68Ga- and 177Lu-labeled PSMA I&T: Optimization of a PSMA targeted theranostic concept and first proof of concept human studies

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ABSTRACT

Based on the high and consistent expression of prostate-specific membrane antigen (PSMA) in metastatic prostate cancer (PC), the goal of this study was the development, preclinical evaluation and first proof of concept investigation of a PSMA-inhibitor for imaging and therapy (PSMA I&T) for $^{68}$Ga-based positron emission tomography (PET) and $^{177}$Lu-based endoradiotherapeutic treatment in patients with metastatic and castration resistant disease.

Methods

PSMA I&T was synthesized in a combined solid phase and solution chemistry strategy. The PSMA-affinity of $^{nat}$Ga/$^{nat}$Lu-PSMA I&T was determined in a competitive binding assay using LNCaP cells. Internalization kinetics of $^{68}$Ga- and $^{177}$Lu-PSMA I&T were investigated using the same cell line, and biodistribution studies were performed in LNCaP-tumor bearing CD-1 nu/nu mice. Initial human PET imaging studies using $^{68}$Ga-PSMA I&T, as well as endoradiotherapeutic treatment of two patients with metastatic PC using $^{177}$Lu-PSMA I&T were carried out.

Results

PSMA I&T and its cold gallium and lutetium analog revealed nanomolar affinity towards PSMA. The DOTAGA-conjugate PSMA I&T allowed fast and high-yield labeling with $^{68}$Ga$^{III}$ and $^{177}$Lu$^{III}$. Uptake of $^{68}$Ga/$^{177}$Lu-PSMA I&T in LNCaP tumor cells is highly efficient and PSMA-specific, as demonstrated by competition studies both in vitro and in vivo. Tumor targeting and tracer kinetics in vivo were fast, with the highest uptake in tumor xenografts and kidneys (both PSMA specific). First human $^{68}$Ga-PSMA I&T PET imaging allowed high contrast detection of bone lesions, lymph node and liver metastases. Endoradiotherapy with $^{177}$Lu-PSMA I&T in two patients was found to be effective and safe with no detectable side effects.

Conclusion

$^{68}$Ga-PSMA I&T shows potential for high-contrast PET imaging of metastatic PC, while its $^{177}$Lu-labeled counterpart exhibits suitable targeting and retention characteristics for successful endoradiotherapeutic treatment. Prospective studies on larger cohorts of patients are warranted and planned.
Key words (3-5): Prostate-specific membrane antigen, PSMA I&T, $^{68}$Ga, $^{177}$Lu, prostate cancer
INTRODUCTION

Besides radiopharmaceuticals that address metabolic processes, approaches focusing on disease specific targets (1, 2) are increasingly important for cancer diagnosis and treatment with radiopharmaceuticals. On PC cells, the cell surface enzyme prostate-specific membrane antigen (PSMA), also known as glutamate carboxypeptidase II or N-acetyl-L-aspartyl-L-glutamate peptidase, is highly up regulated, whereas it shows low or no expression in the normal prostate (3). PSMA expression correlates with the malignancy of the disease, being further increased in metastatic and hormone refractory patients (4). As a consequence, PSMA has attracted the attention as a target for molecular imaging as well as for targeted radioligand therapy, especially in metastatic castrate resistant prostate cancer (mCRPC).

For imaging of PC, a variety of selective small molecule PSMA-inhibitors, labeled with a broad range of radionuclides for PET and single-photon emission computed tomography (SPECT), have been evaluated preclinically in recent years (5-13). Amongst these, ^18^F-DCFBC (N-[N-[S]-1,3-dicarboxypropyl]carbamoyl)-4-^18^F-fluorobenzyl-L-cysteine) (7), ^123^I-MIP-1072 ((S)-2-(3-((S)-1-carboxy-5-(4-iodobenzylamino)pentyl)ureido)pentanedioic acid) and ^123^I-MIP-1095 ((S)-2-(3-((S)-1-carboxy-5-(3-(4-iodophenyl)ureido)pentyl)ureido)pentanedioic acid) (9), Glu-NH-CO-NH-Lys(Ahx)-^68^Ga-HBED-CC (9), and ^99m^Tc-MIP-1404 ((7S,12S,16S)-1-(1-((2-(bis(carboxymethyl)amino)-2-oxoethyl)-1H-imidazol-2-yl)-2-((1-((2-(bis(carboxymethyl)amino)-2-oxoethyl)-1H-imidazol-2-yl)methyl)-9,14-dioxo-2,8,13,15-tetraazaoctadecane-7,12,16,18-tetracarboxylic acid; (12)) have already been successfully applied in first patient PET or SPECT studies (14-17). Despite certain differences between these ligands in overall pharmacokinetics, all these compounds allow sensitive detection of PC lesions, thus improving imaging and therapy planning.

As long as the disease is restricted to the prostate, surgery and radiation therapy exhibit high efficacy (18) in therapy of PC. Treatment options for PC patients with metastatic disease are androgen deprivation therapy and chemotherapy (19). However, these therapies cause potentially serious adverse effects. The calcium mimetic α-emitter ^223^Ra-radium dichloride (Xofigo, Bayer AG) was recently approved. Xofigo is indicated in patients with mCRPC that has spread to bones (improved overall survival by 3.6 months) (20). Thus, there is a major need for additional therapeutic options for patients with soft tissue metastasizing disease.
In-Capromab pendetide (ProstaScint, Cytogen Corporation) is a FDA-approved murine monoclonal antibody for radioimmunoscintigraphy in PC patients (21), which is directed against the intracellular domain of PSMA. To increase uptake and sensitivity (22), antibodies against the extracellular domain, e.g. J591, its humanized analog huJ591 and a minibody version of huJ591 have been developed. For radioimmunotherapy J591 was labeled with β-emitters (90Y, 177Lu) and evaluated in patients with mCRPC (23). Using 177Lu-J591, patient survival could be prolonged for 9.9 months (24).

In a first step towards the development of small molecule PSMA-targeting theranostics with fast pharmacokinetics and high PSMA affinity, we recently introduced (3S,7S)-29,32-dibenzyl-5,13,20,28,31,34-hexaaxo-37-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)-4,6,12,21,27,30,33-heptaazaheptatriacontane-1,3,7,26,37-pentacarboxylic acid (DOTAGA-FFK(Sub-KuE) (13); Fig. 1). In an initial study in patients with mCRPC (25, 26), 177Lu-DOTAGA-FFK(Sub-KuE) administration led – in some patients – to a significant reduction in metastatic tumor load. In animal studies higher metabolic stability and thus improved overall pharmacokinetics of PSMA-inhibitors was achieved by substitution of the L-amino acid linker part (FFK) between the inhibitor and the chelator (13). Thus, the present study was focused on the further optimization of this second-generation theranostics tracer concept by exploiting the potential of the peptidic linker unit to enhance the PSMA-affinity by increasing the lipophilic interaction of the tracer with the PSMA enzyme (8, 27). To this aim, DOTAGA-(I-y)fk(Sub-KuE), termed “PSMA I&T” (for Imaging and Therapy; Fig. 1), was developed and evaluated in detail, both in vitro and in vivo. Based on the promising preclinical data obtained for 68Ga/177Lu-PSMA I&T and to highlight the potential of 68Ga- and 177Lu-PSMA I&T, initial proof of concept in humans is described.
MATERIALS AND METHODS

General
All animal experiments were conducted in accordance with German Animal Welfare Act (Deutsches Tierschutzgesetz, approval #55.2-1-54-2532-71-13). All human studies were approved by the institutional review boards of the participating medical institutions. Patients provided signed informed consent.

Synthesis and radiolabeling
PSMA I&T and its gallium and lutetium complexes were synthesized according to a previously published protocol (13). The synthesis as well as a detailed description of the $^{68}$Ga- and $^{177}$Lu-labeling conditions is given in the supplementary information. The radioiodinated reference ligand ((S)-1-carboxy-5-(4-(-$^{125}$I-iodo-benzamido)pentyl)carbamoyl)-l-glutamic acid (($^{125}$I-BA)KuE) was prepared as described previously (13).

In vitro evaluation
The PSMA$^+$ LNCaP cells (CLS: 300265) were grown in Dulbecco’s modified Eagle medium/Nutrition Mixture F-12 with Glutamax-I (1:1) (DMEM/F-12) (Invitrogen, Germany) supplemented with 10% fetal calf serum. Cultures were maintained at 37 °C in a humified 5% CO$_2$ atmosphere. One day prior to the experiment, cells were harvested using trypsin/ethylenediaminetetraacetate (0.05%/0.02%) in phosphate-buffered saline (PBS), centrifuged and resuspended in culture medium, counted and seeded in 24-well plates.

* Determination of PSMA affinity: The $IC_{50}$ values were determined in a competitive binding assay using LNCaP cells (1.5 * $10^5$ cells in 1 mL/well) and ($^{125}$I-BA)KuE as radioligand as described previously (13). Quantification of the amount of free and bound activity was performed in a $\gamma$-counter (Wallac 1480 WIZARD TM 3™, Perkin Elmer, Inc, Waltham, MA, USA). $IC_{50}$ values were calculated using PRISM 6 software (Graph Pad Software, San Diego, CA).
Cell binding kinetics: Cell binding and internalization kinetics were determined as reported (13). In brief, 1.25 * 10^5 LNCaP cells in poly-l-lysine-coated (PLL) 24-well plates were incubated with radiolabeled PSMA I&T (0.2 nM for ^68^Ga- and 0.5 nM for ^177^Lu-labeled ligands) at 37 °C for 5, 15, 30 and 60 min. The tracers were also incubated in the presence of 10 µM 2-(phosphonomethyl)pentane-1,5-dioic acid (PMPA) solution (blocking) and parallel experiments using the external reference ligand (^125^I-BA)KuE (0.2 nM) were carried out. The amount of radioactivity in the supernatant, PSMA-specifically surface bound (incubation in 250 µL 10 µM PMPA for 10 min at 4 °C) and in the cells (lysed with 250 µL 1 M NaOH) was quantified in a γ-counter.

In vivo evaluation

Animal model: To induce tumor growth, LNCaP cells (app. 10^7 cells/200 µL) were suspended 1/1 in DMEM/F-12 and matrigel (BD Biosciences, Germany) and were inoculated subcutaneously onto the right shoulder of CD-1 nu/nu mice (6-8 weeks, Charles River Laboratories). After 2-4 weeks (males) and 4-6 weeks (females), respectively, tumors had reached 4-8 mm in diameter, and the animals were used for experiments.

Dual tracer biodistribution studies: Both ^68^Ga-PSMA I&T (4.7-6.3 MBq) and ^177^Lu-PSMA I&T (1.7-2.0 MBq) were coinjected into the tail vein of LNCaP-tumor bearing male mice under isoflurane anesthesia. The total injected peptide amount was kept constant at 0.2 nmol in all experiments. At 1 h p.i., the mice were sacrificed, the organs of interest were dissected, and the activity in weighed tissue samples was immediately quantified (^68^Ga). After the decay of ^68^Ga (next day) quantification of ^177^Lu was performed in a γ-counter.

Small animal PET imaging: Imaging studies were performed at a Siemens Inveon small animal PET scanner. For data analysis the Inveon Research Workplace software was used. Into the tail vein of female animals under isoflurane anesthesia 14-18 MBq (0.2 nmol) ^68^Ga-PSMA I&T were injected. Dynamic imaging was performed for 1.5 h after on-bed injection. Static images were recorded at 1 h p.i. with an acquisition time of 15 min. Images were reconstructed using 3D ordered-subsets expectation maximum (OSEM3D) algorithm without scanner and attenuation correction.

^68^Ga/^177^Lu-PSMA I&T
Dosimetry calculation

Female wild type CD-1 mice were injected with 1.3-1.5 MBq $^{177}$Lu-PSMA I&T (33.6-38.7 pmol), sacrificed at 1, 6, 12, 24, 48 and 96 h p.i. (n = 5, respectively). Organs of interest were dissected and the activity in weighed tissue samples was quantified in a $\gamma$-counter. Using the uptake data at different time points an extrapolation of the absorbed doses to humans was performed. The dose extrapolation to humans involved the scaling of the time-integrated activity coefficients and the subsequent calculation of the absorbed doses from the animal biodistribution data using two different methods. Time-integrated activity coefficients were calculated using the software solution NUKFIT (28). The dose calculation was performed for a selected group of organs using OLINDA/EXM V1.1 (29). Details on the methodology used for extrapolating the mouse data to humans are provided in the supplementary information.

$^{68}$Ga-PSMA I&T PET imaging in patients

The patient underwent PET/CT imaging (Biograph mCT PET/CT, Siemens Medical Solution AG) 60 min after intravenous (i.v.) administration of 133.2 MBq $^{68}$Ga-PSMA I&T. CT and reconstruction details are given in the supplementary file. Circular regions of interest (ROIs) were drawn around areas with increased uptake in transaxial slices for calculation of the maximum standardized uptake value ($SUV_{max}$). ROIs were automatically adapted to a three dimensional volume of interest with Syngovia™ (Siemens Medical Solutions, Erlangen, Germany) at a 40% isocontour.

Patient 1, 70 years of age, was diagnosed with PC in 2011 with an initial Gleason score of 10 (5+5). The patient had initially undergone palliative transurethral resection of the prostate, followed by androgen deprivation therapy using abiraterone acetate. Further treatment with docetaxel plus prednisolone was initiated after development of mCRPC with multiple bone metastases displayed by bone scan. The serum total PSA level at the time of imaging was 10.1 ng/mL.
Endoradiotherapy of patients using $^{177}$Lu-PSMA I&T

Two patients with mCRPC were assessed before the $^{177}$Lu-PSMA I&T therapy by $^{68}$Ga-PSMA-HBED-CC PET/CT imaging (Biograph mCT Flow 64, Siemens Medical Solutions AG). Contrast-enhanced PET/CT was performed 1-5 days prior to endoradiotherapy and for follow-up at 65 ± 4 minutes after i.v. administration of 170 ± 23 MBq $^{68}$Ga-PSMA-HBED-CC. Both patients with progressive mCRPC underwent therapy with 5.7 and 8.0 GBq $^{177}$Lu-PSMA I&T, respectively, administered intravenously over 15 min. Complete blood counts, parameters of renal function (serum creatinine, blood urea nitrogen) and liver function (albumin, bilirubin, enzymes), as well as tubular extraction rate measured by $^{99m}$Tc-mercaptoacetyltriglycine scintigraphy were documented before and after therapy. Response to therapy was assessed by $^{68}$Ga-PSMA-HBED-CC PET combined with contrast-enhanced CT 8-10 weeks post-therapy. In addition, biochemical response was documented by means of prostate specific antigen (PSA) monitoring.

Patient 2 was a 68-year-old man with progressive metastatic prostatic adenocarcinoma (Gleason Score 7) and multiple mediastinal lymph node metastases. The 54-year-old patient 3 with adenocarcinoma of the prostate (Gleason Score 9: 4+5) status post hormonal therapy and external beam radiation therapy, presented with progressive mediastinal and retroperitoneal lymph node metastases as well as multifocal osseous lesions.
RESULTS

Synthesis and radiolabeling

PSMA I&T was synthesized in accordance with the protocol described for DOTAGA-ffk(Sub-KuE) (13) and was obtained in 32.4% yield (based on DOTAGA-(I-y)fk) and > 99% purity (220 nm).

For cell studies, manual $^{68}$Ga-labeling of PSMA I&T (3 nmol) resulted in a specific activity of 250-300 GBq/µmol, whereas for animal studies fully automated $^{68}$Ga-labeling (5 nmol) yielded $^{68}$Ga-PSMA I&T in specific activities of 80-120 GBq/µmol. For quantitative $^{177}$Lu-complexation, 24.5 MBq $^{177}$LuIII was reacted with a 4.5-fold molar excess of PSMA I&T yielding $^{177}$Lu-PSMA I&T in specific activities of $\geq$ 27 GBq/µmol.

Tracers for patient application were prepared using a fully automated synthesis module and were obtained in radiochemical yields of 67 ± 10% (non-decay corrected) and radiochemical purities of 98 ± 2% (ITLC-SG strips, Varian). Calculated specific activities were 40.0 MBq/µg (37.8 GBq/µmol) for $^{68}$Ga-PSMA-HBED-CC and 13.6 MBq/µg (20.4 GBq/µmol) for $^{68}$Ga-PSMA I&T, respectively. For $^{177}$Lu-PSMA I&T the radiochemical purity was 99.0 ± 1.0% as determined by RP-HPLC (LiChroCART 250-4, Lichrospher100, RP18, Merck) and specific activities of 40.0 MBq/µg (59.9 GBq/µmol) were achieved.

PSMA binding affinity

The affinity ($IC_{50}$) of PSMA I&T and its natGa- and natLu-complexes towards PSMA (Table 1) was determined in a competitive binding assay using the human prostate carcinoma cell line LNCaP (1.5 · 10^5 cells/well, 1 h) at 4 °C and (125I-BA)KuE (0.2 nM) as the radioligand.

Compared to the second generation DOTAGA-functionalized PSMA-ligands (13), PSMA I&T contains a $\alpha$-Phe-by-3-iodo-$\alpha$-Tyr substitution in the peptidic linker unit (Fig. 1). To be able to assess the influence of this modification on PSMA-affinity, data for DOTAGA-ffk(Sub-KuE) (13) are also included in Table 1. Metal complexation only has negligible effect on PSMA-affinity of PSMA I&T. However, substitution of $\alpha$-Phe by

$^{68}$Ga/$^{177}$Lu-PSMA I&T
d-3-iodo-Tyr in the linker does have beneficiary influence in significantly improving PSMA affinity of the PSMA I&T constructs as compared to the second generation DOTAGA-analogs.

**Internalization kinetics**

To investigate the impact of the increased affinity of $^{68}$Ga/$^{177}$Lu-PSMA I&T on ligand internalization and cell binding, LNCaP cells ($1.25 \cdot 10^5$ cells/well) were incubated with the respective radioligands at 37 °C for different time points up to 1 h. Experiments were performed in the absence (total binding) and presence of 10 µM PMPA (non-specific binding), and a PMPA wash step (10 µM, 10 min, 4 °C) was included to differentiate between specifically bound and internalized activity. To exclude an influence of inter-experimental variations in cell count or cell viability on the absolute amount of bound/internalized tracer, the standard ligand ($^{125}$I-BA)KuE was always assayed in parallel as an external reference, and its cellular uptake in the respective experiments was used for data normalization.

Figure 2A shows the ligand binding kinetics of $^{177}$Lu-PSMA I&T in comparison to $^{177}$Lu-DOTAGA-ffk(Sub-KuE). The internalization of all $^{68}$Ga- and $^{177}$Lu-labeled PSMA-inhibitors investigated in this study is very high and nearly identical. At all time points, 1 to 3% of the total activity is bound to PSMA on the cell membrane (Fig. 2B) and less than 0.5% is non-specifically bound. While $^{177}$Lu-DOTAGA-ffk(Sub-KuE) only shows 44 ± 2% of the cellular uptake of the reference compound ($^{125}$I-BA)KuE in a parallel experiment, internalization of $^{177}$Lu-PSMA I&T is increased to 76 ± 2% of that of ($^{125}$I-BA)KuE. The same trend was observed for the respective $^{68}$Ga-labeled analogs, where the transition from $^{68}$Ga-DOTAGA-ffk(Sub-KuE) to $^{68}$Ga-PSMA I&T leads to an increase in internalization from 42 ± 2% to 59 ± 2% of ($^{125}$I-BA)KuE internalization.

**Dual tracer biodistribution study**

The biodistribution of $^{68}$Ga-PSMA I&T (0.1 nmol) and $^{177}$Lu-PSMA I&T (0.1 nmol) investigated in a dual tracer experiment at 1 h p.i. in LNCaP tumor bearing CD-1 nu/nu mice is summarized in Table 2. Given their nearly identical physicochemical properties, both $^{68}$Ga- and $^{177}$Lu-PSMA I&T show fast and rapid $^{68}$Ga/$^{177}$Lu-PSMA I&T
clearance from the circulation and virtually no background accumulation at 1 h p.i. (Table 2). Interestingly, the increased PSMA-mediated internalization of $^{177}$Lu-PSMA I&T compared to its $^{68}$Ga-analog is well reflected by an enhanced uptake in the PSMA-positive tissues, i.e. lung, spleen, and a significantly ($P < 0.05$) higher kidney and tumor uptake. Therefore tumor/background ratios for $^{177}$Lu-PSMA I&T are increased for almost all organs (Figure 3).

Small-animal PET imaging

As shown on the PET images of LNCaP tumor bearing CD-1 nu/nu mice at 1 h p.i., $^{68}$Ga-PSMA I&T primarily accumulates in the tumor xenograft and the kidneys, which was shown to be PSMA-specific (Fig. 4A). Fast renal excretion is confirmed by significant bladder activity. The time-activity curves (Fig. 4B) derived from dynamic PET data reveal fast uptake kinetics and retention of the tracer in kidney and tumor over the 1.5 h observation period, whereas the activity is rapidly washed out from non-target tissues and compartments like blood (ROI over the heart) and muscle (linear decline in a logarithmic plot).

Dosimetry of $^{177}$Lu-PSMA I&T in mice

Absorbed doses for $^{177}$Lu-PSMA I&T in humans were extrapolated from mouse biodistribution data using two alternative extrapolation methods M1 and M2. Details on the methodology, a list of the time-integrated activity coefficients for a number of organs of relevance for dosimetry (residence times), a full list of the corresponding absorbed doses and information on the absorbed dose by beta particles and photons are given in the supplementary information. The highest time-integrated activity coefficient was observed for the kidneys (M1: 8.3 h and M2: 5.8 h). For all organs, the total absorbed doses are summarized in Table 3 and were lower than $5.9 \cdot 10^{-2}$ mGy/MBq (adrenals M1), except kidneys (2.4 mGy/MBq (M1) or 1.6 mGy/MBq (M2)). Unless more than 9.6 GBq (M1) or 14.4 GBq (M2) are administered to humans, the limiting kidney dose of 23 Gy could be exceeded.

In addition, the effective dose per unit activity in kidneys has been calculated. However, the quantity “effective dose” can only be applied to the description of stochastic radiation effects and organ absorbed
doses of less than 1 Gy. The extrapolated effective doses are $9.6 \times 10^{-3}$ mSv/MBq (M1) and $7.4 \times 10^{-3}$ mSv/MBq (M2). This corresponds to effective doses of 1.9 mSv (M1) and 1.5 mSv (M2) for an administered activity of 200 MBq.

**$^{68}$Ga-PSMA I&T PET imaging in a first patient**

In a first patient suffering from CRPC with multiple metastases $^{68}$Ga-PSMA I&T PET/CT revealed multiple bone, abdominal lymph node and a liver metastasis (Fig. 5A). The primary prostate tumor (SUV$_{\text{max}}$: 65.1, Fig. 5B), as well as periprostatic tissue and urinary bladder invasion was not concealed by radioactivity in the bladder. A 7 mm left perirectal lymph node showed a SUV$_{\text{max}}$ of 15. The liver lesion (Fig. 5C), which was not known before PET scanning, showed a SUV$_{\text{max}}$ of 10.9 and 2 cm in diameter. Further, a sclerosis in a sternal lesion, that had been barely visible in the CT image, exhibited very high $^{68}$Ga-PSMA I&T uptake (SUV$_{\text{max}}$: 76.4; Fig. 5E). Multiple paraaortic and pelvic lymph nodes showed high contrast in $^{68}$Ga-PSMA I&T PET, as shown for a 8 mm sized paraaortic lymph node with a SUV$_{\text{max}}$ of 39.4 in Fig. 5D. Mean SUV$_{\text{max}}$ values of lymph node metastases were 26.4 (range 7–80.4) and of bone metastases were 52.8 (range 22–76.5). The average $^{68}$Ga-PSMA I&T lesion to background ratio was 17.6 for lymph node metastases, 35.2 for bone metastases, and 20.7 for the liver metastasis. Background activity was determined in gluteal musculature (SUV$_{\text{max}}$ = 1.5).

Besides the pathological tracer accumulation, $^{68}$Ga-PSMA I&T showed high uptake in kidneys and salivary glands and low uptake in liver, in spleen and in the proximal segments of the small intestine.

**$^{177}$Lu-PSMA I&T therapy in patients**

Two patients suffering from mCRPC and multiple metastases in bone and lymph nodes, which had been confirmed by baseline $^{68}$Ga-PSMA-HBED-CC PET/CT, were treated with $^{177}$Lu-PSMA I&T. Therapy control was performed using $^{68}$Ga-PSMA-HBED-CC PET/CT to ensure comparability to data from the literature and an objective interpretation of the therapy outcome. The administered mass of PSMA I&T was 142 and 200 µg and the administered activity was 5.7 and 8.0 GBq, respectively. In patient 2 (PSA = 54.2 ng/mL) the mediastinal lymph node metastases (SUV$_{\text{max}}$: 36.5, determined by $^{68}$Ga-PSMA-
HBED-CC PET/CT) exhibited a high uptake of $^{177}$Lu-PSMA I&T (Fig. 6) on post-therapy planar and SPECT/CT images. Therapy was well tolerated and no significant fall in blood counts, renal function (serum creatinine, tubular extraction rate) or in any of the laboratory parameters was found. There was no adverse or clinically detectable pharmacologic effect. During early follow-up no side effects were observed, particularly no dry mouth caused by activity in salivary glands.

The baseline $^{68}$Ga-PSMA-HBED-CC PET/CT scan in patient 3 (PSA: 40.2 ng/mL) demonstrated PSMA-mediated uptake in the primary tumor as well as multiple lymph node and bone metastases (Fig. 7A). The $\text{SUV}_{\text{max}}$ of target lesions were 26.3 in right paraaortic lymph node (transverse PET/CT image in upper row), 25.2 in interaortocaval lymph node (middle row) and 16.4 in the primary tumor in the prostate (lower row). The patient underwent one therapy cycle with 8.0 GBq $^{177}$Lu-PSMA I&T. Follow-up $^{68}$Ga-PSMA-HBED-CC PET/CT (Fig. 7B) 3 months after $^{177}$Lu-PSMA I&T therapy revealed partial remission of many of the intense PSMA positive metastases depicted by $^{68}$Ga-PSMA-HBED-CC PET/CT ($\text{SUV}_{\text{max}}$ values of 3.0, 3.5 and 5.1 in paraaortic, interaortocaval lymph node metastasis and primary tumor, respectively) accompanied by a significant drop in the PSA to 0.7 ng/mL. Clinically, a symptomatic pain relief, especially on the left side of the chest, was reported.
DISCUSSION

In comparison to $^{18}$F-fluoromethylcholine the urea-based PSMA-inhibitor $^{68}$Ga-PSMA-HBED-CC (30) displays significantly improved diagnostic sensitivity and specificity. To meet the urgent need for a targeted therapeutic agent in the treatment of PC, first promising (urea-based) candidates for endoradiotherapy of PC have also been introduced and evaluated in first patient studies (25, 26, 31).

Based on the valuable results of this tracer class both in diagnostic imaging and endoradiotherapy, the aim of this study was the development of a suitable PSMA-targeted theranostic concept, combining straightforward labeling procedures for clinical routine application with optimized PSMA targeting characteristics. Previous studies leading to a first-generation theranostic agent (Fig. 1) have already demonstrated the beneficiary effect of DOTAGA-for-DOTA substitution and of using an all-$\delta$-amino acid peptide linker on PSMA-affinity and metabolic stability and thus uptake and clearance kinetics, respectively (13). Complementing these findings with the substitution of one of the $\delta$-phenylalanine residues in the peptidic linker by 3-iodo-$\delta$-tyrosine for an improved interaction of the tracer molecule with a remote binding site (27), led to PSMA I&T (Fig. 1). As anticipated, this modification resulted in an increased PSMA-affinity of PSMA I&T and its $^{nat}$Ga- and $^{nat}$Lu-complexes compared to their ffk-analogs (Table 1), which is also reflected by an enhanced internalization efficiency of $^{68}$Ga/$^{177}$Lu-PSMA I&T into PSMA-expressing LNCaP cells. Interestingly, although the PSMA-affinity of $^{nat}$Lu-PSMA I&T is only marginally increased compared to $^{nat}$Ga-PSMA I&T, internalization of $^{177}$Lu-PSMA I&T into LNCaP cells is significantly enhanced. This improved targeting efficiency is reflected by an increased uptake of $^{177}$Lu-PSMA I&T in the LNCaP tumor xenografts and thus enhanced tumor to background ratios (Fig. 3). It also shows nearly two-fold higher accumulation in lung, spleen and kidney, all of which are tissues with documented PSMA expression (32). Compared to other promising PSMA-directed tracers, $^{68}$Ga/$^{177}$Lu-PSMA I&T both show a tissue distribution pattern comparable to that of $^{68}$Ga-PSMA-HBED-CC (11, 13) at the same time point. While both compounds show enhanced blood activity levels and liver accumulation compared to $^{99m}$Tc-MIP-1404 (12). The extent of PSMA-unspecific activity retention in blood and liver was significantly lower compared to $^{18}$F-DCFBC (7). This is most probably the result of the excellent metabolic stability and comparably low plasma protein binding of $^{68}$Ga/$^{177}$Lu-PSMA I&T.

$^{68}$Ga/$^{177}$Lu-PSMA I&T
First clinical application of $^{68}$Ga-PSMA I&T in PET/CT successfully demonstrated multiple metastatic foci in different organs and tissues with very high lesion to background ratios of 17.6-35.2 as early as 1 h p.i.. Even very small (mm-range) abdominal lymph node and small bone metastases showed high uptake and were easily detectable (Fig. 5). Comparable to previously reported data for $^{68}$Ga-PSMA-HBED-CC in PET/CT (14), low physiological tracer uptake was observed in liver (33), spleen (33) and intestine (3), and - to a higher extent - in proximal tubules of the kidneys (3) and salivary glands (34), all of which are organs with documented moderate to high PSMA expression. However, the reasons for the observed high tracer uptake into salivary glands is still a matter of debate, since the PSMA expression level would suggest lower uptake.

As, presently, there is no standardized way for extrapolating time-integrated activity coefficients from animal data to humans, we decided applying two methods originally described by Sparks and Aydogan (35). Choosing two different ways of scaling provides an estimate of the extrapolation-related uncertainty in calculating absorbed doses to humans, as can be seen in table 3. Overall, the agreement between the absorbed doses to the organs is acceptable. For most organs, the difference is less than $\pm$ 45%. Major deviations caused by the different ways of extrapolating are for the intestines, the pancreas and the uterus. If, for therapeutic applications, organs-at-risk need to be considered for a first-in-man-study, one would apply the higher of both values as a conservative estimate of the organ absorbed doses.

The extrapolated organ dosimetry for $^{177}$Lu-PSMA I&T shows that the highest absorbed dose per unit activity is expected in the kidneys. The values are about 1.1- to 1.7-fold higher than the corresponding values reported for $^{131}$I-MIP-1095 with 1.5 mGy/MBq (31) and for $^{177}$Lu-J591 with 1.4 mGy/MBq (23). For $^{90}$Y-J591 (23) a value of 4.5 mGy/MBq is reported, which is about two times higher than the extrapolated values for $^{177}$Lu-PSMA I&T. In addition, the calculated absorbed doses per unit activity for other organs (e.g. liver, spleen, heart wall, bone marrow) for $^{131}$I-MIP-1095 (31) and $^{177}$Lu-J591 (23) are at least one order of magnitude higher than the corresponding values for $^{177}$Lu-PSMA I&T. Of course the present extrapolated data need to be confirmed in a dosimetry study in patients, including an assessment of the absorbed doses to tumor lesions in patients.
As its $^{68}$Ga-analog, $^{177}$Lu-PSMA I&T shows high, specific and rapid uptake in all previously identified tumor lesions of the mCRPC patients included in this proof of concept study (Fig. 6). As expected, significant tracer uptake is also observed for kidney, spleen and salivary glands, but also in the small intestine. This is consistent with the PSMA expression levels documented for these tissues (3, 33, 34); for example, $^{177}$Lu-PSMA I&T uptake in the small intestine most likely is the result of PSMA expression in human intestine (3), where the physiological function of PSMA is mediating folate absorption (36). Based on the high PSMA expression in the metastases of mCRPC (37) and the resulting high uptake of $^{177}$Lu-PSMA I&T in these lesions, therapeutically effective doses were delivered to the PC metastases resulting in impressive molecular treatment response. Besides concomitant subjective reduction of pain, both patients demonstrated objective clinical measures of improvement, such as drop of PSA and reduction of disease burden as determined by $^{68}$Ga-PSMA-HBED-CC PET/CT (Fig. 7).

Due to the rapid renal wash-out and blood clearance of $^{177}$Lu-PSMA I&T, no side effects neither in salivary glands nor in kidneys or blood parameters were observed in either of the two treated patients, and treatment was well tolerated. In contrast, after endoradiotherapy with $^{131}$I-MIP-1095 (31) dry mouth and one case of mucositis were reported due to high salivary gland retention of the therapeutic agent. Thus, high contrast in PET imaging and therapeutic effectiveness with no detectable side effects qualifies $^{68}$Ga/$^{177}$Lu-PSMA I&T to be a valid choice for theranostic management of PC.

**CONCLUSION**

$^{68}$Ga-PSMA I&T is a PET tracer of high potential for the detection of metastatic PC and may be useful for stratification and follow-up of patients undergoing radioligand therapy with $^{177}$Lu-PSMA I&T (Theranostics). In a proof of concept study $^{177}$Lu-PSMA I&T endoradiotherapy was feasible, safe and effective in metastatic PC. Subsequent studies have to assess the optimal activity, as well as the amount of peptide administered, potential kidney or salivary gland protection and the need of repeated therapeutic interventions based on patient follow-up.
DISCLOSURE

The authors MW, MScho, RPB, AY, SB, HRK, ML, IK, ME and MSchw have nothing to disclose. JS is an employee and HJW CEO of SCINTOMICS GmbH, Fuerstenfeldbruck, Germany.

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REFERENCES


Figure 1. Chemical structures of DOTAGA-FFK(Sub-KuE), a 1st generation tracer (upper) and PSMA I&T, a 3rd generation tracer (lower panel).
Figure 2. Specific binding kinetics of $^{177}$Lu-PSMA I&T and $^{177}$Lu-DOTAGA-ffk(Sub-KuE) (upper) and specific binding and internalization kinetics of $^{177}$Lu-PSMA I&T at 37 °C to LNCaP cells (lower panel). All data are expressed as mean ± SD.
**Figure 3.** Tumor/organ ratios of dual tracer biodistribution of $^{68}$Ga- and $^{177}$Lu-PSMA I&T at 1 h p.i. in LNCaP-tumor bearing CD-1 nu/nu mice ($n=4$).
Figure 4: PET of LNCaP-tumor bearing CD-1 nu/nu mice. A) Maximum intensity projections (MIP) of static PET scans using 15.8 MBq $^{68}$Ga-PSMA I&T (left) or 14.3 MBq $^{68}$Ga-PSMA I&T coinjected with 8 mg/kg PMPA (middle). B) Time-activity curves (logarithmic plot) for $^{68}$Ga-PSMA I&T (15.4 MBq) derived from dynamic small animal PET data (right panel).
Figure 5. $^{68}$Ga-PSMA I&T PET/CT of patient 1. A. Whole body MIP showing one liver lesion as well as multiple lymph node and bone metastases. B. Transaxial slices show infiltration of a soft-tissue mass with increased tracer uptake in the urinary bladder, and periprostatic tissue. C. Transaxial slices revealing $^{68}$Ga-PSMA I&T uptake in the right lobe of the liver with a hypodense lesion in corresponding CT slice. D. Transaxial slices presenting a small paraaortic lymph node with intense PSMA-expression indicative of a lymph node metastasis. E. Sagittal reformatted CT reveals only minimal sclerosis of a sternal bone metastasis with high $^{68}$Ga-PSMA I&T uptake. All slices are shown on CT (left), PET (middle), and fused PET/CT (right panel).
Figure 6. Patient 2. **A.** MIP of $^{68}$Ga-PSMA-HBED-CC PET/CT (164 MBq, 60 min p.i., left) showed intense tracer accumulation in mediastinal lymph node metastases. **B.** Correspondingly, these mediastinal lymph nodes demonstrated a high $^{177}$Lu-PSMA I&T uptake 47 h after therapy with 5.7 GBq $^{177}$Lu-PSMA I&T.
Figure 7. PET/CT in patient 3. A. Baseline PET/CT 65 min after i.v. administration of 176 MBq $^{68}$Ga-PSMA-HBED-CC. B. Follow-up scan with 180 MBq $^{68}$Ga-PSMA-HBED-CC (60 min. p.i.) performed 3 months after $^{177}$Lu-PSMA I&T therapy (8.0 GBq).
### TABLES

**Table 1:** IC₅₀ values determined in a competitive binding assay (LNCaP, c(¹²⁵I-BA)KuE = 0.2 nM, HBSS + 1% BSA, 1 h, 4 °C). Data are expressed as mean ± SD (n = 3 in three separate determinations).

<table>
<thead>
<tr>
<th>Ligand</th>
<th>IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOTAGA-ffk(Sub-KuE)</td>
<td>13.9 ± 0.4</td>
</tr>
<tr>
<td>natGa-DOTAGA-ffk(Sub-KuE)</td>
<td>15.9 ± 0.5</td>
</tr>
<tr>
<td>natLu-DOTAGA-ffk(Sub-KuE)</td>
<td>13.1 ± 2.2</td>
</tr>
<tr>
<td>PSMA I&amp;T (DOTAGA-(I-y)fk(Sub-KuE))</td>
<td>10.2 ± 3.5</td>
</tr>
<tr>
<td>natGa-PSMA I&amp;T</td>
<td>9.3 ± 3.3</td>
</tr>
<tr>
<td>natLu-PSMA I&amp;T</td>
<td>7.9 ± 2.4</td>
</tr>
</tbody>
</table>
Table 2. Dual-tracer biodistribution (% ID/g) in LNCaP tumor bearing CD-1 nu/nu mice at 1 h p.i. (n = 4).

<table>
<thead>
<tr>
<th>Organ</th>
<th>$^{68}$Ga-PSMA I&amp;T</th>
<th>$^{177}$Lu-PSMA I&amp;T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.45 ± 0.23</td>
<td>0.44 ± 0.19</td>
</tr>
<tr>
<td>Heart</td>
<td>0.26 ± 0.08</td>
<td>0.29 ± 0.08</td>
</tr>
<tr>
<td>Lung</td>
<td>1.49 ± 0.38</td>
<td>1.65 ± 0.56</td>
</tr>
<tr>
<td>Liver</td>
<td>1.00 ± 0.39</td>
<td>1.10 ± 0.41</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.88 ± 1.46</td>
<td>5.85 ± 2.26</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.54 ± 0.15</td>
<td>0.57 ± 0.24</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.42 ± 0.10</td>
<td>0.42 ± 0.14</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.27 ± 0.07</td>
<td>0.69 ± 0.14</td>
</tr>
<tr>
<td>Kidney</td>
<td>53.26 ± 9.02</td>
<td>107.24 ± 15.61</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.35 ± 0.08</td>
<td>0.56 ± 0.36</td>
</tr>
<tr>
<td>Brain</td>
<td>0.03 ± 0.02</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>Bone</td>
<td>0.27 ± 0.08</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>Tumor</td>
<td>4.95 ± 1.57</td>
<td>7.96 ± 1.76</td>
</tr>
</tbody>
</table>
Table 3. Total absorbed doses in different organs (mGy/MBq) after application of $^{177}$Lu-PSMA I&T, calculated by both methods (M1 and M2- see supplementary information).

<table>
<thead>
<tr>
<th>Target organ</th>
<th>Total absorbed dose (mGy/MBq)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>Adrenals</td>
<td>5.85E-02</td>
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<tr>
<td>Brain</td>
<td>5.10E-04</td>
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<td>Breasts</td>
<td>2.50E-04</td>
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<tr>
<td>Gallbladder wall</td>
<td>3.42E-03</td>
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<tr>
<td>LLI wall</td>
<td>5.65E-04</td>
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<tr>
<td>Small intestine</td>
<td>9.25E-03</td>
</tr>
<tr>
<td>Stomach wall</td>
<td>5.56E-03</td>
</tr>
<tr>
<td>ULI wall</td>
<td>1.92E-03</td>
</tr>
<tr>
<td>Heart wall</td>
<td>1.86E-03</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.40E+00</td>
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<td>Liver</td>
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<td>Lung</td>
<td>7.81E-03</td>
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<td>Ovaries</td>
<td>7.36E-04</td>
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<td>Pancreas</td>
<td>1.79E-02</td>
</tr>
<tr>
<td>Red marrow</td>
<td>2.00E-03</td>
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<tr>
<td>Osteogenic cells</td>
<td>4.83E-03</td>
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<tr>
<td>Skin</td>
<td>3.92E-04</td>
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<td>Spleen</td>
<td>3.07E-02</td>
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<td>Testes</td>
<td>8.18E-05</td>
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<tr>
<td>Thymus</td>
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<td>Thyroid</td>
<td>9.28E-05</td>
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<tr>
<td>Urinary bladder wall</td>
<td>2.73E-04</td>
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<td>Uterus</td>
<td>3.33E-02</td>
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<tr>
<td>Total body</td>
<td>1.12E-02</td>
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68Ga- and 177Lu-labeled PSMA I&T: Optimization of a PSMA targeted theranostic concept and first proof of concept human studies


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