Title: A PET imaging agent for evaluating PARP-1 expression in ovarian cancer

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Abstract

Background: Poly(ADP-ribose) polymerase (PARP) inhibitors are effective in a broad population of ovarian cancer patients, however resistance caused by low enzyme expression of the drug target, poly(ADP-ribose) polymerase 1 (PARP-1), remains to be clinically evaluated in this context. We hypothesize that PARP-1 expression is variable in ovarian cancer and can be quantified in primary and metastatic disease using a novel positron emitting tomography (PET) imaging agent.

Methods: We used a translational approach to describe the significance of PET imaging of PARP-1 in ovarian cancer. First, we produced PARP1 KO ovarian cancer cell lines using CRISPR/Cas9 gene editing to test loss of PARP-1 as a resistance mechanism to all clinically used PARP inhibitors. Next, we performed pre-clinical microPET imaging studies using ovarian cancer patient derived xenografts in mouse models. Finally, in a phase 1 PET imaging clinical trial we explored PET imaging as a regional marker of PARP-1 expression in primary and metastatic disease through correlative tissue histology.

Results: We found deletion of PARP1 causes resistance to all PARP inhibitors in vitro and microPET imaging provides proof of concept as an approach to quantify PARP-1 in vivo. Clinically, we observed a spectrum of standard uptake values (SUVs) for PARP-1 in tumors ranging from 2-12. In addition, we found a positive correlation between PET SUVs and fluorescent immunohistochemistry for PARP-1 ($r^2:0.60$).

Conclusions: This work confirms the translational potential of a PARP-1 PET imaging agent and supports future clinical trials to test PARP-1 expression as a method to stratify patients for PARP inhibitor therapy.

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Introduction

Epithelial ovarian cancer (EOC) is the fifth deadliest malignancy in women with greater than 70% of patients presenting with advanced disease. (1) Until the recent emergence of targeted therapeutics, treatment has relied heavily on cytoreductive surgery and platinum/taxane-based chemotherapy. (2) Poly(ADP-ribose) polymerase inhibitors (PARPi) have emerged as promising drug candidates for the treatment of EOC. In current clinical practice, patients are commonly selected for PARPi therapy by testing positive for genetic mutations in genes encoding DNA repair proteins that result in homologous recombination deficiency (HRD), most notably mutations in breast and ovarian cancer susceptibility genes 1 and 2 (BRCA1 and BRCA2). (3, 4) However, only ~50% of patients with HRD respond to PARPi therapy. (3) Moreover, patients without known HRD have also shown a clinical benefit from PARPi, as seen in a recent trial assessing niraparib, olaparib, and rucaparib, as maintenance therapy in platinum-sensitive recurrent ovarian cancer. (5-8) Given not all patients will respond to PARPi, improved clinical tools for predicting patients who will respond are urgently needed.

Numerous clinical trials have led to FDA approval of three PARPi since 2014 and there is continued development of 2 additional drugs within this class. (9-13) Despite growth in the development and application of PARPi the primary drug target poly(ADP-ribose) polymerase-1 (PARP-1) has never been evaluated in vivo, even though loss of expression in vitro is a well-characterized resistance mechanism. (3, 14-19) It was first hypothesized that PARPi work primarily through a synthetic lethality pathway where loss of BRCA1 or BRCA2 combined with chemical inhibition of PARP-1 results in cell death. (20, 21) However, it was later shown that deletion of PARP1 did not result in enhanced sensitivity to a DNA alkylating agent in wild-type cells suggesting loss of catalytic function could not be the only reason for PARPi efficacy. (17-19) Next, Murai et al. demonstrated that all clinically used PARPi have differential abilities to trap PARP-1 on DNA but equally lack cytotoxicity in PARP1 KO -/- cells. (22, 23) While some PARPi are potent PARP-1
trappers, all PARPi target the PARP-1 enzyme, making it a potential pre-requisite biomarker for efficacy.(22-24) Furthermore, PARP-1 has been shown by immunohistochemistry (IHC) to be highly variable in patients with ovarian(25-28), breast(29), and prostate cancer(30), irrespective of BRCA status. Together these studies demonstrate the need for a biomarker technology capable of quantitatively assessing PARP-1 in vivo that could enable patient selection for PARPi therapy.

Current methods to determine PARP-1 expression in clinical tumor specimens are limited and based on immunohistological methods that require invasive procedures such as biopsy or surgery. Association studies of PARP-1 expression by IHC with prognosis and outcome have demonstrated mixed results suggesting inconsistency of staining procedures and antibodies.(25-28) Indeed, there lacks a validated clinical IHC staining protocol for PARP-1 that can be widely and robustly applied in clinical practice.(28) Furthermore, approaches based on tissue sampling inadequately assess the potential heterogeneity of PARP-1 expression in disseminated EOC, a stage of disease highly relevant to PARPi therapy.

Radiotracer technology for the non-invasive imaging of PARP-1 could theoretically overcome the limitations of IHC by quantitatively assessing global PARP-1 expression in primary and disseminated disease.(31, 32) $[^{18}\text{F}]$FluorThanatrace ($[^{18}\text{F}]$FTT) is a radiolabeled small molecule PARPi that is currently approved for clinical use under an investigational new drug application at the University of Pennsylvania, Philadelphia PA, and Washington University, St. Louis MO.(33, 34) $[^{18}\text{F}]$FTT, and its iodinated analogue $[^{125}\text{I}]$KX1, have been shown to correlate with PARP-1 expression through a receptor-ligand effect which stems from their primary pharmacological mechanism of action.(16, 35) As such, $[^{18}\text{F}]$FTT and $[^{125}\text{I}]$KX1 quantify PARP-1 expression, and have the ability to measure drug-target engagement of clinical PARPi by competing with one another for the NAD+ binding pocket on the catalytic sub-domain of PARP-1. Contrary to current methodologies which measure the biochemical product of PARP-1, poly(ADP-ribose) (PAR), this is a direct measurement of drug-target engagement.
In this work, we validate the pre-clinical rationale for measuring PARP-1 expression as a predictive biomarker of response to PARPi and report the first clinical trial studying PARP-1 expression with [{\textsuperscript{18}}F]FTT PET in EOC.

Results

CRISPR/Cas9 deletion of PARP1 in ovarian cancer cells

Using CRISPR/Cas9 gene editing, we mediated the deletion of PARP1 in two ovarian cancer cell lines, one with a BRCA1 mutation and another with BRCA1 promoter methylation (UWB1.289 and OVCAR8). OVCAR8 cells have been previously shown to have reduced BRCA-1 expression attributed to promoter methylation and are sensitive to DNA damaging agents. (36-38) Strikingly, the genetic deletion of PARP1 in EOC cells with a BRCA1 mutation or BRCA1 promoter methylation did not result in synthetic lethality, in that the cells were viable and grew in culture. (21, 39) Identifying the mechanism of viability was beyond the scope of this work, but is being pursued. Using this system, we achieved >90% reduction of PARP-1 expression in polyclonal populations of BRCA1 mutant (UWB1.289) and BRCA1 methylated (OVCAR8) ovarian cancer cells as measured by immunofluorescence (IF) and Western blot analysis (figure 1a, figure 1b, figure s1a, and figure s1b). Cell microscopy studies showed that PARP-1 was indeed absent at the single cell level in polyclonal populations (figure 1a and figure s1a). We also examined PARP-2 and PARP-3 expression by Western blot to investigate off-target effects of single guide RNA’s. We found no differences from control for PARP-2 or PARP-3 expression by Western analysis (figure s1b). Lastly, to determine if PARP-1 expression varies amongst ovarian cell lines with and without BRCA dysfunction (table s1), we measured PARP-1 in multiple cell lines and demonstrated a dynamic range of expression (figure s2a, figure s2b, and table s2).

[{\textsuperscript{125}}I]KX1 radioligand binding measures differences in PARP-1 expression

Using the PARP1 KO cell lines along with several non-modified EOC cell lines, we showed significant differences in the radioligand binding of [{\textsuperscript{125}}I]KX1 relative to PARP-1 expression (figure 1c, figure s2c,
and table s2). Most notably, the deletion of PARP1 reduced radioligand binding of [125I]KX1 in both models. Furthermore, we observed that the radioligand binding of [125I]KX1 was reduced in BRCA functional vs. dysfunctional cells (figure 1c, figure s2c, and table s2). The restoration of BRCA1 in UWB1.289 cells resulted in lower radioligand binding and corresponded to lower PARP-1 expression by IF and Western blot (figure 1a, figure 1b, figure 1c, figure s2a, figure s2b, and figure s2c). These data support in vitro radioligand binding with [125I]KX1 as a method to assay PARP-1 expression.

**Loss of PARP1 blunts PARPi efficacy in ovarian cancer cell lines in vitro**

The genetic deletion of PARP1 resulted in reduced DNA damage and sensitivity to PARPi in vitro. First, we examined isogenic UWB1.289 cells by IF after treatment with olaparib for 24 hrs. We found no change in DNA damage measured by γH2AX foci formation following olaparib treatment in both BRCA1 restored and PARP1 KO cells compared to DMSO controls. In contrast, γH2AX levels increased 2.6x in olaparib-treated UWB1.289 cells compared to DMSO controls (figure 1d). Similarly, the same effect was observed in OVCAR8 cells (figure 1d). Next, the cytotoxicity evaluated for clinically used PARPi showed the loss of PARP1 resulted in equal to or greater resistance compared to restoring BRCA1 in UWB1.289 cells (figure 1e, figure s2d, table s3a, and table s3b). Strikingly, a greater resistance in PARP1 KOs compared to BRCA1 restored from parent controls was observed for the potent PARP trappers, niraparib and talazoparib. The loss of PARP1 resulted in greater than a 1000-fold decrease in sensitivity to talazoparib while restoring BRCA1 had a lesser effect in UWB1.289 cells. OVCAR8 cells also became resistant to PARPi after loss of PARP1. No differences in sensitivity to cisplatin were observed in cell lines after loss of PARP1 indicating there was no change in overall sensitivity to DNA damaging drugs. These data provide direct support for PARP-1 expression as a requirement for PARPi sensitivity in vitro.

**Pre-clinical imaging of PARP-1 Expression**

The aforementioned findings demonstrate the need for dynamic, noninvasive, and quantitative monitoring of PARP-1 expression in patients selected for PARP inhibitor therapy. To this end, proof-
of-concept studies were conducted in pre-clinical mouse patient derived xenograft (PDX) models to confirm the in vitro radioligand binding studies and demonstrate the specificity of $[^{18}\text{F}]$FTT for in vivo quantitative imaging of PARP-1. Significant differences were observed in radiotracer uptake in tumors before and after treatment with olaparib. By administering olaparib (50mg/kg) we observed a reduction in $[^{18}\text{F}]$FTT tumor-to-muscle ratios, a semi-quantitative measure of tracer binding in vivo (figure 2a and figure s3a). In addition, tumor tissue analyzed from untreated and olaparib treated mice by ex-vivo autoradiography was used to confirm microPET findings (figure 2b and figure s3b). Results between microPET imaging and ex-vivo autoradiography were similar. Tumor-to-muscle ratios from microPET imaging before and after olaparib treatment were $4.2 \pm 0.32$ vs. $2.5 \pm 0.11$ (paired t-test, p-value <0.0025, n=4) (Figure 2c). In agreement with microPET results ex-vivo autoradiographs showed a significant difference between untreated and olaparib treated groups that was $5.14 \pm 0.13$ vs. $2.41 \pm 0.18$ (unpaired t-test, p-value <0.0001, n=2, 10 sections/tumor) (figure 2d). Autoradiographs also showed significant differences in tumor and muscle of untreated and olaparib treated groups (unpaired t-test, p-values < 0.0001) (figure s3c).

**Clinical Trial Enrollment**

In this trial 20 patients were enrolled and 10 patients that underwent surgical debulking or biopsy were included in this study. From the 10 patients, 13 tissue specimens were collected for in vitro analysis. Of the 10 patients included in this study, 8 underwent PET/CT imaging (figure 3). One of the 8 patients had a negative $[^{18}\text{F}]$FTT scan and was excluded from the $[^{18}\text{F}]$FTT PET vs. PARP-1 IF correlation.

**PARP-1 immunohistochemistry and autoradiography on clinical specimens**

Radioligand binding with $[^{125}\text{I}]$KX1 was found to co-localize with PARP-1 colormetric IHC (c-IHC) (figure 4a) on adjacent tissue sections, supporting the specificity of the iodinated radiotracer for PARP-1. In addition, a spectrum of PARP-1 expression was observed by fluorescent IHC (f-IHC) and autoradiography (figure 4b and figure s4) providing direct evidence for the wide range of PARP-1
expression in ovarian cancer. Interestingly, PARP-1 co-localized with both tumor cells and lymphocytes in the tumor microenvironment (table s4 and figure s5). PARP-1 expression was also positive in two lymph node samples, with residual disease on pathology, and co-stained with γH2AX. Finally, positive staining for PARP-1 and p53 co-occurred in 77% (10/13) of tissue samples where as positive staining for PARP-1 and γH2AX had 100% (13/13) co-occurrence (table s4 and figure s5).

**Clinical observations with $[^{18}\text{F}]$FTT PET imaging**

Positron-emission tomography (PET) imaging demonstrated $[^{18}\text{F}]$FTT localized to areas of known EOC based on clinical $[^{18}\text{F}]$FDG PET/CT imaging, with low bladder uptake allowing for clear visualization of lesions within the pelvic region (figure 5). Despite hepatobiliary excretion, $[^{18}\text{F}]$FTT uptake was discernable in omental disease in the abdomen (figure 6). Differences in radiotracer uptake were seen between $[^{18}\text{F}]$FTT and $[^{18}\text{F}]$FDG PET, which is commonly used for evaluating tumor response and recurrence in EOC as well as identifying extrapelvic metastases and diagnosis.(40) Such differences suggest these radiotracers provide different and complementary information, concordant with their distinct molecular targets. For example, Patient 2, who had completed 4 cycles of carboplatin and paclitaxel 2 weeks prior to imaging, showed multiple omental lesions by $[^{18}\text{F}]$FTT PET imaging with maximum SUVs ranging from 5-8 that were low on $[^{18}\text{F}]$FDG PET (figure 6a, figure 6b, and table s5). In follow up, patient 2 showed disease progression within 4 months of therapy and was platinum resistant. In contrast, patient 11 had a $BRCA1$ mutation and showed low uptake on both $[^{18}\text{F}]$FTT and $[^{18}\text{F}]$FDG PET after 4 cycles of therapy (carboplatin and paclitaxel) and was platinum sensitive (figure 6c and figure 6d). These results suggest that $[^{18}\text{F}]$FTT PET provides unique molecular information as a biomarker of viable tumor tissue and could be used in the future to guide clinical management in addition to current biomarkers of metabolic activity. Inter-tumor heterogeneity of $[^{18}\text{F}]$FTT was observed in patients (figure s6), further emphasizing a beneficial role of PET imaging compared with standard biopsy approaches for quantifying PARP-1 in local and disseminated disease.
Clinical $[^{18}\text{F}]$FTT PET imaging positively correlates with PARP-1 expression in patient tumors

A spectrum of $[^{18}\text{F}]$FTT uptake was seen in tumors, indicative of varying levels of PARP-1 expression (table s5). Maximum standardized uptake values (SUVs) of tumors ranged from as low as 2 (background) to as high as 12 and a similar range of PARP-1 expression was also observed by immunofluorescence and autoradiography (figure 7a, figure 7b, and table s5). Since, $[^{18}\text{F}]$FTT uptake is a marker or PARP-1 expression this could translate to some tumors expressing up to six times as much PARP-1 as other tumors, with consequently higher drug-target densities for PARPi (figure 7a). Further characterizing $[^{18}\text{F}]$FTT as a measurement of PARP-1 expression, we found positive correlations using linear regression analysis and calculating Pearson coefficients, between PARP-1 f-IHC and $[^{18}\text{F}]$FTT PET imaging, in addition to PARP-1 f-IHC and $[^{125}\text{I}]$KX1 autoradiography ($r^2 = 0.60, 0.79, r = 0.77, 0.89$) (figure 7b and table s5). As expected, there was no correlation observed between PARP-1 f-IHC, $[^{18}\text{F}]$FTT, or $[^{125}\text{I}]$KX1 autoradiography with $[^{18}\text{F}]$FDG, supporting distinct molecular imaging targets for $[^{18}\text{F}]$FTT and $[^{18}\text{F}]$FDG.

Discussion

This translational work further supports PARP-1 as the primary target for PARPi and describes the development of $[^{18}\text{F}]$FTT as a non-invasive measure of regional tumor PARP-1 expression in ovarian cancer patients. *In vitro* radioligand binding of $[^{125}\text{I}]$KX1, the radio-iodinated analog of $[^{18}\text{F}]$FTT, was previously reported as a method to assay PARP-1 expression and was further confirmed in this work.(16) The genetic deletion of PARP1 was explored as a mechanism of PARPi resistance in a BRCA1 mutant and a BRCA1 methylated ovarian cancer cell line, and our findings support the notion that PARP-1 expression is necessary for PARPi sensitivity *in vitro*. Pre-clinical imaging of PARP-1 in mice with $[^{18}\text{F}]$FTT demonstrated the specificity of the radiotracer for its molecular target. Lastly, $[^{18}\text{F}]$FTT was studied in EOC patients in a Phase 1 trial. $[^{18}\text{F}]$FTT demonstrated a wide dynamic range of uptake correlating with *in vitro* measures of PARP-1 expression and the absence of
correlation with $^{18}$F]FDG. In addition, we confirmed the feasibility of $^{18}$F]FTT as a practical radiotracer to image lesions in disseminated omental disease despite bowel uptake. Given our small sample size it is too early to remark on imaging nodal disease involvement with $^{18}$F]FTT although a recent pre-clinical study suggests uptake in malignant nodes is greater than normal lymph nodes in a diffuse large b-cell lymphoma model.(41) It is difficult to extrapolate between diseases but we did observe a single case that showed nodal uptake on $^{18}$F]FDG but not $^{18}$F]FTT and was negative on pathology which is consistent with inflammation. Together these data are important first steps in establishing $^{18}$F]FTT as a novel non-invasive biomarker of PARP-1 expression and lay the foundation for future testing of $^{18}$F]FTT PET in parallel with PARPi clinical trials.

We explored the loss of drug target expression as a mechanism of PARPi resistance by studying the effects of the genetic deletion of PARP1 in BRCA1 mutant and BRCA1 methylated cell lines. While loss of PARP1 in BRCA1 mutant or BRCA1 methylated cell lines has the potential to be synthetically lethal, we found that stable deletion of PARP1 by CRISPR/Cas9 resulted in polyclonal populations of cells where >90% of clones lacked PARP-1 expression and grew in culture. It has been recently shown that OVCAR8 cells are capable of forming RAD51 foci indicating functional HR and could explain why loss of PARP1 was not synthetically lethal in this cell line.(42) The observed viability with UWB1.289 cells is most likely due to the biological redundancy between PARP-1 and PARP-2 or PARP-3. Future studies are ongoing to assess PARP1/PARP2 KO and PARP1/PARP3 KO models to test this hypothesis. In addition, we intend to evaluate loss of PARP1 in isogenic BRCA2 mutants with and without reversion mutations, such as the PEO1/PEO4 cell lines, to test if similar results are found. We also found that lack of PARP-1 expression prevented PARPi’s from inducing DNA damage as measured by γH2AX. These results are consistent with previous reports.(17-19, 22, 23) Loss of PARP1 most likely prevents the DNA damage caused by PARPi induced PARP-1 trapping. This mechanistic understanding is highly relevant to PARPi therapy and
our findings are consistent with what has been previously reported showing PARP-1 as the primary target for PARPi induced DNA damage.(3, 14, 15, 17-19, 23)

In further support, we also observed a greater reduction in PARPi sensitivity with loss of PARP-1 expression compared to BRCA1 restoration in the UWB1.289 isogenic cell lines when treated with the potent PARP trappers, niraparib and talazoparib. This observation may have translational implications since olaparib, rucaparib, and niraparib were recently approved by the FDA as maintenance therapy in platinum-sensitive patients, regardless of BRCA mutation status.(6-8) These clinical reports suggest there is a need for predictive biomarkers for PARPi therapy that go beyond BRCA mutation and HRD testing. The direct measurement of PARPi-target, PARP-1 expression, described in this work offers a complementary approach that could strengthen genomic testing for predicting patient response to PARPi therapy.

We demonstrated that in addition to using radioligand binding as an in vitro assay of PARP-1 expression, the use of PET radiopharmaceuticals as an in vivo assay for PARP-1 is feasible in patients. This work reports the first clinical imaging trial in EOC using a PARP-1 radiotracer, $[^{18}\text{F}]$FTT, and preliminarily establishes $[^{18}\text{F}]$FTT as a non-invasive biomarker of PARP-1. We found a wide spectrum of PARP-1 expression in EOC measured by $[^{18}\text{F}]$FTT PET, with some tumors showing SUVs as high as 12 and as low as 2. Regardless of the exact mechanism in which PARPi induce anticancer effects, all current models require the drug target, PARP-1.(15, 16, 23, 24) This signifies the importance of quantitatively assessing the target of PARPi directly at the site of action by measuring intratumoral PARP-1 in primary and metastatic disease. We have shown in pre-clinical imaging studies that $[^{18}\text{F}]$FTT is capable of quantifying intratumoral PARPi-PARP-1 drug target engagement. This is the only biomarker technology capable of non-invasively measuring PARP-1 and PARPi drug target engagement directly in tumors, in contrast to surrogate assays such as peripheral blood test.(43)
PARP-1 expression may also play a role in predicting inherent or incurred DNA damage burden to identify DNA-repair deficiencies or even treatment response as a functional biomarker of HRD.(44, 45) We are currently entering several clinical trials in both breast and ovarian cancer at the University of Pennsylvania to directly evaluate $[^{18}\text{F}]$FTT as a predictive and pharmacodynamic biomarker of PARPi therapy and other DNA-damaging chemotherapy. These studies should provide the impetus for multi-center clinical trials to fully evaluate the importance of PARP-1 expression as a biomarker for cancer therapy and the technological advantages of $[^{18}\text{F}]$FTT for measuring intratumoral and inter-tumoral PARP-1 expression vs. conventional IHC approaches.

**Methods**

**Study Design**

The objective of this study was to highlight the significance of PARP-1 expression for PARP inhibitor therapy using pre-clinical models of ovarian cancer and perform the first PET imaging study of PARP-1 in ovarian cancer using $[^{18}\text{F}]$FTT with histological correlates to confirm radiotracer uptake as a biomarker of PARP-1 expression. This goal was addressed by using CRISPR/Cas9 to delete PARP1 in models of ovarian cancer, which were then used to test PARPi efficacy *in vitro*. In addition, we used patient-derived orthotopic xenograft models of ovarian cancer to show proof of concept for $[^{18}\text{F}]$FTT microPET imaging of PARP-1 and PARPi drug target engagement *in vivo*. $[^{18}\text{F}]$FTT PET imaging of PARP-1 was performed in ovarian cancer patients and tumor specimens were retrieved at surgical debulking or biopsy for *in vitro* correlative analysis of PARP-1 using $[^{125}\text{I}]$KX1 autoradiography and PARP-1 f-IHC.

All *in vitro* experiments were carried out using standard cell culture technique and were repeated three independent times. The deletion of PARP1 with CRISPR/Cas9 was performed in two ovarian cancer cell lines. In addition, three unique guide RNA’s were used to mediate the deletion of PARP1. Polyclonal populations of PARP1 KO cell lines were used to test PARPi efficacy *in vitro*. An n
= 4 was selected for pre-clinical imaging studies to prevent bias greater than 10% for interpreting and analyzing microPET image results. No data from pre-clinical models was excluded from analyses.

For the clinical portion of this study, 20 EOC patients were enrolled, 10 of which had tissue available for in vitro analysis and 8 out of 10 underwent $^{18}$F]FTT imaging prior to subsequent standard clinical management (figure 3). One of the 8 patients that underwent $^{18}$F]FTT had a negative PET image and was excluded from correlative analysis. A total of 13 tissue specimens were available from the 10 patients and were used for in vitro assays including PARP-1 f-IHC and $^{125}$I\textsuperscript{1}KX1 autoradiography (table s5). All tissue specimens from patients who underwent $^{18}$F]FTT PET imaging were included in correlations between $^{18}$F]FTT PET, $^{18}$F]FDG, $^{125}$I\textsuperscript{1}KX1 autoradiography, and PARP-1 f-IHC. All tissue specimens collected from consented patients were included in correlations between $^{125}$I\textsuperscript{1}KX1 and PARP-1 f-IHC. Patients with and without BRCA1 mutations were enrolled in this study (table s6). All experimental procedures for pre-clinical and clinical work are described in detail in the supplemental information.

**CRISPR/Cas9 deletion of PARP1 in ovarian cancer cells**

OVCA\textsuperscript{R8} cells were a gift from Dr. David M. Livingston, Dana Farber, Boston MA. All other cell lines were available through ATCC or the Basser Center for BRCA at the University of Pennsylvania, Philadelphia PA (table s1). PARP1 was deleted in UWB1.289 and OVCA\textsuperscript{R8} EOC cell lines that have reduced BRCA-1 expression through a deleterious mutation (UWB1.289) or promoter methylation (OVCA8). These mutations are characterized elsewhere in the literature.(46, 47) Using 3 unique guide RNA’s (SI materials and methods), PARP1 deletion was mediated by Cas9 in both cell lines effectively producing 3 unique polyclonal PARP1 KO cell lines for each. Loss of PARP-1 expression was confirmed by IF cell microscopy and Western blot in each polyclonal population. Polyclonal populations were used in all experiments.

$^{125}$I\textsuperscript{1}KX1 radioligand binding measures differences in PARP-1 expression
Using multiple ovarian cancer cell lines, we performed in vitro pharmacological PARP-1 saturation radioligand binding studies to quantify PARP-1 expression. \(^{[125]} I\)KX1 was synthesized and in vitro radioligand binding studies were performed as previously described.\(^{(16)}\) Briefly, increasing concentrations, from 0.01 nM – 10 nM, of \(^{[125]} I\)KX1 were added to a 96 well plate in quadruplicate for each respective cell line and experiments were completed in triplicate.

**Loss of PARP1 promotes PARPi resistance in ovarian cancer cell lines**

Immunofluorescence was used to determine DNA damage induced by olaparib or DMSO control in ovarian cancer cell lines. Using 5 clinically used PARPi, we performed in vitro cell viability assays to determine changes in drug sensitivity based on loss of PARP-1 expression. The experimental procedures were adopted from previous work.\(^{(16)}\) Cisplatin was also evaluated as a control to evaluate changes in sensitivity of DNA damaging agents that occur after loss of PARP1. Cell viability was assessed using CellTiterGlo™ (Promega, WI). Experiments were completed in duplicate three independent times.

**Pre-clinical microPET imaging of PARP-1**

Through serial \(^{18}F\)FTT PET imaging studies, we evaluated PARP-1 drug-target engagement using the FDA-approved PARPi olaparib. PET imaging of PARP-1 drug-target engagement is a non-invasive methodology to directly assess PARP inhibition. Using 2 ovarian cancer patient derived xenograft models in 15-18 week old female NOD SCID mice provided from the Simpkins laboratory at the University of Pennsylvania\(^{(48)}\), including a BRCA1 mutant and wild type, we performed microPET imaging on a Phillips (Netherlands) scanner (n=4 mice). Mice were imaged on day 1 and then olaparib was orally administered daily by gavage at a dose of 50 mg/kg. Mice were then re-imaged on day 4, 1 hour after the 4\(^{th}\) dose of olaparib. A total of 4 doses of olaparib were administered between imaging studies to achieve steady state. Ex-vivo autoradiography studies were performed on mice from PDX model WO-12-2 with and without olaparib treatment (n=6). The difference in tumor-to-
muscle ratios were measured and were representative of the percentage of PARPi drug-target engagement.

**PARP-1 immunohistochemistry and autoradiography on clinical specimens**

Colormetric immunohistochemistry was performed at the University of Pennsylvania Pathology Core for hematoxylin and eosin (HE) and biomarkers, including PARP-1, γH2AX, p-53, LCA, and AE1/3. These biomarkers were chosen due to their relevance to EOC. AE1/3 is a keratin marker and was used to identify tumor cells by c-IHC. In order to determine where in the tumor microenvironment PARP-1 was expressed, we co-stained with LCA, which is a pan lymphocyte marker. In addition, we analyzed common biomarkers present in high grade serous EOC, including p53 and γH2AX. Lastly, PARP-1 was evaluated by f-IHC and $^{[125]}$I]KX1 autoradiography on adjacent sections to determine if the two methods co-localize.

**Clinical $^{[18]}$FTT PET imaging**

The clinical trial included two imaging cohorts: a biodistribution cohort and a dynamic cohort. The patients included in this trial with pathologic correlates were all enrolled in the dynamic cohort. As such, only that protocol is reported in this section. Dynamic scanning was performed for approximately 60 minutes from the time of injection with a field-of-view over the abdomen/pelvis or to include a known site of tumor involvement. Static images from the skull base to mid-thigh were performed starting approximately 90 and 180 minutes after $^{[18]}$FTT injection; these static scans were optional at the discretion of the investigator or study physician. Static images obtained at ~90 minutes were used in the analysis. $^{[18]}$FTT PET/CT imaging was performed on a Phillips Ingenuity PET/CT scanner (Phillips Medical System, Netherlands). Images were reconstructed using standard reconstruction techniques and interpreted by trained radiology readers. Lesions were first located on a contemporaneous clinical $^{[18]}$FDG PET/CT and then located on the $^{[18]}$FTT PET/CT. The maximum standardized uptake value (SUV) was recorded for each scan.

**Statistical analysis**
Data presented are in the form of means ± SEM unless otherwise noted. All graphs were produced and statistically analyzed using Prism version 6.0, GraphPad. The maximum number of PARP binding sites (B$_{\text{max}}$) was calculated by an exponential non-linear curve fit one-site binding hyperbola function. All statistical tests comparing the mean of two groups were two sided. The comparisons of the means from more than two groups were performed using one-way ordinary ANOVA. Dose response curves were fitted using a non-linear sigmoidal dose response curve and effective calculations for 50% reduction in cell viability were calculated. Image analysis was performed using PMOD software (Zurich, Switzerland) and tumor-to-muscle ratios were calculated. Pre-clinical microPET imaging studies comparing tumor uptake of radiotracer were analyzed using paired, two-tailed t-test. Autoradiography studies were analyzed using unpaired two-tailed t-test. We chose an alpha of 0.05 (95% confidence interval) and data was deemed significant if p-values were less than 0.05. For correlations the Pearson correlation co-efficient was calculated and the data was considered statistically significant if the p-value was less than 0.05. To determine the ability of [$^{18}$F]FTT PET maxSUV to predict PARP-1 expression measured by f-IHC we used a linear regression and results were considered statistically significant if different from zero.

**Study Approval**

All animal studies were performed under protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee, Philadelphia PA, and were conducted under guidelines for animal welfare provided by the National Institute of Health (NIH).

This was an open-label, phase 1 trial of [$^{18}$F]FTT PET conducted under an approved Institutional Review Board (IRB) at the University of Pennsylvania, Philadelphia PA, and registered with the FDA as an expanded investigational new drug (Clinical trial identifier: NCT02637934). All subjects were consented for PET imaging and providing clinical tissue specimens for *in vitro* analysis.
Author Contributions
Mehran Makvandi is a translational scientist who designed experiments, performed experiments, collected data, analyzed data, and composed the manuscript. Austin Pantel is a nuclear medicine physician who read PET scans, determined SUV values in lesions for PET tracers, analyzed data, and composed the manuscript. Lauren Schwartz is a clinical pathologist who interpreted tissue histology and evaluated biomarker positivity. Erin Schubert is the clinical coordinator who wrote the clinical protocol. Kuiying Xu is a chemist who synthesized precursors for radiotracers used. Chia-Ju Hsieh is an image analyst who analyzed pre-clinical PET imaging data. Catherine Hou is a biologist who performed in vivo pre-clinical PET imaging studies. Hyoung Kim is a biologist who developed and produced patient derived xenograft models. Chi-Chang Weng is a biologist who performed ex-vivo autoradiography studies and analyzed data. Harrison Winters is a research technician who designed CRISPR/Cas9 guides for PARP-1 and performed in vitro PARP1 KO experiments. Robert Doot is an image analyst who analyzed clinical PET images. Michael D. Farwell is a nuclear medicine physician who administered the radiotracer to patients and interpreted PET images. Daniel A. Pryma is a nuclear medicine physician who administered the radiotracer to patients and interpreted PET images. Roger A. Greenberg is a cancer biologist who designed in vitro experiments and composed the manuscript. David A. Mankoff is a nuclear medicine physician who who administered the radiotracer to patients, designed experiments, interpreted the data, and composed the manuscript. Fiona Simpkins is a obstetrician oncologist who designed patient derived xenograft models, recruited patients, performed surgeries and biopsies, and composed the manuscript. Robert H. Mach is a radiochemist who designed radiotracers, experiments, and composed the manuscript. Lilie Lin is a radiation oncologist who designed experiments, analyzed data, recruited patients, lead the clinical trial, and composed the manuscript.
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References


Figures with legends

**Figure 1:** The characterization of PARP1 KO ovarian cancer cell lines and *in vitro* evaluation of PARP inhibitor efficacy. 

**A)** Immunofluorescence showed PARP-1 was absent in >90% of single cells in PARP1 KO polyclonal populations (ANOVA, p-value <0.0001) and was reduced in BRCA1 restored cells compared to parent control (ANOVA, p-value <0.0001). 

**B)** Polyclonal populations of PARP1 KO cell lines had reduced PARP-1 by Western blot compared to parent control. 

**C)** [125I]KX1 radioligand binding assays showed a significant reduction radiotracer binding in PARP1 KO and UWB1.289 BRCA1 restored cell lines compared to parent control (ANOVA, p-value < 0.0001). 

**D)** Immunofluorescence of olaparib treated UWB1.289 PARP1 KO and UWB1.289 BRCA1 restored cells
showed no increase in γH2AX compared to DMSO controls. While olaparib treated OVCAR8 PARP1 KO G1 and G3 cells showed a 1.3 times (ANOVA, p-value < 0.01 and < 0.001) increase in γH2AX from DMSO controls. This was in contrast to olaparib treated UWB1.289 and OVCAR8 cells that showed a 2.6 times (ANOVA, p-value < 0.0001) and 2.2 times (ANOVA, p-value < 0.0001) increase in γH2AX from DMSO controls. E) Cell viability assays showed that PARP1 KO cells were equally resistant to olaparib compared to BRCA1 restored cells and all clinical PARP inhibitors required PARP-1 for maximum efficacy. Loss of PARP1 caused the greatest change in efficacy for niraparib and talazoparib. Cisplatin sensitivity was used as a positive control and remained unchanged after loss of PARP1. All in vitro experiments were completed three independent times.

* Cell lines shown in figure 1 from left to right are: UWB1.289, UWB1.289 BRCA1 restored, UWB1.289 PARP1 KO G1, UWB1.289 PARP1 KO G2, UWB1.289 PARP1 KO G3, OVCAR8, OVCAR8 PARP1 KO G1, OVCAR8 PARP1 KO G2, and OVCAR8 PARP1 KO G3.

- BRCA1 mutant
+ BRCA1 restored
Figure 2: *In vivo* $[^{18}\text{F}]{\text{FTT}}$ microPET imaging and ex vivo autoradiography of two ovarian cancer patient derived xenografts. **A)** Tumor bearing mice underwent microPET imaging with $[^{18}\text{F}]{\text{FTT}}$ before (top images) and after (bottom images) olaparib treatment. **B)** Ex-vivo autoradiographs of tumor and muscle from olaparib untreated (-) vs. treated (+) mice. **C)** Significant differences were observed in the tumor:muscle ratios calculated before and after olaparib treatment from microPET images, 4.2 ± 0.32 vs. 2.5 ± 0.11 (n=4, parametric paired t-test, p-value <0.0025, n=4). **D)** Ex-vivo autoradiographs of olaparib untreated (-) vs. treated (+) mice (2b) also showed a statistically significant difference between groups 5.14 ± 0.13 vs. 2.41 ± 0.18 (n=2, 10 sections/tumor, parametric unpaired t-test, p-value <0.0001).
**Figure 3:** Diagram overview and flow chart of the pilot clinical trial of \(^{18}\text{F}\)FTT PET/CT imaging in ovarian cancer.

*10 patients that were enrolled and underwent PET imaging were excluded from this sub-analysis due to lack of clinical tissue sample available for correlative studies.*
**Figure 4:** Immunohistochemistry and autoradiography analysis on clinical tissue. Distance bars represent 275 µM. **A)** HE, PARP-1, and [\(^{125}\)I]KX-1 autoradiograph on adjacent tissue section showed co-localization between [\(^{125}\)I]KX1 and PARP-1 c-IHC. **B)** *In vitro* autoradiography showed a difference in PARP-1 expression that was also confirmed by PARP-1 f-IHC. Max intensity by autoradiograph was 0.28 vs. 0.20 µCi/mg and fluorescent intensity of PARP-1 from whole section f-IHC was 9.6 vs. 6.7 RFU.
**Figure 5:** Clinical $[^{18}\text{F}]$FTT and $[^{18}\text{F}]$FDG PET/CT images of ovarian cancer patient with vaginal cuff lesion. Minimal radiotracer in the urinary bladder with $[^{18}\text{F}]$FTT-PET allowed for clear visualization of the lesion (green arrow) with no interference despite some bowel uptake (yellow arrow on $[^{18}\text{F}]$FTT image). Note excreted radiotracer in the bladder on $[^{18}\text{F}]$FDG-PET (yellow arrow on $[^{18}\text{F}]$FDG-PET).
Figure 6: Patients 2 and 11 underwent $[^{18}\text{F}]$FTT PET/CT imaging within two weeks of completing 4 cycles of carboplatin and paclitaxel. Omental metastases in patient 2 showed higher uptake of $[^{18}\text{F}]$FTT than $[^{18}\text{F}]$FDG with maximum SUVs of A) 7.8 vs. 3.4, and B) 5.1 vs. 2.0. Patient 2 was platinum resistant and relapsed within 4 months of therapy. Patient 11 showed low uptake on both $[^{18}\text{F}]$FTT and $[^{18}\text{F}]$FDG with maximum SUVs of C) 2.4 vs. 3.7 and D) 2.3 vs. 2.9. Patient 11 received two additional cycles of chemotherapy and was platinum sensitive.
Figure 7: [$^{18}$F]FTT PET imaging of PARP-1 in ovarian cancer patients and tissue correlates. A) The spectrum of PARP-1 expression as determined by [$^{18}$F]FTT PET/CT imaging with maximum SUV's ranging from approximately 2 to 12. B) We found a positive correlation between PARP-1 immunofluorescence vs. [$^{18}$F]FTT PET or [$^{125}$I]KX1 autoradiography (linear regression, $r^2 = 0.60$, 0.79). No associations were observed between PARP-1 immunofluorescence, [$^{18}$F]FTT imaging, or [$^{125}$I]KX1 autoradiography, and [$^{18}$F]FDG.