

## Review Article

# Targeted $\alpha$ -therapy of prostate cancer using radiolabeled PSMA inhibitors: a game changer in nuclear medicine

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**Abstract:** Prostate cancer (PCa) is one of the most common malignancies in men and is a major contributor to cancer related deaths worldwide. Metastatic spread and disease progression under androgen deprivation therapy signify the onset of metastatic castration resistant prostate cancer (mCRPCa)-the lethal form of the disease, which severely deteriorates the quality of life of patients. Over the last decade, tremendous progress has been made toward identifying appropriate molecular targets that could enable efficient *in vivo* targeting for non-invasive imaging and therapy of mCRPCa. In this context, a promising enzymatic target is prostate specific membrane antigen (PSMA), which is overexpressed on PCa cells, in proportion to the stage and grade of the tumor progression. This is especially relevant for mCRPCa, which has significant overexpression of PSMA. For therapy of mCRPCa, several nuclear medicine clinics all over the world have confirmed that <sup>177</sup>Lu-labeled-PSMA enzyme inhibitors (<sup>177</sup>Lu-PSMA-617 and <sup>177</sup>Lu-PSMA I&T) have a favorable dosimetry and convincing therapeutic response. However, ~30% of patients were found to be short or non-responders and dose escalation was severely limited by chronic hematological toxicity. Such limitations could be better overcome by targeted alpha therapy (TAT) which has the potential to bring a paradigm shift in treatment of mCRPCa patients. This concise review presents an overview of the successes and challenges currently faced in TAT of mCRPCa using radiolabeled PSMA inhibitors. The preclinical and clinical data reported to date are quite promising, and it is expected that this therapeutic modality will play a pivotal role in advanced stage PCa management in the foreseeable future.

**Keywords:** <sup>225</sup>Ac, <sup>211</sup>At, <sup>213</sup>Bi, metastasis, prostate cancer, PSMA, targeted alpha therapy

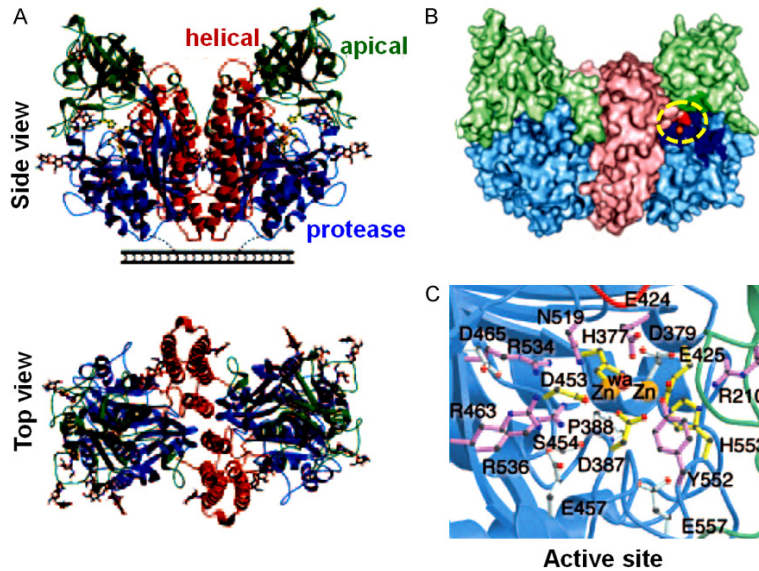
## Introduction

Prostate cancer (PCa) is one of the most common non-cutaneous malignancies in men worldwide, and its incidence has increased substantially in recent years [1-6]. In the United States alone, more than 40,000 men die from PCa every year [7]. The incidence of prostate cancer increases proportionally with age: published post-autopsy data illustrate an incidence of ~12% in the 60-69 year old male age group, which increases to a value of ~48% in 80-89 year old men [8-10]. There is a high probability that cases may be under diagnosed, especially in developing countries, and the numbers are likely to be much higher than reported. Interestingly, incidence of prostate cancer also seems to be dependent on demographics and

racial prevalence, highlighting the influence of environmental factors on occurrence of the disease [11]. PCa has a low incidence in Asia (3-8 per 100,000 men per year), an intermediate incidence in Africa and Eastern Europe, and higher incidence in Western Europe and North America [11, 12]. In many countries, there are clinical recommendations for carrying out PCa screening by using the prostate specific antigen (PSA) test [8]. However, the harms caused by overdiagnosis and overtreatment must also be considered while adhering to these recommendations.

Conventional treatment options for localized PCa include surgery, chemotherapy, cryotherapy, brachytherapy with radioactive seeds, and the use of external radiation which is now being

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**Figure 1.** Structure of PSMA. A. Ribbon diagrams of side and top view of PSMA. B. A surface rendering in which the apical domain is light green, the helical domain is light red, the protease domain is light blue, and zinc ions are orange. The residues facing the substrate-binding cavity are indicated in a darker version of the color matching to the domain from which the residue derives. PSMA active site is encircled. C. Stereoview of the PSMA active site. Zinc ions are orange spheres, and a water molecule is shown as a red sphere. Zinc binding residues are yellow sticks, water- or substrate-binding ligands are purple sticks, and residues with structural roles are light blue sticks. Adapted from Ref. [39] with permission. Copyright 2005 National Academy of Sciences.

aggressively promoted with the use of proton therapy machines using conformal targeting technologies [2, 10, 13-18]. The choice of therapeutic modality is based on the individual cancer characteristics, evaluating histopathology, serum PSA, Gleason score, comorbidity and life expectancy. As such, PCa is a highly assorted disease and it can be heterogeneous in the prostate of the same patient [19, 20]. To optimize therapeutic outcome, especially in high-risk PCa patients, treatment for PCa is moving rapidly toward personalization. Molecular imaging plays an important role in personalized cancer management, as it aims to deliver patient-specific, targeted treatment at the appropriate time [3, 21-25]. Thanks to recent developments in radiopharmaceuticals chemistry and imaging technologies, the role of molecular nuclear medicine for diagnosis, as well as therapy, of prostate cancer is expected to increase significantly in the future [26-33].

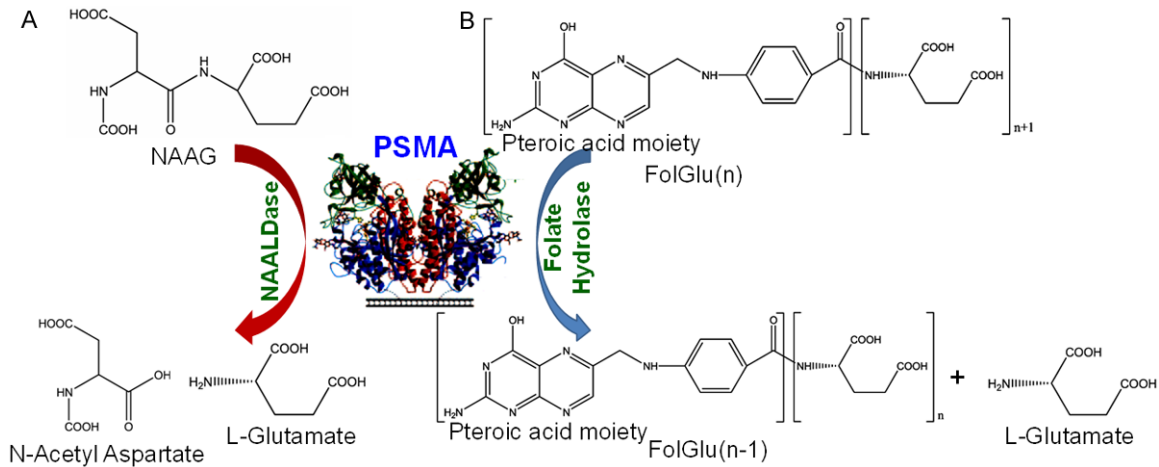
There are several mechanisms by which a radiopharmaceutical may accumulate in cancerous lesions. An enzyme-substrate reaction

taking place at the cellular level with prostate specific membrane antigen (PSMA) targeting enzyme inhibitors is a recently explored strategy that can be utilized for the development of radiopharmaceuticals for imaging and therapy of PCa [10, 34]. PSMA is highly expressed in all types of PCa, and the expression increases with tumor aggressiveness, metastatic disease, and recurrence [34]. Using suitably radiolabeled PSMA inhibitors, PCa can be accurately visualized, characterized and optimally treated according to tumor biology, patient preferences, and survivorship goals [35, 36]. Radiolabeled PSMA inhibitor is the first class of radiopharmaceutical based on an enzyme inhibitor as the targeting agent, with a cellular enzyme target [10]. The name PSMA is somewhat of a misnomer as it is also expressed in the vasculature of other tumors including, bladder, pancreas, lung, and kidney carcinomas [10, 37]. Nevertheless, there has been overwhelming interest in use of radiolabeled analogues of several PSMA inhibitors for their high potential in diagnosis and therapy of PCa, and studies indicate that such agents are quite effective as new clinical options in PCa management [36]. The aim of this review is to provide an overview of the recent advances in targeted  $\alpha$ -therapy of PCa using radiolabeled PSMA inhibitors which is poised to be a game changer in therapeutic nuclear medicine.

### PSMA as an enzymatic target for developing radiopharmaceuticals for prostate cancer

PSMA, also known as folate hydrolase I, glutamate carboxypeptidase II, N-acetyl-L-aspartyl-L-glutamate peptidase I (NAALDase I) or N-acetyl-L-aspartyl-L-glutamate (NAAG) peptidase, shows several biological features which make it an ideal target structure for radiopharmaceutical development [10, 38]. Basically, PSMA is a zinc containing metalloenzyme, having a molecular weight of 100-104 kDa [10].

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**Figure 2.** The enzymatic action of PSMA. A. N-Acetyl-L-aspartyl-L-glutamate (NAAG) is hydrolyzed to aspartate and glutamate. B. Glutamic acid is released from folate polyglutamate resulting in the release of folic acid. After successive release of glutamate, folate is released. Adapted from Ref. [10] with permission. Copyright 2016 Elsevier.

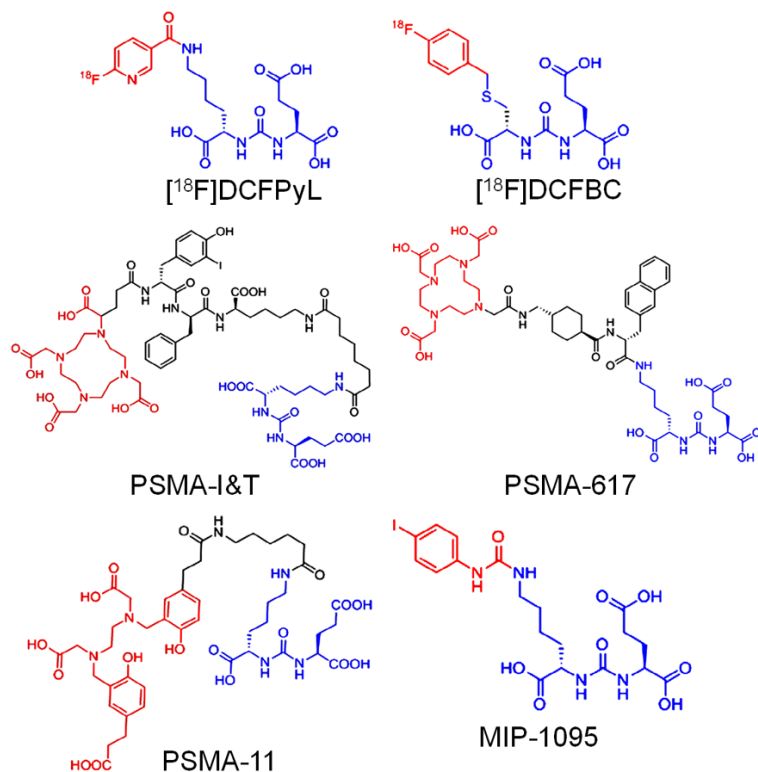
The PSMA structure reveals a symmetric dimer with each polypeptide chain containing three domains: a protease domain (residues 56-116 and 352-591), an apical domain (residues 117-351), and a helical domain (residues 592-750) (Figure 1A) [39]. A large cavity ( $\sim 1,100 \text{ \AA}^2$ ) exists at the periphery of the three domains and this includes a binuclear zinc site (Figure 1B) [39]. Two zinc ions have been observed in this cavity, which has been identified as the substrate binding site (Figure 1C). As mentioned, PSMA shows significant overexpression on most prostate cancer cells, especially those which are in advanced stage, such as mPCa [10]. Several clinical studies have revealed a correlation between PSMA and the stage and grade of the cancer, showing enhanced PSMA levels with higher stages and grading [40-44].

In nuclear medicine practices, two different approaches have been used for targeting PSMA. The first approach takes advantage of the macromolecular protein structure of PSMA to provide specific monoclonal antibodies as targeting vectors [45]. The second approach relies on the enzymatic activity of PSMA and uses radiolabeled enzyme inhibitors or binding agents as target seeking agents [46]. Though both these approaches have been successfully demonstrated for the development of diagnostic and therapeutic radiopharmaceuticals for targeting prostate cancer, the second approach, which utilizes radiolabeled small molecules targeting the enzyme activity of PSMA, is gaining overwhelming popularity recently. This is

because smaller molecules show rapid blood clearance compared to monoclonal antibodies. This leads to a desirable higher target-to-non-target ratio. Additionally, with use of radiolabelled small molecules, after the ligand binds to its membrane-anchored target, internalization occurs via clathrin-coated pits and endocytosis [10]. This results in effective transportation of the bound molecule into the cells, ending up in the late endosomes, leading to increased tumor uptake and retention.

PSMA has two distinct enzyme activities: (a) folate hydrolase and (b) NAALDase, and in both cases the enzymatic role is to release the terminal glutamate residue from the substrate molecules (Figure 2) [10]. The NAALDase activity of PSMA (i.e. hydrolysis of the NAAG substrate to yield aspartate and glutamate) has been explored for the development of radiopharmaceuticals for imaging and therapy of PCa [10]. The NAAG substrate binds to PSMA in the extracellular portion of the enzyme [10]. However, NAAG substrate cannot be used as the targeting molecule for PCa because the substrate molecule gets converted into the product and will not be retained within the cells. Additionally, being small residues, NAAG substrates are likely to get released from the cells and hence will not concentrate in the cancerous lesions. Therefore, modified forms of NAALDase inhibitors mimicking the NAAG substrate were designed and used as PSMA targeting agents for PCa imaging and therapy [47, 48].

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**Figure 3.** Structures of representative urea-based PSMA ligands used in clinical context. Adapted from Ref. [50] with permission. Copyright 2017 Elsevier.

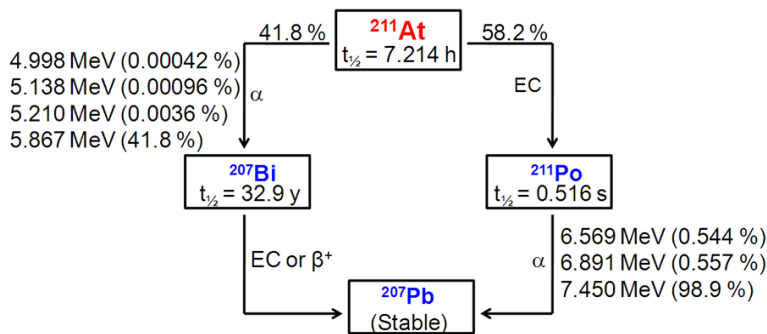
The PSMA ligands based on NAALDase inhibitors are classified into three groups: (a) phosphorous-based compounds (including phosphonate, phosphate, and phosphoramidate), (b) thiol, indole-thiol, hydroxamate and sulfonamide derivatives, and (c) urea based compounds [47, 48]. The phosphorus-based compounds were the first high affinity PSMA ligands with nanomolar inhibitory potency [10]. However, these compounds are highly polar and have a relatively poor pharmacokinetic profile, which limits their clinical application [10, 49]. Subsequently, thiol-based agents were considered a good alternative to phosphorus-containing molecules as they demonstrated enhanced membrane permeability and oral bioavailability [10, 49]. However, metabolic stability and selectivity of these compounds are not adequate to advance them into clinics. In order to overcome these limitations, a series of novel urea-based PSMA ligands has been developed which finds relevance in clinical context [47].

Generally, PSMA ligands consist of three components: (a) the binding motif, (b) a radiolabel

bearing moiety which can be a chelator or a prosthetic group and (c) a linker molecule that connects both binding motif and radiolabel bearing moiety and adjusts the lipophilicity of the agent [50-52]. Among various ligands reported to date, the class consisting of peptidomimetic, urea-based PSMA inhibitors has been most widely studied from clinical perspective (**Figure 3**) [27, 36, 47]. Over the last few years, the number of clinical studies using urea-based PSMA ligands, such as <sup>123/124/131</sup>I-MIP-1072/-1095, [53, 54] <sup>99m</sup>Tc-MIP-1404/-1405, [55] <sup>68</sup>Ga-PSMA-11, [56] [<sup>18</sup>F]-DCFBC, [57] [<sup>18</sup>F]-DCFPyL, [58] <sup>177</sup>Lu-PSMA617, [59] and <sup>177</sup>Lu-PSMA I&T [60] has exponentially increased. Among these agents, today, <sup>68</sup>Ga-PSMA-11 is the most prominent positron emission tomography (PET) radioligand for PET imaging of

PCa [61-66]. Aside from use in diagnostic imaging, radiolabeled PSMA ligands also have potential for use in radionuclide therapy of PCa. Radiolabeling of PSMA ligands with the <sup>131</sup>I, <sup>177</sup>Lu, <sup>90</sup>Y and <sup>188</sup>Re therapeutic radionuclides has also been attempted and results of clinical studies with <sup>177</sup>Lu and <sup>131</sup>I have been reported [10, 67-71]. From a clinical perspective, <sup>177</sup>Lu offers several advantages over <sup>131</sup>I, such as easier synthesis protocol and a lower proportion of  $\gamma$ -radiation, which can potentially result in reduced hospital admission times and lower toxicity compared to <sup>131</sup>I [50]. Therefore, the majority of recent studies have focused on the use of <sup>177</sup>Lu for PSMA-based radioligand therapies, concentrating mainly on efficacy evaluations of <sup>177</sup>Lu-PSMA-617 and <sup>177</sup>Lu-PSMA I&T [60, 72-75]. Since the focus of the present review is on targeted  $\alpha$ -therapy of PCa, for comprehensive discussion on positron emission tomography-computed tomography (PET-CT) imaging of PCa and targeted  $\beta^-$  therapy using <sup>177</sup>Lu labeled PSMA inhibitors, the readers are referred to recent reviews on these topics [24, 43, 44, 46, 76-84].

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**Figure 4.** Simplified decay scheme of  $^{211}\text{At}$ .

### Targeted $\alpha$ -therapy of PCa

Androgen deprivation is the mainstay of therapy for advanced PCa, and this treatment leads to PSA responses and clinical improvements in > 90% of patients [85]. However, this treatment is not curative, and despite initial responses, almost all patients progress to castration-resistant prostate cancer (CRPCa), which keeps growing even when testosterone body levels are very low [86]. Many early-stage prostate cancers need normal levels of testosterone to grow, but CRPCa does not [86]. In many cases, CRPCa demonstrates clinical metastases in bone and other organs of the body, significantly deteriorating the quality of life of patients. This condition is known as metastatic castration-resistant prostate cancer (mCRPCa) [87-89].

Several international nuclear medicine centers have used  $^{177}\text{Lu}$ -PSMA-617 as a potential radiopharmaceutical for therapy of mCRPCa [72, 79, 90-93]. These centers confirmed that  $^{177}\text{Lu}$ -PSMA-617 possesses a favorable dosimetry and shows convincing therapeutic response in terms of both serum PSA level and radiologic findings. However, ~30% of patients were found to be short or non-responders and dose escalation was severely limited by chronic hematological toxicity [94, 95]. In this premise, it has recently been demonstrated that targeted  $\alpha$ -therapy (TAT) with radiolabeled PSMA inhibitors could prevent radioresistance to  $\beta$ -emitters while simultaneously reducing hematological toxicity in PCa patients [96]. After binding at the tumor cell surface, radiolabeled PSMA inhibitors are internalized. This is particularly beneficial for short-range  $\alpha$ -particle radiation, and this is also important for radionuclides that decay into unstable daughter nuclides [97, 98]. In addition, the short tissue

range of  $\alpha$ -radiation offers the prospective of targeting tumor cells which are infiltrating bone marrow, with reduced toxicity compared to  $\beta$ -emitters [97, 98].

### Production of $\alpha$ -emitting radioisotopes for TAT of mCRPCa

The  $\alpha$ -emitting radioisotopes which have been utilized for radiolabeling PSMA inhibitors

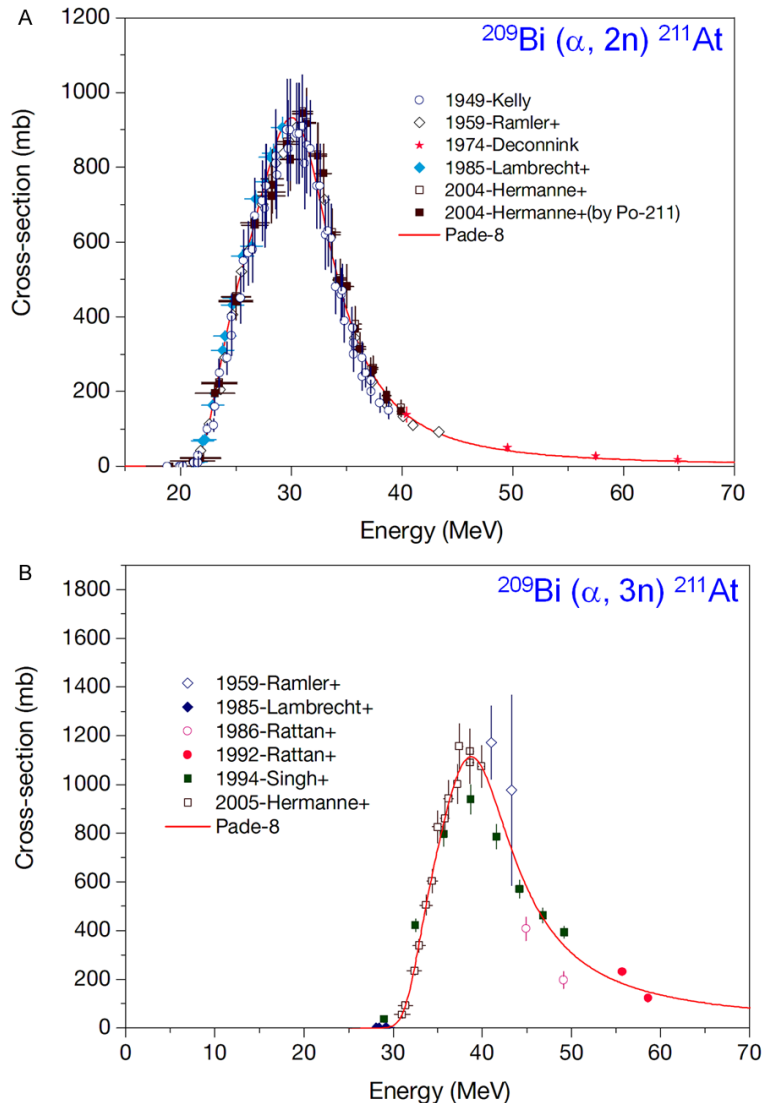
for TAT of mCRPCa are  $^{211}\text{At}$ ,  $^{225}\text{Ac}$  and  $^{213}\text{Bi}$ . The physical characteristics and production methodology of each of these radioisotopes are discussed in the following sections.

#### $^{211}\text{At}$

Astatine-211 is an ideal radionuclide for TAT due to its favorable nuclear decay characteristics [99]. The decay of  $^{211}\text{At}$  follows a branched decay scheme with a half-life of 7.21 hours (**Figure 4**). One branch leads to  $^{207}\text{Bi}$  by emission of an  $\alpha$ -particle. The radioisotope,  $^{207}\text{Bi}$ , decays with a half-life of 33.9 year to  $^{207}\text{Pb}$  via electron capture. The second decay branch occurs via electron capture and leads to formation of  $^{211}\text{Po}$ , which has a half life of 516 milliseconds. Polonium-211 in turn decays to stable  $^{207}\text{Pb}$  by emission of an  $\alpha$ -particle. The result of these two decay pathways is 100%  $\alpha$ -particle emission during the decay of  $^{211}\text{At}$  (5.87 and 7.45 MeV in 42% and 58% of the decays, respectively). A major concern with the clinical utilization of  $^{211}\text{At}$  is the presence of long-lived  $^{207}\text{Bi}$  ( $t_{1/2} = 32.9$  years), and potential for future health issues due to the uptake of this radionuclidic impurity in the bone, liver, and kidneys [100]. However, 347 MBq of  $^{211}\text{At}$ , which is the highest dose that has been administered to a human, [100] leads to only 310 kBq of  $^{207}\text{Bi}$ , making its potential toxicity negligible.

The most common method of  $^{211}\text{At}$  production is by irradiation of natural bismuth ( $^{209}\text{Bi}$ ) target following the nuclear reaction:  $^{209}\text{Bi}(\alpha, 2n)^{211}\text{At}$  [99, 101, 102]. Other production methods such as  $^{209}\text{Bi}(7\text{Li}, 5n)^{211}\text{Rn} \rightarrow ^{211}\text{At}$ ,  $^{209}\text{Bi}(3\text{He}, n)^{211}\text{At}$ ,  $^{\text{nat}}\text{U}(p, x)^{211}\text{At}$  and  $^{234}\text{Th}(p, x)^{211}\text{Rn} \rightarrow ^{211}\text{At}$  have also been investigated [99, 103]. However, these production routes are inefficient and require particle energies in the range

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**Figure 5.** (A) Excitation function for the  $^{209}\text{Bi} (\alpha, 2n) ^{211}\text{At}$  reaction, (B) Excitation function for the  $^{209}\text{Bi} (\alpha, 3n) ^{211}\text{At}$  reaction. Adapted from Ref. [104] with permission. Copyright 2009 International Atomic Energy Agency.

of 160–660 MeV and extensive separation procedures. Therefore, these alternative methods are unreliable with respect to clinical-scale production of  $^{211}\text{At}$  and have very limited scope.

Adopting  $^{209}\text{Bi} (\alpha, 2n) ^{211}\text{At}$  nuclear reaction,  $^{211}\text{At}$  production is possible for  $\alpha$ -beam energies ranging from 21 to greater than 40 MeV, with a maximum cross-section observed for 31 MeV (Figure 5A) [104]. However, one cannot take advantage of the full breadth of the cross section for this nuclear reaction because of concerns regarding co-production of  $^{210}\text{At}$  ( $t_{1/2} = 8.3$  h) by the  $^{209}\text{Bi} (\alpha, 3n) ^{210}\text{At}$  nuclear reaction

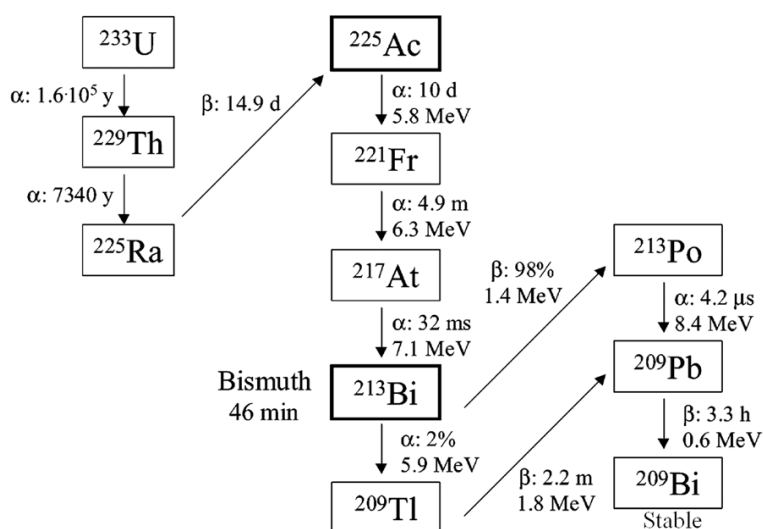
(Figure 5B) [104]. This radioisotope is especially problematic because greater than 99% of its decays results in the production of  $\alpha$ -emitting  $^{210}\text{Po}$  ( $t_{1/2} = 138.4$  d) that can be extremely toxic to the bone marrow [99, 100]. Therefore, the  $\alpha$ -beam energy is generally controlled to 28–29 MeV to limit formation of  $^{210}\text{At}$  while maintaining appreciable yields of  $^{211}\text{At}$ .

In terms of cost, use of natural bismuth is an ideal target material as it precludes the need for procedures to recover and purify the target material. However, thermal properties of bismuth are not ideal, as it has poor thermal conductivity ( $7.97 \text{ W}\cdot\text{K}^{-1}\cdot\text{m}^{-1}$ ) and a low melting point ( $272^\circ\text{C}$ ) [100]. Owing to the relatively high volatility of astatine ( $337^\circ\text{C}$ ), this could lead to vaporization of the produced activity, due to overheating of the target during the irradiation. This overheating has been addressed by target cooling systems using cold gas and water during irradiation. Also, in certain cases, a suitable irradiation angle has been chosen that spreads the beam over a larger impact surface on the bismuth target. For target preparation, metallic bismuth

is generally fused or vaporized onto an aluminum backing plate ( $k = 250 \text{ W m}^{-1} \text{ K}^{-1}$ ), which then may be machined to provide a smooth surface [99, 104]. Backing plates made from copper ( $k = 390 \text{ W m}^{-1} \text{ K}^{-1}$ ) have also been investigated in order to improve the thermal conductivity [99]. However, this has led to lower  $^{211}\text{At}$  isolation yields and also resulted in the co-production of several radionuclidic impurities including  $^{67}\text{Ga}$ ,  $^{66}\text{Ga}$ ,  $^{65}\text{Zn}$  and  $^{69}\text{Ge}$  [99].

After irradiation,  $^{211}\text{At}$  produced must be separated from the bulk target material ( $^{209}\text{Bi}$ ) and traces of co-produced  $^{210}\text{Po}$ . Generally, two

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**Figure 6.** Decay chain of  $^{233}\text{U}$  indicating production and decay of  $^{225}\text{Ac}$  and  $^{213}\text{Bi}$ . Adapted from Ref. [111] with permission. Copyright 2005 American Chemical Society.

methods have been utilized for the separation of  $^{211}\text{At}$  from the irradiated target: (a) dry distillation [105] and (b) liquid-liquid extraction that requires dissolution of the target in an acidic solution [106]. In dry distillation method, the target is placed in a furnace and heated above the boiling point of astatine [105]. Since, the boiling points of bismuth and polonium are  $1564^\circ\text{C}$  and  $962^\circ\text{C}$ , respectively; the furnace temperature is generally set to  $650\text{--}900^\circ\text{C}$ . During this process, while the bismuth and the polonium melt and stay on the support, the volatile astatine is carried away by a stream of gas (generally nitrogen or argon) and trapped at the outlet. The astatine activity is finally obtained by bubbling the stream of gas directly into the solvent of choice, though it can also be captured in capillary tubing cooled in dry ice/ethanol placed at the outlet. In the wet distillation procedure, the irradiated target is dissolved in concentrated nitric acid followed by extraction with di-isopropyl ether [106, 107]. Although the wet distillation method is easy to perform in a hot cell, the choice of the extraction solvent is limited to di-isopropyl ether or analogous solvent, which is an issue because of the associated hazards. Furthermore, some nitric acid is extracted into the organic phase in notable concentrations, which can lead to side reactions during the radiolabeling chemistry [100]. Therefore, the dry distillation procedure is more widely used for radiochemical processing of  $^{211}\text{At}$ .

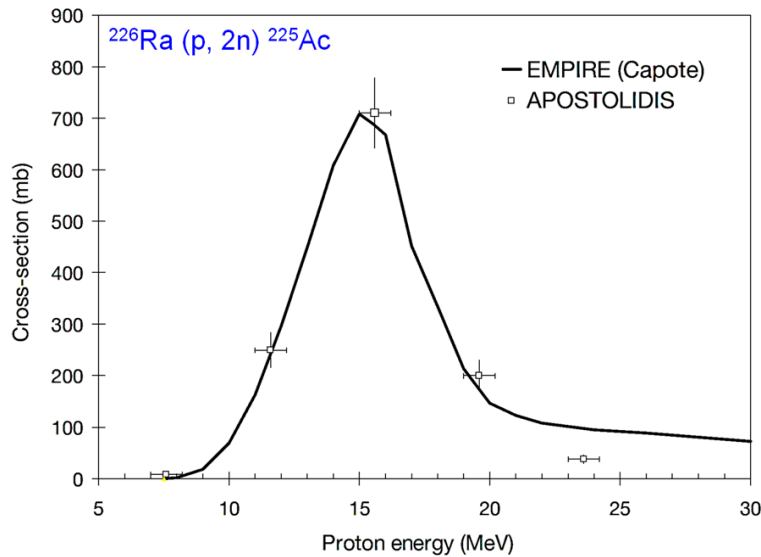
### $^{225}\text{Ac}$

Actinium-225 ( $t_{1/2} = 10$  d) is another  $\alpha$ -emitting radioisotope with immense potential in TAT of mCRPCa [98, 108–110]. The decay of  $^{225}\text{Ac}$  results in 6 daughter products ( $^{221}\text{Fr}$ ,  $^{217}\text{At}$ ,  $^{213}\text{Bi}$ ,  $^{213}\text{Po}$ ,  $^{209}\text{Pb}$ , and  $^{209}\text{Tl}$ ) with several  $\alpha$  and  $\beta$  decays (Figure 6). Despite its excellent nuclear decay characteristics, the widespread use of  $^{225}\text{Ac}$  in TAT has been restricted due to unavailability of the radioisotope. Presently,  $^{225}\text{Ac}$  can be obtained in limited quantities ( $\sim 37$  GBq/y) by radiochemical separation from two  $^{229}\text{Th}$  sources, one located at Oak Ridge National Laboratory (ORNL), USA and

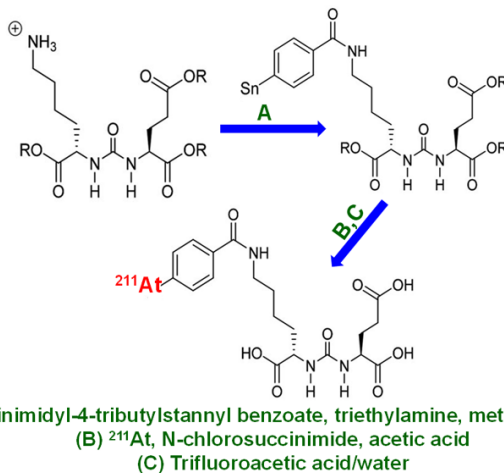
the other at the Institute for Transuranium Elements in Karlsruhe (ITU), Germany (Figure 6) [111, 112]. The  $^{229}\text{Th}$  available at both sites was recovered from  $^{233}\text{U}$ , which has been in long-term storage at ORNL (Figure 6). This  $^{233}\text{U}$  was produced in kilogram quantities during the 1960s by neutron irradiation of  $^{232}\text{Th}$  in molten salt breeder reactors. The radiochemical separation procedure involves two steps: (a) ion exchange separation of  $^{225}\text{Ac}/^{225}\text{Ra}$  from  $^{229}\text{Th}$ , and (b) extraction chromatography separation of  $^{225}\text{Ac}$  from  $^{225}\text{Ra}$  [111, 112]. After the radiochemical separation, clinical grade no-carrier-added (NCA)  $^{225}\text{Ac}$  could be obtained with  $>95\%$  yield.

In order to meet the increasing demand of  $^{225}\text{Ac}$  for clinical studies, alternative ways of production of  $^{225}\text{Ac}$  are being discussed. Among them, high energy proton spallation reaction on natural thorium metal targets has been utilized to produce  $^{225}\text{Ac}$  adopting  $^{232}\text{Th}$  ( $p, 2p, 6n$ )  $^{225}\text{Ac}$  nuclear reaction [113, 114]. In this route, the cross sections for production of  $^{225}\text{Ac}$  range from  $3.6\text{--}16.7$  mb in the incident proton energy range of  $78\text{--}192$  MeV. Accordingly, production of curie quantities of  $^{225}\text{Ac}$  is possible by irradiating a  $^{232}\text{Th}$  target ( $5.0$  g  $\text{cm}^{-2}$ ) for 10 days. Although appreciable activity of  $^{225}\text{Ac}$  can be produced in a single batch, the major limitation of this approach is the unavailability of high energy proton accelerators which must be operated continuously for a prolonged period of 10 days.

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**Figure 7.** Excitation function for the  $^{226}\text{Ra} (p, 2n) ^{225}\text{Ac}$  reaction. Adapted from Ref. [104] with permission. Copyright 2009 International Atomic Energy Agency.



**Figure 8.** Synthesis of  $^{211}\text{At}$ -labeled PSMA inhibitor. R = 5 para-methoxybenzyl. Adapted from Ref. [127] with permission. Copyright 2016 Society of Nuclear Medicine and Molecular Imaging.

In order to circumvent this limitation, it has been proposed to produce  $^{225}\text{Ac}$  via  $^{226}\text{Ra} (p, 2n) ^{225}\text{Ac}$  reaction in a cyclotron [104, 115, 116]. Adopting this reaction, maximum yield of  $^{225}\text{Ac}$  was reached at incident proton energies of 16.8 MeV (Figure 7) and therefore it is possible to produce  $^{225}\text{Ac}$  at many cyclotron facilities in the world by this route. Another alternative method for production of  $^{225}\text{Ac}$  is by the use of linear accelerator (LINAC) by  $^{226}\text{Ra} (\gamma, n) ^{225}\text{Ra} \rightarrow ^{225}\text{Ac}$  nuclear reaction [116]. Though this route is inefficient, it can produce required

quantities of  $^{225}\text{Ac}$  by irradiating a large amount of target in the LINAC. In both these methods, separation of  $^{225}\text{Ac}$  from the  $^{226}\text{Ra}$  target material could be performed using lanthanide extraction resin chromatography [115, 116]. Since the target material used in both these production routes is radioactive and long-lived, additional safety precautions must be taken into consideration during the irradiation process. This may pose regulatory issues, especially in facilities which do not allow use of radioactive material as targets for radioisotope production.

### $^{213}\text{Bi}$

Among the daughter products of  $^{225}\text{Ac}$ ,  $^{213}\text{Bi}$  is well suited for TAT of mCRPCa. Bismuth-213 decays with a  $t_{1/2}$  of 45.6 min and emits an 8.4 MeV  $\alpha$ -particle with a branching ratio of 97.8% (Figure 6) [98, 117, 118]. The decay of  $^{213}\text{Bi}$  follows with the emission of rather low intensity  $\gamma$ -rays [440 keV (26%) and 1566 keV (2%)] which make it an ideal theranostic radioisotope. NCA  $^{213}\text{Bi}$  can be obtained from  $^{225}\text{Ac}/^{213}\text{Bi}$  generator [119, 120]. Due to the 10-day half life of  $^{225}\text{Ac}$ , the useful life time of  $^{225}\text{Ac}/^{213}\text{Bi}$  generators is several weeks. Owing to the 45.6 min half life of  $^{213}\text{Bi}$ , the  $^{225}\text{Ac}/^{213}\text{Bi}$  generator can be eluted several times in a day. Over the last several years, various types of  $^{225}\text{Ac}/^{213}\text{Bi}$  generators have been reported, based on cation and anion exchange, or extraction chromatography [119, 121, 122].

Among the  $^{225}\text{Ac}/^{213}\text{Bi}$  generators reported, the generators based on AG MP-50 cation exchange resin are the most widely used and have been applied for all patient studies with  $^{213}\text{Bi}$  to date [121, 122]. For preparation of the generator, both trivalent cations ( $\text{Ac}^{3+}$  and  $\text{Bi}^{3+}$ ) are effi-



## Targeted $\alpha$ -therapy of prostate cancer using radiolabeled PSMA inhibitors

**Table 1.** Representative examples of radiolabeled PSMA inhibitors used for TAT of mCRPCa in preclinical and clinical settings

Radioisotope	Radiolabeled agent used	Type of study (Preclinical/clinical)	Prostate cancer type	Treatment response	Reference
<sup>211</sup> At	(2S)-2-(3-(1-carboxy-5-(4- <sup>211</sup> At-astatobenzamido) pentyl)ureido)-pentanedioic acid	Preclinical	(PSMA+) PC3 PIP xenograft	Caused significant delay in tumor growth.	[127]
<sup>225</sup> Ac	<sup>225</sup> Ac-PSMA-617	Clinical	mCRPCa	Experienced PSA decline to below the measurable level and showed complete response on PET imaging.	[96]
<sup>213</sup> Bi	<sup>213</sup> Bi-PSMA I&T	Preclinical	PSMA+ LNCaP xenografts	Caused DNA double strand breaks in tumors.	[132]
<sup>213</sup> Bi	<sup>213</sup> Bi-PSMA-617	Clinical	mCRPCa	Experienced decrease in PSA level from 237 $\mu$ g/L to 43 $\mu$ g/L and showed complete response on PET imaging.	[133]

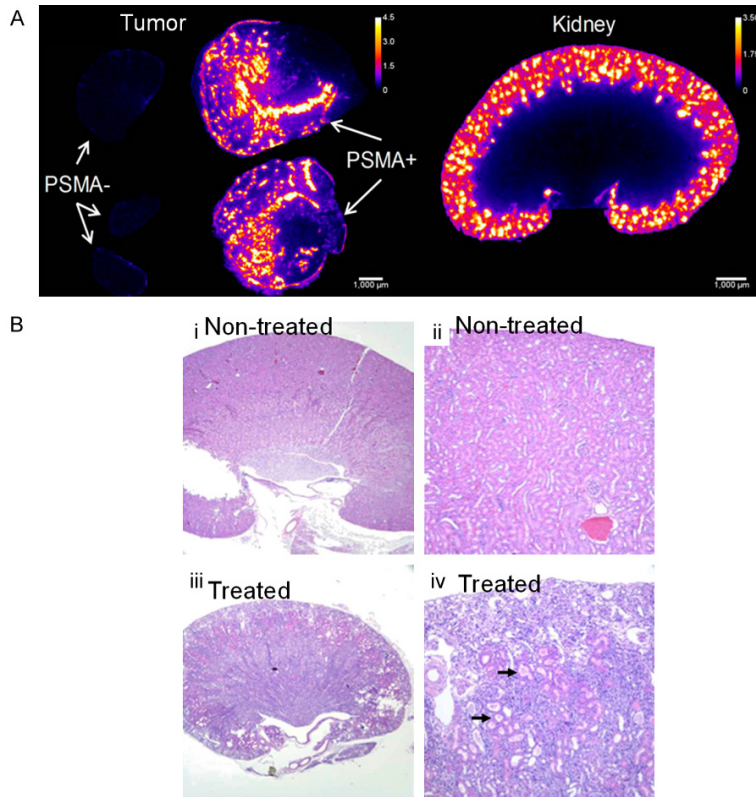
ciently sorbed to AG MP-50 cation exchange resin. As hard Lewis acid, the Bi<sup>3+</sup> cation has a strong affinity to form complexes with sulfur and halogens, especially iodide. The strong affinity of Bi<sup>3+</sup> for complexation with iodide is used for selective elution of <sup>213</sup>Bi from the cation exchange resin as anionic Bi<sub>4</sub><sup>-</sup>/Bi<sub>5</sub><sup>2-</sup> species using a solution of 0.1 M HCl/0.1 M NaI as the eluent. This procedure provided a high yield of <sup>213</sup>Bi elution, low breakthrough of the <sup>225</sup>Ac, and the radioactivity was obtained in a medium amenable for subsequent radiopharmaceutical preparation.

### Radiolabeling techniques

The radiolabeling of biological molecules with radiometals for preparation of radiopharmaceuticals involves an interdisciplinary approach requiring knowledge of coordination chemistry, kinetics and thermodynamics, radiochemistry, synthetic chemistry, and biology/physiology [123-126]. Generally, radiolabeling of PSMA inhibitors with <sup>211</sup>At is performed by adopting conventional radioiodination chemistry, since astatine belongs to the halogen family and has similar chemical properties to iodine. Tin precursors and prosthetic groups have been used to label PSMA inhibitor with <sup>211</sup>At as shown in **Figure 8** [127]. It is pertinent that the carbon-astatine bond in the radiolabeled agent is relatively weak, and the release of free astatine can result in uptake of radioactivity in non-targeted organs. Like iodine, free astatine is taken up in the thyroid, stomach, and macrophage bearing organs such as lung and spleen [127]. Therefore, it is more prudent to radiolabel PSMA inhibitors with  $\alpha$ -emitting radiometals

such as <sup>225</sup>Ac and <sup>213</sup>Bi, involving the use of bifunctional chelators (BFCs) for preparation of radiopharmaceuticals for TAT of PCa.

The popular tetraaza amino carboxylate-based macrocyclic chelator, DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), along with its bifunctional derivatives, form a class of 'gold standards' that have been extensively used for radiolabeling biomolecules with <sup>225</sup>Ac and <sup>213</sup>Bi [123]. Other macrocyclic chelators which have been used for this purpose include {4-[2-(bis-carboxymethylamino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl]-acetic acid (NETA), 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (TCMC), 2-[(carboxymethyl)-[5-(4-nitrophenyl)-1-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]pentan-2-yl]-amino]acetic acid (3p-C-DEPA) and their derivatives [123]. The macrocyclic chelators require minimal physical manipulation during coordination with radiometal ions, as they possess inherently constrained geometries and partially pre-organized metal ion binding sites, thereby decreasing the entropic loss experienced upon metal ion coordination [123]. Complex formation using macrocyclic chelators requires heating at elevated temperatures (80-95°C), however this is not an issue for small molecular weight ligands such as PSMA inhibitors, which remain stable in this temperature range [128]. For all clinical studies reported to date with <sup>225</sup>Ac and <sup>213</sup>Bi for TAT of PCa, [96, 129] DOTA derivatives have been used as the BFCs of choice because of their excellent thermodynamic stability and kinetic rigidity *in vivo*.



**Figure 9.** (A)  $\alpha$ -camera images at 1 h showing relative  $^{211}\text{At}$ -labeled PSMA inhibitor activity concentrations for PSMA+ and PSMA- tumors and kidneys. Scale shows activity concentration relative to whole tumor/kidney average concentration. (B) Renal histopathology from non-treated mouse (i and ii) and mouse treated with 1.5 MBq of  $^{211}\text{At}$ -labeled PSMA inhibitor (iii and iv). Treated kidney showed subcortical atrophy and degenerative loss of proximal tubules (arrows) consistent with late nephropathy due to  $\alpha$ -particle irradiation. Adapted from Ref. [127] with permission. Copyright 2016 Society of Nuclear Medicine and Molecular Imaging.

nogenic survival in PSMA-positive (PSMA+) PC3 PIP and PSMA-negative (PSMA-) PC3 flu human PCa cells after treatment with (2S)-2-(3-(1-carboxy-5-(4- $^{211}\text{At}$ -astatobenzamido) pentyl)ureido)-pentanedioic acid. From this study, it was observed that the uptake in (PSMA+) PC3 PIP cells increased from  $4.9 \pm 0.3\%$  at 0.5 h to  $19.3 \pm 1.0$  at 4 h [127]. When co-incubated with the PSMA inhibitor, the radiotracer uptake was reduced to  $< 2\%$  of the initial dose, indicating PSMA specificity of (2S)-2-(3-(1-carboxy-5-(4- $^{211}\text{At}$ -astatobenzamido) pentyl)ureido)-pentanedioic acid. Also, it was observed that the uptake in PSMA- PC3 flu cells was significantly lower than in PSMA+ PC3 PIP cells, thus further corroborating the PSMA specificity of the radiotracer synthesized. Additionally, decreased clonogenic survival of (PSMA+) PC3 PIP cells after incubation with (2S)-2-(3-(1-carboxy-5-(4- $^{211}\text{At}$ -astatobenzamido) pentyl)ureido)-pentanedioic acid radiotracer was observed.

#### TAT of mCRPCa using radiolabeled PSMA inhibitors

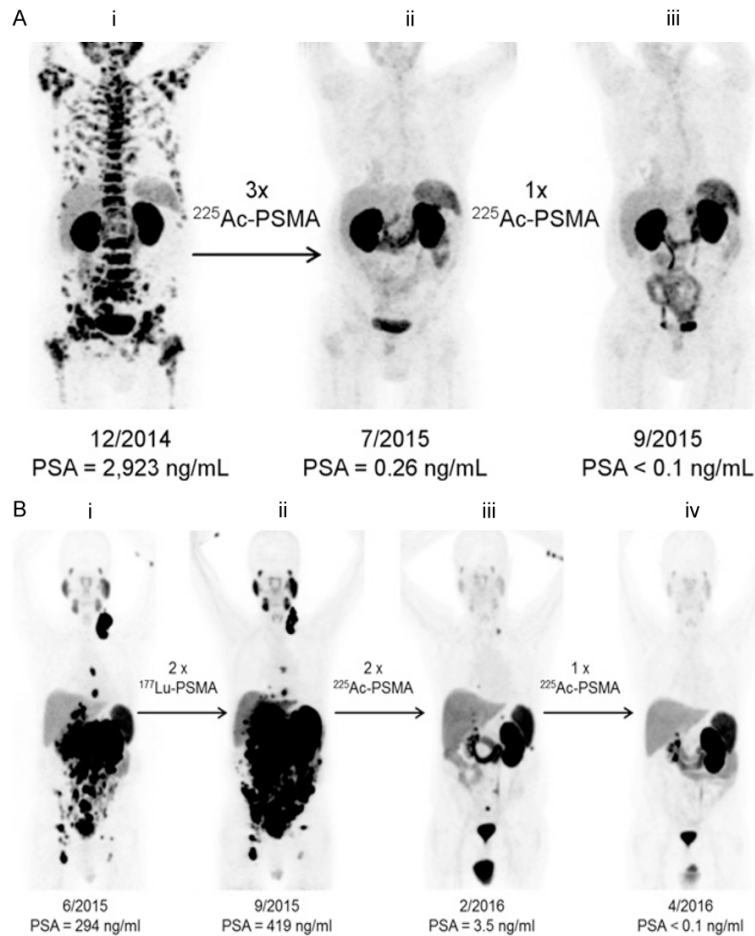
The preclinical and clinical studies related to TAT of mCRPCa with PSMA inhibitors radiolabeled with different  $\alpha$ -emitting radioisotopes are summarized in **Table 1** and described in the following text.

##### $^{211}\text{At}$ -labeled PSMA inhibitor

In an attempt to explore the utility of  $^{211}\text{At}$ -labeled PSMA inhibitor for the treatment of mCRPCa, Kiess et. al. synthesized (2S)-2-(3-(1-carboxy-5-(4- $^{211}\text{At}$ -astatobenzamido) pentyl)ureido)-pentanedioic acid and evaluated its efficacy in preclinical settings [127]. The radiolabeled agent could be synthesized with  $> 60\%$  yield and  $> 98\%$  radiochemical purity. The authors evaluated the cellular uptake and clo-

Biodistribution studies showed significant uptake of (2S)-2-(3-(1-carboxy-5-(4- $^{211}\text{At}$ -astatobenzamido) pentyl)ureido)-pentanedioic acid radiotracer in (PSMA+) PC3 PIP tumors (maximum uptake 28.2% ID/g) and in kidneys ( $> 90\%$  ID/g) [127]. For the PSMA- PC3 flu tumors, maximum uptake was  $2.1 \pm 1.4\%$  ID/g, indicating PSMA specificity of the radiotracer *in vivo* (**Figure 9A**). Microscale kidney dosimetry was done based on  $\alpha$ -camera imaging and a nephron model was developed, which revealed hot spots in the proximal renal tubules (**Figure 9A**). The long-term toxicity studies confirmed that the dose-limiting toxicity was late radiation nephropathy with loss of the proximal tubules. This was demonstrated by both histopathology at necropsy and serial laboratory studies (**Figure 9B**). However, (2S)-2-(3-(1-carboxy-5-(4- $^{211}\text{At}$ -astatobenzamido) pentyl)ureido)-pentane-

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**Figure 10.** (A)  $^{68}\text{Ga}$ -PSMA-11 PET/CT scans of patient A. Pretherapeutic tumor spread (i), restaging 2 months after third cycle of  $^{225}\text{Ac}$ -PSMA-617 (ii), and restaging 2 months after one additional consolidation therapy (iii). (B)  $^{68}\text{Ga}$ -PSMA-11 PET/CT scans of patient B. In comparison to initial tumor spread (i), restaging after 2 cycles of  $\beta$ -emitting  $^{177}\text{Lu}$ -PSMA-617 presented progression (ii). In contrast, restaging after second (iii) and third (iv) cycles of  $^{225}\text{Ac}$ -PSMA-617 therapy presented impressive response. Adapted from Ref. [96] with permission. Copyright 2016 Society of Nuclear Medicine and Molecular Imaging.

dic acid did not show significant dose-limiting hematologic toxicity.

Another cause of concern with this radiotracer is *in vivo* deastatination as indicated by uptake of radioactivity in thyroid, stomach, spleen, and lungs, in the biodistribution studies [127]. Therefore, from the perspective of clinical translation of this class of radiolabeled agents for TAT of mCRPCa, urea-based PSMA inhibitors with more favorable tumor-kidney dose ratios must be identified, and improved radiolabeling techniques must be developed to achieve enhanced *in vivo* stability of the radiolabeled agent. Nevertheless, the present study could

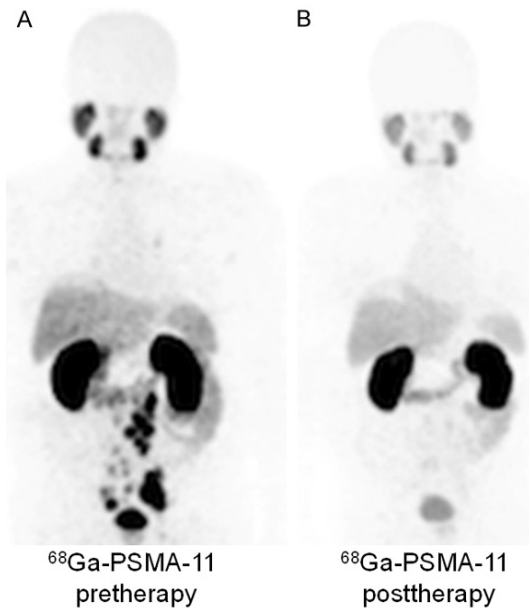
set the stage for development of new  $^{211}\text{At}$ -based agents for TAT of PCa, and it also highlighted the importance of long-term toxicity studies and microscale dosimetry in this context.

### $^{225}\text{Ac}$ -labeled PSMA inhibitor

The first-in-human treatment with an  $\alpha$ -emitting radionuclide ( $^{225}\text{Ac}$ ) labeled PSMA inhibitor was reported by Kratochwil et al [96]. For this purpose, PSMA-617 was radiolabeled with  $^{225}\text{Ac}$  with > 98% radiochemical purity and with specific activity of  $0.17 \pm 0.05$  MBq/nmol. In this study, 2 patients in highly challenging clinical situations were subjected to targeted  $^{225}\text{Ac}$ -PSMA-617 therapy. Among these 2 patients, treatment with  $\beta$ -emitters had contraindicated in one patient (patient A) while the other patient (patient B) was resistant to  $^{177}\text{Lu}$ -PSMA-617. In both the patients,  $^{68}\text{Ga}$ -PSMA-11 PET/CT scans validated the presence of the PSMA+ tumor phenotype (Figure 10). For TAT, a 100-kBq activity dose of  $^{225}\text{Ac}$ -PSMA-617 per kilogram of body weight was administered bimonthly and the PSA response and hematologic toxicity were determined at minimum every

4 weeks. Restaging was done with  $^{68}\text{Ga}$ -PSMA-11 PET/CT (Figure 10). Both patients demonstrated a PSA decline to below the measurable level and showed a complete response on imaging. Also, no significant hematological toxicity was experienced. However, xerostomia was observed as the clinical side effect of this therapy. Despite the impressive therapeutic responses observed in 2 PCa patients in clinically critical situations, investigation of therapeutic modality in larger cohort of PCa patients is warranted to arrive at a definite conclusion.

In a more extensive study, the same group of authors developed a treatment protocol for



**Figure 11.**  $^{68}\text{Ga}$ -PSMA-11 PET/CT scans of patient. (A) Pretherapeutic tumor spread, (B) restaging 11 months after therapy with  $^{213}\text{Bi}$ -PSMA-617. Adapted from Ref. [133] with permission. Copyright 2017 Springer.

$^{225}\text{Ac}$ -PSMA-617  $\alpha$ -radiation therapy in advanced-stage, mCRPCa patients with PSMA positive tumor phenotype [130]. Mainly, end-stage patients who had already exhausted the approved therapeutic options were admitted for the study. The authors calculated a dosimetry estimate on the basis of time-activity curves derived from serially obtained  $^{177}\text{Lu}$ -PSMA-617 scans extrapolated to the physical half-life of  $^{225}\text{Ac}$ . For these calculations, instant decay of unstable daughter nuclides of  $^{225}\text{Ac}$  was assumed. This study revealed mean doses of 2.3 Sv for salivary glands, 0.7 Sv for kidneys, and 0.05 Sv for red marrow that are composed of 99.4%  $\alpha$ , 0.5%  $\beta$ , and 0.1%  $\gamma$  radiation, respectively. Salvage therapies were empirically conducted with 50, 100, 150, 200 kBq/kg dose of  $^{225}\text{Ac}$ -PSMA-617, and treatment and toxicity responses were retrospectively evaluated. It was observed that severe xerostomia became the dose-limiting toxicity if treatment dose of  $^{225}\text{Ac}$ -PSMA-617 exceeded 100 kBq/kg per cycle. At 100 kBq/kg, the duration of PSA decline was < 4 months. However, if TAT was repeated every 2 months, patients experienced additive antitumor effects. Treatment doses of 50 kBq/kg were nontoxic, but the antitumor response was insufficient in these high-tumor-

burden patients. Based on these clinical results, the authors concluded that for mCRPCa patients, a treatment dose of 100 kBq/kg of  $^{225}\text{Ac}$ -PSMA-617 per cycle, repeated every 8 weeks, presents a reasonable balance between toxicity and biochemical response. This therapeutic regimen was used by the same group of authors in a larger cohort of patients, wherein remarkable anti-tumor activity of  $^{225}\text{Ac}$ -PSMA-617 was demonstrated [131]. In this study, swimmer-plot analysis provided first longitudinal indicators that TAT with  $^{225}\text{Ac}$ -PSMA-617 presented clinical efficacy with regard to duration of tumor control. As in the previous study, xerostomia was the main cause to stop the therapy or to reject additional administrations and was in the same facet as non-response. Despite promising attributes, this study indicated that further modifications of the therapeutic protocol with regard to side effects might be essential in order to further improve the therapeutic range.

#### $^{213}\text{Bi}$ -labeled PSMA inhibitor

In a first preclinical study on TAT of PCa using  $^{213}\text{Bi}$ -labeled PSMA inhibitor ( $^{213}\text{Bi}$ -PSMA I&T), Nonnekens et. al. demonstrated that the radiolabeled agent induced DNA double strand breaks in PCa xenografts [132]. The authors prepared  $^{213}\text{Bi}$ -PSMA I&T with > 95% radiolabeling yield with a specific activity of 58 MBq/nmol. *In vitro* studies conducted in PSMA+ LNCaP cells indicated that  $^{213}\text{Bi}$ -PSMA I&T led to increased number of DNA double-strand breaks, detected as 53BP1 and  $\gamma\text{H2AX}$  nuclear foci. The results of the biodistribution studies in mice bearing LNCaP xenografts showed significant tumor uptake of the radiotracer at 1 h p.i., with accumulation in the kidneys. Additionally,  $^{213}\text{Bi}$ -PSMA I&T induced *in vivo* DNA double strand breaks in the tumors, which were detected between 1 hour and 24 hours p.i. The results of this preliminary study set the stage for further evaluation of  $^{213}\text{Bi}$ -labeled PSMA inhibitors with regard to their therapeutic efficacy and toxicity for PCa management.

The first-in-human treatment with  $^{213}\text{Bi}$ -PSMA-617 in a patient with mCRPCa was reported by Sathekege et. al. [133]. In this study, the lone patient was treated with two cycles of  $^{213}\text{Bi}$ -PSMA-617 with a cumulative activity of 592 MBq. Restaging was done with  $^{68}\text{Ga}$ -PSMA PET/CT after 11 months, which showed a

remarkable molecular imaging response (**Figure 11**). Also, the patient had demonstrated a biochemical response (decrease in PSA level from 237 to 43  $\mu\text{g/L}$ ). Despite promising results, detailed clinical evaluation of  $^{213}\text{Bi}$ -PSMA-617 in a large cohort of PCa patients is warranted to arrive at any conclusion. In another recent clinical study, Kratochwil et. al. estimated the radiation dosimetry of  $^{213}\text{Bi}$ -PSMA-617 in PCa patients [129]. The authors used  $^{68}\text{Ga}$  ( $t_{1/2} = 68$  min) as a surrogate nuclide for  $^{213}\text{Bi}$ , enabling high-resolution quantitative  $^{68}\text{Ga}$ -PSMA-617 PET-imaging. Based on this, the extrapolated radiation dosimetry for  $^{213}\text{Bi}$ -PSMA-617 was estimated and its therapeutic index was compared with findings for  $^{225}\text{Ac}$ -PSMA-617. The authors found that the dosimetry of  $^{213}\text{Bi}$ -PSMA-617 was in a range usually considered suitable for clinical use. However, as compared to  $^{225}\text{Ac}$ -PSMA-617 dosimetry, it suffered from higher perfusion-dependent off-target radiation, therefore its therapeutic index for PCa therapy was considered to be inferior. Moreover, due to longer biological half-life of PSMA-617 in dose-limiting organs compared to the physical half-life of  $^{213}\text{Bi}$ , this radionuclide becomes a second choice (after  $^{225}\text{Ac}$ ) for radiolabeling PSMA inhibitors for TAT of PCa [129]. A major limitation of this study is that a different complex ( $^{68}\text{Ga}$ -PSMA-617) was used to estimate the dosimetry of  $^{213}\text{Bi}$ -PSMA-617 in PCa patients. It is worth mentioning that the radiometal in the chelator complex could affect the pharmacokinetics of the radiotracer and these effects can be significant [134]. Nevertheless, the results of this study amply demonstrated that  $^{213}\text{Bi}$ -PSMA-617 is suitable for clinical translation and more detailed studies in larger cohort of patients are warranted.

### Conclusions and future perspectives

TAT using radiolabeled PSMA inhibitors is emerging as a promising new modality for the treatment of mCRPCa. Significant research activity and resulting outstanding progress in production of clinically safe, radionuclidically pure,  $\alpha$ -emitters, design and synthesis of PSMA inhibitors, and suitable linkers forming radiolabeled constructs with appreciable *in vitro* and *in vivo* stability, make it very likely that this modality will become a new line of therapy protocol for advanced stage PCa management. It is worth mentioning that the onset of early clinical trials with PSMA inhibitors radiolabeled

with  $\alpha$ -emitters will definitely enable the nuclear medicine practitioners to come up with extremely effective and highly specific radiopharmaceuticals to target micro metastases in advanced stage PCa patients. With regard to the safety, convenience of handling, transportation logistics and clinical efficacy,  $^{225}\text{Ac}$  is the most advantageous among the radionuclides studied for TAT of PCa. Additionally, the radiolabeling chemistry for preparation of  $^{225}\text{Ac}$ -based radiopharmaceuticals is well established and clinically proven. Nevertheless, production of this radioisotope is still limited to very few countries in the world, and therefore its availability at a reasonable cost is an issue for widespread clinical use.

Over the last decade, a variety of PSMA inhibitors have been synthesized which can be used for preparation of radiopharmaceuticals. For effective clinical utilization, PSMA inhibitor selection should be based on rapid uptake and persistent localization at the target site, with negligible retention in non-targeted tissues. In addition to the normal prostate, low levels of endogenous PSMA expression have also been found in many organs, including the proximal tubules of the kidneys, the lacrimal and salivary glands, the spleen, the liver, the intestinal membranes, the testes, the ovaries, and the brain [135, 136]. Therefore, upon administration of radiolabeled PSMA inhibitor, uptake of small amount of radioactivity also occurs in these normal tissues in addition to cancerous lesions, which might cause unnecessary side effects, especially while using  $\alpha$ -emitting radioisotopes. Based on the results of clinical trials reported to date, the most significant adverse side effect observed with  $^{225}\text{Ac}$ -PSMA-617 is xerostomia, which as such could be avoided using radiolabeled monoclonal antibodies such as J591 (which targets a different epitope) [137-140] instead of PSMA ligands for targeting PCa. However, small molecular agents such as PSMA inhibitors have a critical advantage over much larger constructs, as they clear faster from the blood, and demonstrate increased tumor permeability, allowing them to escape physiological barriers met by larger molecules, such as monoclonal antibodies. Therefore, it is prudent to design and synthesize a new class of PSMA ligands wherein the uptake in these organs can be minimized for enhanced clinical benefits.

## Targeted $\alpha$ -therapy of prostate cancer using radiolabeled PSMA inhibitors

While numerous complications face all new technologies, materializing the opportunities presented by TAT of mCRPCa requires addressing several interdisciplinary challenges. Additionally, there are other complex factors, which include considerable regulatory hurdles in handling  $\alpha$ -emitters in hospital radiopharmacies, a limited potential market (at least initially), lobbying by the manufacturers of conventional PCa therapy agents, lack of reimbursement strategies by the insurance agencies for such novel strategies, and socio-economic factors, which might obstruct widespread translation of this novel therapeutic modality in nuclear medicine clinics. In view of all these challenges, concerted efforts of all stakeholders, which include radiopharmaceutical scientists, nuclear medicine physicians, radiochemists, medical physicists, radiologists, program advisory boards, and regulatory authorities would be required, both to create enthusiasm for developing this new concept and to prevent undesirable messaging based on myths, speculations, exaggeration, and prejudice. This in turn would provide impetus to further clinical research, which might aid toward use of TAT in routine clinical practices for advanced stage PCa management.

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