Journal of Nuclear Medicine, published on February 12, 2021 as doi:10.2967/jnumed.120.243287

Molecular Imaging: PARP-1 and Beyond

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ABSTRACT

The genetic code to life is balanced on a string of DNA that is under constant metabolic and physical stress from environmental forces. Nearly all diseases have a genetic component caused by or resulting in DNA damage that alters biology to drive pathogenesis. Recent advancements in DNA repair biology have led to the development of imaging tools that target DNA damage response and repair proteins. Positron-emission tomography (PET) has been used for early detection of oncogenic processes and monitoring tumor response to chemotherapeutics that target the DNA repair machinery. In the field of precision medicine, imaging tools provide a unique opportunity for patient stratification by directly measuring drug target expression or monitoring therapy to identify early responders. This overview discusses the state-of-the-art on molecular imaging of DNA damage and repair from the past 5 years, with an emphasis on poly [adenosine diphosphate (ADP) ribose] polymerase-1 (PARP-1) as an imaging target and predictive biomarker of response to therapy.

INTRODUCTION

Underlying Biology

DNA molecules preserve and transmit genetic information. Defects in DNA structure and function when unrepaired, can lead to genomic instability and the emergence of disease (*1*). As such, it is imperative to develop molecular imaging technologies that can identify and monitor processes that are inherently involved in regulating DNA integrity to study its role in the pathogenesis of clinical disease. To maintain genomic stability, cells have evolved a number of mechanisms by which proteins can detect and repair damaged DNA. This response consists of (I) detection of damaged DNA (II) recruitment of DNA repair proteins and (III) DNA repair (*1*).

The Role of PARP-1 in DNA Repair and Disease

Poly [adenosine diphosphate (ADP) ribose] polymerase-1 (PARP-1) is a 116 kDa protein (*2*) consisting of three functional domains: DNA-binding domain, auto-modification domain, and catalytic domain (Fig. 1A). It is widely known for its role in the DNA damage response, where upon recognition and association with DNA damage, it catalyzes poly-ADP-ribose (PAR) formation for recruitment of DNA repair proteins to sites of DNA damage (*1*) (Fig. 1B). Besides

its role in DNA repair, PARP-1 activity has been implicated in (I) chromatin modification (II) transcription regulation and (III) RNA modulation (*3*).

In disease settings, extensive DNA damage can trigger PARP-1 hyperactivation leading to redox imbalances and the excess accumulation of the highly branched anionic PAR polymer, all of which promote the dysregulation of critical cell processes (*4*). While moderate genotoxic stimuli facilitate PARP-1 activation and DNA repair, increased PARP-1 activity and expression have been linked to a number of pathological states, with the most prominent being the overexpression of PARP-1 across a diverse set of carcinomas (*5*) – an observation that has led to the recognition of PARP-1 as an attractive imaging target in human malignancies (*5*). PARP-1 is also involved in modulating cancer cell immunogenicity and has been implicated in the regulation of inflammatory response mechanisms, including the modulation of disease-associated genes, such as chemokines, pro-inflammatory mediators, and metabolic factors (*6*). In fact, some of the key pathological features shared by inflammation-associated disorders, such as cancer, heart disease, and diabetes, involve the accumulation of reactive oxygen and nitrogen species resulting in mitochondrial dysfunction, extensive DNA damage, and activation of PARP-1 (Fig. 1C).

PARP Inhibitors

PARP inhibitors (PARPi) induce selective toxicity in homologous recombination (HR)-deficient cancers by disrupting DNA repair pathways. The mechanism of action of these inhibitors revolves around PARP-1 trapping on DNA which creates toxic lesions resulting in mitotic error and cell death (*7*) (Fig. 2A, B). The first oral PARPi, olaparib, received approval from the Food and Drug Administration (FDA) in 2014 as a fourth-line treatment for advanced ovarian cancer with deleterious germline BRCA1/2 mutation (Fig. 2C) (*7*). In 2017, Olaparib was approved for BRCA1/2 mutated platinum sensitive, recurrent, epithelial ovarian, fallopian, and peritoneal cancer. In 2018 it was approved for metastatic, HER-2 negative BRCA1/2 mutated breast cancer and in 2019 Olaparib was approved for BRCA1/2 mutated metastatic pancreatic cancer. Rucaparib was approved in 2016 as a third-line option for the treatment of patients with germline or somatic BRCA1/2-mutated ovarian cancer (Fig. 2D) (*7*). Niraparib was approved a year later (2017) as a maintenance therapy in adults with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who received partial or complete response to platinum-based therapy (Fig. 2E). Recently, Niraparib received additional approval for the treatment of HR-deficient advanced ovarian, fallopian tube, or primary peritoneal cancers following at least three prior chemotherapy regimens (*8,9*). Talazoparib was approved in 2018 for the treatment of germline BRCA1/2-mutated, HER2-negative locally advanced or metastatic breast cancer (Fig. 2F). Veliparib while not FDA-approved for cancer treatment is currently being investigated in multiple ongoing combination therapy clinical studies (Fig. 2G) (*7*). In addition, PARPi are also being investigated in combination with other chemotherapies and immune-checkpoint blockade therapies such as PD-1/PD-L1 and CTLA-4 inhibitors (*10*).

IMAGING PARP-1

PARP-1-targeted imaging tools have allowed for real-time quantification of PARP-1 expression or activity in living systems, along with assessment of target engagement and monitoring of treatment response to PARPi therapy. Specifically, positron emission tomography (PET) imaging of PARP-1 has been established as a successful imaging tool for the non-invasive assessment of physiological PARP-1 levels in patients (*5*). To date, the majority of PET tracers developed for molecular imaging of DNA damage and repair use PARP-1 as a surrogate target.

PARP-1-targeted radiotracers bind the catalytic domain of PARP-1 and compete with NAD^+ for binding in the active site of the enzyme (*5*). Although PARP radiotracers are pharmacological PARPi, they typically occupy less than 1% (estimation extrapolated from human dosimetry data, by converting to units of radioactivity/tissue and applying the average molar activity of ^{18}F-FTT used in clinical studies.) of total PARP enzymes in cells or tissue due to the low concentrations of radiotracers (sub-nanomolar). This tracer property minimizes pharmacological activity to levels that are inconsequential to physiological PARP-1 enzymatic activity.

Since recent reviews (*5,11,12*) have provided in-depth summaries on PARP-1 targeted radiotracers, along with detailed information on the pharmacokinetic properties of these tracers, we focus on PARP-1 tracers (Fig. 3) that are currently undergoing clinical evaluation as well as provide a brief overview on pre-clinical tracers from the past 5 years that target either PARP-1 or alternative DNA damage markers.

PARP-1 Tracers Undergoing Clinical Investigation

18F-FluorThanatrace (^{18}F-FTT) is the most studied PARP radiotracer. Encouraging pre-clinical data with ^{18}F-FTT, prompted clinical translation just two years after it was first reported (*13*). *In vivo* data obtained from microPET imaging studies showed specific uptake of ^{18}F-FTT in multiple mouse models of human breast cancer (*14*) and ovarian cancer patient derived xenografts (*15*). ^{18}F-FTT also showed high tumor uptake 60 min post-injection and reduced uptake following administration of a pharmacological blocking dose of the PARPi olaparib (*14-16*); validating PARP-1 specificity *in vivo* and establishing the role of ^{18}F-FTT for quantifying PARPi drug target engagement.

A landmark trial in ovarian cancer showed ^{18}F-FTT can quantify PARP-1 expression in tumors (*15*). Noteworthy, while radiotracer validation is critical to clinical implementation, these data also showed high inter-patient tumor, inter-tumor, and intra-tumor variability, indicating varying levels of PARP-1 expression (*15*). Strikingly, some patients had tumor standard uptake values (SUVs) that were at background (Fig. 4A) and the patient with highest tumor SUV observed was nearly 6 times greater, indicating higher PARP-1 drug target expression (Fig. 4B). A second landmark trial in breast cancer (*17*), showed differential uptake of ^{18}F-FTT in triple-negative and estrogen receptor positive breast cancer (Fig. 4C). Notably, patients with mutations in BRCA1, showed inter-patient heterogeneity (Fig. 4C) (*17*). Whether low tumor uptake of ^{18}F-FTT is associated with poor response to PARPi remains to be proven – although pre-clinical evidence suggests PARP-1 expression is critical for therapeutic response.

The first clinical trials to assess whether PARPi target engagement are predictive of drug response are currently underway in high-grade serous ovarian cancer patients currently receiving PARPi therapy (NCT02637934, NCT03846167). In these trials, patients are serially imaged, once before initiation of PARPi therapy and at a second time, roughly seven days from the first image after patients have reached steady state with the PARPi. Additionally, there are multiple active and ongoing clinical trials evaluating ^{18}F-FTT in a variety of other cancers, including: glioblastoma (NCT04221061), pancreatic (NCT03492164), prostate (NCT03334500), and suspected epithelial ovarian/ fallopian tube/peritoneal cancer (NCT03604315). Thus far, ^{18}F-FTT imaging in human subjects shows promise as a platform technology to quantify PARP-1 expression *in vivo,* which could be utilized to stratify patient response to PARPi therapy and detect the pharmacodynamics effects of PARPi treatment (*15,18*). As such, these ongoing studies will help determine the clinical utility of ^{18}F-FTT and may allow clinicians to monitor and predict treatment response in patients receiving PARPi therapies (*15*).

The second PARP radiotracer that recently completed a phase I clinical trial is ^{18}F-PARPi (*19*), an ^{18}F-labeled analogue of the FDA approved PARPi olaparib. Pre-clinical PET/CT imaging studies with ^{18}F-PARPi in mice bearing orthotopic brain tumors (U251 MG) and subcutaneous xenografts, indicated 2.2 ± 0.8 %ID/g and 1.82 ± 0.21 %ID/g tumor uptake of ^{18}F-PARPi respectively 2 h post-injection. Similarly, PET/MRI studies showed colocalization of ^{18}F-PARPi signal and the orthotopic tumor. In addition to brain tumors, ^{18}F-PARPi has been studied in mouse models of Non-Small Cell Lung Cancer in order to assess target engagement of olaparib and talazoparib (*20*), in oral cancer models to evaluate its efficacy at delineating tumor tissue (compared to ^{18}F-FDG) (*21,22*), and in B-Cell lymphoma to differentiate between malignant vs. inflamed lymph nodes (*23*). Currently, ^{18}F-PARPi is being investigated in two ongoing clinical trials (NCT03631017, NCT04173104) for imaging PARP-1 expression in head and neck cancer (*21*) as well as brain tumors. Encouraging results from the head and neck cancer phase I study reported that imaging with ^{18}F-PARPi is both feasible and safe (*21*). In addition, PARPi-FL, a fluorescent analogue of ^{18}F-PARPi (*24*) is being evaluated as a fluorescent dye for the detection of tongue and mouth cancer (NCT03085147).

Radiofluorinated Olaparib Analogues from the Past 5 Years

^{18}F-PARPi-FL (*25*) is a dual-modality imaging probe that functions as a fluorescent dye and PET tracer for imaging PARP-1 expression. Surface fluorescence imaging with ^{18}F-PARPi-FL showed the fluorescence capability of the imaging agent and confirmed PARP-1 selectivity; while small animal PET imaging studies on mice bearing xenografts of the human glioblastoma line U87 MG, showed increased tumor-to-muscle (4.0 ± 0.6 %ID/g) and tumor-to-brain uptake (12 ± 2.1) 1 h post-injection. Autoradiographic studies showed a decrease in ^{18}F-PARPi-FL signal 30 min post-treatment with olaparib in U87 MG tumors, when compared to muscle uptake. Thus, confirming its selectivity for PARP-1. However, brain-to-muscle ratio did not yield a significant difference in positive and blocked brains, suggesting that ^{18}F-PARPi-FL had poor brain penetrance.

^{18}F-20 is an olaparib derivative with low nanomolar affinity for PARP-1 in both cell-free and cell-based assays (*26*). In cells, IC_{50} values for ^{18}F-20 were similar to olaparib – with reported values of 1.3 nM and 2.0 nM in G7 and T98G human glioblastoma cells respectively. *Ex vivo* biodistribution studies with ^{18}F-20 in a subcutaneous glioblastoma (U87 MG-Luc2) xenograft murine model, indicated primary hepatobiliary clearance for the tracer (*26*). Biodistribution and PET-magnetic resonance (MR) imaging studies showed specific tumor uptake and retention – with a reported %ID/g of 1.9 ± 0.5 and 3.6 ± 0.5 at 30 min and 120 min, respectively. However, the tracer was reported to exhibit high skeletal uptake, with a reported %ID/g >8.5 – this effect was attributed to potential *in vivo* defluorination and accumulation of radiofluoride in bone tissue.

^{18}F-olaparib is an ^{18}F-labeled isotopolog of olaparib synthesized via a copper-mediated 18F-radiofluorination of aryl boronic esters (*27*). *In vitro* studies in pancreatic ductal adenocarcinoma cells (PSN-1, MIA PaCa-2, and Capan-1) showed increased uptake in cells expressing elevated levels of PARP-1. *In vivo* PET imaging studies in mice bearing PSN-1 xenografts, showed tumor-specific uptake, with a reported %ID/g of 3.2 ± 0.36. In addition, treatment with excess unlabeled olaparib blocked ^{18}F-olaparib uptake, thus confirming target selectivity for PARP-1.

Radioiodinated PARP-1 Tracers

^{131}I-I2-PARPi is a radioiodinated olaparib analog with an IC_{50} of 9 ± 2 nM and demonstrated specific tumor uptake in an orthotopic rodent model of glioblastoma – as determined via SPECT/CT and PET/CT (using a structural analog ^{124}I-I2-PARPi) and supported by *ex vivo* autoradiography on U251 MG brain tumors (*28*). Pre-injection with olaparib blocked ^{131}I-I2-PARPi brain uptake, indicating PARP-1 specificity. However, high deiodination was reported – with a tumor-to-thyroid ratio of 1.82 ± 0.25.

^{125}I-KX1 is an iodinated analog of ^{18}F-FTT that has been used to validate radiotracer uptake as a biomarker of PARP-1 expression by correlating radiotracer maximum binding potential (B_{max}) with PARP-1 expression levels (*29*). *In vitro* studies with ^{125}I-KX1 did not show specific binding in PARP-1 K/O MEF cells and no difference in specific binding was observed in PARP-2 K/O and wildtype MEF cell lines (*29*). Consequently, clinical imaging trials with ^{18}F-FTT have incorporated ^{125}I-KX1 autoradiography on tumor biopsies as a correlative biomarker of PARP-1 expression (*15*), thus highlighting the potential for this diagnostic-correlative biomarker assay pair.

^{125}I-KX-02-019 is an iodinated tracer that is structurally similar to ^{125}I-KX1 with a bicyclic (instead of a tricyclic) benzimidazole (*30*). Murine biodistribution studies with this imaging agent in mice bearing EMT-6 tumors showed measured tumor uptake of 1% ID/g 2 h post-injection (*30*). However, studies in PARP-1 KO and PARP-2 KO cells reported that ^{125}I-KX-02-019 had higher affinity for PARP-2.

ALTERNATIVE TARGETS

Additional DNA damage and repair proteins that have been targeted for molecular imaging include phosphorylated histone variant H2A (γH_2AX*)* and the ataxia-telangiectasia-and Rad3-related (ATR) kinase. γH_2AX accumulate in punctate foci on sites of DNA damage (*31,32*). While ATR kinase, functions as a sensor for DNA damage and plays a key role in activating checkpoint kinase 1 (CHK1), the latter is a serine/threonine kinase that modulates the S and G2 phases of the cell cycle following ATR activation (*33*).

ATR Targeted Radiotracers

^{18}F-ATRi (^{18}F-9) is a radioflourinated version of Ve-821, an ATR inhibitor, with a reported log*P* value of 1.6. Synthesis of this tracer involves a 3-step reaction mixture with a reported radiochemical yield of 30 ± 10% in 120 ± 15 min and a molar activity of 0.0259 MBq/mole. MicroPET imaging studies on mice bearing human glioblastoma (U251 MG) tumor xenografts (*33*) were performed to assess radiopharmaceutical uptake and target selectivity. Results from these studies show that ^{18}F-ATRi had minimal decrease in target-to-non-target ratio after blocking with Ve-821, thus suggesting that ^{18}F-ATRi has poor specificity which could limit its applications as a PET imaging tracer.

γH2AX-Targeted Immunoconjugates

Knight et al., developed an γH2AX-targeted immunoconjugate radiolabeled with Zirconium-89 (^{89}Zr) for *in vivo* PET imaging of γH2AX expression. In order to enhance nuclear localization, the radioconjugate was linked to a penetrating peptide sequence (GRKKRRQRRRPPQGYG) (^{89}Zr-anti-γH2AX-TAT). *In vitro* studies with ^{89}Zr-anti-γH2AX-TAT in human breast cancer cells (MDA-MB-468), showed increased specific signal following irradiation and subsequent DNA damage; specific signal was compared to non-irradiated cells or non-specific IgG controls (*31*). In addition, *in vivo* PET imaging studies in a mouse model of

pancreatic ductal adenocarcinoma showed improved monitoring of treatment response to chemotherapy with ^{89}Zr-anti-γH2AX-TAT when compared to ^{18}F-FDG (*32*). Altogether, these studies provide an alternative molecular imaging biomarker for monitoring the real-time response to DNA damaging agents in tumors.

^{18}F-SuPAR

^{18}F-labeled substrate-based PARP activity radiotracer (^{18}F-SuPAR) is an nicotinamide adenine dinucleotide (NAD) analog with modifications on adenosine C8 and N6 (*34*). *In vitro* and *in vivo* studies show that upon PARP-1 activation, ^{18}F-SuPAR is recognized by PARP-1 and incorporated into PAR chains during active polymerization. In addition, *in vivo* studies on murine models of human breast (MDA-MB-23) and cervical (HeLa) cancers show a time-dependent increase in the uptake and retention of approximately 7.4 MBq dose of ^{18}F-SuPAR in both tumor types following a single radiothererapy dose (5 or 10 Gy); with maximal radiotracer uptake observed at 24 h and 8 h for both MDA-MB-23 and HeLa tumors respectively. However, additional studies are needed to evaluate if non-target enzymes that also use NAD as a cofactor, affect the uptake kinetics of ^{18}F-SuPAR in tumor tissue.

FUTURE DIRECTIONS

Advancements in personalized medicine have led to improved treatment response and promising survival outcomes. Paradigm shifts in the development of chemotherapies have paved the way for the emergence of PARPi. Consequently, the therapeutic compounds that have emerged from this paradigm shift have been repurposed in the areas of molecular imaging to serve as templates for the development of targeted imaging agents. These platform technologies, which utilize the principles of personalized medicine, have evolved into invaluable tools that can be used to track treatment response and disease progression.

In addition, since PARP-1 activity regulates signaling pathways associated with inflammation, an argument can be made to support the development of radioligands for PET imaging of PARP-1 expression and activity in a number of non-oncological disease settings, including cardiovascular disease, diabetes, and neurological disorders. Beyond PARP-1, additional molecular imaging targets have also been identified which may offer an alternative strategy for evaluating DNA damage and cellular stress in tumors with the ultimate goal of identifying patients who would benefit most from chemotherapy and adjuvant treatment options such as radiotherapy.

DISCLOSURE

Robert H. Mach is the lead inventor on the international patents for 18F-FTT – licensed to Trevarx Biomedical, Inc. and co-founder of Trevarx Biomedical, Inc. No other potential conflicts of interest exist.

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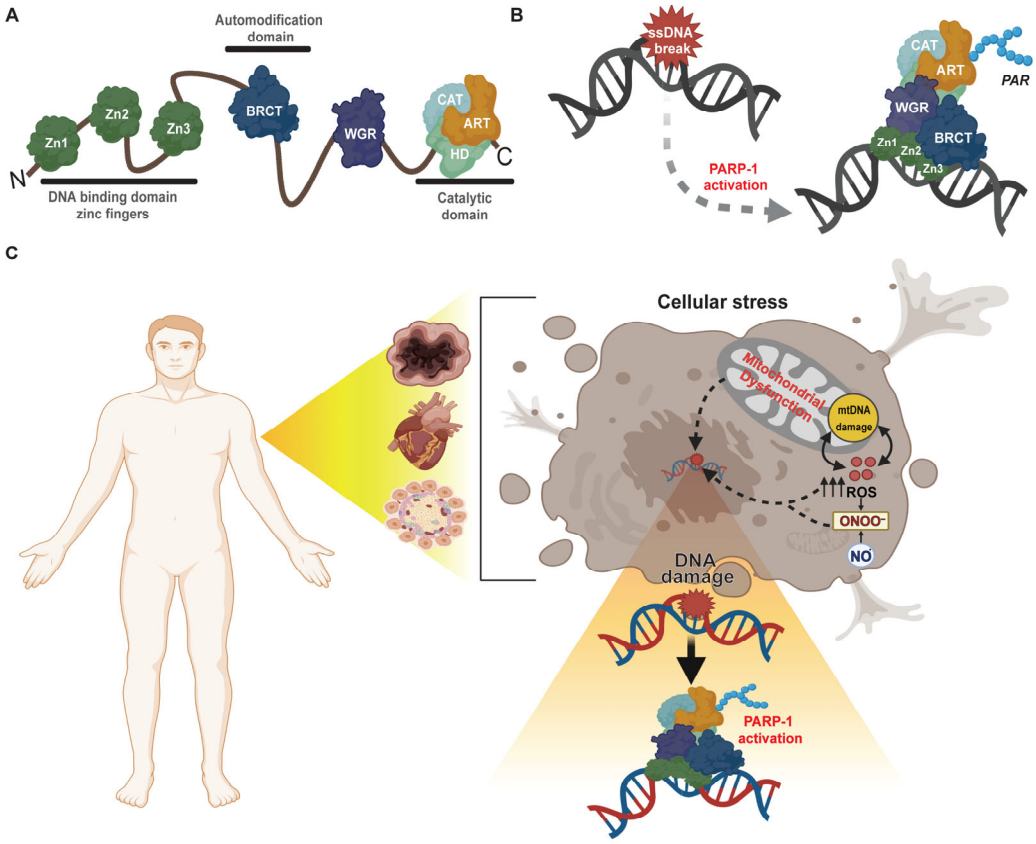

Figure 1. (A) Full length PARP-1 protein. (B) Mechanism of PARP-1 activation mediated by single-stranded DNA breaks. (C) Inflammation-associated diseases promote the accumulation of reactive oxygen species (ROS) and reactive nitrogen species i.e. peroxynitrite (ONOO-). Oxidative and nitrosative stress induce mitochondrial DNA (mtDNA) damage resulting in increased mitochondrial ROS production and mitochondrial dysfunction. The excess accumulation of these reactive species promotes extensive DNA damage and PARP-1 activation.

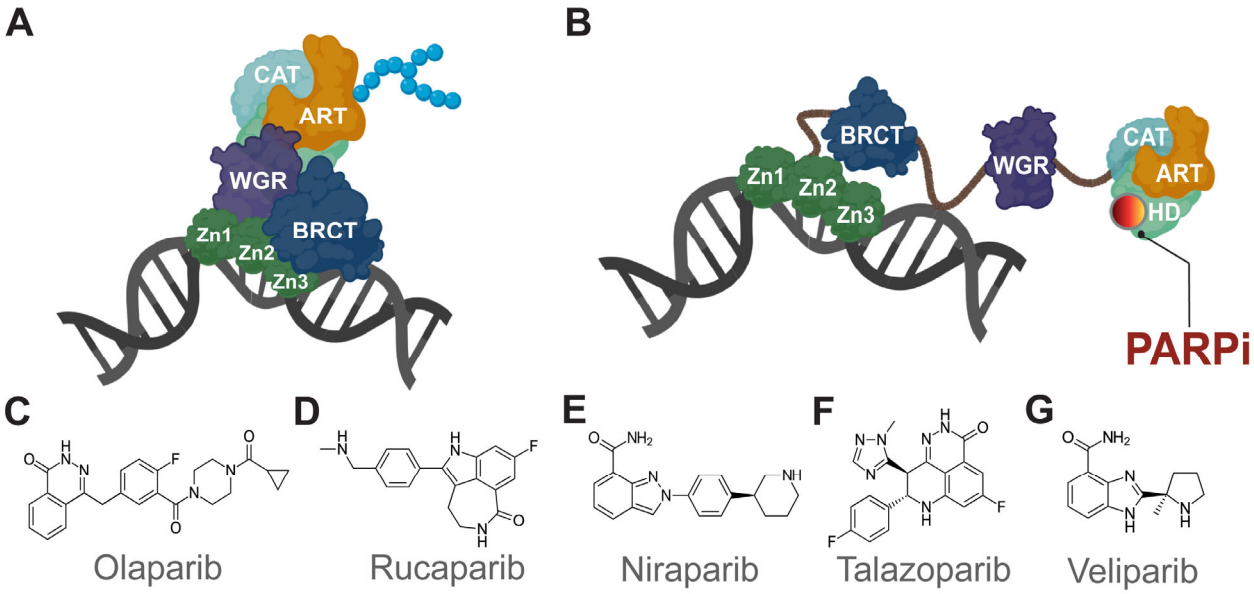

Figure 2. (A) Catalytically active PARP-1 enzyme. (B) PARPi-mediated PARP-1 trapping onto DNA. (C – G) Chemical structures of clinically relevant or approved PARPi.

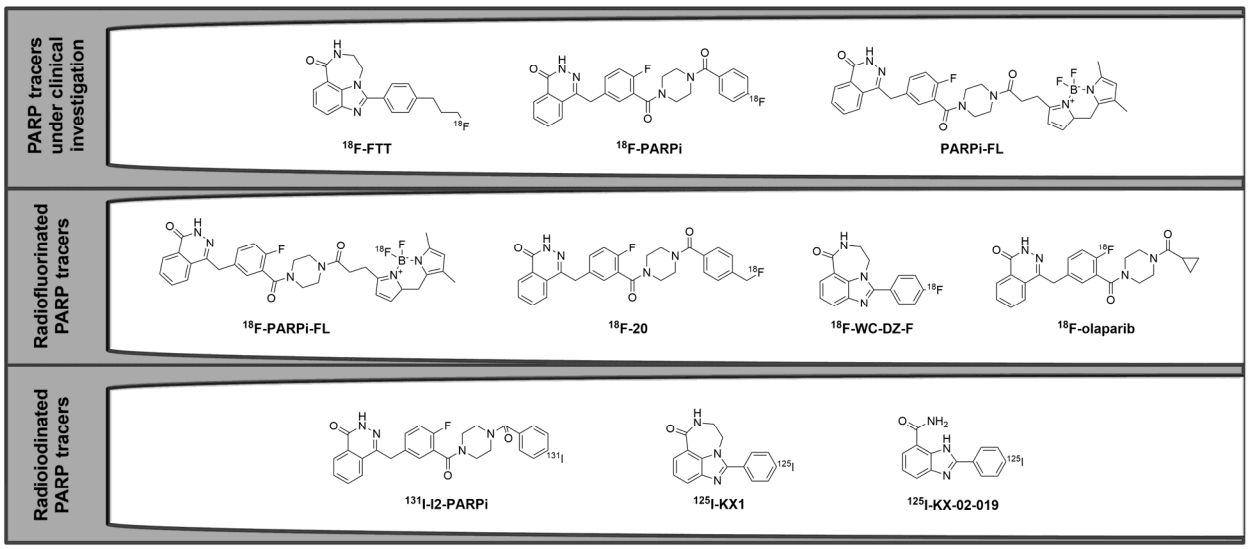

Figure 3. Radiolabeled PARP-1 imaging tracers under clinical (*top panel*) and preclinical investigation.

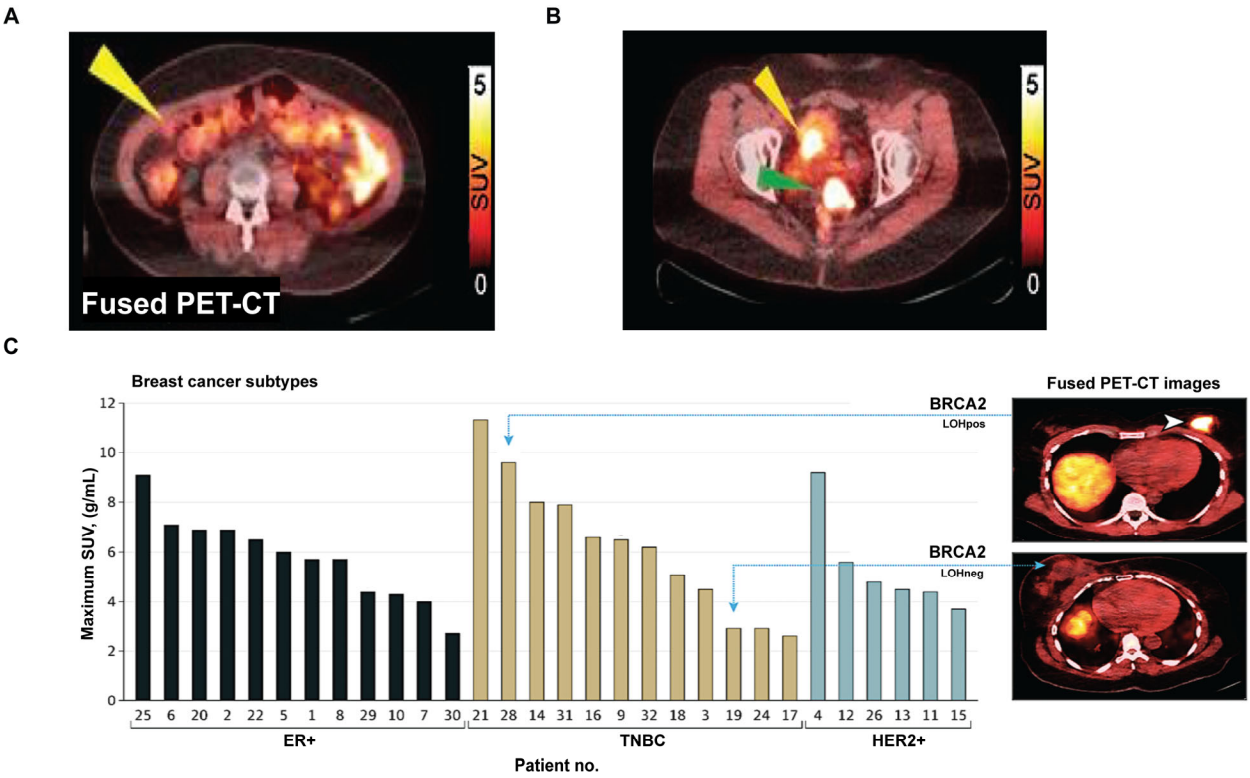

Figure 4. Representative ^{18}F-FTT PET/CT images of ovarian cancer patients with (A) background tumor SUV and (B) high tumor SUV (*15*). Green arrow indicates specific lesion uptake and yellow arrow indicates bowel uptake. (C) ^{18}F-FTT uptake compared across breast cancer subtypes and separated by receptor status. Representative ^{18}F-FTT PET-CT of patient 28 (*top*) and patient 19 (*bottom*), segregated by pathogenic variant carrier status (BRCA2), showing inter-patient tumor variability of PARP-1 expression (*17*).