

## **PARP Inhibitors in Cancer Diagnosis and Therapy**

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## Abbreviations

ATK	Ataxia Telangiectasia and Rad3-related
ATM	Ataxia Telangiectasia Mutated
BCL2	B-Cell Lymphoma 2
CI	Confidence Interval
DLBCL	Diffuse large B cell lymphomas
HER2	Human epidermal growth factor receptor 2
LMO2	LIM Domain only 2
MYCN	MYC oncogene (neuroblastoma)
PIN1	Peptidylprolyl cis/trans Isomerase, NIMA-Interacting 1
PTEN	Phosphatase and tensin homolog
R-CHOP	Rituximab-Cyclophosphamide-Hydroxydaunorubicin-Oncovin-Prednisone

## Abstract

Targeting of poly(ADP-ribose)polymerase (PARP) enzymes has emerged as an effective therapeutic strategy to selectively target cancer cells with deficiencies in homologous recombination (HR) signaling. Currently used to treat *BRCA*-mutated cancers, PARP inhibitors (PARPi) have demonstrated improved outcome in various cancer types as single agents. Ongoing efforts have seen the exploitation of PARPi combination therapies, boosting patient responses as a result of drug synergisms. Despite great successes using PARPi therapy, selecting those patients who will benefit from single agent or combination therapy remains one of the major challenges. Numerous reports have demonstrated that the presence of a *BRCA* mutation does not always result in synthetic lethality with PARPi therapy in treatment-naïve tumors. Cancer cells can also develop resistance to PARPi therapy. Hence, combination therapy may significantly affect the treatment outcomes. In this review, we discuss the development and utilization of PARPi in different cancer types from preclinical models to clinical trials, provide a current overview of the potential uses of PARP imaging agents in cancer therapy, and discuss the use of radiolabeled PARPi as radionuclide therapies.

## Introduction

Targeting DNA damage repair (DDR) signaling is a fast-expanding field for cancer therapy. DNA damage causes genomic instability in cells that require DDR for rescue. DDR signaling triggers activation of repair protein transcription, hence leading to overexpression of the related repair protein and activation of mechanisms for DNA repair (1). Poly(ADP-ribose)polymerase (PARP) is an essential and abundant DNA repair protein, which plays an important role in regulation of various DDR pathways, including Base Excision Repair (BER), Homologous Recombination (HR), classical and alternative Non-Homologous End Joining (NHEJ), Nucleotide Excision Repair (NER), microhomology-mediated end-joining (MMEJ), maintenance of replication fork stability, and Mismatch Repair (MMR) (2). In addition, PARP has been found overexpressed in various types of cancers, such as breast, ovarian and oral cancers than their normal surrounding healthy tissues, making inhibition of PARP activity a promising strategy for cancer therapeutics by disrupting PARP functions, thereby impairing DDR pathways of cancer cells. PARP inhibition for the treatment of breast or ovarian cancers with *BRCA1/2* mutations (deficiencies in the HR) is now a well-established approach. A PARP inhibitor (PARPi) is utilized to impair the process of single-strand break (SSB) repair, thereby converting SSB into double-strand breaks (DSBs) by stalling replication fork (1) or accelerating fork elongation (3), which requiring HR for repair during S-phase (4). Therefore, if HR is defective for example, resulting from mutations in HR genes such as *BRCA1* and 2, DSBs cannot be repaired, and the cell dies, a concept known as synthetic lethality. Over the past decade, four PARPi (olaparib, rucaparib, niraparib, talazoparib) have been FDA-approved for clinical use as single agents, with veliparib expected to receive approval following promising phase 3 trial results (BROCADE3; NCT02163694). The ability

of PARPi to impair DDR processes led to their employment in combination with chemo- and radiotherapy. Increasing attention is also being drawn to radiolabeled versions of PARPi that possibly allow direct visualization of PARP expression, quantification of PARPi biodistribution and tumor delivery, as well as target engagement, thereby potentially improve stratification of patients and enable the monitoring of treatment progress. Additionally, radiolabeled PARPi-based radionuclide therapy agents are being proposed as therapies for PARP-expressing cancers. Here, we present an up-to-date understanding of PARP in DDR, the use of PARP inhibitors as mono- or combination therapies for cancer, and the potential of radiolabeled PARPi in clinical applications.

### **PARP Regulation and Activation in Response to DNA Damage**

PARP enzymes form a 17-member group of mostly nuclear enzymes that share a conserved catalytic domain homologous to ADP-ribosyl-transferases diphtheria toxin (ARTD), which gives the PARP enzyme family its alternative name of ARTD-like enzymes (5). Upon DNA damage, members of the PARP family bind the DNA lesion, become catalytically active, and mediate ADP-ribosylation using  $\text{NAD}^+$  as substrate, leading to rapid recruitment of other DNA repair factors (6). PARP-1, PARP-2 and tankyrase (PARP-5A/5B) produce poly-ADP-ribose (PAR) modifications on target proteins, while other PARPs are responsible for generating mono-ADP-ribose (MAR) and have potential cancer-related functions (7). The evidence to date suggests that only PARP-1, PARP-2 and PARP-3 are activated by DNA breaks and catalyze ADP-ribosylation after DNA damage, with PARP-1 responsible for 80-90% of global PAR synthesis in response to DNA damaging agents (6). Apart from SSB recognition, recent studies showed that PARP-1 can also detect

DSB rapidly, and plays an essential role in regulation of DNA end resection for DSB repair, with recruitment and activation of PARP-1 occurring within 0.1  $\mu$ s after induction of DSBs (6).

PARP-1 is the most studied of the PARP enzyme family by far. Its structure comprises of three zinc finger domains for DNA binding, a BRCA-C-terminus domain (BRCT), a WGR (Trp-Gly-Arg) domain that contributes to but is not required for DNA interaction and catalytic activity regulation in response to DNA damage, and a catalytic domain consisting of a helical subdomain (HD) adjacent to a conserved ADP-ribosyl transferase fold (ART) (8). Compared to PARP-1 (N-terminal region: > 500 residues), PARP-2 and PARP-3 have more compact structures, with shorter N-terminal extensions (70 and 40 residues, respectively) from the WGR domain (Fig. 1A) (9). PARP-1 requires the zinc finger domains for DNA break detection and binding (10), but PARP-2 and PARP-3 rely on their WGR domain for this function (2,11).

Despite differences in DNA damage detection, PARP-1, PARP-2 and PARP-3 share allosteric regulatory activation mechanisms, where binding to damaged DNA leads to an increase of PARylation activity (12). Langelier et al. demonstrated that the HD domain in PARP-1 can selectively and reversibly block or allow access of NAD<sup>+</sup> or the NAD analogue BAD upon DNA binding (13). The HD domain, in its native state, was found primarily in a folded conformation, sterically blocking the catalytic binding site. Upon DNA damage detection, the binding of NAD<sup>+</sup> to the catalytic active site stabilizes the HD domain in its unfolded conformation and strengthens the interaction between the regulatory domains and DNA (Fig. 1B). This reversible blockage of the NAD<sup>+</sup> binding site provides control of NAD<sup>+</sup> access to the catalytic site, regulating the PARylation process.

## PARP Trapping-Induced Cytotoxicity

Clinical PARP inhibitors compete for binding in the NAD<sup>+</sup> binding pocket of the PARP enzymes, and are mostly selective for PARP-1 and PARP-2. However, different PARP inhibitors display markedly different levels of cytotoxicity, attributed to the ability of PARP inhibitors to prevent auto-PARylation of PARP, thereby preventing its dissociation from the broken DNA, thus stabilizing and trapping PARP on the DNA (14). This trapping leads to DSB formation by stalling and collapsing replication forks (15) or accelerating fork elongation (3). Hence, different degrees of trapping (talazoparib > niraparib ≥ olaparib = rucaparib > veliparib (16,17)) lead to large differences in cellular toxicity. Although the trapping abilities of PARPi are dependent on catalytic inhibition and the resulting dissociation rates of PARPi (17,18), reverse allostery effects also play a significant role. Molecular impacts of PARPi binding to PARP-1 corroborated by molecular dynamics simulation, hydrogen-exchange mass spectrometry (HXMS) and X-ray crystallography implied that different PARPi induce diverse degrees of structural deviations in the regulatory domains, thereby positively or negatively affecting the retention of PARP-1 trapped at the DNA damage site (19,20). Three types of PARPi were discerned based on their allosteric impacts on the PARP-1-DNA complex (19): Type I, allosteric pro-retention on DNA, which induces HD conformational changes that reinforce contacts with the DNA break and result in slow release; Type II, with no or mild allosteric effects; and Type III, allosteric pro-release from DNA, which induce HD conformational changes opposite to type I inhibitors (Fig. 1C). These results have illuminated that the *PARP-1* trapping induced by some PARPi, such as type II PARPi (talazoparib and olaparib) and type III PARPi (rucaparib and niraparib), does not rely on the allosteric effect,

indicating that other factors may contribute to the trapping ability of these PARPi, and therefore further investigation is warranted.

## **PARP Inhibitors in Cancer Therapy – Preclinical and Clinical Development**

### **PARPi as Monotherapy**

The efficacy of PARPi monotherapy in HR deficient (HRD) cancers carrying *BRCA1/2* mutations was first demonstrated in cancer cell lines, and later confirmed in patient-derived xenograft (PDX) models. Treatment with olaparib resulted in significant tumor graft regression (21,22) in PDX tumors of *BRCA1*-mutant triple negative breast cancer (TNBC). Other alterations, such as absence of *PTEN* or *ATM* also cause sensitivity of TNBC (23) and small-cell lung cancer (SCLC) (24) to talazoparib treatment *in vivo*, confirming the effectiveness of PARPi beyond germline *BRCA1/2* mutation cancers.

A wealth of clinical data supports the use of PARPi for maintenance treatment in platinum-sensitive high-grade serious ovarian cancer (HGSOC) patients. In the randomized, placebo-controlled, double blind phase 3 SOLO2 trial, patients with relapsed epithelial ovarian, fallopian tube or primary peritoneal cancer carrying a *BRCA1/2* mutation demonstrated a significantly longer median progression-free survival (PFS) under olaparib maintenance treatment than those receiving placebo (19.1 versus 5.5 months, 95% CI 16.3–25.7 vs 5.2–5.8, HR 0.3), without significant detrimental effects on health-related quality of life (HRQoL) (25,26).

The antitumor activity of olaparib was highlighted in the recent phase 3 SOLO3 (NCT02282020) and OlympiAD (27) trials, in germline *BRCA*-mutated ovarian cancer and HER2-negative breast cancer, respectively. Similarly, the ABRAZO study (28), and the latest EMBRACA trial, where patients underwent



talazoparib monotherapy, showed a significant PFS advantage and higher objective response rate (ORR) over standard chemotherapy (8.6 versus 5.6 months, 62.6% versus 27.2%, respectively) with greater HRQoL in favor of talazoparib (29). Several recently reported trials including POLO and PROfound also demonstrated the benefits of PARPi (olaparib or rucaparib) over placebo or androgen receptor (AR)-directed therapy, respectively, in metastatic pancreatic ductal adenocarcinoma (PDAC) patients harboring germline *BRCA* mutation (30) and castration-resistant prostate cancer (mCRPC) patients with confirmed HRD phenotype (31). Ongoing efforts to expand the use of PARPi to a wider population of cancer patients in different settings beyond *BRCA* mutation will undoubtedly expand the potential use of PARPi as monotherapy, and several studies are currently underway (NCT03402841, NCT03286842, NCT02000622).

### **Combination Therapy with PARP Inhibitors**

Heterogeneous responses to single-agent PARPi are frequently observed in both preclinical models and clinical practice, even in *BRCA*-mutated tumors, associated with complex intrinsic or acquired resistance. The mechanisms of PARPi resistance are well-described in several recent reviews, with the majority related to restoration of HR capability (32). Rational combination treatment regimens thus become the next strategy to improve patient outcome e.g. using PARPi with chemotherapy, radiation, targeted drugs or immunotherapy. The choice of partner must be based on the fundamental insights into the molecular pathways that drive tumorigenesis and therapy response. Strategies to optimize the use of PARPi as chemopotentiators or radiosensitizers are being actively pursued to overcome or prevent the development of treatment resistance in patients.

One approach is the combination of genotoxic agents and PARPi, where chemotherapy-induced DNA damage can increase the cellular reliance on DNA repair and hence sensitize tumors to PARPi, such as in (33). Combination of PARPi and ionizing radiation (IR) is another effective strategy to sensitize tumors to radiotherapy, without overt signs of toxicity at least in mice, warranting further its evaluation in ongoing clinical trials (34), despite an early recognition of the limitation of this combinatorial approach in cells (35). Given the relationship between PARP and HR repair (HRR) regulatory pathways, it is not surprising to see the potential benefits of combination treatments between PARPi and DDR signaling-targeting or otherwise genotoxic therapies. The increased reliance of cancer cells on alternative or specific DRR pathways provides an opportunity to pharmacologically induce HR deficiency, which in combination with PARPi achieves synergistic response. Disruption of for example, checkpoint kinase 1 (CHK1) activity with PARP inhibition leads to the accumulation of DSB and induces apoptosis following treatment of PDX mouse models using olaparib/MK8776 (36) or olaparib/prexasertib (37) combinations. Tretinoin, used to treat acute promyelocytic leukemia patients, also acts as an effective HR disrupter and is capable of sensitizing *BRCA*-proficient TNBC to olaparib (38). Other studies involved the use of cyclic dependent kinase (CDK) inhibitors to disrupt DNA damage checkpoint signaling and prevent DNA repair in neuroendocrine prostate cancer (NEPC) (39) and TNBC PDX models (40).

Inhibition of bromodomain-containing protein (BRD4) disrupts DDR signaling by reducing DNA repair proteins levels, thereby sensitizing cancer cells to olaparib in preclinical models (41,42). Treatment of mCRPC PDXs with an androgen receptor (AR) inhibitor enzalutamide induces downregulation of AR-regulated HRR gene expression and sensitizes to PARPi (43). An overview is presented in Table 1.

Promising efficacy of PARPi with genotoxic agents was observed in preclinical models and successfully translated in various cancer types: The phase 3 VELIA study demonstrated the efficacy of concurrent administration of veliparib and carboplatin with paclitaxel (V/CP) followed by veliparib maintenance in advanced HGSOC patients with and without germline *BRCA* mutation. A significant PFS advantage was observed in both *BRCA*-mutant (34.7 versus 22 months, HR 0.44), HR defects cohorts (31.9 versus 20.5 months), and the intention-to-treat population (23.5 versus 17.3 months, HR 0.68) receiving veliparib versus placebo with chemotherapy (44). Similar efficacy was observed in breast cancer patients in phase 2 and 3 studies (45,46).

Other clinical studies showed improved patient outcomes for treatment regimens involving PARPi combined with targeted drugs, such as anti-angiogenic and AR antagonists. The recent FDA approval of olaparib/bevacizumab combination therapy as a first-line maintenance treatment for HGSOC patients regardless of their HRD phenotypes based on the PAOLA-1 trial (47) will undoubtedly encourage additional studies to investigate other combination pairs (such as ICON9; NCT03278717, NCT02502266). For example, the use of olaparib/cediranib showed appreciable benefits in ovarian cancer patients (48). Other promising studies include the combination of abiraterone and olaparib in mCRPC patients, irrespective of HRR status (49). Combination of immune checkpoint inhibitors with PARPi is under active investigation, with only a limited amount of clinical data available to date. The MEDIOLA trial showed initial promise in a subset of ovarian cancer patients (platinum sensitive, germline *BRCA*-mutated) receiving this combination (50), but no improvement in ORR or PFS when a more heterogeneous patient population (without *BRCA* mutation) was considered (51). Notwithstanding, the preliminary outcomes

supported the tolerability of this combination in patients and better patient selections in subsequent trials should help to create clinically meaningful responses (NCT02484404). An overview is presented in Table 2.

### **Radiolabeled PARP Inhibitors: Imaging and Therapy**

Molecular imaging using PARP imaging agents could serve as a powerful tool for functional imaging of PARP *in vivo*. Tagging a PARPi with a radionuclide allows PET ( $^{11}\text{C}$ ,  $^{18}\text{F}$ ) or SPECT ( $^{123}\text{I}$ ) imaging to potential clinical benefits: interrogation of PARP expression levels, PARPi distribution, tumor uptake, and drug-target engagement in a non-invasive manner, which may allow tumor diagnosis and staging, selection of patient subgroups suitable for PARP inhibition, monitor the treatment response, or gauge the emergence of resistance. If, on the other hand, the same chemical scaffold is radiolabeled with a therapeutic radionuclide, emitting alpha ( $^{211}\text{At}$ ), beta ( $^{131}\text{I}$ ), or Auger ( $^{123}\text{I}$ ) radiation, the resulting radionuclide therapy agent is capable of causing cell death by delivering ionizing radiation to PARP-expressing tumor cells.

Over the past 15 years, numerous radiolabeled PARP agents have been developed (Fig. 2A) and undergone characterization *in vitro* and in animal models, with two compounds already evaluated in early phase clinical trials (34,52). [ $^{11}\text{C}$ ]PJ34 was the first radiolabeled PARPi, intended for PET imaging of tissue necrosis with upregulation of PARP-1 expression levels in a rat models of diabetes (53). The radiolabeled imaging agents [ $^{18}\text{F}$ ]BO (54), [ $^{18}\text{F}$ ]PARPi (55), [ $^{123/131}\text{I}$ ]PARPi (56,57), [ $^{18}\text{F}$ ]PARPi-FL (58) and [ $^{64}\text{Cu}$ ]PARPi-DOTA (59), all shared structural similarity to olaparib, and most displayed high specificity toward PARP. Also of note is the development of [ $^{18}\text{F}$ ]olaparib, an  $^{18}\text{F}$ -labeled isotopologue of olaparib, with an

identical chemical structure as the parent molecule, hence with identical pharmacokinetic and dynamic profiles *in vivo* (60). Among the olaparib-derived radiotracers, [ $^{18}\text{F}$ ]PARPi-FL, the dual-modality PARP imaging agent (optical/PET), was used to image drug distribution and drug-target engagement of different PARP inhibitors in different cancer models (61,62), demonstrating the potential application of radiolabeled-PARPi in monitoring of drug distribution and drug-target engagement. Rucaparib-inspired radiolabeled compounds [ $^{18}\text{F}$ ]FE-LS-75 (without publicly available preclinical characterization) (63), [ $^{125}\text{I}$ ]KX1 (64), [ $^{125}\text{I}$ ]KX-02-019 (65), and [ $^{18}\text{F}$ ]FTT (66) also show excellent PARP selectivity.

Moving beyond preclinical models, both [ $^{18}\text{F}$ ]FTT (66,67) and [ $^{18}\text{F}$ ]PARPi (55,68) have been evaluated in different types of cancers in clinical practice. [ $^{18}\text{F}$ ]FTT is currently the subject of phase 1 trials in ovarian patients (66) (Fig 2B), and also able to measure PARP-1 expression in different subtypes of breast cancer patients (estrogen receptor positive, human epidermal growth factor receptor 2-positive and triple negative) (67). [ $^{18}\text{F}$ ]PARPi is currently also in phase 1 trials but for head-and-neck cancer (55) (Fig. 2C). Furthermore, recent clinical data has shown that the utility of [ $^{18}\text{F}$ ]PARPi can be potentially expanded to image brain cancer in patients for treatment monitoring (68). In addition to applying radiolabeled-PARPi in patients for cancer detection, a fluorescent-labeled PARPi, PARPi-FL, was also subjected to clinical studies for the detection of oral carcinoma, and potentially serve as the surgical-margin assessment of epithelial cancer of the upper intestinal tract (69). These translational studies established the encouraging potential of PARP imaging agents in cancer detection for clinical practice.

Although these agents showed promising tumor uptake, they display different affinity and selectivity for PARP-1 and other isoforms, with a range of  $\text{IC}_{50}$  values

(2.3 – 200 nM) as a proxy for PARP affinity (Fig. 2A). Their selectivity toward other PARP family enzymes is not always fully characterized. Combined results indicate that small structural modifications may have significant impact on the target specificity of the compound. The Reiner group showed, for example, that altering the iodination position on the aromatic ring and the length of the linker between the iodinated-aromatic ring and the olaparib core resulted in very different IC<sub>50</sub> values for PARP-1 (57). Similarly, recently reported on the effects of structure modification on tumor uptake and target specificity of the olaparib-derived radiolabeled PARPi [<sup>18</sup>F]olaparib and [<sup>18</sup>F]AZD2461 (70).

The great potential of radiolabeled PARPi has now also expanded to the use of therapeutic radionuclides: <sup>123</sup>I (Auger electron therapy) (71), <sup>131</sup>I (high energy beta particles) (72), and <sup>211</sup>At (alpha radiation) (73). These PARPi-based radionuclide therapy agents have shown some efficacy in brain cancer and neuroblastoma models with little toxicity to normal tissues, making use of the high ionizing potential and short ranges of these particle emitters for cancer treatment (Fig. 3A-3C).

To date, PARPi-derived radiolabeled compounds are based on either olaparib-like structures or rucaparib-like structures. Given that small variations in structure can lead to large differences in PARP IC<sub>50</sub> values and trapping, it may not be assumed that all radiolabeled variants are similar in PARP trapping ability, or elicit the same allosteric effects under the same circumstances. Olaparib and rucaparib have a comparable ability for trapping PARP-1 on DNA via catalytic inhibition, yet their allosteric effects differ significantly, and may even be different for other PARP isoforms. These variances may result in diverse degrees of target engagement and PARP trapping, including PARP inhibitor trapping. This may in turn influence tumor versus normal tissue uptake, affecting imaging contrast and toxicity, of radionuclide

imaging and therapy. Therefore, target specificity and PARP trapping ability of PARPi or their radiolabeled analogues, and target expression should be taken into account in PARP-targeting approaches. For example, high accumulation of  $^{18}\text{F}$ -PARPi in healthy tissues such as lymph nodes, bone marrow, liver and spleen may induce higher toxicity to these organs due to high physiological expression of PARP-1 and PARP-2 (55). Careful consideration is needed in the interpretation of PARPi-derived imaging agents, which cannot necessarily predict the precise behavior of analogous, but non-identical radionuclide therapy compounds. This can of course be overcome by using thera(g)nostic compounds, which allow imaging patient dosimetry calculation and therapy response, as demonstrated by the recent study using [ $^{123}\text{I}$ ]PARPi in glioblastoma models (Fig. 3A) (71).

## Conclusions and Future Prospects

The discovery and development of PARPi in *BRCA*-mutant cancers is arguably the most elegant example of synthetic lethality to target tumor suppression gene loss in clinical practice. PARP inhibitor therapy can be of considerable benefit to a wide variety of cancer patients as a monotherapy and can synergize with appropriately selected drugs to improve treatment outcome. However, before the full potential of mono- and combination therapies may be achieved in clinical practice, it is imperative to recognize the limitations currently faced in the PARP inhibition in cancer therapy. Attention should be paid to identification and validation of predictive biomarkers to predict synthetic lethality, how distinct molecular mechanisms of action of different PARPi, off-target effects due to high physiological PARP-expressing healthy organs, tumor heterogeneity and genetic modifications in patients may lead to unexpected treatment responses. Genetic testing e.g. BRACAnalysis<sup>®</sup> or myChoice<sup>®</sup>

test (Myriad Genetics) to select those patients who will benefit from PARPi treatment has been implemented in many clinical trials, and the use of diagnostic PARPi-based PET imaging may serve as a non-invasive method to monitor patient treatment response or study the degree of drug engagement in tumor (based on PARP-1 expression levels) at any time during the treatment cycle. Proof-of-concept studies using imaging agents to study PARP levels will add value to the interpretation of clinical data. The emergence of PARP-targeting radionuclide therapy represents another treatment strategy that warrants further investigation to explore its potential.

### **Authors' Contribution**

C.Y.C., K.V.T. and B.C. contributed substantially to the design, collecting literature data and writing the article. All authors discussed, reviewed and approved the final manuscript before submission.



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## Figure Legends

**Figure 1:** Allosteric regulation of PARP-1 and effects on PARP-1 allostery by PARPi.

- (A) Schematic representation of multi-domain organization of human PARP-1, PARP-2, and PARP-3. Zinc fingers (Zn1, Zn2, and Zn3); BRCT (BRCA C-terminus); WGR (Trp-Gly-Arg domain); HD (helical domain); ART (ADP-ribosyltransferase).
- (B) Overall mechanism and allosteric regulation of PARP-1 (13). Upon DNA damage, the multi-domains of PARP-1 quickly assemble on the DNA damaged site. The binding of  $\text{NAD}^+$  to the catalytic domain promotes PARylation to produce PAR chains for the recruitment of target proteins (such as DNA ligase, XRCC1, etc.). Auto-PARylation leads to dissociation of PARP-1 from the DNA to allow binding of other repair proteins to the DNA; The HD domain regulates  $\text{NAD}^+$  access and catalytic activity of PARP-1 through a reversible substrate-blocking mechanism. In the absence of damaged DNA, the HD is in folded conformation and sterically blocks the substrate-binding site (the nicotinamide site (N) and the adenosine site (A)) to restrict the access of  $\text{NAD}^+$ . The assembly of DNA-binding domains of PARP-1 on the DNA break interfaces with the HD domain, which promotes an unfolded HD conformation to restore access of  $\text{NAD}^+$  to the catalytic active site. The  $\text{NAD}^+$  binding to the catalytic site drives the HD equilibrium distribution to its unfolded state (purple arrow), thus strengthening the interaction between the regulatory domains and the DNA break.
- (C) Impacts on PARP-1 allostery in DDR by PARPi binding (19). Three distinct types of PARPi based on their allosteric effects on the PARP-1/SSB DNA

complex: Type I PARPi influence PARP-1 allostery and retain PARP-1 on DNA (in green), whereas type III PARPi perturb PARP-1 allostery and release PARP-1 from DNA (in red). Type II PARPi do not influence PARP-1 allostery (in black).

**Figure 2:** Molecular structures and PARP-1 IC<sub>50</sub> or inhibition constant (K<sub>i</sub>) values of PARP-targeted PET imaging agents, and examples of their clinical studies.

- (A) Molecular structures with radionuclide highlighted in red, and PARP-1 IC<sub>50</sub> or inhibition constant (K<sub>i</sub>) values of PARP-targeted PET imaging agents.
- (B) Clinical [<sup>18</sup>F]FTT and [<sup>18</sup>F]FDG PET/CT images of a patient completed 4 cycles of carboplatin and paclitaxel (66). A patient with omental metastases showed higher uptake of [<sup>18</sup>F]FTT than [<sup>18</sup>F]FDG prior (SUV<sub>max</sub>: 5.1 vs 2.0) and after (SUV<sub>max</sub>: 7.8 vs 3.4) chemotherapy. Yellow arrowheads indicate sites of disease. Reproduced with permission.
- (C) A 67-year-old patient with oropharyngeal squamous cell carcinoma (OPSCC) underwent [<sup>18</sup>F]PARPi PET/CT imaging (55). Maximum intensity projections at 30 min, 60 min and 120 min post [<sup>18</sup>F]PARPi injection in the patient with OPSCC. At 120 min, uptake in the primary tumor was higher than lymph nodes (SUV<sub>max</sub>: 4.1 vs 3.6). Green arrow: primary tumor; Red arrow: metastatic lymph node. Reproduced with permission.

**Figure 3:** PARP-targeted therapeutic agents and their preclinical evaluations.

- (A) *In vivo* [<sup>123</sup>I]MAPi SPECT/CT imaging and [<sup>123</sup>I]MAPi efficacy (71). Molecular structure of [<sup>123</sup>I]MAPi; SPECT/CT images of orthotopic GBM model were taken at 18 h after injecting with [<sup>123</sup>I]MAPi (T = tumor, Cl = clearing organs), showing specific tumor uptake of [<sup>123</sup>I]MAPi; Kaplan-Meier

survival curves of vehicle or [ $^{123}\text{I}$ ]MAPi (single injection: 0.37 - 1.11 MBq) treated mice.  $P < 0.01$ . [ $^{123}\text{I}$ ]MAPi treated mice showed improved survival compared to the vehicle treated mice; Cytology staining and MRI imaging of brain tissues of untreated mouse at week 3, vehicle treated mouse at week 7 and [ $^{123}\text{I}$ ]MAPi treated mouse at 14 week after tumor implantation. Reproduced with permission.

(B) *In vivo* therapeutic effect of [ $^{131}\text{I}$ ]PARPi in subcutaneous GBM mouse models (72). Molecular structure of [ $^{131}\text{I}$ ]PARPi; Tumor growth at week 0 and week 2 of the mice treated with PBS, [ $^{127}\text{I}$ ]PARPi or [ $^{131}\text{I}$ ]PARPi. The treatment with [ $^{131}\text{I}$ ]PARPi suppressed tumor growth  $P < 0.01$ . Reproduced with permission.

(C) *In vivo* comparison of single and fractional dosing regimens of [ $^{211}\text{At}$ ]MM4 in IMR-05 xenograft models (73). Molecular structure of [ $^{211}\text{At}$ ]MM4; Tumor growth and Kaplan-Meier curves for IMR-05 tumor-bearing mice treated with single high dose of 1,480 kBq vs. a fractionated dose of 370 kBq twice weekly for a cumulative dose of 1,480 kBq; Immunofluorescence staining for PARP-1 in tumor sections harvested from IMR-05 tumor-bearing mice treated with 370 kBq of [ $^{211}\text{At}$ ]MM4 at 24, 48, and 72 h after treatment, showing upregulation of PARP-1 compared to the control. Reproduced with permission.

**Table 1:** Combination therapies with PARP inhibitors in PDX models.

**Table 2:** Selected clinical trials evaluating PARP inhibitors combinations.

Figures: Figure 1

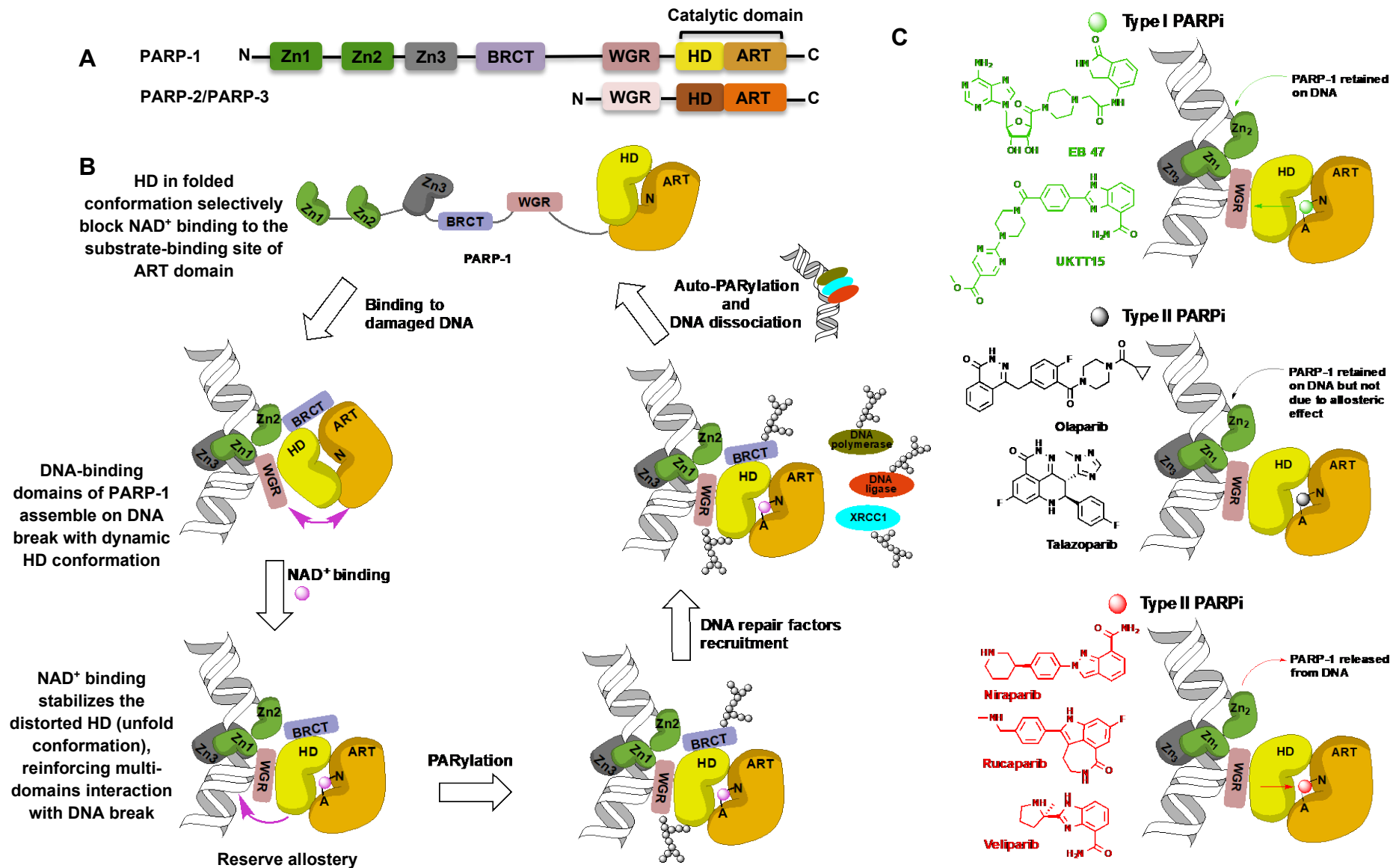


Figure 2

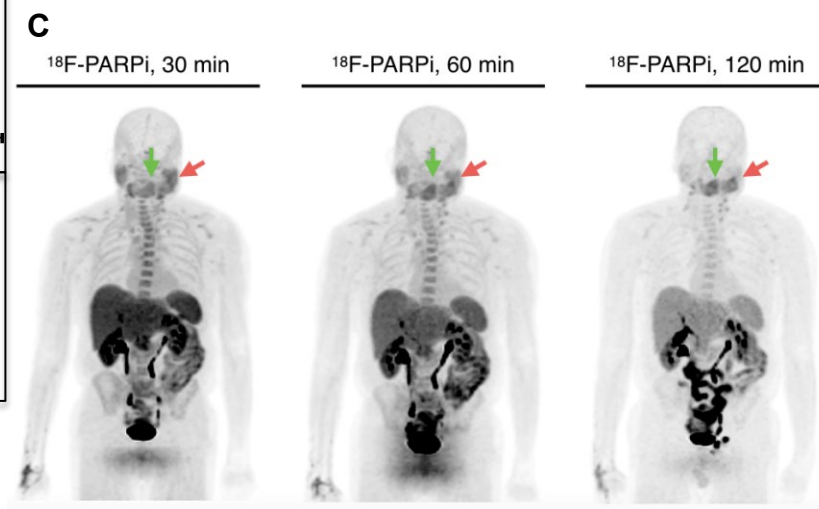
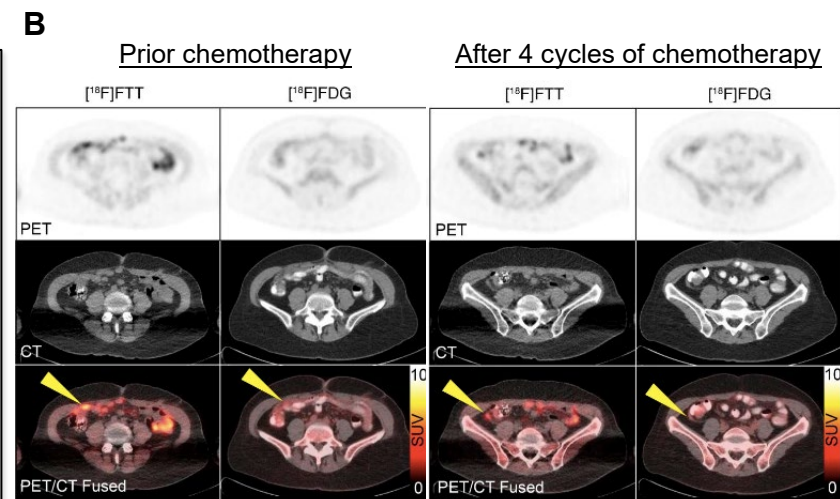
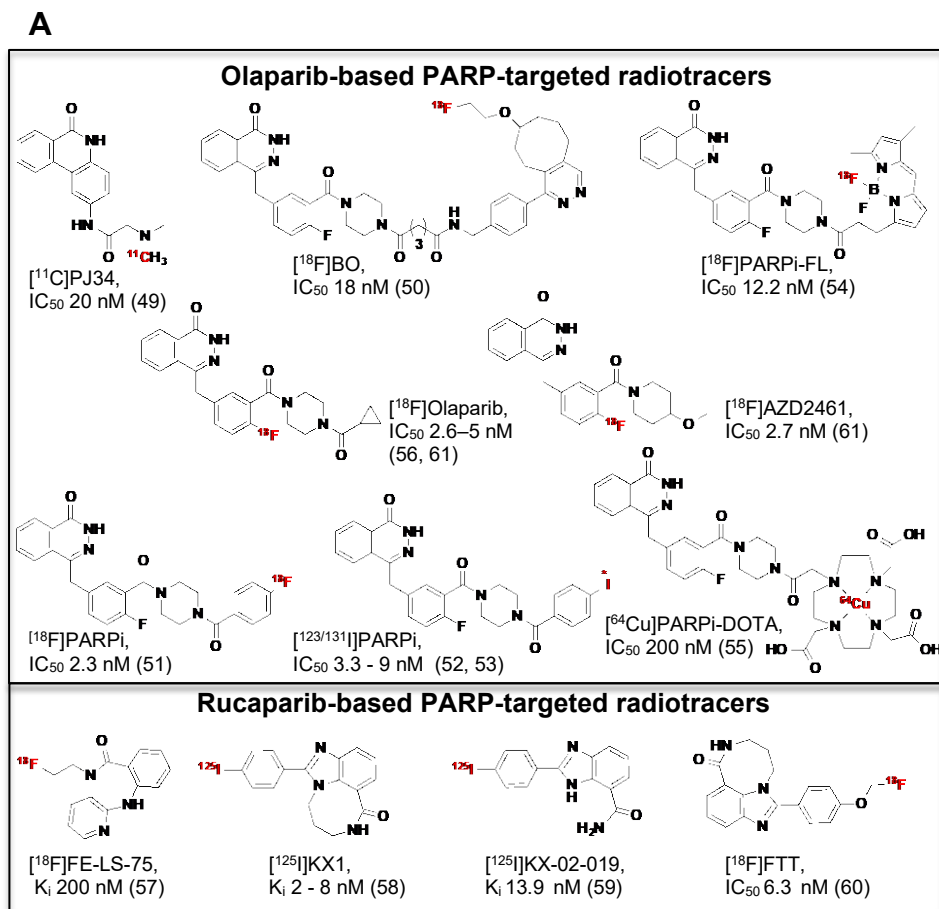
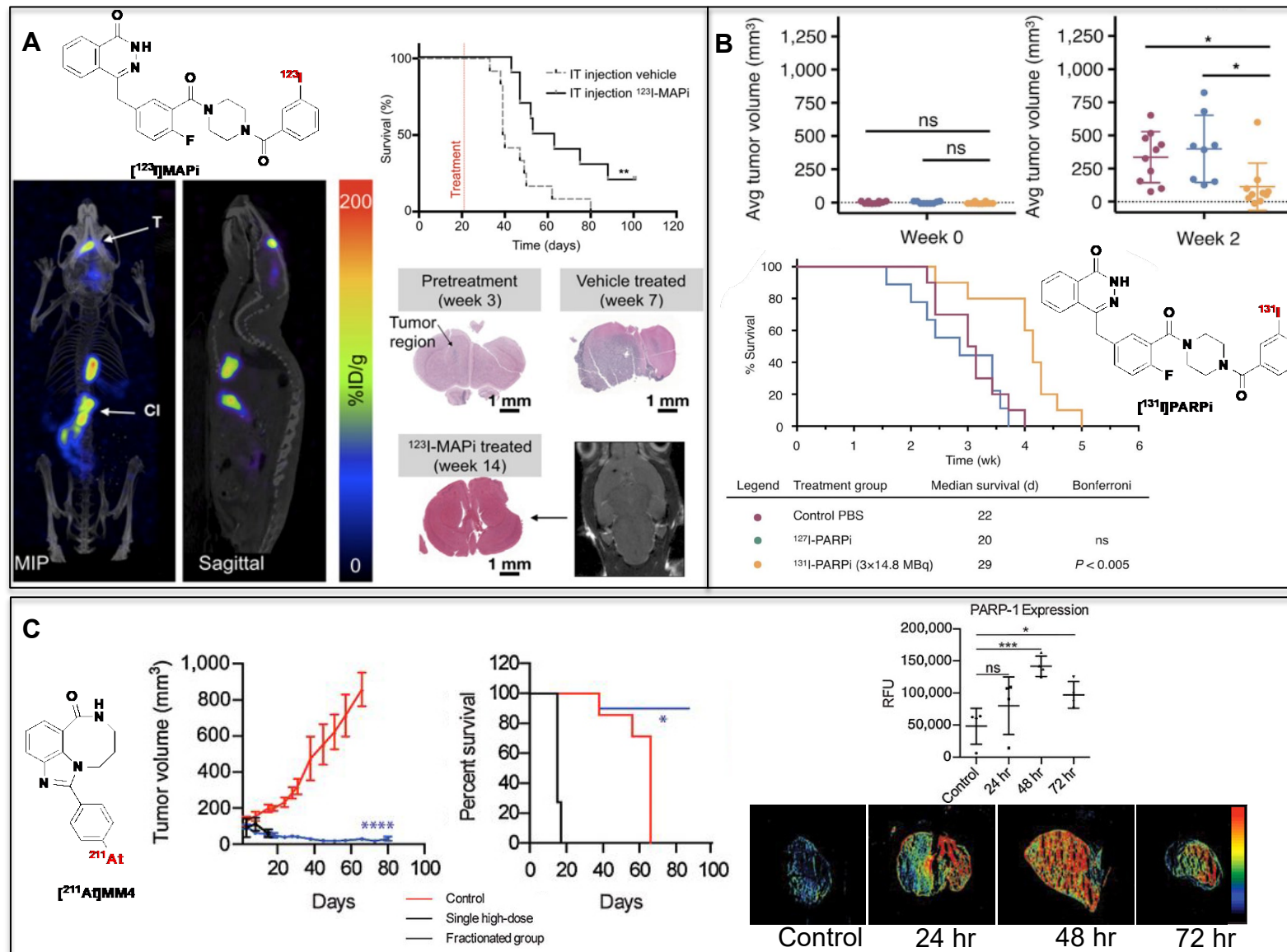


Figure 3:



**Table 1:**

Combination therapy		Additional Target	Treatment	Cancer Type	Publication
Genotoxic	BCL2		Olaparib + Dacarbazine	Uveal Melanoma	(74)
			Olaparib + Navitoclax	Ewing Sarcoma	(75)
	N/A		Olaparib + Chloroquine	Ovarian	(76)
			Talazoparib + TMZ	SCLC	(33)
			Olaparib + Trabectedin	Ewing Sarcoma	(77)
	N/A		Olaparib + Cisplatin	Breast	(78)
			Niraparib + carboplatin + paclitaxel	HGSOC	(79)
IR	N/A		Olaparib + IR	PDAC	(80)
			Olaparib + IR	Renal Cell Carcinoma	(81)
			Talazoparib + IR	SCLC	(82)
			Veliparib + IR	GBM	(83)
Targeted	DDR	ATM	Olaparib + AZD0156	Breast	(78)
		ATR	Olaparib + AZD6738 or MK8776	HGSOC	(36)
		CHK1	Olaparib + Prexasertib	HGSOC	(37)
		CHK2	Talazoparib + Prexasertib	Gastric	(84)
		CDK2/CDK5	Olaparib + Dinaciclib	NEPC	(39)
		CDK12	Veliparib + Dinaciclib	TNBC	(40)
		LMO2	Olaparib + R-CHOP	DLBCL	(85)
		PIN1	Olaparib + Tretinoin	TNBC	(38)
	DNA-PK		Olaparib + AZD7658	Ovarian	(86)
			Olaparib + AZD7659	TNBC	
			Olaparib + AZD7660	NSCLC	
			Olaparib + AZD7661	Head and Neck	
	Anti-oncogenic	BRD4	Olaparib + AZD5153	PDAC	(41)
		BRD5	Olaparib + JQ1	PDAC	(42)
		BRD6	Talazoparib + JQ1	Breast	(41)
		MYCN	Olaparib + Danusertib	NEPC	(87)
		AR	Olaparib + enzalutamide	CRPC	(43)

**Table 2**

Treatment	Phase	Trial Name	Study population	HRD Testing	NCT	Status	Publication
Olaparib + carboplatin + paclitaxel	II	N/A	High-grade serious epithelial ovarian, fallopian tube, or primary peritoneal carcinoma, irrespective of <i>gBRCA1/2</i> status	N/A	NCT01081951	Active, not recruiting	(88)
Veliparib + carboplatin + paclitaxel	III	VELIA/GOG-3005	High-grade serious epithelial ovarian, fallopian tube, or primary peritoneal carcinoma stage III–IV, irrespective of <i>gBRCA1/2</i> status	BRACAnalysis® or myChoice® assay (Myriad Genetics), HRD score $\geq 33$	NCT02470585	Active, not recruiting	(44)
Veliparib + carboplatin + paclitaxel	II	BROCADE	HER2+ locally recurrent or metastatic breast cancer with <i>gBRCA1/2</i> mutation	BRACAnalysis® or myChoice® assay (Myriad Genetics), HRD score undetermined	NCT01506609	Active, not recruiting	(46)
Veliparib + carboplatin + paclitaxel	III	BROCADE3	HER2– locally advanced or metastatic breast cancer with <i>gBRCA1/2</i> mutation	BRACAnalysis® or myChoice® assay (Myriad Genetics), HRD score undetermined	NCT02163694	Active, not recruiting	N/A



Veliparib + carboplatin + paclitaxel	III	BrighTNess	TNBC stage II–III (T1N1–2 or T2–4N0–2), irrespective of <i>gBRCA1/2</i> status	BRACAnalysis <sup>®</sup> assay (Myriad Genetics), HRD score undetermined	NCT02032277	Active, not recruiting	(45)
Veliparib + TMZ	II	N/A	SCLC sensitive or refractory to platinum-based chemotherapy, irrespective of <i>gBRCA1/2</i> status	N/A	NCT01638546	Completed	(89)
Veliparib + cisplatin + etoposide	II	N/A	SCLC with presence of extrathoracic metastatic disease, malignant pleural effusion, and bilateral or contralateral supraclavicular adenopathy, irrespective of <i>gBRCA1/2</i> status	N/A	NCT01642251	Completed	(90)
Veliparib + cisplatin + gemcitabine	II	N/A	Locally advanced and metastatic PDAC stage III–IV, with <i>gBRCA1/2</i> and PALB2 mutation	BRACAnalysis <sup>®</sup> or myChoice <sup>®</sup> assay (Myriad Genetics), HRD score undetermined	NCT01585805	Active, not recruiting	N/A
Veliparib + FOLFIRI	II	N/A	Locally advanced and metastatic PDAC stage IV, irrespective of <i>gBRCA1/2</i> status	N/A	NCT02890355	Active, not recruiting	N/A

Olaparib + Bevacizumab	III	PAOLA-1	High-grade serious epithelial ovarian, fallopian tube, or primary peritoneal carcinoma, irrespective of <i>gBRCA1/2</i> status	myChoice® assay (Myriad Genetics), HRD score $\geq 42$	NCT0247764 4	Active, not recruiting	(47)
Olaparib + Cediranib	I/II	N/A	High-grade serious epithelial ovarian, fallopian tube, or primary peritoneal carcinoma and TNBC, with <i>gBRCA1/2</i> mutation	BRACAnalysis® assay (Myriad Genetics), HRD score undetermined	NCT0111664 8	Active, not recruiting	(48)
Olaparib + Abiraterone	II	N/A	mCRPC, irrespective of <i>gBRCA1/2</i> status	N/A	NCT0197221 7	Active, not recruiting	(49)
Olaparib + Durvalumab + Bevacizumab	I/II	MEDIOLA	Advanced or metastatic solid tumor, irrespective of <i>gBRCA1/2</i> status	BRACAnalysis® or myChoice® assay (Myriad Genetics), HRD score undetermined	NCT0273400 4	Active, not recruiting	(50)
Olaparib + Durvalumab + Bevacizumab	I/II	N/A	Advanced solid tumor or recurrent ovarian, triple-negative breast, lung, prostate and colorectal cancers with <i>gBRCA1/2</i> mutation	BRACAnalysis® or myChoice® assay (Myriad Genetics), HRD score undetermined	NCT0248440 4	Recruiting	(51)

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