Preclinical evaluation of a tailor-made DOTA-conjugated PSMA inhibitor with optimized linker moiety for imaging and endoradiotherapy of prostate cancer

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Footline: Theranostic PSMA inhibitor
ABSTRACT

Despite many advances in the past years, the treatment of metastatic prostate cancer still remains challenging. In recent years, PSMA inhibitors were intensively studied in order to develop low molecular weight ligands for imaging prostate cancer lesions by PET or SPECT. However, the endoradiotherapeutic use of these compounds requires optimization with regard to the radionuclide-chelating agent and the linker moiety between chelator and pharmacophore which influence the overall pharmacokinetic properties of the resulting radioligand. In an effort to realize both detection and optimal treatment of prostate cancer, a tailor-made novel naphthyl-containing DOTA-conjugated PSMA inhibitor has been developed. **Methods:** The peptidomimetic structure was synthesized by solid-phase peptide chemistry and characterised using RP-HPLC and MALDI-MS. Subsequent $^{67/68}$Ga- and $^{177}$Lu-labeling resulted in radiochemical yields (RCY) of >97% or >99%, respectively. Competitive binding and internalization experiments were performed using the PSMA$^+$ LNCaP cell line. The in vivo biodistribution and dynamic small animal $\mu$PET imaging studies were investigated in BALB/c nu/nu mice bearing LNCaP xenografts. **Results:** The chemically modified PSMA inhibitor PSMA-617 demonstrated high radiolytic stability for at least 72 hours. A high inhibition potency ($K_i = 2.34 \pm 2.94$ nM on LNCaP; $K_i = 0.37 \pm 0.21$ nM enzymatically
determined) and highly efficient internalization into LNCaP cells were demonstrated. The µPET measurements showed high tumor-to-background contrasts as early as 1 hour post injection. Organ distribution revealed specific uptake in LNCaP tumors and in the kidneys 1 hour post injection. With regard to therapeutic use, the compound exhibited a rapid clearance from the kidneys from 113.3 ± 24.4 %ID/g at 1 h to 2.13 ± 1.36 %ID/g at 24 h. The favourable pharmacokinetics of the molecule led to tumor-to-background ratios of 1,058 (tumor/blood) and 529 (tumor/muscle), respectively, 24 hours post injection.

**Conclusion:** The here presented tailor-made DOTA-conjugated PSMA inhibitor PSMA-617 is sustainably refined and advanced with respect to its tumor-targeting and pharmacokinetic properties by systematic chemical modification of the linker region. Therefore, this radiotracer is suitable for a first-in-man theranostic application and may help to improve the clinical management of prostate cancer in the future.

**Keywords:** Prostate cancer, PSMA, theranostic radiopharmaceuticals, PET imaging, endoradiotherapy
INTRODUCTION

In western societies prostate cancer (PCa) continues to be the most common cancer in elderly men and the third most frequent cause of cancer-related mortality (1). Consequently, there is a high clinical demand for more effective treatment options in case of metastatic and hormone-refractory prostate cancer. Specific cancer targeting based on low molecular weight radioligands may offer a more accurate and rapid visualization, improved staging, and highly effective endoradiotherapy.

On the basis of small molecules, promising positron emission tomography (PET) radiotracers have been investigated in the last couple of years for the imaging of PCa such as $^{18}$F-fluoro- or $^{11}$C-choline (2,3), $^{18}$F-fluoro- or $^{11}$C-acetate, $^{11}$C-methionine as well as peptidyl radiotracers based on the gastrin-releasing peptide receptor (GRPr) and the Prostate-specific membrane antigen (PSMA) (4-6).

As PSMA is strongly expressed in PCa and upregulated in poorly differentiated, metastatic and hormone-refractory carcinomas (7,8) it represents a highly attractive target in Nuclear Medicine potentially meeting the clinical requirements for an effective therapy of metastatic prostate cancer. In particular urea-based peptidomimetic inhibitors of PSMA were investigated mainly for
diagnosis and shown to image advantageously PSMA-expressing prostate cancer (9-11). Due to low expression levels in healthy tissue, however, PSMA has additionally the potential for high dose endoradiotherapy with minimized radioactivity related side-effects.

An in vivo theranostic approach combines the potential of both diagnosis and therapy in one and the same targeting molecule by either labeling with a diagnostic or a suitable therapeutic radionuclide. The beta emitters such as $^{90}$Y, $^{131}$I and $^{177}$Lu are appropriate candidates for current systemic radionuclide therapy. Both $^{131}$I and $^{177}$Lu emit $\gamma$-radiation in addition to its $\beta^-$ particle, whereas $^{90}$Y is a pure $\beta^-$ emitter (12). Recently, the very promising therapeutic low molecular weight compound $^{131}$I-MIP-1095 was clinically investigated. In this study, twenty-eight men with metastatic castration resistant prostate cancer were treated with $^{131}$I-MIP-1095 (mean injected activity: 4.8 GBq). The treatment has shown a significant impact on the tumor lesions and PSA values and resulted in a reduction of bone pain. However, due to the high fraction of gamma radiation, patients were obliged to stay in the hospital for around one week. In addition mild hematological toxicities were observed (13). Endoradiotherapy with the aforementioned radiometals, however, bear a high potential to improve the clinical situation. For example $^{177}$Lu presents a lower
proportion of γ-radiation which would result in a reduced stay in the hospital and lower hemotoxicity in comparison to $^{131}$I (12).

Thus, a novel theranostic compound was designed consisting of three components: the pharmacophore Glutamate-urea-Lysine, the chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) able to complex both $^{68}$Ga or $^{177}$Lu, and a linker connecting these two entities. The linker turned out to be crucial for high imaging contrasts (14) as the chemical structure determines the internalization potency of the PSMA inhibitors (15). Besides the targeting properties, modifications of the linker might also influence the pharmacokinetic properties of peptidomimetic PSMA inhibitors and, therefore, improve their therapeutic potential (16). This study presents the preclinical evaluation of a tailor-made linker-optimized theranostic PSMA inhibitor with considerably optimized pharmacokinetic and targeting properties. Finally, the first individual clinical diagnostic experience with this novel radiotracer is shown underlining its clinical potential.

MATERIALS AND METHODS

All chemicals, reagents and solvents for the synthesis and analysis of the compound were analytical grade (for radiosynthesis ultrapure and metal-free),
purchased from Sigma Aldrich, Merck, Iris Biotech, or CheMatech and used without further purification.

The synthesis of PSMA-617 is summarized in Figure 1. The peptidomimetic Glutamate-urea-Lysine binding motif (step 1 – 6) and the linker was synthesized by solid-phase peptide chemistry as previously described (17).

The conjugation of the chelator was performed using DOTA-tris(tBu)ester (see supplemental material for detailed synthesis information). The final product (PSMA-617) was cleaved from the resin and deprotected.

The compound (~42% yield) was analyzed and purified by RP-HPLC (Reversed-phase High-performance Liquid Chromatography) and MALDI-MS (Matrix-assisted Laser Desorption/Ionization Mass Spectrometry) (see supplemental material for detailed method information).

$^{68}$Ga [$T_{1/2} = 68 \text{ min}, E_{\beta^+, \text{max}} = 1.9 \text{ MeV (88%)}, E_{\beta^-} = 2.5 \text{ MeV (22%)}, E_{\gamma} = 184 \text{ keV (24%)}, E_{\beta^-} = 296 \text{ keV (22%)}]$ was gained from a $^{68}$Ge/$^{68}$Ga generator based on a pyrogallol resin support (18) as $[^{68}\text{Ga}]\text{GaCl}_3$ in 0.1 M HCl. $^{67}$Ga [$T_{1/2} = 3.26 \text{ d}, E_{\gamma/x} = 94 \text{ keV (40%)}, 184 \text{ keV (24%)}, 296 \text{ keV (22%)}, E_{\beta^-} = 497 \text{ keV (79%)}, E_{\gamma/x} = 113 \text{ keV (6%)}, 208 \text{ keV (11%)}]$ was purchased from MDS Nordion as $[^{67}\text{Ga}]\text{GaCl}_3$ in 0.1 M HCl. $^{177}$Lu [$T_{1/2} = 6.71 \text{ days}, E_{\beta^-, \text{max}} = 497 \text{ keV (79%)}, E_{\gamma/x} = 113 \text{ keV (6%)}, 208 \text{ keV (11%)}]$ was obtained from PerkinElmer as $[^{177}\text{Lu}]\text{LuCl}_3$ in 0.05 M HCl.
Typically, 80–95 μL of 2.4 M HEPES (4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; pH 7.5) was mixed with 20 μL of $[^{68}\text{Ga}]{\text{Ga}}^{3+}$ eluate (~70 MBq) or 5 μL of $[^{67}\text{Ga}]{\text{GaCl}}_3$ (~50 MBq) and adjusted to pH 4.0 with 10–30% NaOH or 0.1 M HCl, respectively. Subsequently, 5 μL of 0.1–1 mM PSMA-617 (0.5–5 nmol) in 0.1 M HEPES was added. The reaction mixture was incubated at 95 °C for 15 min. Radiolabeling was performed without any separation of labeled and non-labeled compound. The specific activity for $[^{68}\text{Ga}]$PSMA-617 was in the range of 14–140 GBq/μmol and for $[^{67}\text{Ga}]$PSMA-617 in the range of 10–100 GBq/μmol. For $^{177}\text{Lu}$-labeling, 112 μL of sodium acetate (0.4 M; pH 5.0), 5 μL of $[^{177}\text{Lu}]{\text{LuCl}}_3$ (~20 MBq) and 5 μL of 0.1–1 mM PSMA-617 (0.5–5 nmol) in 0.1 M HEPES was mixed and incubated for 20 min at 95 °C. The specific activity was in the range of 4–40 GBq/μmol. The radiochemical yield (RCY) was determined by analytical RP-HPLC and RP-TLC (Reversed-phase Thin-layer Chromatography) on silica gel plates (60 RP-18 F_{254S}) with 0.1 M sodium citrate as a mobile phase.

The radiochemical stability of $^{67/68}\text{Ga}$-labeled PSMA-617 in PBS and human serum up to 72 hours at 37 °C was tested by RP-TLC. The lipophilicity was determined via the distribution of $^{67/68}\text{Ga}$-labeled PSMA-617 in the two-phase system n-octanol and HEPES buffer. The serum protein binding was analyzed by gel filtration (see supplemental material for detailed method information).
Both in vitro and in vivo experiments were performed using the PSMA+ LNCaP cell line (androgen-sensitive human lymph node metastatic lesion of prostatic adenocarcinoma, ATCC CRL-1740). The cells were supplemented with 10% fetal calf serum (FCS) and L-glutamine and incubated at 37 °C in an environment of humidified air containing 5% CO₂. For in vivo experiments, 8 week old BALB/c nu/nu mice were subcutaneously inoculated into the right trunk with 5 x 10⁶ LNCaP cells in 50% Matrigel. When the size of tumor was approximately 1 cm³, the radiolabeled compound was injected via the tail vein (ca. 30 MBq, 0.5 nmol for μPET imaging; ca. 1 MBq, 0.06 nmol for organ distribution).

The binding affinity of PSMA-617 was assayed by enzyme-based (NAALADase) and cell-based competitive assays. Data obtained from both experiments were fitted using a nonlinear regression algorithm (GraphPad Prism 5) in order to obtain IC₅₀ (50% inhibitory concentration).

The NAALADase assay is based on a competitive reaction of recombinant human PSMA (rhPSMA; R&D Systems) with non-labeled PSMA-617 and was performed as previously described (17) (see supplemental material for detailed method information).
Meanwhile PSMA-617 became commercially available (CAS No. 9933, PSMA-617, ABX advanced biochemical compounds, chemical characteristics were proved to be identical) and was additionally used as radioligand in the internalization assay. The assay was performed as described previously (19) (see supplemental material for detailed method information).

For μPET imaging with $^{68}$Ga-labeled PSMA-617, a 50 min dynamic scan and a static scan from 100 to 120 min p.i. were performed (see supplemental material for detailed method information).

For organ distribution, the animals were sacrificed after indicated time points (from 1 hour to 24 hours). The distributed radioactivity ($^{67/68}$Ga or $^{177}$Lu, respectively) was measured in all dissected organs and in blood using a gamma counter. The values are expressed as %ID/g.

The first clinical diagnostic study with [$^{68}$Ga]Ga-PSMA-617 PET/CT (1 h p.i.) was performed as previously published (20-22). The administered mass of [$^{68}$Ga]Ga-PSMA-617 was 2 µg. The administered activity was 288 MBq. There were no adverse or other clinically detectable pharmacological effects. No significant changes in vital signs were observed. The outcome from this study will be described in more detail in a further publication.
RESULTS

The chemical analysis is summarized in Table 1. PSMA-617 is stable for at least 6 months as a lyophilized fluffy white powder and also in a solution of DMSO-\textit{d6}, both at -20 °C, as shown by RP-HPLC and MALDI-MS. A precursor amount of 0.5 nmol was labeled with radiogallium with a RCY of >90 % in 15 min at 95 °C, for both $^{67}$Ga or $^{68}$Ga. \mu PET imaging was performed after purification by means of solid phase extraction using SepPak C18 cartridges (Waters). Higher amount of precursor (5 nmol) resulted in a RCY >97 %. Radiolabeling with $^{177}$Lu gained RCY >99 % at low amounts of precursor (0.5 µg, 0.5 nmol).

For determination of the radiolytic stability, PSMA-617 was radiolabeled with $^{68}$Ga or $^{67}$Ga and incubated at 37 °C for 1 or 24 hours, respectively, both in PBS and in human serum. [$^{68}$Ga]Ga-PSMA-617 showed a high stability after 1 hour in PBS and in human serum, respectively, as indicated by TLC. After 24 hours, values demonstrated 1 % of free activity in PBS and <4 % in human serum. Long-term stability with $^{177}$Lu did not reveal any free activity after 1 and 24 hours, neither in PBS nor in serum. After 48 hours <0.4%, and after 72 hours <0.6% of free $^{177}$Lu was measured in serum and <0.2% and <0.6% in PBS, respectively. Stability of the compound was also confirmed via gel filtration
with a Sephadex column. This run did not demonstrate any transfer of activity to human serum proteins after 1 hour incubation at 37 °C.

PSMA-617 revealed nanomolar affinity for PSMA on LNCaP cells ($K_i = 2.34 \pm 2.94 \text{ nM; } n=7$). In addition, the inhibition potency was determined for the $^{68}$Ga- and $^{177}$Lu-labeled PSMA-617, respectively. No significant difference in binding affinity was observed ($K_i = 6.40 \pm 1.02 \text{ nM for } ^{68}\text{Ga-complex; } K_i = 6.91 \pm 1.32 \text{ nM for } ^{177}\text{Lu-complex}$). The binding affinity determined by the enzyme-based NAALADase assay was subnanomolar ($K_i = 0.37 \pm 0.21 \text{ nM; } n=2$). $^{68}$Ga-labeled PSMA-617 was specifically internalized up to $17.67 \pm 4.34 \%\text{IA/10}^6 \text{LNCaP cells (n=3)}$ and $^{177}$Lu-labeled PSMA-617 up to $17.51 \pm 3.99 \%\text{IA/10}^6 \text{LNCaP cells (n=3)}$, both at 37°C.

The time-activity curves obtained from dynamic PET showed high tumor-to-muscle ratio (8.5) at 1 hour post injection (Figure 2A) as well as fast clearance from the kidneys followed by rapid accumulation of radioactivity in the bladder (Figure 2B).

The $\mu$PET slices (Figure 3A,B,C,D) reveal an increasing tumor uptake up to 2 hours post injection and a rapid elimination of radioactivity from other organs, muscles and blood. The rapid kidney excretion is also demonstrated by the
maximum intensity projection (MIP) scans (Figure 4A,B), whereby tumor uptake is maintained (see also Figure 2).

Organ distribution with $^{68}$Ga-labeled PSMA-617 after 1 hour ($n=3$; Figure 5A) revealed a high specific uptake in LNCaP tumors ($8.47 \pm 4.09 \%$ID/g; $0.98 \pm 0.32 \%$ID/g by coinjection of 2-PMPA) and in the kidneys ($113.3 \pm 24.4 \%$ID/g). The high uptake in the kidneys was nearly completely blocked ($2.38 \pm 1.40 \%$ID/g) by coinjection of 2 mg/kg of 2-PMPA. Other organs like liver ($1.17 \pm 0.10 \%$ID/g), lung ($1.41 \pm 0.41 \%$ID/g) and spleen ($2.13 \pm 0.16 \%$ID/g) showed rather low uptake and no blocking effect (data not shown), with the exception of spleen ($0.52 \pm 0.36 \%$ID/g). Tumor-to-background ratios were determined as 7.8 (tumor/blood) and 17.1 (tumor/muscle), respectively, 1 hour post injection.

As compared to the $^{68}$Ga-labeled version, the organ distribution with $^{177}$Lu-labeled PSMA-617 ($n=3$; Figure 5B) showed a similar uptake in the LNCaP tumors ($11.20 \pm 4.17 \%$ID/g; $0.64 \pm 0.07 \%$ID/g by coinjection of 2-PMPA) and in the kidneys ($137.2 \pm 77.8 \%$ID/g; $0.85 \pm 0.22 \%$ID/g by coinjection of 2 mg/kg of 2-PMPA). The uptake of radioactivity in lung ($0.78 \pm 0.17 \%$ID/g; not significant; $P=0.06$) and spleen ($2.98 \pm 1.32 \%$ID/g; not significant; $P=0.25$) demonstrated similar values as for $^{68}$Ga-labeled PSMA-617. The liver uptake
was found to be statistically different (0.22 ± 0.08 %ID/g; P<0.01). Tumor-to-background ratios determined 1 hour post injection showed slightly higher values (tumor/blood: 22.1; tumor/muscle: 25.6) compared to previous organ distribution with $^{68}$Ga-labeled PSMA-617.

Organ distribution with $[^{177}\text{Lu}]$Lu-PSMA-617 (n=3; Figure 5C) showed that the high initial kidney uptake is almost completely cleared (2.13 ± 1.36 %ID/g) within 24 hours while the tumor uptake remained high or even tends to slightly increase (10.58 ± 4.50 %ID/g; not significant P=0.55). Other organs such as liver (0.08 ± 0.03 %ID/g), lung (0.11 ± 0.13 %ID/g) and spleen (0.13 ± 0.05 %ID/g) demonstrated low uptake 24 h post injection. The favourable pharmacokinetics led to very high tumor-to-background ratios (tumor/blood: 1,058; tumor/muscle: 529) at 24 hours post injection.

Side-by-side comparison of $[^{68}\text{Ga}/^{177}\text{Lu}]$Ga/Lu-PSMA-617 ($^{177}\text{Lu}$ for 24 h biodistribution) with the $[^{67/68}\text{Ga}]$Ga-HBED-CC conjugated PSMA inhibitor PSMA-11 ($^{67}\text{Ga}$ for 24 h biodistribution) (17) is presented in Table 2 and 3 and in Figure 6. Major differences were observed in the spleen (2.13 ± 0.16 %ID/g and 17.88 ± 2.87 %ID/g; P<0.01), the kidneys (2.13 ± 1.36 %ID/g and 187.4 ± 25.3 %ID/g; P<0.01) and the tumor (10.58 ± 4.50 %ID/g and 3.20 ± 2.89 %ID/g; P=0.07) at 24 h p.i.. As compared to 1 h p.i., the kidney uptake of
[\textsuperscript{67}Ga]Ga-PSMA-11 was not significantly reduced after 24 h p.i. while [\textsuperscript{177}Lu]Lu-PSMA-617 was nearly completely cleared.

Figure 7 shows the encouraging first introductory human [\textsuperscript{68}Ga]Ga-PSMA-617-PET/CT imaging of a patient with a PSA level of 20 ng/mL presenting with multiple abdominal metastases. The lesions were clearly visualized with high tumor uptake and high tumor-to-background ratios already one hour after intravenous injection.

DISCUSSION

Once a metastatic prostate cancer becomes hormone-refractory there are only a few therapy options with rather poor clinical success left. According to the current medical guidelines, anti-mitotic chemotherapy with Docetaxel is typically recommended. However, the overall benefit for the patient is typically poor due to the reported side-effects and rather short time-to-progression with a reported improvement in overall survival of less than 2.5 months (23).

As a consequence, there is a high clinical demand for more effective, i.e. targeted, systemic therapy strategies. Targeted endoradiotherapy offers the possibility to treat the lesions in a specific and tumor-selective manner by addressing cell surface receptors mainly expressed on malignant cells. As
PSMA is highly expressed on the surface of metastatic and hormone-refractory prostate cancer cells and - besides the kidneys and salivary glands - nearly no expression in healthy tissue is found, a highly effective treatment can be expected by using radiolabeled urea-based small molecule inhibitors of PSMA. Besides many encouraging reports about the diagnostic clinical use of urea-based PSMA inhibitors, Zechmann et al. have recently shown promising therapeutic effects with $^{131}$I-labeled MIP-1095 in patients with hormone-refractory prostate cancer. The radiotracer has shown significant lesion uptake in all patients. The PSA values decreased in 60.7 % of men treated by >50 % and 84.6 % of men with bone pain showed complete or moderate reduction of pain (13).

Despite these highly encouraging preliminary clinical results further developments are necessary to optimize the effectivity of treatment and to minimize the reported side-effects. The combination of the commonly used chelating agent DOTA with the PSMA targeting inhibitors opens the possibility of using the same vector molecule for imaging and therapeutic purposes as DOTA effectively forms complexes with diagnostic ($^{68}$Ga) as well as therapeutic radiometals ($^{90}$Y or $^{177}$Lu). The half-life of $^{68}$Ga matches the pharmacokinetics of low molecular weight molecules with relatively fast diffusion, target localization and blood clearance (24). Therapeutic
radionuclides such as $^{177}$Lu allow scintigraphy and subsequent dosimetry with the same compound. In comparison to $^{131}$I, $^{177}$Lu presents a lower proportion of $\gamma$-radiation which results in a considerably reduced hospitalization which goes along with improved radiation protection and lowered hemotoxicity (25).

In general, theranostics based on radiometals offer targeted and personalized treatment for the patient while minimizing the damage of healthy tissues. From an economic point of view, in vivo theranostics yield an improved outcome for cancer patients, clinical trials can be designed more effectively and efficiently, and finally, individual costs for cancer treatment will be reduced.

Recently, $^{68}$Ga-labeled Glu-urea-Lys-(Ahx)-HBED-CC ([$^{68}$Ga]Ga-PSMA-11) proved to be a very successful tracer for PET-imaging of recurrent prostate cancer (17). It was reported that the chelator HBED-CC seems to interact advantageously with the lipophilic part of the PSMA binding pocket (17,22). However, due to the high selectivity of the complexing agent HBED-CC for $^{68}$Ga the radiotracer is not suitable for radiolabeling with therapeutic radiometals such as $^{177}$Lu or $^{90}$Y. The here aimed linker region is designed in order to elucidate the structure-activity relationships (SAR) and to mimic the proven additional biological interactions of HBED-CC with the binding pocket. Thus, an ideal linker length (26), polarity (27), size, flexibility (28), presence of
aromatic groups (26) and/or hydrophobic functionality distal to the glutamic acid moiety (29) represents an effective strategy to design highly potent PSMA inhibitor based radiotracers.

Based on these considerations, a DOTA-conjugate of a PSMA inhibitor with optimized targeting properties has been designed in this study. The resulting \([^{68}\text{Ga}]\text{Ga-PSMA-617}\) exhibits high binding affinity for PSMA and is effectively internalized. Thus, the biological properties of the novel tracer were significantly improved in comparison with \([^{68}\text{Ga}]\text{Ga-PSMA-11}\) (17). Moreover, dynamic μPET imaging and organ distribution of \([^{68}\text{Ga}]\text{Ga-PSMA-617}\) showed an early enrichment in the bladder (6 min p.i.) and also the maximum kidney uptake was reached as early as 15 min after injection and diminishes substantially already after 20 min, while it is further accumulated and retained in the tumor. The favourable pharmacokinetics led to high tumor-to-background ratios (tumor/blood: 1,058; tumor/muscle: 529) with the \(^{177}\text{Lu}\)-labeled version 24 hours post injection. In contrast to the clinical PET tracer \([^{68}\text{Ga}]\text{Ga-PSMA-11}\) (21), the initial high kidney uptake was nearly completely cleared 24 h post injection.

Taken together, the here presented studies of PSMA-617 disclose that the presence of the naphthylic linker has a significant impact on the tumor-targeting
and biological activity as well as on imaging contrast and pharmacokinetics, properties which are crucial for both high imaging quality and efficient targeted endoradiotherapy. With regard to therapeutic use, the high binding affinity and internalization, prolonged tumor uptake, rapid kidney clearance and high tumor-to-background ratio give clear clinical advantages for PSMA-617 compared to previously published DOTA-based PSMA inhibitors (11,30). In comparison to PSMA-11 (21), PSMA-617 seems to be more attractive for endoradiotherapy due to its higher tumor uptake at later time points, lower spleen accumulation and the highly efficient clearance from the kidneys.

The first individual clinical diagnostic experience with $^{68}$Ga-labeled PSMA-617 is comparable with the recently introduced exclusive PET tracer $[^{68}\text{Ga}]$Ga-PSMA-11. The fast kidney clearance shown with $[^{177}\text{Lu}]$Lu-PSMA-617 encourages us to transfer the compound into the clinical scenario with the aim of a first individual endoradiotherapeutic study. In this connection, a comprehensive first-in-man clinical evaluation of $[^{177}\text{Lu}]$Lu-PSMA-617 is underway (31).

CONCLUSION
Theranostic radiotracers provide patients with targeted and personalized treatment options thereby improving the current clinical treatment options at the same time. The compound PSMA-617 exhibits high PSMA-specific tumor uptake, rapid background clearance as well as fast kidney excretion which gives clear clinical advantages for both high imaging quality and efficient endoradiotherapy of recurrent prostate cancer.

ACKNOWLEDGMENTS

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References


Figure 1: Synthesis of the DOTA-conjugated PSMA inhibitor PSMA-617.
Figure 2: Time-activity-curves for tumor and background (A) and for relevant organs (B) up to 1 h post injection of 0.5 nmol of $^{68}$Ga-labeled PSMA-617. Data are expressed as mean standardized uptake value (SUV).
Figure 3: Whole-body coronal slices from µPET imaging of an athymic male nude mouse bearing LNCaP tumor xenografts. The tumor-targeting efficacy and pharmacokinetic properties were evaluated by injection of 0.5 nmol of the $^{68}$Ga-labeled PSMA-617 (~30 MBq) with following scans after 0-20 min (A), 20-40 min (B), 40-60 min (C) and 120 min (D) p.i..
Figure 4: Whole-body coronal scans as MIP 60 min (A) and 120 min (B) p.i. of $^{68}\text{Ga}$-labeled PSMA-617.
Figure 5: Organ distribution of 0.06 nmol $^{68}$GaGa-PSMA-617 at 1 h p.i. (A), and 0.06 nmol $^{177}$LuLu-PSMA-617 at 1 h p.i. (B) and 24 h p.i. (C), respectively. The specificity was demonstrated by co-injection of 2 mg/kg body weight of 2-PMPA. The
uptake in murine kidneys and in tumor proved to be PSMA-specific. Data is expressed as %ID/g tissue ± SD (n=3).
Figure 6: Organ distribution expressed as %ID/g tissue ± SD (n=3) of 0.06 nmol of 
$[^{68}\text{Ga}]\text{Ga-PSMA-617}$ and $[^{68}\text{Ga}]\text{Ga-PSMA-11}$ 1 h p.i. (A) and $[^{177}\text{Lu}]\text{Lu-PSMA-617}$ and $[^{67}\text{Ga}]\text{Ga-PSMA-11}$ 24 h p.i. (B).
Figure 7: $[^{68}\text{Ga}]\text{Ga-PSMA-617}$ PET/CT (1 h p.i.) demonstrating a very first patient with multiple lymph node metastases. Red arrows point to a representative lesion with a SUVmax of 36.5 and a tumor-to-background ratio of 52.1 one hour p.i.: (A) Contrast enhanced CT, (B) maximum intensity projection (MIP) of the PET scan 1 h p.i., (C) fusion of PET scan and contrast enhanced CT.
Table 1: Analytical data of compound PSMA-617

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<th>$t_r^†$ [min]</th>
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*Retention time of non-labeled ligand on analytical HPLC; †Retention time of $[^{67/68}\text{Ga}]\text{Ga-PSMA-617}$; ‡Retention time of $[^{177}\text{Lu}]\text{Lu-PSMA-617}$; §Mass spectrometry of the non-labeled ligand detected as [M+H]$^+$. 
Table 2: PSMA inhibition potency ($K_i$) of $^{68}$Ga-labeled PSMA-617 and PSMA-11.

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<th>Compound</th>
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<td>[$^{68}$Ga]Ga-PSMA-11</td>
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<td>[$^{68}$Ga]Ga-PSMA-617</td>
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*Data are expresses as mean ± SD (n=3 for PSMA-11; n=7 for PSMA-617).
Table 3: Cell surface binding and internalization of PSMA-617 and PSMA-11.

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<tr>
<th>Compound</th>
<th>Cell surface* [%IA/10⁶ cells]</th>
<th>Lysate* [%IA/10⁶ cells]</th>
</tr>
</thead>
<tbody>
<tr>
<td>[⁶⁸Ga]Ga-PSMA-617</td>
<td>14.81 ± 8.67</td>
<td>17.67 ± 4.34</td>
</tr>
</tbody>
</table>

*Data are expresses as mean ± SD (n=3).