Supplemental Data

General Information

The Fmoc-(9-fluorenylmethoxycarbonyl-) and all other protected amino acid analogs were purchased from Bachem Inc. (Bubendorf, Switzerland), Merck KGaA (Darmstadt, Germany) or Iris Biotech GmbH (Marktredwitz, Germany). The H-Rink amide ChemMatrix® resin (35-100 mesh particle size, 0.4-0.6 mmol/g loading) was purchased from Merck KGaA (Darmstadt, Germany). CheMatech (Dijon, France) provided the chelator DOTA(^{t}Bu)$_3$. Peptide syringes were obtained from VWR International GmbH (Bruchsal, Germany).

All necessary solvents and other organic reagents were purchased from either, Alfa Aesar™ (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany) or VWR International GmbH (Bruchsal, Germany). Solid-phase peptide synthesis was carried out by manual operation using a Scilogex MX-RL-E Analog Rotisserie Tube Rotator (Scilogex®, Rocky Hill, CT, USA). H_2O was used after purification by a Barnstead MicroPure system (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Analytical and preparative reversed-phase high-performance liquid chromatography (RP-HPLC) were performed using Shimadzu gradient systems (Shimadzu Deutschland GmbH, Neufahrn, Germany), each equipped with a SPD-20A UV/Vis detector (220 nm, 254 nm). Different gradients of MeCN (0.1% TFA) in H_2O (0.1% TFA) were used as eluents for all RP-HPLC operations.

For analytical measurements of the precursor, a MultoKrom 100-5 C18 (150 × 4.6 mm) column (CS Chromatographie Service GmbH, Langerwehe, Germany) was used at a flow rate of 1 mL/min. Both, specific gradients and the corresponding retention times t_R as well as the capacity factor *K*' are cited in the text.

Preparative RP-HPLC purification of the precursor and the ^{nat}Lu-labeled AMTG was accomplished using a MultoKrom 100-5 C18 (250 × 20 mm) column (CS Chromatographie GmbH, Langerwehe, Germany) at a constant flow rate of 10 mL/min.

Analytical radio-RP-HPLC was performed using a MultoKrom 100-5 C18 (150 × 4.6 mm) column (CS Chromatographie GmbH, Langerwehe, Germany). For radioactivity detection, the outlet of the UV detector was connected to a HERM LB 500 NaI detector (Berthold Technologies, Bad Wildbad, Germany). For metabolite analysis a FlowStar^2 LB 514 detector (Berthold Technologies, Bad Wildbad, Germany) was additionally connected to the HERM detector. For radio-thin layer chromatography, a Scan-RAM™ Scanner with Laura™ software (LabLogic Systems Ltd., Broomhill, Sheffield, United Kingdom) was used.

For lyophilization, an Alpha 1-2 lyophilizer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) connected to an Edwards nXDS10i oil-free scroll pump (Edwards GmbH, Feldkirchen, Germany) was used.

Electrospray ionization-mass spectra for characterization of the substances were acquired on an expression^L CMS mass spectrometer (Advion Ltd., Harlow, UK).

A Heraeus Pico 17 microcentrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to precipitate residual plasma proteins in serum samples (13,000 rpm, 5 min).

General Procedures (GP) and Execution Protocols

On-Resin Peptide Formation (GP1). The respective side-chain protected Fmoc-AA-OH (1.5 eq.) is dissolved in NMP and pre-activated by adding TBTU (1.5 eq.), HOAt (1.5 eq.) and DIPEA (4.5 eq.). After activation for 10 min, the solution is added to resin-bound free amine peptide and shaken for 1.5 h at room temperature. Subsequently, the resin is washed with NMP (6×20 mL/g resin) and after Fmoc deprotection (GP2), the next amino acid is coupled analogously.

On-Resin Fmoc Deprotection (GP2). The resin-bound Fmoc-peptide is treated with 20% piperidine in NMP (*v*/*v*) for 5 min and subsequently for 15 min. Afterwards, the resin is washed with NMP (6×20 mL/g resin).

Conjugation of Chelator (GP3). The protected chelator DOTA(^{t}Bu)$_3$ (1.5 eq.) is dissolved in NMP and pre-activated by adding TBTU (1.5 eq.), HOAt (1.5 eq.) and DIPEA (4.5 eq.). After activation for 10 min, the solution is added to resin-bound *N*-terminal deprotected peptide (1.0 eq.) and shaken for 3 h at room temperature. Subsequently, the resin is washed with NMP (3×20 mL/g resin) and DCM (3×20 mL/g resin).

Peptide Cleavage from the Resin with Additional Deprotection of acid-Labile Protecting Groups (GP4). The fully protected resin-bound peptide is washed with DCM, afterwards dissolved in a mixture of TFA/TIPS/DCM (*v*/*v*/*v*; 95/2.5/2.5) and shaken for 45 min. The solution is filtered off and the resin is treated in the same way for another 45 min. Both filtrates are combined and concentrated under a stream of nitrogen. After dissolving the residue in MeOH and precipitation in diethyl ether, the liquid is decanted and the remaining solid is dried.

Characterization of AMTG

AMTG was synthesized by standard Fmoc-based SPPS (**GP1**-**4**). After finishing the peptide sequence with slightly modifications within the RM2 sequence, a chelator was coupled at the resin (**GP3**). Thereafter, the peptide was cleaved (**GP4**) and purified by RP-HPLC.

AMTG (DOTA-Pip-phe-Gln-*α*-Me-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$)

AMTG. RP-HPLC (20→35% MeCN in 20 min): t_R = 15.2 min, *K*‘ = 8.50.

Calculated monoisotopic mass ($C_{79}H_{120}N_{20}O_{19}$): 1652.9, found: m/z = 551.6 $[M+3H]^{3+}$, 826.6 $[M+2H]^{2+}$, 1652.7 $[M+H]^{+}$.

[^{nat}Lu]Lu-AMTG. RP-HPLC (20→35% MeCN in 20 min): t_R = 15.2 min, *K*‘ = 8.50.

Calculated monoisotopic mass ($C_{79}H_{117}LuN_{20}O_{19}$): 1824.8, found: m/z = 609.6 $[M+3H]^{3+}$, 914.0 $[M+2H]^{2+}$.

Non-radioactive Complexation

2.5 mg of the purified AMTG precursor (1.0 eq., 1.51 µmol) as well as 189 µL of [^{nat}Lu]$LuCl_3$ (20 mM in Tracepur® H_2O, 2.5 eq.) were diluted with Tracepur® H_2O to a final volume of 500 µL and heated to 90°C for 30 min. Following purification via RP-HPLC [^{nat}Lu]Lu-AMTG was lyophilized, and the product was used as reference compound for HPLC measurements to verify the identity of [^{177}Lu]Lu-AMTG and its retention time during serum stability studies.

Radiolabeling

The synthesis of [^{177}Lu]Lu-AMTG according to Good Manufacturing Practice for application in humans was carried out manually using AMTG precursor and non-carrier-added [^{177}Lu]$LuCl_3$ (acquired from ITM Isotope Technologies Munich SE or Billev Pharma ApS). A buffer solution was prepared (27 mg sodium acetate trihydrate in 1 mL sterile 0.1 M hydrochloric acid). The AMTG precursor was dissolved in 700 µL of the buffer solution and transferred to the delivery vial of the [^{177}Lu]$LuCl_3$. Subsequently, 100 mg of sodium ascorbate were dissolved in 300 µL buffer and added to the reaction vial. The solution was heated to 110°C for 20 min. Afterwards, the reaction mixture was diluted with a 0.9% sodium chloride solution to a volume of 10 mL, followed by sterile filtration using the Cathivex-GV (Merck KGaA, Darmstadt, Germany). The filter was then washed with additional 5 mL 0.9% sodium chloride.

Quality control included a thin-layer chromatography scan (iTLC-SG, 0.1 M sodium citrate solution (pH = 5), Agilent, Santa Clara, CA, USA) as well as a reversed-phase high-performance liquid chromatography measurement, using [^{nat}Lu]Lu-AMTG as the corresponding reference compound to verify radioligand identity via comparative retention time analysis. Furthermore, a sterile filter integrity test and a limulus amebocyte lysate were performed.

Patient Information

Patient 1 was a 70-year-old patient who was diagnosed 9 years ago (histology and Gleason-Score unknown, BRCA-1/2 wild type). Metastases occurred after 3 years in soft tissue and bone. Patient was treated with GnRH agonist in metastasized hormone sensitive stage and with first line docetaxel, second line [^{223}Ra]$RaCl_2$ and third line enzalutamide in castration resistant stage. Patient was classified as unfit for a further chemotherapy by his oncologist because of his general condition. PSMA-PET/CT didn't show any tracer uptake at any time. [^{68}Ga]Ga-AMTG PET/CT showed high tracer uptake in disseminated tumor lesions of the bone and in multiple lymph nodes, so [^{177}Lu]Lu-AMTG-RPT was offered.

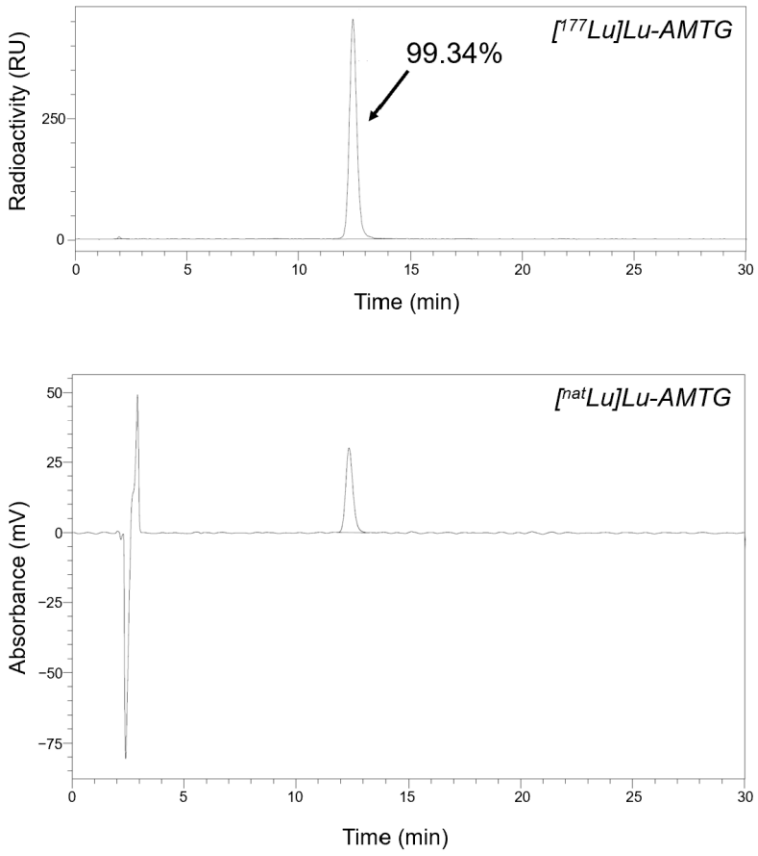
Patient 2 was a 79-year-old patient diagnosed 10 years ago with locally advanced prostate cancer (Gleason-Score 5+4=9) and initially treated with prostatectomy (R1), GnRH agonist and local radiation therapy. One year ago, he suffered from a local recurrence with histologically confirmed neuroendocrine differentiation. A first line therapy was started with cisplatin and etoposide and was stopped after 4 cycles because of a severe leucopenia and a polyneuropathy, for that no further chemotherapy was possible. Second line therapy was performed with abiraterone and olaparib. In [^{68}Ga]Ga-PSMA PET/CT progression was seen with multiple lymph node metastases in the thoracic, abdominal and iliac region. A further [^{18}F]F-FDG-PET/CT showed high tracer uptake and additional metastases, so a [^{68}Ga]Ga-AMTG PET/CT was added. AMTG-PET showed all lesions positive and more lesions than PSMA-PET, so [^{177}Lu]Lu-AMTG-RPT was offered.

Patient 3 was a 65-year-old patient with diagnosis of a locally advanced prostate cancer (Gleason-Score 5+4=9) and lymph node metastases 6 years ago and underwent prostatectomy, lymphadenectomy and adjuvant radiation therapy as well as GnRH analog. 2 years later PSA was increasing and a [^{68}Ga]Ga-PSMA PET/CT was performed, showing PSMA positive osseous metastases. First line therapy with abiraterone and second line therapy with docetaxel was performed as well as intermittent radiation therapy of bone metastases in the ribs and pelvis.

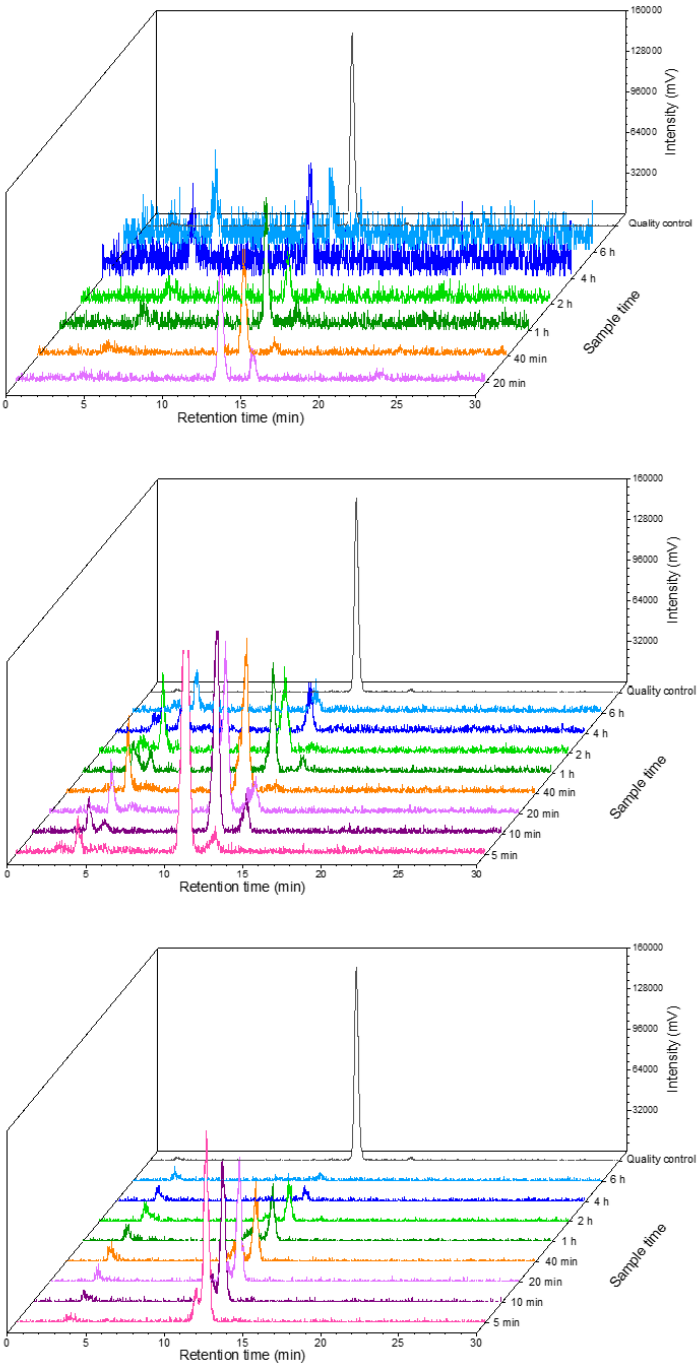
Nevertheless, PSMA PET/CT showed progression of bone metastases. Two cycles of [^{177}Lu]Lu-PSMA-617-RPT were performed with continuous progression of bone metastases. Fourth line chemotherapy was not possible because of polyneuropathy. [^{68}Ga]Ga-AMTG PET/CT showed no PSMA-negative metastases but a more intensive uptake in known PSMA positive tumor lesions. A [^{177}Lu]Lu-AMTG-RPT was offered.

Patient 4 was a 52-year old patient who was first diagnosed with prostate cancer and synchronous metastases of bone and liver 2 years ago and was treated with GnRH analog and multiple therapy lines (docetaxel, abiraterone, olaparib, enzalutamide, two cycles of [^{177}Lu]Lu-PSMA-617-RPT, cabazitaxel, carboplatin and cisplatin). Nevertheless, he had a PSMA doubling time <1 month showing an aggressive tumor biology. Elevated neuroendocrine blood markers (neuron-specific enolase, chromogranin A) suggested a neuroendocrine tumor differentiation. [^{68}Ga]Ga-AMTG PET/CT was performed and showed a high GRPR expression in the metastases. A [^{177}Lu]Lu-AMTG-RPT was offered.

Supplemental Data



SUPPLEMENTAL FIGURE 1. Exemplary chromatograms confirming compound identity and integrity for [^{177}Lu]Lu-AMTG and [^{nat}Lu]Lu-AMTG, as analyzed by analytical (radio-)RP-HPLC (MultoHigh 100 RP18-5µ, 5 µm, 250 x 4 mm, CS Chromatographie GmbH, Langerwehe, Germany; 26% MeCN in H_2O + 0.1% TFA in 30 min).



SUPPLEMENTAL FIGURE 2.

Radio-RP-HPLC chromatograms showing intact [^{177}Lu]Lu-AMTG as well as two metabolites in human serum of different patients over time. Respective retention times for [^{177}Lu]Lu-AMTG and its metabolites changed slightly for patient 2, 3 and 4 compared with patient 1. Co-injection of [^{nat}Lu]Lu-AMTG confirmed identity and integrity of intact [^{177}Lu]Lu-AMTG for the serum samples of each individual patient. Patient 2: t_R ([^{177}Lu]Lu-AMTG) ~13.0 min, metabolites: t_R~5.0 min and t_R~15.2 min; Patient 3: t_R ([^{177}Lu]Lu-AMTG) ~10.8 min, metabolites: t_R~3.9 min and t_R~12.7 min; Patient 4: t_R ([^{177}Lu]Lu-AMTG) ~12.1 min, metabolites: t_R~3.25 min and t_R~11.4 min.

SUPPLEMENTAL TABLE 1. In vivo serum stability of [^{177}Lu]Lu-AMTG in four patients with mCRPC by means of the percentage of intact radiopharmaceutical at distinct time points after intravenous injection of 7.6±0.1 GBq of [^{177}Lu]Lu-AMTG.

Time after injection (min)	Amount of intact radiopharmaceutical (%)				
	Patient 1	Patient 2	Patient 3	Patient 4	Mean ± SD
5	85.4	n.d.	86.0	83.5	85.0 ± 1.1
10	78.8	n.d.	76.2	74.5	76.5 ± 1.8
20	78.3	81.7	70.5	71.8	75.6 ± 4.6
40	73.0	73.7	73.7	59.5	70.0 ± 6.1
60	70.2	65.6	60.6	53.0	62.4 ± 6.4
120	58.2	54.6	53.1	50.6	54.1 ± 2.7
240	54.8	53.8	41.4	49.3	49.8 ± 5.3
360	35.3	40.8	37.2	37.0	37.6 ± 2.0