life, ^{152}Tb is theoretically suited for radiolabelling of precursors with slow biokinetics, such as albumin binders or antibodies. Together with the therapeutic $^{149/161}$Tb isotopes, it forms a true theranostic pair. Due to their favorable properties, Terbium isotopes are expected to gather major interest for future nuclear medicine applications.(65)

3.3 β- therapy nuclides

3.3.1 ^{90}Y

^{90}Y has been used for many decades for radioligand therapy. ^{90}Y has suitable half- life for slow antibody kinetics. Further, it emits high energy electrons with a maximum range of 2cm in soft tissue, suitable for treatment of bulky metastases. However, only one ^{90}Y labelled antibody (Zevalin®) for treatment of lymphoma was approved throughout this time, but its marketing authorisation in the EU finally lapsed in 2024, after cessation of distribution due to non- medical reasons. Other ^{90}Y-labelled antibodies for the treatment of lymphoma and leukaemia are under investigation in clinical trials.(66-69)

3.3.2 ^{177}Lu

Throughout the last years, ^{177}Lu has become the main radionuclide for targeted radioligand therapy applications. ^{177}Lu has a significantly lower β^- decay energy and resulting path length compared to those of ^{90}Y and is therefore believed to be more suitable for smaller metastases. The acquisition of Endocyte and AAA by Novartis and subsequent marketing of Pluvicto®and Lutathera® are considered major milestones in the development of radioligand therapy. Further, a variety of approved radionuclide precursors for radiolabelling are available (e.g. Endolucin Beta®) and worldwide supply with this radionuclide is established. (70)

3.3.3 ^{67}Cu

^{67}Cu has similar decay characteristics as ^{177}Lu but a shorter half- life. It is a potential candidate for similar indications, such as peptide- receptor radiotherapy (PRRT) or radioimmunotherapy and has been investigated in a few recent trials. It can be produced in cyclotron and therefore may spread the risk of sudden radionuclide shortages, e.g. due to ^{177}Lu reactor shutdowns. (71, 72)

3.3.4 ^{161}Tb

^{161}Tb also shows similar decay characteristics as ^{177}Lu but emits additional conversion and Auger-Meitner electrons. Therefore, a higher energy deposition especially in small tumors is expected to occur. Direct preclinical comparisons of a folate precursor labelled with either ^{161}Tb or ^{177}Lu showed superiority of the ^{161}Tb compound.(73)

3.4 α therapy nuclides

3.4.1 ^{225}Ac

One of the most promising radionuclides to overcome radiation resistance to medium-energy electrons from ^{177}Lu decay is ^{225}Ac. With a half-life of 9.92 d, it decays via a decay chain, under the emission of a net number of 4 a-particles with an average energy of 6.7 MeV and 2 β^- particles. The emitted a-particles travel only a few cell diameters while depositing about 80 keV/mm of tissue.

There, a-particles cause lethal, irreparable double-strand breaks. The shorter pathlength of a-particles may also protect surrounding healthy tissue and therefore spare bone marrow reserves in disseminated disease. (26, 74)

3.4.2 ^{212}Pb

^{212}Pb is the leading candidate for future alpha therapy applications apart from ^{225}Ac. It also decays via a decay chain (Figure 26) and in comparison with ^{225}Ac, it has a considerably shorter $t_{1/2}$ of 10.64h. As opposed to 225Ac, the first decay event is emission of a β^- particle, which could potentially lead to better dose deposition due to retention of the daughter nuclide ^{212}Bi in the chelator, until the first α particle emission by ^{208}Tl or ^{212}Po. (75)

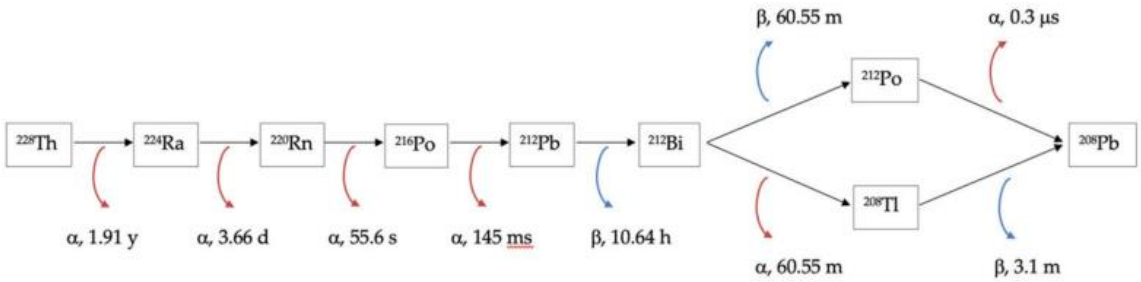


Figure 23 ^{212}Pb decay chain, taken from(75)

3.4.3 ^{149}Tb

This terbium isotope is unique due to its alpha emissions, short half- life and additional positron emission. Therefore, it is suitable for the radiolabelling of small molecules with fast biodistribution and clearance and enables additional PET imaging. Theoretically, this could be economically effective due to reduced restaging PET scans, as a 2 in 1 solution. As opposed to ^{225}Ac and ^{212}Pb, its daughters decay without alpha emissions and therefore less recoil related radiation doses to off- target organs are expected. However, multiple radioactive daughters are formed in the decay chain of 149Tb and may cause issues regarding radiation protection as well as radiotoxicity.(76)

4. Conclusion

The unique characteristics of radiopharmaceuticals influence every stage of drug development and the entire product life cycle. These include time-critical production, the effects of ionizing radiation, environmental considerations, and the rapid emergence of novel radionuclides that require state-of-the-art technologies to accurately assess their clinical and pharmaceutical properties. The approval of Pluvicto® and Lutathera® marked the beginning of a new era in nuclear medicine and radiopharmaceutical drug development. Continuous education, training, and technological advancement are essential to keep pace with this rapidly evolving field.

List of Tables

List of Figures

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