Molecular Imaging: PARP-1 and Beyond

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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. PET has been used for early detection of oncogenic processes and monitoring of 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 ribose]polymerase-1 as an imaging target and predictive biomarker of response to therapy.

Key Words: molecular imaging; PET/CT; radiopharmaceuticals; DNA; PARP-1; imaging

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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 several mechanisms by which proteins can detect and repair damaged DNA. This response consists of detection of damaged DNA, recruitment of DNA repair proteins, and repair of DNA (*1*).

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THE ROLE OF PARP-1 IN DNA REPAIR AND DISEASE

Poly(adenosine diphosphate ribose)polymerase-1 (PARP-1) is a 116-kDa protein (*2*) consisting of 3 functional domains: DNA-binding domain, automodification domain, and catalytic domain (Fig. 1A). PARP-1 is widely known for its role in the DNA damage response; on recognition and association with DNA damage, it catalyzes poly(adenosine diphosphate-ribose) formation for recruitment of DNA repair proteins to sites of DNA damage (Fig. 1B) (*1*). Besides its role in DNA repair, PARP-1 activity has been implicated in chromatin modification, transcription regulation, and RNA modulation (*3*).

In disease settings, extensive DNA damage can trigger PARP-1 hyperactivation, leading to redox imbalances and excess accumulation of the highly branched anionic poly(adenosine diphosphate-ribose) polymer, all of which promote the dysregulation of critical cell processes (*4*). Although moderate genotoxic stimuli facilitate PARP-1 activation and DNA repair, increased PARP-1 activity and expression have been linked to several pathologic states, with the most prominent being the overexpression of PARP-1 across a diverse set of carcinomas (*5*)—an observation that has led to 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, proinflammatory mediators, and metabolic factors (*6*). In fact, some of the key pathologic 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 (PARPIs) induce selective toxicity in homologous recombination–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 (Figs. 2A and 2B) (*7*). The first oral PARPI, olaparib, received approval from the Food and Drug Administration in 2014 as a fourth-line treatment for advanced

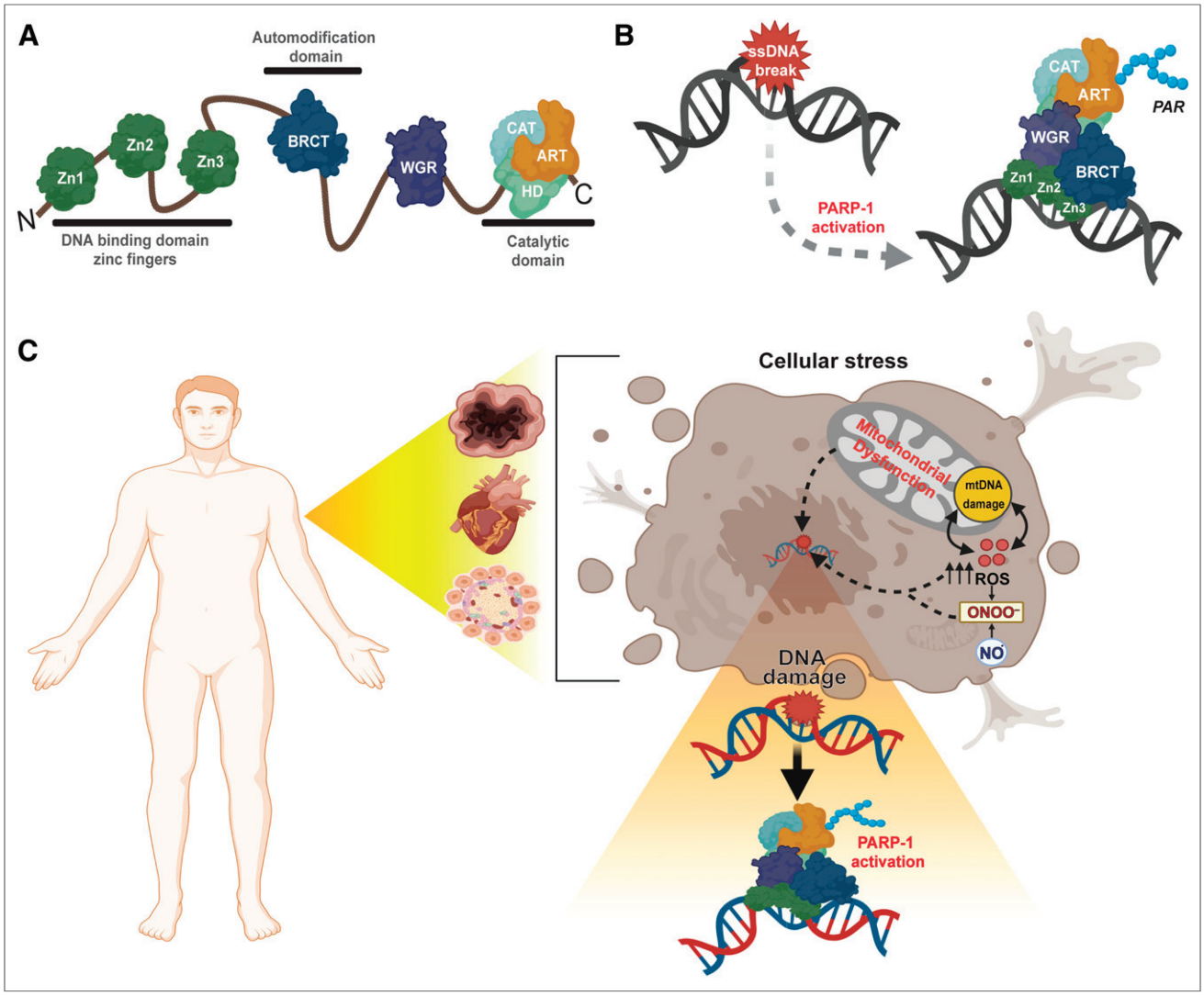

FIGURE 1. (A) Full-length PARP-1 protein. (B) Mechanism of PARP-1 activation mediated by single-stranded DNA (ssDNA) breaks. (C) Inflammation-associated diseases promote accumulation of reactive oxygen species (ROS) and reactive nitrogen species—such as, peroxynitrite (ONOO−). Oxidative and nitrosative stress induce mitochondrial DNA (mtDNA) damage resulting in increased mitochondrial ROS production and mitochondrial dysfunction. Excess accumulation of these reactive species promotes extensive DNA damage and PARP-1 activation.

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 human epidermal growth factor receptor 2–negative BRCA1/2-mutated breast cancer, and in 2019 it 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 had a partial or complete response to platinum-based therapy (Fig. 2E). Recently, niraparib received additional approval for the treatment of homologous recombination–deficient advanced ovarian, fallopian tube, or primary peritoneal cancers after at least 3 prior chemotherapy regimens (*8,9*). Talazoparib was approved in 2018 for the treatment of germline BRCA1/2-mutated human epidermal growth factor receptor 2–negative locally advanced or metastatic breast cancer (Fig. 2F). Veliparib, although not Food and Drug Administration–approved for cancer treatment, is currently being investigated in multiple ongoing combination-therapy clinical studies (Fig. 2G) (*7*). In addition, PARPIs are also being investigated in combination with other chemotherapies and immune-checkpoint blockade therapies such as programmed cell death protein 1/programmed cell death ligand 1 and cytotoxic T-lymphocyte–associated protein 4 inhibitors (*10*).

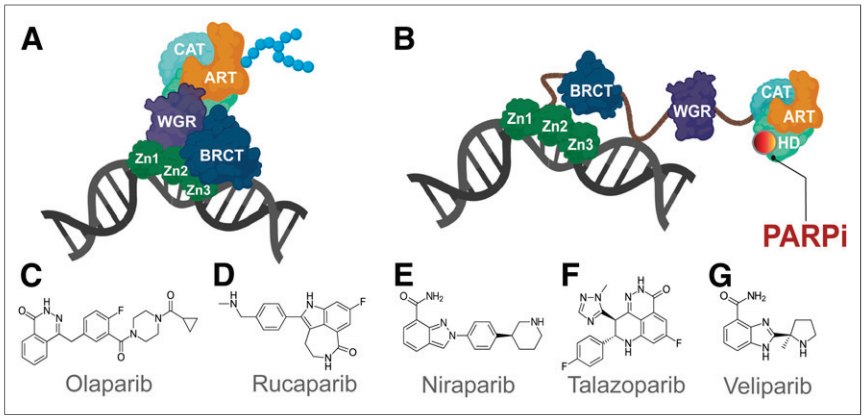

FIGURE 2. (A) Catalytically active PARP-1 enzyme. (B) PARPI-mediated PARP-1 trapping onto DNA. (C–G) Chemical structures of clinically relevant or approved PARPIs.

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, PET imaging of PARP-1 has been established as a successful imaging tool for the noninvasive assessment of physiologic PARP-1 levels in patients (*5*). To date, most 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 nicotinamide adenine dinucleotide for binding in the active site of the enzyme (*5*). Although PARP radiotracers are pharmacologic PARPIs, they typically occupy less than 1% (estimation extrapolated from human dosimetry data, by converting to units of radioactivity per tissue and applying the average molar activity of ^{18}F-fluorthanatrace [^{18}F-FTT] used in clinical studies) of total PARP enzymes in cells or tissue because of the low concentrations of radiotracers (subnanomolar). This tracer property minimizes pharmacologic activity to levels that are inconsequential to physiologic PARP-1 enzymatic activity.

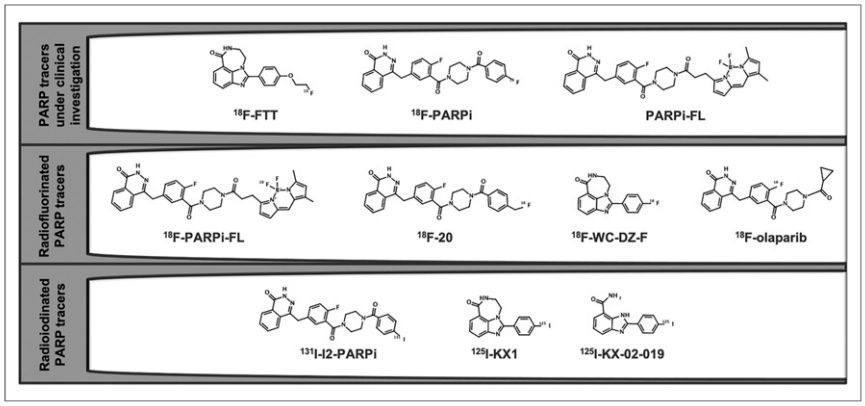

FIGURE 3. Radiolabeled PARP-1 imaging tracers under clinical and preclinical investigation.

Because 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. We also provide a brief overview on preclinical tracers from the past 5 years that target either PARP-1 or alternative DNA damage markers.

PARP-1 Tracers Undergoing Clinical Investigation

^{18}F-FTT is the most studied PARP radiotracer. Encouraging preclinical data with ^{18}F-FTT prompted clinical translation just 2 years after it was first reported (*13*). In vivo data obtained from small-animal PET 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 after injection and reduced uptake after administration of a pharmacologic 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 that ^{18}F-FTT can quantify PARP-1 expression in tumors (*15*). Noteworthy, although radiotracer validation is critical to clinical implementation, these data also showed high interpatient tumor, intertumor, and intratumor variability, indicating varying levels of PARP-1 expression (*15*). Strikingly, some patients had tumor SUVs that were at the background level (Fig. 4A), and the SUV in the patient with the highest observed tumor SUV was nearly 6 times greater, indicating higher PARP-1 drug target expression (Fig. 4B). A second landmark trial on 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 interpatient heterogeneity (Fig. 4C) (*17*). Whether low tumor uptake of ^{18}F-FTT is associated with a poor response to PARPI remains to be proven, although preclinical evidence suggests that PARP-1 expression is critical for a therapeutic response.

The first clinical trials to assess whether PARPI target engagement is predictive of drug response are currently under way in high-grade serous ovarian cancer patients currently receiving PARPI therapy (NCT02637934 and NCT03846167). In these trials, patients

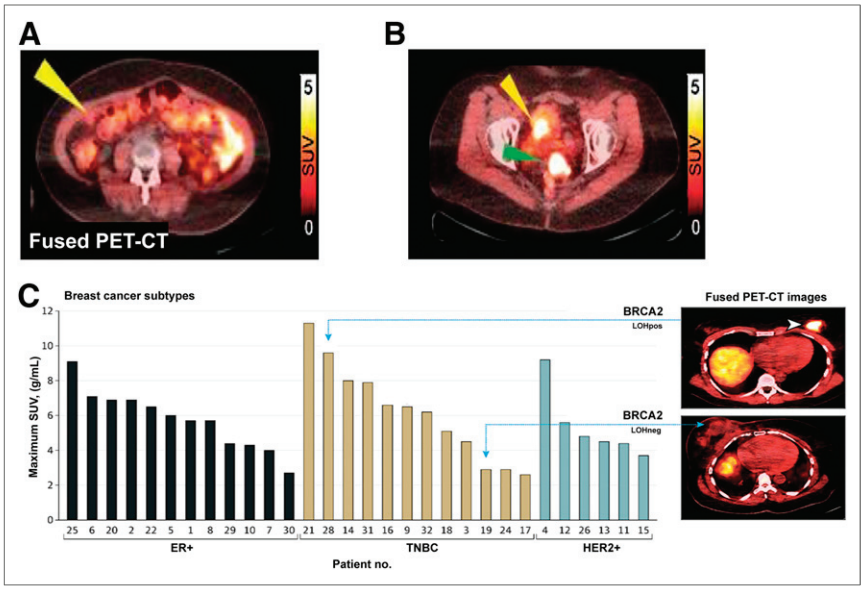

FIGURE 4. Representative ^{18}F-FTT PET/CT images of ovarian cancer patients with background tumor SUV (A) and high tumor SUV (B) (*15*). Green arrowhead indicates specific lesion uptake, and yellow arrowhead indicates bowel uptake. (C) ^{18}F-FTT uptake compared across breast cancer subtypes and separated by receptor status. Representative ^{18}F-FTT PET/CT images of patients 28 (top) and 19 (bottom), segregated by pathogenic variant carrier status (BRCA2), show interpatient tumor variability of PARP-1 expression (*17*). ER+ = estrogen receptor–positive; HER2+ = human epidermal growth factor receptor 2–positive; LOHneg = loss-of-heterozygosity–negative; LOHpos = loss-of-heterozygosity–positive; TNBC = triple-negative breast cancer.

are serially imaged, once before initiation of PARPI therapy and at a second time roughly 7 d later, after the 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 cancer (NCT03492164), prostate cancer (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 that might be used 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 analog of the Food and Drug Administration–approved PARPI olaparib. Preclinical PET/CT imaging studies on mice indicated that 2 h after injection of ^{18}F-PARPI, uptake in orthotopic brain tumors (U251 MG) and subcutaneous xenografts was 2.2 ± 0.8 percentage injected dose (%ID)/g and 1.82 ± 0.21 %ID/g, respectively. Similarly, PET/MRI studies showed colocalization of ^{18}F-PARPI signal and the orthotopic tumor. In addition to being studied in brain tumors, ^{18}F-PARPI has been studied in mouse models of non–small cell lung cancer to assess target engagement of olaparib and talazoparib (*20*), in oral cancer models to evaluate its efficacy at delineating tumor tissue (compared with ^{18}F-FDG) (*21,22*), and in B-cell lymphoma to differentiate between malignant and inflamed lymph nodes (*23*). Currently, ^{18}F-PARPI is being investigated in 2 ongoing clinical trials (NCT03631017 and NCT04173104) for imaging PARP-1 expression in head and neck cancer (*21*) and in brain tumors. Encouraging results from the head and neck cancer phase I study indicated that imaging with ^{18}F-PARPI is both feasible and safe (*21*). In addition, PARPI-FL, a fluorescent analog of ^{18}F-PARPI (*24*), is being evaluated as a fluorescent dye for the detection of tongue and mouth cancer (NCT03085147).

Radiofluorinated Olaparib Analogs 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, and small-animal PET imaging studies on mice bearing xenografts of the human glioblastoma line U87 MG showed increased tumor-to-muscle uptake (4.0 ± 0.6 %ID/g) and tumor-to-brain uptake (12 ± 2.1) 1 h after injection. Autoradiographic studies showed a decrease in ^{18}F-PARPI-FL signal 30 min after treatment with olaparib in U87 MG tumors, when compared with muscle uptake, thus confirming its selectivity for PARP-1. However, the brain-to-muscle ratio did not yield a significant difference between 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, half-maximal inhibitory concentrations for ^{18}F-20 were similar to those for olaparib, with reported values of 1.3 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/MRI studies showed specific tumor uptake and retention, with a reported %ID/g of 1.9 ± 0.5 and 3.6 ± 0.5 at 30 and 120 min, respectively. However, the tracer showed high skeletal uptake, with a reported %ID/g of more than 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 ^{18}F-radiofluorination of aryl boronic esters (*27*). In vitro studies on 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 on 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 a half-maximal inhibitory concentration of 9 ± 2 nM and with 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*). Preinjection 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 with PARP-1 expression levels (*29*). In vitro studies with ^{125}I-KX1 did not show specific binding in PARP-1 knockout MEF cells, and no difference in specific binding was observed in PARP-2 knockout and wild-type MEF cell lines (*29*). Consequently, clinical imaging trials with ^{18}F-FTT have incorporated ^{125}I-KX1 autoradiography into 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*). Biodistribution studies with this imaging agent in mice bearing EMT-6 tumors showed measured tumor uptake of 1 %ID/g 2 h after injection (*30*). However, studies on PARP-1 knockout and PARP-2 knockout 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 accumulates in punctate foci on sites of DNA damage (*31,32*). Although ATR kinase functions as a sensor for DNA damage and plays a key role in activating checkpoint kinase 1, the latter is a serine/threonine kinase that modulates the S and G2 phases of the cell cycle after ATR activation (*33*).

^{18}F-ATR–Targeted Radiotracers

^{18}F-ATR inhibitor is a radioflourinated version of Ve-821 (3-amino-6-[4-(methlsulfonyl)phenyl)-*N*-phenyl-2-pyrazinecarboxamide) 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. Small-animal PET imaging studies on mice bearing human glioblastoma (U251 MG) tumor xenografts (*33*) were performed to assess radiopharmaceutical uptake and target selectivity. These studies showed that ^{18}F-ATR inhibitor had a minimal decrease in target-to-nontarget ratio after blocking with Ve-821, thus suggesting that ^{18}F-ATR inhibitor has poor specificity, which could limit its applications as a PET imaging tracer.

γH2AX-Targeted Immunoconjugates

Knight et al. developed a γH2AX-targeted immunoconjugate radiolabeled with ^{89}Zr for in vivo PET imaging of γH2AX expression. 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 on human breast cancer cells (MDA-MB-468) showed increased specific signal after irradiation and subsequent DNA damage; specific signal was compared with nonirradiated cells or nonspecific IgG controls (*31*). In addition, in vivo PET imaging studies on a mouse model of pancreatic ductal adenocarcinoma showed improved monitoring of treatment response to chemotherapy with ^{89}Zr-anti-γH2AX-TAT when compared with ^{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 a nicotinamide adenine dinucleotide analog with modifications on adenosine C8 and N6 (*34*). In vitro and in vivo studies show that on PARP-1 activation, ^{18}F-SuPAR is recognized by PARP-1 and incorporated into poly(adenosine diphosphate-ribose) chains during active polymerization. In addition, in vivo studies on murine models of human breast cancer (MDA-MB-23) and cervical cancer (HeLa) show a time-dependent increase in the uptake and retention of an approximately 7.4-MBq dose of ^{18}F-SuPAR in both tumor types after a single radiotherapy dose (5 or 10 Gy), with maximal radiotracer uptake observed at 24 and 8 h for MDA-MB-23 and HeLa tumors, respectively. However, additional studies are needed to evaluate whether nontarget enzymes that also use nicotinamide adenine dinucleotide 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 PARPIs. 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 use the principles of personalized medicine, have

evolved into invaluable tools that can be used to track treatment response and disease progression.

In addition, because PARP-1 activity regulates signaling pathways associated with inflammation, an argument can be made in support of the development of radioligands for PET imaging of PARP-1 expression and activity in several nononcologic disease settings, including cardiovascular disease, diabetes, and neurologic disorders. Beyond PARP-1, additional molecular imaging targets have also been identified that 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 ^{18}F-FTT (licensed to Trevarx Biomedical, Inc.) and a cofounder of Trevarx Biomedical, Inc. No other potential conflict of interest relevant to this article was reported.

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