PARP-1 Expression Quantified by [¹⁸F]FluorThanatrace: A Biomarker of Response to PARP Inhibition Adjuvant to Radiation Therapy

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Abstract

Introduction: Poly (ADP-ribose) polymerase 1 (PARP-1) is the major target of clinical PARP inhibitors and is a potential predictive biomarker for response to therapy. Due to the limited success of PARP inhibitors as monotherapy, investigators have shifted the clinical role of PARP inhibitors to the adjuvant setting. In this study, we evaluate the radiotracer [¹⁸F]FluorThanatrace ([¹⁸F]FTT) as a marker of PARP expression *in vitro* and the associated biological implications of PARP-1 expression in PARP inhibitor treatment adjuvant to radiation therapy.

Materials and Methods: SNU-251 (*BRCA1*-mutant) and SKOV3 (*BRCA1*-WT) cell lines were evaluated *in vitro* by using the radiotracer [¹⁸F]FTT. Pharmacological binding assays were performed at baseline and were correlated with PARP-1 protein expression measured by Western blot protein analysis. Cell viability and clonogenic assays were used to characterize *in vitro* cytotoxicity for treatments, including: PARP inhibitors alone, radiation alone, and PARP inhibitor adjuvant to radiation. Western blot protein analysis was used to assess response to treatment by using γ H2AX to measure DNA damage and PAR to measure the catalytic inhibition of PARP.

Results: [¹⁸F]FTT was capable of measuring PARP-1 protein expression *in vitro* and corresponded to Western blot protein analysis at baseline. The addition of a PARP inhibitor enhanced radiation effects in both cell lines; however, a greater synergy was observed in the SNU-251 cell line that expresses a *BRCA1* mutation and homologous recombination deficiency. Western blot protein analysis showed that the addition of a PARP inhibitor adjuvant to radiation increases DNA damage in both cell lines and reduces PARP enzymatic activity as measured by PAR.

Conclusions: In this work, we found that PARP-1 expression positively corresponds *in vitro* to the response of PARP inhibitors in combination with radiation therapy in ovarian cancer.

Keywords: PARP-1, ovarian cancer, DNA damage, PARP inhibitor, *BRCA1*

Introduction

Poly(ADP-ribose) Polymerase 1 (PARP-1) is one of the most abundantly expressed nuclear enzymes and plays a key role in cell signaling, DNA damage response, and a variety of other biological pathways.^{1–3} PARP-1 has been a promising target for cancer therapy; however, clinical trials utilizing PARP inhibitors for monotherapy have shown

mixed response rates.⁴ These pitfalls have directed PARP inhibitors to the adjuvant setting where combinatorial approaches offer greater therapeutic efficacy with limited toxicity.⁵ As clinical trials continue to report promising results regarding PARP inhibitors in the adjuvant setting, there becomes a greater demand for pharmacokinetic and pharmacodynamic biomarkers to predict and assess response to therapy.⁴

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Multiple genetic biomarkers have been identified for their potential to select patients for PARP inhibitor therapy.^{6–9} These have predominately involved mutations of genes that encode for proteins within the homologous recombination (HR) DNA repair pathway that confer sensitivity to PARP inhibitors. Historically, the main genes of interest have been the breast and ovarian cancer susceptibility genes 1 and 2 (*BRCA1/2*).¹⁰ Recently, the *BRCA1/2* genes have been found to be identical to genes previously discovered within the Fanconi's anemia pathway, and they give rise to 18 additional genes that can lead to HR deficiency.¹¹ HR is one of the primary mechanisms for repairing DNA double-stranded breaks, and it utilizes complementary single-stranded DNA as a template for repair. Through HR, the genomic information is conserved, preventing DNA repair-associated mutagenesis. In the absence of HR, DNA repair shifts to non-homologous end joining (NHEJ), which results in the direct ligation of double-stranded DNA regardless of sequence homology. This process results in chromosomal aberrations and genomic instability promoting cell death. Preclinical studies have confirmed this mechanism, and it was shown that HR-deficient cells are only sensitive to PARP inhibition when NHEJ is functional.^{12,13} Despite promising clinical advantages of being able to identify HRD through genetic mutations in patients, a perfect correlation between HRD and PARP inhibitor efficacy has not been established.^{14,15}

PARP-1 expression has been evaluated through histological studies correlating overexpression with survival outcomes.^{14–19} These studies have shown that in ovarian cancer, high PARP-1 expression is associated with decreased overall patient survival. This warrants PARP-1 expression as a suitable prognostic biomarker. We propose that PARP-1 expression is the single most important biomarker to determine PARP inhibitor efficacy due to PARP-1 being required for the pharmacological mechanism of action of PARP inhibitors. It has been recently established that PARP radiotracers can quantitatively assess the expression of PARP-1 both *in vitro* and *in vivo*.²⁰ These radiotracers are analogs of PARP inhibitors that competitively and reversibly bind to the NAD⁺ active site on the catalytic domain of PARP-1.²¹ We have previously shown that these radiotracers bind to PARP *in vivo* by using breast cancer tumor models and can be competitively blocked by using a clinical PARP inhibitor.^{22,23}

In this study, we explored a PARP-1 PET imaging agent [¹⁸F]FluorThanatrace ([¹⁸F]FTT) in two ovarian cancer cell lines, SNU-251 and SKOV3. In addition, we investigated the biological characteristics of each cell line in the setting of PARP inhibitor treatment adjuvant to radiation therapy. The cell lines used in this study were selected based on the genetic profile as either ovarian cancer *BRCA1* mutant (SNU-251) or *BRCA1* functional-wild type (SKOV3). We hypothesize that [¹⁸F]FTT uptake *in vitro* will correspond to PARP-1 expression, and ovarian cancer cells with higher PARP-1 expression will be more sensitive to PARP inhibition adjuvant to radiation therapy.

Materials and Methods

Radiochemistry: [¹⁸F]FTT synthesis

[¹⁸F]FTT was synthesized by using the AllinOne (TRASIS, Belgium) fully automated chemistry module. Briefly, 1 mL

of a solution containing 7 mg of cryptand and 2 mg potassium carbonate was used to elute ¹⁸F/F[−] from an ion exchange QMA cartridge. The resulting solution was then azeotropically dried with acetonitrile at 100°C. Next, 0.8–1.0 mg of tosylate precursor dissolved in dimethylformamide was added to the reaction vessel and was heated at 105°C for 10 minutes. The reaction mixture was then purified by semipreparative high-performance liquid chromatography by using a 40:60 methanol:water mobile phase, and [¹⁸F]FTT was isolated. The product peak was then diluted with water to reduce the methanol concentration to <10% and was trapped on a C-18 Sep-Pak (Waters, Waltham, MA). The Sep-Pak was then rinsed with 10 mL of water to remove residual methanol solvent and was eluted in 1 mL of 200 proof ethanol. Radiosynthesis was done in accordance with current Good Manufacturing Practices (cGMP) for PET radiopharmaceuticals.²³

Cell culture

Ovarian cancer cell lines SNU-251 and SKOV3 were used in this study. SNU-251 cells express a deleterious mutation of the *BRCA1* gene. This mutation confers DNA repair deficiency through deletion of the *BRCA1* protein that is essential for functional HR-mediated DNA repair. SKOV3 cells were acquired from ATCC, and SNU-251 cells were acquired from the Bassett Center for BRCA. Both cell lines were cultured in Roswell Park Memorial Institute Medium 1640 10% fetal bovine serum (FBS) and 1% Pen/Strep. These conditions were kept constant in all experiments except for radiosensitivity clonogenic assays, which required SNU-251 cells to be grown in Dulbecco's Modified Eagle Medium 10% FBS and 1% Pen/Strep.

Radiotracer PARP-1 saturation

SNU-251 or SKOV3 cells were each seeded in 96-well plates at 50,000 cells/well 24 hours before radioligand binding studies were performed. On the day of the study, [¹⁸F]FTT was produced and was diluted in phosphate buffered saline (PBS) with Ca²⁺ and Mg²⁺, pH 7.3. Next, varying concentrations of radiotracer solutions were added to wells and allowed to reach equilibrium for 1 hour. Ten micromolar of olaparib (Selleckchem, Houston, TX) was used to determine nonspecific binding. At 1 hour, radiotracer solutions were aspirated from the wells. After removal of the radiotracer solutions, wells were washed with 200 μ L of PBS. Next, radioactivity was assayed on a Perkin Elmer Wizard gamma counter (Waltham, MA). Data were plotted by using a nonlinear fit one-site binding hyperbola in Prism version 6.0 (La Jolla, CA).

PARP inhibitor sensitivity

We studied the relative sensitivity of SNU-251 and SKOV3 cell lines to clinically used PARP inhibitors, talazoparib, and olaparib. SNU-251 and SKOV3 cells were seeded at a density of 1000 cells/well in a 96-well format 24 hours before the addition of either PARP inhibitor. On the day of treatment, the media was removed and fresh media containing varying concentrations of PARP inhibitors were added. Cells were incubated in the presence of PARP inhibitors continuously for 7 days. At the end of the

treatment, solutions were aspirated and CellTiter Glo® (Promega, Madison, WI) was added. Next, plates were measured for luminescence on a Perkin Elmer Enspire™ multimode plate reader. Data were normalized to healthy controls and fitted by using a nonlinear sigmoidal dose-response curve, Prism version 6.0. Effective concentrations for 50% growth inhibition were calculated as part of the data analysis.

Radiosensitivity

To study the radiosensitivity of SNU-251 and SKOV3 ovarian cancer cells, we performed clonogenic assays. First, the plating efficiency of each cell line was determined by seeding 100, 250, or 500 cells into 60 mm dishes and allowing colonies to form over 2 to 3 weeks. SKOV3 feeder cells irradiated at 12 Gy were used to facilitate colony formation in both cell lines (10–20,000/dish). Next, 500,000 cells were seeded in a 60 mm dish 24 hours before irradiation. Each dish received a different dose of radiation, and cells were returned to the incubator for 1 hour. Next, cells were trypsinized and reseeded at appropriate concentrations based on the radiation dose delivered, resulting in ~100 colonies formed in each dish. Cells were then returned to the incubator, and colonies were allowed to form over the span of 2 to 3 weeks. After the colonies were greater than 50 cells, they were stained with trypan blue and manually counted. Experiments were repeated with a pulse exposure of PARP inhibitors 1 hour before and 2 hours after irradiation. Cells were then trypsinized and reseeded in fresh growth media as previously described. Data were plotted by using Prism version 6.0.

Western blot protein analysis

Whole cell lysate preparation. One to three million SKOV3 or SNU-251 cells were lysed in RIPA buffer (Cat. No. 89900; Thermo Fisher Scientific) with protease and phosphatase inhibitors (P8340, P2850, P5726; Sigma Aldrich) on ice for 30 minutes. Next, cell lysates were sonicated and centrifuged at 14,000 rpm for 20 minutes at 4°C. Samples were then assayed for protein concentration by using the DC BioRad (Hercules, CA) absorbance assay. Samples were diluted to a final concentration with 25% Laemmli buffer (BioRad). All buffers used during Western blot analysis were approximately at physiological pH, 7.3.

Nuclear protein extraction. Nuclear proteins were extracted from SNU-251 and SKOV-3 cells that were irradiated with or without talazoparib. First, cell pellets were weighed and recorded. One to three million cells were lysed for 20 minutes on ice in a solution of 115 mM KCl, 5 mM NaCl, 1 mM KH₂PO₄, 20 mM HEPES, and 3 mM MgCl₂, at pH 7.3, with protease and phosphatase inhibitors (Sigma Aldrich) at a volume 10× the total pellet weight. Next, cells were centrifuged at 3000 rpm for 5 minutes and the supernatant was discarded. The pellet was resuspended in the lysing solution described earlier with 0.5% NP40 on ice for 5 minutes in a volume 5× the pellet weight. Lastly, nuclear proteins were extracted by using urea at 5× volume equal to the original pellet weight. The resulting solutions were then centrifuged at 12,000 rpm, and the supernatant was col-

lected. The final solution was diluted by 25% with glycerol for storage at –75°C.

Gel electrophoresis was performed by using a BioRad system on 4%–20% Mini-PROTEAN® TGX™ precast gels. Proteins were then transferred to polyvinylidene fluoride membrane by using a BioRad Turbo Transfer system. Membranes were blocked for 1 hour in Odyssey blocking buffer. Primary antibodies were incubated at 4°C overnight. Anti-BRCA-1 (C-20; Santa Cruz Biotechnology) and anti-phospho-histone H2AX(Ser139) (20E3; Cell Signaling and Technology) were incubated in PBS with 0.2% tween 20. Anti-PARP (46D11; CST) and anti-Histone H3 (D1H2; CST) were incubated in Odyssey blocking buffer with 0.2% tween 20. Membranes were washed, and fluorescent secondary antibodies were incubated with membranes at room temperature for 1 hour. All membranes were read on a LiCor Odyssey Imager (Lincoln, NE), and regions of interest quantifying fluorescent signals were produced. Antibody incubations were performed in triplicate.

Results

Radiochemistry: [¹⁸F]FTT synthesis

[¹⁸F]FTT was successfully synthesized on a fully automated synthesis module. The entire synthesis required 55 minutes and gave 50%–60% decay-corrected isolation yield (100–200 mCi). The decay-corrected yield was calculated by first measuring the initial ¹⁸F transferred to the product vial by a radiometric detector and then the final product measured by a dose calibrator. The radioactivity of the final product was then divided by the starting amount of radioactivity and multiplied by 100 to convert to a percent. The radiochemical and chemical purity was >90% with a specific activity >2200 Ci/mmol. [¹⁸F]FTT was manufactured in accordance with cGMP guidelines for positron-emission tomography radiopharmaceuticals and was suitable for human use.

Radiotracer PARP-1 saturation

SNU-251 cells showed a higher level of PARP-1 binding that was determined by the specific to nonspecific binding ratio (Fig. 1a, b). SNU-251 and SKOV3 cells differed by a factor of ~5. In this study, we have shown the SNU-251 cells had a higher threshold for PARP-1 binding sites over the SKOV3 cells. Baseline western protein analysis is presented in Figure 1c and is in agreement with radiotracer binding data showing that PARP-1 expression in SNU-251 cells is elevated almost four times that in SKOV3 cells.

PARP inhibitor sensitivity

SNU-251 and SKOV3 cells were evaluated side by side for relative sensitivity to PARP inhibitors olaparib and talazoparib. We observed that the SNU-251 cell line was 10- and 100-fold more sensitive to olaparib and talazoparib than SKOV3 cells. Dose-response curves for each cell line-specific drug treatment are presented in Figure 2a. The effective concentration required to inhibit growth by 50% (EC₅₀) is listed in Table 1.

FIG. 1. (a) This bar graph represents the SBR of [¹⁸F]FTT measured at 1 nM. A statistically significant difference was observed in radiotracer binding between the two cell lines (*p* < 0.005). Experiments were performed in triplicate. (b) Here, data are plotted for [¹⁸F]FTT saturation experiments as a nonlinear regression hyperbolic curve fit. SBR was extracted from this data set. (c) Western blots for relative protein expression are presented for SNU-251 and SKOV3 cell lines. The bar graph represents the immunofluorescent intensity measured from each protein band quantified on the Western blot. PARP, poly (ADP-ribose) polymerase; RFU, relative fluorescent units; SBR, specific binding ratio.

Radiosensitivity

SNU-251 cells were more sensitive to radiation compared with the SKOV3. Doses required to decrease the survival fraction of 10% (SF10) for each cell line are

listed in Table 1. As expected, there was a greater level of synergy in the SNU-251 cells compared with SKOV3 cells with the transient addition of a PARP inhibitor during irradiation. See Figure 2b for clonogenic survival curves.

FIG. 2. (a) Dose-response curves are presented for cell viability growth inhibition assays. The leftward shift in the curve for SNU-251 cells compared with SKOV3 cells shows that the SNU-251 cells are more sensitive to PARP inhibitors. (b) This graph represents the logarithmic plot of clonogenic assays performed in SNU-251 and SKOV3 cells that are treated with radiation or PARP inhibitor plus radiation.

TABLE 1. VALUES LISTED BELOW REPRESENT DIFFERENT TREATMENTS EVALUATED IN SNU-251 AND SKOV3 CELLS

Treatment	SNU-251	SKOV3
PARP inhibitor single-agent EC ₅₀ (nM) ± SE ^a		
Olaparib	714 ± 157	8427 ± 2100
Talazoparib	4 ± 1	386 ± 101
Radiosensitivity SF ₁₀ (Gy) ^b		
Irradiated	3.8	6
PARP inhibitor adjuvant to radiation SF ₁₀ (Gy) ^c		
1 μM Talazoparib	2	4.8
10 μM Talazoparib	1.4	4.4

^aEffective concentrations for 50% reduction in cell growth for single-agent PARP inhibitors.

^b10% survival fraction for irradiated cells.

^c10% survival fraction for PARP inhibitor adjuvant to radiation. PARP, poly (ADP-ribose) polymerase.

Western blot protein analysis

We evaluated protein expression at baseline by using whole cell homogenate and found that the SNU-251 cell line exhibited a higher expression of PARP-1. These data correspond with [¹⁸F]FTT binding data and show that SNU-251 cells express a higher amount of target protein. BRCA-1

expression was only found in the SKOV3 cells, and it confirms the deleterious mutation in SNU-251 cells. Histone H3 was used as a loading control throughout Western blot protein analysis. Relative protein expression at baseline is presented in Figure 1c. In addition, we evaluated protein expression postradiation or transient PARP inhibition to confirm the synergistic effects observed in clonogenic radiosensitivity assays. In agreement with our clonogenic assays, we observed a greater increase in DNA damage defined by γH2AX in the presence of a PARP inhibitor in both cell lines compared with baseline. PARP activity measured by PAR was decreased in all PARP inhibitor-treated cells, showing that the 3 hour incubation period was sufficient for blocking enzymatic activity. Western blots are presented in Figure 3a, and triplicate experiments corresponding to blot analysis are presented in Figure 3b.

Discussion

Recently, PARP inhibitors have experienced a renewed emphasis in the treatment of multiple cancers, including breast, ovarian, and prostate.^{5,24} Although each cancer is unique when defined by tissue of origin and genetic mutations, the underlying biochemical function of PARP enzymes remains universal. Past and current clinical trials have focused on genetic signatures, specifically HR deficiencies, to predict patient response to PARP inhibitor

FIG. 3. (a) Representative Western blots performed after radiation or PARP inhibitor plus radiation. Each treatment has been numbered after cell line type and corresponds in this figure (a, b). Proteins analyzed are indicated to the left of the blot, and a + sign below the blot indicates whether treatment was given. (b) Bar graphs representing the relative protein analysis for γH2AX and PAR are performed in triplicate.

therapy. These methods have shown that the determination of functional HR is much more difficult than simply identifying a potential gene that could render the DNA repair pathway dysfunctional. For example, even with single mutations within the HR pathway, there could also be bypass mechanisms that restore HR as seen with mutations in 53BP1.²⁵ This complicates the determination of HR function and, in turn, results in difficulty in predicting patient outcomes with PARP inhibitor therapy. It is also known that HR deficiency is not the only reason that cancer cells are sensitive to PARP inhibition but also functional NHEJ is required.¹³ This adds another level of complexity when defining patient genetic signatures that will have a favorable response to PARP inhibitor therapy. Each of these approaches has focused on the downstream effector proteins, with little consideration to the primary drug target.

All PARP inhibitors bind to the NAD⁺ binding pocket within the PARP-1 enzyme, and to a lesser extent PARP-2 or PARP-3, preventing the biochemical synthesis and signaling of PAR. The first and possibly most important molecular property to define in cancer patients who will receive PARP inhibitor therapy is PARP protein expression. It has been shown that even in the absence of functional HR, PARP1, or PARP2, knockout cells are resistant to PARP inhibitors.^{26,27} Without target protein expression, the drug cannot exert the desired pharmacological effect. Identification of patients who do not express PARP-1 would allow healthcare providers to switch to alternative treatment options before therapeutic failure and disease progression.

By utilizing a PARP-1 radiotracer, clinicians can quantify PARP-1 expression with PET imaging.²⁰ This offers multiple advantages to current methods of characterizing PARP expression. With this technology, clinicians can characterize PARP-1 expression in the entire tumor and at distant sites of metastasis. Current methodologies to characterize PARP-1 expression, such as immunohistochemistry (IHC), are limited by sampling bias and nonquantitative results. Furthermore, since PET is a noninvasive procedure, it can be repeated throughout the course of PARP inhibitor therapy, providing longitudinal information on PARP-1 expression. In contrast, patient biopsies are typically not repeated due to the invasive nature of the procedure, so IHC data represent snapshots in time that may or may not be relevant to the current disease state.

Using PET imaging, clinicians can identify patients who have high expression of PARP-1. This technology would not replace current methods of characterizing genetic signatures of HRD but would be complementary as a functional test. As patient care becomes more personalized, methodologies for identifying molecular targets of emerging therapeutics become essential to the advancement of medicine.

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Disclosure Statement

No competing financial interests exist.

References

1. Barkauskaite E, Jankevicius G, Ahel I. Structures and mechanisms of enzymes employed in the synthesis and degradation of PARP-dependent protein ADP-ribosylation. *Mol Cell* 2015;58:935.
2. Feng FY, de Bono JS, Rubin MA, et al. Chromatin to clinic: The molecular rationale for PARP1 inhibitor function. *Mol Cell* 2015;58:925.
3. Krishnakumar R, Kraus WL. The PARP side of the nucleus: Molecular actions, physiological outcomes, and clinical targets. *Mol Cell* 2010;39:8.
4. Livraghi L, Garber JE. PARP inhibitors in the management of breast cancer: Current data and future prospects. *BMC Med* 2015;13:188.
5. Sonnenblick A, de Azambuja E, Azim HA Jr., et al. An update on PARP inhibitors—Moving to the adjuvant setting. *Nat Rev Clin Oncol* 2015;12:27.
6. Wang ZC, Birkbak NJ, Culhane AC, et al. Profiles of genomic instability in high-grade serous ovarian cancer predict treatment outcome. *Clin Cancer Res* 2012;18:5806.
7. Timms KM, Abkevich V, Hughes E, et al. Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. *Breast Cancer Res* 2014;16:475.
8. Cardnell RJ, Feng Y, Diao L, et al. Proteomic markers of DNA repair and PI3K pathway activation predict response to the PARP inhibitor BMN 673 in small cell lung cancer. *Clin Cancer Res* 2013;19:6322.
9. Michels J, Vitale I, Saparbaev M, et al. Predictive biomarkers for cancer therapy with PARP inhibitors. *Oncogene* 2014;33:3894.
10. Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 2005;434:913.
11. Schwab RA, Niemuszczysz J, Shah F, et al. The Fanconi Anemia Pathway Maintains Genome Stability by Coordinating Replication and Transcription. *Mol Cell* 2015;60:351.
12. Somyajit K, Mishra A, Jameei A, et al. Enhanced non-homologous end joining contributes toward synthetic lethality of pathological RAD51C mutants with poly (ADP-ribose) polymerase. *Carcinogenesis* 2015;36:13.
13. Patel AG, Sarkaria JN, Kaufmann SH. Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. *Proc Natl Acad Sci U S A* 2011;108:3406.
14. Green AR, Caracappa D, Benhasouna AA, et al. Biological and clinical significance of PARP1 protein expression in breast cancer. *Breast Cancer Res Treat* 2015;149:353.
15. Domagala P, Huzarski T, Lubinski J, et al. PARP-1 expression in breast cancer including BRCA1-associated, triple negative and basal-like tumors: Possible implications for PARP-1 inhibitor therapy. *Breast Cancer Res Treat* 2011;127:861.
16. Gan A, Green AR, Nolan CC, et al. Poly(adenosine diphosphate-ribose) polymerase expression in BRCA-proficient ovarian high-grade serous carcinoma; association with patient survival. *Hum Pathol* 2013;44:1638.

17. Ozretic L, Rhiem K, Huss S, et al. High nuclear poly(adenosine diphosphate-ribose) polymerase expression is predictive for BRCA1- and BRCA2-deficient breast cancer. *J Clin Oncol* 2011;29:4586.
18. Rojo F, Garcia-Parra J, Zazo S, et al. Nuclear PARP-1 protein overexpression is associated with poor overall survival in early breast cancer. *Ann Oncol* 2012;23:1156.
19. Stanley J, Klepczyk L, Keene K, et al. PARP1 and phospho-p65 protein expression is increased in human HER2-positive breast cancers. *Breast Cancer Res Treat* 2015;150:569.
20. Makvandi M, Xu K, Lieberman BP, et al. A radio-tracer 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.
21. Tu Z, Chu W, Zhang J, et al. Synthesis and *in vivo* evaluation of [¹¹C]PJ34, a potential radiotracer for imaging the role of PARP-1 in necrosis. *Nucl Med Biol* 2005; 32:437.
22. Edmonds CE, Makvandi M, Lieberman BP, 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.
23. Zhou D, Chu W, Xu J, et al. Synthesis, [(1)(8)F] 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.
24. Mateo J, Carreira S, Sandhu S, et al. DNA-repair defects and olaparib in metastatic prostate cancer. *N Engl J Med* 2015;373:1697.
25. Bunting SF, Callen E, Wong N, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* 2010;141:243.
26. Murai J, Huang SY, Das BB, et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. *Cancer Res* 2012;72:5588.
27. Murai J, Huang SY, Renaud A, et al. Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. *Mol Cancer Ther* 2014;13:433.