Published in final edited form as:

Mol Imaging Biol. 2017 December ; 19(6): 944–951. doi:10.1007/s11307-017-1091-9.

Repeatability of [⁶⁸Ga] DKFZ11-PSMA PET Scans for Detecting Prostate-Specific Membrane Antigen-Positive Prostate Cancer

Joseph R. Osborne^{1,4}, Teja M. Kalidindi², Blesida J. Punzalan², Kishore Gangangari^{1,5}, Daniel E. Spratt³, Wolfgang A. Weber¹, Steven M. Larson^{1,2,4}, and Naga Vara Kishore Pillarsetty^{1,4}

¹Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY

²Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY

³Department of Radiation Oncology, University of Michigan, Ann Arbor, MI

⁴Department of Radiology, Weill Cornell Medical College, New York, NY

⁵Department of Chemistry, Hunter College and PhD Program in Chemistry, The Graduate Center of the City University of New York, New York, NY

Abstract

Purpose—We studied the effect of varying specific activity of [⁶⁸Ga]DKFZ-PSMA11 ([⁶⁸Ga]DP11) on repeated imaging of prostate-specific membrane antigen-positive (PSMA+) xenograft tumors.

Procedures—Athymic nude mice bearing PC3-PIP (PSMA+) and PC3 (PSMA–) bilateral flank tumors were assessed to study intra- and inter-day repeatability of [⁶⁸Ga]DP11 imaging in mice administered [⁶⁸Ga]DP11 or [⁶⁷Ga]DP11 (as a dilution tracer) using imaging and biodistribution studies.

Results—Region-of-interest (ROI) analysis of the [⁶⁸Ga]DP11 imaging study indicated that the uptake was constant on same day or consecutive days. Prior imaging with [⁶⁸Ga]DP11 did not significantly influence the subsequent uptake of [⁶⁸Ga]DP11. Uptake of [⁶⁸Ga]DP11 (60 min) and [⁶⁷Ga]DP11 (24 h) in PC3-PIP tumors was 12.37 ± 4.19 %ID/g and 12.49 ± 6.88 %ID/g, respectively; [⁶⁸Ga]DP11 was 13.83 ± 3.77 and 17.76 ± 1.84 on same-day and 15.98 ± 5.82 %ID/g on second-day imaging.

Conclusions—This study demonstrates that [⁶⁸Ga]DP11, in a given PSMA+ lesion, is constant under several same-day or serial-day imaging conditions.

Corresponding Authors: Joseph R. Osborne, MD, PhD, Department of Radiology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, Box 77, New York, NY 10065, Phone: (212) 639-8001, Fax: (212) 717-3263, osbornej@mskcc.org; Naga Vara Kishore Pillarsetty, PhD Department of Radiology, Memorial Sloan Kettering Cancer Center, Zuckerman Research Center, 417 East 68th Street, New York, NY 10065, pillarsn@mskcc.org.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Keywords

Gallium-68; PSMA; prostate cancer; repeatability; positron emission tomography; mice; specific activity

Introduction

Prostate cancer (PCa) is the second most lethal cancer among men in the U.S. with 26,120 estimated deaths in 2016 [1]. To address this problem, significant advances have been made in diagnostic imaging, which have aimed to characterize primary prostatic lesions, correlate with the clinical state of disease, and monitor response to therapy [2]. The current standard-of-care imaging, however, cannot adequately monitor the spread of disease from the primary site to regional lymph nodes and distant metastatic sites or annotate the genomic evolution of disease during this process. As the detection of metastatic lesions and spread is crucial for the staging, management, and disease-specific survival of prostate cancer, an imaging biomarker-based solution appears necessary.

Prostate-specific membrane antigen (PSMA) is a candidate antigen on which to target a biomarker-based strategy. It is an extracellular glutamate carboxypeptidase II and a zinc metalloenzyme that catalyzes the hydrolysis of C-terminal glutamate in peptides. More importantly, it is highly expressed in both primary and metastatic prostate cancer lesions, correlating with the stage/grade of the PCa [3]. PSMA was originally detected in the androgen-dependent prostate cancer cell line LNCaP and was thought to be confined to prostate tumors, but subsequent studies have revealed that PSMA is expressed in the neovasculature of many other tumors [4]. Regardless, this relative selectivity still makes PSMA a very attractive target for developing targeted imaging and therapeutic agents. Accordingly, several biological vectors including antibodies [5], peptides [6], small molecules, and particles have been developed and evaluated as potential carriers for imaging and therapeutic entities.

Urea-glutamate-based small molecule antagonist probes have recently gained favor among other alternatives, as their molecular weight, ease of labeling, fast clearance, and ability to label with short-lived isotopes (e.g., Ga-68 and F-18) permit repetitive imaging. A variety of Positron Emission Tomography (PET)/Single Photon Emission Computed Tomography probes using I-123, Ga-68, and F-18 have been developed and evaluated in both preclinical and clinical settings [7–12]. Recently, [⁶⁸Ga]DKFZ11-PSMA developed by Eder and colleagues [13] has gained significant popularity due to its ease of labeling and superior performance *in vivo* in detecting both primary and metastatic prostate cancer lesions. The tracer demonstrated 100% sensitivity and specificity in a cohort study of 319 patients [14]. These studies indicate that [⁶⁸Ga]DKFZ11-PSMA ([⁶⁸Ga]DP11) has true potential to be used worldwide for detecting prostate cancer in patient populations.

Ga-68 is a PET isotope and therefore [⁶⁸Ga]DP11 has the potential to quantify receptor density on each individual lesion. The theoretical maximum specific activity (SA) of Ga-68 based radiopharmaceuticals is approximately 2764 Ci/μmol. However, with the current synthetic protocols used in clinical production, the typical apparent SAs (e.g., [⁶⁸Ga]DP11 +

free DKFZ11-PSMA or [^{68}Ga]DOTA-TOC + DOTA-TOC, etc.) are closer to 0.5–1 Ci/ μmol due to the presence of uncomplexed peptide. This translates to about 5–10 nmoles for each patient for a 10 mCi dose. Due to the high amount of ligand administered during scanning, it is possible that the ligand can residualize on the receptors and affect the quantification on the subsequent scan. With increasing use of [^{68}Ga]DP11 as a scouting scan for treatment with [^{177}Lu]DKFZ-PSMA-617 [15] or [^{225}Ac]DKFZ-PSMA-617 [16], it is important to understand the effect of prior imaging with [^{68}Ga]DP11 (if any) on the uptake of the therapeutic radiopharmaceutical.

While many studies have focused on optimizing the radiosynthesis and findings on the ability to detect lesions in patient populations using [^{68}Ga]DP11 have been published, so far none of the studies have addressed the question of reproducibility of scans. For a receptor-targeted radiopharmaceutical, residual activity can impact the subsequent scan. This is important if PSMA-based imaging is being used for measuring response to therapy on a routine basis [17]. This is also important for PSMA-targeted therapeutic approaches that leverage these urea-glutamate-based small molecules for delivering toxins or therapeutic radionuclides [18], as receptor availability is one of the critical limiting factors. In the current manuscript, we have addressed using PSMA-expressing PC3-PIP xenografts, specifically whether imaging these tumors with [^{68}Ga]DP11 affects subsequent serial scans performed at 24 and 48 h.

Materials and Methods

All starting materials, solvents, and reagents were purchased from commercial sources (Sigma-Aldrich, St. Louis, MO and Thermo Fisher Scientific, Waltham, MA) and used without further purification. The radiochemical precursor DFKZ-PSMA11 (catalog number 9920.0001) was purchased from ABX (Radeberg, Germany) and dissolved in chelexed water to achieve a concentration of 1 mg/ml, and 10 μg (10 μl) was aliquoted in 1.5 ml microcentrifuge tubes and stored at $-20\text{ }^{\circ}\text{C}$ and used for reactions. All solvents used for high-performance liquid chromatography (HPLC) analysis and purification were purchased from Fisher Scientific (HPLC grade). For radiosynthesis, TraceSELECT[®] or TraceSELECT[®] Ultra (Sigma-Aldrich) grade reagents were used. The reagents included KOH, acetic acid, 30% HCl, KCl, and CH₃CN. Water ($>18.2\text{ M}\Omega\text{ cm}^{-1}$ at $25\text{ }^{\circ}\text{C}$) was obtained from an Alpha-Q Ultrapure water system (Millipore, Bedford, MA). C-18 reversed phase cartridge StrataTM-X (#8B-S100-TAK, 33 μm Polymeric Reversed Phase C-18, 30 mg/1mL) and HPLC column Luna (00G-4252-E0, 250 $\times$ 4.6 mm, 5 μm , 100 $\text{\AA}$, C-18 with TMS end capping) were purchased from Phenomenex (Torrance, CA).

Cell Lines

PC-3 cell lines were obtained from the American Type Culture Collection (Manassas, VA) and cultured in F12-K medium with 10% FCS, 2mM L-Glutamine, 1.5g/l Sodium Bicarbonate, and penicillin-streptomycin. PC3-PIP cells were kindly provided by Dr. Martin Pomper (Johns Hopkins University, Baltimore, MD) and maintained under RPMI 1640 medium with 10% FCS and penicillin-streptomycin.

Radiosynthesis

Gallium-68 was eluted from the ANSTO generator using 0.3 N HCl, trapped on a cation exchange column (Biorad resin) using an automated elution system and eluted from the ion exchange resin as [⁶⁸Ga]K[Ga(OH)₄] using 0.5 M KOH solution in 600 µl volume [19].

To neutralize and acidify the solution, 26 µl of glacial acetic acid (>3 µL per 100 µl of 0.5 M KOH) was added to 500 µl eluate. The pH was <5 (pH paper) and about 50 µl of NaOAc buffer (2 mM, pH 4.2) was added. [⁶⁸Ga]DP11 was synthesized by adding 20 µg of DKFZ-PSMA11 ligand (947 MW, 21.1 nmoles) to a 600 µl solution of [⁶⁸Ga]Ga(OAc)₃ (~555 MBq/15 mCi) in NaOAc (0.5 M, pH 4.5) buffer and incubating the reaction mixture for 10 min at 37 °C (Fig. 1). The compound was purified by passing through a Strata™-X cartridge (#8B-S100-TAK, 33µm Polymeric Reversed Phase C-18, 30 mg/1 ml) preconditioned with 1 ml of 95% ethanol and 2.5 ml of pure water. The cartridge was then rinsed with 3 ml of water to remove any residual free Ga-68 and finally, the purified Ga-68 peptide conjugate was eluted in 500 µl of ethanol (95% in saline). The purity of the compound was analyzed using HPLC on a C18 column (Phenomenex Luna, 250 × 4.6 mm, 5 m, 110 Å) using a solvent of 22% CH₃CN in 0.1% trifluoroacetic acid solution in the water. The product was concentrated under reduced pressure to reduce ethanol content and formulated in 0.9% saline.

For the synthesis of [⁶⁷Ga] DP11, [⁶⁷Ga]GaCl₃ (74 MBq/2 mCi) in 0.1 N HCl was added to an Eppendorf tube containing 30 µg of DKFZ-PSMA11 (947 MW, 31.6 nmoles) and allowed to react at 37 °C for 120 min. HPLC analysis (using the conditions described above) was performed to ensure complete complexation of gallium and the product was diluted using PBS and used without any further purification.

Xenografts

All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of Memorial Sloan Kettering Cancer Center and followed National Institutes of Health guidelines for animal welfare. Bilateral tumor subcutaneous xenografts of PC3-PIP (PSMA+) and PC3 (PSMA-) were established for imaging and biodistribution studies. Slow-growing PC3 tumors xenografts were first established by administration of 5 × 10⁶ cells in 200 µl (1:1 F12-K medium and Matrigel®) on the left flank under 2% isoflurane anesthesia and allowed to reach a volume of 150 mm³, which needed approximately 6–10 days. This was followed by inoculation of fast-growing PC3-PIP tumor cells (5 × 10⁶ cells in 20 µl in 1:1 RPMI-1640 and Matrigel®) on the right flank of the mice under 2% isoflurane anesthesia. By day 30–35, the tumors were ready for imaging and biodistribution studies.

Small Animal PET Imaging

Small animal PET imaging studies were performed on a dedicated small-animal PET scanner (Focus 120 microPET; Siemens Medical Solutions USA, Inc.). Mice were maintained under 2% isoflurane anesthesia in oxygen at 2 L/min during the entire scanning period. Athymic nude mice bearing PC3-PIP (PSMA+) and PC3 (PSMA-) bilateral tumor xenografts were used for imaging and biodistribution studies. For the repeatability study,

Group 1 (Fig. 1) mice were administered [^{68}Ga]DP11 *i.v.* via the tail vein either on two alternate (days 1 and 3) and Group 2 were injected and imaged three consecutive days (days 1, 2, and 3). Mice were anesthetized and imaged 60 min post-administration using a small animal PET scanner (Focus 120, Siemens, Knoxville, TN). Group 3 mice were imaged twice in one day with ~ 7.4 MBq (200 μCi) [^{68}Ga]DP11 containing about ~ 0.37 MBq (10 μCi) of [^{67}Ga]DP11 per injection.

An energy window of 350–700 keV and a coincidence timing window of 6 ns were used. The imaging data were corrected for non-uniformity of the scanner response, dead time count losses, and physical decay to the time of injection, but not for attenuation, scatter, or partial-volume averaging. The spatial resolution of the Focus 120 scanner at the center of the field of view is ~ 1.6 mm full-width at half maximum. The count-rates in the reconstructed images were converted to percentage injected dose per gram of tissue (%ID/g) using a calibration factor (0.037 MBq/ml/cps/voxel) derived by imaging a mouse-size water-equivalent phantom containing Ga-68.

Group 1 mice also received [^{67}Ga]-DP11 on day 2 and were sacrificed on day 3, 60 min post-administration, and all the organs were collected. To ensure reproducibility of the imaging studies, we administered the same amount (~ 5.5 MBq/150 μCi) of [^{68}Ga]DP11 with SA of about 22.9 GBq/ μmole (620 mCi/ μmole). For measuring intra-day variability, Group 3 mice were imaged with [^{67}Ga]DP11 in the morning and underwent repeated imaging with 7.4 MBq (200 μCi) [^{68}Ga]DP11 at 7 h after the first injection.

Region of interest (ROI) image analysis was performed using ASIPRO VM[®] software, which was provided by the vendor. A total of 18–20 2D ROIs were drawn around the tumor using the free trace setting across the coronal plane. The activity, volume, and %ID/cc details generated by the software on these slices was used for data analysis.

Biodistribution Studies

For terminal biodistribution studies, mice with PC3 and PC3-PIP bilateral tumors were injected with ~ 5.5 MBq (150 μCi) of [^{68}Ga]DP11 in 200 μl of saline via the tail vein. For Group 1 animals on day 2, mice were administered 2.75 MBq (75 μCi) of [^{67}Ga]DP11 (2.29 GBq/ μmole /62 mCi/ μmole) via the tail vein. The exact dose delivered to each animal was determined by measuring radioactivity in the syringe before and after administration in an energy-calibrated dose calibrator (CRC-15R). The animals were euthanized at different time points, organs harvested, and radioactivity measured in a calibrated γ -counter (1480 Wizard 3 Auto γ -counter) using a 400 to 600-keV energy window for Ga-68 and 70 to 320-keV energy window for Ga-67 and decay correction. Gallium-67 counts were collected 24 h to allow decay of Ga-68. The counts were converted into activity, and %ID/g was calculated by dividing by decay-corrected injected activity and weight of the organ. A minimum of five animals was used for each time point and the values are reported as mean $\pm$ SD of mean.

Results

Radiosynthesis

[⁶⁸Ga]DP11 was synthesized in >95% radiochemical yield (non d.c.) with radiochemical purity of >99% at an SA of 26.3 ± 2.2 GBq/μmole (711.1 ± 81.2 mCi/μmole; n = 5) at the end of synthesis (EOS). No optimization to increase the SA was attempted. Though the crude product yields were consistently >98%, purification was performed to ensure reproducibility of the experiments. [⁶⁷Ga]DP11 was synthesized in >99.5% radiochemical yield with a radiochemical purity of >99%, at an SA of ~2.34 GBq/μmole (63.3 mCi/μmole) at the EOS.

PET Imaging Study

Small animal PET imaging was performed on mice bearing PC3-PIP (R) and PC3 (L) bilateral tumors to evaluate the repeatability of imaging with [⁶⁸Ga]DP11 on consecutive days. Figure 2 shows representative coronal and transaxial slices of PET images obtained using the same mice for three consecutive days. As expected, only PSMA+ PC3-PIP tumors displayed uptake, whereas PC3 tumors had minimal or no uptake. Qualitatively, the images indicated that accumulation of the tracer in the tumors was virtually the same on the three consecutive days. Additionally, we observed that the kidneys have the highest uptake with rapid clearance from the rest of the organs.

To obtain quantitative information, ROI analysis of the imaging study in mice bearing PC3-PIP (R) tumors indicated that the uptake was constant during the imaging period of three consecutive days. PC3 tumors could not be visualized or delineated with the [⁶⁸Ga]DP11 tracer and therefore we could not perform ROI analysis, so only the biodistribution study was performed. Figure 3 shows the performance of the [⁶⁸Ga]DP11 in PC3-PIP tumors on consecutive imaging days, demonstrating constant uptake. To minimize the influence of parameters of inter-tumor heterogeneity, size, body weight, etc. of the mice, we compared the relative uptake of the tracer in each mouse. Figure 4 demonstrates this minimal variation in the mean %ID/g values of [⁶⁸Ga]DP11 in PC3-PIP tumors.

Biodistribution Studies

Biodistribution studies in Group 1 indicate that uptake of both [⁶⁸Ga]DP11 (60 min) and [⁶⁷Ga]DP11 (24 h) in PC3-PIP tumors is similar, with values of 12.37 ± 4.19 and 12.49 ± 6.88 %ID/g (Table 1). Both of these values are comparable to uptake values in PC3-PIP tumors in Group 2 mice, which exhibited uptake values of 15.98 ± 5.82 %ID/g. The corresponding values in PC3 tumors were about 1.08 ± 0.17 and 0.41 ± 0.13 %ID/g and for blood, the values were 4.33 ± 0.58 and 0.58 ± 0.25 %ID/g. The results are summarized in Table 1. Biodistribution studies in PC3-PIP tumor bearing mice administered with [^{67/68}Ga]DP11 on the same day (Group 3) indicates that the uptake in tumor is 13.82 ± 3.77 and 17.76 ± 1.84 in the morning and evening, respectively (Fig. 4). Additionally, the uptake in other organs remains fairly constant in both the studies (Table 2).

Discussion

Many emerging imaging modalities and agents designed to evaluate prostate cancer metastatic lesions in the most appropriate clinical context are currently under evaluation. It is well understood that the current standard-of-care imaging agents are either non-quantitative or do not report information relevant to the clinical setting. Of the FDA-approved molecular imaging (PET) agents in the U.S., the currently approved radiopharmaceuticals ($[^{11}\text{C}]\text{Choline}$ and $[^{18}\text{F}]\text{FACBC}$) also have well-documented limitations regarding specificity and stage-specific information. Investigational PSMA small molecule antagonists have been used extensively outside the U.S. and in more limited research protocols in the U.S., but challenges remain in getting FDA approval and developing a biomarker-based imaging strategy for PCa. Conceptually, the success of a small antibody fragment Phase I trial [20] at our institution is an intriguing prelude to the use of shorter-lived small molecule tracers, which addresses a persistent desire for a similar but cost-effective alternative.

We believe there may be a cost-effective path to utilizing $[^{68}\text{Ga}]\text{DP11}$ and have therefore examined some of the salient features (repeatability) of $[^{68}\text{Ga}]\text{DP11}$ in small animals. This process involves several components, but almost all rely on the agent being dispensed from a single source to a hub of satellite imaging sites. To accomplish this task, the agent must be able to provide reliable quantitative information under a variety of conditions seen in complex trials of human subjects when the goal is viable information for both clinicians and regulatory authorities.

The biodistribution results demonstrate at least two important points. The first is that, as expected, the choice of gallium label did not affect the binding, internalization, or residualization of the antagonist in this context. This was an important point that allowed us to complete an inter- and intra-day repeat analysis with the knowledge that we were not perturbing the expression of the antigen on the cell surface. Secondly, the biodistribution of the Ga-68 on the same and subsequent days confirmed our hypothesis with the candidate radiotracer and allowed the proper context to look at the high and low SA values without the confound of altered expression levels or altered interaction with the radiotracer antagonist.

For an investigational imaging agent to succeed, numerous hurdles must be addressed as it is examined as a clinical trial agent. For $[^{68}\text{Ga}]\text{DP11}$, its production at multiple sites could be a serious impediment with respect to both scientific and economic constraints. Here we address the issue of SA and repeatability of uptake in a murine model.

Based on our study, we can also conclude that a patient on a PSMA-targeted diagnostic protocol can be administered the corresponding PSMA-targeting therapeutic agent (e.g., $[^{177}\text{Lu}]\text{DKFZ-PSMA617}$ or $[^{225}\text{Ac}]\text{DKFZ-PSMA617}$) on the same day without adversely affecting the target of the therapeutic tracer. Furthermore, a second diagnostic PSMA scan at a later date is likely to be an accurate reflection of the expression of the target in that individual with respect to the initial pre-treatment scan. While this concept would require additional validation, the premise of this statement is reasonable based on these data. It is particularly relevant with respect to Hope et al., where the decrease of PSMA uptake in

response to AR inhibitor therapy is dependent on the repeatability of the measure. Taken together, a repeatability study is necessary in human subjects with this radiotracer to solidify this premise that PSMA-directed imaging will be a good reporter for response to therapy when treatment of the tumor is thought to parallel a decline in the antigen.

Conclusions

The results of the current study clearly establish that the uptake of [^{68}Ga]DP11 in PSMA-expressing tumors remains fairly constant over repeated imaging procedures on consecutive days. Therefore, [^{68}Ga]DP11 can be considered for human subject imaging trials where a change in PSMA expression over time as a marker of response to therapy (such as [^{177}Lu]DKFZ-PSMA-617) is desired. These data also infer the ability to image over a range of SA levels. This characteristic has long-range implications for the practical delivery of this tracer from a single source for future multicenter human trials [21–22]. Therefore, we believe that [^{68}Ga]DP11 based imaging has a promising translational path based on critical radiotracer qualities needed to survive the practical rigors of clinical trials [21, 23].

Acknowledgments

This study was funded by National Cancer Institute grants R01 CA207645 (JO, NP, WW, SML), R21 CA153177-03 (JO, NP), and P50 CA86483 (SM); the Center for Targeted Radioimmunotherapy and Diagnosis; and the Ludwig Center for Cancer Immunotherapy of MSK (NP, SML, TK, KG). Technical services provided by the MSK Small-Animal Imaging Core Facility were supported in part by National Institutes of Health grant R24 CA83084 and National Institutes of Health/National Cancer Institute Cancer Center Support Grant P30 CA008748. Support from the Radiochemistry group at the Australian Nuclear Science and Technology Organization (ANSTO) for providing the gallium-68 generator is gratefully acknowledged. Funding from an MSK Department of Radiology seed grant is also gratefully acknowledged.

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin.* 2017; 67:7–30. [PubMed: 28055103]
2. Carroll PR, Parsons JK, Andriole G, et al. NCCN guidelines insights: prostate cancer early detection, version 2.2016. *J Natl Compr Canc Netw.* 2016; 14:509–519. [PubMed: 27160230]
3. Smith-Jones PM, Vallabhaajosula S, Goldsmith SJ, et al. In vitro characterization of radiolabeled monoclonal antibodies specific for the extracellular domain of prostate-specific membrane antigen. *Cancer Res.* 2000; 60:5237–5243. [PubMed: 11016653]
4. Foss CA, Mease RC, Cho SY, et al. GCPII imaging and cancer. *Curr Med Chem.* 2012; 19:1346–1359. [PubMed: 22304713]
5. Fung EK, Cheal SM, Fareedy SB, et al. Targeting of radiolabeled J591 antibody to PSMA-expressing tumors: optimization of imaging and therapy based on non-linear compartmental modeling. *EJNMMI Res.* 2016; 6:7. [PubMed: 26801327]
6. Banerjee SR, Pullambhatla M, Byun Y, et al. ^{68}Ga -labeled inhibitors of prostate-specific membrane antigen (PSMA) for imaging prostate cancer. *J Med Chem.* 2010; 53:5333–5341. [PubMed: 20568777]
7. Afshar-Oromieh A, Haberkorn U, Hadaschik B, et al. PET/MRI with a ^{68}Ga -PSMA ligand for the detection of prostate cancer. *Eur J Nucl Med Mol Imaging.* 2013; 40:1629–1630. [PubMed: 23817686]
8. Barrett JA, Coleman RE, Goldsmith SJ, et al. First-in-man evaluation of 2 high-affinity PSMA-avid small molecules for imaging prostate cancer. *J Nucl Med.* 2013; 54:380–387. [PubMed: 23303962]

9. Chen Y, Foss CA, Byun Y, et al. Radiohalogenated prostate-specific membrane antigen (PSMA)-based ureas as imaging agents for prostate cancer. *J Med Chem*. 2008; 51:7933–7943. [PubMed: 19053825]
10. Cho SY, Gage KL, Mease RC, et al. Biodistribution, tumor detection, and radiation dosimetry of ^{18}F -DCFBC, a low-molecular-weight inhibitor of prostate-specific membrane antigen, in patients with metastatic prostate cancer. *J Nucl Med*. 2012; 53:1883–1891. [PubMed: 23203246]
11. Maresca KP, Hillier SM, Femia FJ, et al. A series of halogenated heterodimeric inhibitors of prostate specific membrane antigen (PSMA) as radiolabeled probes for targeting prostate cancer. *J Med Chem*. 2009; 52:347–357. [PubMed: 19111054]
12. Pandit-Taskar N, O'Donoghue JA, Beylertgil V, et al. ^{89}Zr -huJ591 immuno-PET imaging in patients with advanced metastatic prostate cancer. *Eur J Nucl Med Mol Imaging*. 2014; 41:2093–2105. [PubMed: 25143071]
13. Eder M, Schafer M, Bauder-Wust U, et al. ^{68}Ga -complex lipophilicity and the targeting property of a urea-based PSMA inhibitor for PET imaging. *Bioconjug Chem*. 2012; 23:688–697. [PubMed: 22369515]
14. Afshar-Oromieh A, Avtzi E, Giesel FL, et al. The diagnostic value of PET/CT imaging with the ^{68}Ga -labelled PSMA ligand HBED-CC in the diagnosis of recurrent prostate cancer. *Eur J Nucl Med Mol Imaging*. 2015; 42:197–209. [PubMed: 25411132]
15. Yadav MP, Ballal S, Tripathi M, et al. ^{177}Lu -DKFZ-PSMA-617 therapy in metastatic castration resistant prostate cancer: safety, efficacy, and quality of life assessment. *Eur J Nucl Med Mol Imaging*. 2017; 44:81–91. [PubMed: 27506431]
16. Kratochwil C, Bruchertseifer F, Giesel FL, et al. ^{225}Ac -PSMA-617 for PSMA-targeted α -radiation therapy of metastatic castration-resistant prostate cancer. *J Nucl Med*. 2016; 57:1941–1944. [PubMed: 27390158]
17. Hillier SM, Kern AM, Maresca KP, et al. ^{123}I -MIP-1072, a small-molecule inhibitor of prostate-specific membrane antigen, is effective at monitoring tumor response to taxane therapy. *J Nucl Med*. 2011; 52:1087–1093. [PubMed: 21680691]
18. Kabasakal L, AbuQbeitah M, Aygun A, et al. Pre-therapeutic dosimetry of normal organs and tissues of (^{177}Lu) -PSMA-617 prostate-specific membrane antigen (PSMA) inhibitor in patients with castration-resistant prostate cancer. *Eur J Nucl Med Mol Imaging*. 2015; 42:1976–1983. [PubMed: 26227531]
19. Oehlke E, Le VS, Lengkeek N, et al. Influence of metal ions on the $(6)(8)\text{Ga}$ -labeling of DOTATATE. *Appl Radiat Isot*. 2013; 82:232–238. [PubMed: 24056001]
20. Pandit-Taskar N, O'Donoghue JA, Ruan S, et al. First-in-human imaging with ^{89}Zr -Df-IAB2M anti-PSMA minibody in patients with metastatic prostate cancer: pharmacokinetics, biodistribution, dosimetry, and lesion uptake. *J Nucl Med*. 2016; 57:1858–1864. [PubMed: 27516450]
21. Weber WA, Gatsonis CA, Mozley PD, et al. Repeatability of ^{18}F -FDG PET/CT in advanced non-small cell lung cancer: prospective assessment in 2 multicenter trials. *J Nucl Med*. 2015; 56:1137–43. [PubMed: 25908829]
22. Desseroit MC, Tixier F, Weber WA, et al. Reliability of PET/CT shape and heterogeneity features in functional and morphologic components of non-small cell lung cancer tumors: a repeatability analysis in a prospective multicenter cohort. *J Nucl Med*. 2017; 58:406–411. [PubMed: 27765856]
23. Lodge MA, Holdhoff M, Leal JP, et al. Repeatability of (^{18}F) -FLT PET in a multicenter study of patients with high-grade glioma. *J Nucl Med*. 2017; 58:393–398. [PubMed: 27688473]

Figure 1.

Three groups were used to better define the effect of previous imaging on uptake of $[^{68}\text{Ga}]\text{DP11}$ in PC3-PIP tumor xenograft-bearing mice. Subsequent figures demonstrate quantitation of these metrics.

Figure 2.
Representative PET images of mice bearing PC3-PIP xenografts imaged with $[^{68}\text{Ga}]\text{DP11}$ on three consecutive days.

Figure 3. Normalized uptake values in mice bearing PC3-PIP tumor xenografts that were imaged using [^{68}Ga]DP11 either consecutively for three days (Group 2, mice 4, 5, and 6) or alternative days with [^{68}Ga]DP11 and administered with [^{67}Ga]DP11 on day 2 (Group 1, mice 1, 2, and 3) 1 h post-administration of tracer.

Figure 4.

Absolute uptake values (%ID/g) of [⁶⁸Ga]DP11 1 h post-administration in mice bearing PC3-PIP tumor xenografts that were imaged 7 h apart on the same day (Group 3).

Table 1

Biodistribution data indicating uptake values in different organs including negative control tumor (PC3) in Group 1 and Group 2 mice administered with [^{68}Ga]DP11 on three consecutive days. [^{67}Ga]DP11 was administered in Group 1 mice on day 2 to act as surrogate for [^{68}Ga]DP11. We expected uptake in tumor to remain fairly constant because of the internalization and residualization of the radiotracer.

Organ	[^{68}Ga]DP11 60 min (Group 1)	[^{67}Ga]DP11 24 h (Group 1)	[^{68}Ga]DP11 60 min (Group 2)
	%ID/g	%ID/g	%ID/g
Blood	0.05 $\pm$ 0.03	0.01 $\pm$ 0.00	0.08 $\pm$ 0.03
PC3-PIP	12.37 $\pm$ 4.19	12.49 $\pm$ 6.88	15.98 $\pm$ 5.82
PC3	0.26 $\pm$ 0.18	0.03 $\pm$ 0.02	0.26 $\pm$ 0.12
Heart	0.12 $\pm$ 0.09	0.03 $\pm$ 0.02	0.20 $\pm$ 0.06
Lungs	0.40 $\pm$ 0.17	0.16 $\pm$ 0.06	0.74 $\pm$ 0.20
Liver	0.15 $\pm$ 0.11	0.11 $\pm$ 0.04	0.20 $\pm$ 0.09
Spleen	2.84 $\pm$ 2.47	0.62 $\pm$ 0.29	3.72 $\pm$ 1.35
Stomach	0.11 $\pm$ 0.31	0.12 $\pm$ 0.04	0.16 $\pm$ 0.07
Small Intestine	0.06 $\pm$ 0.04	0.03 $\pm$ 0.02	0.12 $\pm$ 0.07
Large Intestine	1.08 $\pm$ 2.43	0.45 $\pm$ 0.24	0.12 $\pm$ 0.05
Kidneys	39.23 $\pm$ 14.77	21.23 $\pm$ 3.83	83.17 $\pm$ 24.60
Muscle	0.20 $\pm$ 0.17	0.03 $\pm$ 0.01	0.27 $\pm$ 0.15
Bone	0.22 $\pm$ 0.15	0.03 $\pm$ 0.02	0.46 $\pm$ 0.22
Tail	0.22 $\pm$ 0.20	0.12 $\pm$ 0.08	0.54 $\pm$ 0.35

Table 2

Biodistribution data indicating uptake values in different Group 3 mice administered [^{68}Ga]DP11 on the same day (7 h apart). [^{67}Ga]-DP11 was used as a dilution tracer for [^{68}Ga]DP11 (2.5%) for the earlier study to enable measurement of absolute uptake of [^{68}Ga]DP11 in the morning.

Organ	Mean	STDEV	Mean	STDEV
Blood	0.031929	0.006623	0.651253	0.07195
PC3-PIP	13.82959	3.776571	17.76399	1.844276
Heart	0.084408	0.054297	0.454322	0.05573
Lungs	0.157713	0.052001	0.788761	0.082985
Liver	0.196009	0.017852	1.182716	0.373648
Spleen	0.67524	0.340853	2.481662	0.559527
Stomach	1.682106	3.257165	0.204175	0.146409
Small Intestine	0.265369	0.2601	0.77066	0.657567
Large Intestine	0.765827	0.697253	0.103875	0.007722
Kidneys	30.07516	13.54364	15.03973	5.63248
Muscle	0.068856	0.038716	0.227362	0.12529
Bone	0.107844	0.033198	0.337378	0.133774
Tail	0.155515	0.065016	2.950146	2.48132