SUPPLEMENTAL INFORMATION

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Abbreviations

ACN	Acetonitrile
BSA	Bovine Serum Albumin
DTPA	Diethylenetriamine pentaacetate
CT	Computed Tomography
Cu	Copper
DOTAGA	1,4,7,10-Tetraazacyclododecane,1-glutaric acid-4,7,10-acetic acid
F	Fluorine
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
Ga	Gallium
HPLC	High Performance Liquid Chromatography
IC_{50}	Half Maximal Inhibitory Concentration
IA/g	Injected activity / gram
LC/MS	Liquid Chromatography / Mass Spectrometry
Lu	Lutetium
MEM EBS	Minimum Essential Medium Eagle
MIP	Maximum Intensity Projection
NEAA	Non-essential amino acids
NODAGA	1,4,7-triazacyclononane,1-glutaric acid-4,7-acetic acid
PBS	Phosphate-Buffered Saline
PCa	Prostate Cancer
PET	Positron Emission Tomography
p.i.	Post Injection
2-PMPA	2-(phosphonomethyl)pentanedioic acid
PSMA	Prostate Specific Membrane Antigen
SD	Standard Deviation
SUV	Standardized Uptake Value

Reagents and Procedures

DOTAGA-PSMA-I&T (PSMA I&T) and NODAGA-PSMA-I&T were custom-made by piChem (Raaba-Grambach, Austria). PSMA-11 and ^{nat}Ga-PSMA-11 were purchased from ABX (Radeberg, Germany). [^{18}F]PSMA-1007 was produced at the University Hospital Zurich (Zurich, Switzerland), under GMP for human use. [^{68}Ga]$GaCl_3$ was eluted from a IGG100 ^{68}Ge/^{68}Ga-generator from Eckert & Ziegler (Berlin, Germany). [^{64}Cu]$CuCl_2$ was provided by the University Hospital Tübingen, Germany. All reagents and solvents were purchased from common suppliers in HPLC or analytical grade.

Liquid chromatography mass spectrometry (LC/MS) was run on a LCMS-2020 Shimadzu system equipped with a Waters X Bridge C18 column (4.6 x 150 mm, 5 µm particle size). The gradient used was 15-65% solvent *B* in 15 min (*A* = H_2O [0.1%TFA], *B* = ACN [0.1% TFA]) at a flow rate of 2.0 mL/min. Radio-HPLC was performed on an Agilent 1260 Infinity system connected to a GABI radioactivity-HPLC-flow-monitor γ-spectrometer (Elysia-raytest, Straubenhardt, Germany). Radioligands were analyzed on a Macherey Nagel Nucleodur ISIS C18 (250 × 4.6 mm, 5 µm particle size) column using the gradient 25-55 % *B* in 15 min (*A* = H_2O [0.1%TFA], *B* = ACN [0.1% TFA]) with a flow rate of 1 mL/min. [^{68}Ga]Ga-PSMA-11 was analyzed using the gradient 5-50 % *B* in 15 min (*A* = H_2O [0.1%TFA], *B* = ACN [0.1% TFA]) with a flow rate of 2 mL/min. Quantitative γ-counting was carried out on a Cobra 5003 γ-system well counter from Packard Instruments (Meriden, CT, USA).

Cell line

Lymph Node Carcinoma of the Prostate (LNCaP) cells were purchased from Cell Lines Service GmbH (Eppelheim, Germany) and cultured in Minimum Essential Medium Eagle (MEM EBS) supplemented with 10% FBS and 1% penicillin (10’000 IU/mL) – streptomycin (10’000 µg/mL), L-glutamine (200 mM), sodium pyruvate (100 mM) and MEM non-essential amino acids (NEAA) at 37°C/5% CO_2.

Radiolabeling and metalation

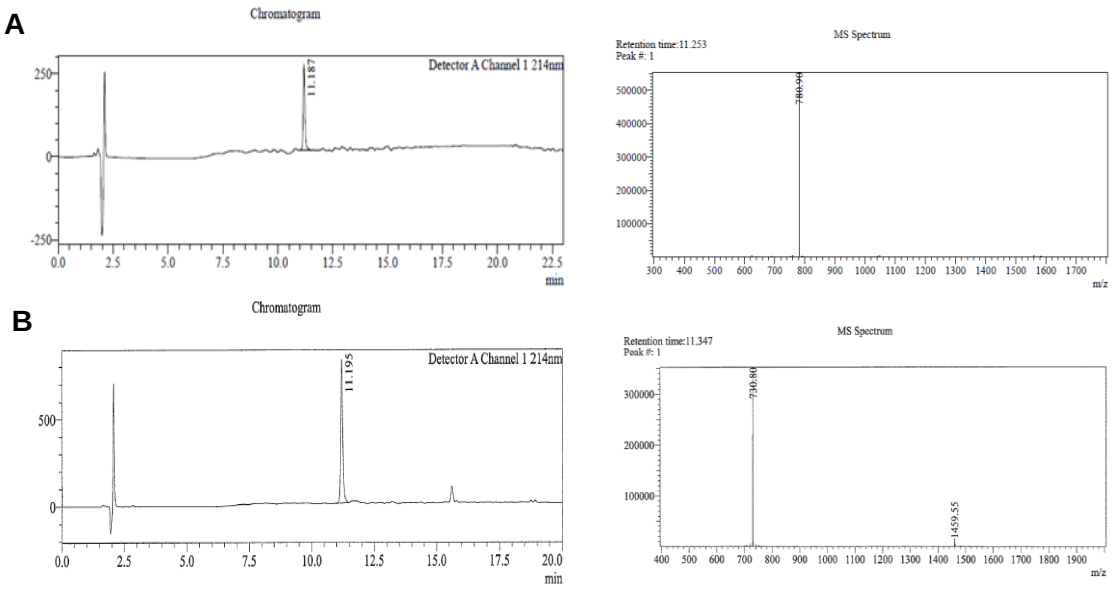
^{61}Cu-labeling: An aliquot of DOTAGA-PSMA-I&T and NODAGA-PSMA-I&T (3-6 nmol, 1 mg/mL in TraceSelect water) was diluted in 0.25-0.30 mL of ammonium (or sodium) acetate (0.5 M pH 8), followed by the addition of 0.05-0.3 mL [^{61}Cu]$CuCl_2$ in 0.05 M HCl (30-150 MBq). The reaction mixture was incubated at 95°C for DOTAGA-PSMA-I&T for 15 min and at room temperature (approx. 20-25°C) for NODAGA-PSMA-I&T from 5 to 15 min. The pH of the reaction mixture was between 5 and 6. Both ligands were labeled with ^{61}Cu in very high yield and purity. No further purification step was necessary to remove uncomplexed ^{61}Cu from the reaction mixture, allowing direct use of the formed radiotracer.

^{64}Cu-labeling: NODAGA-PSMA-I&T was labeled with ^{64}Cu, following an identical procedure as described above for ^{61}Cu.

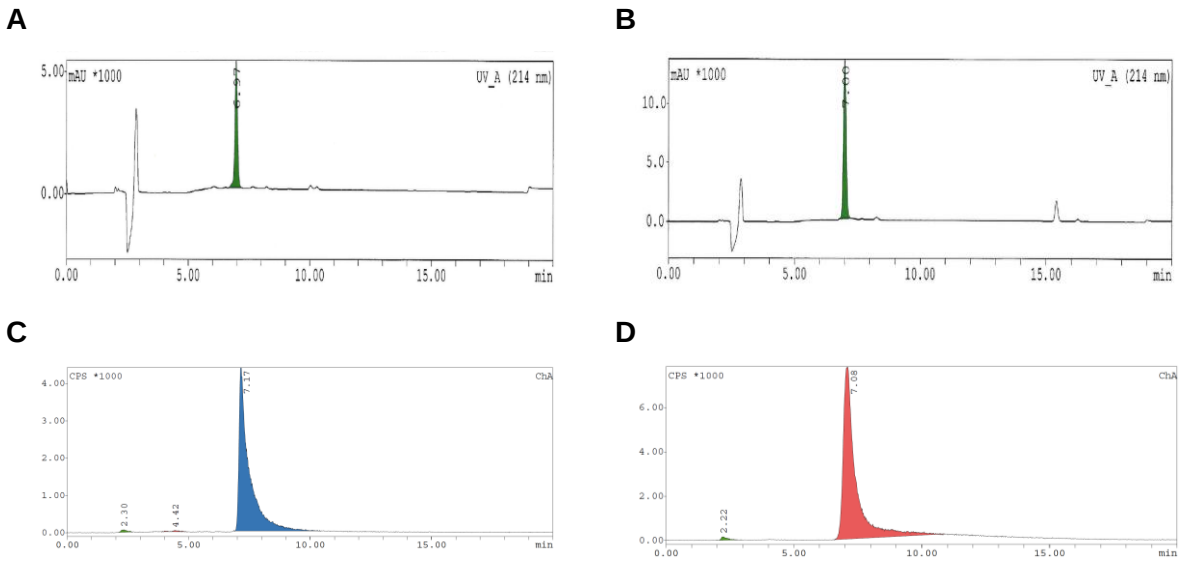
^{nat}Cu complexation: The ^{nat}Cu complexes were prepared by incubating 1-1.5 mg of DOTAGA-PSMA-I&T and NODAGA-PSMA-I&T with 1.5-fold excess of $^{nat}CuCl_2$ under the same condition described above for ^{61}Cu-labeling. Free metal ions were eliminated via SepPak C-18 cartridge (Waters), pre-conditioned with methanol and water. The reaction mixture was loaded on the cartridge, the free $^{nat}Cu^{2+}$ was eluted with water while the ^{nat}Cu-complexes were eluted with methanol, evaporated to dryness, re-dissolved in water and lyophilized.

^{68}Ga-labeling: [^{68}Ga]Ga-DOTAGA-PSMA-I&T, [^{68}Ga]Ga-NODAGA-PSMA-I&T and [^{68}Ga]Ga-PSMA-11 were prepared by incubating 3-6 nmol of the corresponding conjugate (1 mg/mL in TraceSelect water) in 0.25-0.30 mL sodium acetate (0.2 M, pH 4-4.5) with 0.075-0.30 mL [^{68}Ga]$GaCl_3$ in 0.1 M HCl (50-200 MBq).

ESI/MS and HPLC profiles



Supplemental Figure 1. Representative UV-chromatograms (left side) and mass spectrum (right side) of ^{nat}Cu-DOTAGA-PSMA-I&T (**A**) and ^{nat}Cu-NODAGA-PSMA-I&T (**B**) analyzed on a LCMS-2020 Shimadzu system, as described in Reagents and Procedures above.



Supplemental Figure 2. Representative UV-chromatogram of ^{nat}Cu-DOTAGA-PSMA I&T (**A**) and ^{nat}Cu-NODAGA-PSMA I&T (**B**) and radio-chromatograms of [^{61}Cu]Cu-DOTAGA-PSMA-I&T (**C**) and [^{61}Cu]Cu-NODAGA-PSMA-I&T (**D**) on an Agilent 1260 Infinity system, as described in Reagents and Procedures above.

Supplemental Table 1. Analytical data of the ^{nat}Cu- and ^{nat}Ga-complexes analyzed on a LCMS-2020 Shimadzu system, as described in Reagents and Procedures above.

Radioligand	Purity (%)	m/z calc.	m/z obs.	t_R (min)
^{nat}Cu-DOTAGA-PSMA-I&T	97	780.9	780.9	11.19
^{nat}Cu-NODAGA-PSMA-I&T	93	730.4	730.8	11.20
^{nat}Ga-PSMA-11	99	507.9	508.3	10.34

m/z: mass number/charge number, t_R: retention time

Supplemental Table 2. Radiochemical purity (RCP) and retention time (t_R) of the ^{61}Cu-labeled tracers, their corresponding ^{68}Ga-counterparts and the reference [^{68}Ga]Ga-PSMA-11, as determined by radio-HPLC, on an Agilent 1260 Infinity system, as described in Reagents and Procedures above. The stability of the ^{61}Cu-labeled tracers in the reaction buffer (no product formulation) was measured after 2h and after 4h at room temperature.

Radioligand	RCP (%)	t_R (min)	Stability (%) @ 2h	Stability (%) @ 4h
[^{61}Cu]Cu-DOTAGA-PSMA-I&T	97.4 ± 2.3	7.2 ± 0.2	94.9 ± 0.7	90.3 ± 0.6
[^{61}Cu]Cu-NODAGA-PSMA-I&T	98.2 ± 1.9	7.0 ± 0.3	97.8 ± 0.1	96.8 ± 0.1
[^{68}Ga]Ga-DOTAGA-PSMA-I&T	97.2 ± 5.7	6.9 ± 0.4	n.d.	n.d.
[^{68}Ga]Ga-NODAGA-PSMA-I&T	98.1 ± 3.2	7.1 ± 0.1	n.d.	n.d.
[^{68}Ga]Ga-PSMA-11*	98.8 ± 0.8	7.0 ± 0.3*	n.d.	n.d.

***** analyzed using different gradient (see Reagents and Procedures above); n.d. not determined

Determination of lipophilicity (log *D*)

The distribution coefficient (log *D*) was determined by the shake-flask method. In a pre-lubricated Eppendorf tube, a pre-saturated mixture of 500 µL of 1-octanol and 500 µL of PBS (phosphate-buffered saline) at pH 7.4 was added. An aliquot of 10 pmol/10 µL of the radiotracer was added to this mixture, shaken vigorously for 30 min, and then centrifuged at 3000 rcf for 10 min to achieve phase separation. Aliquots of 100 µL were removed from the 1-octanol and from the PBS phases, and the activity was measured in a γ-counter. The distribution coefficient was calculated as the average log ratio value of the radioactivity in the organic fraction and PBS fraction.

Supplemental Table 3. Lipophilicity (log $D_{O/PBS\ pH7.4}$) of ^{61}Cu-labeled tracers in comparison to their corresponding ^{68}Ga-labeled tracers and the reference tracers [^{68}Ga]Ga-PSMA-11 and [^{18}F]PSMA-1007. Results are mean ± SD from a minimum of two separate experiments, each in triplicates.

Radioligand	**log $D_{O/PBS\ pH7.4}$**
[^{61}Cu]Cu-DOTAGA-PSMA-I&T	-2.69 ± 0.44
[^{61}Cu]Cu-NODAGA-PSMA-I&T	-2.95 ± 0.08
[^{68}Ga]Ga-DOTAGA-PSMA-I&T	-2.79 ± 0.41
[^{68}Ga]Ga-NODAGA-PSMA-I&T	-2.85 ± 0.29
[^{68}Ga]Ga-PSMA-11	-3.89 ± 0.19
[^{18}F]PSMA-1007	-3.02 ± 0.11

Affinity determination (IC_{50}) via competition binding assay

The radioiodinated ((S)-1-carboxy-5-(4-(-^{125}I-iodo-benzamido)pentyl)carbamoyl)-L-glutamic acid ([^{125}I-BA]KuE, Chelatec, Saint-Herblain, France) was used as reference radioligand. The affinity of ^{nat}Cu-DOTAGA-PSMA-I&T and ^{nat}Cu-NODAGA-PSMA-I&T versus ^{nat}Ga-PSMA-11 was measured via the determination of the half maximal inhibitory concentration (IC_{50}), which corresponds to the concentration of the test ligand causing 50% inhibition of the specific binding of the reference radioligand. The assay was performed on LNCaP cells seeded in 24-well plates (1.5 x 10^5 cells/well). The cells were incubated with increased concentrations of each metalated ligand (ranging from 0.1 up to 100 nM) in the presence of 0.2 nM [^{125}I-BA]KuE. After 1 hour incubation on ice, the unbound (free) [^{125}I-BA]KuE was collected by removing the medium and the cells were detached with NaOH 1 M for counting (bound radioligand). Non-specific binding was defined as the amount of binding activity in the presence of the blocking agent 2-(phosphonomethyl)pentanedioic acid (2-PMPA) in high excess (10 µM).

The values were fitted using GraphPad Prism 9 and the IC_{50} values were determined using the "log(inhibitor) *vs* response" equation.

In vitro cellular uptake and distribution

LNCaP cells were seeded in 6-well plates at a concentration of 0.8-1x10^6 cells/well in 1 mL of culture medium 24 h before the experiment. The cells were then preconditioned with culture medium without supplements at 37°C for 60 min and incubated with the radiotracer (0.5 nM) either alone or in the presence of 2-PMPA (10 µM) to measure non-specific uptake. Cellular uptake was interrupted at different time points (5, 15, 30 and 60 min), by washing twice with ice-cold PBS. Cell surface-bound radioligand was obtained by washing cells twice with ice-cold glycine buffer (pH 2.8), followed by a collection of the internalized fraction with 1 M NaOH at 37°C. The activity in each fraction was measured in a γ-counter (Cobra II). The results were expressed as the mean ± standard deviation of the percentage of the applied radioactivity, after subtracting the non-specific uptake. The results are presented in the **Supplemental Table 4**.

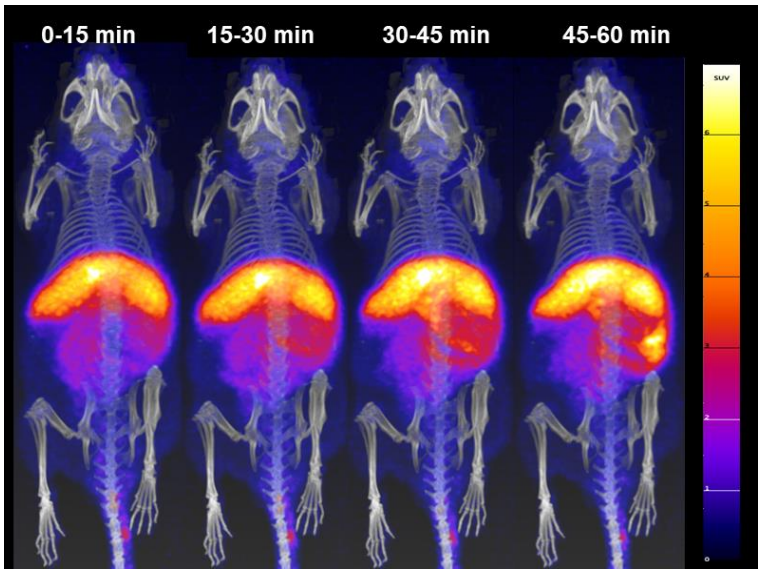
Supplemental Table 4. Cellular uptake and distribution between cell surface (cell membrane) bound and internalized fractions of the ^{61}Cu-labeled tracers in comparison to their ^{68}Ga-counterparts and the reference [^{68}Ga]Ga-PSMA-11. The values are expressed as % of the applied activity and refer to the specific uptake calculated after subtracting the non-specific uptake (measured in the presence of 10 µM 2-PMPA) from the total uptake (specific = total – nonspecific). Then results are from minimum two separate experiments per radioligand, each in triplicates.

Time Point [min]	[^{61}Cu]Cu-DOTAGA-PSMA-I&T	[^{61}Cu]Cu-NODAGA-PSMA-I&T	[^{68}Ga]Ga-DOTAGA-PSMA-I&T	[^{68}Ga]Ga-NODAGA-PSMA-I&T	[^{68}Ga]Ga-PSMA-11
Cell surface bound fraction					
5	3.7 ± 0.5	4.3 ± 0.7	4.3 ± 0.2	4.7 ± 0.9	5.3 ± 1.6
15	8.1 ± 0.7	8.2 ± 1.0	8.6 ± 0.8	7.4 ± 1.2	10.9 ± 1.8
30	11.6 ± 1.0	10.7 ± 1.0	9.5 ± 0.5	8.8 ± 1.3	13.8 ± 1.1
60	13.4 ± 0.8	10.8 ± 1.8	10.8 ± 1.1	9.3 ± 1.9	15.3 ± 1.2
120	14.7 ± 1.0	10.2 ± 1.8	10.0 ± 0.5	n.d.	16.6 ± 0.2
Internalized fraction					
5	0.7 ± 0.1	1.2 ± 0.2	0.7 ± 0.1	0.6 ± 0.3	0.4 ± 0.2
15	2.8 ± 0.2	3.9 ± 0.4	2.6 ± 0.2	2.1 ± 0.5	2.4 ± 1.3
30	6.2 ± 0.5	7.0 ± 1.1	5.0 ± 0.2	4.4 ± 0.6	5.6 ± 1.0
60	13.3 ± 0.5	11.7 ± 1.6	9.8 ± 1.3	8.8 ± 1.0	11.4 ± 0.6
120	21.3 ± 0.9	17.6 ± 2.8	15.4 ± 2.1	n.d.	20.5 ± 0.8

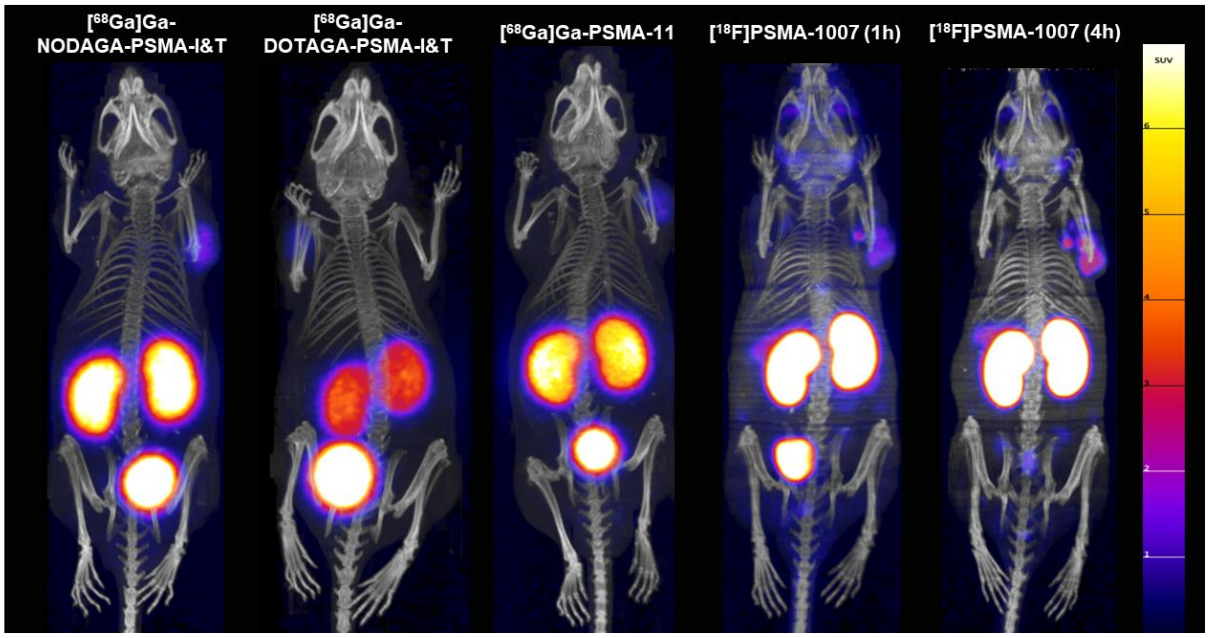
n.d. not determined

PET/CT imaging studies

The PET images were acquired in list mode using a small animal PET scanner (β-CUBE, Molecubes, Ghent, Belgium) with a spatial resolution of 0.85 mm and an axial field-of-view of 13 cm. Dynamic PET scans were acquired for 60 min. All PET scans were decay corrected and reconstructed into a 192 × 192 × 384 matrix by an ordered subsets maximization expectation (OSEM) algorithm using 30 iterations, a voxel size of 400 × 400 × 400 µm a 15 min per frame. CT data was used to apply attenuation correction on the PET data. The CT was imaged supine, head first, using the NanoSPECT/CTTM scanner (Bioscan Inc.). Topograms and helical CT scans of the whole mouse were first acquired using the following parameters: X-ray tube current: 177 µA, X-ray tube voltage 45 kVp, 90 seconds and 180 frames per rotation, pitch 1. CT images were reconstructed using CTReco (version r1.146), with a standard filtered back projection algorithm (exact cone beam) and post-filtered (RamLak, 100 % frequency cut-off), resulting in a pixel size of 0.2 mm. Co-registered PET/CT images were visualized using maximum intensity projection (MIP) with VivoQuant software (version 4.0.). The PET images were converted to standard uptake value (SUV) considering the tissue concentration of the radioligand by the activity injected and the body weight of the mice.



Supplemental Figure 3. Maximum intensity projections (MIPs) of dynamic PET/CT scans of [^{61}Cu]$CuCl_2$ (7 MBq) in non-tumor bearing male nude mice from 0 to 1h p.i., in 15 min frames. SUV: standard uptake value.



Supplemental Figure 4. Maximum intensity projections (MIPs) PET/CT scans at 1h after injection of ^{68}Ga-labeled tracers (100 µL/400 pmol/6-9 MBq) or [^{18}F]PSMA-1007 (100 µL/70 pmol/15 MBq) in LNCaP xenografts. A PET/CT scan of [^{18}F]PSMA-1007 was also acquired at 4 hours p.i., as indicated in the figure. SUV: standard uptake value.

Biodistribution studies

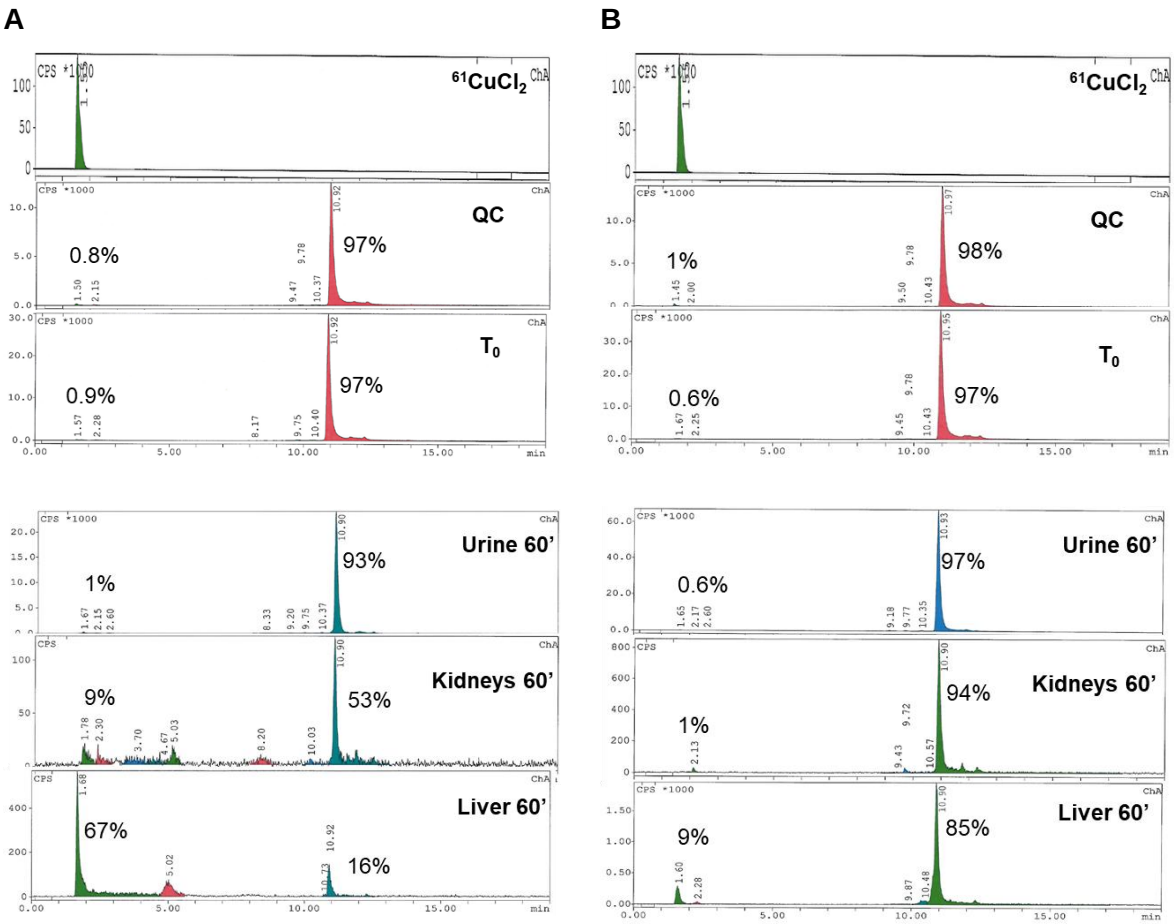
Mice were randomized (4-8/group) based on their tumor volume and injected intravenously with the tested radioligand. [^{61}Cu]Cu-DOTAGA-PSMA-I&T or [^{61}Cu]Cu-NODAGA-PSMA-I&T (100 µL/200 pmol/2-3 MBq), [^{68}Ga]Ga-DOTAGA-PSMA-I&T, [^{68}Ga]Ga-NODAGA-PSMA-I&T, [^{68}Ga]Ga-PSMA-11 (100 µL/200 pmol/3-5 MBq) and [^{18}F]PSMA-1007 (100 µL/70 pmol/15 MBq). The mice were euthanized at the time-point of investigation (1 h or 4 h p.i.) by CO_2 asphyxiation. Organs of interest and blood were collected, rinsed of excess blood, blotted dry, weighed, and counted in a γ-counter. The samples were counted against a suitably diluted aliquot of the injected solution as the standard.

Supplemental Table 5. Biodistribution of [^{68}Ga]Ga-DOTAGA-PSMA-I&T and [^{68}Ga]Ga-NODAGA-PSMA-I&T (100 µL/200 pmol/3-5 MBq) in LNCaP xenografts at 1 hour p.i.. Results are expressed as mean of the % injected activity per gram of tissue (%IA/g) ± standard deviation (SD) of n=5-6 mice/group.

Organ	[^{68}Ga]Ga-DOTAGA-PSMA-I&T	[^{68}Ga]Ga-NODAGA-PSMA-I&T
Blood	0.22 ± 0.08	0.31 ± 0.08
Heart	0.19 ± 0.08	0.29 ± 0.14
Lung	1.03 ± 0.52	1.41 ± 0.42
Liver	0.16 ± 0.08	0.21 ± 0.07
Pancreas	0.28 ± 0.08	0.51 ± 0.14
Spleen	2.11 ± 0.33	7.15 ± 1.99
Stomach	0.38 ± 0.16	0.54 ± 0.15
Intestine	0.32 ± 0.13	1.09 ± 0.68
Adrenals	6.49 ± 2.57	13.7 ± 3.9
Kidneys	81.3 ± 7.8	147 ± 59
Muscles	0.33 ± 0.21	0.85 ± 0.50
Femur	0.90 ± 0.38	2.19 ± 1.33
Salivary glands	0.70 ± 0.33	1.11 ± 0.31
Tumor	10.3 ± 2.7	9.77 ± 3.12

In vivo metabolic studies

The *in vivo* stability of [^{61}Cu]Cu-DOTAGA-PSMA-I&T and [^{61}Cu]Cu-NODAGA-PSMA-I&T was assessed in healthy BALB/c mice after intravenous injection of each radioligand (100 µL/400 pmol/ 8-9 MBq). The mice were euthanized at 1h p.i. by CO_2, followed by urine sample collection in test tubes containing methanol (v/v 1:2). The mixture was vortexed, and subsequently centrifuged at 15'000 g for 15 min at 4°C. Liver and kidney samples were quickly removed after euthanasia and were transferred to ice-cooled polypropylene tubes containing 1:1 2-PMPA (400 µM in PBS) and 1% ReadyShield® Protease Inhibitor Cocktail (Sigma-Aldrich). The tissues were homogenized using an Ultra-Turrax homogenizer, followed by centrifugation at 15'000 g for 15 min at 4°C. The supernatant of each sample was collected, mixed with methanol (v/v 1:2) and centrifuged again for additional 15 min. Aliquots of the supernatant obtained from the urine, kidney or liver homogenate were diluted 1:1 with H_2O up 1 mL and analyzed by radio-RP-HPLC on an Agilent 1260 Infinity system (see Reagents and Procedures above). The samples were analyzed on a Proteo Jupiter C12 (5 µm, 250x4.6 mm) column using the gradient 5-50% *B* in 15 min (*A* = H_2O [0.1% TFA], *B* = ACN [0.1% TFA]) with a flow rate of 2 mL/min. [^{61}Cu]$CuCl_2$ was used as reference control.



Supplemental Figure 5. Radio-RP-HPLC chromatograms of the *in vivo* metabolic stability study of [^{61}Cu]Cu-DOTAGA-PSMA-I&T (A) and [^{61}Cu]Cu-NODAGA-PSMA-I&T (B) at 1h p.i.. QC represents the quality control of the radiotracer before injection. T_0 represents the radio-chromatogram of the radiotracer treated under the same conditions, as the biological samples (same matrix). The reported % refers to the intact radiotracer.

Pharmacokinetics for dosimetry estimates of [^{61}Cu]Cu-NODAGA-PSMA-I&T

Pharmacokinetic studies were performed in healthy BALB/c mice (n=5/group) from 1 up to 24 hours after injection of 100 µL/200 pmol/4 MBq of [^{64}Cu]Cu-NODAGA-PSMA I&T. The biodistribution at the investigated time points was performed as described previously. The biodistribution data at 1 and 4 h p.i. were combined with the data obtained with [^{61}Cu]Cu-NODAGA-PSMA-I&T in LNCaP xenogratfs at 1 hour and 4 hours p.i., as the biodistribution in nude mice was the same as in the healthy BALB/c mice. The results are presented in the **Supplement Table 6**.

The non-decay corrected biodistribution data for ^{61}Cu ($t_{1/2}$=3.33 h) were used to generate time-activity curves for [^{61}Cu]Cu-NODAGA-PSMA-I&T. A linear relationship between the blood and the red marrow residence times was assumed for estimating the red marrow radiation dose. The proportionality factor was the ratio between the red marrow mass and the blood mass in humans. OLINDA/EXM 1.0 was used to integrate the fitted time-activity curves and to estimate the organ and effective doses using the whole-body adult male model. The approximation of the human salivary glands' weight of 30 g was made, given that salivary glands is not included in the human phantom. The radiation dose estimate for salivary glands was 8.27E-02 mGy/MBq. For all calculations, the assumption was made that the mouse biodistribution, determined as the %IA/organ, was the same as the human biodistribution.

Supplemental Table 6. Biodistribution data of [$^{61/64}$Cu]Cu-NODAGA-PSMA-I&T at 1 and 4 hours p.i. in BALB/c mice and LNCaP xenografted nude mice (combined) and [^{64}Cu]Cu-NODAGA-PSMA-I&T at 12 and 24 hours p.i. in BALB/c mice. Results are expressed as mean of the % injected activity per gram of tissue (%IA/g) ± standard deviation (SD).

Organ	1 h	4 h	12 h	24 h
Blood	0.33 ± 0.09	0.11 ± 0.03	0.11 ± 0.01	0.08 ± 0.01
Heart	0.49 ± 0.12	0.25 ± 0.06	0.26 ± 0.05	0.21 ± 0.02
Lung	1.49 ± 0.45	0.68 ± 0.22	0.52 ± 0.11	0.32 ± 0.12
Liver	1.12 ± 0.25	0.88 ± 0.25	0.96 ± 0.18	0.84 ± 0.11
Pancreas	1.37 ± 0.90	0.55 ± 0.16	0.28 ± 0.04	0.17 ± 0.03
Spleen	9.33 ± 3.81	2.35 ± 1.45	1.40 ± 0.57	0.58 ± 0.15
Stomach	1.12 ± 0.15	0.72 ± 0.15	0.52 ± 0.03	0.31 ± 0.05
Intestine	2.11 ± 0.85	1.12 ± 0.48	0.94 ± 0.41	0.46 ± 0.11
Adrenal	14.4 ± 4.4	7.31 ± 2.76	2.99 ± 0.93	1.17 ± 0.22
Kidneys	124 ± 21	94 ± 12	60 ± 9	16.1 ± 4.9
Muscles	0.99 ± 0.30	0.45 ± 0.18	0.25 ± 0.05	0.08 ± 0.02
Femur	2.48 ± 0.97	1.34 ± 0.38	0.50 ± 0.10	0.20 ± 0.05
Salivary glands	1.89 ± 0.28	0.58 ± 0.12	0.39 ± 0.05	0.22 ± 0.05

Manufacturing of [^{61}Cu]Cu-NODAGA-PSMA-I&T for human use

The manufacture of [^{61}Cu]Cu-NODAGA-PSMA-I&T for human use took place at the premises of the Department of Nuclear Medicine at the Klinikum rechts der Isar. The reaction was carried out in a GE Healthcare FASTlab 2 module. A 40 µg (28 nmol) aliquot of lyophilised NODAGA-PSMA-I&T (GPM grade, piChem, Austria) was dissolved up to 6 mL in 0.5 M sodium acetate (pH 8) + 20 µg/mL ascorbic acid and transferred to a reaction vial. Then, 3 mL of [^{61}Cu]$CuCl_2$ in 0.05 M hydrochloric acid (77 MBq/mL) were added to the NODAGA-PSMA-I&T solution, reaching pH 5.8. The obtained reaction solution was incubated for 10 min at room temperature (approx. 20-25°C) and dispensed to the product vial (20 mL sterile evacuated vial) over a sterile Cathivex-GV 25 mm PVDF 0.22 µm filter. The product was finally diluted with 0.9% sodium chloride for injection (B. Braun, Germany) up to 12.5 mL. Quality controls results are reported in **Supplemental Table 7**. [^{61}Cu]Cu-NODAGA-PSMA-I&T was produced with high radiochemical purity (99.6%). All the reagents used were trace metal grade.

Supplemental Table 7. Specifications of [^{61}Cu]Cu-NODAGA-PSMA-I&T.

Parameter	Test method	Specifications	Results
Appearance	Visual inspection	Colourless, clear solution	Colourless, clear solution
Bacterial endotoxin content	LAL test	<17.5 EU/mL	<0.5 EU/mL
Free [^{61}Cu]Cu	Radio-TLC test	≤5%	2.53%
pH	pH strips	5.0-7.0	5.8
Radioactive concentration	Dose calibrator	8-15 MBq/mL	14.8 MBq/mL
Radiochemical purity	Radio-TLC test Radio-HPLC test	≥95% ≥95%	97.47% 99.6%
Radionuclidic identity	Gamma-spectrometry	Peaks at 511±30 keV and 656±30 keV	Peaks at 516 keV and 660 keV
Radionuclidic purity* [^{56,57,58,60}Co]Co	Gamma-spectrometry	≤0.01%	0.0001%
Radionuclidic purity* [^{61}Cu]Cu	Gamma-spectrometry	≥99.99%	99.9999%
Sterile filter integrity	Bubble point test	3.0-4.5 bar	3.8 bar
Sterility*	Sterility test	No microbial growth	No microbial growth

**after release*

Physical properties of ^{61}Cu *vs* ^{64}Cu

The physical properties of the two copper radioisotopes for PET imaging, namely ^{61}Cu and ^{64}Cu, are compared in the **Supplemental Table 8**.

Supplemental Table 8. Physical properties of ^{61}Cu *vs* ^{64}Cu.

Physical properties	**^{61}Cu**	**^{64}Cu**
Half-life (hr)	3.34	12.7
Decay, yield (%)	β^+ 61% EC 39%	β^+ 17.9% EC 43.5% β^- 39.0%
$E\beta^+_{max}$ / $E\beta^+_{mean}$ (keV)	1216 / 500	653 / 278
Max / Mean β^+ range in water (mm)	5.2 / 1.3 mm	2.5 / 0.7 mm

Stability of [^{61}Cu]Cu-NODAGA-PSMA-I&T in saline

The stability of [^{61}Cu]Cu-NODAGA-PSMA-I&T synthesized in GMP grade, as described above for human use, was determined at room temperature after dilution with saline (0.9% sodium chloride, B. Braun, Germany) at an activity concentration of 20 MBq/mL. For this purpose, the radiochemical purity was assessed at 3, 6 and 9 hours after synthesis (t=0) by radio-HPLC.

The results are presented in **Supplemental Table 9** and illustrated very high stability, with the radiochemical purity remaining unchanged up to 9 h after end of synthesis.

Supplemental Table 9. Stability data of [^{61}Cu]Cu-NODAGA-PSMA-I&T in saline solution at an activity concentration of 20 MBq/mL, stored at room temperature (n=3). The results are expressed as radiochemical purity (RCP) determined via radio-RP-HPLC.

Time	**0 h**	**3 h**	**6 h**	**9 h**
RCP	99.0 ± 0.2%	99.5 ± 0.2%	99.0 ± 0.5%	99.1 ± 0.5%