

1 Glossary

Acronym	Full name
ADT	Androgen-deprivation therapy
BSA	Bovine serum albumin
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
Micro-PET/CT	Micro-positron emission tomography/computed tomography
PBS	Phosphate-buffered saline
PSA	Prostate specific antigen
PSMA	Prostate-specific membrane antigen
Radio-HPLC	Radio-high-performance liquid chromatography
Radio-TLC	Radio-thin layer chromatography

2 Materials

All commercially obtained chemicals were of analytical grade and were used without further purification. DOTA-NT-20.3 peptide [Ac-Lys(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)-Pro-(NMe-Arg)-Arg-Pro-Tyr-Tle-Leu] was kindly provided by Iason GmbH (Graz, Austria). PSMA-11 and NT were purchased from ABX (Radeberg, Germany) and Aladdin (Shanghai, China), respectively. Sodium acetate and hydrochloric acid were obtained from Merck (Darmstadt, Germany). 0.9% sodium chloride injection was provided by Otsuka (China). An ITG Ge-68/Ga-68 Generator and iQS-theranostics synthesizer (iQS-TS) automated module (ITG GmbH, Munich, Germany) were used. The peptide was analyzed using a Shimadzu HPLC system with a C18 column (5 μ m, 250 mm \times 10 mm; Waters Xbridge C18, Milford, MA, USA). ⁶⁸Ga-radioactivity (400-600 keV) was measured with a gamma counter (Wizard 2; PerkinElmer, USA). Imaging *in vivo* was carried out with Inveon micro-PET/CT (Siemens Medical Solutions, Germany) and reconstructed with Inveon Research Work Station.

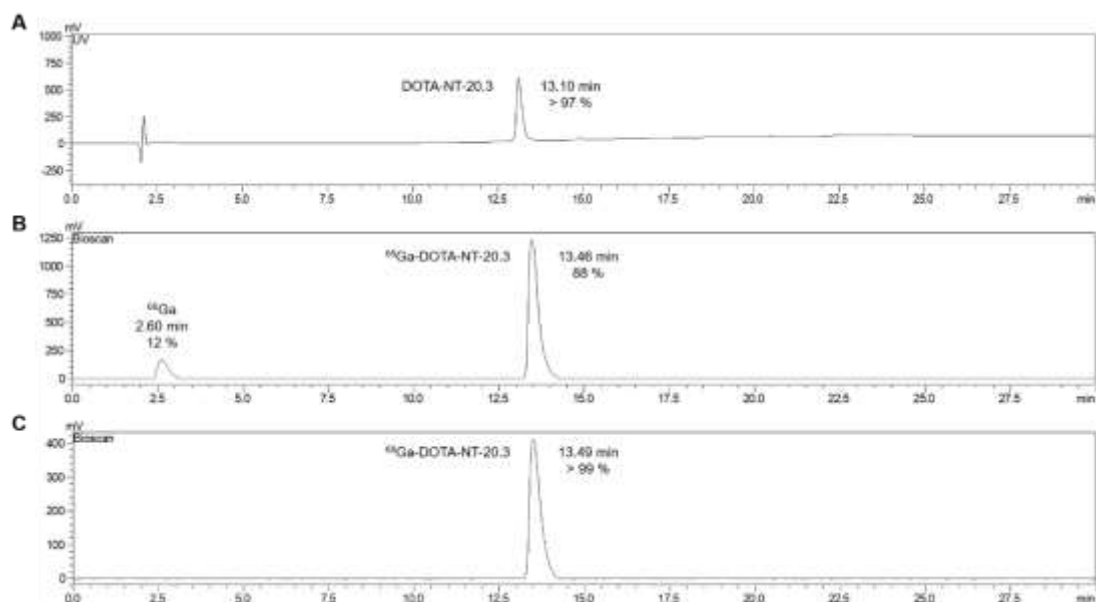
16 Tumor Cell Lines and Mouse Models

Human prostate adenocarcinoma PC3 and LNCap cells were obtained from the Cell Bank of Shanghai Institutes for Biological Sciences and cultured in medium containing 10 % fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin–streptomycin solution 100 U/mL (Beyotime, Shanghai, China). All cell lines were incubated at 37 °C in a humidified incubator with 5 % CO₂ and used for subsequent experiments during logarithmic growth. Animal experiments were performed using (4–6)-week-old male

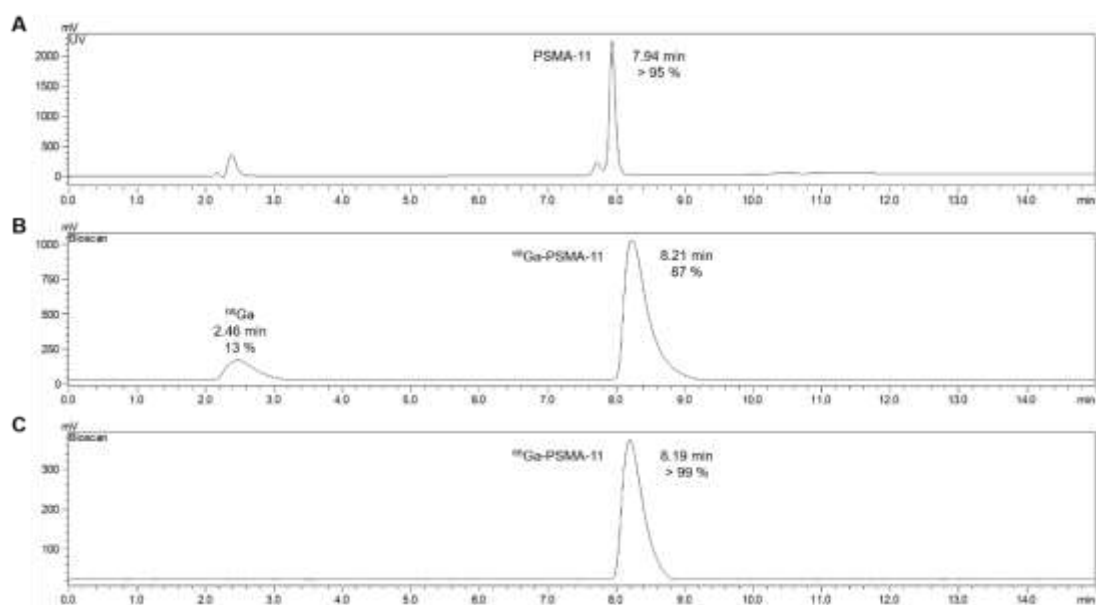
BALB/c nude mice (18-25g, BioHermes, Wuxi, China). To establish PCa models, mice were injected in their right armpits with a single-cell suspension of 2×10^6 PC3 or LNCap cells. When the tumor volume reached 200mm^3 , the mice were subjected to PET imaging and biodistribution analysis. Isoflurane (RWD, Shenzhen, China) served as anesthetic for animal use.

Quality Control

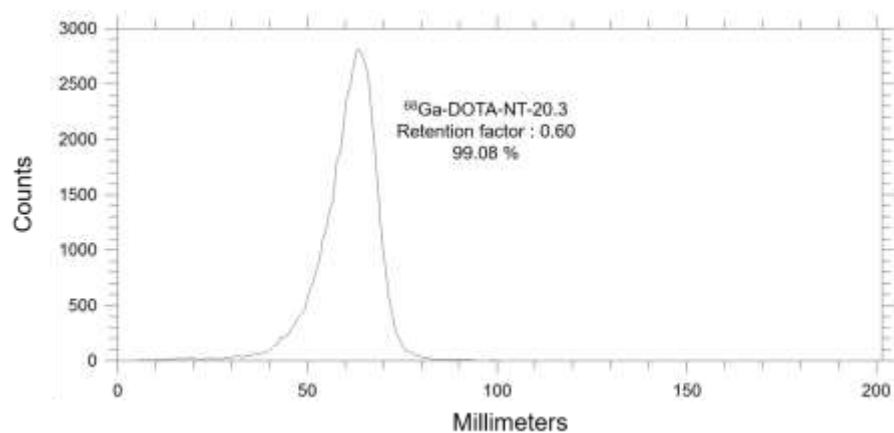
The radiolabeling yield and radiochemical purity of ^{68}Ga -DOTA-NT-20.3 and ^{68}Ga -PSMA-11 were detected with a flow-count radio-HPLC detector (B-FC-1000F, Bioscan, DC, USA). Elution was performed with a mixture of A (0.1% trifluoroacetic acid (TFA) in water) and B (0.1% TFA in acetonitrile) with a flow rate of 1 mL/min. The linear gradient elution of ^{68}Ga -DOTA-NT-20.3 started from 100%–90% A for 5 min, and then changed linearly to 90%–20% A for 15 min. Finally, the mobile phase returned linearly to 100% B at the run end, and the retention time of ^{68}Ga -DOTA-NT-20.3 was 13.61 ± 0.12 min. ^{68}Ga -PSMA-11 elution was carried out with a linear gradient of A from 80 % to 20% within 15 min, and the retention time was 8.16 ± 0.13 min. Radiochemical purity of ^{68}Ga -DOTA-NT-20.3 was also accessed by a B-AR-2000 radio-TLC imaging scanner (Bioscan) using the silica gel impregnated iTLC-SG-Glass microfiber chromatography papers (Agilent, CA, USA). The developing solvent was methanol and 1 M sodium acetate (1:1), and the retention factor of ^{68}Ga -DOTA-NT-20.3 was 0.60 ± 0.02 .



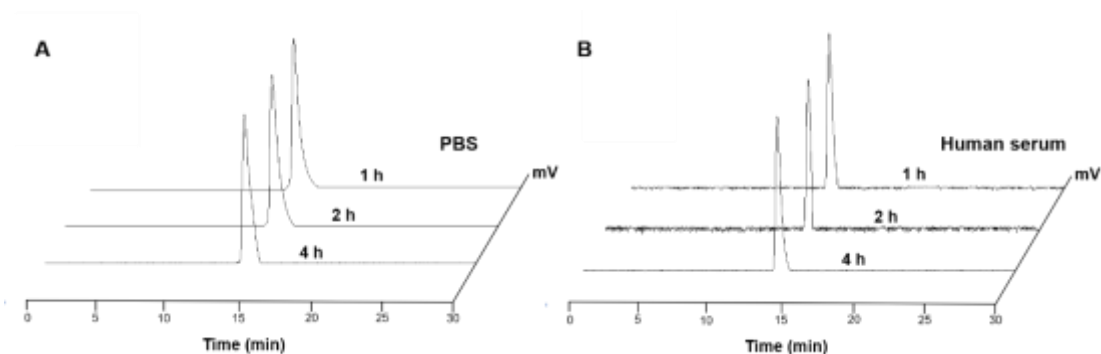
Supplemental Figure 1. (A) HPLC analysis of DOTA-NT-20.3. (B, C) Radiolabeling yield and radiochemical purity of ^{68}Ga -DOTA-NT-20.3 analyzed by radio-HPLC.



Supplemental Figure 2. (A) HPLC analysis of PSMA-11. (B, C) Radiolabeling yield and radiochemical purity of ^{68}Ga -PSMA-11 analyzed by radio-HPLC.



Supplemental Figure 3. Radiochemical purity of ^{68}Ga -DOTA-NT-20.3 analyzed by radio-TLC.



Supplemental Figure 4. In vitro stability of ^{68}Ga -DOTA-NT-20.3 in PBS (A) and human serum (B), respectively, 1, 2, and 4 h after incubation.