Published in final edited form as:

Nucl Med Biol. 2018 November ; 66: 26–31. doi:10.1016/j.nucmedbio.2018.08.003.

Preliminary evaluation of a novel ^{18}F -labeled PARP-1 ligand for PET imaging of PARP-1 expression in prostate cancer

Dong Zhou^{a,*}, Jinbin Xu^a, Cedric Mpyo^b, Wenhua Chu^a, Sung Hoon Kim^c, Huifangjie Li^a, Buck E. Rogers^b, and John A. Katzenellenbogen^c

^aDepartment of Radiology, School of Medicine, Washington University in Saint Louis, Saint Louis, MO 63110, United States of America

^bRadiation Oncology, School of Medicine, Washington University in Saint Louis, Saint Louis, MO 63110, United States of America

^cDepartment of Chemistry, University of Illinois at Urbana-Champaign, IL 61801, United States of America

Abstract

Introduction: Poly (ADP-ribose) polymerase-1 (PARP-1) plays many roles in prostate cancer (PC), such as mediating DNA damage repair, transcriptional regulation and nuclear hormone receptor signaling. Because of this, PARP-1 has been targeted for therapy in PC, and non-invasive imaging of PARP-1 could help predict which patients are likely to respond to such therapy. Several PARP-1 positron emission tomography (PET) imaging agents have been developed and show promise for imaging PARP-1 expression in breast, brain, and lung cancer in small animals, but not as yet in prostate cancer. [^{18}F]WC-DZ-F is an analogue of [^{18}F]Fluor Thanatrace (FTT) and [^{125}I]KX1, which are well-established PARP-1 ligands for measuring PARP-1 expression. Herein, we evaluated the potential of [^{18}F]WC-DZ-F for the imaging PARP-1 expression in PC.

Methods: [^{18}F]WC-DZ-F was synthesized by a two-step sequence. [^{18}F]WC-DZ-F was evaluated by in vitro uptake studies in PC-3 cells and by in vivo biodistribution and microPET imaging using PC-3 tumor xenografts. Ex vivo autoradiography of PC-3 tumors after microPET imaging was also performed.

Results: [^{18}F]WC-DZ-F has high, PARP-1-specific uptake in PC-3 cells. In the microPET imaging study, [^{18}F]WC-DZ-F accumulated in PC-3 xenograft tumors over 2 h, and the uptake was significantly reduced by blocking with olaparib. PC-3 tumors were clearly visualized in microPET images, and the imaging results were further confirmed by autoradiography of PC-3 tumors ex vivo. In the biodistribution study [^{18}F]WC-DZ-F washed out quickly from most tissues within 2 h, except for the liver in which the uptake was not blockable by olaparib.

Conclusions: We synthesized a novel PARP-1 radioligand, [^{18}F]WC-DZ-F. The preliminary evaluation of [^{18}F]WC-DZ-F indicates that it is a suitable PET imaging agent for measuring PARP-1 expression in prostate cancer and should be applicable to other types of cancers.

*Corresponding author at: 510 S. Kingshighway Blvd, Saint Louis, MO 63110, United States of America. zhou@wustl.edu (D. Zhou).

Keywords

PARP-1; PET imaging; Prostate cancer; Cell uptake; Biodistribution

1. Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1) is an abundant and ubiquitous enzyme found predominantly in the nucleus in association with chromatin, where it participates in DNA repair and regulation of gene expression [1,2]. PARP-1 has been actively pursued as a therapeutic target for cancer treatment [3], by inducing synthetic lethality in cancers that are deficient in certain DNA repair mechanisms and that depend on PARP-1 activity for survival [4]. Clinical trials using PARP inhibitors, Olaparib, Rucaparib, Veliparib, Talazoparib and Niraparib have shown encouraging results in the treatment of various cancers, including ovarian and breast. Recently, clinical data suggested that men with either germline or somatic mutations in DNA repair pathways can derive therapeutic benefit from PARP inhibition [5,6]. Preclinical data also suggest that PARP inhibition may produce benefits beyond patients with DNA repair-defective tumors by targeting chromatin binding and gene transcription [7]. Besides its role in DNA repair, PARP-1 has many other activities in prostate cancer, including transcriptional regulation, which may contribute to oncogenic signaling and cancer progression [8]. Because of the importance of PARP-1 as a target for cancer treatment, several nuclear imaging agents have been reported, and they show potential for imaging PARP-1 expression in breast cancer, brain cancer and lung cancer in small animals, but imaging in prostate cancer has not yet been demonstrated [9–19]. Nuclear imaging of PARP-1 expression in prostate cancer can provide direct information about PARP-1 and its levels in prostate tumors and might provide better prognostic and predictive information to improve selection of patients most likely to benefit from PARP-1 therapies for prostate as well as other cancers.

PARP-1 inhibitors have been labeled with fluorine-18 [10,16] and radioiodine [14,15,17,18] as potential agents for nuclear imaging and therapies (Fig. 1). The first in-human studies of [¹⁸F]FluorThanatrace ([¹⁸F]FTT) at Washington University suggest that [¹⁸F]FTT uptake reflects PARP expression [20]. Recently, it has been shown that PARP PET imaging using [¹⁸F]FPARPi can quantify target engagement of several FDA approved PARP-1 inhibitors in lung cancer in vitro and in vivo [19]. Despite these promising results, however, several important criteria for targeted imaging have not been met, especially for imaging PARP-1 expression in metastatic castration-resistant prostate cancer (mCRPC). Challenges include high and blockable uptake in spleen, pancreas, bone and marrow in small animals [19,20]. Most PARP inhibitors reported so far mimic the NAD⁺ substrate of PARP-1 and competitively binding to the catalytic domain of PARP-1. A large hydrophobic pocket adjacent to the nicotinamide binding site [21] allows the addition of functionality to these inhibitors to improve their potency and properties as imaging agents. By comparing the in vivo uptake of [¹⁸F]FPARPi [10] and [¹³¹I]I2-PARPi [14] in orthotopic brain tumors (2% ID/g vs 0.2% ID/g at 2 h post injection), we hypothesized that replacing the iodine in KX1 with fluorine-18 may provide a PET tracer with better pharmacokinetics for imaging

prostate cancer. In this paper, we report the preliminary evaluation of a novel PARP-1 radioligand, [^{18}F]WC-DZ-F, for PET imaging of PARP-1 expression in prostate cancer.

2. Methods

All animal studies were performed under the Guide for the Care and Use of Laboratory Animals through the Washington University Animal Studies Committee.

2.1. Synthesis of WC-DZ-F

WC-DZ-F was synthesized from the diamine precursor **1** and 4-fluorobenzaldehyde **2** in the presence of 10% Pd/C in methanol, according to literature (Fig. 2) [22] with some modifications. Methanol (10 mL) was degassed by refluxing under argon atmosphere for 30 min in the reaction vessel. Diamine precursor **1** (195 mg, 1.1 μmol), 4-fluorobenzaldehyde **2** (0.14 mL, 1.32 μmol), and 10% Pd/C (50 mg) were added. The reaction mixture was stirred in an oil bath (100 $^{\circ}\text{C}$) under argon atmosphere, and the reaction was checked by TLC, which indicated the completion of the reaction within 1 h. The reaction mixture was filtered through a pad of celite and followed by rinsing with hot methanol (3×10 mL). Methanol was removed under reduced pressure and the residue was purified by silica gel chromatograph (5% methanol/ethyl acetate) to afford off-white solid (278 mg) in 90% yield. mp (265–266 $^{\circ}\text{C}$). ^1H NMR (400 MHz, DMSO d_6) δ 8.50 (t, $J = 4.4$ Hz, 1H), 7.98–7.91 (m, 4H), 7.47 (t, $J = 8.4$ Hz, 2H), 7.40 (t, $J = 7.6$ Hz, 1H), 4.49 (m, 2H), 3.58 (m, 2H). ^{13}C NMR (100 MHz, DMSO d_6) δ 167.7, 164.8, 162.3, 153.3, 143.6, 132.9, 132.5, 132.4, 126.5, 125.9, 123.4, 122.1, 118.2, 116.3, 116.1, 50.9, 40.8.

2.2. Radiosynthesis

Into a 10 mL Pyrex tube containing precursor **3** (4 mg, 12.8 μmol) and $\text{K}_2\text{CO}_3/\text{K}_{222}$ (5.7 mg, 6.4 μmol) was added [^{18}F]tosyl fluoride (0.95 GBq) in acetonitrile (0.5 mL), the detail of which will be published elsewhere. In brief, it is generated by passing a solution of tosyl chloride through an anion exchange cartridge, in which [^{18}F]fluoride was trapped and dried by acetonitrile. The reaction mixture was shaken and heated in an oil bath at 102 $^{\circ}\text{C}$ for 8 min. At room temperature, the reaction mixture was treated by passing through a Waters MCX cartridge (100 mg in 1 mL cartridge, pretreated with 3 mL acetonitrile) and eluted into a 10 mL Pyrex tube containing precursor **1** (2.6 mg, 14.7 μmol) and 10% Pd/C (6 mg) in methanol (0.5 mL). The first reaction tube and the MCX cartridge were rinsed with acetonitrile (0.5 mL) and loaded onto the second tube, which was then capped firmly and heated at 120 $^{\circ}\text{C}$ for 20 min. The reaction mixture was passed through a pad of Celite (pretreated with acetonitrile), and the reaction tube and Celite were rinsed with acetonitrile (2 mL). All the eluted solution was collected in a tube, and solvents were removed under a flow of nitrogen at 100 $^{\circ}\text{C}$. The residue was dissolved in HPLC mobile phase (3 mL), and injected onto HPLC via a nylon filter. After HPLC purification, [^{18}F]WC-DZ-F was collected (0.35 GBq) and diluted in water (40 mL). [^{18}F]WC-DZ-F was extracted from the solution by a standard solid phase extract procedure using a Waters C18 classic Sep-Pak and was formulated in 10% ethanol/saline.

2.3. Cell uptake

Twenty four hours before the uptake assays, aliquots of PC-3 cells suspended in culture medium were added to each well of a 6-well cell culture plates and allowed to reach log phase growth, with approximately 60–80% confluence at the time of the uptake assay. Cell culture medium was removed, and freshly prepared cell culture medium containing [^{18}F]WC-DZ-F was added to each well. The concentration [^{18}F]WC-DZ-F was calculated based on the amount of radioactivity and specific activity of [^{18}F]WC-DZ-F. The cells were incubated for 1, 5, 30, 60, and 120 min at 37 °C and then rinsed twice with 1 mL ice cold PBS. The cells were detached and harvested after treatment with 500 μL 0.05% EDTA/trypsin for 2 min, and the protein concentration of the cell suspension was determined by a DC protein assay. A Wizard gamma counter was used to count the radioactive uptake, and the uptake was expressed as the total dose %/mg protein. Non-specific cell uptake was determined using cells in the presence 50 μM olaparib per well.

2.4. Biodistribution

The biodistribution study was performed in mature male C57BL/6 mice (N = 5/group). Each group was intravenously injected with [^{18}F]WC-DZ-F (0.74 MBq) in ethanol/saline (100 μL) via the tail vein. In the blocking group, olaparib (5 mg/kg) in 10% ethanol/saline (100 μL) was intravenously injected via the tail vein 10 min prior to the injection of [^{18}F]WC-DZ-F. At the specified time point, the mice were sacrificed, and the blood, lungs, liver, spleen, kidneys, muscle, heart, brain, bone, bone marrow, pancreas, fat and prostate were harvested, weighed, and counted in a γ -counter. The percentage injected dose per gram of tissue (%ID/g) was determined by decay correction of the [^{18}F]WC-DZ-F for each sample, normalized to a standard of known weight, which was representative of the injected dose.

2.5. Xenograft model

Mature SCID mice from Charles River Laboratories are allowed to acclimate in an AALAC accredited housing facility for at least 1 week prior to tumor implantation for these imaging studies. Male SCID mice (7 weeks old, n = 4) were implanted subcutaneously in the right shoulder with 1×10^7 PC-3 prostate cancer cells. The tumors were allowed to grow for 18–19 days before the imaging studies.

2.6. MicroPET and autoradiograph

Tumor-bearing mice (baseline n = 2; blocking n = 2) were placed in an induction chamber containing ~2% isoflurane/oxygen and then secured to a custom quadruple bed for placement of tail vein catheters; anesthesia was maintained via nose-cone at ~1% isoflurane/oxygen for the dynamic imaging procedure. The mice were injected with 3.7 MBq of [^{18}F]WC-DZ-F and scanned for 0–120 min using Inveon PET/CT scanners. The standard uptake values (SUVs) were generated from manually drawn regions of interests for tumors and surrounding ‘background’ tissue. Blocking and displacement studies were carried out by injection of olaparib (5 mg/kg) i.v. in 10% ethanol/saline (100 μL) 10 min before or 10 min after injection of [^{18}F]WC-DZ-F, respectively.

After microPET imaging, the mice were sacrificed and the tumors were harvested and frozen at -80°C . Several sections ($20\ \mu\text{m}$ in thickness) of tumor were prepared, and autoradiography was performed using a Beta Imager 2000Z digital beta imaging system. Quantitative analysis of the autoradiography was done with the beta-Image Plus software. Regions of interest were outlined and results were expressed as dpm/mm^2 .

2.7. Metabolism study

Metabolism study of $[^{18}\text{F}]\text{WC-DZ-F}$ in blood and liver of C57BL/6 male mice (5, 30 and 120 min, $n = 2$) was carried out using the protocol for $[^{18}\text{F}]\text{FTT}$, previously reported by us [20].

3. Results

3.1. Synthesis and radiosynthesis of WC-DZ-F

The same method was used to synthesize **WC-DZ-F** and to radiosynthesize $[^{18}\text{F}]\text{WC-DZ-F}$, starting from 4-fluorobenzaldehyde and $[^{18}\text{F}]4\text{-fluorobenzaldehyde}$, respectively. Under optimized conditions, $[^{18}\text{F}]\text{WC-DZ-F}$ was prepared in over 50% yield (Decay corrected), with a molar activity of around $37\ \text{GBq}/\mu\text{mol}$ and radiochemical purity of 99%. $[^{18}\text{F}]\text{WC-DZ-F}$ is stable in 10% ethanol/saline ($0.09\ \text{GBq}/\text{mL}$) for over 4 h at room temperature determined by HPLC and radio-TLC.

3.2. Cell uptake

PARP-1 specific uptake of $[^{18}\text{F}]\text{WC-DZ-F}$ was observed in PC-3 human prostate cancer cell line as demonstrated by the blocking with olaparib ($50\ \mu\text{M}$). The uptake appears to be dependent on the concentration of $[^{18}\text{F}]\text{WC-DZ-F}$. A lower concentration of $[^{18}\text{F}]\text{WC-DZ-F}$ resulted in higher uptake in PC-3 cells, but both are reduced to the same level by olaparib (Fig. 3).

3.3. Biodistribution

In C57BL/6 male mice, $[^{18}\text{F}]\text{WC-DZ-F}$ distributed quickly throughout the whole body (Table S1), but then washed out, reaching a low level in most of the tissues within 2 h post injection (Fig. 4). Liver has the highest uptake at 2 h, but it was not cleared with time or blocked by olaparib, indicating that this uptake is not PARP-1 mediated. The uptake in spleen, kidney, marrow, and pancreas are low ($1\text{--}0.5\%\text{ID}/\text{g}$); the uptake in brain and prostate are very low ($<0.5\%\text{ID}/\text{g}$). There is no specific uptake of $[^{18}\text{F}]\text{WC-DZ-F}$ in any tissues as observed by the blocking with olaparib, except for the spleen (0.92 ± 0.16 vs $0.35 \pm 0.13\%\text{ID}/\text{g}$, $P < 0.01$), marrow (0.54 ± 0.25 vs $0.15 \pm 0.04\%\text{ID}/\text{g}$, $P < 0.01$), and prostate (0.25 ± 0.11 vs $0.09 \pm 0.04\%\text{ID}/\text{g}$, $P < 0.05$).

3.4. MicroPET and autoradiography

In the microPET imaging of a PC-3 xenograft tumor mice, the tumor is clearly visible and the uptake is blocked and displaced by olaparib (Fig. 5a). According to the time activity curve (Fig. 5b), $[^{18}\text{F}]\text{WC-DZ-F}$ accumulated in the tumor over the two hours of the experiment, while half of the uptake was reduced gradually over the time due to the blocking

by olaparib. The results are further confirmed by ex vivo autoradiography of tumors post imaging (Fig. 5c). Quantitative analysis of the autoradiography gave the same percentage of reduction (Fig. 5d).

3.5. Metabolism study

The metabolism study in C57BL/6 male mice demonstrated the stability of [^{18}F]WC-DZ-F in blood ($94.5 \pm 0.5\%$, $78.5 \pm 9.5\%$ and $65 \pm 7\%$ at 5, 30 and 120 min, respectively) and in liver ($91 \pm 2\%$, $77.5 \pm 0.5\%$ and $51.5 \pm 0.5\%$ at 5, 30 and 120 min, respectively).

4. Discussion

[^{18}F]WC-DZ-F is an analogue of [^{18}F]FTT [16] and [^{125}I]KX1 [17] (Fig. 1), which are based on the benzimidazole NU1085 and its relatives AG14361 and rucaparib. WC-DZ-F is a potent PARP-1 inhibitor with a reported K_i of 6 nM [23], in the same range as reported for AG14361, KX1, Olaparib, and Talazoparib [17,23]. It has been demonstrated that [^{18}F]FTT and [^{125}I]KX1 can be used to measure PARP-1 expression specifically in vitro and in vivo [17,20,24]. However, [^{125}I]KX1 did not deliver satisfactory in vivo results (slow washout in tissues and no-blocking in tumors), which could be attributed to the bulky and lipophilic iodine atom. As expected, the PARP-1 specificity of [^{18}F]WC-DZ-F uptake was demonstrated in our cell uptake study, which used olaparib as a blocking agent. Olaparib is a clinical PARP-1 inhibitor, and WC-DZ-F should have the same binding profiles as olaparib [14].

Over-expression of PARP-1 in prostate cancer is observed in humans [25], and our studies also showed high and PARP-1 specific uptake of the well-studied [^{18}F]FTT and [^{18}F]FPARPi in PC-3 and LNCaP prostate cancer cells (Fig. S1). Similar cell uptake of [^{18}F]WC-DZ-F in PC-3 cells was observed (Fig. 3). The cell uptake of [^{18}F]WC-DZ-F in MDA-MB-231, which is a breast cancer cell line with low PARP-1 expression (Fig. S2) was lower than that in PC-3. These observations are consistent with the overexpression of PARP-1 in prostate cancer and suggest that PARP-1 is a good target for PET imaging in this disease. The biodistribution of [^{18}F]WC-DZ-F in normal male C57BL/6 mice showed that its tissue clearance is favorable for PET imaging in prostate cancer, and it is different from that reported for [^{18}F]FTT, [^{125}I]KX1 and [^{18}F] PARPi. [^{125}I]KX1 is still retained in the body at high level at 2 h post injection [17], while [^{18}F]FTT has high uptake in the spleen, pancreas and bone (but low in bone marrow) [20], and [^{18}F]PARPi has high to very high uptake in the bone marrow (around 3% ID/g in SCID mice and 12% ID/g in nude mice 2 h post injection) [19], which is consistent with the reported bone marrow toxicity of olaparib [26]. These biodistribution and clearance problems will result in a high radiation dose to these tissues, which is likely to limit the number of PET scans for use in monitoring PARP-1 expression during PARP-1 inhibition treatment. The small size of [^{18}F]WC-DZ-F (MW 281.29) with a logP of 1.96 ± 0.01 [27] gives [^{18}F]WC-DZ-F favorable pharmacokinetics as PET imaging agent.

In the microPET imaging study, [^{18}F]WC-DZ-F accumulated and remained in the target tumor (close to 4% ID/c.c. at 2 h post injection) but readily washed out of other organs and tissues over two hours post injection, except for the liver. The good rate of washout from

non-target tissues is a favorable feature for a PET imaging agent. The result of the blocking/displacement studies indicates that the uptake of [^{18}F]WC-DZ-F in the tumor is a reversible process, which is consistent with the dynamic nature of PARP-1 action. The extent to which the in vivo uptake of [^{18}F]WC-DZ-F in PC-3 tumors is blocked by olaparib is similar to that of the well-studied [^{18}F]PPARi in lung cancer at the same dose of olaparib (50% vs 59% at 5 mg/kg of olaparib) [19]. It is worthwhile to note that the time activity curve of [^{18}F]WC-DZ-F in PC-3 tumor (Fig. 5b) is similar to those of [^{18}F]FTT in breast cancer cell lines MDA-MB-231 (low PARP-1 expression) and MDA-MB-436 (high PARP-1 expression) [16].

As expected, [^{18}F]WC-DZ-F is much more stable than [^{18}F]FTT in vivo (94.5% vs 55% at 5 min in blood and 78.5% vs 13% at 30 min) [20]. This sharp difference should be contributed to the more metabolically liable fluoroethoxy group in FTT.

[^{18}F]WC-DZ-F was radio synthesized via a two-step method, which is not ideal for automated production. Optimization of the radiosynthesis of [^{18}F]WC-DZ-F using copper-mediated radiofluorination of a boron precursor [28] is under way, and preliminary results were promising. So far, the specificity of [^{18}F]WC-DZ-F for PARP-1/PARP-2 has not been investigated. Further investigation of [^{18}F]WC-DZ-F is warranted because of its promising in vitro uptake and in vivo biodistribution and PET imaging properties.

5. Conclusions

We synthesized a novel PARP-1 radioligand, [^{18}F]WC-DZ-F. Evaluation of [^{18}F]WC-DZ-F by cell uptake studies, microPET imaging and postPET autoradiography, and biodistribution studies indicates that [^{18}F]WC-DZ-F is a suitable PET imaging agent for measuring PARP-1 expression in prostate cancer and has the potential for use in other types of cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

This work was supported by the National Institutes of Health R01CA025836 (JAK). We thank Robert Dennett and Brian Wingbermuehle for the production of [^{18}F]fluoride and the staff of the Washington University Small Animal Imaging Facility for their technical assistance.

References

- [1]. Do K, Chen AP. Molecular pathways: targeting PARP in cancer treatment. Clin Cancer Res 2013;19:977–84. [PubMed: 23269547]
- [2]. Schiewer MJ, Knudsen KE. Transcriptional roles of PARP1 in cancer. Mol Cancer Res 2014;12:1069–80. [PubMed: 24916104]
- [3]. Ferraris DV. Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. From concept to clinic. J Med Chem 2010;53:4561–84. [PubMed: 20364863]
- [4]. Basu B, Sandhu SK, de Bono JS. PARP inhibitors: mechanism of action and their potential role in the prevention and treatment of cancer. Drugs 2012;72:1579–90. [PubMed: 22834679]

- [5]. Fong PC, Boss DS, Yap TA, Tutt A, Wu PJ, Mergui-Roelvink M, et al. Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009;361:123–34. [PubMed: 19553641]
- [6]. Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, et al. DNA-repair defects and olaparib in metastatic prostate cancer. *N Engl J Med* 2015;373:1697–708. [PubMed: 26510020]
- [7]. Feng FY, De Bono JS, Rubin MA, Knudsen KE. Chromatin to clinic: the molecular rationale for PARP1 inhibitor function. *Mol Cell* 2015;58:925–34. [PubMed: 26091341]
- [8]. Deshmukh D, Qiu Y. Role of PARP-1 in prostate cancer. *Am J Clin Exp Urol* 2015;3:1–12. [PubMed: 26069882]
- [9]. Carlucci G, Carney B, Brand C, Kossatz S, Irwin CP, Carlin SD, et al. Dual-modality optical/PET imaging of PARP1 in glioblastoma. *Mol Imaging Biol* 2015;17:848–55. [PubMed: 25895168]
- [10]. Carney B, Carlucci G, Salinas B, Di Gialleonardo V, Kossatz S, Vansteene A, et al. Non-invasive PET imaging of PARP1 expression in glioblastoma models. *Mol Imaging Biol* 2016;18:386–92. [PubMed: 26493053]
- [11]. Edmonds CE, Makvandi M, Lieberman BP, Xu K, Zeng C, Li S, et al. [(18)F] FluorThanatrace uptake as a marker of PARP1 expression and activity in breast cancer. *Am J Nucl Med Mol Imaging* 2016;6:94–101. [PubMed: 27069769]
- [12]. Keliher EJ, Reiner T, Turetsky A, Hilderbrand SA, Weissleder R. High-yielding, two-step 18F labeling strategy for 18F-PARP1 inhibitors. *ChemMedChem* 2011;6:424–7. [PubMed: 21360818]
- [13]. Reiner T, Keliher EJ, Earley S, Marinelli B, Weissleder R. Synthesis and in vivo imaging of a 18F-labeled PARP1 inhibitor using a chemically orthogonal scavenger-assisted high-performance method. *Angew Chem Int Ed Engl* 2011;50:1922–5. [PubMed: 21328671]
- [14]. Salinas B, Irwin CP, Kossatz S, Bolaender A, Chiosis G, Pillarsetty N, et al. Radioiodinated PARP1 tracers for glioblastoma imaging. *EJNMMI Res* 2015;5:123. [PubMed: 26337803]
- [15]. Zmuda F, Malviya G, Blair A, Boyd M, Chalmers AJ, Sutherland A, et al. Synthesis and evaluation of a radioiodinated tracer with specificity for poly(ADP-ribose) polymerase-1 (PARP-1) in vivo. *J Med Chem* 2015;58:8683–93. [PubMed: 26469301]
- [16]. Zhou D, Chu WH, Xu JB, Jones LA, Peng X, Li SH, et al. Synthesis, [F-18] radiolabeling, and evaluation of poly (ADP-ribose) polymerase-1 (PARP-1) inhibitors for in vivo imaging of PARP-1 using positron emission tomography. *Bioorg Med Chem* 2014; 22:1700–7. [PubMed: 24503274]
- [17]. Makvandi M, Xu KY, Lieberman BP, Anderson RC, Efron SS, Winters HD, et al. A radiotracer strategy to quantify PARP-1 expression in vivo provides a biomarker that can enable patient selection for PARP inhibitor therapy. *Cancer Res* 2016;76: 4516–24. [PubMed: 27261505]
- [18]. Anderson RC, Makvandi M, Xu KY, Lieberman BP, Zeng CB, Pryma DA, et al. Iodinated benzimidazole PARP radiotracer for evaluating PARP1/2 expression in vitro and in vivo. *Nucl Med Biol* 2016;43:752–8. [PubMed: 27689533]
- [19]. Carney B, Kossatz S, Lok BH, Schneeberger V, Gangangari KK, Pillarsetty NVK, et al. Target engagement imaging of PARP inhibitors in small-cell lung cancer. *Nat Commun* 2018;9:176. [PubMed: 29330466]
- [20]. Michel LS, Dyroff S, Brooks FJ, Spayd KJ, Lim S, Engle JT, et al. PET of poly (ADP-ribose) polymerase activity in cancer: preclinical assessment and first in-human studies. *Radiology* 2017;282:453–63. [PubMed: 27841728]
- [21]. Kinoshita T, Nakanishi I, Warizaya M, Iwashita A, Kido Y, Hattori K, et al. Inhibitor-induced structural change of the active site of human poly(ADP-ribose) polymerase. *FEBS Lett* 2004;556:43–6. [PubMed: 14706823]
- [22]. Ferraris DV, Li J-HL, Kalish V, Zhang J. US7915280.
- [23]. Thomas HD, Calabrese CR, Batey MA, Canan S, Hostomsky Z, Kyle S, et al. Preclinical selection of a novel poly(ADP-ribose) polymerase inhibitor for clinical trial. *Mol Cancer Ther* 2007;6:945–56. [PubMed: 17363489]

- [24]. Edmonds CE, Makvandi M, Lieberman BP, Xu KY, Zeng CB, Li SH, et al. [F-18] FluorThanatrace uptake as a marker of PARP1 expression and activity in breast cancer. *Am J Nucl Med Mol Imaging* 2016;6:94–101. [PubMed: 27069769]
- [25]. Salemi M, Galia A, Fraggetta F, La Corte C, Pepe P, La Vignera S, et al. Poly (ADP-ri-bose) polymerase 1 protein expression in normal and neoplastic prostatic tissue. *Eur J Histochem* 2013;57:e13. [PubMed: 23807292]
- [26]. van der Noll R, Marchetti S, Steeghs N, Beijnen JH, Mergui-Roelvink MWJ, Harms E, et al. Long-term safety and anti-tumour activity of olaparib monotherapy after combination with carboplatin and paclitaxel in patients with advanced breast, ovarian or fallopian tube cancer. *Br J Cancer* 2015;113:396–402. [PubMed: 26180927]
- [27]. Wilson AA, Jin L, Garcia A, DaSilva JN, Houle S. An admonition when measuring the lipophilicity of radiotracers using counting techniques. *Appl Radiat Isot* 2001;54:203–8. [PubMed: 11200881]
- [28]. Zischler J, Kolks N, Modemann D, Neumaier B, Zlatopolskiy BD. Alcohol-enhanced Cu-mediated radiofluorination. *Chemistry* 2017;23:3251–6. [PubMed: 27943464]

Fig. 1.
PARP-1 radio-ligands.

Fig. 2. Synthesis and radiosynthesis of WC-DZ-F/ ^{18}F WC-DZ-F (a. 10% Pd/C, MeOH/ MeCN; b. ^{18}F TsF, K₂CO₃, K₂₂₂, MeCN).

Fig. 3. Cell uptake of [¹⁸F]WC-DZ-F in PC-3 cells and the blocking study by olaparib (50 μM). (*P* value <0.05).

Fig. 4.

Biodistribution of [¹⁸F]**WC-DZ-F** (0.74 MBq) in C57BL/6 male mice (5 per group) at 2 h post injection (blocked by olaparib (5 mg/kg), administered 10 min prior to injection of [¹⁸F]**WC-DZ-F**). *P* value: * <0.05, ** <0.01, all others >0.05.

Fig. 5. (a) MicroPET imaging of PC-3 xenograft tumors ($[^{18}\text{F}]\text{WC-DZ-F}$ (3.7 MBq); Anesthetized by isoflurane; 2 h of scanning; Blocked by olaparib (5 mg/kg in 10% ethanol/saline, 10 min (T1) before and 10 min (T3) after injection of radioactivity); (b) Time activity curve of tumors; (c) Ex vivo autoradiographs of PC-3 tumors (T2 and T4 as controls, T1 and T3 as blocking and displacement, respectively); (d) Quantitative analysis of autoradiography of PC-3 tumors. Tumor size: T1 (11 $\times$ 7), T2 (9 $\times$ 7), T3 (9 $\times$ 7), T4 (16 $\times$ 7), unit: mm.