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PSMA-Targeted Radiopharmaceuticals for Imaging and Therapy

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As described in more detail in other contributions in this issue of *Seminars in Nuclear Medicine*, prostate-specific membrane antigen (PSMA) has become one of the most promising molecular targets in nuclear medicine. Due to its overexpression on prostate cancer cells in proportion to the stage and grade of tumour progression, especially in androgen-independent, advanced and metastatic disease, various tracers for the detection and treatment of prostate cancer by means of radioligand imaging, radioligand therapy or radioguided surgery have been developed and transferred to clinical applications. Even though monoclonal antibodies were investigated and introduced as first PSMA-targeted probes, the inherent advantage of fast tumour uptake and rapid excretion of small molecules has shifted the research focus during the last decade to low molecular weight inhibitors with high affinity to PSMA, such as [^{18}F]FDCFPyL, [^{18}F]PSMA-1007, [^{68}Ga]PSMA-HBED, [^{177}Lu]PSMA-617, [^{177}Lu]PSMA-I&T, [^{99m}Tc]MIP-1404 or [^{99m}Tc]PSMA I&S, to mention only a few. Due to the plethora of such PSMA probes described during the last years, this review aims to give an overview over the specific characteristics of those radiopharmaceuticals that have already found widespread clinical application. In addition, recently introduced concepts such as PSMA-tracers with increased plasma protein binding, are discussed.
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Introduction

With ca 164,690 estimated new cases (9.5% of all new cancer cases) in the US (worldwide about 1000,000) in 2018 and 29,430 estimated deaths (4.8% of all cancer deaths) in the US (worldwide about 300,0000) in 2018, prostate cancer is one of the most frequent cancers and cause of cancer−related deaths among men.[1-3]

Depending on the stage and severity of the disease, various imaging methods, such as CT, bone scintigraphy, and trans rectal ultrasound, as well as more recently introduced methods such as whole-body magnetic resonance imaging, multiparametric MRI, SPECT and PET have been suggested for the evaluation of prostate cancer.

However, the specificity of the existing imaging modalities used in the assessment of metastatic burden is limited. With respect to nuclear imaging, the sensitivity of [^{11}C]choline,[4] [^{18}F]fluoromethylcholine,[5] and [^{18}F]fluoroethylcholine[6] was found to be limited and suboptimal, especially in patients with rising but low PSA levels (<1.5 ng/mL).

Due to the high expression of prostate-specific membrane antigen (PSMA), also named glutamate carboxypeptidase II (GCPII), *N*-acetyl-L-aspartyl-L-glutamate peptidase I or NAAG peptidase, on the cell membrane of prostate cancer cells and based on first urea-based compounds designed by Kozikowski et al.[7] to inhibit GCPII in the brain, various low molecular weight PSMA inhibitors (ca 0.5-1.5 kD) have been developed with the aim to provide more sensitive radiopharmaceuticals for prostate cancer imaging. These small-molecule PSMA inhibitors are zinc binding structures such as ureas, phosphonates, phosphates, phosphoramidates, to mention only a few, that interact with the binuclear zinc active site of PSMA that is indispensable for the GCPII hydrolytic activity and coordinated by the side chains of His377, Asp387, Glu425, Asp453, and His553. Formally, the entire PSMA substrate binding cavity of PSMA can be divided by this binuclear zinc active site into two 'halves', the S1' and the S1 pocket. Although S1' is quite specific for binding of glutamate and glutamate-like residues, the S1' pocket exploits both polar and non-polar interactions for binding of substrates or inhibitors. Consequently, some more hydrophobic glutamate-like functionalities have

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been used for the development of inhibitors capable to penetrate the blood–brain barrier.[8,9]

With respect to substrate binding, the S1 pocket of GCPII is quite specific for the negatively charged amino acids glutamate and aspartate. This specificity is based on the so-called 'arginine patch' formed by the cationic Arg534, Arg536, and Arg463 side chains of PSMA. The S1 pocket has been described as a flexible funnel with an 8 Å base at the active-site zinc ions and a rim at a distance 20 Å with a diameter of approximately 20 Å. Barinka et al. identified a hydrophobic accessory pocket near the S1 site.[10] This pocket with an approximate diameter of 7 Å × 8.5 Å × 9 Å, which is opened only by suitable positioning of Arg463 and Arg536, can significantly improve binding interaction of inhibitors. In addition, Zhang et al.[11] described a previously unknown arene-binding site on PSMA, that is formed by the indole side chain of Trp541 and the guanidinium group of Arg511, whose additional targeting can drastically enhance small molecule binding affinity. This arene-binding side is exploited by various radiolabelled PSMA inhibitors with longer spacers consisting of Phe, iodo-Phe, or Nal residues, but also plays a major role for the binding of PSMA-HBED-CC (DKFZ-PSMA-11) (Fig. 1) by interaction with the aromatic residues of the HBED chelator.

In summary, binding of a glutamate or glutamate-like residue to the S1' pocket and simultaneous inhibition of the binuclear zinc active site are indispensable characteristics and minimum requirements for PSMA-inhibitors, whereas interaction with one or more of the other key structures described above is optional and can significantly improve binding affinity. An example of an inhibitor with the smallest possible structure (only S1'- and Zn-binding) is 2-[(^{18})F]fluoro-4-(phosphonomethyl)-pentanedioic acid ([^{18}F]BAY1075553)[12] (Fig. 1). In direct comparison with ^{18}F-labelled choline in a small cohort of patients, [^{18}F]FCH PET produced results superior to those seen with [^{18}F]BAY1075553 PET, particularly with respect to detecting lymph node and bone marrow metastases. This example can be taken as indication that clinically relevant PSMA inhibitors should comprise additional structural features to exploit interaction with the additional binding pockets provided by PSMA.[13]

Initiated by a series of urea-based inhibitors of GCP II synthesized by Kozikowski et al.[7] in 2001 and triggered by the first evaluation of one of these small molecule inhibitors, [^{11}C]MeCys-C(O)-Glu ([^{11}C]MCG) (Fig. 1), in mice and baboons to investigate GCP II expression in the CNS by Pomper et al.,[14] a huge variety of PSMA-targeted tracers has been developed. To get a more detailed overview on the binding modes of PSMA inhibitors, reference is made to some excellent and comprehensive recent reviews on PSMA-binding molecules as potential radiopharmaceuticals and optical probes for targeted imaging as well as radioligand therapy.[15-20] In the following sections, only those

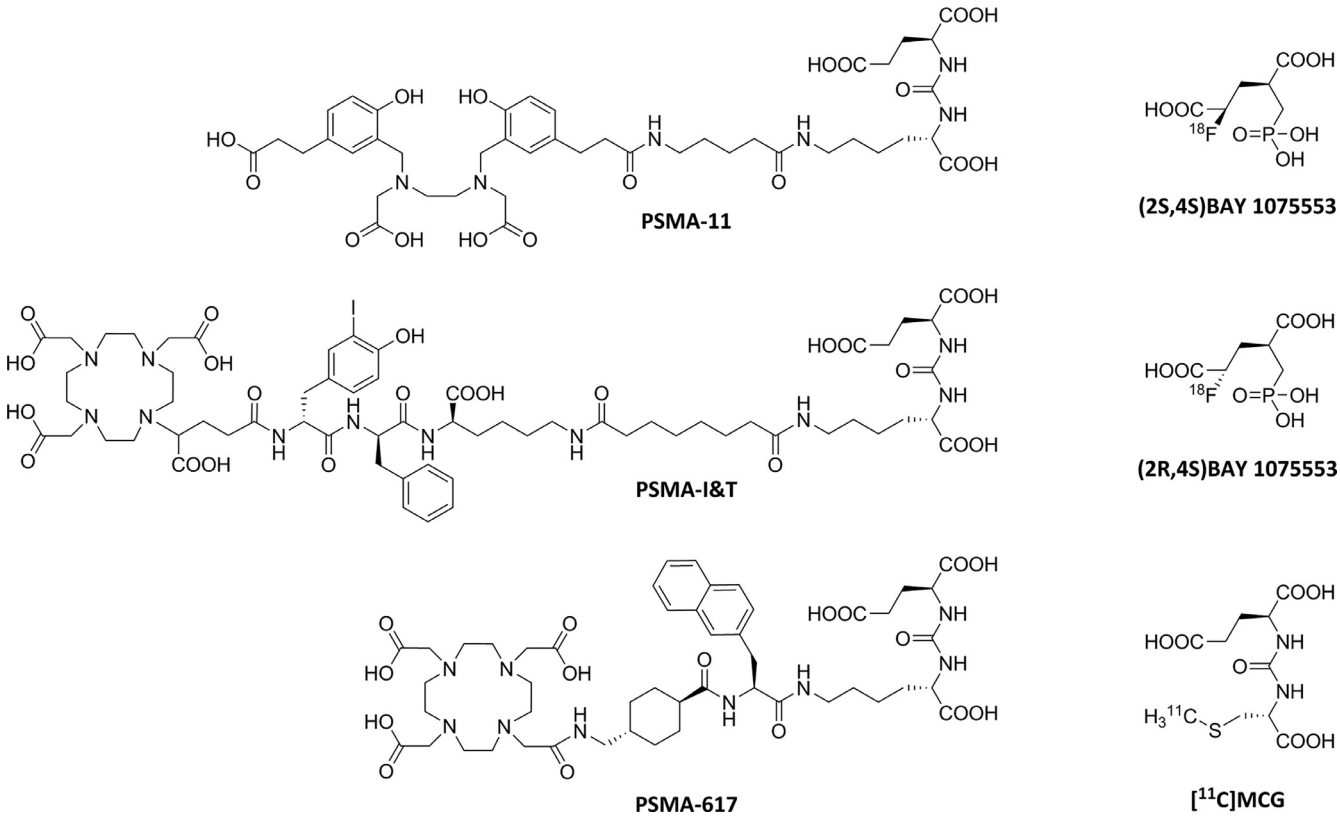

Figure 1 Selected small molecule-based PSMA-binding inhibitors: PSMA-11 for ^{68}Ga-labeling; the first theranostic tracers PSMA-I&T and PSMA-617 for labelling with ^{68}Ga, ^{177}Lu, ^{225}Ac, ^{111}In, and other radiometals suitable for complexation with DOTAGA- and DOTA-chelator; [^{18}F]BAY1075553, a 90/10 mixture of the (2S,4S)- and (2R,4S)-isomer, probably representing the smallest possible structure of a PSMA inhibitor, and [^{11}C]MCG, the first small molecule PSMA inhibitor for PET imaging (in mice and baboons).

PSMA-binding radiopharmaceuticals that have already found clinical application are discussed further.

Radiometallated PSMA-Targeted Small Molecules

Despite some earlier studies with radiolabelled PSMA tracers in humans, the development of ^{68}Ga-PSMA-11 (also called ^{68}Ga-DKFZ-PSMA-11 or ^{68}Ga-PSMA-HBED-CC) (Fig. 1) by Eder and Haberkorn[21] and the subsequent first clinical evaluation by Haberkorn and Afshar-Oromieh[22,23] at the German Cancer Research Centre and the University Hospital Heidelberg can be regarded as major milestone in the clinical transfer and utilisation of small molecule-based PSMA targeted probes for PET imaging of metastasized prostate cancer. PSMA-11 consists of a Glu-urea-Lys inhibitor motif conjugated with the highly efficient and Ga-specific acyclic chelator N,N′-*bis*[2-*hydroxy*-5-(*carboxyethyl*)*benzyl*]-ethylenediamine-N,N′- diacetic acid (HBED-CC) via an aminohexanoic acid spacer. Later it became clear that the HBED chelator in PSMA-11 with its two substituted phenol rings interacts with the remote arene-binding site on PSMA, while the corresponding DOTA analogue showed lower affinity and almost negligible internalization into PSMA$^+$ LNCaP cells. In addition, HBED can be labelled with ^{68}Ga at low concentrations even at room temperature. Thus, after its initial and successful preclinical evaluation, ^{68}Ga-PSMA-11 fulfilled all criteria for a highly promising PSMA targeted radiopharmaceutical and was consequently rapidly transferred into first proof-of-concept studies in men.[24,25]

A third compound coevaluated with PSMA-11 and its DOTA analogue by Eder et al.[21] consisted of the same EuK-inhibitor motif, coupled to a suberyl-N$_\varepsilon$-Lys-linker, followed by a Phe−Phe dipeptide for interaction with the arene binding side a terminal DOTA chelator. Similar to the DOTA analogue of PSMA-11, this compound had comparably low affinity and was only negligibly internalized into LNCaP cells.

Due to the excellent imaging results already obtained in the initial evaluation of [^{68}Ga]PSMA-11, we took up the challenge of a rapid development and clinical transfer of a therapeutic analogue. To overcome the limitations of the above mentioned EuK-sub-N$_\varepsilon$-Lys-Phe-Phe-DOTA compound and to improve the in vivo stability, the N$_\varepsilon$-Lys-Phe-Phe-residues were substituted by the corresponding D-amino acids, and DOTA was replaced by the DOTAGA chelator, thus introducing another negative charge at this part of the molecule. Based on these optimizations, the very first PSMA-targeted ^{177}Lu-radioligand therapy was successfully carried out in Bad Berka in April 2013.[26] Since DOTAGA also allows equally efficient radiolabelling with ^{68}Ga, this first theranostic PSMA-targeted compound was named PSMA I&T (Imaging and Therapy)[27,28] (Fig. 1), and was thereafter used in several centres, both for imaging with ^{68}Ga and radioligand therapy (RLT) with ^{177}Lu.[29-31]

Simultaneously, Benešová et al.[32,33] investigated and evaluated a series of DOTA-conjugated EuK-based inhibitors with optimized linker structure. Based on preclinical (affinity, internalization rate) and biodistribution data in LNCaP tumour bearing mice, EuK-Nal-tranexamyl-DOTA (PSMA-617) (Fig. 1) was selected as most promising agent. Compared to ^{177}Lu-PSMA I&T, ^{177}Lu-PSMA-617 showed significantly lower uptake in the kidneys of mice, while tumour uptake and overall bisdistribution in mice were quite similar.

While the low kidney uptake of PSMA-617 in comparison to PSMA I&T has often been referred in the literature as an important criterion and dosimetric advantage in subsequent studies in men, a direct comparison of both tracers in patients impressively demonstrated almost identical kidney clearance kinetics of ^{177}Lu-PSMA I&T and ^{177}Lu-PSMA-617 in men.[26] Whether this significant species differences between mice and men in the context of the kidney retention of PSMA-targeted tracers is generally valid, needs further investigation. Nevertheless, high kidney uptake of PSMA-targeted probes in mice should not be overvalued and regarded as an exclusion criterion for clinical translation.

A retrospective multicentric clinical trial on 145 patients with a total of 248 therapy cycles was started in 2015 to evaluate the efficacy and safety of ^{177}Lu-PSMA-617 in patients with metastatic prostate cancer.[34] The study demonstrated favourable safety and high efficacy that exceed those of other third-line systemic therapies in comparable patient cohorts. Based on these on further promising clinical data, Endocyte (USA) acquired the rights on PSMA-617 from ABX Biochemical Compounds (GER) in October 2017 to enter a phase III study with ^{177}Lu-PSMA-617 (phase III global VISION clinical trial in men with mCRPC). In October 2018, Novartis (CH) announced to plan a merger with Endocyte in early 2019. Thus, after the acquisition of Advanced Accelerator Applications by Novartis in October 2017, Novartis´ therapeutic PSMA-pipeline consists of at least two promising candidates, since AAA also inlicenced the theranostic PSMA-compound PSMA-SR6 with undisclosed structure from Johns Hopkins University in January 2016.

As already established for other peptide or peptide-like ligands, DOTA and DOTAGA-conjugated ligands can and are commonly labelled with a diversity of other radionuclides of potential interest, such as ^{44}Sc,[35-37] ^{68}Ga, ^{111}In,[38] Schottelius for imaging, ^{47}Sc, ^{90}Y,[39,40] ^{161}Tb[41] or ^{177}Lu for β-therapy or ^{225}Ac,[42-44] ^{213}Bi,[45,46] ^{149}Tb or ^{212}Pb[47] for α-therapy, to mention only a few. In some other cases, such as copper-labelled compounds, alternative and more kinetically stable complexes with for example, NOTA, CB-TE2A, PCTA and others are used.[48] Since especially the clinical studies with ^{225}Ac for targeted α-therapy of prostate cancer have been carried out with those tracers, that is, PSMA−617, that have also been used for ^{177}Lu-based β-therapy, we omitted to discuss these small molecules in this context again.

18F-Labelled PSMA-Targeted Small Molecules

[^{18}F]DCFBC (N-[N-[(S)-1,3-*dicarboxypropyl*]carbamoyl]-4-[^{18}]F-fluorobenzyl-L-cysteine)[49] (Fig. 2) was the first ^{18}F-labeled compound that has been successfully evaluated in men.[50-53]

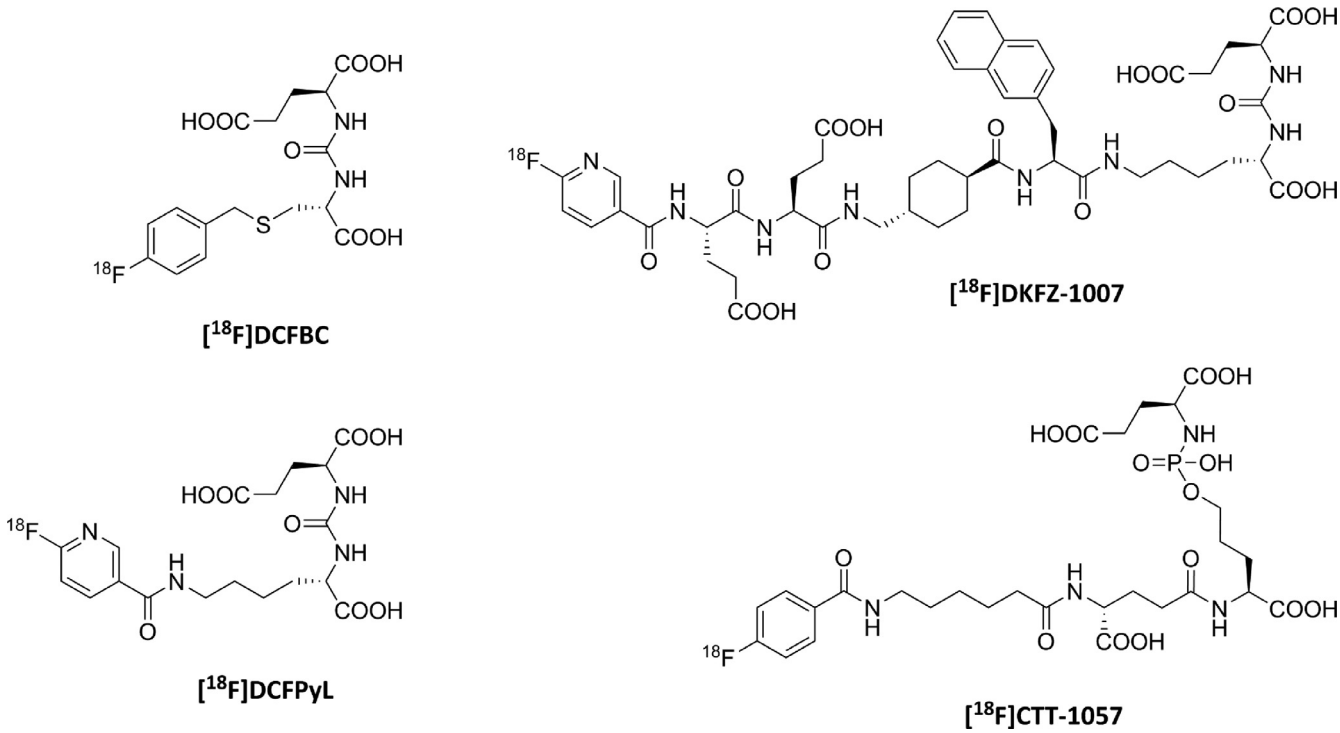

Figure 2 ^{18}F-labelled PSMA-inhibitors that have already been used for clinical imaging of patients with metastasized prostate cancer. [^{18}F]DCFBC with slow clearance kinetics and low tumour/background ratios; the second generation compounds with fast clearance: [^{18}F]DCFPyL (renal excretion, low activity in the liver, fast appearance of activity in the bladder), [^{18}F]DKFZ-1007 (predominantly renal excretion, higher liver uptake, no activity transfer to the bladder) and [^{18}F]CTT-1057 with pharmacokinetics quite similar to [^{18}F]DCFPyL.

Although some interesting studies have been carried out with this tracer, [^{18}F]DCFBC suffers from its slow blood clearance (high background activity) and associated limitations for early imaging with high sensitivity. These problems were overcome with next generation compound [^{18}F]DCFPyL (Fig. 2), which is hydrophilic and thus shows fast renal excretion.[54] In early 2019, 39 clinical studies with [^{18}F]DCFPyL were registered in the ClinicalTrials.gov database. First data from the OSPREY 2301 Study to examine the diagnostic performance of [^{18}F]DCFPyL to detect prostate cancer in pelvic lymph nodes in patients with high risk locally advanced prostate cancer as well as distant metastases in patients with metastatic or recurrent prostate cancer have been published in October 2018 by Progenics Pharmaceuticals (US). In a recently published direct comparison of [^{18}F]DCFPyL with [^{68}Ga]PSMA-11, [^{18}F]DCFPyL was found to perform equally well as ^{68}Ga-PSMA-11, while offering the advantages of large scale productions.[55] Noteworthy, PET images were acquired 1 hour p.i. with [^{68}Ga]PSMA-11 and 2 hours after injection of [^{18}F]DCFPyL, respectively.

Similar to [^{68}Ga]PSMA-11, [^{18}F]DCFPyL is rapidly cleared by renal excretion and thus also shows rapid appearance of activity in the ureters and the bladder.

[^{18}F]DKFZ-1007 (Fig. 2), a compound structurally related to PSMA-617, represents another tracer developed by the Group at the German Cancer Research Centre. Like PSMA-617, PSMA-1007 contains the same EuK-inhibitor motif conjugated with Nal, but the tranexamyl-linker is replaced by a 4-caboxy-benzylamine residue followed by two glutamic acid residues – to enhance the hydrophilicity – conjugated with 6-^{18}F-fluoronicotinic acid.[56] Whereas the initial synthesis consisted of the production of the activated ^{18}F-labelled prosthetic group that was subsequently coupled to the non-protected precursor, followed by HPLC-purification of the product (overall 1.5%-6.0% non-decay-corrected yield overall, 45 minutes),[56] an improved one-step production by direct ^{18}F-fluorination of the unprotected 6-trimetylammonium-nictotinyl precursor and subsequent cartridge separation was recently established.[57] Using the novel one-step procedure, the authors reported to be able to produce [^{18}F]PSMA-1007 in radiochemical yields from 25% to 80% in less than 55 minutes (batches up to 50 GBq).

In contrast to [^{68}Ga]PSMA-11 and [^{18}F]DCFPyL and despite predominant renal clearance, [^{18}F]DKFZ-1007 does not appear in the ureters and bladder within the imaging time interval. Compared to [^{18}F]DCFPyL, this advantage of [^{18}F]DKFZ-1007 comes at the price of a higher liver uptake, which is caused by its higher lipophilicity. In another study, [^{18}F]DCFPyL and [^{18}F]DKFZ-1007 were intra-individually compared in a cohort of 12 patients.[58] All patients were imaged 2 hours p.i. of about 250 MBq of [^{18}F]DCFPyL (six patients) or [^{18}F]PSMA-1007 (six patients), and were imaged 48 hours later under otherwise identical conditions with the second tracer. No statistically significant differences between the SUV_{max} of [^{18}F]DCFPyL and [^{18}F]PSMA-1007 in local tumour, lymph node metastases, and bone metastases were observed. Although excellent imaging quality was achieved with both tracers, [^{18}F]DCFPyL showed significantly higher

uptake in kidneys, urinary bladder, and unexpectedly also in lacrimal glands. In contrast, significantly higher uptake of [^{18}F]PSMA-1007 was found in muscle, submandibular and sublingual glands, spleen, pancreas, liver, and gallbladder. In addition the authors reported that the non-urinary excretion of [^{18}F]PSMA-1007 might offer some advantage with regard to delineation of local recurrence or pelvic lymph node metastasis in selected patients, whereas the lower hepatic background of [^{18}F]DCFPyL might favour this tracer in rare late stage cases with liver metastases.[58]

[^{18}F]CTT1057 (Fig. 2) is another ^{18}F-labelled radiopharmaceutical that has been licensed by pharmaceutical industry (February 2018, by Advanced Accelerator Applications) for further development in clinical trials. [^{18}F]CTT1057 is a phosphoramidate-based inhibitor for PSMA that showed high affinity and suitable in vivo characteristics in an experimental PSMA^{+} CWR22Rv1 animal model.[59,60] It has been selected from a series of compounds with different linkers between the terminal 4-[^{18}F]fluorobenzoyl residue and the inhibitor part. Docking studies revealed that an additional aminohexanoic residue in the linker part significantly enhance PSMA affinity by enabling the 4-[^{18}F]fluorobenzoyl prosthetic group to interact with the arene-binding groove of PSMA. Production of [^{18}F]CTT1057 is performed in a two-step process starting from the production of succinimidyl-4-[^{18}F]fluorobenzoate that is coupled to the primary amine precursor followed by purification (overall 95 minutes, 12 ± 5% decay corrected yield).[61] The phase I clinical trial for evaluation of the safety, pharmacokinetics, and ^{18}F-radiation dosimetry of [^{18}F]CTT1057 in 20 patients was completed in August 2018 and has been recently published.[62] The average total effective dose was 0.023 mSv/MBq. [^{18}F]CTT1057 was found to be a promising phosphoramidate PSMA-targeting PET radiopharmaceutical that demonstrates similar biodistribution to urea-based PSMA-targeted agents with potentially lower radiation exposure of the kidneys and salivary glands. Based on the imaging data presented and the unexpectedly slow excretion kinetics of [^{18}F]CTT1057, imaging at 90 minutes or later seems to be a prerequisite for high contrast imaging (low background). Similar to [^{18}F]DCFPyL, [^{18}F]CTT1057 exhibits almost exclusive and fast renal excretion with activity in the ureters and the bladder already a few minutes after injection.

It should also be mentioned here that PSMA-11 (PSMA-HED-CC) has also been used for ^{18}F-labelling[63] by means of [^{18}F]FAl^{2+} complexation and even automated syntheses has been developed.[64,65] The authors conclude that labelling of PSMA-11 is feasible, but needs careful control of the labelling and especially the formulation conditions. To the best of our knowledge, studies on the clinical evaluation of [^{18}F]AlF-PSMA-11 have not been published so far.

^{99m}Tc-Labelled PSMA-Targeted Small Molecules

The first ^{99m}Tc-labelled PSMA probe that was published in 2007 by Mishra et al.[66] was an adamantine trimerized phosphonate-based small molecule with 3 nM affinity binding to PSMA. The trimeric conjugate was labelled with ^{99m}Tc by means of a mercaptoacetyl-seryl-seryl-serine sequence conjugated as forth decoration unit to the adamantine core structure. Among many further developments, Babich and colleagues developed a series of novel and promising ^{99m}Tc/Re-tricarbonyl radiolabelled inhibitors of the glutamate-urea-lysine- (Glu-urea-Lys) and glutamate-urea-glutamate- (Glu-urea-Glu) type generated by coupling of pyridine and imidazole-based single amino acid chelates via spacers with different length to the pharmacophore.[67] In a subsequent comparative preclinical evaluation, the four most promising ^{99m}Tc-candidates (MIP-1404, MIP-1428, MIP-1427 and MIP-1405) (Fig. 3) were evaluated in vitro and in vivo.[68] Although all compounds were found to bind with high specificity and low nM affinity to PSMA, the two compounds with the carboxyl-rich TIM chelator (four carboxylates, MIP-1404 and MIP-1428) showed faster excretion and less unspecific binding when compared with the two compounds with the CIM chelator (with two carboxylates, MIP-1427 and MIP-1405). Based on this comparison, the authors concluded that ^{99m}Tc-MIP-1404 exhibited the best combination of high tumour uptake and rapid clearance from kidney and non-target tissues. Consequently, ^{99m}Tc-MIP-1404 was further evaluated in a series of clinical studies[69-74] that confirmed the high potential of this tracer for clinical SPECT imaging. Recently, a phase III clinical trial to evaluate the specificity of ^{99m}Tc-MIP-1404 imaging to identify patients without clinically significant prostate cancer and its sensitivity to identify patients with clinically significant disease by Progenics Pharmaceuticals was completed (Sept. 2018). The study dosed 471 patients in the US and Canada. ^{99m}Tc-MIP-1404 detected clinically meaningful prostate cancer with specificity ranging 71%-75%, the co-primary endpoint of sensitivity was not met.

In a very detailed investigation, Banerjee and colleagues at the Johns Hopkins University compared three different ^{99m}Tc-labelling methods ((a) [^{99m}Tc(CO)$_3$]$^{+}$ labelling, (b) N_xS_y-based chelating and ´classical´ agents with varying charge and polarity for the ^{99m}Tc-oxo core, and (c) a ^{99m}Tc-organo-hydrazine-labelled radioligand) and evaluated the effect of the chelator on the biodistribution and tumour uptake kinetics of 12 urea-based PSMA-targeted inhibitors.[75] Amongst these compounds, one of the ^{99m}Tc(I)-Tricarbonyl-labelled analogues provided the highest PSMA-specific tumour uptake and demonstrated the lowest retention in normal tissues including kidney 2 hours p.i..

After first successful evaluation of [^{111}In]PSMA I&T for radioguided surgery,[76–78] the Munich group initiated the development of a [^{99m}Tc]PSMA ligand to overcome the costly ^{111}In-labelling and to provide a ligand with improved characteristics for intraoperative detection of single and atypically localized lymph node metastasis by means of a small and sensitive handheld γ-probe. Based on the general ligand design of PSMA-I&T, PSMA-I&S (for Imaging and Surgery) (Fig. 3) was specifically modified and optimized for application as a PSMA-targeted intraoperative probe. This involved the substitution of the N-terminal DOTAGA chelator by

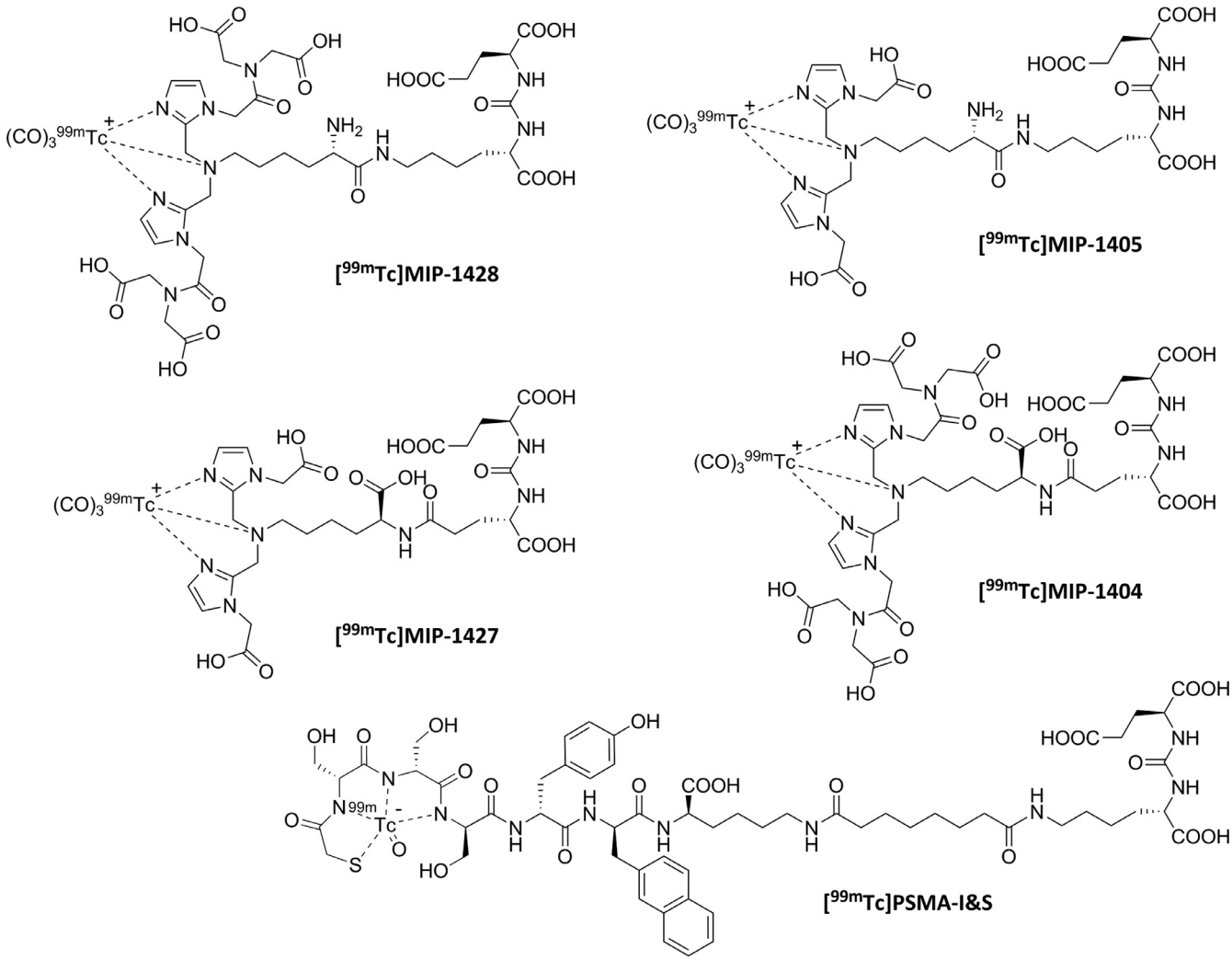

Figure 3 ^{99m}Tc-labelled PSMA-inhibitors MIP-1428, 1404, 1405 and 1427. MIP-1428 and MIP-1405 are Glu-urea-Lys-(=EuK) based inhibitors, whereas MIP-1427 and MIP-1404 are using the Glu-urea-Glu (EuE-) inhibitor structure. Due to its favourable properties in preclinical studies and proof of concept studies in men, [^{99m}Tc]MIP-1404 has been further evaluated in clinical trials.

mas_3 (mercaptoacetyl-(D-Ser)$_3$-) and the use of an alternative peptidic linker unit (y-nal-k instead of (3-iodo-tyr)-f-k). The all-D-amino acid chelator was used to further improve the metabolic stability of the ^{99m}Tc-chelate, and the alternative peptide linker was introduced to enhance interaction with the remote arene binding site[79] and to support plasma protein binding of [^{99m}Tc]PSMA-I&S and thus to delay blood clearance, with the aim of achieving maximal tracer uptake in the metastatic lymph nodes prior to surgery. Although this compound was originally designed to ideally match the typical workflow of a radioguided surgery (injection of the ^{99m}Tc-compound in the late afternoon; radioguided surgery in the early morning of the following day), [^{99m}Tc]PSMA-I&S has also found widespread application for SPECT Imaging at 3-5 hours p.i. with unexpectedly high contrast and imaging quality. [^{99m}Tc]PSMA I&S radioguided surgery has rapidly been established at various hospitals and was reported to be of high value for successful intraoperative detection and removal of metastatic lesions in PC patients scheduled for salvage surgery. Nevertheless, its long-term impact on outcome still needs to be evaluated.[80-82]

Radioiodinated PSMA-Targeted Small Molecules

The first radioiodinated PSMA tracer, urea-bridged 3-[^{125}I]iodo-L-tyrosine and glutamic acid, was developed by the Pomper group in 2005.[83] However, despite its higher affinity (1.5 nM), the tumour uptake of this first iodinated tracer was significantly lower than that of the co-evaluated [^{11}C]DCMC (3.1 nM; urea-bridged S-[^{11}C]methyl-L-cyctein and glutamic acid). In a further study, this group developed [^{125}I]DCIBzl (urea-bridged N^{ε}-4-[^{125}I]iodobenzoyl-L-Lys and L-Glu)[84] (Fig. 4). Due to its 10 pM affinity to PSMA, this ligand is extensively used as reference compound in competitive binding assays in the development of novel PSMA radiopharmaceuticals. In 2009, Babich and colleagues described and

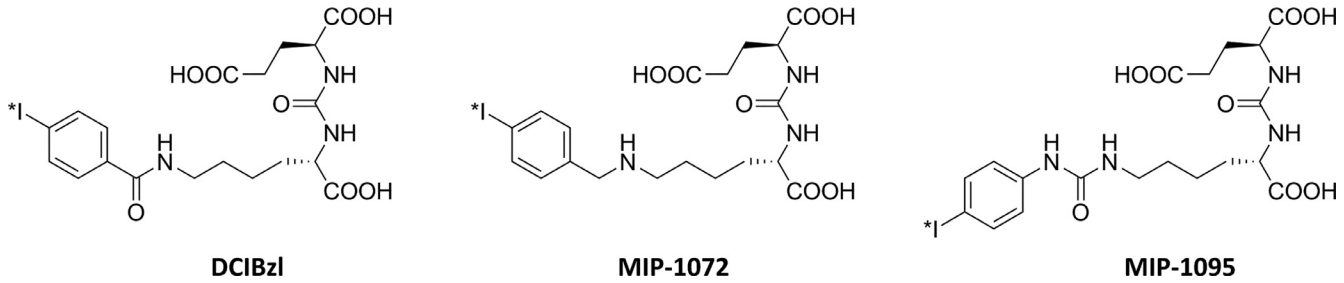

Figure 4 Iodinated PSMA-inhibitors DClBzl, MIP-1072 and MIP-1095. DClBzL is a compound with very high affinity to PSMA and thus is often used as ^{125}I-labeled reference compound in competitive binding assays. Based on the pharmacokinetics of both compounds in preclinical and first human studies, ^{123}I-labelled MIP-1072 was further evaluated as a diagnostic agent, whereas ^{131}I-labelled MIP-1095 has been evaluated for radioligand therapy.

evaluated a series of novel radiohalogenated benzyl- and phenylureido-conjugated Lys-urea-Glu-based PSMA ligands,[85] some of which have later been investigated in a variety of clinical investigations. Two of the most promising compounds from this series, MIP-1072 and MIP-1095 showed high affinity to PSMA (Ki values of 0.24 ± 0.14 and 4.6 ± 1.6 nM, respectively). Biodistribution studies in LNCaP tumour−bearing mice with ^{123}I−labelled versions of both compounds resulted in remarkable tumour uptake values of 17.4 ± 6.3%ID/g at 1 hour p.i. and 5.03 ± 1.35%ID/g at 24 hours for MIP-1072 and 34.3 ± 12.7%ID/g at 4 hour p.i. and 29.1 ± 15.1%ID/g at 24 hours for MIP-1095. Both compound were evaluated in clinical studies,[86] with MIP-1095 being the radioiodinated inhibitor of choice for subsequent therapy studies in men, whereas MIP-1072 has been further evaluated for SPECT imaging.[87] Unfortunately it was found, that the best therapeutic effect was achieved by the first therapy cycle with [^{125}I]MIP-1095 (PSA decline of ≥50% in 70.6% of the patients), while the subsequent therapy cycles were significantly less effective.[88]

New Concepts

Concepts to Decrease the Salivary Gland Uptake PSMA Inhibitors

To further improve targeted radioligand therapy of metastasized prostate cancer, some new concepts are under investigation. Salivary gland toxicity (dry mouth syndrome) is regarded as the main limitations during targeted α-RLT. To overcome these shortcomings, sialendoscopy with dilatation, saline irrigation and steroid injection,[89] intraparenchymal injections of botulinum toxin[90] and other drugs, external cooling of the salivary glands with icepacks and other methods have been investigated in patients with some, but limited success. It seems that not only inflammation, but also a direct effect of radiation is a potential cause of dry mouth after targeted α-therapy. It was recently reported, that intraperitoneal injection of up to 164 mg/kg monosodium glutamate reduces the uptake of ^{68}Ga-PSMA-11 in salivary glands and kidneys in LNCaP tumour-bearing mice,[91] while tumour uptake is almost unaffected. Although inhibition of kidney uptake with 2-phosphonomethylpentanedioic acid (PMPA) (Fig. 5) has already been investigated in mouse models,[92] Mayer and colleagues recently developed orally available prodrugs of (2-PMPA).[93] For their best candidate product JHU-2545 (Fig. 5), in which both the phosphonate and α-carboxylate are protected with isopropyloxycarbonyloxymethyl residues, the desired in vivo release of 2-PMPA after oral administration was demonstrated both in mice and dogs. In a first study in a small number of mCRPC patients[94] intravenous injection of 10 mg JHU-2545 15 minutes prior to injection of ^{177}Lu-PSMA-617 increased the metastases and/or salivary gland dose ratio to 350%-550% of control values, and the metastases and/or kidney dose ratio to between 190% and 650% of control. The authors conclude that, based on available dosimetry, JHU-2545 increases the cumulative allowable ^{177}Lu-PSMA617 dose by two to sixfold.[94]

Concepts to Increase the Overall Tumour Uptake by Plasma Protein Binding

Increasing the plasma protein binding of pharmaceuticals can be an effective strategy to decrease clearance rate while improving specific uptake of drugs.[95] For this purpose, conjugation of an albumin binding domain[96] or low-molecular-weight albumin binding moieties has been investigated to improve the tissue distribution of the corresponding biovectors.[96,97] Consequently, the use of PSMA-inhibitors with increased albumin binding and decelerated clearance kinetics has been suggested as promising approach to improve tumour uptake of therapeutic PSMA-ligands.[98-107] Among

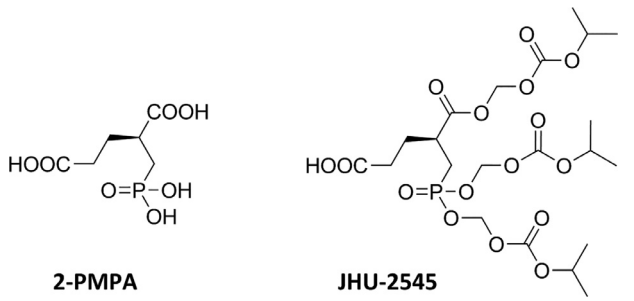

Figure 5 The PSMA inhibitor PMPA and the PMPA prodrug JHU-2545. With the aim to reduce activity uptake in the salivary gland and the kidneys, both compounds have been used for pretreatment prior to administration of radiolabelled PSMA inhibitors.

others, one approach currently investigated by several groups aims at exploiting the high albumin binding of Evans Blue and its analogues for the modulation of the clearance kinetics of therapeutic PSMA-inhibitors,[108] such as PSMA-617.[109] The improved pharmacokinetics of one of the modified PSMA-617 compounds, named EB-PSMA-617, resulted in significantly higher accumulation in PSMA+ PC3-PIP tumours and highly effective RLT. In a first proof-of-concept study in nine patients with mCRPC,[110] the number of disintegrations of all tumour lesions (MBq-h/MBq/g) in the ^{177}Lu-EB-PSMA-617 group was about 2.15-fold higher than that in patients receiving ^{177}Lu-PSMA-617, with the ^{177}Lu-EB-PSMA-617/^{177}Lu-PSMA-617-ratios of organ doses also being significantly increased (salivary glands: 5.1, kidneys: 6.1, osteogenic cells: 6.8 or red marrow: 6.5; ratios of the effective doses: 5.5). Further studies are required to demonstrate whether the positive effect of prolonged clearance kinetics on the tumour uptake of therapeutic PSMA inhibitors by enhanced albumin binding can increase the therapeutic window and thus the efficacy of RLT, or whether the parallel increase of undesirable tracer uptake in dose limiting tissues will generally overcompensate the enhanced tumour uptake.

Radiohybrid PSMA-Inhibitors

With the aim to provide a platform technology that allows for fast and efficient labelling of peptide and peptide-like radiopharmaceuticals such as PSMA inhibitors with either ^{18}F or radiometals, a unique and novel class of radiopharmaceuticals named radiohybrid PSMA-ligands (rhPSMA) has recently been developed, preclinically evaluated and transferred into clinical studies. A unique feature of rhPSMA-ligands is that these ligands contain both covalently bonded fluorine and a metal complex, with one of them alternatively being radioactive and the other non-radioactive (eg, [^{18}F][^{nat}Ga]rhPSMA or [^{19}F][^{68}Ga]rhPSMA, or [^{18}F][^{nat}Lu]rhPSMA or [^{19}F][^{177}Lu]rhPSMA). As chemically identical twins, rhPSMA ligands can be used as twins for PET-imaging with either ^{18}F or ^{68}Ga or to truly bridge imaging and therapy, for example, when applying the ^{18}F-twin for pretherapeutic PET imaging, dosimetry and/or therapy monitoring, while using the ^{177}Lu-twin for the corresponding RLT. Taking into account that application of recently developed [^{18}F][^{nat}Ga]rhPSMA-7.3 in >1600 patients with metastasized prostate cancer demonstrated excellent imaging properties, the corresponding [^{19}F][^{68}Ga]rhPSMA-7.3 tracer would allow to easily transfer this experience to ^{68}Ga-PSMA PET. Beyond these unique features, the large scale production of ^{18}F-labelled rhPSMA-ligands in clinical GMP environments is completed within <15 minutes [*citations to be added in the proofs*]. Radiohybrid PSMAs, developed at the Technical University Munich, have been licensed by Blue Earth Diagnostics (May 2019) for further clinical development.

Conclusions

During the last years, the development of PSMA-targeted pharmaceuticals has become one of the most active and dynamic radiopharmaceutical research fields. These academic research activities are paralleled and supported by heretofore unknown commitments of industrial companies and focused clinical development programs. Based on these activities it can be reasonably assumed that the first approved PSMA-targeted radiopharmaceutical, most probably a ^{99m}Tc- or ^{18}F-labelled PSMA-inhibitor, will appear within the next 2 years. From both a clinical and a chemical point of view, it seems justified to conclude that with respect to PSMA-targeted *imaging agents*, the limits of what is chemically feasible and necessary concerning further tracer improvements are virtually exhausted. In contrast, a 'second generation' of therapeutic agents with optimized pharmacokinetic profiles and lower uptake in non-tumour tissue could pave the way towards more efficient RLT strategies, for example, targeted α-therapy and combination therapies.

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