General

All protected amino acid analogs and Tritylchloride polystyrene (TCP) resin (100-200 mesh; loading: 1.6 mmol/g) were purchased from Iris Biotech (Marktredwitz, Germany) or Bachem (Bubendorf, Switzerland). S-Trityl-Mercaptoacetic acid was obtained from AnaSpec (Seraing, Belgium). All other organic reagents and solvents were purchased from SigmaAldrich (Munich, Germany) or CLN (Freising, Germany). Solid phase peptide synthesis (SPPS) was carried out manually using an Intelli-Mixer syringe shaker (Neolab, Heidelberg). Analytical reversed-phase high performance liquid chromatography (RP-HPLC) was performed on a Nucleosil 100 C 18 (5 µm, 125 x 4.0 mm) column (CS GmbH, Langerwehe, Germany). For preparative RP-HPLC, a Multospher 100 RP 18-5 (250 x 20 mm) column (CS GmbH, Langerwehe, Germany) was used. Analytical and preparative RP-HPLC were performed on a Sykam gradient HPLC System (Sykam GmbH, Eresing, Germany). The peptides were eluted with 0.1 % TFA (v/v) in H₂O (Solvent A) and 0.1 % TFA in acetonitrile (Solvent B) with a constant flow of 1 or 10 mL/min for analytical and preparative HPLC, respectively. Specific gradients, retention times tᵣ as well as capacity factors K’ are cited in the text. For UV detection, a 206 PHD UV-Vis detector (Linear™ Instruments Corporation, Reno, USA) was used. Radio-HPLC for ((¹²⁵)I-I-BA)KuE was performed on a Nucleosil 100 C 18 (5 µm, 125 x 4.0) column using a NaI(Tl) well-type scintillation counter from EG & G Ortect (München, Germany) for radioactivity detection. Analytical Radio-HPLC of ⁹⁹ᵐTc-labeled derivatives was carried out using a Nucleosil 100 C 18 (5 µm, 125 x 4.0) column (flow: 1.5 mL/min) on a Shimadzu HPLC system equipped with a NaI(Tl) scintillation detector (2” x 2”) and a SPD M20A diode array UV/Vis detector. Radio-TLC analysis was carried out using a B-FC-3600 TLC Scanner (Bioscan, Washington, USA). ESI-MS spectra were recorded using a Varian 500-MS IT mass spectrometer (Agilent Technologies, Santa Clara, USA).
**Analytical data:**

**PSMA-I&S (mas₂-y-nal-k(Sub-KuE, 5):**

HPLC (20-40% B in 15 min): $t_R = 8.9$ min, $K^\prime = 6.4$.

Calculated monoisotopic mass ($C_{59}H_{82}N_{10}O_{21}S$): 1298.5; found: $m/z = 1299.9$ [M+H]⁺, 1321.9 [M+Na]⁺, 1337.5 [M+K]⁺

**MAS₂-y-nal-k(Sub-KuE) (6):**

HPLC (20-40% B in 15 min): $t_R = 10.7$ min, $K^\prime = 7.91$.

Calculated monoisotopic mass ($C_{59}H_{82}N_{10}O_{21}S$): 1298.5; found: $m/z = 1300.1$ [M+H]⁺, 1321.9 [M+Na]⁺, 1337.8 [M + K]⁺

**natRe-PSMA-I&S (natRe-mas₂-y-nal-k(Sub-KuE)) (natRe-5):**

HPLC (20-40% B in 15 min): $t_R = 10.3$ min, $K^\prime = 5.05$. Calculated monoisotopic mass ($C_{59}H_{78}N_{10}O_{22}ReS$): 1498.5; found: $m/z = 1499.3$ [M+H]⁺, 1521.3 [M+Na]⁺.

**Radiolabeling**

**Radioiodination**

Radiosynthesis of the reference radioligand ($^{125}$I)-I-BA)KuE via oxidative destannylation of the corresponding protected tributylstannyl precursor was performed as published previously [1-4].

**$^{99m}$Tc-labeling**

Standard labeling protocol: For $^{99m}$Tc-labeling, the precursor was dissolved in 0.5 M ammonium acetate buffer, pH 8. To 50 µL of the precursor solution (20-30 nmol peptide) 50 µL of 0.25 M ammonium acetate buffer and 20 µL disodium tartrate buffer (50 mg/mL in 0.5 M ammonium acetate buffer) were added. Upon addition of $^{99m}$Tc-pertechnetate (1-5 mL, 1000 – 1200 MBq), 10 µL of a freshly prepared and argon degassed solution of stannous chloride dihydrate (4 mg/mL) in ascorbic acid/HCl (3 mg/mL ascorbic acid in 10 mM HCl) were added. The final pH of the reaction mixture was 7-7.5. After heating to 90°C for 20 min, the reaction mixture was cooled and diluted with 10 mL of water/acetic acid (95/5, v/v), and the solution was passed through a C-18 cartridge. Upon purging with 10 mL of water, the labeled peptide was eluted from the cartridge with 1 mL EtOH containing 0.5 % acetic acid. Fractions with the highest activity were pooled and diluted with PBS (EtOH content < 6% v/v). Finally, for patient administration, the labeled peptide solution was sterile filtered (0.22 µm sterile Filter, Cathivex, Merck Millipore, Germany).
**Kit formulation:** For the preparation of the lyophilized kit, 25 nmol peptide in 100 µL sodium hydrogen phosphate buffer and 40 µL sodium tartrate buffer were added to a glass vial. Subsequently, 10 µL of a freshly prepared, nitrogen-purged stannous chloride dihydrate solution was added (4 mg/mL in ascorbic acid/HCl solution). The kit was freeze-dried, filled with argon and stored at -20°C. For $^{99m}\text{Tc}$-labeling, 1-5 mL $^{99m}\text{Tc}$-pertechnetate in saline were added, and the kit was heated to 90°C for 20 min. The final pH of the reaction mixture was 7. For final formulation of the radiopharmaceutical, sterile isotonic saline was added to obtain a total volume of 10 mL, and the solution was sterile filtered before administration.

**Radio-HPLC**

$^{99m}\text{Tc}$-mas$_3\gamma$-nal-k(Sub-KuE) ($^{99m}\text{Tc}$-$5 = ^{99m}\text{Tc}$-PSMA-$i$&$S$):

HPLC (5-95% B in 15 min, 220 nm): $t_R = 6.5$ min, $K' = 4.0$.

$^{99m}\text{Tc}$-MAS$_3\gamma$-nal-k(Sub-KuE) ($^{99m}\text{Tc}$-$6$):

HPLC (5-95% B in 20 min, 220 nm): $t_R = 6.5$ min, $K' = 4.0$.

**Radio-TLC**

Radio-TLC analysis for reaction and quality control was carried out using two different TLC systems. To determine the extent of $^{99m}\text{Tc}$-colloid formation, ITLC using silica impregnated TLC paper (Agilent, Waldbronn, Germany) and MeOH/1M ammonium acetate (1/1, v/v)) as a mobile phase was performed. The amount of free $^{99m}\text{Tc}$-pertechnetate was determined using RP-TLC plates (Merck Millipore, Darmstadt, Germany) and MeCN/water (1% TFA, 30/70 (v/v)) as mobile phase.