INHIBITING PARP AS A STRATEGIC TARGET IN CANCER

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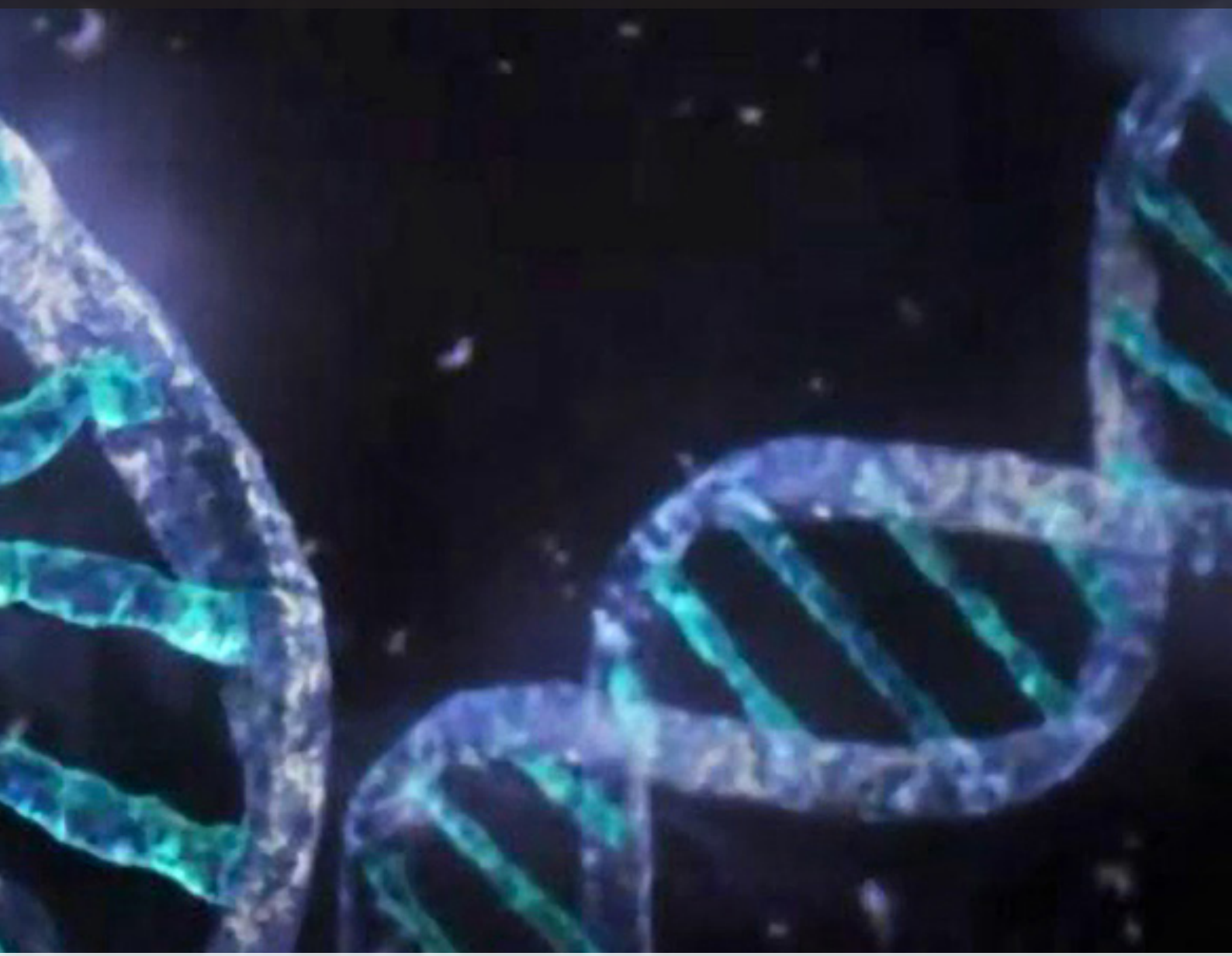

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INHIBITING PARP AS A STRATEGIC TARGET IN CANCER

Topic Editors:
Christina Annunziata, National Cancer Institute, USA
Kristin Zorn, University of Arkansas for Medical Sciences, USA

Damage to the DNA double helix - Image by NASA

Poly-ADP ribose polymerase (PARP) proteins are critical mediators of DNA repair. Many traditional anti-cancer chemotherapy agents overwhelm a cell's ability to repair DNA damage in order to kill proliferating malignant cells. Recent evidence suggests that cancers within and across tissue types have specific defects in DNA repair pathways, and that these defects may predispose for sensitivity and resistance to various classes of cytotoxic agents. Breast, ovarian and other cancers develop in the setting of inherited DNA repair deficiency, and these cancers may be more sensitive to cytotoxic agents that induce DNA strand breaks, as well as to inhibitors of PARP activity. A series of recent clinical trials has tested whether PARP inhibitors can achieve synthetic lethality in hereditary DNA repair-deficient tumors. At the current time, mutation of BRCA serves as a potential, but not comprehensive, biomarker to predict response to PARP inhibitor therapy. Mechanisms of resistance to PARP inhibitors are only recently being uncovered. Future studies seek to identify sporadic cancers that harbor genomic instability rendering susceptibility to PARP inhibitors that compound lethal DNA damage.

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EDITORIAL
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Editorial: Inhibiting PARP as a Strategic Target in Cancer

Kristin K. Zorn[1]* *and Christina M. Annunziata*[2]

[1] Department of Obstetrics and Gyencology and Divison of Genetics, University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2] National Cancer Institute, Bethesda, MD, USA

Keywords: PARP inhibitors, poly(ADP-ribose) polymerase, DNA repair, PARP-1, BRCA1 protein, cancer therapy

The Editorial on the Research Topic

Inhibiting PARP as a Strategic Target in Cancer

When Christina Annunziata and I embarked on guest editing an e-journal about poly(ADP-ribose) polymerase (PARP) inhibitors for cancer therapy, our goal was to capture how one of the most promising, rationally developed therapies had become increasingly complex in clinical use. We recruited an outstanding group of researchers to help in this effort. We organize their contributions into two broad categories, those with a more basic science approach and those with a more clinical approach, although these are not without overlap.

Beginning with the more mechanistic contributions, "The Elephant and the Blind Men: Making Sense of PARP Inhibitors in Homologous Recombination Deficient Tumor Cells" by De Lorenzo et al. provides an excellent review of the main models proposed to explain the synthetic lethality seen with PARP inhibition and deficiency in homologous recombination. Limitations of the models are highlighted, along with the potential impact that our knowledge gaps might have on clinical application of PARP inhibition. This article provides the groundwork for critical consideration of the other papers in this compilation.

In "Strategic Combination of DNA Damaging Agent and PARP Inhibitor Results in Enhanced Cytotoxicity," Horton and Wilson describe using a mouse embryonic fibroblast cell culture model to better understand how PARP-1 inhibition impacts cell killing in combination with various chemotherapeutic agents. They report that the chemistry of the DNA repair intermediate that is formed is critical to PARP inhibitor-induced sensitization. This level of detail complements that presented by Steffen et al. who contributed "Structural Implications for Selective Targeting of PARPs." The authors delve into the question of whether PARP inhibition should be targeted to all PARPs through binding to conserved regions or more selective in targeting specific PARPs. The answer has the potential to impact both PARP inhibitor effectiveness and toxicity.

Poly(ADP-ribose) polymerase enzymes are most widely recognized for their roles in single-strand DNA repair, especially when complementing the double-strand repair that is dependent on BRCA proteins. It is important to realize, however, that the PARP enzymes have other functions in the cell. In "Beyond DNA Repair: Additional Functions of PARP-1 in Cancer," Weaver and Yang broaden our focus on PARP inhibition from the typically discussed DNA damage response to other processes, such as inflammation, angiogenesis, cellular metabolism, and cell death, that are critical to cancer biology. This information helps interpret some side effects of the PARP inhibitors in the clinic and hints at pathways to co-target in the future. In the group of more clinical contributions, Shah et al. build on this theme by discussing the clinical impact of our lack of complete understanding of the mechanism of action of PARP inhibitors in their contribution, "PARP Inhibitors in Cancer Therapy: Magic Bullets but Moving Targets."

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Giuseppe Giaccone, Georgetown University, USA

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***Correspondence:**
Kristin K. Zorn
kkzorn@uams.edu

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Key perspectives on the clinical development process of PARP inhibitor development unfold in additional articles included in the compilation. Ricks et al. discuss the development of PARP inhibitors from the perspective of the US Food and Drug Administration. In "Successes and Challenges of PARP Inhibitors in Cancer Therapy," they provide insight into the regulatory aspects of the development process from the phase 0 veliparib trial to the current approval of olaparib for limited clinical use. Adding to this series, Burgess and Puhalla discuss clinical trials from their perspective as academic physicians. They have contributed an in-depth review of the use of PARP inhibitors in clinical trials to date in breast and ovarian cancer. Their review in "BRCA 1/2-Mutation Related and Sporadic Breast and Ovarian Cancers: More Alike than Different" provides a window into the biologic factors that might influence response to PARP inhibition. Additional detail is teased out, with relevance to women's malignancies, in "The Role of PARP Inhibitors in the Treatment of Gynecologic Malignancies." Here, Reinbolt and Hays review the data on the use of PARP inhibitors in ovarian, endometrial, and cervical cancer, and discuss their vision regarding future directions for their development. O'Sullivan et al. provide a complementary direction in "Beyond Breast and Ovarian Cancers: PARP Inhibitors for BRCA Mutation-Associated and BRCA-Like Solid Tumors." Importantly, they broaden potential application with respect to tumor type, but focus on the molecular aspects that may help optimize PARP inhibitor use in a defined patient population. Specifically, these authors review the data for PARP inhibitor use in solid tumors other than breast and ovarian cancer, helping to frame the potential for expanded use in the future.

This collection of articles addresses the role of PARP inhibition in cancer therapy, from both basic science and clinical research perspectives. The integration of bench and bedside aspects is vital for moving the field forward to the most efficacious use of these agents. While our knowledge of PARP inhibitors has grown substantially in a relatively short amount of time, critical issues, such as mechanisms of action, appropriate therapeutic combinations, limiting short- and long-term toxicity, and defining the ideal patient population, remain to be resolved. We have compiled these articles to stimulate thoughts and discussion regarding this promising line of therapy, and expedite the successful application to patients.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contribution to the work, and approved it for publication.

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REVIEW ARTICLE
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The elephant and the blind men: making sense of PARP inhibitors in homologous recombination deficient tumor cells

Silvana B. De Lorenzo[1†], Anand G. Patel[1,2†], Rachel M. Hurley[2] and Scott H. Kaufmann[1,2]*

[1] *Division of Oncology Research, Mayo Clinic, Rochester, MN, USA*
[2] *Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA*

Edited by:
Christina Annunziata, National Cancer Institute, USA

Reviewed by:
Marco Barchi, University of Rome Tor Vergata, Italy
Michael J. Metzger, Columbia University, USA

***Correspondence:**
Scott H. Kaufmann, Division of Oncology Research, Mayo Clinic, Gonda 19-212, 200 First Street, SW, Rochester, MN 55905, USA
e-mail: kaufmann.scott@mayo.edu

† Silvana B. De Lorenzo and Anand G. Patel have contributed equally to this work.

Poly(ADP-ribose) polymerase 1 (PARP1) is an important component of the base excision repair (BER) pathway as well as a regulator of homologous recombination (HR) and non-homologous end-joining (NHEJ). Previous studies have demonstrated that treatment of HR-deficient cells with PARP inhibitors results in stalled and collapsed replication forks. Consequently, HR-deficient cells are extremely sensitive to PARP inhibitors. Several explanations have been advanced to explain this so-called synthetic lethality between HR deficiency and PARP inhibition: (i) reduction of BER activity leading to enhanced DNA double-strand breaks, which accumulate in the absence of HR; (ii) trapping of inhibited PARP1 at sites of DNA damage, which prevents access of other repair proteins; (iii) failure to initiate HR by poly(ADP-ribose) polymer-dependent BRCA1 recruitment; and (iv) activation of the NHEJ pathway, which selectively induces error-prone repair in HR-deficient cells. Here we review evidence regarding these various explanations for the ability of PARP inhibitors to selectively kill HR-deficient cancer cells and discuss their potential implications.

Keywords: PARP inhibitor, synthetic lethality, non-homologous end joining, homologous recombination, BRCA1, BRCA2, ovarian cancer, breast cancer

INTRODUCTION

Poly(ADP-ribose) polymerase (PARP) inhibitors are currently undergoing extensive testing as potential anticancer agents (1–13). These drugs were initially developed as modulating agents that could enhance the cytotoxicity of DNA damaging treatments such as ionizing radiation and temozolomide (1, 12, 14). Interest in these agents was heightened by the demonstration that *BRCA1*- and *BRCA2*- (*BRCA1/2*-) mutant cancer cells are selectively killed by single-agent PARP inhibitor treatment (15, 16). Consistent with these preclinical observations, the PARP inhibitor olaparib has exhibited substantial single-agent activity in *BRCA1/2*-mutant breast and ovarian cancer (17–21). Nonetheless, fewer than 50% of patients with *BRCA1/2*-mutant cancers respond to these drugs, raising important questions about identifying patients most likely to derive benefit from PARP inhibition (22, 23). With this in mind, extensive efforts have been directed at further refining the mechanism of cytotoxicity of PARP inhibitors and elucidating mechanisms of resistance.

To provide a context for discussing the selective killing of BRCA1/2-deficient cells by PARP inhibitors, we first briefly outline what is known about the PARP family of enzymes and the repair of DNA double-strand breaks. We then describe and discuss four models that have been proposed to account for the selective killing of homologous recombination (HR)-deficient cells by PARP inhibitors.

PARPs: A FAMILY OF ADP-RIBOSYLTRANSFERASES

The molecular biology and biochemistry of the PARP family of ADP-ribosyltransferases have been extensively reviewed elsewhere (24–33) and will only briefly be summarized here. Originally described in the 1960s (34–36), PARP1 is the founding member of a family of enzymes (37, 38) that transfer ADP-ribose moieties from the dinucleotide NAD^+ to polypeptide acceptors, thereby catalyzing either mono- or poly(ADP-ribosyl)ation of polypeptide substrates (24, 39, 40). Although 18 members of the PARP family have been identified in mammalian cells (24, 25), only 6 are known to synthesize poly(ADP-ribose) polymers (1, 25, 41). Three of these family members, PARP1, PARP2, and PARP3, have been implicated in DNA repair (31). Of these, PARP1 is the most abundant (up to 10^6 copies/nucleus) and has been shown to play critical roles in DNA repair, epigenetic modification of chromatin, regulation of genomic stability, modulation of cellular energy pools, the regulation of transcription, and a distinct form of cell death termed parthanatos (25–32, 42).

Although other PARPs might play an important role in the response to PARP inhibitors (43), existing models of PARP inhibitor-induced cytotoxicity emphasize the role of PARP1. Moreover, despite the well-established effects of PARP1 modulation on transcription (28), chromatin structure (26, 28, 44), and energy metabolism (1, 30, 33), current explanations for the lethality of PARP inhibition in HR-deficient cells focus solely on the role of PARP1 in DNA repair.

In response to certain types of DNA damage – particularly DNA nicks and double-strand breaks – PARP1 catalytic activity increases as much as 500-fold (41, 45, 46). This activation reflects a recently described conformational change that is transmitted from the DNA binding domains at the N-terminus of the PARP1 molecule through intervening domains to the catalytic domain at the

C-terminus, resulting in altered alignment of critical residues in the active site (41, 47, 48). Once activated, PARP1 adds poly(ADP-ribose) moieties to a wide range of nuclear proteins, including histones, topoisomerases, and other non-histone chromatin proteins, although PARP1 itself is the major protein that is covalently modified (41, 49). The resulting poly(ADP-ribose) polymers not only alter the function of the covalently modified proteins (49–52), but also serve as a new binding site for other nuclear proteins (32, 41, 53–55).

Through this ability to synthesize poly(ADP-ribose) polymer, which covalently or non-covalently interacts with a variety of nuclear proteins, PARP1 contributes to a number of different steps in DNA damage response pathways. In its most extensively studied role, PARP1 is essential for base excision repair (BER) (56–58), a process involving the removal of a single damaged base and subsequent restoration of DNA integrity (59, 60). After recruitment to the damaged DNA, PARP1 recruits the scaffolding protein X-ray cross complementing protein 1 (XRCC1) (57, 61), which in turn binds to various BER proteins, bringing together a variety of components required for efficient repair of different base lesions (59, 62).

The involvement of PARP1 in DNA repair is not limited to XRCC1 recruitment during BER. PARP1 has also been reported to play a critical role in HR (63–65), including recruitment of MRE11 and NBS1 to DNA double-strand breaks (66), and to competitively inhibit the classical non-homologous end-joining (NHEJ) pathway by preventing Ku binding to free DNA ends (67). In addition, PARP1 plays a critical role in restarting replication forks that stall as a consequence of nucleotide depletion or collisions with bulky lesions (68–71). Any or all of these roles of PARP1 in DNA repair might be important in understanding the cellular effects of PARP inhibitors.

HOMOLOGOUS RECOMBINATION

In order to understand the models that currently describe the action of PARP inhibitors in HR-deficient cells, we also briefly review the process of HR itself. When DNA double-strand breaks form, two pathways compete to repair them (**Figure 1**): HR, which is a high fidelity pathway, and NHEJ, which is error-prone. According to current understanding (60, 72, 73), the HR pathway is activated when components of the MRN (MRE11/Rad50/Nbs1) complex bind to DNA double-strand breaks. In brief, Nbs1 brings its binding partners MRE11 and Rad50 to the nucleus, where the complex binds to double strand breaks (74). This MRN complex then recruits phosphorylated CtIP, which activates the exonuclease activity of MRE11 (75–78). After activated MRE11 resects one strand of the DNA to generate relatively short 3′ single-stranded DNA (ssDNA) tails, two different exonucleases, ExoI and DNA2, extend the single-stranded tails to a length of several thousand basepairs by continuing the resection (79, 80). The resulting ssDNA is rapidly bound by the ssDNA binding protein replication protein A (RPA), which is then replaced by Rad51 to form a nucleofilament as described in greater detail below. This Rad51-ssDNA complex facilitates homology searching and invasion of the ssDNA into homologous duplex DNA sequences of its sister chromatid. Once the resected ends are annealed to complementary strands, intervening sequence is synthesized using the intact strand as a template and ligated into place (81).

A critical step in the HR pathway is the loading of Rad51 onto ssDNA. This step is the culmination of a long series of reactions (**Figure 1**) that are triggered in response to DNA damage (72, 82). Once the MRN complex binds to DNA double-strand breaks, it also recruits and activates the DNA damage-activated kinase ATM, resulting in ATM autophosphorylation followed by sequential phosphorylation and recruitment of the histone variant H2AX, the "mediator" (scaffold) protein MDC-1, and several other proteins, including the tumor suppressor protein BRCA1, to sites of DNA damage (73, 82). Partner and localizer of BRCA2 (PALB2) binds to the C-terminus of BRCA1 and N-terminus of BRCA2, creating a bridge to recruit BRCA2 to sites of DNA damage. BRCA2 then binds phosphorylated Rad51, targeting active Rad51 to the ssDNA (83).

This entire HR process is tightly linked to cell cycle progression in multiple ways (84). First, BRCA2 and Rad51 are only expressed in S and G2 phases of the cell cycle, making HR impossible in G1 (76). Second, the cyclin-dependent kinase CDK2, which is active primarily at the G1/S transition and in S phase, catalyzes a priming phosphorylation of CtIP that is required before DNA damage can induce CtIP binding to MRN and subsequent MRE11-initiated end resection (85, 86). Finally, G0 and G1 cells have not replicated their DNA and, therefore, lack sister chromatids that provide homologous sequences for HR.

HR DEFICIENCY DEFINES CERTAIN MALIGNANCIES

The complex HR process can be interrupted at any of a number of steps. In particular, HR fails to occur efficiently if genes encoding components of the MRN complex, CtIP, ATM, MDC-1, H2AX, PALB2, BRCA1, BRCA2, or Rad51 are silenced or mutated at critical residues. Mutations that disable these proteins, as well as other participants in the HR process, are often found in cancers (73). In high-grade serous ovarian cancer, for example, *BRCA1* and *BRCA2* mutations are found in roughly 15% of cases, with mutations in another dozen or more HR genes found in an additional 10–15% of cases (87–89). While some of these mutations are familial, as many as half appear to be sporadic (89, 90). These mutations and the resulting genomic instability are a hallmark of high-grade serous ovarian cancer (90). Likewise, mutations in *BRCA1, BRCA2, PALB2*, and other components with the HR pathway are common in familial and certain subtypes of sporadic breast cancer, particularly triple negative breast cancer (91–93). *PTEN* is deleted or silenced in over 50% of endometrial cancers and a substantial fraction of glioblastomas and prostate cancers (94–97).

Early studies found that BRCA1- or BRCA2-deficient cells are hypersensitive to PARP inhibitors (15, 16). In particular, cells lacking BRCA1 or BRCA2 were more susceptible to PARP inhibitor-induced apoptosis and showed more profound growth inhibition when treated as xenografts in nude mice (15, 16). Subsequent investigation demonstrated that cells deficient in other HR components, including NBS1, ATM, ATR, Chk1, Chk2, Rad51, Rad54, FANCD2, FANCA, PALB2, or FANCC, are also hypersensitive to PARP inhibitors (98–100). Moreover, cells lacking the lipid phosphatase PTEN were shown to be deficient in Rad51 expression (101, 102), also leading to PARP inhibitor sensitivity

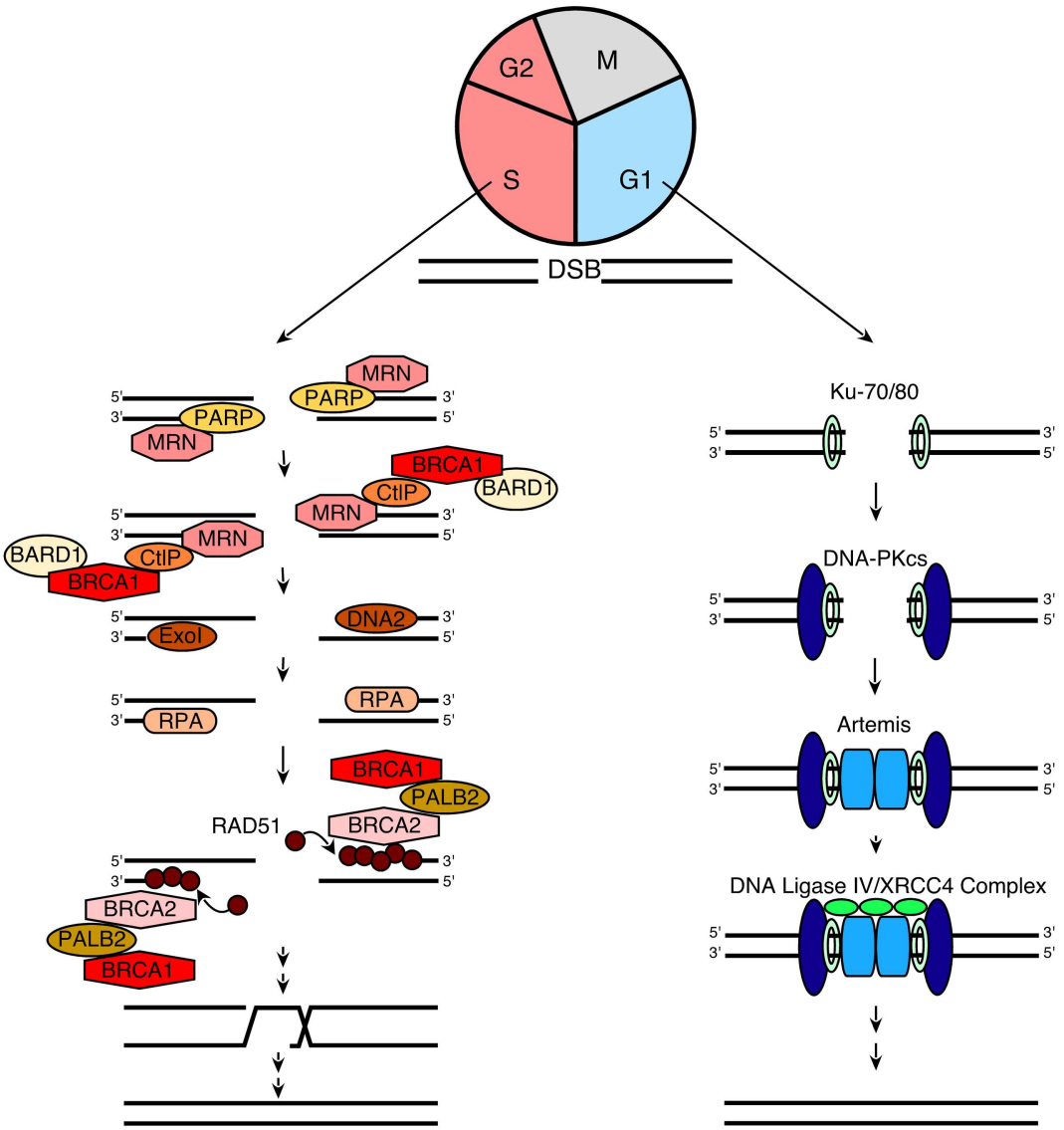

FIGURE 1 | A simplified model for NHEJ and HR. When a DNA double-strand break (DSB) occurs during G1, it is repaired via NHEJ (right). This process involves the following steps: (1) the Ku70/80 heterodimer detects and binds to the DSB; (2) Ku70/80 bound to the DSB recruits DNA-PKcs; (3) DNA-PKcs undergoes autophosphorylation, favoring the processing of DNA ends by Artemis; and (4) the XRCC4/DNA ligase IV complex ligates the processed DNA ends. Additional details regarding NHEJ can be found in refs (109–111). In contrast, when a DSB occurs during the S and G2 phases of the cell cycle, repair occurs preferentially via the HR pathway (left), which involves the following steps: (1) PARP1 binds to the DSB (48) and competes with Ku binding to DNA ends (67); (2) the MRN complex is recruited (66) to the DSB (together with CtIP and BRCA1/BARD1) and mediates the initial stages of DSB resection; (3) extensive end resection is catalyzed by EXO1 and DNA2/BLM (79, 80), resulting in long stretches of ssDNA; (4) this ssDNA is coated by RPA; (5) the BRCA2/PALB2/BRCA1 complex facilitates replacement of RPA with Rad51 (73, 81); (6) RAD51 filaments induce strand invasion into homologous DNA sequences; (7) DNA polymerization occurs using the sister chromatid as a template; and (8) resolution of the resulting complexes produces an exact copy of the template where the DSB was generated. Additional details of the HR process can be found in Refs. (60, 72, 73).

(102). Accordingly, the demonstration that PARP inhibitors are active, relatively non-toxic anticancer agents (17–21) led to substantial enthusiasm for developing these agents to treat a variety of neoplasms that exhibit HR deficiency.

Given the tantalizing preclinical and early clinical activity of PARP inhibitors in HR-deficient tumors, there has also been substantial interest in inducing a state of temporary HR deficiency in hopes of sensitizing cancers that lack inactivating mutations in the Fanconi anemia (FA)/HR pathway. Previous studies have demonstrated that this can be accomplished by treating cells with epidermal growth factor receptor inhibitors (103) or cyclin-dependent kinase inhibitors (104), which promote BRCA1 trafficking from the nucleus to the cytoplasm; phosphatidylinositol-3 kinase inhibitors, which downregulate Rad51 (105) or BRCA1 and

BRCA2 (106); ATR inhibitors, which diminish replication stress-induced activation of cell cycle checkpoints and repair (107), or even possibly PARP inhibitors themselves (108). Whether this pharmacological inhibition of HR will sensitize cancer cells in the clinical setting as effectively as inactivating mutations in FA/HR pathway genes remains to be determined.

NHEJ AS AN ALTERNATIVE MECHANISM OF DNA REPAIR

In addition to HR, which is a high fidelity repair process, cells also can employ the more error-prone NHEJ pathway to repair double-strand breaks. In essence, NHEJ is a process that detects free DNA ends, trims incompatible DNA, and directly ligates the double helix to restore DNA integrity (**Figure 1**). As reviewed elsewhere (109–111), this process involves initial binding of the Ku70/Ku80 heterodimer to free DNA ends, resulting in recruitment of the large serine/threonine kinase DNA-PKcs. Once bound to the DNA terminus, DNA-PKcs phosphorylates itself as well as a number of enzymes that can process DNA ends, including the nuclease Artemis, polynucleotide kinase phosphorylase, and DNA polymerases. Finally, the DNA ends are ligated by the DNA ligase IV/XRCC4 complex. Because cells in G1 lack both the DNA substrate and much of the protein machinery required for HR, NHEJ is the major pathway used for DNA double-strand break repair during G0 and G1. Moreover, this pathway is thought to play a major role in DNA repair when HR is impaired.

Previous studies have demonstrated that the NHEJ pathway is regulated in a number of ways. First, a complex containing the large scaffolding protein 53BP1 and its binding partner Rif1 inhibits accumulation of BRCA1 and the HR regulator CtIP at sites of DNA damage, thereby facilitating NHEJ in preference to HR (112–115). Second, ATM-mediated phosphorylation modulates the activity of the NHEJ nuclease Artemis (111). Third, Ku70, Ku80, and DNA-PKcs have all been previously identified as binding partners of poly(ADP-ribose) polymer (pADPr) (54, 57); and more recent studies suggest that other NHEJ components such as XRCC4 and Artemis also interact with pADPr (55). Additional studies have indicated that pADPr inhibits the NHEJ pathway, providing a starting point for one of the models describing the cytotoxicity of PARP inhibitors (15, 116).

CHOICE BETWEEN HR AND NHEJ

Several factors determine whether a DNA double-strand break is repaired by HR or NHEJ (117, 118). The lack of BRCA2, Rad51, and a suitable sister chromatid as a template prevent HR during the G0 and G1 phases of the cell cycle. During S and G2 phases, on the other hand, there is a competition between HR and NHEJ. For example, Ku70 and Ku80 binding impairs double-strand break end resection, whereas resection prevents binding of the Ku70/Ku80 complex (119, 120). Additional studies have shown that MRN plays a primary role in removing or displacing Ku from DNA ends to allow resection to take place. When damage occurs during the G1 phase of the cell cycle, the 53BP1/Rif1 complex restricts CtIP recruitment and stimulation of MRE11-mediated resection as described above, thereby facilitating NHEJ (112–115). During the S and G2 phases of the cell cycle, on the other hand, Rif1 is inhibited by a BRCA1-CtIP complex, allowing HR to occur. These competing interactions illustrate the complexity of processes that regulate DNA repair and provide an explanation for the observation that mechanisms involved in DNA double-strand break repair shift from NHEJ to HR during S phase (121).

CURRENT EXPLANATIONS FOR THE SELECTIVE CYTOTOXICITY OF PARP INHIBITORS IN HR-DEFICIENT CELLS

The seminal observation that PARP inhibitors selectively kill *BRCA1/2*-deficient cells in preclinical models (15, 16) was rapidly followed by the demonstration that PARP inhibitors exhibit clinical activity against *BRCA1/2*-mutant tumors (17–20). At least four different explanations have been advanced to explain this so-called synthetic lethality.

BER INHIBITION

Because PARP1 plays a critical role in BER (122, 123), initial explanations for the ability of PARP inhibitors to selectively kill HR-deficient cells focused on the interplay between BER and HR. According to this classical view [**Figure 2A**, see also Ref. (124, 125)], DNA damage induced by reactive oxygen species or replication errors results in DNA single-strand breaks, which ordinarily would be repaired by the BER pathway. Inhibition of PARP is postulated to cause persistence of these single-strand breaks, which are then converted to DNA double-strand breast as a consequence of interactions with transcription complexes and advancing replication forks. In HR proficient cells these DNA double-strand breaks would be repaired by HR. In the absence of BRCA1, BRCA2, or other HR components, however, impaired repair would result in persistence of these breaks and lethality. Accordingly, cells with fully active PARP1 or an intact HR pathway (*BRCA1/2* wild type cells) would be expected to survive these endogenous DNA insults, whereas cells with an HR defect treated with a PARP inhibitor would not (124, 125).

TRAPPING OF PARP1 AT SITES OF DNA DAMAGE

An alternative model suggests that PARP1 becomes trapped on DNA in the presence of PARP inhibitors, thereby diminishing access of other repair proteins to damaged DNA. This model (**Figure 2B**) is based on some of the well-established characteristics of PARP1 reviewed above. In particular, PARP1 contains N-terminal zinc fingers that recognize damaged DNA, permitting PARP1 binding to various lesions (126), and increased pADPr synthesis (48, 127, 128). While PARP covalently modifies a wide range of substrates, most of the resulting pADPr is covalently bound to PARP1 itself (129), increasing the negative charge of the enzyme and eventually causing its dissociation from the DNA (51).

Studies performed over 20 years ago demonstrated that catalytically inactive PARP1, e.g., PARP1 lacking its substrate NAD^+, inhibits DNA repair under cell-free conditions (51). Additional experiments showed that the DNA binding domain of PARP1, which is able to recognize damaged DNA but not catalyze pADPr formation, also acts as a dominant negative to enhance the cytotoxicity of certain DNA damaging treatments in intact cells (130, 131). PARP1 that has been catalytically inactivated by treatment with an effective small molecule inhibitor would likewise be expected to inhibit repair. This mechanism has recently been found to account for the ability of PARP inhibitors to enhance the cytotoxicity of the topoisomerase I poison topotecan (132) and the DNA methylating

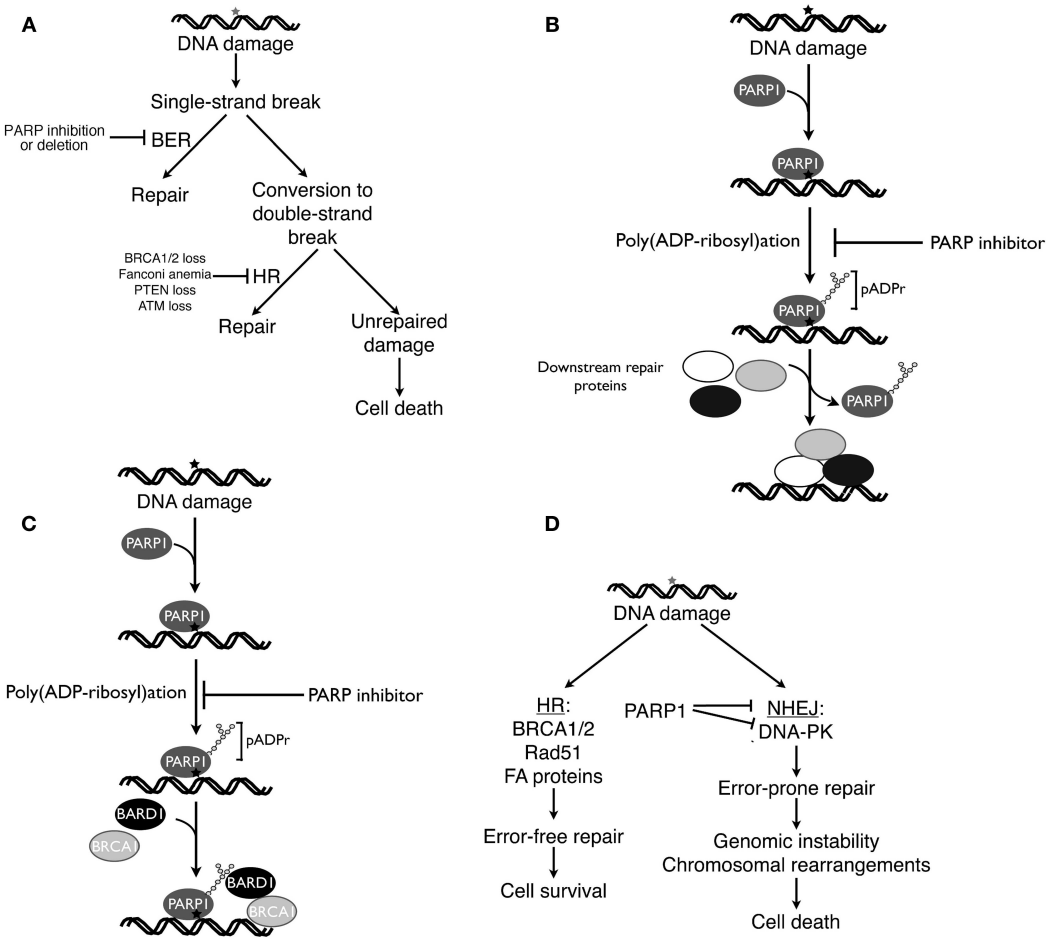

FIGURE 2 | Four current models of PARP inhibitor-induced cancer cell killing. **(A)**, classical explanation of PARP inhibitor cytotoxicity in HR-deficient cells (124, 125). As described in the text, endogenous DNA damage is thought to result in DNA single-strand breaks, which ordinarily would be repaired by base excision repair (BER). If PARP inhibitors prevent BER, then persistent single-strand breaks are thought to be converted to DNA double-strand breaks, which would be repaired by HR in HR-proficient cells but remain unrepaired in HR-deficient cells. **(B)** Model emphasizing trapping of inhibited PARP1 at sites of DNA damage. According to this model, PARP1 binds to damaged DNA, synthesizes polymer, and then is released from the DNA so that repair enzymes can bind (51). Building on these observations, this model postulates that PARP inhibition results in failure of PARP1 to dissociate from sites of damage, leading to diminished access of other repair proteins, inhibited repair, and cell death. **(C)** Model emphasizing impaired recruitment of mutated BRCA1 in the presence of PARP inhibitors. As described by Li and Yu (134), recruitment of BRCA1 to DNA double-strand breaks requires both rapid binding of the BRCA1 binding partner BARD1 to pADPr and subsequent binding of a BRCA1-containing complex to phosphorylated H2AX at the break. Mutations that impair recruitment of the BRCA1-containing complex to phosphorylated H2AX render BRCA1 localization to sites of damage more dependent on the BARD1-pADPr interaction and, therefore, more sensitive to PARP inhibitors. **(D)**, model emphasizing the role of activated NHEJ in PARP inhibitor killing. When DNA double-strand breaks occur, HR preferentially repairs them. In HR-deficient cells, however, double-strand breaks are more frequently repaired by the error-prone NHEJ pathway, resulting in mutations, chromosomal rearrangements, and NHEJ-mediated cell death. PARP inhibitors accelerate this process by removing a brake on NHEJ (116). **(A,D)** are modified from Patel et al. (116).

agent methylmethane sulfonate (MMS) (133). Extrapolating from these observations, it has been suggested that trapping of PARP1 at sites of endogenous DNA damage might account for the ability of PARP inhibitors to kill HR-deficient cells (**Figure 2B**).

DEFECTS IN RECRUITMENT OF BRCA1 TO SITES OF DNA DAMAGE

Li and Yu recently reported that recruitment and retention of BRCA1 at sites of DNA damage reflects two different processes, (i) an initial interaction between poly(ADP-ribose) polymer at the damage site and the BRCT domain of the BRCA1 binding partner BARD1 and (ii) subsequent slower binding of a BRCA1-containing protein complex to phosphorylated histone H2AX at the damage site (134). Mutations that impair BARD1 interactions with poly(ADP-ribose) polymer, BARD1-BRCA1 complex formation, or binding of the BRCA1-containing protein complex to phosphorylated H2AX all reduce survival after DNA damage. Moreover, in the presence of PARP inhibitors, the initial rapid recruitment of the BARD1-BRCA1 complex to sites of DNA damage is impaired, making the cells more dependent on phospho-H2AX-mediated BRCA1 recruitment. Conversely, when mutations in the BRCT domain of BRCA1 impair participation of BRCA1 in the complex that interacts with phospho-H2AX, recruitment of BRCA1 to

sites of DNA damage becomes dependent on poly(ADP-ribose)-mediated recruitment of BARD1 (134), providing another model to explain synthetic lethality between *BRCA1* mutations and PARP inhibitor treatment (**Figure 2C**).

NHEJ ACTIVATION

Although PARP1 clearly plays an important role in BER (14, 122), it is important to emphasize that PARP also regulates other repair processes (1, 30, 123, 135) as described above. Earlier observations suggested that a variety of DNA repair proteins, including Ku70, Ku80, and DNA-PKcs, can be regulated by ADP-ribosylation (135). In particular, Ku70, Ku80, DNA-PKcs, and more recently Artemis were identified as pADPr binding proteins (53–55). Moreover, the interactions of Ku70 and Ku80 with pADPr inhibit classical NHEJ (67, 136–138). These observations prompted several groups to examine the potential contribution of NHEJ pathway activation to PARP inhibitor-induced killing of HR-deficient cells.

Collectively, these studies have provided several pieces of evidence suggesting an important role for NHEJ activation in PARP inhibitor-induced killing. PARP inhibitor treatment results in DNA-PKcs activation in HR-deficient cells, as manifested by DNA-PKcs autophosphorylation and phosphorylation of the downstream substrate H2AX in a DNA-PK-dependent fashion (116). This PARP inhibitor-induced DNA-PKcs activation is accompanied by increased NHEJ activity as indicated by assays for repair of a plasmid that has a DNA double-strand break (116). Moreover, PARP inhibitors selectively induce chromosomal rearrangements and mutations in HR-deficient cells (15, 116). Importantly, this PARP inhibitor-induced increase in chromosomal rearrangements and mutations is diminished by simultaneous treatment of HR-deficient cells with a selective DNA-PK inhibitor (116). Likewise, the cytotoxicity of PARP inhibitors is diminished by manipulations that diminish NHEJ activation, including Ku80 siRNA (116), DNA-PKcs inhibition (116), or DNA-PKcs deficiency (116, 139, 140). Based on these results, a model for PARP inhibitor-induced cytotoxicity that emphasizes activation of the NHEJ pathway has been proposed (**Figure 2D**). In this model, some endogenous source of DNA damage results in DNA double-strand breaks. If cells are HR proficient, the HR pathway repairs this damage with high fidelity. If cells are HR deficient, however, then end resection-dependent NHEJ is activated (116) and contributes to error-prone repair that results in mutations and chromosomal rearrangements (**Figure 2D**).

Consistent with this model, deletion of 53BP1, which is required for NHEJ pathway activation, leads to PARP inhibitor resistance (141). Likewise, 53BP1 loss was shown to rescue the lethality of deleterious *BRCA1* mutation in mouse models (142, 143), suggesting that *BRCA1* deficiency kills mouse cells by activating NHEJ.

THE ELEPHANT AND THE BLIND MEN

Like the blind men examining the elephant, each of these models emphasizes a different aspect of PARP1 biology. Just as none of the blind men in the parable could provide a complete description of the elephant, we believe that the present models explain certain facets of PARP inhibitor-induced lethality but also leave some questions unanswered.

The role of poly(ADP-ribose) polymers in recruitment of BRCA1 to sites of DNA damage

The observations summarized in **Figure 2C** provide substantial new insight into the recruitment of BRCA1 to sites of DNA damage. Nonetheless, this model fails to explain PARP inhibitor sensitivity of HR-deficient cells in general. As the authors themselves point out, this model cannot explain the enhanced PARP inhibitor sensitivity of cells that totally lack BRCA1 (as opposed to expressing a BRCT domain mutant). Moreover, it is unclear how this model accounts for the synthetic lethality observed when cells lacking BRCA2, Rad51, or other downstream components of the FA/HR pathway are treated with PARP inhibitors (15, 98).

Trapping of PARP1 at sites of DNA damage

We are concerned that the model shown in **Figure 2B** also fails to account for critical observations regarding PARP inhibitor-induced killing. In particular, this model is a classical enzyme poisoning model, where the inhibited enzyme becomes an agent that contributes to cellular demise. This type of model, for example, accounts for the cytotoxicity of topoisomerase I poisons such as camptothecin (144). For this class of drugs, the poisoning model accounts for a number of critical observations: (i) loss of the target enzyme is not lethal (145, 146); and (ii) because the lethality results from the cytotoxic action of the inhibited enzyme rather than the inhibition of product production, the killing effect is observed at concentrations far below those that inhibit all activity of the enzyme (144). Importantly, this type of model accurately predicts that elevated expression of the target enzyme will increase the lethality of drugs that poison the enzyme and diminished expression of the target enzyme will decrease the lethality of the poisons (144).

Recent reports suggest that PARP inhibitors sensitize to certain DNA damaging agents by poisoning PARP1 (**Figure 2B**) as proposed by Lindahl and coworkers two decades ago (51). In particular, it has been reported that cells selected for resistance to the DNA methylating agent temozolomide in combination with the PARP inhibitor veliparib express markedly diminished levels of PARP1 (147). As the authors point out, this is difficult to explain if PARP inhibitors are sensitizing cells by diminishing total cellular levels of poly(ADP-ribose) polymer below a critical threshold (catalytic inhibition) but are readily understood by the poisoning model put forward in **Figure 2B**. Likewise, recent studies of topoisomerase I poison/PARP inhibitor combinations are also compatible with this type of PARP1 poisoning model (132). In particular, PARP1 downregulation or knockout abolishes the ability of the PARP1 inhibitor veliparib to sensitize cells to topotecan or camptothecin, establishing PARP1 as the critical target for this sensitization. Importantly, however, PARP1 knockdown or knockout does not result in cells that are hypersensitive to camptothecin or topotecan (132). Instead, $Parp1^{-/-}$ cells and $Parp1^{+/+}$ cells exhibit identical camptothecin sensitivity in the absence of PARP inhibitors (132), suggesting that PARP1 catalytic activity is not essential for camptothecin resistance. *Parp1* gene deletion likewise protects chicken DT40 cells from the methylating

agent MMS in combination with PARP inhibitors without rendering the cells hypersensitive to MMS alone (133), suggesting that PARP1 catalytic activity is also not required for MMS resistance. Consistent with a poisoning model, further experiments examining the topoisomerase I poison/PARP inhibitor combination have shown that transfection of *Parp1*$^{-/-}$ cells with catalytically inactive PARP1 or the isolated PARP1 DNA binding domain sensitizes to camptothecin just like treating *Parp1*$^{+/+}$ cells with a PARP inhibitor (132). Collectively, these observations suggest that trapping of inhibited PARP1 on damaged DNA, which has previously been reported to prevent access of repair complexes (51), contributes to the cytotoxicity of certain types of drug-induced DNA lesions (133, 147, 148) as illustrated in **Figure 2B**.

On the other hand, it is difficult to see how the poisoning model in **Figure 2B** can account for the synthetic lethality between HR deficiency and PARP inhibition. As described above, this type of model in which the inhibited enzyme is the lethal agent predicts that cells lacking PARP1 will be resistant to PARP inhibitors and cells containing elevated PARP1 levels will be hypersensitive. Contrary to this prediction, a number of groups have demonstrated that PARP1 downregulation kills BRCA1/2-deficient cells (15, 16, 116), suggesting that PARP inhibitors are killing *BRCA1/2*-deficient cells by diminishing the production of poly(ADP-ribose) polymer rather than trapping PARP1 at sites of DNA damage.

BER inhibition

In contrast to the preceding model, the classical model that focuses on the role of PARP1 in BER (**Figure 2A**) is consistent with the observation that PARP knockdown kills HR-deficient cells. It should also be acknowledged that this model provided part of the rationale for testing PARP inhibitors in BRCA2-deficient cells in the first place (16). Nonetheless, this model makes several predictions that have been difficult to verify experimentally.

First, the model predicts that DNA ss breaks will accumulate after PARP inhibition. Work by Helleday and coworkers, however, has demonstrated no induction of ss breaks by PARP inhibitors (149, 150). It is, of course, possible that the putative PARP inhibitor-induced ss breaks are converted to DNA double-strand breaks so rapidly that they are not detected. Further study of this issue, perhaps with more sensitive assays for DNA ss breaks, appears to be warranted.

A second issue relates to the reported effects of XRCC1 knockdown. If ss break repair is playing a critical role in the cytotoxicity of PARP inhibitors, then the effect of downregulating other ss break repair components such as the scaffolding protein XRCC1 immediately downstream of PARP1 (151) should recapitulate the effect of PARP1 downregulation. However, XRCC1 downregulation has no impact on survival of *BRCA2*-mutant PEO1 ovarian cancer cells, whereas PARP1 downregulation is cytotoxic (116). Importantly, the XRCC1 knockdown was sufficient to sensitize the cells to MMS, suggesting that BER had been inhibited. These results imply that PARP1 exerts a role outside of ss break repair in HR-deficient cells (116).

Collectively, these observations call into question the suggestion that PARP inhibitors are inducing so-called synthetic lethality in the setting of HR by inhibiting ss break repair. Further testing of additional predictions of the model shown in **Figure 2A** is clearly needed.

NHEJ activation

As indicated above, a number of observations suggest that NHEJ plays a critical role in PARP inhibitor-induced killing (15, 116, 139–141). The model shown in **Figure 2D**, which emphasizes the role of PARP in regulating NHEJ, is consistent with these observations. Nonetheless, a number of questions about this model also remain unanswered.

First, it is unclear whether all components of the NHEJ pathway contribute equally to PARP inhibitor sensitivity. Available studies only show what happens if 53BP1, Ku80, or DNA-PKcs is disabled. In view of observations that "atypical" NHEJ can occur in the absence of certain components (110), it remains to be determined whether loss of Artemis, XRCC4, ligase 4, or other NHEJ components has the same impact on PARP inhibitor sensitivity.

Second, the available data suggest that inhibiting the NHEJ pathway diminishes cytotoxicity of PARP inhibitors in HR-deficient cells. However, additional research is needed to determine how these cells survive and repair DNA double-strand breaks if HR and NHEJ are both disabled.

Third, preclinical and clinical studies have suggested that PARP inhibitors are particularly effective in tumors that have deleterious mutations in HR pathway genes such as *BRCA1* and *BRCA2*. In contrast, tumors such as triple negative breast cancer that have *BRCA1/2* gene methylation appear to be less sensitive. It is unclear whether this reflects incomplete inhibition of the HR pathway by methylation, or whether NHEJ pathway genes might also be methylated in these tumors, leading to a repair status similar to *BRCA2*-mutant cells in which NHEJ components have been downregulated.

Finally, the model summarized in **Figure 2D** fails to specify the source of DNA damage that activates the NHEJ pathway. Given the importance of this putative damage to PARP inhibitor-induced killing, this question clearly warrants further study.

Should the models be combined?

Like the blind men in the parable, perhaps we can better understand the true nature of the elephant by merging several incomplete pictures. For example, it has been suggested (150) that inhibition of ss break repair (**Figure 2A**) might generate the DNA double-strand breaks (**Figure 2D**) that activate NHEJ and contribute to the cytotoxicity of PARP inhibitors. This would certainly be consistent with some of the known roles of PARP1 in DNA repair described above. On the other hand, the failure of PARP inhibitors to increase DNA ss breaks (149), like the failure of XRCC1 downregulation to reproduce the effects of PARP1 downregulation in BRCA2-deficient cells (116), raises concern that the hybrid model might not adequately account for the DNA damage that contributes to NHEJ-mediated killing. Given the other roles of PARP1, e.g., in restarting stalled replication forks (68–71), it is equally plausible that PARP inhibitor-induced collapse of stalled replication forks or disruption of some other PARP1-mediated process provides the DNA double-strand breaks that trigger NHEJ. Clearly, like the blind men, we require additional information to generate a coherent picture.

TRANSLATION TO THE CLINIC: WHY THE CORRECT MECHANISM MATTERS

In contrast to chronic myelogenous leukemia, where the vast majority of patients respond to a Bcr/Abl kinase inhibitor (152), or BRAF V600E-mutant melanoma, where the response to vemurafenib is also above 50% (153, 154), early studies have suggested that PARP inhibitors have only a 30–40% response rate in *BRCA1/2*-mutant ovarian and breast cancers (19–21). In an era of increasingly personalized cancer treatment, a less than 50% chance of responding to a supposedly tailored therapy is somewhat disconcerting (22). By understanding the mechanistic basis for the synthetic lethality between HR deficiency and PARP inhibition, it might be possible to better understand why some HR-deficient cancers respond and others do not.

The models described above make different predictions about the cancers most likely to benefit from PARP inhibitor therapy. For example, the poisoning model shown in **Figure 2C** predicts that HR-deficient tumors with elevated PARP1 levels should be hypersensitive to PARP inhibitors. In contrast, the models shown in **Figures 2A,D**, which emphasize catalytic inhibition of PARP1 as the triggering event, predict that HR-deficient tumors with lower PARP1 levels will, if all other factors are equal, be more sensitive to PARP1 inhibitors because they will require less drug to decrease poly(ADP-ribose) polymer levels below a critical threshold. The model shown in **Figure 2D** further predicts that HR-deficient cancers with diminished levels of NHEJ proteins will be relatively resistant to PARP inhibitors, whereas the model in **Figure 2A** predicts that HR-deficient cancers with diminished levels of NHEJ proteins will be more sensitive to PARP inhibitors because they are dependent on NHEJ for repair of DNA double-strand breaks in the absence of HR.

In order to understand why some HR-deficient cancers respond to PARP inhibitors and others do not, these predictions need to be tested in the clinic. In addition, it will also be important to assess the relationship between response and more classical determinants of drug sensitivity such as levels of the target enzyme PARP1 or drug uptake and efflux.

In order for these correlative studies to proceed, it will be important for patients enrolling in PARP inhibitor trials to undergo biopsies prior to drug treatment to determine the status of DNA repair pathway genes. Whenever possible, investigators are also encouraged to obtain additional biopsies at the time of progression in order to determine the properties of cells that have resisted PARP inhibitor treatment. In this way, future studies can potentially allow identification of patients most likely to benefit from PARP inhibitor treatment.

In summary, current models describing the mechanistic basis for selective killing of HR-deficient cells by PARP inhibitors emphasize different aspects of PARP1 biology. Just as the blind men needed more information to make sense of the elephant, we need additional information in order to understand the action of these promising new agents. Given the need to improve the therapeutic outcomes for patients with HR-deficient tumors such as high-grade serous ovarian cancer, as well as the tantalizing activity of PARP inhibitors in this setting, further preclinical and clinical efforts to understand this new class of agents appear to be warranted.

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Strategic combination of DNA-damaging agent and PARP inhibitor results in enhanced cytotoxicity

Julie K. Horton and Samuel H. Wilson*

Laboratory of Structural Biology, NIEHS, National Institutes of Health, Research Triangle Park, NC, USA

Edited by:
Christina Annunziata, National Cancer Institute, USA

Reviewed by:
Eddie Reed, University of South Alabama, USA
Martijn Luijsterburg, Leiden University Medical Center, Netherlands

****Correspondence:***
Samuel H. Wilson, Laboratory of Structural Biology, NIEHS, National Institutes of Health, 111 T.W. Alexander Drive, P.O. Box 12233, Research Triangle Park, NC 29909-2233, USA
e-mail: wilson5@niehs.nih.gov

PARP inhibitors (PARPi) are under clinical trial for combination cancer chemotherapy. In the presence of a PARPi, PARP-1 binds DNA strand breaks but cannot produce poly(ADP-ribose) polymers or undergo auto-poly(ADP-ribosyl)ation. DNA binding is persistent, hindering DNA repair. Methylated bases formed as a result of cellular exposure to DNA-methylating agents are repaired by DNA polymerase β (pol β)-dependent base excision repair (BER) producing a 5′-deoxyribose phosphate (5′-dRP) repair intermediate. PARP-1 binds and is activated by the 5′-dRP, and PARPi-mediated sensitization to methylating agents is considerable, especially in pol β-deficient cells. Cells deficient in the BER factor XRCC1 are less sensitized by PARPi than are wild-type cells. PARPi sensitization is reduced in cells expressing forms of XRCC1 deficient in interaction with either pol β or PARP-1. In contrast, agents producing oxidative DNA damage and 3′- rather than 5′-repair intermediates are modestly PARPi sensitized. We summarize PARPi experiments in mouse fibroblasts and confirm the importance of the 5′-dRP repair intermediate and functional pol β and XRCC1 proteins. Understanding the chemistry of repair is key to enhancing the clinical success of PARPi.

Keywords: DNA polymerase β, XRCC1, PARP-1, PARP inhibitors, base excision repair

BACKGROUND

Clinical trials suggest that PARP inhibitors (PARPi) may represent an opportunity to gain selective killing of cancer cells, since the cytotoxic effects make use of deficiencies in cellular DNA repair systems that are distinctive for individual tumor cells versus normal tissues (1, 2). But it has proved difficult to design chemotherapy regimes because of toxic side effects such as myelosuppression. Information enabling prediction of PARPi effects is not easy to gain from the literature and may not be well recognized in the community. We suggest that understanding PARPi effects in model systems, such as mouse embryonic fibroblast (MEF) cells in culture, will be informative for considering strategies in cancer chemotherapy. We have discussed this viewpoint in a recent article (3). Here, we summarize current experiments with the aim of understanding the roles of PARP in mammalian cell DNA repair and how the presence of the inhibited PARP-1 protein during base excision repair (BER) may promote cell killing. The level of cell killing observed with DNA-damaging agents is modulated by co-treatment with a PARPi and by expression of other BER proteins such as XRCC1 and pol β, and we will outline a model to explain these effects. Selection of specific chemotherapeutic agents combined with specific repair deficiencies in patients may prove to be extremely beneficial.

BER OF BASE DAMAGE AND BINDING OF PARP-1 TO INTERMEDIATES OF BER

The mammalian BER pathway is important for the removal of single base lesions in double-stranded genomic DNA. Base damage can arise through spontaneous base loss from DNA or from base alkylation and oxidation from both endogenous and exogenous sources. Methyl methanesulfonate (MMS) is a directly acting DNA-methylating agent causing alkylation of base nitrogens (e.g., 7-methylguanine), whereas the oxidizing agent peroxynitrite produces reactive oxygen species (ROS) that oxidize DNA bases resulting in the promutagenic DNA lesion 8-oxoguanine and other base lesions. During single-nucleotide BER of a methylated base, repair is initiated by a lesion-specific monofunctional glycosylase (i.e., *N*-methylpurine DNA glycosylase; MPG), that removes the damaged base leaving an abasic (AP) site in double-stranded DNA. The DNA backbone is then incised 5′ of the AP site by AP endonuclease 1 (APE1) resulting in a 1-nucleotide (nt) gap with margins of 3′-OH and 5′-deoxyribose phosphate (dRP) groups. DNA polymerase β (pol β) binds to this repair intermediate, removes the 5′-dRP group and performs single-nucleotide gap filling DNA synthesis. Many of the glycosylases specific for oxidative DNA damage (e.g., 8-oxoguanine DNA glycosylase; OGG1) are bifunctional enzymes that have an associated AP lyase activity in addition to their glycosylase activity. After base removal, this activity cleaves the DNA backbone 3′ to the abasic site leaving 3′-dRP and 5′-PO_4 margins in a single-nucleotide gap. APE1 is able to remove the 3′-blocking group leaving a 3′-OH-containing substrate suitable for DNA synthesis and ligation. In this BER sub-pathway there will be no formation of a 5′-deoxyribose-containing blocking group or requirement for pol β-dependent dRP lyase tailoring activity to enable DNA ligation (4).

PARP-1 is an abundant nuclear protein involved in DNA damage recognition. It can bind to AP sites and single-strand breaks in DNA, including the 5′-dRP-containing intermediate of BER of MMS-induced damage. Once bound to DNA, PARP-1 becomes catalytically activated synthesizing poly(ADP-ribose) (PAR) polymers from NAD^+, and resulting in poly(ADP-ribosyl)ation of itself, as well as other proteins involved in DNA repair and

chromatin remodeling (5, 6). PARP-1, the first discovered member of a family of proteins, is responsible for the majority of cellular PARP activity after DNA damage. Following auto-modification, PARP-1 can interact with other BER proteins such as XRCC1 and pol β enabling their recruitment to the damage site (7, 8). A recent publication has suggested that PARP-1 recruits XRCC1 to single-strand break repair, but not to sites of oxidative damage BER (9). This may be due the absence of 5′-dRP intermediate formation during oxidative damage (8-oxoguanine) repair (4).

In the case of methylation damage, after removal of the abasic site sugar by pol β lyase activity and completion of repair by pol β gap filling and DNA ligation, PARP-1 dissociates from DNA, and the PAR glycosidic bonds are rapidly cleaved, primarily by poly(ADP-ribose) glycohydrolase (PARG) (10). In earlier photoaffinity labeling studies, PARP-1 was identified as the predominant BER intermediate-binding factor in the MEF cell extract (11). Use of other binding ligands revealed PARP-1 binding specificity for the 5′-dRP-containing BER intermediate with much less binding when an alternate BER intermediate without the 5′-dRP group was used (12). The results are consistent with a biological role for an interaction between PARP-1 and the 5′-dRP-containing BER intermediate. Additionally, as discussed below and elsewhere (3), and in agreement with the *in vitro* studies, we find that the cytotoxic effects of cellular PARP inhibition correlate very well with the presence of the 5′-dRP group in the BER intermediate.

PARP INHIBITION AND HYPERSENSITIVITY TO DNA DAMAGE

In the presence of a catalytic inhibitor, PARP-1 can still bind to DNA damage sites, but auto-ribosylation is prevented (1). In its inhibited and inactivated state, PARP-1 binding to DNA is stabilized, hindering the BER process (13). We have proposed that the DNA-bound and inhibited PARP-1 molecule results in cytotoxicity due to formation of replication-dependent double-strand breaks (DSBs) (14).

Experiments in MMS-treated MEFs demonstrated that PAR synthesis was completely inhibited by the PARPi 4-amino-1,8-naphthalimide (4-AN) (15, 16). Wild-type (WT) MEFs are highly (40-fold) sensitized to MMS and to the methylating chemotherapeutic agent temozolomide (TMZ) by 4-AN co-treatment (17). Positive TMZ/PARPi potentiation data have been reported in a number of other systems, e.g., human tumor cell lines and xenografts (18, 19), and this combination has been successful in phase I clinical trials in patients with solid tumors (20) or melanoma (21). Additionally, a recently reported phase II study of an inhibitory dose of a PARPi with TMZ in metastatic melanoma provided evidence for chemopotentiation and increased disease-free survival (22). The authors suggest the need for a phase III trial comparing TMZ with TMZ + PARPi, also for evaluation of DNA repair capacity in patients to identify those most likely to benefit from this combination.

In contrast to the results with TMZ and MMS, co-treatment with 4-AN has minimal effect (1.1-fold sensitization) on cellular sensitivity to the reactive oxidant peroxynitrite (17). This agent results in oxidative DNA modifications including 8-oxoguanine, 8-nitroguanine and single-strand breaks (23). Repair of 8-oxoguanine initiated by the bifunctional OGG1 is not expected to produce the 5′-dRP blocked repair intermediate. Thus, a key difference in BER following treatment with these two agents (MMS and peroxynitrite) is initiation by a monofunctional versus a bifunctional glycosylase. Only in the former case (repair of MMS damage by a monofunctional glycosylase) will there be formation of a repair intermediate with a 5′-sugar phosphate blocking group. The results emphasize that the presence of the 5′-dRP blocking group is critical for binding PARP-1 and for observing PARPi-mediated sensitization to DNA damage.

PARP INHIBITOR EFFECTS IN BER PROTEIN-DEFICIENT AND DEFECTIVE CELLS

The most notable phenotype of pol β null MEFs is hypersensitivity to S_N2 alkylating agents such as MMS, and to S_N1 alkylating agents such as the chemotherapeutic methylating agent TMZ (24, 25). Hypersensitivity to these agents in pol β-deficient mouse fibroblasts can be reversed by expression of either the full-length protein or the 8 kDa dRP lyase domain with 5′-dRP gap-tailoring activity (26). XRCC1-deficient cells are extremely hypersensitive to monofunctional methylating agents including MMS and TMZ (4). XRCC1 interacts with a number of repair proteins and binding to PARP-1 is critical for recruitment of XRCC1 to damaged sites in DNA. Thus, in PARP-1-deficient cells, recruitment of XRCC1 is hindered (7). The interaction between the amino-terminal domain (NTD) of XRCC1 and the polymerase domain of pol β is essential for recruitment of pol β to sites of damaged DNA (27). Hypersensitivity to MMS can be reversed by transfection of full-length WT XRCC1 protein into *Xrcc1*$^{-/-}$ cells (28), but as observed previously in CHO cells (29), only partial reversal is observed following expression of a mutant protein (V88R) that does not interact with pol β. Likewise, there is no rescue of hypersensitivity following expression of the L360R mutant XRCC1 protein that has disrupted folding of the BRCT I domain and interrupted interaction with PARP-1 (30, 31). The results suggest that interactions between PARP-1, XRCC1, and pol β are required for the protective effects of XRCC1 and pol β against MMS and TMZ exposures.

A high level of sensitization to MMS and TMZ is observed in both *pol* β$^{+/+}$ and *pol* β$^{-/-}$ MEFs following combination treatment with 4-AN. Interestingly, the level of sensitization of *pol*β$^{-/-}$ cells is at least double that observed in *pol*β$^{+/+}$ cells (**Figure 1A**). Thus, when utilizing the TMZ + PARPi combination, pol β null cells become considerably more TMZ-sensitive than WT cells. Similar pol β-dependent results were obtained with other agents (MMS, MNU) that result in DNA damage repaired by monofunctional glycosylase-initiated BER. We propose that through its role in removing the 5′-dRP intermediate, pol β is able to regulate the PARPi-mediated sensitization in TMZ cytotoxicity. There have been numerous reports of cancer related pol β single-nucleotide polymorphisms (32, 33). Expression of a dRP lyase inactivating mutation would be a critical biomarker for enhancement of TMZ + PARPi cytotoxicity. Additionally, current assays for dRP repair intermediates are used with cell culture models in laboratory research, but have not yet been adapted for clinical use. Such adaptation of these techniques represents an opportunity for translational research. Ongoing studies will address this question.

In contrast *Xrcc1*$^{+/+}$ WT cells are more highly sensitized (two to threefold) to MMS and TMZ than are *Xrcc1*$^{-/-}$ cells

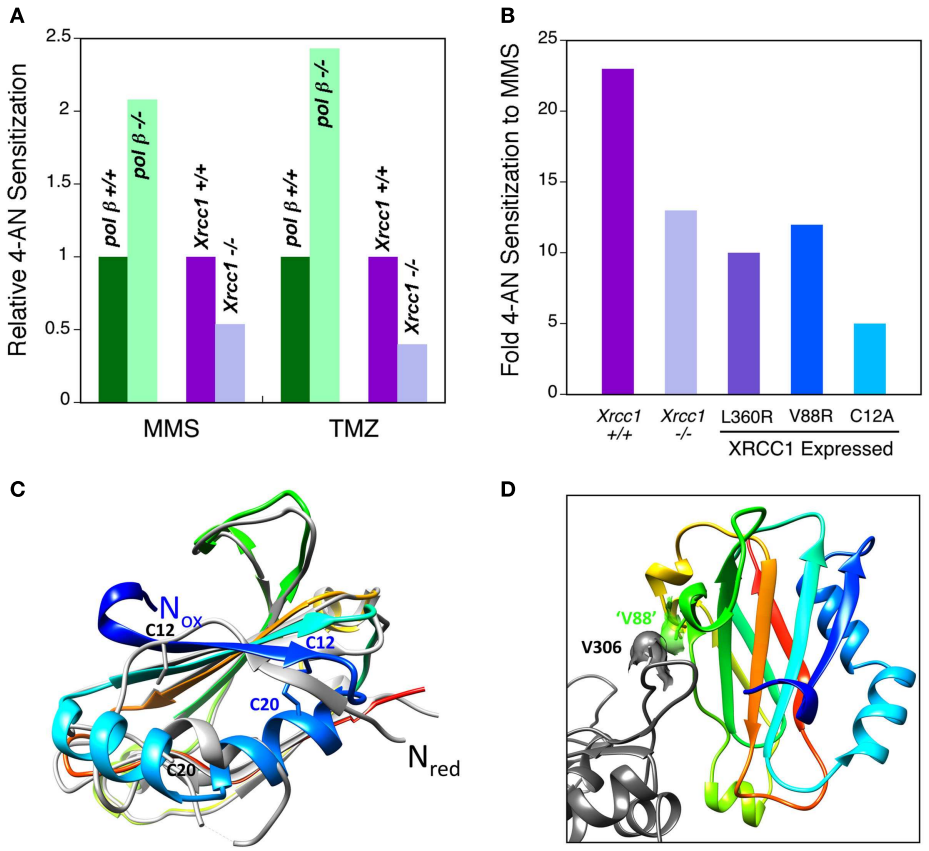

FIGURE 1 | PARPi-mediated sensitization to MMS and TMZ and ability of XRCC1 to interact with pol β. **(A)** Relative sensitization in wild-type and repair protein-deficient MEFs (as indicated) by a 24 h exposure to the PARPi 4-AN. Pol β-deficient cells are more highly sensitized than the wild-type line (green), while XRCC1-deficient cells are less sensitized (purple). **(B)** Level of PARPi-mediated sensitization to MMS in *Xrcc1*$^{+/+}$ (WT) and *Xrcc1*$^{-/-}$ (null) MEFs, and in XRCC1 null cells expressing mutated XRCC1 proteins (L360R, V88R and C12A) as indicated. **(C)** The XRCC1 NTD has been crystallized in two forms: oxidized and reduced (34). An overlay of the oxidized (colored, PDB ID 3LQC) and reduced (light gray, PDB ID 3K75) forms indicates that the amino-termini are on opposite sides of this domain (N_{ox} and N_{red}, respectively). Accordingly, the interactions around the amino-termini are very different for these two forms. The cysteine residues (C12 and C20, respectively) that participate in disulfide bond formation in the oxidized form are indicated. **(D)** 'V88' (green) of mouse NTD forms a hydrophobic interaction with V306 (gray) of pol β. This portion of the pol β-binding interface is similar for both the oxidized and reduced forms of the NTD, and includes the hydrophobic interaction of XRCC1 'V88' with V306 of pol β. V88 corresponds to V86 of the structurally characterized human NTD of XRCC1. Replacing this valine with arginine (V88R) significantly reduces the interaction between these proteins (28).

(**Figures 1A,B**). Thus, the interaction between XRCC1 and PARP-1 proteins appears to be required for the strongest PARP-inhibitor-mediated sensitization. Expression of WT XRCC1 will stabilize the protein complex through its accessory protein functions, and this will allow for more efficient PARP binding to the 5′-dRP-containing BER intermediate. Another possibility, that XRCC1 may modulate the dRP lyase activity of pol β, is being tested in the laboratory. Sensitization in cells expressing the L360R mutated XRCC1 protein without interaction with PARP-1 (30, 31) was similar to that in *Xrcc1*$^{-/-}$ cells (**Figure 1B**), consistent with the proposal that the interaction between XRCC1 and PARP-1 enables the sensitization. In *Xrcc1*$^{-/-}$ cells expressing an XRCC1 mutant (V88R) that is compromised in its ability to bind pol β, sensitization to MMS was also about half of the level observed in WT cells (**Figure 1B**).

Pol β and XRCC1 interact through a redox-sensitive binding interface in the N-terminal domain (NTD) of XRCC1 (34), and equal levels of both oxidized and reduced forms of the full-length protein are found in untreated WT MEFs (28). Structural characterization of both oxidized and reduced forms of the XRCC1 NTD reveal that they have distinct conformations (**Figure 1C**) and a different pol β functional interaction, with the oxidized form binding tighter to pol β (34). The disulfide bond between C12 and C20 required for stabilizing the oxidized form is evident in the structure shown, whereas C12 and C20 are far apart in the reduced form (**Figure 1C**). Nevertheless, some portions of the pol β-binding interface are similar for both the oxidized and reduced forms of the NTD, and this includes the hydrophobic interaction between V306 (**Figure 1D**) of pol β and V88 of mouse XRCC1 NTD ("V88"). Cells expressing C12A XRCC1 protein locked in the reduced state are equally as MMS resistant as

WT cells (28). However, cells expressing reduced C12A XRCC1 have a considerably lower level of PARPi-mediated sensitization than WT cells (5- and 23-fold, respectively) (**Figure 1B**). These results are consistent with the requirement for tight XRCC1-pol β interaction for strong PARPi-mediated sensitization (V88R in **Figure 1B**). However, the extremely low PARPi-mediated sensitization in the cells expressing the reduced XRCC1 protein suggests there may be additional XRCC1 effects linked to its ability to take the oxidized form.

Pol β null cells are minimally hypersensitive to DNA oxidants such as peroxynitrite, IR, and bleomycin where repair of oxidative DNA damage does not involve significant formation of an intermediate with a 5′-sugar phosphate. The low PARPi sensitization observed in WT cells for peroxynitrite co-treatment was also seen in pol β-deficient cells (17), and similar data (≤3-fold sensitization) were obtained for clinically utilized IR and the radiomimetic agent bleomycin. Bleomycin results in formation of ROS, oxidized sugars and abasic sites with 3′-blocking groups such as 3′-phosphoglycolate (35), and repair may involve pol β and BER, but the 5′-sugar phosphate blocking group is not abundantly formed. Again the results suggest a requirement for a 5′-sugar phosphate-containing repair intermediate for significant cellular hypersensitivity in pol β-deficient cells. Similarly, despite the hypersensitivity of *Xrcc1*$^{-/-}$ cells to methylating agents, only low-level hypersensitivity is observed to oxidative DNA damage (4).

Taken together, these results are consistent with a correlation between formation of the 5′-dRP blocking group and the degree of PARPi-mediated sensitization. In the absence of pol β, cells will be deficient in the 5′-dRP gap-tailoring activity, allowing for enhanced binding of PARP-1 to DNA damage and for more PARPi-mediated sensitization. These cells therefore demonstrate the concept of synthetic lethality occurring under conditions of PARP inhibition in the presence of pol β-deficiency. The notion of synthetic lethality explains the vulnerability of cells that are deficient in one pathway in repair (here pol β-mediated BER) and then have repair additionally blocked by a chemical agent (e.g., a PARPi). A similar well-appreciated situation occurs when PARPi are used in BRCA- and other homologous recombination-deficient cells and tumors (36–38). The expression level of specific repair proteins is expected to modulate the degree of PARPi-mediated sensitization. The chemistry of DNA damage and repair also regulates PARPi effects, since in the absence of the 5′-dRP group-containing repair intermediate, there is minimal PARPi-mediated sensitization.

MODEL FOR PARP INHIBITOR-MEDIATED CELL KILLING

PARP inhibitors have become valuable in chemotherapy as part of a combination regime or as monotherapy. In MEF model systems, the magnitude of the cell killing effect of a PARPi in combination with a genotoxic agent is dependent on the chemistry of the DNA repair intermediate. Inhibition of PARP when it is bound to a 5′-dRP group-containing intermediate results in a dramatic cell sensitization. In contrast, if the repair intermediate does not have the 5′-dRP group, both PARP-1 binding and inhibitor-mediated sensitization are minimal.

A schematic model consistent with these results is shown in **Figure 2**. It is important to note that the current results do not prove this model, but instead the model is useful as a framework for designing future experiments. The model illustrates a replication fork colliding with the BER repair protein complex bound at the 5′-dRP-containing site in double-stranded genomic DNA. The replication fork moves in the direction of the arrow and becomes stalled at the protein complex, consisting of PARP-1, pol β, and XRCC1, among other proteins not shown in the image. Replication fork stalling is proposed to lead to fork collapse, DSB formation, and eventually to cell death. Thus, fork stalling is proportional to cell killing, at least in the context of this model. The model predicts that in the absence of inhibited PARP-1 or the 5′-dRP group, the protein complex will not form.

Pol β is able to remove the 5′-dRP group from repair intermediates. In pol β null BER-deficient MEFs, excess 5′-dRP

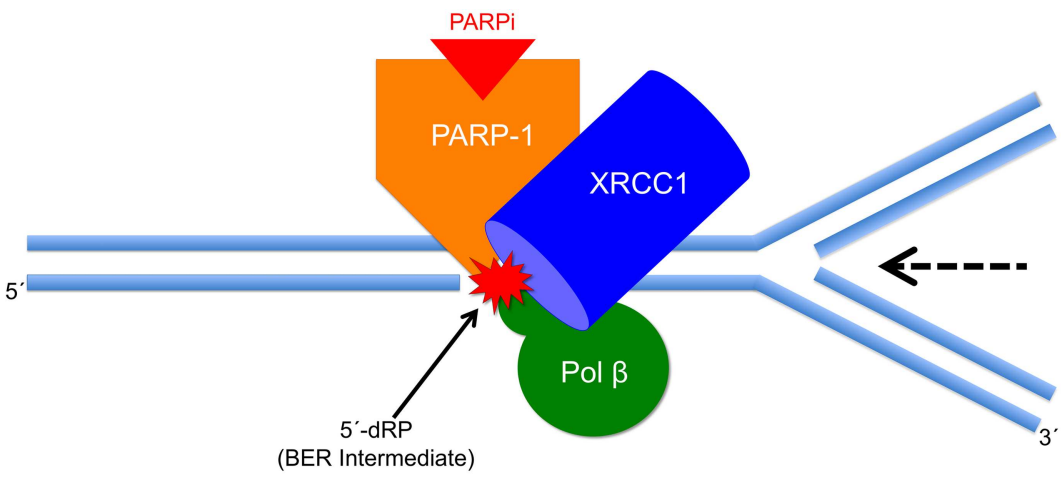

FIGURE 2 | Schematic model illustrating PARPi-mediated cell killing. Shown is a replication fork colliding with the BER repair complex bound at the 5′-dRP of the BER intermediate. The replication fork moves in the direction indicated by the arrow and becomes stalled at the protein complex. We propose that stalling leads to replication fork collapse, DSB formation, and cell death.

group-containing intermediates may accumulate, and PARP-1 binding and PARPi-mediated sensitization will be considerable. The model illustrates that the dRP group is key for PARP-1 binding, such that in the absence of pol β dRP lyase activity, there is more PARP-1 binding and more PARPi-induced cell killing. In the absence of XRCC1, pol β binding at damaged DNA is decreased and this is expected to lead to diminished dRP group removal and more cell killing. Further, the model predicts that in the absence of XRCC1 the stability of the complex will be reduced, and consequently the replication fork may be able to bypass the complex without stalling. The weaker affinity of the reduced form of XRCC1 for pol β is consistent with a less stable overall complex, more replication fork bypass, and less PARPi-mediated cell killing as observed experimentally. The results are consistent with this prediction in that the absence of XRCC1 expression, or less binding of XRCC1 to PARP-1 or pol β, is associated with lower PARPi-mediated sensitization.

In summary, PARPi are under study for use in cancer chemotherapy and here we report that the ability for PARPi-induced sensitization in model mammalian cell lines (mouse fibroblasts) correlates with the chemistry of DNA repair intermediates. Surprisingly, we find that in the absence of the 5′-dRP group-containing repair intermediate, there is minimal PARPi-mediated sensitization. Additionally, we show that the presence of functional BER factors pol β and XRCC1 regulate PARPi-induced sensitization, but this is only under conditions where the 5′-dRP group is formed.

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The role of PARP inhibitors in the treatment of gynecologic malignancies

Raquel E. Reinbolt and John L. Hays*

Division of Medical Oncology, Department of Internal Medicine, The James Comprehensive Cancer Center, The Ohio State University Wexner Medical Center, Columbus, OH, USA

Edited by:
Kristin Zorn, Magee-Womens Hospital of UPMC, USA

Reviewed by:
Maria Felice Brizzi, University of Torino, Italy
Jian Lu, Johns Hopkins University, USA

****Correspondence:***
John L. Hays, Division of Medical Oncology, The James Comprehensive Cancer Center, 588 Biomedical Research Tower, 460 W 12th Avenue, Columbus, OH 43210, USA
e-mail: john.hays@osumc.edu

Gynecologic malignancies annually account for over 91,000 new cancer cases and approximately 28,000 deaths in the United States. Although there have been advancements in cytotoxic chemotherapies, there has not been significant improvement in overall survival in these patients. While targeted therapies have shown some benefit in many solid tumors, further development of these agents is needed for the treatment of gynecologic malignancies. Poly(ADP-ribose) polymerase (PARP) catalyzes the polyADP-ribosylation of proteins involved in DNA repair. Inhibitors of PARP were originally developed for cancers with homologous recombination deficiencies, such as those harboring mutations in *BRCA1* or *BRCA2* genes. However, pre-clinical research and clinical trials have suggested that the activity of PARP inhibitors is not limited to those with *BRCA* mutations. PARP inhibitors may have activity in cancers deficient in other DNA repair genes, signaling pathways that mitigate DNA repair, or in combination with DNA-damaging agents independent of DNA repair dysfunction. Currently there are seven different PARP inhibitors in clinical development for cancer. While there has been promising clinical activity for some of these agents, there are still significant unanswered questions regarding their use. Going forward, specific questions that must be answered include timing of therapy, use in combination with cytotoxic agents or as single-agent maintenance therapy, and whether there is a predictive biomarker that can be used with PARP inhibition. Even with large strides in the treatment of many gynecologic malignancies in recent years, it is imperative that we develop newer agents and methods to identify patients that may benefit from these compounds. The focus of this review will be on pre-clinical data, current clinical trials, and the future of PARP inhibitors in the treatment of ovarian, endometrial, and cervical cancer.

Keywords: ovarian cancer, cervical cancer, endometrial cancer, PARP inhibitor, olaparib, veliparib, rucaparib, niraparib

INTRODUCTION

Gynecologic malignancies annually account for over 91,000 new cancer cases and approximately 28,000 deaths in the United States (1). Effective screening for cervical cancer is available in many parts of the world, but there is no effective screening for endometrial or ovarian cancer. Many women with ovarian cancer, therefore, present with advanced stage disease for which cure is rare. Endometrial cancer is more commonly diagnosed early on, as patients are often symptomatic with postmenopausal bleeding. While there have been advancements in the development and administration of cytotoxic chemotherapies, there has not been significant improvement in overall survival in these patients. It is imperative that novel and effective treatment strategies are developed. Although targeted therapies have shown occasional benefit in some solid tumors, these agents have been largely ineffective for the treatment of gynecologic malignancies.

One area of recent interest in targeted therapies for many cancers has been the development of poly(ADP-ribose) polymerase (PARP) inhibitors. PARP catalyzes the polyADP-ribosylation of proteins involved in DNA repair. Inhibitors of PARP were shown to be highly selective for cancer cells that harbor homologous recombination (HR) deficiencies, such as those harboring mutations in *BRCA1* or *BRCA2* genes (2). PARP inhibitors cause an increase in single strand breaks (SSBs) in DNA that, if left unrepaired, will lead to double strand breaks (DSBs) when encountered by replication forks (3, 4). In the laboratory, HR-deficient cells are unable to maintain genomic integrity in the presence of a large number of DNA DSBs and are, therefore, exquisitely sensitive to PARP inhibition. This synthetic lethal interaction between PARP and BRCA has been proposed as a potential explanation for the

Abbreviations: ATP, adenosine triphosphate; ATM, ataxia telangiectasia-mutated; BER, base excision repair; DL, dose level; DSB, double strand breaks; EEC, endometrioid endometrial carcinoma; FANC, Fanconi anemia complementation group; HBOC, hereditary breast and ovarian cancer; HR, homologous recombination; MRE11, mitotic recombination 11; MSI, microsatellite instability; MTD, maximum tolerated dose; NAD^+, nicotinamide adenine dinucleotide; NSB1, Nijmegen breakage syndrome; ORR, objective response rate; PAR, poly(ADP) ribose; PARP, poly(ADP-ribose) polymerase; PFS, progression free survival; PI3K, phosphatidylinositide 3-kinase; PHTS, PTEN hamartoma tumor syndromes; PMBCs, peripheral blood mononuclear cells; RECIST, response evaluation criteria in solid tumors; SSBs, single strand breaks; TNBC, triple negative breast cancer; VEGFR, vascular endothelial growth factor receptor.

sensitivity of *BRCA* mutation cell lines to PARP inhibition. Preclinical research and clinical trials, however, have suggested that the activity of PARP inhibitors is not limited to those with *BRCA* mutations. PARP inhibitors may demonstrate synthetic lethality in cancers deficient in other proteins that mitigate DNA repair (5). McCabe et al. examined the effects of PARP inhibition on various cell lines deficient in RAD51, Fanconi anemia complementation group (FANC), and Nijmegen breakage syndrome 1 (NBS1), amongst other proteins involved in HR, and found that mutations of these individual proteins induced sensitivity to PARP (6). These findings suggest that the notion of synthetic lethality may be more broadly applied to cancers with an impaired HR pathway, not just those with *BRCA* mutations. This concept is frequently referred to as "BRCAness" or "BRCA-like" (7). The inhibition of SSB repair by PARP inhibition may also be sufficient to enhance the anti-cancer activity in combination with DNA-damaging agents independent of dysfunction in DNA repair pathways (8).

The combination of phosphatase and tensin homolog (PTEN)-deficient cells and PARP inhibition is another area of potential synergistic activity. *PTEN* encodes for a phosphatase that negatively regulates the phosphatidylinositide 3-kinase (PI3K)/AKT/mTOR pathway, which is important for cell proliferation and survival (9, 10) and also plays a poorly understood role in the expression of the DNA repair protein RAD51 and in the functionality of HR. Both *in vitro* and *in vivo* studies have demonstrated sensitivity of PTEN-deficient cells to PARP inhibitors (11–13). Thus, PARP inhibition may benefit patients with malignancies in which there is decreased PTEN expression, such as endometrial cancer, glioblastoma, malignant melanoma, prostate, breast, lung, and colorectal cancers (11).

Currently, there are multiple PARP inhibitors in clinical development for cancer. While there has been promising clinical activity for some of these agents, there are still significant unanswered questions regarding their use. Going forward, specific questions that must be answered include: timing of therapy, use in combination with cytotoxic agents or as a single-agent, maintenance therapy, and the existence of predictive biomarker(s) that can be used with PARP inhibition. Even with large strides in the treatment of many gynecologic malignancies in recent years, it is imperative that we develop newer agents and methods to identify patients that may benefit from these compounds.

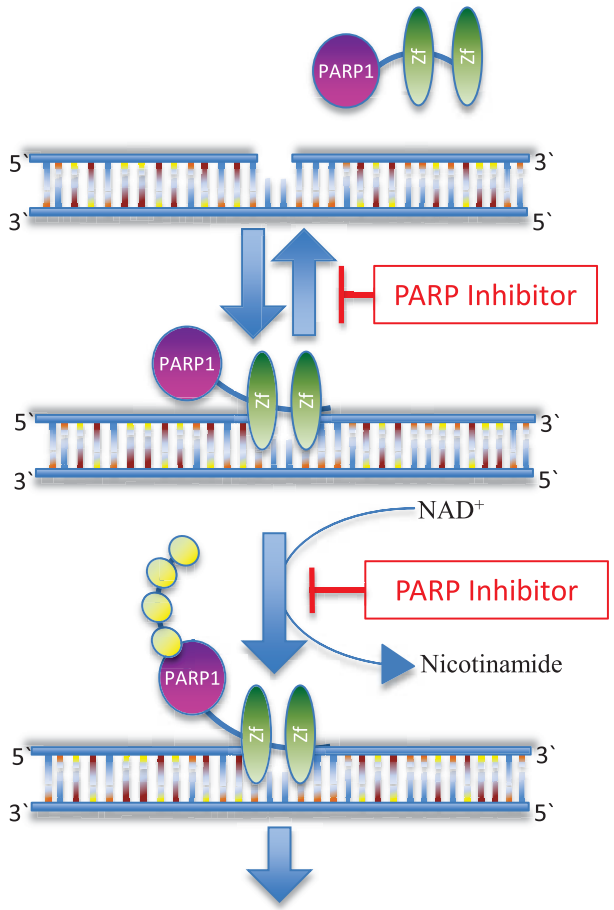

FIGURE 1 | Inhibition of PARP function. PARP1 recognizes and binds to sites of DNA damage through its Zn-finger domains (Zf). PARP inhibitors can trap PARP1 on damaged DNA in a complex that is possibly more toxic than unrepaired single strand DNA breaks (28). PARP inhibitors also block the enzymatic activity of the enzyme thereby inhibiting poly(ADP-ribosyl)ation, which in turn blocks recruitment of downstream repair proteins (114).

POLY(ADP-RIBOSE) POLYMERASE

Base excision repair (BER) is one of multiple critical pathways that maintain genome integrity in all cells, specifically in the recognition and repair of SSBs (14, 15). PARP is a family of 17 proteins that play an important role in DNA repair pathways. The most well studied member of the family, PARP1, is critical in the BER pathway for DNA SSBs. It detects and binds single strand DNA damage sites through its zinc finger domains, next attaching poly(ADP) ribose (PAR) moieties on itself and other proteins that have been recruited to the damage site (**Figure 1**). If there is excessive DNA damage, such as is seen with ischemia, PARP1 becomes hyperactivated. This heightened activity results in high levels of PAR and the depletion of nicotinamide adenine dinucleotide (NAD^+) and adenosine triphosphate (ATP) (16), and ultimately, cell death termed parthanatos (17). PARP is also involved in the repair of DSBs (18) and the recruitment of additional repair proteins like ataxia telangiectasia-mutated (ATM) and mitotic recombination 11 (MRE11), both of which are integral to the HR process (19, 20).

PARP1 was first reported in 1963 (21), but its anti-cancer utility was not fully realized until 1980. At that time, Durkacz et al. demonstrated that early-generation PARP inhibitors not only hindered DNA repair, but also enhanced the cytotoxic effects of DNA methylating agents in murine leukemia (22). Kupper et al. demonstrated the enhancement of the cytotoxic effects of gamma-irradiation after reduction of active PARP through overexpression of a dominant negative mutant of PARP that recognizes and binds damaged DNA, but does not possess the catalytic activity of the enzyme (23). More recently, PARP moved into the spotlight with the discovery that PARP inhibition in both cancer cell lines (2, 24) and human tumors (25) lacking BRCA1 or BRCA2 is selectively cytotoxic compared to non-mutation containing

tumors. One rationale for this efficacy is a principle termed synthetic lethality, a condition by which deletion or inactivation of only one of two genes (either *BRCA* or *PARP*) would not cause cell death, but deletion or inactivation of two genes in combination (both *BRCA* and *PARP*) is lethal. If PARP1 and PARP2 are inhibited, SSBs typically repaired by BER remain unresolved and when encountered by a replication fork, lead to the accumulation of DSBs (26). BRCA1- or BRCA2-deficient cells lack the ability to effectively complete HR and repair DNA DSBs. This double hit by impairment of both BRCA and PARP functionality ultimately results in genomic instability and cell death. Conferring a potential therapeutic benefit, cell death appears to be limited to homozygous target tissues (i.e., tumor), since most *BRCA* patients carry only one copy of the wild-type *BRCA* gene and there is no apparent effect on cells heterozygous for *BRCA* mutations (2). These observations have been exploited in the treatment of cancers associated with *BRCA* mutations, such as hereditary breast and ovarian cancer (HBOC), and even endometrial cancers (27).

Recently, Murai et al. suggest that the action of PARP inhibition is not only a function of how well the inhibitors disrupt the enzymatic activity, but that certain inhibitors also trap PARP1 on damaged DNA, thereby blocking repair (28). Interestingly, these studies showed that the potency in trapping PARP1 varied among agents, independent of their catalytic inhibitory properties. Clearly, additional investigation is warranted to better understand the intricacies inherent to PARP inhibition pathway and ultimately, advance drug development.

HEREDITARY BREAST AND OVARIAN CANCER AND BRCA

Hereditary breast and ovarian cancer is typically characterized by the onset of breast cancer at a young age, a strong family history of both breast and ovarian cancer, as well as an autosomal dominant inheritance pattern. Fallopian tube and primary peritoneal cancers also fall into this hereditary spectrum and are included under the ovarian cancer designation. An increased chance of bilateral cancers (e.g., both breasts), the development of both breast and ovarian cancer, and/or an increased incidence of other cancers (pancreas, prostate, etc.) may also be seen in this syndrome. In ovarian cancer, 10% of patients have a genetic predisposition. However, in those patients with a family history of ovarian cancer, the rate of *BRCA1* mutations is 80 and 15% for *BRCA2* mutations (29). More recently with the use of a massively parallel sequencing approach, Walsh et al. identified that closer to 24% of serous ovarian cancer patients have a germline DNA repair defect, over 30% of these were in patients without a family history of breast or ovarian cancer (30). The use of this broader assay is a promising method for detecting germline mutations with greater sensitivity and at decreased cost. Approximately 5–10% of all breast cancers and up to 25–40% of breast cancers in young patients (<35 years old) are hereditary. An estimated 3–8% of all breast cases and 30–40% of familial cases are likely caused by *BRCA1* and *BRCA2* mutations.

Individuals with a *BRCA* mutation have an increased risk of developing ovarian cancer up to 63% by some estimates, and breast cancer by up to 87% (31). Patients with *BRCA1* breast tumors tend to have a higher histologic grade, medullary histopathology, and are more likely than sporadic (non-*BRCA* mutant) tumors to be estrogen receptor negative, progesterone-receptor negative, and HER2/neu overexpression negative or "triple negative" (TNBC) (32). Ovarian cancers associated with *BRCA1* mutation are more often serous adenocarcinomas (90%) compared to women without this mutation (50%) (33–36). Although largely derived from retrospective or indirect data, most studies have not identified a significant survival difference between individuals with *BRCA* mutation-associated breast cancer versus controls (37–44). However, patients with high-grade serous ovarian carcinoma associated with a *BRCA* mutation tend to have a better prognosis than sporadic cases (45, 46). This improved prognosis may be related to *BRCA*-mutated cells' impaired DNA repair mechanism, lending these lesions greater sensitivity to cytotoxic chemotherapy, especially with platinum-based agents (47, 48). Based on the high selective lethality of *BRCA*-mutated cancer cells to PARP inhibitors, multiple studies have been undertaken to establish efficacy in gynecologic malignancies.

THE ROLE OF PARP INHIBITORS IN OVARIAN CANCER

Although it ranks as the ninth most common cancer among women, excluding non-melanoma skin cancers, ovarian cancer is the fifth most deadly cancer in females and accounts for more deaths than any other cancer of the female reproductive tract in the United States (1). Since the symptoms of disease are typically non-specific, ovarian cancer is often detected in advanced stages when the chance of cure is low. Given its insidious nature and the lethality of the disease, novel therapies are needed to improve overall survival in ovarian cancer patients.

In *BRCA* mutation-associated ovarian cancers, multiple investigations have been completed or are presently underway to establish the clinical activity of PARP inhibition in these mutational carriers. Sixty patients with refractory solid tumors were enrolled in a phase I trial of the PARP inhibitor olaparib (KU-0059436/AZD2281); the study was enriched for patients with *BRCA* mutations (25). In addition to establishing the maximum tolerated dose (MTD) of olaparib at 400 mg bid and observing only minimal adverse effects (primarily fatigue and gastrointestinal), it was noted that only *BRCA* mutation carriers had a significant objective tumor response. Out of 19 patients, 9 had a partial response (PR) (47%) and remarkably, 8 of which were ovarian cancer patients. Twelve of these patients (63%) had either radiological or tumor-marker responses or stable disease for $\geq$4 months. In an expanded cohort of the same trial, 50 patients with *BRCA1*/2 mutation-associated ovarian, primary peritoneal, and fallopian tube cancers were found to have a clinical benefit rate of 46%, including 40% that experienced a Response Evaluation Criteria in Solid Tumors (RECIST) radiologic or CA125 response (49). The median duration of response was 28 weeks. Another key finding was the overall clinical benefit rate was correlated with platinum sensitivity. Platinum-resistant and refractory patients had a 46 and 23% respective benefit rate versus 69% in the platinum-sensitive population ($P = 0.038$). The study also reported statistically significant associations between the overall platinum-free interval and antitumor response, as well as between platinum sensitivity and the maximum percentage change from radiologic baseline tumor size and from baseline CA125 after olaparib treatment.

In a phase 2 international, multicenter fashion, two sequential cohorts of women with confirmed *BRCA1* or *2* mutations and

recurrent disease were given either olaparib at 400 mg twice daily ($n = 33$) or 100 mg twice daily ($n = 24$) (50). The primary efficacy endpoint was objective response rate (ORR). In the 400 mg twice-daily cohort, ORR was 11 of 33 patients (33%; 95% CI 20–51); in the 100 mg twice daily cohort, ORR was 3 of 24 patients (13%; 95% CI 4–31). The most common toxicities experienced included nausea, anemia, and fatigue and were mild in the majority of cases. This phase 2 study provided positive proof of concept for the efficacy and tolerability of olaparib in advanced *BRCA*-mutated ovarian cancer.

Stemming from these initial reports, Kaye et al. designed a phase II, open-label, randomized, international study to assess the safety and efficacy of different doses (200 or 400 mg) of olaparib given twice daily versus intravenous liposomal doxorubicin given monthly in patients with *BRCA*-related ovarian cancer who had failed prior platinum-based chemotherapy (51). A statistically significant higher combined RECIST and CA125 rate of response for olaparib 400 mg twice daily compared to liposomal doxorubicin was noted. It did not find a significant difference in progression free survival (PFS) between the groups, with a reported median PFS of 7.1 months for liposomal doxorubicin, 6.5 months for the 200 mg olaparib cohort, and 8.8 months for the 400 mg olaparib cohort. There were roughly twice as many ≥grade 3 toxicities seen with liposomal doxorubicin compared to the PARP inhibitor. While this study did not show a statistically significant improvement in PFS between olaparib and liposomal doxorubicin, there was a much greater PFS with liposomal doxorubicin (7.1 months) than had been reported in historical data. Gordon et al. demonstrated PFS was only 4 months for liposomal doxorubicin compared to topotecan in a phase III randomized study of recurrent ovarian cancer (52). A recently reported phase III trial by Colombo et al. also demonstrated a similar PFS (3.7 months) for liposomal doxorubicin (53). Although the ability to draw comparisons between studies is limited, Kaye et al. reported PFS with liposomal doxorubicin is still within the 95% CI of historical controls, which suggests that this difference may simply reflect random variation within the population (54).

In addition to their use in *BRCA* mutation-associated ovarian cancer, PARP inhibitors are also being investigated in non-mutation carrier (or *BRCA* wild-type) ovarian cancers. Using PARP inhibitors in such a scenario is based on the idea that there is a HR DNA repair defect, but no germline *BRCA1/2* mutation in up to 50% of ovarian cancers (7, 11, 46, 55). Several studies have exploited this concept. Gelmon et al. conducted a phase II trial with high-grade serous/undifferentiated ovarian cancer with unknown *BRCA* status or *BRCA*-negative disease (56) and an additional reference group with known germline *BRCA* mutations. Patients were treated with olaparib 400 mg twice daily. The ORR in *BRCA*-mutants ($n = 17$) was 41% (95% CI 22–64) with median PFS of 221 days (95% CI 106–383), while *BRCA* mutation negative patients had an ORR of 24% ($n = 46$; 95% CI 14–38) and PFS of 192 days (95% CI 109–267). In a *post hoc* exploratory analysis, the ORR in patients with platinum-sensitive ovarian cancer was 50% (10 of 20) in the *BRCA*-negative cohort and 60% (3 of 5) in the *BRCA*-mutant cohort. In platinum-resistant ovarian cancers, 33 and 4% of patients with *BRCA* mutation positive and *BRCA*-mutant negative status respectively had responses. Observed toxicities were similar to those described in previous studies. This trial's findings were noteworthy, as they solidified the clinical utility of PARP inhibition in sporadic ovarian cancer. Further, these results suggest that platinum sensitivity may be used as a surrogate marker for HR deficiency. Results of a phase I study of niraparib (MK4827), an oral PARP inhibitor shown to induce selective lethality in HR repair deficient tumors with *BRCA* loss or non-*BRCA* HR defects (57), was given to a small cohort of patients enriched for *BRCA*-deficient and sporadic cancers associated with HR repair defects (58). Thirty-nine patients were treated at 7 successive dose levels; 11 of these patients were *BRCA* mutation carriers. Although results are only available in abstract form, the study reported that three patients with serous ovarian cancer had prolonged RECIST PR (one sporadic platinum-sensitive, two *BRCA*-deficient ovarian cancers). Disease stabilization was observed for >44 weeks in the sporadic serous ovarian cancer patient and for >16 weeks in the two patients with *BRCA*-deficient disease. In another phase II study with the PARP inhibitor rucaparib (AG-014699/PF-0136738), 41 patients with either breast (17) or ovarian (24) cancer and known *BRCA* deficiencies were given rucaparib as monotherapy and followed for ORR (59). Preliminary findings included a clinical benefit rate of 32%, but an ORR of 5% (2/38). However, 26% (10/38) achieved stable disease for ≥4 months and three patients remained on study for >54 weeks. The final results from these two ongoing studies are anxiously awaited.

Another larger, randomized, double-blind, placebo-controlled, phase II trial evaluated maintenance treatment with olaparib in patients with platinum-sensitive, relapsed, high-grade serous ovarian cancer (60). Included patients had received ≥2 platinum-based regimens and were required to have had a partial or complete response to their most recent platinum-based therapy. Two-hundred and sixty five patients were randomized to receive olaparib at 400 mg twice daily or placebo (136 olaparib arm, 129 placebo). *BRCA* mutational status was similar between the two groups. PFS was significantly longer in the olaparib arm than placebo (8.4 versus 4.8 months); however, there was no difference in overall survival at the first interim analysis. Interestingly, subgroup analysis revealed that regardless of *BRCA* mutational status, the olaparib cohort had a decreased risk for progression. Toxicities were overall mild in the olaparib group; most adverse events were grade 1 or 2 and typically included nausea, fatigue, vomiting, and anemia. These findings again support the argument that platinum sensitivity is a useful clinical marker for olaparib sensitivity. Further, this investigation recapitulates the role of PARP inhibitors in the ovarian cancer population, regardless of *BRCA* mutational status, and underscores the need for development of relevant biomarkers that predict HR deficiency in the setting of *BRCA* mutations or no known genetic abnormalities. Fortunately, there are multiple ongoing trials investigating the relationship between PARP inhibition and ovarian cancer that will hopefully clarify some of these uncertainties (**Table 1**).

PARP INHIBITORS IN ENDOMETRIAL CANCER

Endometrial cancer is the fourth most common cancer in women and the most commonly diagnosed gynecologic malignancy. An estimated 90% of the cases are sporadic and 10% have a genetic

Table 1 | Active clinical trials investigating PARP inhibitors in gynecologic malignancies.

Agent	Clinical trial identifier[E]	Trial description	Phase	Combination or monotherapy
Olaparib[A]	NCT01237067	Olaparib in combination with carboplatin for refractory/recurrent women's cancers	1	Combination
	NCT01116648	Olaparib in combination with cediranib for recurrent ovarian or TNBC	1/2	Combination
	NCT01445418	Olaparib with carboplatin to treat breast and ovarian cancer	1	Combination
	NCT01623349	Olaparib with BKM120 in recurrent TNBC or high-grade serous ovarian cancer	1	Combination
	NCT01650376	Olaparib with carboplatin and paclitaxel in relapsed ovarian cancer	1b	Combination
	NCT00782574	Olaparib with cisplatin in advanced solid tumors	1	Combination
	NCT00628251	Olaparib versus doxorubicin in advanced BRCA1/2 ovarian cancer patients who have failed previous platinum-therapy	2	Monotherapy
	NCT01844986	Olaparib in BRCA-mutated ovarian cancer patients following first line platinum-based chemotherapy	3	Monotherapy
	NCT01078662	Olaparib in advanced cancers with a confirmed BRCA1/2 mutation	2	Monotherapy
	NCT01874353	Olaparib in BCRA mutated ovarian cancer patients after complete or partial response to platinum chemotherapy	3	Monotherapy
	NCT00516373	Olaparib in ovarian cancer	1	Monotherapy
Veliparib[B]	NCT00989651; GOG-9923	Veliparib in combination with carboplatin, paclitaxel, bevacizumab for newly diagnosed ovarian, fallopian tube, or primary peritoneal cancer	1	Combination
	NCT01306032	Veliparib with cyclophosphamide in refractory BRCA-positive ovarian, primary peritoneal, ovarian high-grade serous carcinoma, fallopian tube cancer, TNBC, low-grade non-Hodgkin's lymphoma	2	Combination
	NCT01459380; GOG 9927	Veliparib in combination with doxorubicin, carboplatin, and bevacizumab	1	Combination
	NCT01281852; GOG-0076HH	Veliparib with cisplatin and paclitaxel in patients with advanced, persistent, or recurrent cervical cancer	1/2	Combination
	NCT01145430	Veliparib and doxorubicin for recurrent ovarian, fallopian tube, and primary peritoneal cancers or metastatic breast cancer	1	Combination
	NCT01266447; GOG 127-W	Veliparib, topotecan, and filgrastim or pegfilgrastim in patients with persistent/recurrent cervical cancer	2	Combination
	NCT01690598	Veliparib with topotecan in patients with platinum-resistant or partially platinum-sensitive relapse of epithelial ovarian cancer with negative or unknown BRCA status	1/2	Combination
	NCT01012817	Veliparib with topotecan in relapsed/refractory or primary peritoneal cancer after prior first line platinum-therapy	2	Combination
	NCT01113957	Veliparib with temozolomide versus doxorubicin alone in ovarian cancer	2	Combination
	NCT01749397	Veliparib and floxuridine in metastatic epithelial ovarian, primary peritoneal, or fallopian tube cancer	1	Combination
	NCT01540565; GOG-0280	Veliparib in persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer patients with a BRCA2 mutation	2	Monotherapy
	NCT00892736	Veliparib monotherapy for patients with BRCA1/2 -mutated cancer, including platinum-refractory ovarian, fallopian tube, or primary peritoneal cancer; or basal-like breast cancer	1	Monotherapy
	NCT01472783	Veliparib for patients with BRCA mutation and platinum-resistant or partially sensitive relapse of epithelial ovarian cancer	1/2	Monotherapy
BMN 673	NCT01286987	BMN 673 in advanced or recurrent solid tumors, including epithelial and ovarian cancers	1	Monotherapy
Niraparib[C]	NCT01847274	Niraparib versus placebo in platinum-sensitive ovarian cancer	3	Monotherapy
Rucaparib[D]	NCT01009190	Rucaparib with carboplatin in advanced solid tumors	1	Combination
	NCT01482715	Rucaparib in patients with BRCA mutation breast or ovarian cancer, or other solid tumor	1/2	Monotherapy
	NCT00664781	Rucaparib in metastatic breast cancer or ovarian cancer	2	Monotherapy

[A] Olaparib, also known as AZD2281.

[B] Veliparib, also known as ABT-888.

[C] Niraparib, also known as MK-4827.

[D] Rucaparib, also known as AG-014699; PF-01367338.

[E] All clinical trials are found at www.clinicaltrials.gov and listed according to their NCT identifier. Last accessed 2013 June 19.

origin. Endometrioid adenocarcinoma and serous carcinoma are the most prevalent histological types, while endometrial clear cell and mucinous carcinomas only account for approximately 5% of all cases (61). Since many patients are symptomatic early in their disease course, the majority of endometrial cancers (approximately 75%) are detected in the initial stages when the disease remains confined to the uterus (61). However, a significant amount of women still experience advanced disease, for which systemic treatment options are limited, toxicities high, and responses often short-lived (62, 63). There is a pressing need for targeted therapies that will yield a greater efficacy and be better tolerated.

A variety of different molecular defects linked to the development of endometrial cancer are described. In endometrioid endometrial carcinoma (EEC), also known as type I endometrial cancer, microsatellite instability (MSI) and mutations in the *PTEN*, *K-ras*, *PIK3CA*, and β-*catenin* genes are reported (64). As previously discussed, *PTEN* is a tumor suppressor gene that is involved in DNA repair mechanisms, as well as in the inhibition of the PI3K/AKT/mTOR pathway; *PTEN*-deficient cells are sensitive to PARP inhibitors (11–13). Rare syndromes collectively known as the PTEN hamartoma tumor syndromes (PHTS) are linked to germline mutations in *PTEN* (65, 66). Outside of PHTS, *PTEN* is altered in up to 83% of endometrioid carcinomas versus only 10% in serous and clear cell cancers (67–71). Dedes et al. demonstrated that *PTEN*-deficient EEC cells had a greater sensitivity to PARP inhibition than wild-type EEC *PTEN* cell lines (12). Given the heightened prevalence of *PTEN* deficiency in EEC superimposed on these laboratory studies demonstrating sensitivity to PARP inhibition, clinical studies are now in progress. A case report describing a 58-year-old female with metastatic endometrioid endometrial adenocarcinoma who had previously demonstrated exquisite sensitivity to platinum-containing regimens, was given olaparib as part of a phase I trial (72, 73). Prior to trial participation, brain metastases were found. However, after 10 weeks on trial, the patient had a significant reduction in the size of the brain metastases without other intervention and also reported improvement in tumor-related symptoms. Unfortunately, the patient had objective disease progression after 8 months on olaparib therapy. Her tumor was biopsied and verified to be negative for *BRCA* mutation, but positive for loss of *PTEN*. Although only an isolated report, this case study coupled with compelling pre-clinical data, provides a strong rationale for larger clinical trials. A phase 2, randomized, placebo-controlled trial comparing olaparib versus best supportive care or progesterone in advanced endometrial cancer was planned, but unfortunately, was unable to be opened. In addition to EEC, serous endometrial cancers appear to have a similar genetic background to serous ovarian carcinoma, including hallmarks of deficiency in DNA repair as well as frequent mutations in *TP53*, *PIK3CA*, *K-RAS*, and *ERBB2* (74). These tumors may prove to be another rational target for PARP inhibition.

PARP INHIBITORS IN CERVICAL CANCER

As the third most common cancer worldwide, cervical cancer has an annual incidence of 530,000 cases, with 250,000 deaths expected (75). It is the second leading cause of death in women from the ages of 20–39 (76). Fortunately, the incidence of this cancer in most developed countries has decreased by 70% over the past 50 years due to improved screening methods with cervical cytology (77). More recently, HPV vaccination has aided in the detection and subsequent prevention of high-risk HPV subtypes, which are the culprit for most cervical cancers (78–82). For advanced disease, chemotherapy remains the standard of care. Similar to the experience in endometrial cancer, such therapy typically does not yield durable responses or cure (83).

The use of PARP inhibitors in cervical cancer has only recently been explored in the pre-clinical arena. Along with non-small cell lung cancer, mesothelioma, and ovarian cancer cell lines, Michels et al. created cervical cancer (HeLa) cell lines resistant to cisplatin (84). Upon further study, these lines were found to have high levels of PAR and PARP1, with PARP1 constitutively hyperactivated. Exposure of the cells to pharmacologic PARP inhibition resulted in cell death. Hence, this work hints at another role for PARP inhibition, in the treatment of cisplatin-resistant cervical cancers. Interestingly, this group also observed that elevated levels of PAR identified in PARP1-overexpressing tumor cells and xenografts predicted response to PARP inhibition *in vitro* and *in vivo* more accurately than PARP1 expression itself, suggesting PAR may be a reasonable biomarker of response to PARP inhibitor therapy in cervical cancer. A phase I trial is presently enrolling patients with cervical cancer along with other gynecological malignancies to investigate the combination of olaparib with carboplatin in refractory or recurrent disease (NCT01237067; see **Table 1**). Another phase 1/2 trial is investigating the use of veliparib with cisplatin and paclitaxel in advanced, persistent, or recurrent cervical cancer (NCT01281852; **Table 1**). Additional pre-clinical and clinical investigation will hopefully reveal even more promising applications for PARP inhibition in cervical cancer.

FUTURE DIRECTIONS

Poly(ADP-ribose) polymerase inhibitors are an exciting new class of agents that have already demonstrated promising pre-clinical and clinical activity in a variety of malignancies. Nevertheless, the full potential of PARP inhibition in cancer has not yet been realized. In addition to single-agent use, PARP inhibitors have been studied in combination with a number of different chemotherapies, anti-angiogenic agents, as well as with ionizing radiation. Other areas of active investigation include the development of markers that will predict clinical benefit from PARP inhibition, as well as the identification of resistance mechanisms to PARP inhibitor therapy.

Chemotherapies known to induce DNA strand breaks, especially SSBs, are of particular interest for combination studies. In the case of methylating agents, activation of BER elicits therapy resistance (85). A large body of pre-clinical *in vivo* and *in vitro* studies demonstrates the addition of a PARP inhibitor may sensitize cells to DNA-damaging agents and further delay the development of treatment resistance (8, 85–93). These studies were conducted with a wide variety of chemotherapeutic agents, including topoisomerase I inhibitors, platinum agents, as well as DNA alkylating agents. Human trials combining PARP inhibitors and chemotherapy agents for sporadic and *BRCA*-associated gynecologic malignancies are underway, but few have reached maturity (NCT01445418, NCT01237067; see **Table 1**). Promising data has come from Oza et al., who conducted a

multicenter phase II study that compared the efficacy of olaparib plus paclitaxel/carboplatin followed by olaparib maintenance therapy versus paclitaxel/carboplatin alone with no further therapy in patients with platinum-sensitive recurrent serous ovarian cancer (94). Importantly, the *BRCA* status was unknown for the majority of the patients. In arm A, patients received six, 21-day cycles of olaparib (200 mg twice daily) with paclitaxel (175 mg/m^2 IV, day 1) and carboplatin (AUC 4 IV, day 1), followed by olaparib maintenance therapy at a dose of 400 mg twice daily in a continuous fashion versus in arm B, the standard dose of carboplatin (AUC 6 IV, day 1) and paclitaxel (175 mg/m^2 IV, day 1) without the PARP inhibitor. Patients receiving olaparib had a significant improvement in PFS versus chemotherapy alone. OS data was felt to be immature, but preliminarily showed similar results between the two arms (64 versus 58%). In the combination phase, both arms had generally similar toxicity profiles, with nausea, fatigue, and alopecia the most common adverse events experienced. During the maintenance phase (olaparib monotherapy versus no further therapy), side effects were consistent with the known monotherapy side effect profile of PARP inhibitors. In a smaller phase I dose escalation trial, olaparib was added to carboplatin in *BRCA1*/2 mutational carriers with breast or ovarian cancer (95). Therapy was administered in a 3 × 3 dose escalation fashion: oral olaparib at 100 or 200 mg every 12 h [dose level (DL) 1/2] with IV carboplatin AUC 3 on day 8 then every 21 days; DL6–9 gave olaparib days 1–7 at 200 then 400 mg every 12 h, with carboplatin AUC 3 on day 2 then escalation to AUC 5 (no DL3–5). From the preliminary results, bone marrow suppression was the observed dose limiting toxicity. Of the 23 evaluable ovarian cancer patients, PR was seen in 8/23, disease stabilization occurred in 11/23. Overall, the ovarian cancer cohort had a clinical benefit of 83%. Clearly, the results of these studies are intriguing; data from similar combination trials is eagerly anticipated.

In addition to chemotherapeutic agents, PARP inhibitors are also being combined with anti-angiogenic agents. The rationale behind this combination is based on the observation that vascular endothelial growth factor receptor (VEGFR) inhibition may lead to increased DNA damage through downregulation of DNA repair proteins, including ERCC1 and XRCC1 (96, 97). Stemming from pre-clinical data supporting the relationship between PARP inhibition and the VEGF pathway (98–100), several phase I studies are presently underway. The phase 1 study of ABT-888 (veliparib) in combination with carboplatin, paclitaxel, and bevacizumab as first-line treatment for stage II-IV ovarian cancer is actively enrolling patients (NCT00989651; **Table 1**). Another phase I trial of olaparib in combination with cediranib, a VEGFR inhibitor, is also open to recurrent ovarian or TNBC patients (NCT01116648; **Table 1**). Trial investigators are exploring the toxicities and recommended phase 2 dosing of the dual therapy. From a preliminary report, myelosuppression was dose limiting at the highest dose level (cediranib 30 mg daily/olaparib 400 mg twice daily) (101). Although unconfirmed, the study also notes a 56% response rate in enrolled ovarian cancer patients. These results are encouraging; additional efficacy data will be forthcoming (**Table 1**).

Due to PARP's ability to inhibit multiple processes related to DNA repair, combining PARP inhibition with ionizing radiation is a logical combination. Pre-clinical studies confirm that PARP inhibition acts to sensitize malignant cells to radiation (88, 102). Several laboratories have also shown that PARP 1 knockout mice have an enhanced sensitivity to gamma-radiation (103, 104). In mouse colon cancer xenografts, veliparib coupled with irradiation resulted in prolonged survival from 23 to 36 days, and in one mouse, a complete response (8). At the present time, there are no active clinical trials investigating the combination of radiation therapy with PARP inhibition in gynecologic malignancies. However, there are active trials investigating this dual therapy in other diseases like breast cancer (NCT01477489) (105) and glioblastoma multiforme (NCT00687765) (106). Enrollment of gynecologic malignancy patients into similar trials is important since radiation plays a significant role in the treatment of cervical and endometrial cancer.

As evidenced by the discussed clinical data, many patients benefit from PARP inhibitor therapy, though the degree of response varies and sometimes there is no observed clinical benefit. A predictive marker that not only evaluates the drug's pharmacodynamic effects, but can also identify who might benefit from therapy may help guide treatment decisions. Several attempts have been made to meet this objective. Duan et al. described a triple stain immunofluorescence assay looking at FANCD2, DAPI, and Ki67 as a means for measuring the functional competency of the Fanconi anemia pathway in proliferating cells in formalin fixed tumor tissue from patient biopsies across multiple tumor types (5). This stain is now being tested in a prospective fashion to select patients for a phase 1 clinical trial using veliparib alone or in combination with mitomycin-C (NCT01017640). The use of massively parallel sequencing analysis (e.g., BROCA) in a prospectively designed trial should also be investigated as this may capture a larger percentage of patients likely to be sensitive to PARP inhibition compared to relying on BRCA1/2 mutational analysis alone (30). Mukhopadhyay et al. developed a method of measuring HR function by quantifying RAD51 foci via immunofluorescence-based assays of ascitic fluid (107). They subsequently correlated *in vitro* cytotoxicity of the PARP inhibitor rucaparib with the HR status from these culture results. They correlated their *in vitro* results to patients whom were treated with platinum-based chemotherapy; tumor progression and OS were prospectively compared between HR-competent versus HR-deficient patients (108). Interestingly, patients who were HR-deficient, as established by assay analysis, had lower rates of tumor progression at 6 months and a higher median survival. From these results, the authors suggest that the RAD51 assay successfully identified those patients with HR deficiency and hence, may better predict which patients will have the best response to PARP inhibition. In addition to ascitic fluid, collection of peripheral blood mononuclear cells (PBMCs) as a surrogate tissue to monitor drug actions may be preferable to tumor biopsy collection, as it is less invasive and multiple samples may be longitudinally obtained. In order to better characterize the pharmacodynamic profile of the PARP inhibitor ABT-888, Ji et al. developed an immunoassay for measuring PAR incorporation in both tumor biopsies and PBMCs (109). In this study, considerable inter-individual and inter-sample heterogeneity in PAR levels was observed. Given these findings, it is not surprising that the trial comparing cyclophosphamide with veliparib presented

a 50% reduction in PAR levels in 90% of patient PBMCs and 80% reduction in tumor biopsies across all dose levels (110). A larger phase II follow up study with this combination is ongoing (NCT01306032; **Table 1**). Though limited conclusions may be drawn from this experience, one must consider the possibility that PAR levels did not correlate well with actual PARP inhibitor activity (111). Ongoing genomic microarray analysis of patients involved in trials using olaparib may give useful insight into genetic signatures that may predict response. Regardless, these results underscore the need to identify a validated method of quantifying PARP inhibitor activity that corresponds to actual clinical outcome.

As with the majority of anti-cancer agents, tumors may develop acquired resistance to PARP inhibitor therapy. There are several proposed mechanisms of resistance, and likely many more that have not yet been described. One potential means is the restoration of HR secondary to a gain of function mutation in the *BRCA2* allele via elimination of the c.6174delT mutation (112). Resistance secondary to up regulation of the *ABCB1a/b* gene that encodes for a P-glycoprotein efflux pump is also described with long-term use of the PARP inhibitor olaparib. Reversal of resistance occurred with co-administration of a P-glycoprotein inhibitor (113). These are just two examples of methods of resistance and certainly the success of PARP inhibitor therapy in the future will rely on further analysis of resistance patterns and subsequent therapy modifications.

CONCLUSION

Gynecologic malignancies represent a significant challenge in women's health. When discovered in advanced stages, few successful therapeutic interventions are available to patients. Therefore, the development of novel agents like PARP inhibitors is essential. Already recognized as a promising agent in the treatment of *BRCA*-related malignancy, initial phase I and II studies confirm the activity of PARP inhibitors in ovarian, endometrial, and cervical cancers. As we learn more about these targeted agents through ongoing trials, it will be important to identify which population of patients may benefit the most from PARP inhibitor therapy and in what manner, as monotherapy or in combination. Whether it is in the neoadjuvant, adjuvant, or maintenance setting, the timing of therapy that will procure the greatest clinical benefit is also unknown. Clearly, PARP inhibitors are an exciting new class of targeted agents for the treatment of ovarian, endometrial, and cervical cancers.

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PARP inhibitors in cancer therapy: magic bullets but moving targets

Girish M. Shah[1], Mihaela Robu[1], Nupur K. Purohit[1], Jyotika Rajawat[1], Lucio Tentori[2] and Grazia Graziani[2]*

[1] *Laboratory for Skin Cancer Research, CHU-Q (CHUL) Research Centre, Laval University, Quebec City, QC, Canada*
[2] *Department of System Medicine, University of Rome "Tor Vergata", Rome, Italy*
**Correspondence: girish.shah@crchul.ulaval.ca*

Edited by:
Christina Annunziata, National Cancer Institute, USA

Reviewed by:
Daekyu Sun, University of Arizona, USA
Michael Witcher, McGill University, Canada

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The pharmacological inhibitors of poly(ADP-ribose) polymerase-1 (PARP-1) have reached the first milestone toward their inclusion in the arsenal of anti-cancer drugs by showing consistent benefits in clinical trials against BRCA-mutant cancers that are deficient in the homologous recombination repair (HRR) of DNA double strand breaks (DSB) (1, 2). PARP inhibitors (PARPi) also potentiate therapeutic efficacy of ionizing radiation and some chemotherapeutic agents (1). These effects of PARPi were initially linked to inhibition of the role of PARP-1 in base excision repair (BER) of DNA damaged by endogenous or exogenous agents, resulting in accumulation of single strand breaks (SSB), which upon conversion to toxic DSB lesions would kill cancer cells deficient in DSB repair (1, 3, 4). However, PARPi lethality in HRR-deficient cancers can also be explained by other mechanisms not involving a direct effect of PARPi on BER [reviewed in Ref. (5, 6)]. In addition, therapeutic benefits of PARPi with agents such as carboplatin in HRR-proficient and -deficient tumors [reviewed in Ref. (1, 7)], simply cannot be explained by BER inhibitory effect of PARPi. Therefore, PARPi are like magic bullets that can kill cancer cells under different circumstances, but to comprehend their global scope and limitations, here we discuss the full range of their targets and the possible impact of broad specificity of current PARPi during prolonged therapy of cancer patients.

MECHANISMS OF ACTION OF PARPi IN CANCER THERAPY: MAGIC BULLETS BUT MOVING TARGETS

It is not surprising that the mechanism of action of PARPi in killing cancer cells still remains an open question, because its principal target PARP-1 is a multifunctional protein implicated in various cellular responses to DNA damage ranging from different pathways of DNA repair and cell death to stress signaling, transcription, and genomic stability (8, 9), all of which could be affected by PARPi and thus influence outcome of cancer therapies. Following are various possibly overlapping mechanisms for the anti-cancer effect of PARPi.

BER/HRR NEXUS FOR SYNTHETIC LETHALITY OF PARPi IN BRCA-MUTANT CANCERS

It was first demonstrated by two teams (3, 4) that two individually non-lethal conditions, i.e., PARPi-mediated inhibition of PARP-1 and BRCA mutation-induced HRR deficiency in cancer cell, would become synthetic lethal when combined in a single cell [reviewed in Ref. (1, 5, 10, 11)] (**Figure 1A**). This model focuses on the role of PARP-1 in BER, the pathway that repairs abasic sites and SSB that are constantly created in the mammalian genome by endogenous oxidants. When PARPi suppress the role of PARP-1 in BER, the unrepaired SSB would accumulate and collapse the DNA replication fork to form potentially lethal DSB. The normal cells would survive by repairing these DSB by HRR, but the HRR-deficient BRCA-mutants would die due to unrepaired DSB or possibly due to excessive reliance on the error-prone non-homologous end-joining (NHEJ) repair pathway to remove DSB (**Figure 1A**). This model also covers minor variations of the central theme as reviewed recently (1, 10) (**Figure 1A**). For example, tumors with other conditions that cause HRR deficiency or "BRCAness" phenotype would also be susceptible to PARPi. It permits inclusion of PARP-2 and its role in BER as target of PARPi, because most current PARPi also inhibit PARP-2 (10). It also explains the potentiating effect of PARPi in the combination therapy with radiation or chemicals, such as temozolomide, irinotecan, or topotecan, because DNA damage caused by these agents is also repaired by BER.

ALTERNATIVE TARGETS OF PARPi IN BRCA-MUTANT CANCERS

However, the above mechanism is inadequate to explain all the effects of PARPi seen in BRCA-mutant cancers, which could be explained by the effect of PARPi on alternate targets, as reviewed earlier (5, 6, 10) (**Figure 1A**). In brief, (i) PARPi could be trapping PARP-1 or PARP-2 to SSB with resultant PARP-SSB complex that would be more toxic than unrepaired SSB or even knockdown of PARPs (5, 12). (ii) PARPi could act via upregulation of NHEJ pathway, which would presumably cause genomic instability and eventual lethality (13). (iii) PARPi could suppress the role of PARP-1 in reactivating DNA replication forks (5). Thus, apart from BER/HRR nexus, there could be NHEJ/HRR or DNA replication/HRR nexus to explain PARPi lethality in BRCA-mutant cancers.

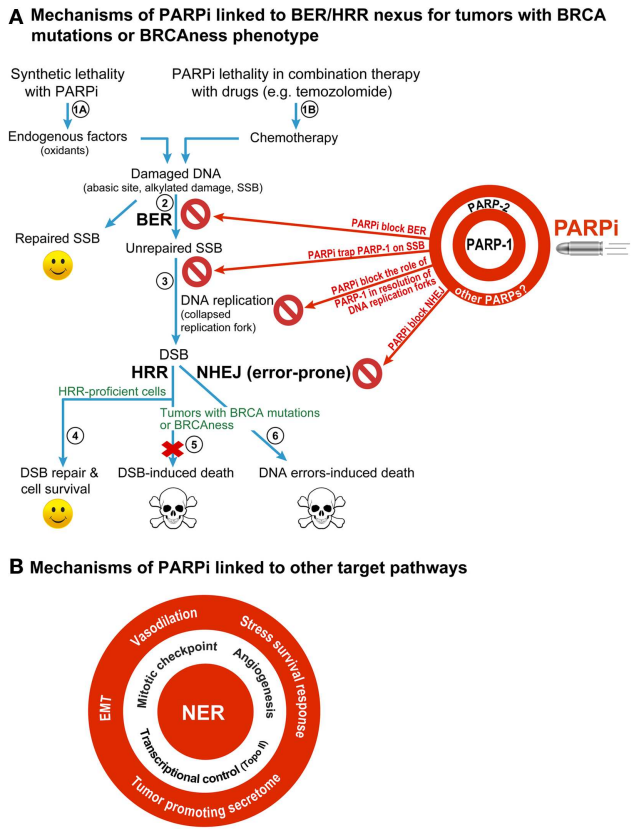

FIGURE 1 | Different mechanisms for therapeutic efficacy of PARPi in cancers. **(A)** BER/HRR model: this model focuses on the role of PARP-1, the principal target of PARPi, in BER that removes abasic sites and SSB created constantly in the mammalian genome by endogenous oxidants (steps 1A). During BER, the binding of PARP-1 to SSB leads to stimulation of its catalytic activity of forming polymers of ADP-ribose (PAR) from its substrate NAD^+. The PAR and PARP-1 interact with and recruit the key BER scaffold protein XRCC1, whereas PAR-modified PARP-1 loses its affinity to bind to SSB and vacates the site for BER to continue. When PARPi suppress the role of PARP-1 in BER (step 2), the unrepaired SSB would accumulate and collapse the DNA replication fork to form potentially lethal DSB (step 3). The normal cells would survive by repairing these DSB by HRR (step 4), but the HRR-deficient BRCA-mutants would die due to unrepaired DSB (step 5) or possibly due to excessive reliance on the error-prone NHEJ repair pathway to remove DSB (step 6). This BER/HRR nexus also explains the effectiveness of combination therapy of PARPi with drugs that cause DNA damage that is repaired by BER (step 1B). Since PARP-2 is also known to play a role in BER, and since current PARPi are also known to inhibit PARP-2, the effect of PARPi may also be mediated by targeting of the functions of PARP-2 in BER, as shown on the target board along with PARP-1. In addition, the inhibitory effect of PARPi on other PARPs could also influence therapeutic efficacy of PARPi (see target board), although their contribution to BER/HRR mediated therapeutic effect of PARPi is not yet fully assessed. **(B)** Other targets of PARPi that can confer therapeutic benefits of PARPi: PARPi could also be effective anticancer agents by targeting the role of PARP-1 in other DNA repair pathways, such as NER; or other cellular pathways, such as control over cell cycle, tumor angiogenesis, transcription, epithelial-mesenchymal transition (EMT), stress survival response, vasodilation, or tumor-promoting secretome.

EXPANDING UNIVERSE OF POTENTIAL TARGETS OF PARPi

Therapeutic effectiveness of PARPi seen with some drugs cannot be explained by any of the above models, e.g., the potentiating effects of PARPi on the platinum-based drugs such as carboplatin, cisplatin, or oxaliplatin on HRR-deficient or -proficient tumors [reviewed in Ref. (1, 7)] (**Figure 1B**). These observations were further supported by recent studies showing the potentiating effect of PARPi veliparib on carboplatin treatment of patients with BRCA-mutant breast cancers (14) or carboplatin and phosphoinositide 3-kinase mTOR inhibitor treatment of mouse xenografts of BRCA-competent triple negative breast cancer cells (15). Since platinum compounds cause DNA damage that is largely repaired by the nucleotide excision repair (NER) pathway and not BER, we need to think beyond BER for an explanation. Moreover, BER was shown to mediate toxicity of cisplatin by competing with the repair of cisplatin inter-strand cross-links and DSB caused by these links (16). Therefore, if PARPi effect was mainly via inhibition of BER, we should have observed less and not more toxicity of cisplatin.

One possible explanation is that PARPi could be causing vasodilation (**Figure 1B**) to improve intra-tumoral delivery of platinum drugs (1), although it needs to be confirmed if this generalized effect could also potentiate other drugs. On the other hand, recently discovered roles of PARP-1 in improving the efficiency of NER-mediated removal of UV-induced DNA damage (17–19) provides a more handy explanation for the PARPi-induced potentiation of platinum compound-based drugs, which also cause DNA damage that is repaired by NER (**Figure 1B**). This NER targeting effect of PARPi alone can account for death of HRR-proficient tumors, as seen in clinical trials [reviewed in Ref. (1, 7)] and supported by *in vitro* results showing that PARP-1 depletion (20) or inhibition (19) decreases clonogenic survival of UV-exposed human skin fibroblasts with no reported HRR-deficiencies. Of course, PARPi could have an additional effect in this model due to suppression of the role of PARP-1 in HRR pathway (21). In addition, in the PARPi-treated BRCA-mutant HRR-deficient tumors, the unrepaired DNA damage by platinum drugs could collapse the DNA replication fork to form DSB and cause lethality. Thus, the NER effect alone or NER-HRR nexus could be possible explanations for the lethality of PARPi/platinum compounds in HRR-proficient or -deficient tumors.

The clinical and preclinical studies have also revealed other targets of PARPi in cancer therapies that are linked to various roles of their multifunctional target PARP-1 in following cellular processes (**Figure 1B**). (i) Transcriptional control of drug-target genes: PARPi have been shown to increase toxicity of topoisomerase II-poison doxorubicin *in vitro* (22) or in xenografted tumors in mice (23). This effect could be

due to doxorubicin-induced decrease in expression and activity of PARP-1 (24) or PARPi-mediated increase in expression of topoisomerase II, because the transcription activator Sp1 loses its affinity for the topoisomerase II-promoter region upon modification by polymer of ADP-ribose (PAR) created by the activated PARP-1 (22). (ii) Mitotic checkpoint: the beneficial effects of PARPi with microtubule stabilizing mitotic inhibitor paclitaxel in patients with recurrent metastatic gastric cancers with BRCAness phenotype (25) could be linked to suppression of the role of PARP-1 in maintaining the mitotic checkpoint via PARylation of itself or the mitotic checkpoint protein CHFR (26, 27). An abrogation of mitotic checkpoint would kill cancer cells, because they will be forced to divide before resolution of the damage. (iii) Tumor-promoting secretome: PARPi-mediated suppression of the role of PARP-1 in elaborating tumor-promoting secretome containing cytokines and growth factors has been suggested as a cause for decreasing the resistance to another mitotic inhibitor docetaxel (28). (iv) Angiogenesis: the role of PARP-1 in promoting angiogenesis that fuels the growth of tumors can also be target of PARPi, because PARP-1 depletion or PARPi reduce vessel formation (29) and expression of markers of angiogenesis in melanoma (30) or endothelial cells (31). (v) Epithelial-mesenchymal transition (EMT) and metastasis: PARPi or PARP-1 depletion-induced reduction in aggressiveness and growth of metastatic melanoma in animal studies (30, 31) along with decreased markers for EMT (31, 32) suggest that the increase in progression-free survival of PARPi-treated patients could be due to reduction in the proliferation rate of the primary tumor and repression of its metastatic potential. (vi) Stress survival response: finally, cancer cells respond to any therapy by elaborating various stress responses to survive; and PARP-1 and its product PAR play key roles in these stress responses (9). Hence the suppression of pro-survival stress responses could explain the effectiveness of PARPi with any anti-cancer drug. An expanding list of potential targets of PARPi provides us with a much larger vision of the future applications of PARPi in cancer therapy.

BROAD SPECIFICITY OF PARPi: A KEY ISSUE FOR THE FUTURE OF PARPi THERAPY

There are two basic issues arising from the broad specificity of current PARPi.

(a) PARPi can inhibit more than one PARP ("they are bazookas not bullets"): many of the current PARPi in clinical trials display strong binding to PARPs 1–4 (33), and inhibit both PARP-1 and 2 at clinically relevant concentrations (10). Most studies assume that the effect of PARPi on both PARP-1 and 2 is important for therapy; however, this may not be the case. In fact, some studies using specific knockdown of PARPs showed that only the knockdown of PARP-1, but not PARP-2, replicates: (i) the synthetic lethal effect of PARPi on BRCA2 mutant cells (3); (ii) potentiation of cisplatin by PARPi in BRCA-proficient triple negative breast cancer cells (34); and (iii) sensitization of melanoma cells *in vitro* to temozolomide (35). On the other hand, the effect of PARPi on gemcitabine in the above breast cancer cells was replicated by PARP-2 knockdown and not PARP-1 knockdown (34). In contrast, the siRNA for PARP-1 could specifically prevent the growth of BRCA-deficient ovarian cancer cell-derived tumors in mice (36). Since the double knockout of PARP-1 and PARP-2 is embryonic lethal (37), we must verify the assumption that gratuitous inhibition of unrelated PARPs has no effect on the end-results.

(b) Indiscriminate inhibition of all the roles of a given PARP by PARPi ("we are nuking the entire PARP-landscape"): PARP-1, the principal target of PARPi, is a multifunctional protein that is implicated not only in DNA repair but also in various forms of cell death, transcription, epigenetic control of gene expression, and chromatin remodeling (8, 38). Hence even if we were to develop novel PARPi to specifically inhibit only PARP-1, it will still shut down most if not all the functions of PARP-1. Similar arguments can be made for PARPi-mediated suppression of different roles of PARP-2. Although adverse genomic consequences of PARPi therapy have not yet been reported, we need to consider that prolonged PARPi therapy may cause genome instability because PARP-$1^{-/-}$ mouse embryonic fibroblasts have a tendency to become tetraploid (39, 40), and the susceptibility of PARP-$1^{-/-}$ female mice to develop mammary carcinoma is enhanced if p53 is also mutated, a phenomenon frequently observed in cancers (41). In effect, PARPi are the magic bullets, but instead of doing precision targeting with them for the desired effect, we are simply nuking the entire spectrum of functions of that target PARP, which could result in unintended consequence during maintenance (prolonged) therapy with PARPi including survival of damaged cancer cells, development of secondary tumors as a consequence of genomic instability and resistance to PARPi. Thus, while the current broad specificity PARPi work properly for short-term cancer therapy, there is a need for development of new and more specific PARPi that are unique not only for a given PARP but also for a given function of that PARP related to its anti-cancer effect.

It is heartening that PARPi have shown some clinical benefit for BRCA-mutant cancer patients in clinical trials as monotherapy or as a combination therapy, but we need to do a lot more to understand the therapeutic effect of PARPi to establish them firmly in the arsenal of anti-tumor agents against variety of cancers.

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Beyond DNA repair: additional functions of PARP-1 in cancer

Alice N. Weaver [1] ***and Eddy S. Yang*** [1,2,3] *******

[1] *Department of Radiation Oncology, School of Medicine, The University of Alabama at Birmingham, Birmingham, AL, USA*
[2] *Department of Cell, Developmental, and Integrative Biology, School of Medicine, The University of Alabama at Birmingham, Birmingham, AL, USA*
[3] *Department of Pharmacology and Toxicology, School of Medicine, The University of Alabama at Birmingham, Birmingham, AL, USA*

Edited by:
Christina Annunziata, National Cancer Institute, USA

Reviewed by:
Xu-Dong Zhu, McMaster University, Canada
Wen-Ming Yang, National Chung Hsing University, Taiwan

****Correspondence:***
Eddy S. Yang, Department of Radiation Oncology, School of Medicine, The University of Alabama at Birmingham, 176F HSROC Suite 2232N, 1700 6th Avenue South Birmingham, AL 35249-6832, USA
e-mail: eyang@uab.edu

Poly(ADP-ribose) polymerases (PARPs) are DNA-dependent nuclear enzymes that transfer negatively charged ADP-ribose moieties from cellular nicotinamide-adenine-dinucleotide (NAD^+) to a variety of protein substrates, altering protein–protein and protein-DNA interactions. The most studied of these enzymes is poly(ADP-ribose) polymerase-1 (PARP-1), which is an excellent therapeutic target in cancer due to its pivotal role in the DNA damage response. Clinical studies have shown susceptibility to PARP inhibitors in DNA repair defective cancers with only mild adverse side effects. Interestingly, additional studies are emerging which demonstrate a role for this therapy in DNA repair proficient tumors through a variety of mechanisms. In this review, we will discuss additional functions of PARP-1 – including regulation of inflammatory mediators, cellular energetics and death pathways, gene transcription, sex hormone- and ERK-mediated signaling, and mitosis – and the role these PARP-1-mediated processes play in oncogenesis, cancer progression, and the development of therapeutic resistance. As PARP-1 can act in both a pro- and anti-tumor manner depending on the context, it is important to consider the global effects of this protein in determining when, and how, to best use PARP inhibitors in anticancer therapy.

Keywords: PARP-1, PARP inhibitors, NF-κB, genetic transcription, sex hormone signaling, ERK signaling, angiogenesis, mitotic spindle

INTRODUCTION

Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme which binds DNA via two zinc finger motifs and transfers chains of ADP-ribosyl moieties (PARs) from nicotinamide-adenine-dinucleotide (NAD^+) to chromatin-associated acceptor proteins, including PARP-1 itself. This post-translational modification plays an important role in promoting DNA repair by releasing PARP-1 from DNA and allowing for recruitment of proteins involved in both base excisional repair (BER) and homologous recombination (HR) (1). Accordingly, PARP-1 is an attractive anticancer target, and poly(ADP-ribose) polymerase (PARP) inhibitors have been identified as chemo- and radiation-sensitizing agents in an array of cancers (2–5), including our report on the sensitization of head and neck cancer to radiotherapy following PARP inhibition (6). Perhaps the most well-known tumoricidal effects of PARP inhibitors are in BRCA-mutated cancers, which harbor DNA repair defects and become dependent on PARP-1-mediated repair for survival. Two landmark studies (7, 8) found inhibition of PARP-1 in cells containing BRCA mutations resulted in the generation of chromatid breaks, G2 cell cycle arrest, and enhancement of apoptosis, results which have been confirmed in early phase clinical trials (9, 10).

Interestingly, recent studies also show potential efficacy of PARP inhibition in sporadic tumors lacking DNA repair defects. A clinical study of the PARP inhibitor olaparib in women with heavily pretreated high-grade serous ovarian cancer without germline BRCA1/2 mutations resulted in objective responses in 11/46 (24%) (11), indicating there may be additional determinants of sensitivity to PARP inhibition. Pre-clinical studies have identified susceptibility to PARP inhibition alone in HR-proficient HER2-positive breast cancer, pancreatic cancer, prostate cancer, Ewing's sarcoma, small cell lung carcinoma, and neuroblastoma, among others (12–17). These reports demonstrate the existence of non-DNA repair functions of PARP-1 that may be targetable for cancer treatment. It is thus becoming increasingly apparent that a number of PARP-1-mediated cellular processes influence characteristics of tumor development, progression, and treatment response, including several of the eight "hallmarks of cancer" proposed by Hanahan and Weinberg (18) (**Figure 1**). In this review, we will discuss cancer-related functions of PARP-1 – including regulation of inflammatory mediators through NF-κB, cell death and energetics, ERK-mediated tumor progression and invasion, mitosis, gene transcription, and sex hormone signaling – and examples of how these functions may be exploited to expand the patient population potentially benefiting from treatment with PARP inhibitors.

NF-κB-MEDIATED TUMOR-PROMOTING INFLAMMATION

In multiple cancers, including breast, prostate, and head and neck among others, the NF-κB signaling pathway undergoes a loss of regulation resulting in constitutive activation (19). Briefly, NF-κB is a family of transcription factors including RelA/p65, RelB, c-Rel, p50, and p52, which exist as homo- and hetero-dimers. DNA-binding affinity and DNA sequence specificity is dependent on the composition of the dimer. Inhibitory proteins bind NF-κB dimers

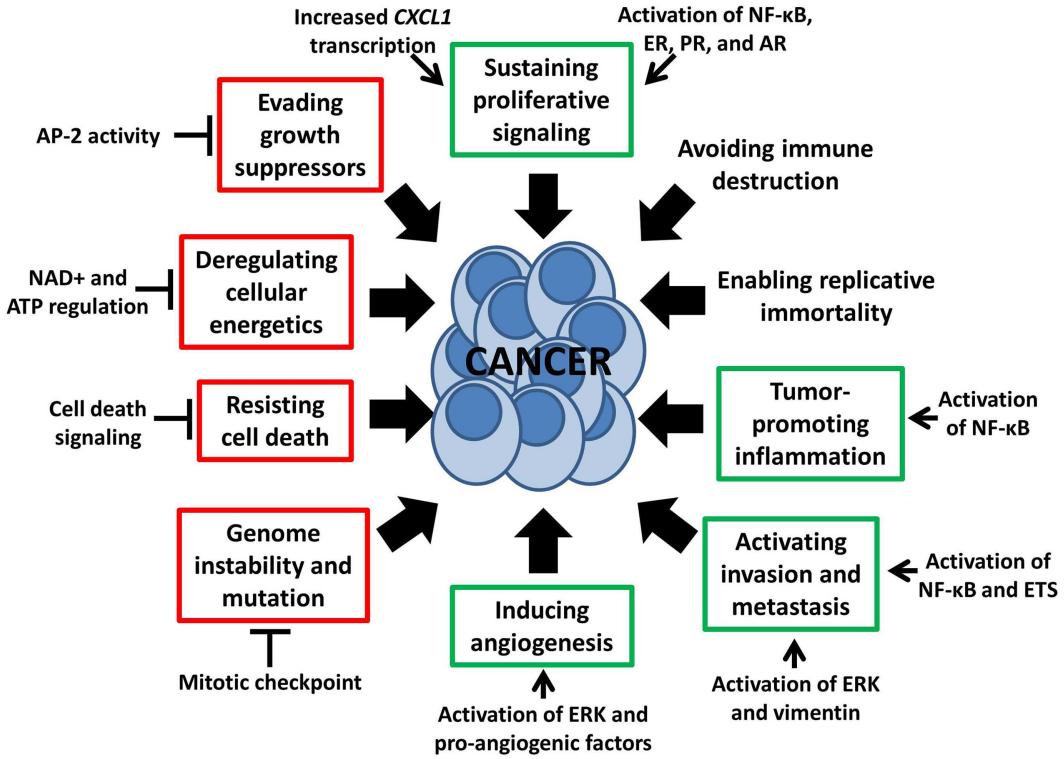

FIGURE 1 | Non-DNA repair functions of PARP-1 influence the "hallmarks of cancer" (18). This schematic depicts multiple PARP-1-mediated processes which either stimulate or inhibit six of the eight "hallmarks of cancer," as indicated by green and red boxes respectively. These hallmarks, proposed by Hanahan and Weinberg, are malignant characteristics that provide a framework for understanding the biology of cancer.

and sequester them in the cytosol in the absence of a stimulus; pathway activation causes proteasomal degradation of inhibitors, allowing the dimer to translocate to the nucleus and activate pro-inflammatory transcription programs. Although NF-κB signaling mediates the acute immune response responsible for targeting and eliminating cancerous cells, chronic inflammation mediated by this "hallmark" pathway can lead to the malignant phenotype (**Figure 1**), facilitating escape from immune surveillance, cancer survival, metastasis, and angiogenesis (20).

Activation of NF-κB can be regulated by PARP-1 via multiple mechanisms (**Figure 2**). First, PARP-1 directly interacts with histone acetyl-transferases p300 and CREB-binding protein (CBP) to synergistically co-activate NF-κB-dependent gene expression. In response to inflammatory stimuli, p300/CBP acetylates PARP-1 at specific lysine residues. This modification is necessary for PARP-1-p50 interaction, enhancement of p300–p50 interaction, and co-activation of NF-κB-mediated transcription programs (21, 22). Co-activation is negatively regulated by the activity of class I histone deacetylases (HDACs) (22) and SUMO1/3-mediated SUMOylation of the automodification domain of PARP-1 (23). Second, enzymatic activation of PARP-1 variably affects NF-κB, with outcomes dependent on the identity of the PAR acceptor protein. AutoPARylation of PARP-1 following detection of DNA strand breaks promotes the formation of a "signalosome" containing IKKγ (NEMO), the regulatory subunit of a NF-κB inhibitory complex, along with PIASγ, and ATM. Chains of PAR on activated PARP-1 provide the scaffold needed for SUMOylation of IKKγ by the PIASγ PAR binding motif, leading to activation of IKK and NF-κB (24). The effects of PARylation on NF-κB itself are less clear, with different sources reporting decreased, increased, or unaffected DNA-binding activity (25–27). Taken together, these studies demonstrate a strong role for PARP-1 in regulating NF-κB activity.

The interaction between PARP-1 and the NF-κB pathway promotes production of pro-inflammatory cytokines such as TNFα, IL-6, INFγ, E-selectin, and ICAM-1, as well as expression of nitric oxide synthase (28–30); PARP inhibition has been shown to attenuate upregulation of these factors in response to inflammatory stimuli (28, 29). Furthermore, PARP inhibition may also prevent inflammation-associated adverse side effects of traditional chemotherapeutics (31), supporting the use of PARP inhibitors in multidrug regimens. Loss of PARP-1 activity not only decreases pro-tumor inflammation, but also inhibits two related hallmarks of cancer through anti-inflammatory mechanisms: proliferative signaling (32) and metastasis (33, 34) (**Figure 1**).

Recently, we discovered an unexpected sensitivity to PARP inhibition in DNA repair proficient HER2-positive breast cancer cells through attenuation of NF-κB-mediated signaling (13). HER2 over-expressing cancers have activated NF-κB, which acts to block apoptosis and possibly mediate resistance to HER2-targeted drugs (35). In HER2-positive breast cancer cells, treatment with PARP inhibitor significantly reduced the expression of NF-κB activator

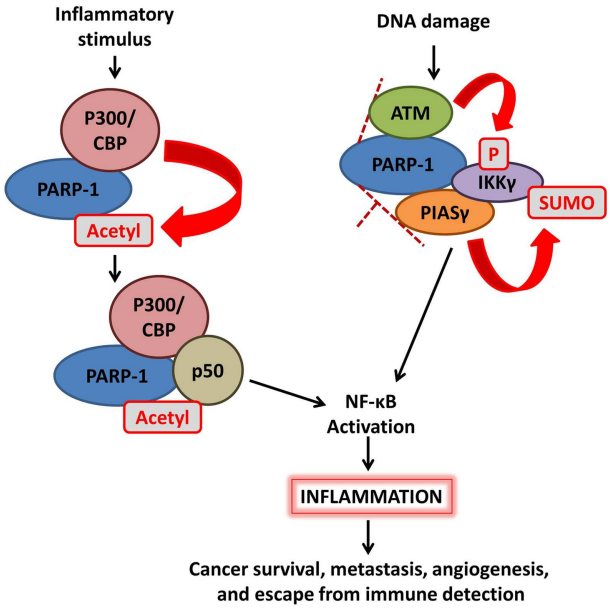

FIGURE 2 | Poly(ADP-ribose) polymerase-1 mediates activation of NF-κB signaling. (Left) inflammatory stimulation triggers p300/CBP acetylation of PARP-1, enhancing the interaction between p50 and PARP-1 as well as the p50–p300 interaction; this ultimately leads to activation of NF-κB. (Right) DNA damage detection promotes the formation of a complex including PARP-1, ATM, PIASγ, and IKKγ (NEMO); chains of PAR on PARP-1 provide a structure upon which PIASγ SUMOylates IKKγ, leading to NF-κB activation.

IKKα and phosphorylated p65 while increasing inhibitory IkBα. These events resulted in decreased NF-κB transcriptional activity in HER2-positive, but not HER2-negative, breast cancer cells (13). Furthermore, overexpression of HER2 alone was sufficient to confer sensitivity to PARP inhibitor, suggesting synthetic lethality with PARP inhibition in tumors that are oncogene-addicted to HER2 signaling through NF-κB. This study represents a specific application of PARP-1-regulated NF-κB signaling to cancer therapy, one that may soon be expanded into a clinical trial.

CELLULAR ENERGETICS AND CELL DEATH

Cancer cells are characterized by excessive proliferation, impaired cell death signaling, and deregulated metabolism (**Figure 1**). These features are often mediated by altered mitochondrial activity coupled with inactivation of apoptotic signaling through decreased expression of pro-apoptotic factors like p53 or overexpression of anti-apoptotic factors like Bcl-x. Integrity of regulatory pathways for cell death and metabolism is important for response to many cancer treatment modalities, as well as in cancer imaging and diagnostics. Cellular energetics and death signaling are heavily regulated by PARP-1, allowing activity of this protein to serve as a switch between cell fates and to affect both tumor proliferation and therapeutic response.

In response to damage stimuli, activated PARP-1 acts early in the apoptosis initiation pathway to stabilize p53 and facilitate its function (36). If damage is excessive, high levels of PAR synthesis by PARP-1 deplete its NAD^+ substrate; additional interactions between PARP-1 and NMNAT-1, a NAD^+ synthase, and SIRT1, a NAD^+-dependent protein deacetylase, further contribute to PARP-1 as a controller of NAD^+ availability and, thus, NAD-dependent metabolic reactions. ATP-dependent NAD^+ salvage saps cellular ATP stores, resulting in energy deprivation and, eventually, energy crisis-induced necrosis (**Figure 3**). Furthermore, PARP-1-mediated PARylation may inactivate caspase-8 and reduce caspase-mediated apoptotic signaling (37). Hyperactivation of PARP-1 and accumulation of PAR can also cause translocation of PAR to the cytosol, where it interacts with the outer mitochondrial surface. Here it binds apoptosis inducing factor (AIF) and induces its release and translocation to the nucleus, ultimately resulting in large-scale DNA fragmentation and a novel PARP-1-dependent cell death mechanism known as "parthanatos" (38). To prevent these events, activated caspases cleave PARP-1 into two fragments: an 89-kDa C-terminal fragment with low levels of catalytic activity and a 24-kDa N-terminal peptide which inhibits the catalytic activity of uncleaved nuclear PARP-1. Conservation of NAD^+ and, thus, ATP allows the cell to undergo programed cell death (39–41). Accordingly, inhibition of PARP-1 preserves ATP levels, improves antioxidant status, and normalizes anti-apoptotic Bcl-x levels in the kidney following chemotherapy-induced injury (42, 43).

Poly(ADP-ribose) polymerase-1 also regulates the classical necroptotic pathway mediated by the death promoting MAP kinase, c-Jun N-terminal kinase (JNK). This signaling network is activated in many cancers and has been implicated as a driver of both tumor development and treatment response (44, 45). PARP-1 downregulates MAP kinase phosphatase MKP-1 expression and inhibits the survival kinase Akt, both of which activate JNK (46, 47), suggesting potential benefit for PARP inhibition in tumors with elevated JNK activity. JNK1 mediates phosphorylation and sustained activation of PARP-1, creating a feed-forward regulatory loop (48). In conjunction, PARP-1-induced depletion of ATP stimulates AMP-activated protein kinase (AMPK) while inhibiting mTOR to promote autophagy, yet another cell death pathway important in cancer survival and treatment response (49). Pharmacologic inhibition of PARP-1 promotes Akt activity and mTOR signaling resulting in decreased cell death (50), although these results are contradicted by a recent report showing PHLPP1-mediated downregulation of Akt activity and increased cell death following PARP inhibition (51).

Clinically, targeting the role of PARP-1 in cell death pathways appears to be complex. PARP-1 inhibition may reduce PAR-mediated inactivation of caspase-8, sensitizing cancer cells to tumor necrosis factor-related apoptosis-induced ligand (TRAIL) therapy (37). Additionally, inhibition of PARP-1 prevented cisplatin- and methotrexate-induced ATP depletion and nephrotoxicity (42, 43), as well as imatinib (Gleevec)-induced JNK activation and cardiotoxicity (52), without significantly affecting the anticancer activity of these agents. However, activation of the Akt survival pathway may counteract the cytotoxic effects of PARP inhibition and cause resistance to therapy (47), suggesting Akt pathway inhibition may enhance PARP inhibition in anti-tumor therapy. Despite these complexities, the influence of PARP-1 on metabolic co-factors and cell death signaling is significant, and further studies examining the role of PARP inhibition in manipulating these processes is warranted.

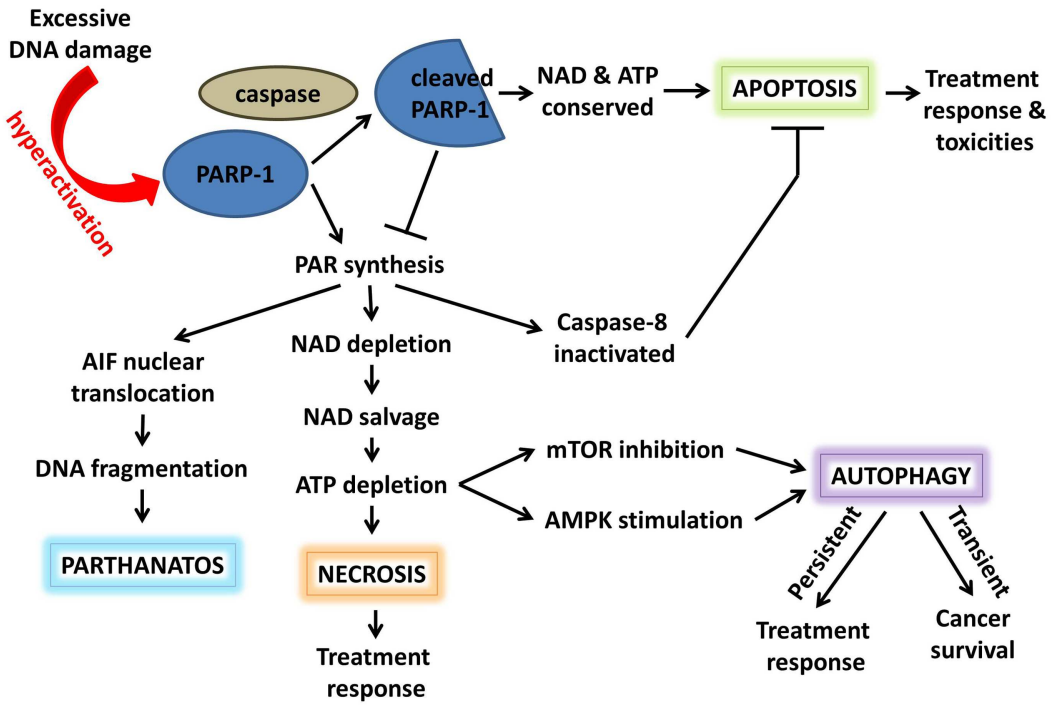

FIGURE 3 | Poly(ADP-ribose) polymerase-1 acts as a switch between cell fates. Hyperactivation of PARP-1 and PAR synthesis depletes NAD and, subsequently, ATP. Elevated PAR can promote necrosis, autophagy, or AIF-induced parthanatos. In addition, PARylation inactivates caspase-8, inhibiting apoptotic signaling. Alternatively, activated caspases can cleave PARP-1; the resulting cleavage product inhibits uncleaved PARP-1, conserving NAD/ATP, and promoting apoptosis. These cell death pathways play a role in both cancer survival and response to anticancer therapy.

ERK-MEDIATED ANGIOGENESIS AND METASTASIS

In addition to the JNK-mediated signaling described previously, a second family of MAP kinases known as extracellular signal-regulated kinases or ERKs is involved not only in cell death determination but also in tumor progression, angiogenesis, and metastasis. ERK activation is pivotal in cancer cell survival through upregulation of anti-apoptotic proteins and inhibition of caspase activity (53). Inhibition of this pathway by targeting ERK or MEK, which is immediately upstream of ERK in signaling, has been associated with suppression of ovarian tumor growth (54), reduced metastatic potential of melanoma cells (55), and increased sensitivity to cytotoxic agents (56). Recent studies indicate an important role for PARP-1 in promoting ERK signaling.

Poly(ADP-ribose) polymerase-1 is activated and autoPARylated by a direct interaction with phosphorylated ERK2 (pERK2), resulting in enhanced pERK2-catalyzed phosphorylation of target transcription factors and increased gene expression (57). Furthermore, PARP inhibition causes loss of ERK2 stimulation by decreasing the activity of critical pro-angiogenic factors including vascular endothelial growth factor (VEGF), transmembrane signaling protein syndecan-4 (SDC-4), platelet/endothelial cell adhesion molecule (PECAM1/CD31), and hypoxia inducible factor (HIF). This ultimately results in reduced angiogenesis and inflammation (58–62). The effects of PARP-1 on ERK signaling are further enhanced by PARP-1-mediated transcription of vimentin, an intermediary angiogenic filament upregulated in tumor vasculature and pivotal for the endothelial-to-mesenchymal transition characteristic of metastasis (63). Pharmacologic inhibition of PARP reverted this transition, correlating with a reduction in the number and size of metastatic melanoma foci in a mouse model (63).

Collectively, these studies indicate PARP-1 directly fosters ERK signaling in addition to mediating separate but parallel signaling pathways reinforcing the same end result of increased angiogenesis and metastasis, two tumor-promoting features (**Figure 1**). As such, PARP inhibition may be effective in blocking the ERK signaling network or increasing activity of ERK/MEK inhibitors, agents already shown to be efficacious in acute myeloid leukemia, multiple myeloma, melanoma, colorectal, breast, lung, and pancreatic cancers (64–68). Furthermore, selective ERK inhibition induces tumor regression in MEK inhibitor-resistant models (67), raising the question of whether PARP inhibition could be similarly effective in either MEK or ERK-resistant tumors due to its proximity in the signaling pathway. As MEK, ERK, and PARP inhibitors have only recently entered early phase clinical trials, it will be some time before we know which patients benefit most from these drugs, either alone or in combination, but their interaction warrants further investigation.

MITOTIC REGULATION

The high proliferation rate of cancer cells is a result not only of decreased cell death but also of improperly regulated cell cycling, allowing evasion of growth suppressing signals. Although multiple cell cycle checkpoints can be impaired in cancer, the mitotic

or spindle assembly checkpoint is of great importance both in tumorigenesis and as an anticancer target. This point of regulation, which is responsible for ensuring appropriate chromosome segregation, is required for cell viability. Cells with a weakened mitotic checkpoint are capable of survival but do not maintain proper chromosome segregation, resulting in genomic instability and aneuploidy. These are common features of tumor cells and may even act as drivers in cancer development (**Figure 1**). PARP-1 can act on many mediators of cell cycle progression through its effects on gene expression (68), which will be detailed in a later section. However, direct regulation of the mitotic checkpoint by PARP-1 is another important factor that may be targetable in cancer treatment.

Recent reports suggest multiple roles for PARP-1 in the structural machinery of mitosis. First, PAR, which is primarily synthesized by PARP-1, is required for assembly and function of the bipolar spindle (69). In addition, PARP-1 both localizes to and PARylates proteins at centromeres and centrosomes during mitosis (70, 71). PARP-1 also mediates PARylation of p53, which is responsible for regulating centrosome duplication and monitoring chromosomal stability (71). Loss of PARP-1activity is associated with mislocalization of centromeric and centrosomal proteins, resulting in incomplete synapsis of homologous chromosomes, defective chromatin modifications, and failure to maintain metaphase arrest, indicating loss of mitotic checkpoint integrity (71, 72). Similarly, inhibition of PARP-1 is associated with genomic instability characterized by reduced stringency of mitotic checkpoints, centrosome hyperamplification, and chromosomal aneuploidy, the most common characteristic of solid tumors (71, 73, 74).

Furthermore, PARP-1 has been shown to interact with the E3 ubiquitin ligase, CHFR, a tumor suppressor with an important role in the early mitotic checkpoint. Binding of these two proteins results in degradation of PARP-1 and cell cycle arrest in prophase, an effect stimulated by the microtubule inhibitor docetaxel resulting in resistance to this drug in CHFR-over-expressing cancer cells. Concomitant use of a PARP inhibitor with docetaxel significantly increased apoptosis in these cells, suggesting a role for PARP inhibition in sensitizing cancers with high CHFR activity to microtubule inhibitors (75).

GENE TRANSCRIPTION

The clinical characteristics of cancer, including growth, metastatic potential, and response to treatment, are greatly influenced by dysregulation of gene transcription. Gene expression profiles are currently being utilized as tumor biomarkers, indicators of treatment sensitivity or resistance, and prognostic predictors. In the future, there may even be a role for therapeutic agents that reactivate a silenced tumor suppressor or silence an activated oncogene. In total, 3.5% of the transcriptome is regulated by PARP-1 with 60–70% positively regulated (76), including genes involved in tumor promotion such as *JUND*, *MDM2*, *HGF*, *FLT1* (VEGFR1), *EGFR*, *HIF2A* (EPAS1), *SPP1* (OPN), *MMP28*, *ANGPT2*, and *PDGF* (77). As discussed below and shown in **Figure 4**, this regulation can occur broadly through interactions with nucleosomes and modification of chromatin, can be gene specific through interactions with promoters and binding factors, or can result as a combination of the two, as binding of PARP-1 to nucleosomes mediates its localization to specific target gene promoters (78, 79).

CHROMATIN STRUCTURE

One mechanism by which PARP-1 alters gene expression is through regulation of chromatin structure and, thus, DNA accessibility. Simultaneous binding of multiple neighboring nucleosomes by PARP-1 compacts chromatin into a supranucleosomal structure, repressing gene transcription (79). This structural change is further stimulated by histone deacetylation mediated by a complex consisting of PARP-1, ATP-dependent helicase Brg1 (SmarcA4), and HDACs (80). Conversely, PARylation of core histones promotes charge repulsion-induced relaxation of chromatin and recruitment of transcription machinery (81–83). PARP-1-mediated PARylation also results in disassociation of linker histone H1, a repressor of RNA polymerase II-mediated transcription; accordingly, higher proportions of PARP-1:H1 indicate active promoters (84), suggesting potential utility of PARP-1 as a biomarker for actively transcribed genes. Although these outcomes can be separated by PARP-1 activity (protein binding versus enzymatic function), pharmacologic inhibition of PARP affect both actions, indicating manipulation of chromatin accessibility through PARP-1 is not currently an option for cancer therapy.

METHYLATION PATTERNS

Along with chromatin structure, methylation patterns also play a large role in determining DNA accessibility. Alterations in DNA methylation are commonly found in many cancers and serve as a functional equivalent to a gene mutation in the process of tumorigenesis. Inhibition of PARP-1 is associated with transcriptional silencing through accumulation of DNA methylation and CpG island hypermethylation throughout the genome (85). This effect may be mediated by dimerization of PARP-1 with CCCTC-binding factor (CTCF), a chromatin insulator which binds to hypomethylated DNA regions. As the CTCF-PARP-1 interaction is PAR-dependent, decreased PAR following PARP inhibition abrogates this function (86, 87). Loss of CTCF-PARP-1 complex activity results in transcriptional silencing of multiple loci including tumor suppressors *CDKN2A-INK4* (p16), *CDH1* (e-cadherin), and *P19ARF* (88, 89).

Poly(ADP-ribose) polymerase-1 can also hinder DNA methylation by dimerization with DNA (cytosine-5-)-methyltransferase 1 (DNMT1), a methyltransferase found overexpressed in gastrointestinal tract carcinomas, resulting in inhibition of its methyltransferase activity (85, 90). In contrast, PARP-1 binding and PARylation of the *Dnmt1* promoter actually enhances its transcription by preventing methylation-induced silencing (91). The reduced catalytic efficiency of PARylated DNMT1 may come as a result of negatively charged PARylated PARP-1 out-competing DNA for binding with DNMT1 (92). Interestingly, PARP-1-DNMT1 can form a ternary complex with CTCF at unmethylated CTCF-target sites in a PAR-dependent manner. Loss of PAR from this complex causes dissociation of PARP-1 and CTCF, allowing the still-bound DNMT1 to methylate the site and inhibit transcription (92).

Although some specific tumor suppressors are mentioned above as being affected by PARP-1-mediated chromatin insulation, the activity of PARP-1 in regulating DNA methylation patterns

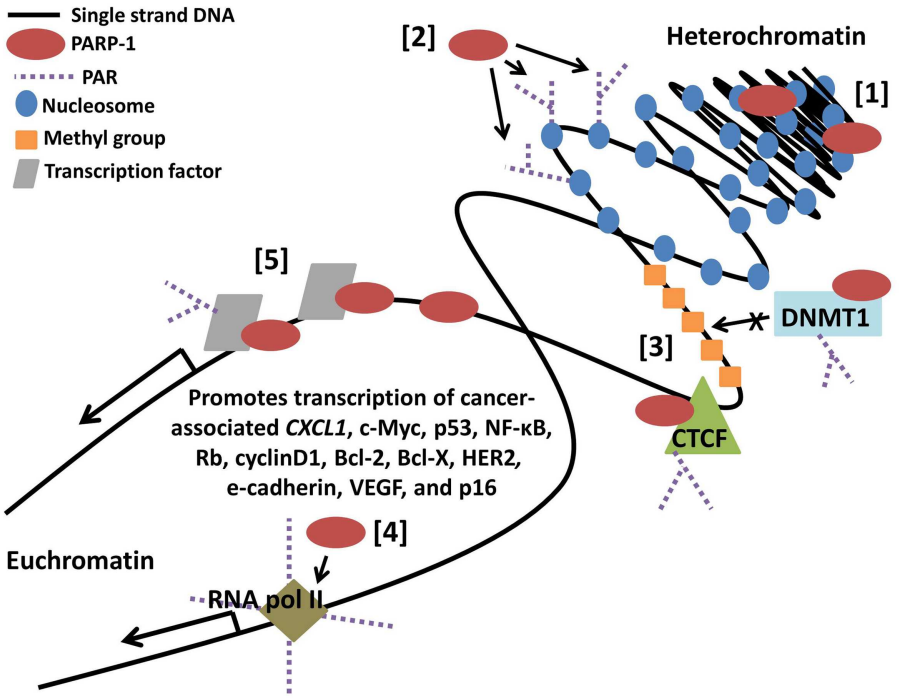

FIGURE 4 | Poly(ADP-ribose) polymerase-1-regulates gene transcription through multiple mechanisms. [1] PARP-1 binds neighboring nucleosomes resulting in chromatin compaction. [2] PARP-1 PARylation of core histones mediates chromatin relaxation. [3] PARP-1 promotes hypomethylation of DNA by enhancing the chromatin insulator activity of CCCTC-binding factor (CTCF) while inhibiting methyltransferase activity of DNMT1. [4] PARP-1 promotes loading and retention of RNA polymerase II at active promoters. [5] PARP-1 binds regulatory DNA sequences and transcription factors, PARylates transcription factors, and recruits additional regulatory binding proteins in a target gene specific manner.

at specific genes or genic regions is largely unknown. As such, it is difficult to predict the effect of PARP inhibition on cancer growth and progression through this mechanism. However, with the advent of genomic profiling, it has recently become possible to identify methylation changes specific to certain cancer subtypes. Anticancer agents with epigenetic modifying activity, such as DNA methyltransferase inhibitors, are being investigated in these cancers and show promising results, especially in hematologic malignancies (93). The effect of PARP inhibition on epimutations has not been studied, but the reports described above suggest PARP inhibitors could have similar applicability.

RNA POLYMERASE II ACTIVITY

Poly(ADP-ribose) polymerase-1 can also promote transcription in a more sequence-specific manner by positively regulating RNA polymerase II activity at active promoters. This occurs through: (1) PARylation-induced exclusion of histone demethylase KDM5B, maintaining levels of activating histone mark K3K4me3 (82), (2) PARylation-induced dissociation of the DEK repressor, promoting loading of the RNA polymerase II mediator complex (94), and (3) creation of a PAR scaffold for retention of RNA polymerase II (95). Surprisingly, a recent report showed that inhibition of PARP-1 enzymatic activity was associated with increased H3K4me3, resulting in upregulation of sodium iodide symporter transcription and elevated radio-iodine uptake in thyroid cancer cell lines (96). This contradictory work may result from target gene specific functions of PARP-1, as the previously cited studies were focused on genes known to be positively regulated by PARP-1. However, it does illustrate the need for greater understanding of PARP-1 involvement at active gene promoters, as well as the potential for manipulating PARP-1-mediated transcription to enhance efficacy of cancer therapy.

DNA AND TRANSCRIPTION FACTOR BINDING

Gene expression can be further regulated by direct interactions between PARP-1 and DNA elements or binding factors. PARP-1 acts as a promoter-specific switch at target genes, facilitating the release of inhibitory co-regulators and recruitment of stimulatory co-regulators (97, 98). PARP-1 binding of the NF-κB immediate upstream region (IUR) element activates transcription of *CXCL1*, which encodes melanoma growth stimulatory activity protein and is overexpressed in the progression of malignant melanoma (99). Binding of PARP-1 to the transcription factor E2F-1 increases E2F-1 promoter activity and expression of the E2F-1-responsive oncogene *Myc* (c-Myc) (100). PARP-1 expression and activity are also required for cancer cell invasion (**Figure 1**) mediated by ETS transcription factors – whose fusion products drive Ewing's sarcoma, acute myeloid leukemia, and prostate cancer – and the Ewing's sarcoma fusion protein EWS-FLI (14, 15). While PARP-1 interaction with these factors promotes pro-tumor signaling, other interactions have the opposite effect. PARP-1 suppresses self-inhibition of AP-2, a transcription factor that negatively regulates

Table 1 | Summary of reported non-DNA repair functions of PARP-1 with potential clinical correlations.

PARP-1 function	Effect	Model system studied	Clinical applicability of PARP inhibition
Binding histone acetyl-transferases p300/CBP	Co-activation of NF-κB (pro-inflammatory)	*In vitro* and *in vivo* HER2$^+$ breast cancer cell lines	May inhibit cancer metastasis; cytotoxicity in HER2-positive breast cancer specifically (13, 21, 22)
Binding DNMT1	Enhances *Dnmt1* transcription, inhibits methyltransferase activity	*In vitro* mouse fibroblasts	May have activity in DNMT1-overexpressing colorectal, gastric, and hepatic carcinomas (85, 91, 92)
Binding pERK2	Promotes target gene transcription	*In vitro* endothelial cells	May inhibit cancer growth and metastasis (58)
Binding CHFR	Prophase arrest, resistance to microtubule inhibitors	*In vitro* gastric carcinoma cell lines	Re-sensitizes CHFR-expressing cancers to microtubule inhibitor therapy (75)
Downregulation of MKP-1 and inhibition of Akt	Activation of JNK	*In vitro* hepatocytes	May have activity in tumors with high JNK activity (46, 47)
AutoPARylation	Activation of NF-κB (pro-inflammatory)	*In vitro* and *in vivo* HER2$^+$ breast cancer cell lines	May inhibit cancer metastasis; cytotoxicity in HER2-positive breast cancer specifically (13)
Caspase-8 PARylation	Impaired apoptotic signaling	*In vitro* and *in vivo* pancreatic cancer cell lines	Sensitizes cancer cells to TRAIL therapy (37)
PARylation	ATP depletion, promotes necrosis and autophagy	Mouse and rat kidney and heart studies	Prevents cell death mediated toxicities of multiple chemotherapy agents (42, 43, 52)
PARylation of transcription regulators	Promotes transcription	*In vitro* thyroid cancer cell lines	Upregulates NaI symporter transcription leading to increased radio-iodine uptake in thyroid cancer (96)
Androgen receptor PARylation	Increases androgen receptor activity	*In vivo* and *ex vivo* prostate cancer cells	Sensitizes prostate cancer to androgen depletion, enhances effects of anti-androgen therapy, delays onset of resistance to anti-androgen therapy (110)
ETS and EWS-FLI PARylation	Promotes transcription of target genes	*In vivo* and *in vitro* prostate cancer and sarcoma cells	Cytotoxicity in ETS-prostate cancer and EWS-FLI Ewing's sarcoma specifically (14, 15)
Vimentin promoter PARylation	Promotes transcription	*In vitro* melanoma cells and *in vivo* melanoma model	Inhibits cancer metastasis (63)
Interaction with VEGF, SDC-4, PECAM1/CD31, HIF promoters	Promotes transcription	*In vitro* endothelial cells	Inhibits tumor angiogenesis (58–62)

cell cycle and proliferation (101). Increased AP-2 expression suppresses cancer cell growth (102) and may inhibit *ras* oncogene-mediated transformation (101), effects likely diminished by PARP inhibition (**Figure 1**). PARP-1 has also been shown to bind the inhibitory element of COX-2, which mediates inflammation and promotes VEGF-mediated pro-angiogenesis pathways activated in cancer cells (103, 104).

Instances of PARP-1-mediated enzymatic activity affecting specific transcription factors or genes often translate to a clear role for PARP-1 inhibitors as anticancer agents, even in monotherapy. For example, ETS-positive prostate tumors and EWS-FLI-positive Ewing's sarcomas are highly sensitive to PARP inhibitors (14, 15). However, PARP-1 has multiple and diverse functions involving both PARylation activity and DNA-binding capability. Enzymatic inhibition, which decreases PARP-1 self PARylation, actually increases DNA binding and may be detrimental in some cancers, such as the malignant melanoma example given above. A greater understanding of the relative effects of PARP-1 on transcriptional activity is needed in order to select tumors with a molecular profile conducive to pharmacologic inhibition through this mechanism.

SEX HORMONE SIGNALING

Sex hormones have been implicated in development, progression, and treatment sensitivity of prostate, breast, gynecologic, and colon cancers. Sex steroid effects are mediated through their receptors, which act as transcription factors in steroid-responsive tissues. Any of the multiple levels of regulation controlling these signaling pathways can become impaired, leading to abnormal proliferative responses characteristic of cancer progression (**Figure 1**). Similar to PARP-1-mediated regulation of transcription factor activity, PARP-1 plays a role in regulating three of the sex hormone receptors most commonly linked to cancer: estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR).

Approximately 80% of breast carcinomas are positive for ER, identifying ER-targeted therapies as excellent, although not unfailable, treatment options in these cancers (105). PARP-1 interacts

with the ERa isoform both directly and through estradiol-induced PARylation to enhance binding of ERa and other activating factors to target gene promoters (106, 107), suggesting PARP inhibition may enhance the activity of ER-targeted agents. A similar interaction occurs between PARP-1 and PR: PARP-1 binding of PR, as well as hormone-activated CDK2-induced PR PARylation, acts to stimulate cancer cell proliferation (108). PARP-1 regulation of PR activity is of great interest in endometrioid carcinomas specifically, as expression of PARP-1 and PR is positively correlated at each pathologic stage of this cancer (109). However, the effects of PARP inhibition in endometrial cancer have yet to be determined.

Recently, a report detailing the strong interaction between PARP-1 and AR has generated much excitement over the potential for PARP inhibitors in prostate cancer treatment. Human prostatic adenocarcinoma, a cancer highly resistant to standard therapies, is reliant on AR activity for growth and survival. Accordingly, AR-targeted therapies are the primary treatment for these patients. Unfortunately, there are multiple mechanisms for AR reactivation leading to tumor recurrence, a lethal phenotype known as castration-resistant prostate cancer. PARP-1 enzymatic activity, which is significantly upregulated in castration-resistant prostate cancer, promotes both AR chromatin binding and transcription factor functions. Although PARP-1 does localize with AR to regulatory sites of AR-target genes, the two proteins appear to be members of separate complexes at these loci. Inhibition of PARP-1 *in vivo*: (1) depletes both PARP-1 and AR at target genes, (2) significantly reduces expression of target genes, including protumorigenic *ets* genes referenced previously, (3) sensitizes both castration-resistant and castration-sensitive prostate cancer cells to genotoxic insult and androgen depletion, (4) enhances the antitumor effects of anti-androgen therapy, and (5) delays onset of resistance to anti-androgen therapy. *Ex vivo* studies of castration resistance prostate tumors displayed a significant anti-tumor response to both veliparib and olaparib, two well-known PARP inhibitors, that correlates with reduced AR activity (110). These results suggest PARP inhibitors have the potential to significantly enhance existing prostate cancer therapy and improve outcomes for patients with castration-resistant tumors.

PROMISE AND CHALLENGES

Poly(ADP-ribose) polymerase inhibitors are exciting new drugs that are easily delivered, can be highly efficacious, and are associated with few side effects. Mild nausea is commonly reported, with rare instances of more serious symptoms such as temporary cognitive deficits and myelosuppression. While ongoing clinical trials are focused on exploiting the role of PARP-1 in DNA repair, we have identified in this review multiple targetable functions of PARP-1 that are not dependent on HR defects (**Figures 1–4**; **Table 1**). One of the challenges in broadening the use of PARP inhibitors in anticancer therapy is more efficient identification of patients who may respond to these drugs. Some ongoing clinical trials include analysis of protein expression – including HR proteins, NF-κB, and PARP-1 itself – in relation to clinical response in search for potential biomarkers of sensitivity. However, the list of candidates is extensive and will continue to grow as additional functions of PARP-1 are discovered. Banking tumor biopsies from patients enrolled in PARP-1 clinical trials will greatly expedite the development of a panel of biomarkers, as will increased use of cancer genome sequencing and microarray technologies. Another challenge will be in identifying and overcoming mechanisms of resistance to PARP inhibition. For example, a second BRCA mutation or a deletion of the original mutation can cause reversion to HR-proficiency and resistance to PARP inhibitors in BRCA-mutated cancers (111). As the majority of clinical applications proposed here are theoretical or in pre-clinical development, associated mechanisms of resistance are entirely unknown, although development of such resistance is practically assured. Thirdly, many of the functions discussed here are effected by PARP-1 binding rather than enzymatic activity. Currently available PARP inhibitors act at the catalytic site of PARP-1, which does result in some degree of altered binding capacity via changes in autoPARylation status. However, treatment with PARP inhibitors may not effectively inhibit specific PARP-1 interactions, or may require different dosing. It will be important to study the various clinically available agents to determine if, and to what extent, binding domains are affected. Despite these obstacles, PARP inhibition is an extremely promising anticancer strategy and, as the first agents near completion of phase III trials, it will be exciting to see the magnitude of impact PARP inhibitors will have in clinical practice.

AUTHOR CONTRIBUTIONS

Alice N. Weaver and Eddy S. Yang conceptualized the topic. Alice N. Weaver conducted the literature review and wrote the article. Eddy S. Yang critically revised the article and provided guidance and supervision.

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Structural implications for selective targeting of PARPs

Jamin D. Steffen[1], Jonathan R. Brody[2], Roger S. Armen[3] and John M. Pascal[1]*

[1] *Department of Biochemistry and Molecular Biology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA*
[2] *Department of Surgery, Division of Surgical Research, Jefferson Pancreas, Biliary, and Related Cancer Center, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA*
[3] *Department of Pharmaceutical Sciences, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA*

Edited by:
Christina Annunziata, National Cancer Institute, USA

Reviewed by:
Dana Victor Ferraris, Johns Hopkins University Brain Science Institute, USA
Myron Jacobson, University of North Texas Health Science Center, USA

Correspondence:
John M. Pascal, Department of Biochemistry and Molecular Biology, Kimmel Cancer Center, Thomas Jefferson University, 233 South 10th Street, BLSB 804, Philadelphia, PA 19107, USA
e-mail: john.pascal@jefferson.edu

Poly(ADP-ribose) polymerases (PARPs) are a family of enzymes that use NAD^+ as a substrate to synthesize polymers of ADP-ribose (PAR) as post-translational modifications of proteins. PARPs have important cellular roles that include preserving genomic integrity, telomere maintenance, transcriptional regulation, and cell fate determination. The diverse biological roles of PARPs have made them attractive therapeutic targets, which have fueled the pursuit of small molecule PARP inhibitors. The design of PARP inhibitors has matured over the past several years resulting in several lead candidates in clinical trials. PARP inhibitors are mainly used in clinical trials to treat cancer, particularly as sensitizing agents in combination with traditional chemotherapy to reduce side effects. An exciting aspect of PARP inhibitors is that they are also used to selectivity kill tumors with deficiencies in DNA repair proteins (e.g., BRCA1/2) through an approach termed "synthetic lethality." In the midst of the tremendous efforts that have brought PARP inhibitors to the forefront of modern chemotherapy, most clinically used PARP inhibitors bind to conserved regions that permits cross-selectivity with other PARPs containing homologous catalytic domains. Thus, the differences between therapeutic effects and adverse effects stemming from pan-PARP inhibition compared to selective inhibition are not well understood. In this review, we discuss current literature that has found ways to gain selectivity for one PARP over another. We furthermore provide insights into targeting other domains that make up PARPs, and how new classes of drugs that target these domains could provide a high degree of selectivity by affecting specific cellular functions. A clear understanding of the inhibition profiles of PARP inhibitors will not only enhance our understanding of the biology of individual PARPs, but may provide improved therapeutic options for patients.

Keywords: PARP, selectivity, structure, inhibitor design

INTRODUCTION

ADP-ribosyltransferases (ARTs) comprise a family of structurally conserved enzymes that catalytically cleave NAD^+ and transfer the ADP-ribose moiety to acceptor residues of target proteins (1). Poly(ADP-ribosyl) polymerases (PARPs) are a subset of the ART family that continue this reaction to create long chains of linear and/or branched poly(ADP-ribose) (PAR). Currently, only the first six members of this family (ARTs 1–6) are regarded as having poly(ADP-ribosyl)ation activity: PARP-1, PARP-2, PARP-3, PARP-4 (vPARP), PARP-5a (TNKS1), and PARP-5b (TNKS2) (**Figure 1**). The remaining ARTs 7–17, although originally considered PARPs (PARPs 6–16) (2), are only capable of producing mono-ADP-ribose modifications and are referred to as mono-ARTs (MARTs). ARTs 9 (PARP-9; BAL-1) and 13 (PARP-13) have yet to confirm any sort of catalytic activity like PARPs or MARTs. The degree of ADP-ribosylation in cells is not only controlled by ARTs, but also by PARG and ADP-ribosyl hydrolases that reverse this modification [recently reviewed in Ref. (3)].

Poly(ADP-ribose) polymerase-1 has emerged as a prominent target in chemotherapy due to its important role in maintenance of genomic integrity. Its functional roles in the DNA damage response and cell fate determination have fueled development of PARP-1 inhibitors. Some of these compounds have entered clinical trials with promising therapeutic applications toward treatment of cancer. In combination with DNA damaging agents (e.g., temozolomide, cisplatin) or irradiation, PARP-1 inhibitors are effective chemosensitizers (4). As monotherapy, PARP-1 inhibitors selectively kill tumors harboring DNA repair deficiencies such as genetic deletion of genes involved in the BRCA1 and BRCA2 homologous recombination DNA repair pathway (5, 6). This phenomenon referred to as "synthetic lethality" has attracted clinical attention and has paved the way for a "personalized" approach to cancer therapy (7).

Originally PARP-1 was the only known enzyme with poly(ADP-ribosylation) activity, but as other PARPs began to emerge the selectivity of PARP-1 inhibitors were called into question and now they are typically referred to as PARP inhibitors. In fact, 185 PARP inhibitors were recently evaluated for binding to the catalytic domain of several different PARPs, and revealed binding profiles demonstrating a lack of specificity for any given PARP (8). Where PARPs 1–3 seem to have an important role in maintaining genomic integrity, other PARPs have roles such as telomere replication and

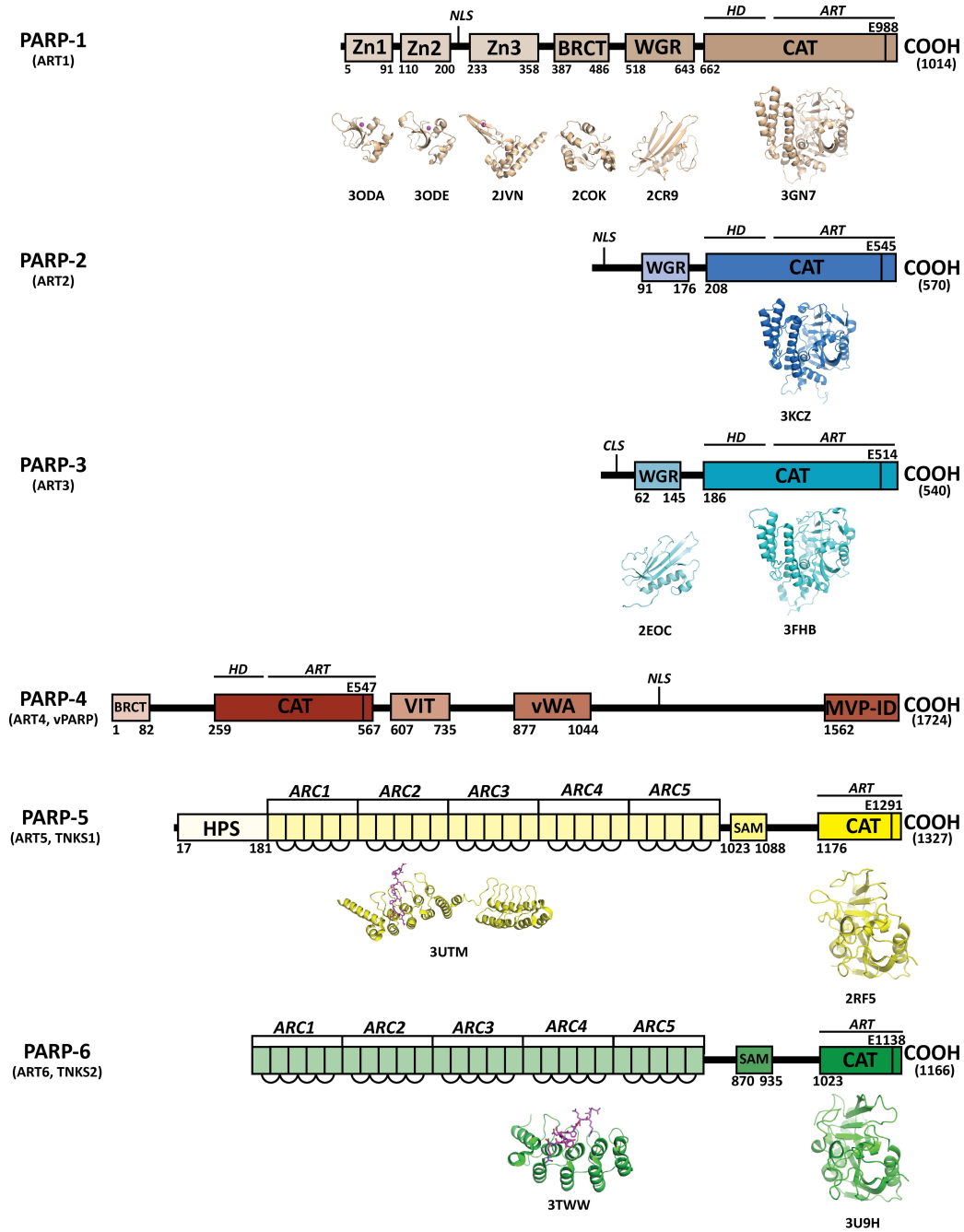


FIGURE 1 | Domains of human PARPs. A sequence and structural representation of the six *bona fide* PARPs. Each PARP has a catalytic domain containing an ADP-ribosyltransferase domain (ART) and conserved catalytic glutamic acid residue. In addition PARPs 1–4 contain a helical domain (HD) that serves in allosteric regulation. PARPs 1–3 contain a WGR domain, which is important in DNA-dependent catalytic activation. The breast cancer susceptibility protein-1 C-terminus (BRCT) domain is commonly found in DNA repair and checkpoint proteins, and resides in the automodification domain of PARP-1, and is also present in PARP-4. Zinc-fingers Zn1 and Zn2 of PARP-1 are important in binding DNA, while the third zinc-finger (Zn3) is important in DNA-dependent catalytic activation. Other domains and sequences represented include: centriole-localization signal (CLS), vault protein inter-alpha-trypsin (VIT), von Willebrand type A (vWA), major vault particle interaction domain (MVP-ID), His-Pro-Ser region (HPS), ankyrin repeat clusters (ARCs), sterile alpha motif (SAM), and nuclear localization signal (NLS).

cellular transport (9, 10). With such a large family of enzymes carrying out distinct biological functions, drug targeting of the conserved catalytic site of PARPs has raised questions concerning intended pharmacological outcomes. This has led some groups to pursue development of PARP inhibitors with increased selectivity to better understand the biology of targeting individual PARPs.

The aim of this review is to describe the structural relationships among PARPs and the drug design efforts that have found ways to engineer PARP selectivity. We bring attention to non-catalytic domains that are contained within PARPs, and how targeting these domains could provide increased selectivity. The differences in therapeutic benefit and unwanted side effects of selective PARP inhibition versus pan-PARP inhibition is not well understood, and the development and use of more selective agents will ultimately help answer these important questions concerning PARP inhibitors as chemotherapy. For clarity and relevance purposes, all structural comparisons regarding residues and numbering are described based on human PARP-1 unless otherwise noted. The locations of key binding or catalytic site residues have been given position numbers in the text and figures to help guide the viewer through the structural comparisons.

STRUCTURAL SIMILARITIES AND DIFFERENCES AMONG PARPs

Poly(ADP-ribose) polymerases are multi-domain proteins that are related through their highly conserved ART domain (**Figure 1**). Outside of the ART domain, distinct domain architectures quickly differentiate the structure and function of each PARP. The catalytic domain crystal structures have been solved for all current PARPs except for PARP-4 (vPARP). The crystal structures of some non-catalytic domains of PARPs have been solved, although there is no crystallographic data on any full-length PARP. The closest to a full-length structure is a catalytically active complex of PARP-1 essential domains bound to DNA damage (11).

CATALYTIC DOMAIN

While the pairwise sequence identity among the catalytic domains of human PARPs is under 50%, their structures are highly conserved (**Figure 1**). The PARP catalytic domain contains an ART domain composed of a donor site with a β-α-loop-β-α signature motif that binds NAD^+, an acceptor site where ADP-ribose chains are extended, and a helical domain (HD) present in PARPs 1–4 and some MARTs (**Figure 2A**). Although there is no crystal structure of NAD^+ bound to a human PARP, the diphtheria toxin structure (PDB: 1TOX) of NAD^+ bound to a bacterial ART domain (12) along with homology modeling of PARP-1 (13) provides insight into the likely binding mode. Within the donor site is a nicotinamide-binding pocket and an ADP-ribose binding pocket. PARPs share an H-Y-E triad sequence motif in their active site that is altered in MARTs. These residues along with other residues conserved among PARPs are critical for the initiation, elongation, and in some instances branching of PAR synthesis (14). Substrate binding in the acceptor site is also not completely understood, since the only structural data shows a portion of a bound non-hydrolyzable NAD^+ analog (carba-NAD, cNAD) that provides insights into how PAR might bind (15).

HELICAL DOMAIN

The HD consists of six α-helices (A through F) that form a hydrophobic core, with helix αA contributing to the fold of the ART domain (**Figure 2A**, HD region). The HD structures of PARP-2 and PARP-3 superimpose with PARP-1 very well, and overall have a high sequence similarity (**Figure 2B**). In PARPs 1–3 (and likely PARP-4) helix αF is adjacent to the donor NAD^+ binding site. In PARP-1, structural rearrangement of the N-terminal Zn1, Zn3, and WGR domains in response to DNA damage detection causes a destabilization of the HD that ultimately triggers catalytic hyperactivation (11, 16). While PARP-4 has a putative HD based on sequence alignment, tankyrases do not contain a HD. Outside of PARP-1 DNA-dependent activation, other mechanisms that could destabilize the HD remain unknown. DNA-independent PARP-1 activation from phosphorylation has been reported (17), but the mechanisms that trigger catalytic activation are unclear.

ART DOMAIN – DONOR SITE

In the PARP catalyzed reaction, the co-substrate NAD^+ binds to the ART domain and "donates" the ADP-ribose portion to an amino acid residue or a growing PAR chain (**Figure 2A**, donor site). The donor site is also the site where PARP inhibitors bind. The donor site is composed of a nicotinamide-binding pocket (NI site), a phosphate binding site (PH site), and an adenine-ribose binding site (AD site) (**Figure 2D**). The NI site consists of a structural motif that is highly conserved among PARPs: two tyrosine residues that form a π–π stacking interaction with the nicotinamide ring (**Figure 2D**, positions 14 and 17), and a hydrogen-bond network between a serine hydroxyl (position 16) and glycine backbone atoms (position 6) with the carboxamide of NAD^+. In the AD site of PARP-1 (**Figure 2D**), main-chain atoms of Gly876 (position 10) and Arg878 (position 11), and side-chains of Asp770 (position 3), His862 (position 5), and Ser864 (position 7) are predicted to interact with the adenosine portion of NAD^+. In the PH site (**Figure 2D**), Asp766 (position 2) and Glu763 (position 1) are situated near the pyrophosphate group of NAD^+. Based on modeling predictions, the catalytic conserved residues (H-Y-E motif) residing at the NI site include Glu988 (position 18) that binds to the 2′-hydroxyl group of the nicotinamide ribose positioning NAD^+ for nucleophilic attack by the acceptor substrate (**Figure 2D**, NI site), His862 (position 5) that binds to the 2′ adenine-ribose hydroxyl (**Figure 2D**, AD site), and Tyr896 (position 14) that stacks with the nicotinamide ring (**Figure 2D**, NI site). Similarly, the rest of the donor site is very much the same among PARPs 1–3 with a few minor variations (**Figures 2B,C**): (i) in the NI site Ser864 (position 7) is replaced with Thr386 (PARP-3), (ii) in the PH site Glu763 (position 1) is replaced with Gln319 (PARP-2), Asp284 (PARP-3), and Arg354 (PARP-4), and (iii) Asp766 in PARP-1 (position 2) extends to Glu322 (PARP-2), Leu287 (PARP-3), and Val357 (PARP-4). Other observations near the donor site that could influence drug selectivity include variations in PARP-3 with respect to PARP-1, such as Val390/Asn868 (position 8) and Met402/Ala880 (position 12).

Like PARPs 1–3, tankyrases contain an ART domain with the catalytic signature (H-Y-E) motif including the active glutamic acid residue essential for PAR synthesis. The NI site is very similar, however since tankyrases do not have an HD domain to form the outer wall of the AD and PH site, residues vary greatly in these regions. Instead, the donor site loop (D-loop, **Figure 2A**) of tankyrases helps form this outer wall creating a more restricted environment in its closed conformation. Perhaps the most interesting feature of the tankyrase catalytic domains is that they contain a CHCC-type zinc-finger that is not known to be present

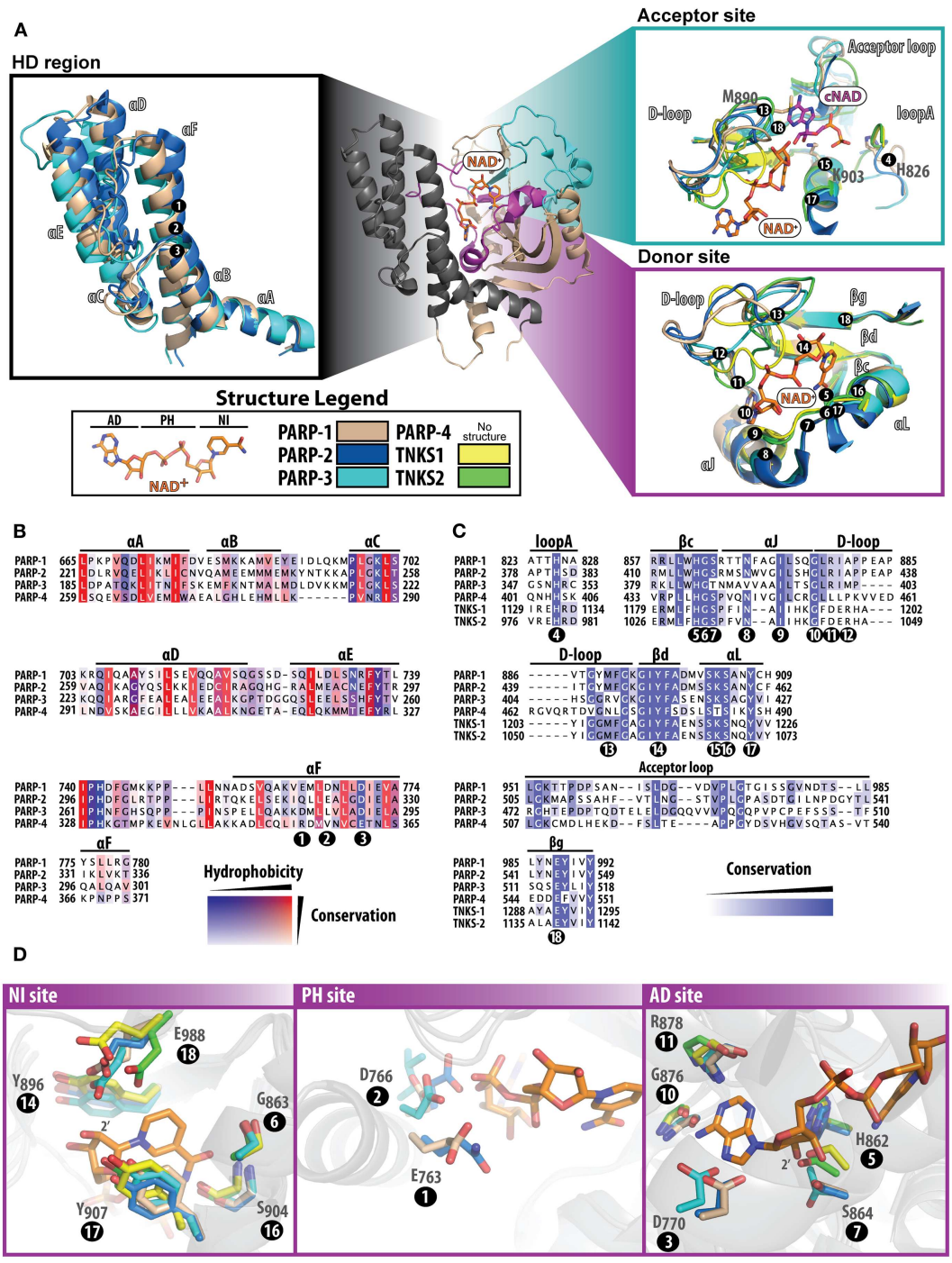


FIGURE 2 | Structure and sequence comparisons of the PARP catalytic domain. The PARP-1 catalytic domain [**(A)**; center] is used as a template to compare specific regions among other PARPs. All other PARPs were structurally aligned using Pymol (www.pymol.org/). In **(A)**, all numbering positions corresponding to the protein sequence are labeled at the Cα of the residue in PARP-1. The helical domain [**(A)**; left] present in PARPs 1–4, consists of six alpha helices numbered A–F. At the core of this domain are several hydrophobic residues, which are highly conserved among PARPs **(B)**. The acceptor and donor sites [**(A)**; right] display binding of NAD^+ (modeled) and the ADP portion of co-crystallized carba-NAD (cNAD) (PDB ID: 1A26). The donor site that binds NAD^+ is highly conserved **(C)** among all PARPs, although the acceptor site is much less conserved (acceptor loop and loop A). The D-loop assumes varying structural conformations and is also less conserved, which is an indication of where selectivity may be best achieved. The donor site is composed of three regions that bind to NAD^+ **(D)** the NI site (left), the PH site (middle), and the AD site (right). Multiple sequence alignments were carried out using ClustalW2 [(83); www.ebi.ac.uk/Tools/msa/clustalw2/], and the sequences of human PARPs were analyzed using Jalview [(84); www.jalview.org/]. Structures used for comparisons include: PARP-1 (PDB ID: 3GN7), PARP-2 (PDB ID: 3KCZ), PARP-3 (PDB ID: 3FHB), TNKS1 (PDB ID: 2RF5), and TNKS2 (PDB ID: 3U9H).

in any other ART domain (18). The importance of this motif is only speculative, but could be used for structural stability or mediating protein or DNA interactions. The sequence identity between TNKS1 and TNKS2 are highly conserved, with variable residues located mostly outside of the NAD^+ binding site.

ART DOMAIN – ACCEPTOR SITE

Despite the lack of structural data on substrates bound to the acceptor site of PARPs, a structure has been reported for a transition state analog of NAD^+ bound to the acceptor site of chicken PARP-1 (15). From this structure of bound cNAD (**Figure 2A**, Acceptor site), it can be projected that His826 (position 4), Lys903 (position 15) and the backbone amides of 985 and 986 form a H-bond network with the acceptor PAR pyrophosphates. The ribose hydroxyl groups H-bond to Tyr907 (position 17) and Glu988 (position 18), and the adenine base stacks against Met890 (position 13). These residues are conserved in other PARPs with the exception of PARP-3 that does not contain the Met890, which is replaced with an arginine (408) that forms a salt bridge with Asp455 (19). This amino acid change could contribute to the smaller polymers produced by PARP-3 (20, 21). A highly variable region among PARPs is in the acceptor loop (**Figure 2A**, acceptor site and **Figure 2C**). PARP-2 has a similar alignment as PARP-1 but contains an additional three residues in this loop, most notably an additional tyrosine residue (Tyr539) that projects into the acceptor site based on the structure of mouse PARP-2 (22). Both tankyrases have a much shorter acceptor loop and diverge in their structural alignment with PARP-1. These differences in the acceptor loop across PARPs could potentially specify a preference for particular proteins that are targeted for modification.

ART DOMAIN – D-LOOP

The D-loop lines the donor site and partially the acceptor site, and represents structural diversity among PARPs due to variations in conformations observed across structures (**Figure 2A**). The D-loop in PARPs 1 and 2 are near identical; in contrast, the D-loop of PARP-3 (Gly398-Lys411) is smaller than PARP-1, which leaves the donor site more open (19). The major differences comparing PARP-3 to PARP-1 include the Met402/Ala880 (position 12) and Gly406/Tyr889 changes. The D-loop of tankyrases is frequently observed in a closed conformation, which blocks the NAD^+ binding site, although it is likely that this loop is dynamic to allow NAD^+ access (18). The sequence conservation between tankyrases is very similar, although in structures of TNKS1 the D-loop is positioned closer to the nicotinamide-binding pocket and in TNKS2 it closes near the ADP-ribose binding pocket. The differences between TNKS1 and TNKS2 may reflect an inherent mobility of the tankyrase D-loops.

NON-CATALYTIC DOMAINS

Poly(ADP-ribose) polymerase-1 is the founding member and most studied of the PARP family. PARP-1 and PARP-3 are the only PARPs for which structures of all domains are known (**Figure 1**). PARP-1 has a modular domain architecture comprising five domains in addition to the catalytic domain: N-terminal Zn1 and Zn2 domains which are homologous zinc-finger domains that recognize damaged DNA ends (23), a third zinc-finger domain (Zn3) that is important in DNA-dependent activation (24), a central BRCA C-terminus-like fold (BRCT) domain that mediates protein–protein interactions and serves as a substrate for PAR automodification (25), and a tryptophan-glycine-arginine (WGR) domain that interacts with DNA and is important for DNA-dependent activation.

As in PARP-1, both PARP-2 and PARP-3 share a homologous WGR domain positioned N-terminal to the catalytic domain. In PARP-1 the WGR domain is important for DNA-dependent activation and interacts with DNA (11). The function of WGR in PARP-2 and PARP-3 is not well evaluated, although it likely interacts with DNA based on homology to PARP-1. Neither PARP-2 or PARP-3 have zinc-finger binding domains or a BRCT domain, but PARP-2 has a highly basic N-terminal region that could mediate interaction with DNA.

Originally characterized by its association with major vault protein (MVP) through its MVP interaction domain (MVP-ID) (26), the structure and function of PARP-4 is one of the least understood of the PARPs. Other PARP-4 domains include vault protein inter-alpha-trypsin (VIT) and von Willebrand type A (vWA) domains that are also found together in the inter-alpha-trypsin inhibitor (ITI) family, but are not completely understood in connection with PARP-4. It is not known to contain zinc-fingers or a WGR domain, but contains an N-terminal BRCT domain homologous to PARP-1 (26).

While tankyrases contain a catalytic domain that is capable of producing PAR, they do not share any other domains with the other PARPs. With regard to the PARPs, tankyrases have the following unique domains: an ankyrin repeat region that binds acceptor proteins, a sterile alpha motif (SAM) domain that mediates oligomerization, and a histidine-proline-serine rich (HPS) domain unique to TNKS1 with unknown function (9). The series of ankyrin repeats are arranged into five ankyrin repeat clusters (ARCs). With the exception of ARC3, each ARC is reported to bind acceptor proteins that carry the tankyrase consensus binding sequence RXXPDG (27). The tankyrase targets, Axin1 and peptides derived from several other target proteins, have been co-crystallized with individual ARCs, and the structures illustrate the key features of the binding interaction (28, 29). The overall conformation of the five ARCs and possible structural arrangements upon mediating protein–protein interactions is not currently understood. TNKS2 is nearly identical to TNKS1 except that it does not have an HPS region and has a seven amino acid insertion after the ankyrin repeat region with unknown importance (30).

DEVELOPMENT OF SELECTIVE PARP INHIBITORS

Nearly 30 years ago inhibitors of PARP-1 were discovered, and shown to sensitize cells to DNA damaging agents (31). These early PARP inhibitors, such as the benzamides and isoquinolinones, established a core pharmacophore from which future PARP inhibitors would build (32, 33). Co-crystallization of the catalytic domain of chicken PARP-1 with these inhibitors showed anchoring into the nicotinamide-binding pocket of PARP-1, consistent with the nicotinamide-mimicking pharmacophore (13, 34). The carboxamide functional group of nicotinamide makes three hydrogen-bond interactions with the serine hydroxyl and glycine

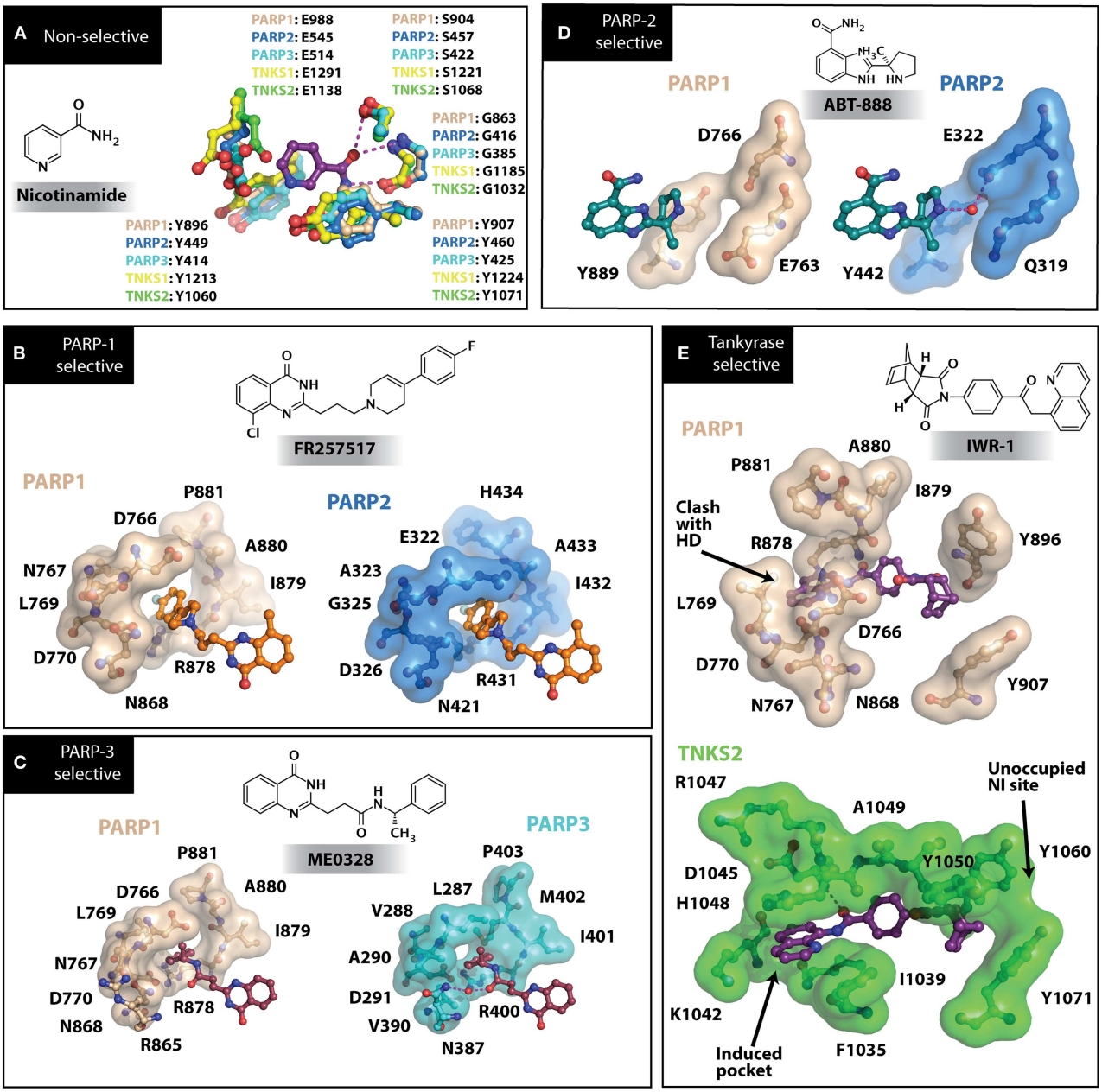


FIGURE 3 | PARP inhibitors bound to the catalytic domain of PARPs. Non-selective inhibitors such as nicotinamide [**(A)**, non-selective] only interact with the nicotinamide pocket (NI site), which is a highly conserved region. Most developed PARP inhibitors have been designed to bind the NI site and adjacent sites to gain potency and selectivity. The compound FR257517 contains a fluorophenyl that reaches into the ADP-ribose binding site (AD site) of PARP-1 (PDB ID: 1UK0) to gain selectivity [**(B)**, PARP-1 selective]. An aligned PARP-2 structure (PDB ID: 3KCZ) shows how the AD site is very similar to that of PARP-1, but the increased hydrophobicity of the PARP-1 AD site is attributed to the observed PARP-1 selectivity. Compounds that interact with E322 of PARP-2 (PDB ID: 3KJD) can gain selectivity over PARP-1 due to the differences in distance between this acidic side-chain and drug heteroatoms [**(D)**, PARP-2 selective]. PARP-3 (PDB ID: 4GV4) has a structurally similar AD site as PARPs 1 and 2, although residue variation creates an environment distinct in polarity that guides selectivity [**(C)**, PARP-3 selective]. Tankyrase inhibitors often demonstrate a much higher window of selectivity from PARPs 1–4, although selectivity between TNKS1 and TNKS2 is difficult to obtain. IWR-1 is a non-traditional PARP inhibitor in that it does not target the nicotinamide site of TNKS2 [**(E)**, Tankyrase selective]. PARP-1 (PDB ID: 1UK0) was aligned with the co-crystallized TNKS2 structure containing IWR-1 (3UA9) to demonstrate that the quinoline ring clashes into the AD site of PARP-1 due to the presence of its helical domain.

backbone atoms of the NI site, and the benzene ring makes π–π stacking interactions with surrounding tyrosine residues (**Figure 3A**). The chemotherapeutic potential of PARP inhibitors prompted medicinal chemistry efforts aimed at designing newer PARP inhibitors with improved potency and pharmacokinetic properties. These efforts spurred development of several small

molecule nicotinamide-like scaffolds with modified side groups reaching outside of the pocket and into regions such as the donor AD site that improved potency, selectivity, and bioavailability (**Table 1**). For more information on this development the reader is referred to an in-depth review focusing on the optimization of PARP inhibitors (35).

SELECTIVITY BETWEEN PARP-1 AND OTHER PARPs

By the late 90s, the identification of a second PARP, termed PARP-2, was reported (36). Since PARP-2 carries out the same catalysis as PARP-1, uses the same co-substrate, and is highly homologous, it is not surprising that most PARP inhibitors show similar inhibition potency between both PARPs. The nicotinamide pockets of PARP-1 and PARP-2 are nearly identical, and there are only minor differences in their ADP-ribose binding pockets. The minor sequence variation, Glu763/Gln319 in HD helix αF, and the presence of Tyr539 of PARP-2 in the acceptor loop, have been noted as important differences in which selectivity could be achieved (22).

Soon after the discovery of PARP-2, several nicotinamide-mimicking inhibitors discovered through a high-throughput cell-based assay identified that most had similar inhibition between PARP-1 and PARP-2, although minor selectivity was noted with certain compounds (37). These findings demonstrated that PARP selectivity could be achieved despite nearly identical binding sites. Although infrequently reported, most compounds that inhibit PARP-1 have little to no preference for PARP-1 over PARP-2. Attempts to improve selectivity resulted in nicotinamide-based compounds that also target outside of the NI site. The quinazolinone-based inhibitor (FR257517) binds the PARP-1 nicotinamide pocket and further interacts with Asn767, Asp770, Asp766, Asn868, and Ala880 in the AD site through its extended substitution (38) (**Figure 3B**). Interestingly, the extended portion of the molecule induces a conformational change in Arg878 that opens a new hydrophobic pocket surrounded by residues Leu769, Ile879, and Pro881 (**Figure 3B**). It is thought that a Leu769/Gly325 variation in the induced hydrophobic pocket creates a more hydrophobic environment in PARP-1, which is why this compound is 10-fold more selective for PARP-1 (39, 40). Further modifications of this compound near the NI site accomplished selectivity for PARP-1 up to 39-fold, indicating that selectivity may also be adjusted through modifications near the nicotinamide pocket. Another example is an isoquinolindione compound (BYK204165) that was identified with a 100-fold PARP-1/PARP-2 selectivity (41). Unfortunately there is no co-crystal structure data of this compound to understand this preference.

Most inhibitors developed target PARP-1 and PARP-2 closely, but there are also varying degrees of selectivity for the other PARPs due to the similarities in active sites (although much less frequently reported). Small, basic PARP inhibitors that target the nicotinamide site (such as 3-amino-benzamide) are very unselective across PARPs, and even MARTs. Potent PARP-1 inhibitors with bulky side groups or extensions typically gain selectivity against other PARPs (especially the tankyrases) due to steric clash that can be easily rationalized considering the noticeable structural differences outside of the NI site (**Figure 2A**).

SPECIFIC PARP INHIBITORS

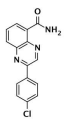
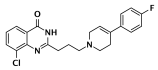
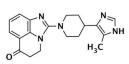
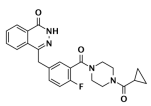
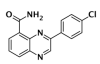
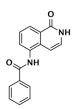
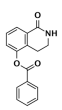
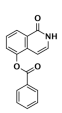
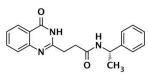
Poly(ADP-ribose) polymerases-2 selective inhibition was seen early on with quinoxaline based inhibitors (39). Preference for PARP-2 over PARP-1 is seen based on residue variations between the two. The modified quinoxaline phenyl ring of compound 2 (**Table 1**) more favorably interacts with the space between Gln319 and Glu322 in PARP-2 over the Glu763 and Asp766 in PARP-1 (as seen in **Figure 3D** for ABT-888). Also, PARP-2 forms a water-mediated hydrogen-bond with the inhibitor through its acidic residue Glu322, which is not formed by PARP-1, thus creating a stronger affinity for PARP-2. In PARP-1 this residue is a shorter Asp766 residue that is further from the NI site, which may explain the preference for PARP-2 selectivity through a closer, thus stronger interaction (40). Crystallographic studies of ABT-888 also suggest a closer proximity of Glu335 over Asp766 in PARP-1 to the side group N-heteroatom of ABT-888 setting up a potentially more favorable interaction (**Figure 3D**) (42). Interaction with this acidic residue is essential for potency in many compounds, and may in part explain the near 1000-fold higher selectivity of ABT-888 for PARP-1 and PARP-2 over TNKS1 and TNKS2, which do not have this residue (43).

A library of isoquinolinone derivatives was reported to display selectivity for PARP-2 up to 60-fold (44). This discrimination is thought to be due to a single residue variation of Glu763 in PARP-1 to Gln319 in PARP-2. Interestingly, desaturation of the nicotinamide-mimicking portion also increased PARP-2 selectivity, indicating that even though these sites are highly conserved, small steric effects can have a significant impact on selectivity (44).

Although there is a lack of data on PARP-3 inhibition, recently reported quinazoline derivatives, such as ME0328 (**Table 1**; **Figure 3C**), have been shown to have up to sevenfold selectivity for PARP-3 over PARP-1 (45). These compounds anchor into the NI site and extend into the AD donor site of PARP-3, which is slightly larger and more hydrophobic. Differences in polarity and geometry of the AD sites of PARP-3 and PARP-1 are likely guiding factors in the observed selective inhibition. Co-crystallization studies of PARP inhibitors with PARP-3 also indicate that the sequence variation and D-loop conformation changes in the AD site create distinguishing environments for designing PARP-3 selective inhibitors (19). Modifications of the core scaffold that reach out into the acceptor site could target Arg408 (which is a methionine residue in other PARPs) in order to achieve selectivity.

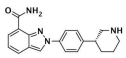
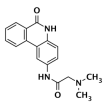
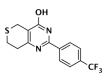
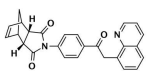
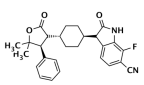
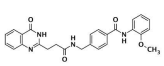
Due to the smaller and more hydrophobic donor site of tankyrases, selectivity over other PARPs can be more easily achieved. The first selective tankyrase inhibitor to be discovered was XAV939, which binds the nicotinamide pocket (46) and has a 200-fold selectivity over PARP-1 (43). Therapeutic interest in tankyrases prompted high-throughput screening (HTS) assays leading to the discovery of IWR-1 (47), JW55 (48), and flavones (49) as specific tankyrase inhibitors. IWR-1 and IWR-2 are non-traditional inhibitors that bind to the AD and PH site but not the NI site of tankyrases, but still block NAD^+ binding (**Figure 3E**) (50, 51). IWR compounds bind to the donor site of TNKS1 making H-bond interactions with Tyr1213 and Asp1198 (Tyr1060 and Asp1045 in TNKS2), and stacking interactions between Phe1198 and His1201 (Phe1035 and His1048 in TNKS2) (51, 52). In co-crystal structures, rearrangement of the

Table 1 | Selectivity of PARP inhibitors. Published IC50 values of PARP inhibitors that have been tested against multiple PARPs.

PARP-1 SELECTIVE										
Compound	**Structure**	**Class**	**PARP-1**	**PARP-2**	**PARP-3**	**PARP-4**	**TNKS1**	**TNKS2**	**Selectivity[a]**	**Reference**
			IC50 (nM)							
DR2313		Thiopyrano pyrimidine	200	2,400					12	Nakajima et al. (81)
1		Quinoxaline	30	90					3	Sunderland et al. (85)
FR257517		Quinazolinone	13	500					39	Ishida et al. (40)
BYK204165		Isoquinolindione	45	4,000					89	Eltze et al. (41)
BYK49187		Imidazoquinolinone	4	20					5	Eltze et al. (41)
BYK20370		Imidazopyridine	400	2,000					5	Eltze et al. (41)
PARP-2 SELECTIVE										
Compound	**Structure**	**Class**	**PARP-1**	**PARP-2**	**PARP-3**	**PARP-4**	**TNKS1**	**TNKS2**	**Selectivity[b]**	**Reference**
			IC50 (nM)							
Olaparib (AZD-2281) (KU-0059436)		Pthalazinone	5	1			1,500		5	Menear et al. (86)
Veliparib (ABT-888)		Benzimidazole	5 8.3	2 11			14,970	6,519	2.5 0.75	Penning et al. (87) Huang et al. (43)
2		Quinoxaline	101	8					13	Ishida et al. (40)
3		Isoquinolinone	13,900	1,500					9	Sunderland et al. (85)
4		Isoquinolinone	13,000	800					16	Pellicciari et al. (44)
5		Isoquinolinone	9,000	150					60	Pellicciari et al. (44)
PARP-3 SELECTIVE										
Compound	**Structure**	**Class**	**PARP-1**	**PARP-2**	**PARP-3**	**PARP-4**	**TNKS1**	**TNKS2**	**Selectivity[c]**	**Reference**
			IC50 (nM)							
ME0328		Quinazolinone	6,300	10,800	890	>30,000	>30,000	>30,000	7	Lindgren et al. (45)

(Continued)

Table 1 | Continued

PARP-1 AND PARP-2 SELECTIVE										
Compound	**Structure**	**Class**	**PARP-1**	**PARP-2**	**PARP-3**	**PARP-4**	**TNKS1**	**TNKS2**	**Selectivity[d]**	**Reference**
			IC50 (nM)							
GPI6150		Isoquinolinone	∼100	∼100					n.d.[f]	Zhang et al. (82)
Niraparib (MK-4827)		Indazole	3.8	2.1	1,300	330	570		87–342	Jones et al. (88)
5-AIQ		Isoquinolinone	940	1,050					n.d.[f]	Sunderland et al. (85)
PJ-34		Phenanthridine	600	1,000					n.d.[f]	Pellicciari et al. (44)
DPQ		Isoquinolinone	4,500	5,300					n.d.[f]	Pellicciari et al. (44)
6		Benzo-naphthyridinone	1	1	50	440	3,500		50–3,500	Torrisi et al. (89)
TANKYRASE SELECTIVE										
Compound	**Structure**	**Class**	**PARP-1**	**PARP-2**	**PARP-3**	**PARP-4**	**TNKS1**	**TNKS2**	**Selectivity[e]**	**Reference**
			IC50 (nM)							
XAV939		Pyrimidinone	2,194	114			11	4	200	Huang et al. (43)
			620				14	8	44	Karlberg et al. (53)
			120	46	>10,000		11	8	11	Larsson et al. (90)
7		Isoquinolinone	>10,000	>10,000	>10,000		860	52	12	Larsson et al. (90)
IWR-1		Tetrahydro-Phthalimide	>18,750	>18,750			131	56	>143	Huang et al. (43)
			>85,000	>170,000			150	39	>567	Bregman et al. (54, 55)
8		Oxazolidinone	>85,000	>170,000			1		>85,000	Bregman et al. (54, 55)
9		Quinazolinone		931			8	2	116	Bregman et al. (54, 55)

[a] *Fold selectivity for PARP-1 vs. PARP-2 (PARP-2 IC50 / PARP-1 IC50).*

[b] *Fold selectivity for PARP-2 vs. PARP-1 (PARP-1 IC50 / PARP-2 IC50).*

[c] *Fold selectivity for PARP-3 vs. PARP-1 (PARP-1 IC50 / PARP-3 IC50).*

[d] *Fold selectivity for PARP-1 vs. PARP-3, PARP-4, and TNKS1 (PARP-1 IC50 / PARP-3, PARP-4, or TNKS1 IC50).*

[e] *Fold selectivity for TNKS1 vs. PARP-1 (PARP-1 IC50 / TNKS1 IC50).*

[f] *Not determined.*

tankyrase D-loop (Ala1202–Ala1210 in TNKS1; Ala1049–Ala1057 in TNKS2) is observed in which Tyr1203 (Tyr1050 in TNKS2) flips outward allowing access to the binding site, and movement of Phe1198 (Phe1035 in TNS2) creates an induced pocket that accommodates binding (18, 51, 52). In the absence of inhibitor or NAD^+, Tyr1203 lies across the NAD^+ binding pocket and forms a hydrogen-bond to the main-chain of Tyr1224 (Y1071 in TNKS2), which effectively blocks access of NAD^+ to the binding pocket.

The opening of this site is similar to the effects seen with XAV939 binding to TNKS2 and TNKS1 (46, 53). In PARPs 1–4 the outer wall is formed in part by the HD that would creates steric clash with these compound (as observed with the aligned PARP-1 structure in **Figure 3E**). While this molecule is a useful tool for selective tankyrase inhibition, it suffers from poor cellular potency and efforts are being made to improve its potency and pharmacokinetic and pharmacodynamic properties (compounds 8 and 9 from **Table 1**) (54, 55).

OTHER PARP INHIBITORS THAT DO NOT MIMIC NICOTINAMIDE

While tankyrase inhibitors appear to be paving the way for non-nicotinamide-based PARP inhibitors, we make note of a few other non-traditional scaffolds. For example, imidazoquinolinones and imidazopyridine based compounds do not contain the carboxamide feature, but are potent inhibitors of both PARPs 1 and 2 (41). These compounds inhibit competitively, meaning they block NAD^+ from binding and thus would likely have similar challenges as most PARP inhibitors in optimizing selectivity.

Metabolites of coumarin derivatives made way for C-nitroso derivatives that irreversibly inhibited PARP-1. These compounds were observed to eject the zinc ion from the first zinc-finger domain (Zn1), presumably through oxidation of the coordinating cysteine residues resulting in disulfide bond formation (56). This mechanism was noted to act selectively on Zn1 and not Zn2, which fell in line with the loss in catalytic activity but remaining DNA binding (57). These compounds showed promising chemotherapeutic potential as they induced apoptosis in human tumor cells (58). Further development of this molecule resulted in 4-iodo-3-nitroso-benzamide (INO_2BA; iniparib), a clinical candidate that showed clinical benefit in treating metastatic triple negative breast cancer (TNBC) (59); however a larger phase 3 trial failed to reproduce prolonged survival in TNBC. Iniparib was later demonstrated to have poor selectivity and potency for PARP-1 zinc-fingers (60, 61), and thus is not a "*bona fide*" PARP inhibitor. Unfortunately this drug provided an inaccurate representation of true PARP inhibitors to the community, and its failure does not reflect the therapeutic potential of PARP inhibitors.

POTENTIAL FOR ALTERNATIVE INHIBITORS AS ISOFORM SPECIFIC PARP INHIBITORS

High-throughput screening for PARP catalytic site inhibitors and substrate mimicry are two typical strategies taken to develop new PARP inhibitors. When PARP selectivity is desired, chemical manipulations by side group modification or scaffold optimization are used to target the slight differences in the NAD^+ binding site. With development of new screening assays, we will be capable of searching for compounds that inhibit non-catalytic domains of PARPs. For example, our group has recently developed an HTS assay to detect allosteric regulation of PARP-1 (62). Since the domains involved in allosteric regulation are unique to PARP-1, identified inhibitors would likely be highly selective. In addition to isoform specificity, inhibition of allosteric regulation may only affect certain functions of PARP-1. For instance, we find that inhibition of allosteric regulation affects DNA-dependent activation without affecting androgen receptor-mediated transcriptional activities. It is likely that other PARP-1 mediated functions would also not be affected by disruption in allosteric regulation, which could be beneficial in terms of pharmacological efficacy and adverse effects.

Structural characterization of PARP non-catalytic domains in complex with protein or DNA has provided grounds for rational drug design approaches. Despite difficulties in development of inhibitors that target protein–protein or protein–DNA interfaces, identification of clustered protein interface regions of high-affinity, known as "hot spots," has been a guiding concept in the inhibition of protein interactions with small molecules (63). From the structure of the essential domains of PARP-1 in complex with DNA damage, there are several domain–domain interfaces that form critical contacts that are required for PARP-1 activation (**Figure 4**). All-atom molecular modeling analysis of the energetic contribution of individual residues to these protein–protein interfaces predicts that hot spots exist between the domains of PARP-1 (unpublished data). Our analysis using the CHARMM force field and the GBMV implicit solvent model (64, 65) suggested that the majority of binding free energy between the Zn1 and Zn3 domain comes from a few local residues (e.g., R78 and W79 of Zn1). Interestingly, mutation of either of these residues is detrimental to PARP-1 DNA-dependent catalytic activity (62). A small nearby hydrophobic groove exists next to these residues, which could potentially bind and disrupt the interaction between the Zn1 and Zn3 domains (**Figure 4**). Moving forward, a better understanding of the dynamics of PARP-1 domain arrangements in a cell-based context will be important in any kind of rational drug design approach that targets interdomain interfaces. Furthermore, additional structural studies that can locate the positions of the Zn2 and BRCT domain might also reveal additional domain interfaces.

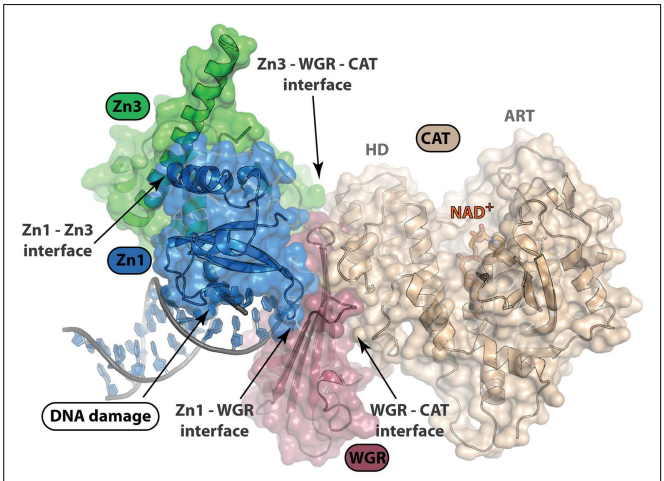


FIGURE 4 | Structure of PARP-1 in complex with DNA damage. PARP-1 binds DNA damage and activates catalytic activity nearly 500-fold. Only four of the six domains of PARP-1 (Zn1, Zn3, WGR, and CAT) are essential for DNA-dependent PARP-1 activation. This structure depicts complex formation and protein–protein interactions between domains upon DNA damage recognition (PDB ID: 4DQY). Disruption of these interdomain protein interfaces could be of interest in selective, allosteric targeting of PARP-1. An understanding of the arrangement of PARP-1 domain architecture in the absence of DNA damage recognition will be important for rational drug design efforts targeting protein interactions.

Another strategy to target PARPs specifically is through the acceptor site in the catalytic domain. This region likely forms contacts with target proteins to be modified with ADP-ribose. The diversity of the region in comparison to the NAD^+ binding site among PARPs presents a greater potential to achieve selectivity. Unfortunately, the differences in protein target recognition among PARPs are not well understood. It is likely that both sequence and structure play a part in target recognition. We do know that glutamic acid, aspartic acid, and lysine residues are the preferred amino acids that get modified by PARPs (66–70). Small peptides with an ADP-ribose modified glutamic acid or lysine residue could serve as a prototype scaffold for development of such inhibitors.

PERSPECTIVE ON THE THERAPEUTIC POTENTIAL OF PAN-PARP INHIBITORS VERSUS SELECTIVE PARP INHIBITORS

Comparisons between the effects of pan-PARP inhibitors and selective PARP inhibitors are largely unknown. In the case of PARP-1, the roles of recognition of DNA damage and repair in the base excision pathway are well established (71). Generation of single-strand breaks (SSBs) tend to accumulate in cells treated with PARP inhibitors, but this is not the case in cells treated with PARP-1 siRNA (72). RNAi technology however, requires careful interpretation since it is a knockdown and not a complete knockout, and even weak PARP-1 activity is enough for efficient DNA repair (73). The residual DNA repair activities of PARP-2 could explain SSB accumulation in cells treated with a PARP inhibitor (that inhibits both PARP-1 and PARP-2), and not in the case of PARP-1 depletion. Another model explains the retention of SSBs by proposing that PARP inhibitors trap PARP-1 and PARP-2 on SSB intermediates and prevent proper repair (72, 74, 75).

In terms of therapeutic potential, PARP inhibitors are more effective at killing BRCA deficient cells than with PARP-1 knockdown (5, 6). A number of clinical trials (Phase I–II) testing PARP inhibitors (with proven activity against either PARP-1 alone or both PARP-1 and 2) singly or in combination with chemotherapy are ongoing (76). Some clinical trials are upfront selecting for patients with known BRCA-deficiency or assessing biomarkers in a retrospective manner; and early reports suggest that selected BRCA-mutant patients do gain the best clinical benefit (77). The selectivity and usefulness of leading clinical PARP inhibitors (veliparib, olaparib, rucaparib) will soon become apparent as clinical trials successfully accrue patients. Moreover, as the research community discovers more BRCA2-related genes (such as the Fanconi Anemia genes) and pathways disrupted in cancers (78) two new opportunities will be: (i) to select patients' tumors that would be optimal for a synthetic lethal approach using PARP inhibitors and (ii) defining new targets within this pathway (79). Additionally, we are hopeful that with an in-depth understanding of the structure-function of each PARP family member, better and more specific targeting strategies will emerge. Finally, we may be better able to enhance PARP inhibitor-based therapies by taking into account the interplay between the DNA damage response and cell cycle dynamics (e.g., WEE1 inhibitors) (80).

CONCLUSION

Over 40 years of research invested from groups worldwide has advanced our understanding of poly(ADP-ribosyl)ation in cancer, identifying PARP-1 as a promising therapeutic target. As the family originating with PARP-1 has grown into a superfamily of PARPs and related MARTs, new therapeutic opportunities have surfaced along with new therapeutic challenges. Since most PARP inhibitors have varying selectivity among PARPs (8), interpretation of biological effects can present difficulties. Only recently have we begun to understand how different PARP inhibitors affect individual PARP function, and whether added therapeutic benefits result from pan-PARP inhibition remains to be determined.

Selectivity of compounds for one PARP over another is infrequently shown, although selectivity between PARP1 and PARP2, and in some instances other PARPs, is becoming more frequently reported. The use of selective agents will be extremely important in understanding each PARPs function. For example, the selectivity of compounds between PARP1, PARP2, and PARP3 is especially needed to clarify roles in response to DNA damage. Methods for screening the family of PARPs has become more prevalent, which will help accelerate the development of selective inhibitors. Cross-inhibition with other enzymes that use NAD^+ as a substrate or cofactor (such as ADP-ribosylcyclases and sirtuins) is an important concern, but is not typically seen (81, 82).

On the road to PARP selective inhibitors, most efforts will likely continue to focus on modifications of the nicotinamide-based inhibitors. The newer tankyrase selective compounds (such as IWR-1) that target the AD and PH sites but not the NI site present exciting new alternatives to nicotinamide-based inhibitors. It will be interesting to see if similar approaches are effective in other PARPs to promote selectivity. The acceptor sites among PARPs contain varying degrees of differences, which could guide the specificity of modifying target proteins. Targeting features of this region, such as the unique Arg408 residue in PARP-3, could be another way to obtain selectivity. Finally, we bring attention to targeting non-catalytic domains as a route to achieving selectivity. PARPs are the most diverse outside of their catalytic domain, and it is becoming increasingly appreciated that these domains make DNA and protein interactions important for proper function. Targeting non-catalytic domains may even allow us to target specific PARP functions, opening up a new dimension of therapeutic opportunities.

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BRCA 1/2-mutation related and sporadic breast and ovarian cancers: more alike than different

Melissa Burgess and Shannon Puhalla*

Department of Medical Oncology, University of Pittsburgh Medical Center, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

Edited by:
Kristin Zorn, Magee-Womens Hospital of UPMC, USA

Reviewed by:
Jian Lu, Johns Hopkins University, USA
Christine Walsh, Cedars-Sinai Medical Center, USA
Bhavana Pothuri, New York University School of Medicine, USA

****Correspondence:***
Shannon Puhalla, University of Pittsburgh Medical Center, University of Pittsburgh Cancer Institute, Magee-Women's Hospital, 300 Halket Street, Pittsburgh, PA 15213, USA
e-mail: puhallasl@mail.magee.edu

No longer is histology solely predictive of cancer treatment and outcome. There is an increasing influence of tumor genomic characteristics on therapeutic options. Both breast and ovarian cancers are at higher risk of development in patients with *BRCA 1/2*-germline mutations. Recent data from The Cancer Genome Atlas and others have shown a number of genomic similarities between triple negative breast cancers (TNBCs) and ovarian cancers. Recently, poly (ADP-ribose) polymerase (PARP) inhibitors have shown promising activity in hereditary *BRCA 1/2*-mutated and sporadic breast and ovarian cancers. In this review, we will summarize the current literature regarding the genomic and phenotypic similarities between *BRCA 1/2*-mutation related cancers, sporadic TNBCs, and sporadic ovarian cancers. We will also review Phase I, II, and III data using PARP inhibitors for these malignancies and compare and contrast the results with respect to histology.

Keywords: *BRCA 1/2*-mutations, breast cancer, ovarian cancer, BRCAness, PARP inhibitor, reversion mutations

INTRODUCTION

BRCA1 and 2 proteins play integral functions in DNA homologous recombination repair (HRR). In normal cells, the HRR pathway is activated in response to DNA double-stranded breaks (1). In *BRCA 1/2*-deficient cells, HRR is faulty secondary to loss of *BRCA* function, and therefore, other more error-prone DNA repair pathways are activated. These less perfect mechanisms are felt to be accountable, in part, for carcinogenesis. Similarly, tumors with defective HRR mechanisms are more susceptible to the direct DNA damaging effects of chemotherapy.

Homologous recombination repair dysfunction can be exploited as a therapeutic strategy by the use of poly (ADP-ribose) polymerase (PARP) inhibitors, which inhibit PARP proteins, most commonly PARP1 and 2. As part of the base excision repair (BER) pathway, PARP1 attaches long polymers of ADP-ribose on itself, so that, XRCC1 and other repair proteins have the ability to rapidly locate single-stranded DNA breaks (2–4). Newer evidence reveals that the exact role of PARP1 in the BER pathway is perhaps more indirect and not yet clearly defined (5). Recent studies have also shown that PARP1 is more versatile, and has been implicated in other DNA repair pathways, such as the non-homologous end-joining (NHEJ) repair pathway (6, 7).

Several mechanisms by which PARP inhibition in HRR-deficient cells lead to cell death have been investigated. Most notably, the concept of synthetic lethality explains combinatory lethal effects of BER and HR repair dysfunction, whereas alone, HR or BER pathway disruptions are not lethal to the tumor cell (8). Additionally, other potential mechanisms have been explored including trapping of inhibited PARP1 at sites of DNA damage preventing other repair proteins access, failure to initiate HRR by PARP-dependent BRCA1 recruitment, and activation of the error-prone NHEJ repair pathway leading to genomic instability and subsequent cell death (9). Knowledge of PARP activity has led to effective treatment strategies for *BRCA 1/2*-germline mutation related tumors.

BRCA 1/2-MUTATED OVARIAN AND BREAST CANCER

BRCA 1/2-mutation related ovarian and breast cancers account for 5–10% of all female ovarian and breast cancers (10, 11). Ovarian cancers in the setting of *BRCA 1/2*-germline mutations can present with more aggressive, high-grade histologies, but are frequently responsive to chemotherapy, particularly platinum-based regimens, leading to an improved 5 years survival (12). The chemotherapy-sensitive mechanism is felt to be related to the intimate relationship between BRCA 1/2 proteins and defective HRR, as discussed above. Recent studies have demonstrated that women with *BRCA*-related ovarian cancers fare much better than sporadic ovarian cancers (13–16). A study, published by the National Israeli Study of Ovarian Cancer, showed women with *BRCA* mutations had a median survival of 55.7 months compared to 37.9 months in sporadic ovarian cancers ($p = 0.002$) (15). This may be in part explained by the standard use of carboplatin-based therapies for ovarian malignancies as the DNA damage induced by the platinum should be more efficacious in the DNA repair-deficient *BRCA*-related tumors.

Contrary to the more convincing outcomes in *BRCA 1/2*-related ovarian cancers, the outcomes of *BRCA* mutation-related breast cancers are less clear. Women with *BRCA1* mutations typically develop breast cancer at an earlier age than *BRCA2*-related and sporadic breast cancers. *BRCA1*-related breast cancers tend to also be higher grade, hormone receptor-negative, and HER-2-negative, or "triple negative" (17), and also frequently express a basal phenotype (18–26). Patients with *BRCA*-mutated breast cancers generally respond to therapy as well as sporadic cancers;

however, the risk of second ipsilateral or contralateral primaries may be as high as 3–5% per year, compared to 0.5–1% per year risk, seen in sporadic breast cancers (17). In contrast to ovarian cancer, platinum chemotherapy is not standardly administered to patients with breast cancer. The use of platinum agents has been evaluated in a small series which have demonstrated high efficacy in breast cancer in particular in the setting of a *BRCA* mutation. Silver et al. evaluated the use of neoadjuvant platinum-containing chemotherapy in patients with triple negative breast cancer (TNBC) ($N = 28$), and found those more likely to be platinum-sensitive were those with low *BRCA1* gene expression (27). Likewise, in *BRCA*-mutated breast cancer patients who received cisplatin in the neoadjuvant setting showed a high rate of pathologic complete response (pCR) in a small series. Ten of 12 patients achieved pCR (83%). When non-platinum-containing regimens were used, the pCR rate was 14% (28). These studies highlight the rationale to further explore the use of platinum-containing regimens, specifically for patients with TNBC and *BRCA* mutations.

BRCAness: SPORADIC TRIPLE NEGATIVE BREAST CANCERS

Triple negative breast cancers account for ~20% of all breast cancers and are associated with an aggressive clinical picture (20, 25, 29). Due to lack of hormone receptor or HER-2 expression, and no other known target for tailored therapy, the only current treatment option is chemotherapy. Over 80% of hereditary *BRCA1*-mutated cancers are TNBCs. Several studies have investigated a potential role for *BRCA1* inactivation in sporadic TNBC given the similar clinical outcomes and histological characteristics among these cancers and hereditary *BRCA1*-mutated breast cancers. Breast cancers developing in patients with *BRCA1* mutations, in addition to frequently being triple negative, also often express basal markers (18–22, 25, 26). Gene microarray expression profiling has shown considerable similarities between *BRCA1*-mutated tumors and basal tumors (25). This shared phenotype has been termed "BRCAness" (26). What is unknown is whether the basal phenotype is a result of the *BRCA* loss or if the *BRCA* loss results in the basal phenotype (6).

Recently, Lehmann and colleagues delved further into the characterization of TNBC. They performed an analysis of gene expression profiles of 587 TNBC cases and identified six separate subtypes of TNBC. These six subtypes were: basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) subtype. Additional analysis of TNBC cell lines, representative of each of these identified subsets, revealed differential responses to various therapeutic agents. Both the BL1 and BL2 groups showed increased gene expression involved in DNA damage response, and showed higher response to cisplatin (30). In a follow-up study, Masuda et al. presented neoadjuvant chemotherapy response data in each of the aforementioned TNBC subtypes (31). In 130 TNBC patients, who received standard anthracycline- and taxane-based chemotherapy, the BL1 subtype achieved a pCR most frequently (52%). In contrast, the pCR in the BL2 subtype was 0%. The molecular differences in BL1 and BL2 may explain these differential responses. Specifically, the BL1 subtype involves the cell cycle, DNA replication reactome, and the *BRCA* pathway, among others, whereas the BL2 subtype involves growth factor, glycolysis, and gluconeogenesis pathways. This work demonstrates that even within "basal-like breast cancer (BLBC)," there may be a great deal of heterogeneity.

Telli and colleagues recently presented a study evaluating gemcitabine, carboplatin, and iniparib, a compound initially believed to have PARP inhibitory effects, in the neoadjuvant treatment of triple negative and *BRCA*-mutated breast cancer (32). This study demonstrated a pCR of 36% overall, with a pCR in *BRCA 1/2*-mutation carriers of 47%. Furthermore, patients who were both triple negative and had a *BRCA 1/2*-mutation, had a pCR of 56%. Although only 10 patients were classified as BL1 or BL2, there were an equal number of responders and non-responders to the neoadjuvant platinum regimen. It is also notable that only one patient classified as basal-like had a known *BRCA* mutation, whereas, there were *BRCA*-mutated tumors that were classified as IM, M, MSL, and unspecified (32). Although basal-like TNBC has become nearly synonymous with BRCAness, this study found that the basal-like subtype of TNBC was neither particularly responsive to the treatment combination, nor had a higher number of *BRCA*-germline mutations. In this study, the homologous recombination deficiency (HRD) score appeared to be more predictive of platinum response, as compared to TNBC intrinsic subtyping (30). The HRD assay has been developed to evaluate for loss of heterozygosity (LOH), which has been shown to be predictive of response to platinum in *BRCA*-related and sporadic cancers (33). While, this data is hypothesis-generating and thought-provoking, larger, prospective studies will be needed before any formal conclusions can be drawn.

In sporadic basal tumors, there are data that show reduced BRCA1 mRNA expression. It is felt that epigenetic modification of the *BRCA* gene, such as promoter hypermethylation, is responsible for this (34–36). Interestingly, no tumors showed both *BRCA1* mutation and *BRCA1* promoter methylation suggesting that these events are mutually exclusive in The Cancer Genome Atlas (TCGA) research network data (37). The association between *BRCA1*-mutated and BLBCs provides an important rationale to include this frequently encountered patient population in studies geared toward manipulation of the characteristic faulty DNA repair mechanisms in *BRCA1*-mutated tumors. As we move into an era where genomic analyses of tumors is becoming the norm, it will be important to link the genome, methylome, and proteome to clinical characteristics and outcomes.

BRCAness: SPORADIC HIGH-GRADE SEROUS OVARIAN CANCERS

Similarly, there are many commonalities among *BRCA 1/2*-mutated cancers and sporadic epithelial ovarian cancers (EOCs). Although only 5–10% of ovarian cancers are directly attributable to a germline mutation in *BRCA1* or *2*, there is a growing body of evidence to suggest that additional mechanisms of *BRCA* dysfunction are involved in the pathogenesis of ovarian cancer (26, 38, 39). One study demonstrated alterations of *BRCA1* and/or *2* in up to 82% of examined ovarian cancers ($n = 92$) (40). Methylation of the *BRCA1* promoter has been demonstrated in up to 14% of sporadic breast and up to 30% of sporadic ovarian cancers (26, 35, 41–46). LOH has been described in ovarian tumors and

Table 1 | Selected PARP inhibitor trials in *BRCA 1/2*-mutated (*BRCA*^{mut}) breast cancers.

Trial	Study population	PARP inhibitor	Comparison therapy	Clinical responses[a]
Phase I De Bono et al. (71) NCT01286987	Advanced *BRCA*^{mut} tumors ($N = 39$, of which 8 BC)	BMN 673	None	*BRCA*^{mut} BC ORR: 2/6
Phase I Sandhu et al. (68) NCT00749502	Advanced solid tumors/hematologic malignancies ($N = 100$, of which 12 BC, including 4 *BRCA*^{mut})	Niraparib	None	*BRCA*^{mut} BC PR: 2/4
Phase I Fong et al. (62) NCT00516373	Advanced solid tumors $N = 60$, of which 9 BC, including 3 with *BRCA*^{mut}	Olaparib	None	*BRCA*^{mut} BC CR: 1/3 SD: 1/3
Phase II Gelmon et al. (65) NCT00679783	Recur, advanced *BRCA*^{mut} OC ($N = 17$)/BC ($N = 10$), or *BRCA*^{wt} HGS and/or undifferentiated OC ($N = 47$)/TNBC ($N = 16$)	Olaparib	None	*BRCA*^{mut} BC CR + PR: 0/8 SD: 5/8
Phase II Kaufman et al. (89) NCT01078662	*BRCA*^{mut} solid tumors (BC, $N = 62$, OC, $N = 193$)	Olaparib	None	*BRCA*^{mut} BC CR: 0/62 PR: 8/62 SD: 29/62 PFS rate: 29% for 6 months OS rate: 44.7% for 12 months
Phase II Tutt et al. (64) NCT00494234 ICEBERG 1	*BRCA*^{mut} advanced BC ($N = 27$)	Olaparib	None	ORR: 11/27 CR: 1/27 PR: 10/27 PFS: 5.7 months
Phase I Lee et al. (72) NCT00647062, NCT01445418	Met or unresect *BRCA*^{mut} BC and EOC ($N = 45$, of which 8 BC)	Olaparib + carboplatin	None	*BRCA*^{mut} BC CR: 1/8 PR: 6/8 SD: 1/8
Phase I van der Noll et al. (90) NCT00516724	Advanced solid tumors [$N = 87$, including BC (26%) and OC (7%), of which 12 *BRCA*^{mut}]	Olaparib + carboplatin ± paclitaxel	None	*BRCA*^{mut} CR: 17%[b] PR: 33%[b]
Phase I Liu et al. (82) NCT01116648	Recur or advanced EOC/TNBC $N = 28$, of which 3 *BRCA*^{mut} BC	Olaparib + cediranib (angiogenesis inhibitor)	None	*BRCA*^{mut} BC ORR: 0/3
Phase I/II Kristeleit et al. (69) NCT01482715	Advanced solid tumors and relapsed PSens *BRCA*^{mut} OC $N = 29$, of which 17 BC and 7 OC, including *BRCA*^{mut} tumors	Rucaparib	None	*BRCA*^{mut} BC PR: 1/17 SD: 10/29 (of which 4 were BC, also 7/10 were *BRCA*^{mut})[b]
Phase I Huggins-Puhalla et al. (91) NCT00892736	Advanced *BRCA*^{mut} solid tumors ($N = 38$, of which 12 BC), or *BRCA*^{wt} BLBC or OC	Veliparib	None	*BRCA*^{mut} BC PR: 1/12 SD: 10/38[b]
Phase I Ramaswamy et al. (92) NCT01251874	Met or unresect *BRCA*^{mut} BC, or *BRCA*^{wt} TNBC and other BCs $N = 38$, of which 6 *BRCA*^{mut} and 7 FA^{def}	Veliparib + carboplatin	None	*BRCA*^{mut} BC PR: 2/6 SD: 4/6 PR: 8/38[b] SD: 17/38[b]

(Continued)

Table 1 | Continued

Trial	Study population	PARP inhibitor	Comparison therapy	Clinical responses[a]
Phase I Somlo et al. (93) NCT01149083	Met or unresect *BRCA*^{mut} BC $N = 28$	Veliparib + carboplatin	None	CR: 3/26[b] PR: 9/26 SD: 7/26 PFS: 7.8 months
Phase I Rodler et al. (94) NCT01104259	Met *BRCA*^{mut} BC or recur and/or met *BRCA*^{wt} TNBC $N = 18$, of which 5 *BRCA1/2*^{mut}	Veliparib + cisplatin and vinorelbine	None	*BRCA*^{mut} BC PR: 3/5 PR: 6/11[b] SD: 5/11[b]
Phase I Tan et al. (95) NCT00740805	Met BC $N = 11$, of which 3 *BRCA2*^{mut}	Veliparib + cyclophosphamide and doxorubicin	None	PR: 2/11 (both *BRCA2*^{mut}) SD: 6/11 (of which 1 *BRCA2*^{mut})
Phase II Isakoff et al. (96) NCT01009788	Met *BRCA*^{mut} BC (expansion cohort, $N = 24$)	Veliparib + temozolomide	None	CR: 1/24 PR: 2/24 SD: 7/24

[a] *Data include only patients with measurable disease.*

[b] *Collective data reported.*

BC, breast cancer; ORR, objective response rate; PR, partial response; CR, complete response; SD, stable disease; recur, recurrent; OC, ovarian cancer; BRCA^{wt}*, BRCA-wild type; HGS, high-grade serous; TNBC, triple negative breast cancer; PFS, progression-free survival; OS, overall survival; met, metastatic; unresect, unresectable; EOC, epithelial ovarian cancer; PSens, platinum-sensitive; BLBC, basal-like breast cancer; FA*^{def}*, fanconi anemia pathway deficiency.*

may have multiple possible mechanisms leading to malignancy including co-existing LOH of *BRCA1* and *p53*, and hypermethylation acting in a synergistic fashion (33, 47–51). In contrast, *BRCA2* methylation has not been found to be a significant contributor (39, 52). Identifying and manipulating these *BRCA*-like deficiencies in DNA repair in sporadic ovarian cancers is of great importance and provides rationale for including these patients in clinical trials designed for *BRCA*-related malignancies.

Another important mechanism of BRCAness in ovarian cancers is the presence of somatic mutations in *BRCA1* and *2* (53). Hennessy and colleagues performed *BRCA1/2* sequencing on 235 unselected ovarian cancers and found that 19% of the sample had detectable mutations in *BRCA1* ($N = 31$) or *BRCA2* ($N = 13$). In the 28 samples, where germline DNA was also available, 42.9% of the *BRCA1* mutations and 28.6% of the *BRCA2* mutations were somatic. Of interest, somatic *BRCA 1/2*-mutations in breast cancer appear to be less frequent. In the TCGA BLBC cohort, about 20% had either germline ($N = 12$) or somatic ($N = 8$) *BRCA 1/2*-mutations. Another study evaluated 77 TNBC samples and only one harbored a somatic *BRCA* mutation (54). This potentially explains the seemingly higher activity of single agent PARP inhibitors, discussed later, in sporadic ovarian cancer as compared to sporadic TNBC.

GENOMIC SIMILARITIES: BASAL-LIKE BREAST CANCER AND HIGH-GRADE SEROUS OVARIAN CANCERS

The Cancer Genome Atlas network recently published findings again demonstrating the four distinct molecular signatures in breast cancer from diverse genetic and epigenetic alterations: luminal A, luminal B, basal-like, and HER-2 enriched subtypes (55). Strikingly, BLBCs were notably different than the other three subtypes based on comprehensive analyses using multiple platforms. As expected, these cancers also often (80%) lacked expression of ER, PR, and HER-2 identifying as TNBCs. Specifically, most BLBCs showed a high frequency of *TP53* deleterious mutations (80%), as well as, loss of *RB1* and *BRCA1*. *PIK3CA* mutations (~9%) were also a common feature of BLBC. Analyses also highlighted increased *MYC* activation as a BLBC characteristic.

The BLBC mutation spectrum reported in the TCGA was similar to that identified in previously described serous ovarian cancers (56) and BLBC were more similar to serous ovarian carcinomas than to other subtypes of breast cancer. One gene, in particular, *TP53*, had a >10% mutation frequency in both basal-like breast and serous ovarian cancers. As well, both tumors when compared to luminal showed increased *BRCA1* inactivation, *RB1* loss, cyclin E1 amplification, high expression of *AKT3*, and *MYC* amplification. These molecular commonalities strongly suggest shared driving events in tumorigenesis, and similarly, show support for shared treatment strategies for TNBCs and high-grade serous ovarian cancers. Of note, *p53* mutations have been described to have high frequency in *BRCA* mutation-related cancers as well (57, 58).

PARP INHIBITORS: PRECLINICAL ERA

Bryant et al. and Farmer et al. demonstrated synthetic lethality in *BRCA2*-deficient cells with the use of two different PARP inhibitors (59, 60). PARP inhibitors have also shown efficacy preclinically in cells lacking other HRR proteins, such as *RAD51*, *ATR*, *ATM*, *CHK1*, and *FANCA* or *FANCC* (61). These studies have given basis for clinical trials in both *BRCA*-deficient cancer

Table 2 | Selected PARP inhibitor trials in *BRCA 1/2*-mutated (*BRCA*^{mut}) ovarian cancers.

Trial	Study population	PARP inhibitor	Comparison therapy	Clinical responses[a]
Phase I De Bono et al. (71) NCT01286987	Advanced *BRCA*^{mut} tumors ($N = 39$, of which 8 BC and 23 OC)	BMN 673	None	*BRCA*^{mut} OC ORR: 11/17
Phase I Sandhu et al. (68) NCT00749502	Advanced solid tumors/hematologic malignancies ($N = 100$, of which 49 OC, including 22 *BRCA*^{mut})	Niraparib	None	*BRCA*^{mut} OC PR: 8/20
Phase I Fong et al. (62) NCT00516373	Advanced solid tumors $N = 60$, of which 21 OC, including 16 with *BRCA*^{mut}	Olaparib	None	*BRCA*^{mut} OC PR: 8/15 SD: 1/15
Phase II Gelmon et al. (65) NCT00679783	Recur, advanced *BRCA*^{mut} OC ($N = 17$)/BCs ($N = 10$), or *BRCA*^{wt} HGS and/or undifferentiated OC ($N = 47$)/TNBC ($N = 16$)	Olaparib	None	*BRCA*^{mut} OC CR: 0/17 PR: 7/17 SD: 6/17
Phase II Kaye et al. (66) NCT00628251	Advanced PRef or PRes *BRCA*^{mut} OC	Olaparib	Liposomal doxorubicin	Olaparib 200 mg twice daily PFS: 6.5 months ORR: 25% Olaparib 400 mg twice daily PFS: 8.8 months ORR: 31% Liposomal doxorubicin: PFS: 7.1 months ORR: 18%
Phase II Kaufman et al. (89) NCT01078662	*BRCA*^{mut} solid tumors (BC, $N = 62$, OC, $N = 193$)	Olaparib	None	*BRCA*^{mut} OC CR: 6/193 PR: 54/193 SD: 78/193 PFS rate: 54.6% for 6 months OS rate: 64.4% for 12 months
Phase II Audeh et al. (63) NCT00494442	Advanced *BRCA*^{mut} OC	Olaparib	None	ORR: 11/33 CR: 2/33 PR: 9/33 PFS: 5.8 months
Phase I Lee et al. (72) NCT00647062, NCT01445418	Met or unresect *BRCA*^{mut} BC and EOC ($N = 45$, of which 37 OC)	Olaparib + carboplatin	None	*BRCA*^{mut} OC CR: 0/34 PR: 15/34 SD: 14/34
Phase I van der Noll et al. (90) NCT00516724	Advanced solid tumors $N = 87$, including BC (26%) and OC (7%), of which 12 *BRCA*^{mut}	Olaparib + carboplatin ± paclitaxel	None	*BRCA*^{mut} CR: 17%[b] PR: 33%[b]
Phase I Liu et al. (82) NCT01116648	Recur or advanced EOC/TNBC $N = 28$, of which 12 *BRCA*^{mut} OC	Olaparib + cediranib (angiogenesis inhibitor)	None	*BRCA*^{mut} OC CR: 1/11 PR: 4/11
Phase I/II Kristeleit et al. (69) NCT01482715	Advanced solid tumors and relapsed PSens *BRCA*^{mut} OC $N = 29$, of which 17 BC and 7 OC, including *BRCA*^{mut} tumors	Rucaparib	None	*BRCA*^{mut} OC PR: 1/7 SD: 10/29 (of which 5 were OC, also 7 were *BRCA*^{mut})[b] CR + PR + SD: 6/7 in OC

(Continued)

Table 2 | Continued

Trial	Study population	PARP inhibitor	Comparison therapy	Clinical responses[a]
Phase I Huggins-Puhalla et al. (91) NCT00892736	Advanced *BRCA*^{mut} solid tumors ($N = 38$, of which 20 OC), or *BRCA*^{wt} BLBC or OC	Veliparib	None	*BRCA*^{mut} OC PR: 1/20 SD: 10/38[b]
Phase II Kummar et al. (97) NCT01306032	Refractory progressive *BRCA*^{mut} OC or HGS OC	Veliparib (V) + cyclophosphamide (C) $N = 36$	Cyclophosphamide (C) $N = 38$	V + C: PR: 3/36[b] C: PR: 5/38[b]
Phase I Bell-McGuinn et al. (98) NCT01063816	Met or unresect solid tumors $N = 59$, of which 39 OC, 24 of 39 OC *BRCA*^{mut}	Veliparib + carboplatin and gemcitabine	None	CR: 2/59[b] PR: 11/59[b] Of 13 responses, 8 *BRCA*^{mut} OC, 3 other OC

[a] *Data include only patients with measurable disease.*

[b] *Collective data reported.*

BC, breast cancer; OC, ovarian cancer; ORR, objective response rate; PR, partial response; SD, stable disease; recur, recurrent; BRCA^{wt}, BRCA-wild type; HGS, high-grade serous; TNBC, triple negative breast cancer; PRef, platinum-refractory; PRes, platinum-resistant; PFS, progression free survival; OS, overall survival; CR, complete response; met, metastatic; unresect, unresectable; EOC, epithelial ovarian cancer; PSens, platinum-sensitive; BLBC, basal-like breast cancer.

populations, as well as, those with malignancies sharing qualities of BRCAness or HRR-deficiency, such as basal-like or TNBC and serous ovarian cancer.

PARP INHIBITORS IN CLINICAL TRIALS

BRCA 1/2-MUTATION STUDIES

The first published Phase I study evaluating PARP inhibitors in the clinic used olaparib (AZD2281) enrolling patients with varying malignancies (**Tables 1** and **2**) (62). An expansion cohort of *BRCA*-positive ovarian, breast, and prostate cancer patients was enrolled at the recommended Phase II dose of 400 mg twice daily. Nearly half of the evaluable patients had an objective response (19 patients, 47%). Results from this pivotal study showed olaparib was generally well tolerated. From here, two Phase II proof-of-concept trials (ICEBERG 1 and 2) (**Tables 1** and **2**) confirmed activity in both *BRCA*-mutated ovarian and breast cancers, with olaparib at 400 mg twice daily [ORR 11/33 (33%) and 11/27 (41%), respectively], with low overall toxicities (63, 64).

Olaparib was also evaluated in patients with sporadic cancers displaying a presumed BRCAness phenotype. Gelmon et al. performed a non-randomized Phase II trial using olaparib in heavily treated high-grade serous or undifferentiated ovarian carcinomas and TNBCs (65) (**Tables 1–4**). Stratified by *BRCA* mutation status, both *BRCA*-mutated and *BRCA*-wild type ovarian carcinoma patients showed response to olaparib. In contrast, neither *BRCA*-mutated nor sporadic breast cancer patients demonstrated significant response to olaparib. Potential explanations for these mixed results include that not all TNBCs have a *BRCA*-like phenotype, so there may have been some heterogeneity to this population (30).

In a population of *BRCA*-positive recurrent ovarian cancer patients with a platinum-free interval of $\leq$12 months, olaparib was compared to pegylated liposomal doxorubicin (PLD) in a randomized Phase II trial ($N = 97$) (66) (**Table 2**). Progression free survival (PFS) was not statistically significantly different for olaparib 200 or 400 mg twice daily (combined or individually) versus PLD (PFS 6.5 versus 8.8 versus 7.1 months, respectively). Where the PFS and ORR were consistent with prior studies for olaparib at 400 mg twice daily, the efficacy of PLD was higher than expected when compared with previous trials. Toxicity profiles were distinct between olaparib (nausea, vomiting, and fatigue) and PLD (stomatitis and palmar-plantar erythrodysesthesia), and overall, the drugs were well tolerated. Although olaparib did not show an improvement in PFS over chemotherapy, these results show that targeted therapy with a PARP inhibitor is as effective as chemotherapy, with potential for improved tolerability.

Other PARP inhibitors have also been studied in clinical trials including niraparib (MK4827) in both *BRCA*-positive and sporadic tumors. This compound's mechanism of action includes PARP inhibition via a novel PARP trapping mechanism (67). A Phase I study utilizing niraparib monotherapy was recently published that established a maximum tolerated dose of 300 mg/day ($N = 100$) (68) (**Table 1**). Dose-limiting toxicities (DLTs) were reported in the first cycle including grade 4 thrombocytopenia at a dose of 400 mg/day. Non-hematologic DLTs included grade 3 fatigue and grade 3 pneumonitis at lower doses (30 and 60 mg/day, respectively). Common treatment-related effects were anemia, nausea, fatigue, thrombocytopenia, anorexia, neutropenia, constipation, and vomiting, but were predominantly grade 1 or 2. There were anti-tumor responses seen in the *BRCA*-mutated breast and ovarian cancer population, and these were recorded at doses $>$60 mg/day. Results from this study show promise for this newer PARP inhibitor and currently there are multiple Phase III trials recruiting in *BRCA*-positive breast and ovarian, and sporadic ovarian cancer populations (NCT01905592, NCT01847274) (**Tables 5** and **6**).

Rucaparib (CO-338/AG-014699, also previously PF-01367338) was recently evaluated in Phase I and II studies in advanced solid tumors, including *BRCA*-positive breast and ovarian cancers. The PARP inhibitor as monotherapy and in combinations with cytotoxic chemotherapy is under investigation. In a standard

Table 3 | Selected PARP inhibitor trials in sporadic breast cancers.

Trial	Study population	PARP inhibitor	Comparison therapy	Clinical responses[a]
Phase II Gelmon et al. (65) NCT00679783	Recur, advanced *BRCA*^{mut} OC ($N = 17$)/BCs ($N = 10$), or *BRCA*^{wt} HGS, and/or undifferentiated OC ($N = 47$)/TNBC ($N = 16$)	Olaparib	None	*BRCA*^{wt} TNBC CR + PR: 0/15 SD: 2/15
Phase I Lee et al. (99) NCT01237067	Refractory or recur BC ($N = 4$) and OC	Olaparib + carboplatin	None	BC PR: 3/4 SD: 1/4
Phase I van der Noll et al. (90) NCT00516724	Advanced solid tumors $N = 87$, including BC (26%) and OC (7%), of which 12 *BRCA*^{mut}	Olaparib + carboplatin ± paclitaxel	None	ORR: 14/87 (16%)[b] CR: 5% PR: 11% SD: 28%
Phase I Liu et al. (82) NCT01116648	Recur or advanced EOC/TNBC $N = 28$, of which 8 BC	Olaparib + cediranib (angiogenesis inhibitor)	None	BC ORR: 0/7 SD: 2/7
Phase I Balmana et al. (100) NCT00782574	Advanced solid tumors $N = 54$, of which 42 BC	Olaparib + cisplatin	None	CR: 1/54[b] PR: 17/54[b] SD: 23/54[b]
Phase I Dent et al. (76) NCT00707707	Met TNBC $N = 19$	Olaparib + paclitaxel	None	PR: 7/19 SD: 1/19
Phase I Huggins-Puhalla et al. (91) NCT00892736	Advanced *BRCA*^{mut} solid tumors, or *BRCA*^{wt} tumors ($N = 25$, of which 21 BLBC)	Veliparib	None	*BRCA*^{wt} BLBC PR: 1/21 *BRCA*^{wt} SD: 7/25[b]
Phase I Kummar et al. (101) NCT00810966	Refractory solid tumors/lymphoma $N = 35$, including BC and OC	Veliparib	Cyclophosphamide	PR: 7/35[b] SD: 6/35[b]
Phase I Ramaswamy et al. (92) NCT01251874	Met or unresect *BRCA*^{mut} BC, or *BRCA*^{wt} TNBC and other BCs $N = 38$, of which 6 *BRCA*^{mut} and 7 FA^{def}	Veliparib + carboplatin	None	PR: 8/38 SD: 17/38 FA^{def} PR: 2/7 SD: 5/7
Phase I Bell-McGuinn et al. (98) NCT01063816	Met or unresect solid tumors $N = 59$, of which 10 BC	Veliparib + carboplatin and gemcitabine	None	CR: 2/59[b] PR: 11/59[b] Of 13 responses, 8 *BRCA*^{mut} OC, 3 other OC, 2 others
Phase I Appleman et al. (102) NCT00535119	Advanced solid tumors including BC $N = 68$, of which 14 BC	Veliparib + carboplatin and paclitaxel	None	BC CR: 3/14 PR: 5/14
Phase I Puhalla et al. (80) NCT01281150	Met or unresect solid tumors, including BC (Q1 week, $N = 10$ TNBC, Q3 week, $N = 9$ TNBC)	Veliparib + carboplatin and paclitaxel	None	TNBC (Q1 week), CR: 2/10, PR: 3/10, SD: 3/10 (Q3 week), CR: 3/9, PR: 4/9, SD: 1/9
Phase I Rodler et al. (94) NCT01104259	Met *BRCA*^{mut} BC or recur and/or met *BRCA*^{wt} TNBC $N = 18$, of which 5 *BRCA1/2*^{mut}	Veliparib + cisplatin and vinorelbine	None	PR: 6/11[b] SD: 5/11[b]

(Continued)

Table 3 | Continued

Trial	Study population	PARP inhibitor	Comparison therapy	Clinical responses[a]
Phase I Tan et al. (95) NCT00740805	Met BC $N = 11$, of which 3 *BRCA2*^{mut}	Veliparib + cyclophosphamide and doxorubicin	None	PR: 2/11 (both *BRCA2*^{mut}) SD: 6/11 (of which 1 *BRCA2*^{mut})

[a] *Data include only patients with measurable disease.*

[b] *Collective data reported.*

recur, recurrent; BRCA^{mut}, mutated BRCA; OC, ovarian cancer; BC, breast cancer; BRCA^{wt}, BRCA-wild type; HGS, high-grade serous; TNBC, triple negative breast cancer; CR, complete response; PR, partial response; SD, stable disease; ORR, objective response rate; EOC, epithelial ovarian cancer; met, metastatic; BLBC, basal-like breast cancer; FA^{def}, fanconi anemia pathway deficiency.

dose-escalation fashion, a Phase I/II study (**Tables 1** and **2**) is currently evaluating rucaparib monotherapy in advanced solid tumors ($N = 29$) including ovarian/primary peritoneal ($N = 7$) and breast ($N = 17$) cancer patients (69). Thus far, no DLTs at 360 mg twice daily (study not yet complete) have been reported. To date, two PRs were seen in one *BRCA*-positive ovarian cancer, and one *BRCA*-positive breast cancer patient at 300 mg daily dosing during the sixth week of therapy. Ten additional patients (ovarian $N = 5$, breast $N = 4$, and colorectal $N = 1$) have experienced stable disease (SD) at >12 weeks so far; seven of which are *BRCA*-positive. Overall the disease control rate (PR + SD > 12 weeks) for ovarian cancer patients is 86% (6/7). Further results are anticipated from this study. These promising results to date have supported ARIEL2, a Phase II study of rucaparib in platinum-sensitive, relapsed, high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer patients, which is currently recruiting patients (**Table 6**).

BMN 673, a novel, highly potent PARP 1/2 inhibitor, demonstrated high efficacy in preclinical studies (70). BMN 673 elicits DNA repair biomarkers at much lower concentrations [PARP1 half maximal inhibitory concentration (IC_{50}) <1 nmol/L] than earlier generation PARP inhibitors, i.e., olaparib, veliparib, and rucaparib. Its anti-tumor activity has been tested *in vitro* and in xenograft cancer models, as monotherapy and in combination. Anti-tumor activity was seen in *BRCA1*, *BRCA2*, and *PTEN* deficient cells with a 20 to more than 200-fold greater potency than existing PARP 1/2 inhibitors. Synergism was also seen when BMN 673 was combined with temozolomide, SN38, or platinum drugs. Thus far, BMN 673 has been the most specific PARP inhibitor in its class.

The first in-human Phase I, clinical trial using BMN 673 in solid tumor patients was recently presented at ASCO 2013 (71) (**Tables 1** and **2**). Patients with advanced solid tumors defective in DNA repair, including *BRCA*-mutated breast ($N = 6$), and ovarian ($N = 17$) cancer patients, were eligible for the stage II expansion phase at the maximum tolerated dose of 1000 mcg daily. In total, 39 patients with advanced solid tumors were enrolled, including those tumors with deleterious *BRCA* mutations. Thrombocytopenia was dose-limiting and occurred in three patients at doses 900 or 1100 mcg daily. Most potential treatment-related adverse events (AEs) were grade 1/2 and included fatigue, nausea, flatulence, anemia, neutropenia, thrombocytopenia, and alopecia. Objective responses were seen in 11/17 *BRCA*-mutated ovarian/primary peritoneal cancer patients and 2/6 *BRCA*-mutated breast cancer patients. Based on these encouraging results, the recommended dose, 1000 mcg daily, will be studied in a Phase III trial in *BRCA*-carrier metastatic or locally advanced breast cancer patients (NCT01945775) (**Table 5**).

In addition to the single agent studies described above, PARP inhibitors have been combined with chemotherapy in *BRCA* mutation-related malignancies. Lee et al. in a Phase I/Ib study, utilized olaparib, in combination with carboplatin, in a standard dose-escalation study design in *BRCA 1/2*-mutated breast and ovarian cancers ($N = 45$) (72) (**Tables 1** and **2**). The recommended Phase II dose was 400 mg twice daily for 14 days with carboplatin AUC 5. As noted in several other trials utilizing olaparib, and other PARP inhibitors, myelosuppression was frequently present with grade 3/4 AEs (neutropenia 42%), as well as, thrombocytopenia (20%), anemia (13%), carboplatin-hypersensitivity (9%), and fatigue (7%). Responses included one CR in a breast cancer patient that was durable (duration of 17 months), and a PR in 15/34 (44%) ovarian cancer (duration 3–28+ months) and 6/8 breast cancer (duration 5–24+ months) patients. Prolonged SD was seen in 14/34 (41%) ovarian cancer patients for as long as 25 months and for 11 months in a breast cancer patient. Remarkably, the overall clinical benefit rate was 100% in breast cancer patients and 85% in ovarian cancer patients. A summary of Phase I–III studies utilizing PARP inhibitors in *BRCA* 1/2-mutated breast and ovarian cancers can be found in **Tables 1** and **2**.

SPORADIC BREAST AND OVARIAN CANCER TRIALS

The earliest trials reported for sporadic TNBCs evaluated iniparib (BSI-201) in combination with gemcitabine and carboplatin. The Phase II trials showed promising anti-tumor activity, prolonged median progression-free survival, and median overall survival (OS) with minimal overall toxicity (73). Disappointingly, the results were not significant in the Phase III trial (74). There are a number of potential explanations for the lack of efficacy seen in the Phase III study, including the heterogeneity within the subtypes of TNBC. Importantly, it was discovered that iniparib was actually not a PARP inhibitor, at physiologic concentrations. Rather, iniparib was shown to cause telomere-centric DNA damage (75).

There are also a number of reported and ongoing studies with "true" PARP inhibitors in sporadic TNBCs, although, only a few studies that have been published in final format. A Phase I/II study of mention explored the use of olaparib in combination with

Table 4 | Selected PARP inhibitor trials in sporadic ovarian cancers.

Trial	Study population	PARP inhibitor	Comparison therapy	Clinical responses[a]
Phase II Gelmon et al. (65) NCT00679783	Recur, advanced *BRCA*^{mut} OC ($N = 17$)/BCs ($N = 10$), or *BRCA*^{wt} HGS and/or undifferentiated OC ($N = 47$)/TNBC ($N = 16$)	Olaparib	None	*BRCA*^{wt} OC CR: 0/46 PR: 11/46 SD: 18/46
Phase II Ledermann et al. (81) NCT00753545	Relapsed PSens serous OC after two courses of platinum-based chemotherapy	Olaparib	Placebo	PFS: 8.4 months OS 29.7 months ORR: 12.3% ORR + SD: 52.9%
Phase I Lee et al. (99) NCT01237067	Refractory or recur BC ($N = 4$) and OC ($N = 23$)	Olaparib + carboplatin	None	OC PR: 8/23 SD: 11/23
Phase I van der Noll et al. (90) NCT00516724	Advanced solid tumors $N = 87$, including BC (26%) and OC (7%), of which 12 *BRCA*^{mut}	Olaparib + carboplatin ± paclitaxel	None	ORR: 14/87 (16%)[b] CR: 5% PR: 11% SD: 28%
Phase II Oza et al. (103) NCT01081951	Advanced PSens serous OC	Olaparib + carboplatin, paclitaxel	Carboplatin, paclitaxel alone	PFS: 12.2 months ORR: 64%
Phase I Liu et al. (82) NCT01116648	Recur or advanced EOC/TNBC $N = 28$, of which 20 OC	Olaparib + cediranib (angiogenesis inhibitor)	None	OC CR: 1/18[b] PR: 7/18[b] SD: 3/18[b]
Phase I Balmana et al. (100) NCT00782574	Advanced solid tumors $N = 54$, of which 10 OC	Olaparib + cisplatin	None	CR: 1/54[b] PR: 17/54[b] SD: 23/54[b]
Phase I Molife et al. (104) NCT01009190	Advanced solid tumors ($N = 23$, of which 6 OC)	Rucaparib + carboplatin	None	OC PR: 1/6 SD: 2/6
Phase I Huggins-Puhalla et al. (91) NCT00892736	Advanced *BRCA*^{mut} solid tumors, or *BRCA*^{wt} tumors ($N = 25$, of which 4 OC)	Veliparib	None	*BRCA*^{wt} SD: 7/25[b]
Phase I Kummar et al. (101) NCT00810966	Refractory solid tumors/lymphoma $N = 35$, including BC and OC	Veliparib	Cyclophosphamide	PR: 7/35[b] SD: 6/35[b]
Phase II Kummar et al. (97) NCT01306032	Refractory progressive *BRCA*^{mut} OC or HGS OC	Veliparib (V) + cyclophosphamide (C) $N = 36$	Cyclophosphamide (C) $N = 38$	V + C: PR: 3/36[b] C: PR: 5/38[b]
Phase I Bell-McGuinn et al. (98) NCT01063816	Met or unresect solid tumors $N = 59$, of which 39 OC, 24 of 39 *BRCA*^{mut}	Veliparib + carboplatin and gemcitabine	None	CR: 2/59[b] PR: 11/59[b] Of 13 responses, 8 *BRCA*^{mut} OC, 3 other OC, 2 others

[a] *Data include only patients with measurable disease.*

[b] *Collective data reported.*

recur, recurrent; BRCA^{mut}, mutated BRCA; OC, ovarian cancer; BC, breast cancer; BRCA^{wt}, BRCA-wild type; HGS, high-grade serous; TNBC, triple negative breast cancer; CR, complete response; PR, partial response; SD, stable disease; PSens, platinum-sensitive; PFS, progression free survival; OS, overall survival; ORR, objective response rate; EOC, epithelial ovarian cancer; BLBC, basal-like breast cancer; met, metastatic; unresect, unresectable.

Table 5 | Ongoing or future PARP inhibitor trials in *BRCA 1/2*-mutated (*BRCA*^{mut}) breast and ovarian cancers.

Trial	Study population	PARP inhibitor	Comparison therapy	ClinicalTrials.gov status
Phase III	Met or unresect *BRCA*^{mut} BC	BMN 673	Physician's choice – capecitabine, eribulin, gemcitabine, or vinorelbine	NCT01945775 Recruiting
Phase III	HER-2 negative met or advanced *BRCA*^{mut} BC	Niraparib	Physician's choice (select from four active comparators)	NCT01905592 (BRAVO) Not yet open for recruitment
Phase III	PSens *BRCA*^{mut} or HGS OC w/prior CR and second CR/PR	Niraparib (maintenance)	Placebo	NCT01847274 Recruiting
Phase III	PSens *BRCA*^{mut} (stage III or IV) OC in first CR/PR	Olaparib (maintenance)	Placebo	NCT01844986 Not yet open for recruitment
Phase III	Relapsed PSens *BRCA*^{mut} OC w/prior CR and second CR/PR	Olaparib (maintenance)	Placebo	NCT01874353 Not yet open for recruitment
Phase II	Met or locally advanced *BRCA*^{mut} BC/OC	Rucaparib	None	NCT00664781 Active, not recruiting
Phase II Miller et al. (105)	*BRCA*^{mut} BC or *BRCA*^{wt} TNBC w/residual disease in adjuvant setting (after NAC/surgery)	Rucaparib + cisplatin	Cisplatin	NCT01074970 Ongoing, not recruiting
Phase I	Met or unresect *BRCA*^{mut} BC and OC	Veliparib	None	NCT01853306 Recruiting
Phase I/II	Relapsed PRes or partially PSens *BRCA*^{mut} EOC	Veliparib	None	NCT01472783 Veli-BRCA Recruiting
Phase II Isakoff et al. (106)	Met or advanced *BRCA*^{mut} BC	Veliparib Three arms, plus temozolomide, or carboplatin, paclitaxel	Placebo and carboplatin, paclitaxel	NCT01506609 Recruiting
Phase II Coleman et al. (107)	Advanced or recur *BRCA*^{mut} EOC	Veliparib	None	NCT01540565 Ongoing, not recruiting
Phase I	*BRCA*^{mut} solid tumors (e.g., BC and OC)	Veliparib + oxaliplatin and capecitabine	None	NCT01233505 Recruiting
Phase I	Met or unresect *BRCA*^{mut} BC and OC	Veliparib + temozolomide	None	NCT00526617 Completed

met, metastatic; unresect, unresectable; BC, breast cancer, PSen, platinum-sensitive; HGS, high-grade serous; OC, ovarian cancer; CR, complete response; PR, partial response; BRCA^{wt}, BRCA-wild type; TNBC, triple negative breast cancer; NAC, neoadjuvant chemotherapy; PRes, platinum-resistant; EOC, epithelial ovarian cancer; recur, recurrent.

paclitaxel in the first or second-line setting for metastatic TNBC patients ($N = 19$) (76) (**Table 3**). Notably, patients were treated with olaparib 200 mg daily with paclitaxel 90 mg/m^2 weekly for 3 of 4 weeks and 15 of the patients had had previous taxane-based therapy. Thirty-seven percent of patients had a PR, although, there were significant dose modifications due to the greater than expected rate of neutropenia, even despite use of growth factor support. While taxanes are proven agents in TNBC (77–79), this class is not typically thought to be a potentiating agent for PARP inhibitors. Most studies have used a platinum agent for potentiation, exploiting the DNA damage/dysfunctional DNA repair pathways concept. Perhaps utilizing two agents that are active in different parts of the cell cycle would potentially target more tumor cells, overall, including those in different phases of growth. Additionally, the utility of PARP inhibitor/taxane-based combination may have potentially overcome taxane resistance. There are ongoing studies with platinum and taxane combinations with a PARP inhibitor. Early looks at efficacy are promising (80).

Similarly in ovarian cancer, there have been a number of studies evaluating PARP inhibitors with chemotherapy, including in the maintenance setting. Ledermann et al. studied olaparib in the maintenance setting after second CR in platinum-sensitive recurrent serous ovarian cancer patients. This was a Phase II, randomized, double-blinded, placebo-controlled trial ($N = 265$)

Table 6 | Ongoing or future PARP inhibitor trials in sporadic breast and ovarian cancers.

Trial	Study population	PARP inhibitor	Comparison therapy	ClinicalTrials.gov status
Phase III	PSens *BRCA*^{mut} or HGS OC w/prior CR and second CR/PR	Niraparib (maintenance)	Placebo	NCT01847274 Recruiting
Phase I	Recur TNBC/HGS OC	Olaparib + BKM120 (PI3 kinase inhibitor)	None	NCT01623349 Recruiting
Phase I	Met or unresect TNBC/serous EOC	Olaparib + carboplatin	None	NCT01445418 Recruiting
Phase I/Ib	Relapsed stage III or IV OC	Olaparib + carboplatin and paclitaxel	None	NCT01650376 Recruiting
Phase II	Relapsed recur PSens high-grade EOC	Rucaparib	None	NCT01891344 (ARIEL2) Recruiting
Phase II Miller et al. (105)	*BRCA*^{mut} BC or *BRCA*^{wt} TNBC w/residual disease in adjuvant setting (after NAC/surgery)	Rucaparib + cisplatin	Cisplatin	NCT01074970 Ongoing, not recruiting
Phase I Pothuri et al. (108)	Recur or residual EOC/met TNBC	Veliparib	Pegylated liposomal doxorubicin	NCT01145430 Recruiting
Phase I	Recur met or locally advanced unresect solid tumors (e.g., BC/OCs) with organ dysfunction	Veliparib	Carboplatin and paclitaxel	NCT01366144 Recruiting
Phase I	Recur OC Two arms + doxorubicin, carboplatin, and bevacizumab	Veliparib	None	NCT01459380 Recruiting
Phase I	Node-positive BC with incomplete response to NAC	Veliparib	Radiation therapy	NCT01618357 Recruiting
Phase I	Recur stage IV EOC	Veliparib + intraperitoneal floxuridine (FUDR)	None	NCT01749397 Recruiting
Phase I	Newly diagnosed stage II–IV optimally or suboptimally debulked OC	Veliparib + paclitaxel, carboplatin, bevacizumab Two parallel arms	None	NCT00989651 Recruiting
Phase II Avery et al. (109)	Stage IIA, IIIA–C TNBC	Veliparib + paclitaxel + carboplatin, followed by doxorubicin, cyclophosphamide (neoadjuvant)	Paclitaxel, carboplatin, followed by doxorubicin, cyclophosphamide	NCT01818063 Recruiting
Phase II	Recur HGS OC	Veliparib + temozolomide	Pegylated liposomal doxorubicin	NCT01113957 Completed
Phase I/II	Recurrent, relapsed PRes or part PSens OC	Veliparib + topotecan	None	NCT01690598 Recruiting
Phase II	Recur advanced non-PSens OC	Veliparib + topotecan	None	NCT01012817 Recruiting

PSen, platinum-sensitive; BRCA^{mut}, BRCA 1/2-mutated; HGS, high-grade serous; OC, ovarian cancer; CR, complete response; PR, partial response; recur, recurrent; TNBC, triple negative breast cancer; met, metastatic; unresect, unresectable; EOC, epithelial ovarian cancer; BC, breast cancer; BRCA^{wt}, BRCA-wild type; NAC, neoadjuvant chemotherapy; PRes, platinum-resistant.

(81) (**Table 4**). Median PFS was statistically significant between the groups, 8.4 versus 4.8 months, in the olaparib and placebo arms, respectively ($p < 0.001$). OS was not significantly different (29.7 versus 29.9 months in the olaparib and placebo groups, respectively). Further studies are needed to identify a population of patients that may experience greater clinical benefit, such as those with *BRCA 1/2*-mutations or those with a BRCAness phenotype.

Combination therapies with PARP inhibitors have also been investigated in sporadic ovarian and breast cancers, specifically with other novel targeted agents. Cediranib, an anti-angiogenesis agent, was studied with olaparib in recurrent epithelial ovarian or TNBCs ($N = 28$, 20 ovarian and 8 breast) (82) (**Tables 1–4**). Patients were enrolled to four dose levels and the recommended Phase II dose was cediranib 30 mg daily and olaparib 200 mg twice daily was based on one occurrence of grade 4 neutropenia ($\geq$4 days) and one of grade 4 thrombocytopenia with dosages of cediranib 30 mg daily and olaparib 400 mg twice daily. Seventy-five percent of patients experienced grade 3 or higher toxicities with grade 3 hypertension and fatigue, occurring in 25 and 18% of subjects, respectively. Despite the frequent hematologic and non-hematologic toxicities, the ORR was 44% in the evaluable ovarian cancer population ($N = 18$). Sixty-one percent of ovarian patients had clinical benefit (including those with SD). None of the breast cancer patients experienced clinical response, but two patients had SD for >24 weeks. A summary of Phase I–III studies utilizing PARP inhibitors in sporadic breast and ovarian cancers can be found in **Tables 3** and **4**.

PLATINUM AND PARP INHIBITOR RESISTANCE

BRCA 1/2-deficient cancers are known to be hypersensitive to platinum agents which are thought account for, in part, better overall prognosis for those patients with *BRCA 1/2*-germline mutation-related breast and ovarian cancer. Not all patients respond to platinum, however, and indeed, it is likely that the majority of tumors will eventually become platinum-resistant. Additionally, not all patients with *BRCA 1/2*-germline mutations or those with an expected BRCAness phenotype respond to PARP inhibition. Several mechanisms of resistance to both agents have been hypothesized and are likely to be multifactorial in etiology. Current evidence suggests that secondary mutations occur in the *BRCA1* or *BRCA2* gene restoring the wild type *BRCA 1/2* open reading frame which may provide return of DNA repair through a functional HR pathway. These reversion mutations are thought to lead to platinum resistance, as well as PARP inhibitor resistance (83–87). It is imperative that these secondary mutations are identified to help modulate therapeutic management of these populations. Of interest, PARP inhibitor resistance may, in fact, not affect subsequent therapy response, including subsequent platinum regimens (88).

CONCLUSION

Poly (ADP-ribose) polymerase inhibitors have shown promising activity as both monotherapy and in combination with cytotoxic chemotherapy in BRCA 1/2-mutated cancers. More recently, this concept has been implicated in sporadic high-grade serous ovarian cancers and TNBCs. Like platinum agents, PARP inhibitors have been efficacious in this population. Published data from the TCGA network further support this therapeutic strategy by showcasing the genomic similarities between high-grade serous ovarian cancers and TNBCs. It may be worthwhile in the future to study new drug therapies in tandem in these two populations. New strategies are needed to combat tumor resistance mechanisms, such as secondary mutations that revert *BRCA* genes to wild type, to both platinum agents and PARP inhibitors. Future directions for PARP inhibition include when best to use these agents, in what combinations, and precisely, how to define the optimal populations that will get the most benefit.

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Beyond breast and ovarian cancers: PARP inhibitors for BRCA mutation-associated and BRCA-like solid tumors

Ciara C. O'Sullivan[1], Dominic H. Moon[1,2], Elise C. Kohn[1] and Jung-Min Lee[1]*

[1] *Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA*
[2] *Medical Research Scholars Program, National Institutes of Health, Bethesda, MD, USA*

Edited by:
Kristin Zorn, Magee-Womens Hospital of UPMC, USA

Reviewed by:
Silvia Soddu, Regina Elena Cancer Institute, Italy
Lin Liu, Nankai University, China

***Correspondence:**
Jung-Min Lee, Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, 10 Center Dr. MSC 1906, Building 10, Room 12N/226, Bethesda, MD 20892, USA
e-mail: leej6@mail.nih.gov

Poly(ADP-ribose) polymerase inhibitors (PARPi) have shown clinical activity in patients with germline BRCA1/2 mutation (gBRCAm)-associated breast and ovarian cancers. Accumulating evidence suggests that PARPi may have a wider application in the treatment of cancers defective in DNA damage repair pathways, such as prostate, lung, endometrial, and pancreatic cancers. Several PARPi are currently in phase I/II clinical investigation, as single-agents and/or combination therapy in these solid tumors. Understanding more about the molecular abnormalities involved in BRCA-like phenotype in solid tumors beyond breast and ovarian cancers, exploring novel therapeutic trial strategies and drug combinations, and defining potential predictive biomarkers are critical to expanding the scope of PARPi therapy. This will improve clinical outcome in advanced solid tumors. Here, we briefly review the preclinical data and clinical development of PARPi, and discuss its future development in solid tumors beyond gBRCAm-associated breast and ovarian cancers.

Keywords: poly(ADP-ribose) polymerase inhibitors, solid tumors, BRCA mutation, BRCA-like, DNA damage repair pathway

INTRODUCTION

Increasing understanding of the cellular aberrations inherent to cancer cells has allowed the development of therapies targeting biological pathways. This approach has been an important step toward individualization of therapy for germline BRCA1/2 mutation (gBRCAm)-associated breast and ovarian cancers (1, 2). The clinical development of poly(ADP-ribose) polymerase inhibitors (PARPi), with their selective mechanisms of action involving the DNA damage repair pathways, is an example of this strategy. Early clinical trials have shown significant single-agent activity of PARPi in gBRCAm-associated breast and ovarian cancers (3–5). Response rates (RR) of 31–40% have been reported in gBRCAm ovarian cancer patients with measureable recurrent disease, and the RR and duration of response to PARPi monotherapy has been associated with platinum sensitivity (6, 7). Emerging evidence suggests that PARPi is an effective therapeutic strategy in subsets of other malignancies that have gBRCAm, such as melanoma, prostate, and pancreatic cancers. BRCA-like tumors have molecular and clinical characteristics in common with tumors occurring in patients with gBRCAm, which may have implications for PARPi-based therapy (8). Additionally, there is a potential therapeutic role for PARP inhibition in a wider subgroup of solid tumors that may have defective homologous recombination (HR) (9). Therefore, the utility of PARPi in other solid tumors is potentially greater than was previously envisioned (8).

PARPi have shown to enhance cytotoxicity in combination with DNA methylating agents (10, 11), topoisomerase inhibitors (12, 13), platinums (14, 15), alkylating agents (14), and radiation (16, 17) in numerous preclinical studies. These preclinical findings are being explored in clinical trials to elucidate the role of PARPi as chemo- and radiosensitizers in various tumor types (18). A large number of clinical trials are exploring the efficacy of combination strategies in malignancies such as non-small cell lung cancer (NSCLC), squamous cell cancer of the head and neck (HNSCC), esophageal, and colorectal cancers (CRCs) (**Tables 1** and **2**); the results of several phase I and II trials have already been reported (**Table 3**). These data suggest further clinical exploration of PARPi as monotherapy or combinations is warranted in patients not only with gBRCAm-associated breast or ovarian cancer, but also in solid tumors with HR dysfunction.

gBRCAm-associated and BRCA-like tumors are rare subsets of advanced solid tumors. Approximately 5–10% of breast (27) and 10–15% of ovarian cancers (28) occur in the setting of a hereditary cancer syndrome, the most common of which is a gBRCAm (29). This occurs less frequently in other solid tumors. Approximately 5% of cutaneous melanoma and gastric cancers are related to gBRCAm and 5–19% cases of familial pancreatic cancer are attributed to a gBRCAm (30, 31). Furthermore, gBRCAm are very rare events in patients with prostate cancer and NSCLC. gBRCAm are present in 0.44–1.2% of prostate cancer cases (32, 33). The overall incidence of gBRCAm in patients with NSCLC has not been reported from large trials; only 3 patients (2.7%) were noted to have a gBRCAm in a study of 110 Jewish men with epithelial growth factor receptor (EGFR) mutant-NSCLC (34). These subgroups of tumors with germline HR dysfunction constitute a rare population with recognized unmet therapeutic needs, and may be sensitive to treatment with PARPi. Additionally, there are significant unanswered questions of their use in solid tumors that have molecular and clinical characteristics in common with gBRCAm-associated tumors. Advances have been made in identifying new therapeutic targets and analyzing response to novel treatments

Table 1 | PARPi in clinical development (excluding breast and ovarian cancer) (19).

Name	Treatment	Cancer types	Phase
Olaparib (AstraZeneca)	Monotherapy Combination with chemotherapy Combination with RT Combination with targeted therapies	GBM, prostate, ES, NSCLC, CRC, and gastric cancer Esophageal cancer and HNSCC	I/II
Rucaparib (Clovis)	Combination with chemotherapy	AST	I
Veliparib (Abbott)	Monotherapy Combination with chemotherapy Combination with RT Combination with targeted therapies	gBRCAm prostate cancer, HNSCC, NSCLC, SCLC, pancreatic cancer, biliary cancers, HCC, rectal cancer, cervical cancer, CRPC, and CNS malignancies	I/II
CEP-9722 (Cephalon)	Monotherapy Combination with chemotherapy	AST	I
E7016 (EISAI)	Combination with chemotherapy	Melanoma and AST	I/II
BMN-673 (BioMarin)	Monotherapy	AST	I

GBM, glioblastoma multiforme; ES, Ewing's sarcoma; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; CRC, colorectal cancer; AST, advanced solid tumors; HNSCC, head and neck squamous cell cancer; CRPC, castrate resistant prostate cancer; HCC, hepatocellular cancer; CNS, central nervous system; n/a, not applicable.

in these patient subgroups and this has led to an explosion of PARPi-based clinical trials extending the patient cohort to include BRCA-like tumors.

PARP FUNCTION AND INHIBITION IN DNA DAMAGE REPAIR PATHWAYS

DNA damage can occur through various mechanisms from environmental factors such as ultraviolet rays, ionizing radiation, and genotoxic chemicals, to endogenous processes including generation of reactive oxygen species and replication (35). Highly complex and intertwined repair pathways have evolved to provide broad and redundant mechanisms to address damaged DNA: mismatch repair (MMR), base excision repair (BER), and nucleotide excision repair (NER) for a low fidelity single strand DNA break (SSB) repair mechanism, and HR and non-homologous end-joining (NHEJ) for double-strand DNA breaks (DSBs) (36). The different repair mechanisms are orchestrated by numerous enzymes to ensure the integrity of DNA essential for cell survival.

PARP are a family of enzymes that catalyze nicotinamide adenine dinucleotide (NAD^+)-dependent ADP-ribosylation of DNA. PARP1 is the best characterized member of the PARP family, and PARP2 has a similar structure and function with varying affinity for substrates (37). PARP1 has been implicated in several DNA repair mechanisms including the repair of SSBs through the BER pathway. It recognizes and binds to DNA sites with SSB via its DNA binding domain, then subsequently synthesizes poly(ADP-ribose) (PAR) by transferring ADP-ribose molecules from NAD^+ to itself and other acceptor proteins (38). This activates the formation of a DNA repair complex consisting of multiple repair proteins, including DNA ligase III and X-ray repair cross-complementing 1 (XRCC1) (39). The PARylated PARP1 dissociates from DNA as the negative charge of PAR decreases its affinity for DNA, and poly(ADP-ribose) glycohydrolase then degrades the PAR on PARP1 (40). PARP has been shown to have a direct involvement in DSB repair in addition to its role in preventing DSB formation by promoting BER. In PARP1-deficient cells, ATM-kinase function is compromised leading to a reduction in DNA DSB in response to radiation, indicating a role of PARP1 in ATM activation and HR (38, 41). PARP1 has been shown to reduce DSB formation by sensing stalled replication forks and recruiting MRE11 for end processing to initiate HR (42). Increased PARP1 expression and/or activity in tumor cells have been demonstrated in many tumor types (43, 44). Thus, HR dysfunction sensitizes cells to PARP inhibition leading to further chromosomal instability, cell cycle arrest, and apoptosis (45, 46).

PARPi are a class of drugs designed to compete with NAD^+ for the substrate binding site of PARP, acting as an effective catalytic inhibitor (47). PARP inhibition has been shown to induce phosphorylation of DNA-dependent protein kinase (DNA-Pk), to further stimulate error-prone NHEJ in HR-deficient cells (44, 48, 49). More recently, another mechanism of action of PARPi involving PARP1-trapping has been proposed (50). PARPi have been shown to trap PARP1 and PARP2 while in complex with damaged DNA, resulting in cytotoxic consequences (51). Trapped PARP prevents its availability for repair function and secondarily causes replication and transcription fork blockade, and subsequent DNA breakage. This mechanism of action may be important to the clinical activity of the PARPi class. The potency in trapping PARP differs markedly among PARPi, with niraparib (MK-4827) and olaparib having greater potency than veliparib. This pattern is not correlated with the catalytic inhibitory properties of each drug. These findings suggest that PARPi have several mechanisms of action and multiple targets in the DNA repair pathway to potentially induce cancer cell death (**Figure 1**).

Table 2 | Trials of PARPi in solid tumors (excluding breast and ovarian cancers).

Malignancy	PARPi	Combination agent(s)	Phase
GI			
Pancreatic	Olaparib Veliparib	Chemotherapy Cisplatin Gemcitabine Gemcitabine/IMRT Monotherapy (gBRCAm pancreatic cancer) Modified FOLFOX 6	I/II
Pancreatic, biliary, urothelial and NSCLC	Veliparib	Cisplatin and gemcitabine	I
Liver	Veliparib	Cisplatin and gemcitabine	I
Colorectal cancer	Veliparib Olaparib Veliparib	TMZ Irinotecan Capecitabine and RT	I/II
Colorectal cancer stratified by MSI	Olaparib	N/A	I/II
Esophageal cancer	Olaparib	RT	I
Gastric cancer	Veliparib Olaparib	FOLFIRI Paclitaxel	I/II
LUNG			
NSCLC (surgically unresectable)	Olaparib Veliparib	Concurrent RT ± cisplatin RT Carboplatin/paclitaxel Cisplatin/gemcitabine	I/II
EGFR mutation positive advanced NSCLC	Olaparib	Gefitinib ± olaparib	I/II
SCLC	Veliparib	Cisplatin/etoposide TMZ	I/II
GENITOURINARY			
CRPC	Veliparib	Abiraterone and prednisone TMZ	I/II
	Olaparib	N/A	II
GYNECOLOGIC			
Cervical cancer	Veliparib	Cisplatin and paclitaxel Topotecan Carboplatin and paclitaxel	I/II
Uterine carcinosarcoma	Veliparib	Carboplatin and paclitaxel	II
CENTRAL NERVOUS SYSTEM			
GBM	Olaparib Veliparib	TMZ TMZ	I I/II
Brain metastases	Veliparib	WBRT	I/II
DPG	Veliparib	RT TMZ	I/II
Refractory CNS tumors	Veliparib	TMZ	I
HEAD AND NECK			
HNSCC	Veliparib	RT Docetaxel 5-FU	I/II

(Continued)

Table 2 | Continued

Malignancy	PARPi	Combination agent(s)	Phase
SARCOMA			
Ewing's sarcoma	Olaparib	N/A	II
SKIN CANCER			
Melanoma	Veliparib	TMZ	II
	E7016	TMZ	
ADVANCED SOLID TUMORS			
	Veliparib	Carboplatin and gemcitabine	I/II
		Gemcitabine	
		Carboplatin and paclitaxel	
		Mitomycin C	
		Capecitabine and oxaliplatin	
		Cyclophosphamide	
	Olaparib	Cisplatin/gemcitabine	
		PLD	
		Topotecan	
	Niraparib	Monotherapy	
	CEP-9722	Monotherapy	
	BMN-673	Monotherapy	

IMRT, intensity modulated radiotherapy; NSCLC, non-small cell lung cancer; RT, radiotherapy; MSI, microsatellite instability; CRPC, castrate resistant prostate cancer; SCLC, small cell lung cancer; GBM, glioblastoma multiforme; DPG, diffuse pontine glioma; HNSCC, squamous cell carcinoma of the head and neck; 5-FU, 5-fluorouracil; PLD, pegylated liposomal doxorubicin.

PARP INHIBITION IN gBRCAm AND BRCA-LIKE SOLID TUMORS

Understanding DNA repair biology has allowed the identification of patient subsets with high potential for response to PARPi treatment. The marked susceptibility of patients with gBRCAm has validated gBRCAm as a predictive biomarker for PARPi response in breast and ovarian cancer patients. In a series of pivotal preclinical studies, PARPi were noted to cause selective cytotoxicity for *in vitro* and *in vivo* models of BRCA-deficient cells (52, 53). Additionally, PARPi attenuates tumor formation in embryonic stem cell-derived teratocarcinoma xenograft models (46). These findings were translated into a phase I clinical trial of the PARPi, olaparib, in recurrent breast, ovarian, and prostate cancer patients with gBRCAm (4), initiating a new era of possibilities for the use of PARPi as single-agent therapy to treat gBRCAm-associated cancers.

The BRCA-like behavior has been described based on clinical and molecular features that parallel gBRCAm-associated cancers' characteristics. The major clinical BRCA-like behavior identified is susceptibility to platinums and other DNA-damaging agents (54–56). Some of the molecular events described in BRCA-like behavior include epigenetic silencing of BRCA1 through promoter methylation (57–59) and overexpression of EMSY, suppressing BRCA2 transcription (60). In addition, loss or disruption of proteins necessary for HR such as RAD51, ATM, ATR, CHK1, CHK2, FANCD2, and FANCA (53, 61–64) are observed in a variety of tumors (8, 65–71), and may confer sensitivity to PARPi (8, 53). Defects in translesion synthesis (TLS) also contribute to carcinogenesis but confer sensitivity to DNA-damaging agents (72, 73), requiring further investigation on sensitivity to PARPi.

Homozygous mutation in the PTEN tumor suppressor gene may also lead to HR dysfunction (74). Increased PARPi sensitivity was shown in a series of cell lines with PTEN mutation or haploinsufficiency, and confirmed in xenograft models using olaparib (74). There is also clinical evidence that olaparib may have a therapeutic utility in PTEN-deficient endometrial cancer (75, 76). Further studies are needed to investigate whether PTEN loss can serve as a potential biomarker for PARPi sensitivity (77–79). Future studies should focus on DNA profiling and the use of predictive biomarkers to select those tumors which are more likely to respond to PARPi. Ongoing research suggests HR deficiency, rather than a specific mutation in the BRCA genes, may be the main driver of cytotoxicity of PARP inhibition (45).

TRIALS WITH PARPi IN gBRCAm AND/OR BRCA-LIKE ADVANCED SOLID TUMORS

MALIGNANT MELANOMA

Little is known about the underlying cause of hereditary cancer predisposition in melanoma and its impact on the prognosis and therapeutic decisions. Cutaneous melanoma has been associated with mutations in the BRCA2 gene although there are only a few cases reported for uveal melanoma in BRCA2 mutation carriers (80). In recent years, the advent of BRAF V600E inhibitors (e.g., vemurafenib) and anti-CTLA4 antibodies (e.g., ipilimumab) has significantly improved outcomes in patients with metastatic melanoma (81–83), with a median duration of response of 8 and 16 months, respectively (84, 85). However, most patients eventually progress and some do not tolerate therapy due to immune-related side effects, indicating the need to develop other therapeutic strategies.

Table 3 | PARPi trials for which tumor response rates have been reported.

PARPi	Patient cohort	Combination	Drug and schedule	Toxicity	Response
Rucaparib (20) (phase I)	AST Melanoma (32 pts)	TMZ	D1: rucaparib 12 mg/m^2 IV D1–5: TMZ 200 mg/m^2 PO q 28 day cycle	No DLT Myelosuppression (13%) At MTD	CR: 1/32 pts (melanoma) PR: 2/32 pts (1 melanoma; 1 desmoid tumor) SD: 7/32 pts-6 mo or greater
Olaparib (19) (phase I)	Melanoma (40 pts)	Dacarbazine	D1–7: olaparib (20–200 mg) PO BID D1: (cycle 2 day 2): dacarbazine (600–800 mg/m^2 IV) q 21 day cycle MTD: 100 mg olaparib PO BID and dacarbazine 600 mg/m^2 IV	Grade 3 hypophosphatemia-1 pt Grade 3 neutropenia-1 pt Grade 4 neutropenia-2 pt	CR: 0/40 pts PR: 2/40 pts SD: 8/40 pts
Olaparib (21) (phase I)	AST (19 pts)	Topotecan	D1–3: topotecan 0.5–1.0 mg/m^2 IV Olaparib (50–200 mg PO BID) q 21 day cycle	DLTs 16% Grade 3 thrombocytopenia-1 pt Grade 4 neutropenia-2 pts Treatment related death-1 pt (pneumonia)	CR: 0/19 pts PR: 1/19 pts SD:4/19 pts RECIST RR = 37%
Olaparib (22) (phase I)	AST (12 pts)	N/A	D1–28: olaparib (100–400 mg PO BID)	No DLTs Grade 3 toxicity in 16% Anemia-8% Elevated AST-8%	CR: 0/12 PR: 1/12 pts-13 mo SD: 4/12 > 8 weeks (unknown gBRCAm status)
Veliparib (23) (phase I)	AST (35 pts)	MCP	D1–21: cyclophosphamide 50 mg daily PO Olaparib (20 mg daily × 7 days >80 mg daily q 21 days cycle) MTD: veliparib 60 mg daily and cyclophosphamide 50 mg once daily	DLTs-6% Grade 3 ileus-1 pt Grade 4 respiratory Failure and death-1 pt Lymphopenia-34.3%	CR: 0/35 pts PR: 7/35 pts (gBRCAm) SD: 6/35 (3 BRCA+)
INO-1001 (phase Ib)	Melanoma (12 pts)	TMZ	D1–5: TMZ 200 mg/m^2 IV daily and INO-1001 (100–400 mg IV q 12 h) × 10 doses, q 28 day cycle MTD: INO-1001 = 400 mg	Anemia-17% Grade 4 hepatotoxicity-8% Grade 4 hematologic toxicity-58% Grade 3 myelosuppression	CR:0/12 pts PR:1/12 pts SD:4/12 pts RR = 4.2% CBR = 41.6%
Rucaparib (24) (phase II)	Melanoma (40 pts)	TMZ	D1–5: TMZ 200 mg/m^2 and rucaparib 12 mg/m^2 IV, q 28 day cycle	Grade 4 thrombocytopenia-12%	10% PR (4/40 pts) SD-4/40 pts
Veliparib (25) (phase I/II)	Pancreatic cancer (18/28 pts evaluable at time of reporting)	Modified FOLFOX6	Phase 1 dose-escalation: veliparib 40–100 mg BID D1–7, q 14 day cycle Phase II-two parallel groups; first line and untreated	Grade 3 neutropenia-1 pt Grade 5 neutropenia-1 pt Grade 3 lymphopenia-1 pt Grade 3 anemia-1 pt	11 pts (First line) RR-18% PFS-3.9 mo OS-7.4 mo

(Continued)

Table 3 | Continued

PARPi	Patient cohort	Combination	Drug and schedule	Toxicity	Response
					Seven patients (pre-treated) RR-14% PFS-1.8 mo OS-5.4 mo CR-1/18 pts PR-1/18 pts
Veliparib (26) (phase II)	Colorectal cancer (47 pts)	TMZ	D1–5: TMZ 150 mg/m^2 PO daily D1–7: veliparib 40 mg BID q 28-day cycle	Grade 3 A/E in 5 pts (myelosuppression)	ORR (CR + PR)-5% 2/47 pts-PR, 0/47 pts = CR CR + PR + SD = 23% Median TTP = 11 weeks; 23 weeks for pts with controlled disease

DLT, dose limiting toxicity; MTD, maximum tolerated dose; CR, complete response; PR, partial response; SD, stable disease; IV, intravenously; PO, orally; D, days; mo, months; q, every; BID, twice a day; RECIST, response evaluation criteria in solid tumors; TMZ, temozolomide; AST, aspartate transaminase; pt(s), patient(s); MCP, metronomic cyclophosphamide; PFS, progression-free survival; OS, overall survival; ORR, overall response rate; TTP, time to progression.

PARPi have multiple targets in DNA repair pathways that can potentially promote cancer cell death. In the setting of melanoma, altered expression or new mutations in DNA MMR genes, MLH1 and MSH2, have been reported in brain metastases (86). A melanoma cell line (MZ7), derived from a patient who received dacarbazine therapy, exhibited a high level of resistance to temozolomide (TMZ) without expressing *O*(6)-methylguanine-DNA methyltransferase (MGMT), which was related to impaired expression of MSH2 and MSH6 (87). PARP inhibition with INO-1001 has been shown to restore sensitivity to TMZ in an MMR-deficient xenograft model of malignant melanoma (88), and another PARPi, GPI 15427, enhanced TMZ anti-tumor activity in various cancers, including metastatic melanoma in an orthotopic xenograft mouse model (24). These preclinical studies provide evidence that MMR loss of function is a potential predictive biomarker of PARPi responsiveness in patients with metastatic melanoma.

A number of clinical trials of PARPi in melanoma patients have been conducted or are ongoing although they have not specifically addressed the frequency of HR dysfunction/gBRCAm in their populations. Bedikian et al. reported the results of a phase IB study of intravenous INO-1001 and oral TMZ in unselected patients with unresectable stage III or IV melanoma (89). The dose limiting toxicities (DLTs) were elevation of liver transaminases and myelosuppression at the 400-mg dose of INO-1001. Of the 12 patients enrolled, 1 patient had a partial response (PR) and 4 patients had stable disease (SD). Several phase II studies using PARPi either as a single-agent or in combination with chemotherapy, radiotherapy, or targeted therapy are summarized in **Table 3**. A phase II trial sought to evaluate the combination of rucaparib and TMZ in patients with metastatic malignant melanoma (90). The disease-control rate was 40% (8/20), where four patients attained a PR and four others had prolonged SD. In total, 12 of the 40 patients required a dose reduction of TMZ secondary to myelosuppression (90). Another phase II study evaluated treatment with rucaparib 12 mg/m^2 and TMZ 200 mg/m^2 in patients with advanced melanoma. Myelosuppression was again noted, with 25 patients (54%) requiring a 25% dose reduction in TMZ. The RR was 17.4%, with median time to progression and OS of 3.5 and 9.9 months, respectively. This study demonstrated that TMZ could safely be given with a PARP-inhibitory dose (PID) of rucaparib (12 mg/m^2), based on 74–97% inhibition in PARP of peripheral blood mononuclear cells (PBMCs). This resulted in an increase in PFS compared with historical controls (91). Phase I and II trials evaluating E7016 in combination with TMZ in patients with advanced solid tumors and malignant melanoma are ongoing (92, 93). Eligibility criteria for the phase II study include BRAF wild-type status and no prior treatment with TMZ or PARPi. As substantial progress has been made in the management of malignant melanoma in recent years (94), it remains to be seen whether PARPi will be added to the treatment armamentarium.

PANCREATIC CANCER

Hereditary pancreatic cancer is rare and extremely heterogeneous, and it accounts for approximately 2% of all pancreatic cancer cases. The major component of hereditary pancreatic cancer is the familial pancreatic cancer syndrome. Although up to 20% of

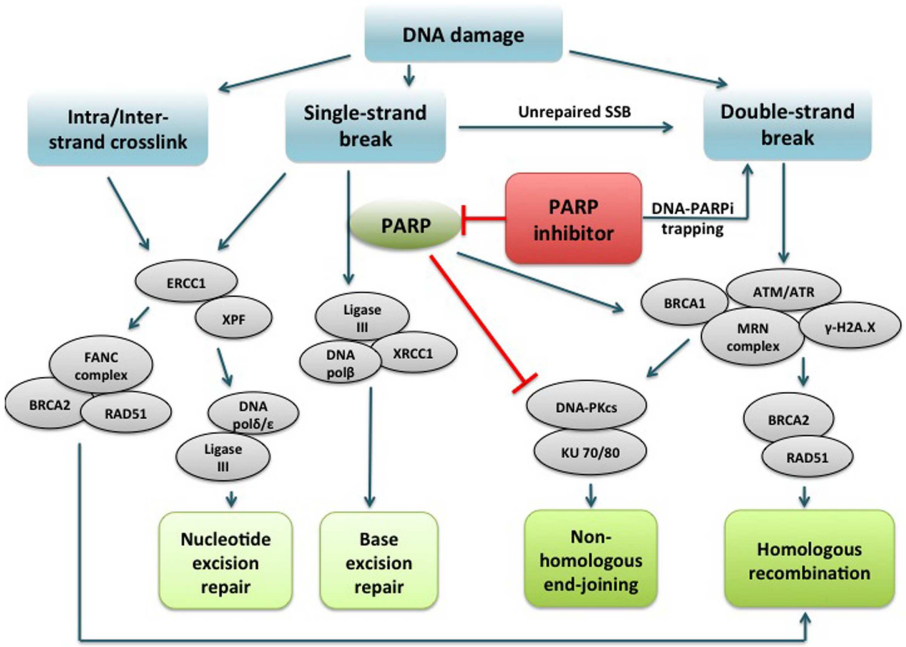

FIGURE 1 | PARP1 binds to DNA single strand break and catalyzes poly(ADP-ribosyl)ation of itself and acceptor proteins, which facilitates recruitment of DNA repair proteins. In addition to its reported role in base excision repair, PARP1 plays a role in activating ATM necessary for homologous recombination and inactivating DNA-dependent protein kinase, a key component of non-homologous end-joining. PARP inhibitors directly interfere with the above functions of PARP1. In addition, PARP inhibitors have been shown to trap PARP1 on damaged DNA, leading to replication and transcription fork blockage and subsequent double-strand DNA breakage. Repair of intra/interstrand crosslinks through nucleotide excision repair or homologous recombination are also important components of the DNA repair system, and whether defects in these repair pathways can confer sensitivity to PARPi are under investigation. PARP, poly(ADP-ribose) polymerase; PARPi, PARP inhibitor; DNA polβ/δ/ε, DNA polymerase beta/delta/epsilon; XRCC1, X-ray repair cross-complementing protein 1; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; KU 70/80, a.k.a XRCC6/5 (X-ray repair cross-complementing protein 6/5); ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; γ-H2A.X, gamma-histone H2A member X; RAD51, RAD51 homolog (*S. cerevisiae*); ERCC1, DNA excision repair protein ERCC1; XPF, DNA repair endonclease XPF (xeroderma pigmentosum group F-complementing protein); FANC, Fanconi anemia.

hereditary pancreatic cancer cases are associated with germline mutations in BRCA2, CDKN2A, PRSS1, STKI1, or MMR genes, the major underlying gene defects are still unknown (95). BRCA2 mutation prevalence in familial pancreatic cancer patients varies between 5 and 19% (30), and a BRCA2 mutation increases the risk of developing pancreatic cancer by approximately 3.5-fold (96). The unique biology of cancer cells with BRCA mutations offers potential therapeutic advantages with agents such as platinums. However, one case series report patients with gBRCAm did not reveal a benefit to first line platinum chemotherapy in the treatment of advanced pancreatic cancer (97), although this needs to be further evaluated in a selected study for pancreatic cancer with gBRCAm. Preclinical studies have shown single-agent activity of PARPi (98), as well as radiosensitization in combination with chemoradiation in BRCA2-deficient pancreatic cells (25). Studies are ongoing to examine single-agent and combination PARPi therapy in BRCA2 mutant pancreatic cancers.

Interim results from an ongoing phase II study of olaparib monotherapy in gBRCAm-associated advanced solid cancers were recently reported (99). Nearly 8% of the patients (23/298) had advanced/recurrent pancreatic cancer. A RR of 5/23 (21.7%) was noted, with eight patients achieving SD. This yielded a clinical benefit rate of 57% in gBRCAm-associated pancreatic cancer patients. Pishvaian et al. reported a phase I study of veliparib with concurrent FOLFOX chemotherapy in patients with metastatic pancreatic cancer (100). Twenty-eight patients were enrolled in the trial and at the time of review, data were available for 18 patients. For the 11 patients who were treated in the first line setting, RR was 18%, with a PFS and OS of 3.9 and 7.4 months, respectively (**Table 3**). Therefore, the investigators concluded that the experimental combination regimen could be given safely, and was modestly active (100). These data support further evaluation of PARPi either as different combinations or more potent PARPi with chemotherapy and/or other targeted agents combination in this subgroup of pancreatic cancer patients.

PROSTATE CANCER

Germline BRCA2 mutation confers the highest genetic risk of prostate cancer known to date at 8.6-fold in men ≤65 years, whereas the effect of BRCA1 is more modest at 3.4-fold (32, 33, 101, 102). Prostate cancer in patients with gBRCAm tends to be more aggressive, with a higher likelihood of nodal involvement and distant metastasis with inferior survival outcomes (103). Trials analyzing the response of these patients to DNA-damaging agents, such as platinums, and identifying the therapeutic targets of this subgroup are urgently needed.

Single-agent olaparib has demonstrated activity in patients with gBRCAm castration resistant prostate cancer (CRPC). A phase I olaparib study by Fong et al. reports one gBRCA2m patient treated with single-agent olaparib who sustained a CR lasting in excess of 2 years (4). Recently, Sandhu et al. presented clinical data on four patients with advanced gBRCAm CRPC, three of whom were treated with olaparib and one with niraparib (104). Two patients on olaparib showed prostate-specific antigen (PSA) and radiologic responses lasting 26 and 34 months, respectively, while the third patient had SD for 10 months. The patient on niraparib exhibited primary resistance with development of a new liver lesion and a rise in PSA of nearly threefold at the time of the first reassessment. Translational studies revealed positive ERG staining by immunohistochemistry, and ERG rearrangements by FISH, as well as either heterozygous or homozygous PTEN allelic loss in all four cases. Subsets of patients with CRPC are also known to manifest increased PARP activity (105). This potentially opens another avenue for therapy utilizing PARPi, although gBRCAm is a very rare event in prostate cancer.

Gene fusion between the ERG proto-oncogene and TMPRSS2 promoter is a major genomic alteration observed in approximately 50% of prostate cancers. Formation of the TMPRSS2-ERG fusion gene causes aberrant androgen-dependent ERG expression (106) and promotes tumorigenesis (107). Preclinical studies have shown that PARP1 directly interacts with ERG to inhibit ETS gene fusion protein activity. In turn, inhibition of PARP1 reduces ETS-positive, but not ETS-negative, prostate cancer xenograft growth (108). This may be a useful predictive biomarker for PARPi sensitivity.

Other preclinical studies include radiosensitization by rucaparib, most evident in PTEN-deficient prostate cancer cells containing the TMPRSS2-ERG fusion gene (109). However, no association was noted between loss of PTEN expression by immunohistochemistry and ETS rearrangements by FISH, with radiologic assessment of the anti-tumor activity of niraparib in 18 patients with prostate cancer (110). The HR/PARP synthetic lethality model may be more widely applicable in prostate cancer with germline or somatic inactivating mutations in the HR DNA repair genes, CHK2, BRIPI/FANCJ, NBS1, BRCA1, and ATM, collectively thought to occur in 20–25% of prostate cancer cases. Recently, a phase II study of olaparib in unselected patients with CRPC was initiated (111).

Veliparib has also been investigated and shown to enhance the anti-tumor activity of TMZ in prostate cancer xenografts, yielding tumor size reduction in TMZ-resistant PC3-Leu prostate cancer mice (112). This formed the rationale for testing the efficacy and safety of veliparib and TMZ in 26 patients with metastatic CRPC (113). Grade III/IV thrombocytopenia was noted in 15% of patients. Two patients had a confirmed PSA response and four patients had SD for at least 4 months. The median PFS and OS were 2.1 (95% CI: 1.8, 3.9) and 9.1 (95% CI: 5.5, 11.7) months, respectively. This study suggested veliparib and TMZ are tolerated well, but with limited clinical activity. Future trials will explore the use of different chemotherapy agents in combination with higher doses of veliparib. Overall, further evaluation of biochemical changes or predictive biomarkers in response to PARPi in advanced prostate cancer is needed.

COLON CANCER

Preclinical data suggest the utility of PARPi in tumors deficient in HR and displaying microsatellite instability (MSI) due to mutations in the coding microsatellites of the MRE11A and hRAD50 genes involved in DNA DSB repair (114). Preferential cytotoxicity to the PARP1 inhibitor ABT-888 was seen in MSI cell lines containing mutant copies of MRE11A, compared with wild-type or microsatellite stable (MSS) cells (115). In a recent study, the observed ability of MSH3 to protect against DSB was exploited by the combination of oxaliplatin and a PARPi, which produced a synergistic cytotoxic effect against CRC cells (116). Another study reporting high correlation between MRE11 mutations and MSI in CRC cell lines as well as primary tumors, found that PARPi preferentially kills MSI cell lines harboring MRE11 mutations (115). The data suggest a role for PARPi in MSI-CRC treatment, providing a rationale for clinical studies in this subset of patients.

Dozens of potential PARPi have been screened *in vitro* and *in vivo* to select candidates for clinical evaluation as a chemosensitizer in CRC (117). A phase II trial is currently evaluating the efficacy of olaparib in metastatic CRC (mCRC) stratified for MSI status (118). Twenty-two patients with MSI-negative tumors were enrolled and received a mean number of two cycles. Preliminary data indicate no single-agent activity of olaparib against non-MSI-high (MSI-H) mCRC. Accrual of MSI-H mCRC patients continues, along with active biomarker analysis. Other clinical trials of PARPi in MSI-CRC are in progress.

Studies have evaluated and validated veliparib as a sensitizer to irinotecan, oxaliplatin, and radiation therapy (RT) in CRC cells (26, 119). Several phase II studies are evaluating the role of PARPi as a chemosensitizer in patients with advanced and mCRC, irrespective of MSI status (**Table 2**). Pishvaian et al. (120) conducted a single arm, open label phase II study in patients with unresectable or mCRC. Patients were treated with TMZ (150 mg/m^2 orally daily) days 1–5, and veliparib (40 mg orally twice a day) days 1–7 of each 28-day cycle. Immunohistochemistry was performed on archived tumor samples to quantify MMR and PTEN protein expression. The combination of veliparib and TMZ was well tolerated in the 47 patients treated, with a disease-control rate of 23%. The results of immunohistochemistry for the MMR and PTEN proteins from 45 archived tumor samples are not yet reported. It was concluded that, in a heavily pre-treated population of patients with mCRC, the combination of veliparib and TMZ can be safely given, and displayed limited clinical activity.

LUNG CANCER

Reduced BRCA1 mRNA and protein expression levels have been observed in up to 44% of NSCLC, occurring through various mechanisms such as promoter hypermethylation (121). One study showed that BRCA1 silencing increased susceptibility to olaparib treatment in NSCLC cell lines (122), providing evidence for possible clinical application in this subset of NSCLCs. A future study will assess the utility of olaparib in delaying the time to disease progression in patients with advanced NSCLC who have responded to initial chemotherapy (123). The role of PTEN mutation and its effect on the susceptibility to PARPi is an area of continued research in lung and other malignancies. Up to 9% of NSCLCs

have a somatic mutation in PTEN. Olaparib has yielded additive activity with cisplatin in homozygous deleted PTEN-deficient NSCLC cells and xenograft models (79). Another gene involved in DNA repair, excision repair cross-complementation group 1 (ERCC1), is a key component of NER and the main mechanism for removing platinum–DNA adducts (124). Preclinical studies have explored this repair pathway, demonstrating synergy of olaparib and veliparib with cisplatin in NSCLC cell lines with low ERCC1 expression levels (125, 126). PARPi have also been explored preclinically in combination with other DNA-damaging modalities such as RT (16).

The role of PARPi in patients with EGFR mutant NSCLC has been studied in a phase IB study of olaparib and the EGFR tyrosine kinase inhibitor (TKI) gefitinib (127). It was noted that high BRCA1 mRNA expression is associated with a shorter PFS in EGFR-mutated patients treated with erlotinib. To date, 18 patients have received treatment at four different dose levels of olaparib ranging 100–200 mg twice daily dose, and 200–250 mg three times daily dose. DLT was grade 3 anemia observed at dose level 4 (250 mg three times daily). Of the 17 patients in whom a disease response could be evaluated, 7 (41.1%) had a PR. All of the patients who responded were EGFR TKI naive. Another seven patients (41.1%), most of whom received prior treatment, had documented SD, and three patients (17.6%), all of whom had prior EGFR TKI treatment, progressed. The observed anti-tumor activity will be further evaluated in EGFR TKI treatment-naive patients with EGFR-mutated NSCLC; a phase II randomized trial comparing the efficacy of olaparib and gefitinib versus gefitinib alone was launched in July 2013.

Multiple studies are also exploring the role of PARPi in combination with chemotherapy and/or RT in NSCLC. A phase I dose-escalation trial of olaparib and concurrent RT, with or without cisplatin, is ongoing in patients with advanced NSCLC (128). SWOG 1206, a phase I/II trial, is evaluating the use of veliparib with or without RT and carboplatin/paclitaxel in patients with inoperable stage III NSCLC. Several similar studies involving other combinations of PARPi ± chemotherapy and/or RT are ongoing in patients with NSCLC (**Table 2**). Ultimately, combining PARPi with cisplatin or radiotherapy may prove to be a useful strategy in the treatment of NSCLC.

EWING'S SARCOMA

PARPi has preclinically shown anti-tumor activity in the treatment of Ewing's sarcoma. Gene fusions involving Ewing's sarcoma breakpoint region 1 (EWS) and ETS transcription factors have been implicated in abnormal proliferation, invasion, and tumorigenesis (129). PARP inhibition has been evaluated as an effective treatment option for Ewing's sarcoma with EWS–FLI1 or EWS–ERG genomic fusions in xenograft models (130), and olaparib has been shown to have potent activity in cell lines with a EWS/FLI1 translocation (131). Additionally, a study in preclinical models showed synergy between PARPi and TMZ (130) in the treatment of Ewing's sarcoma cell lines. Currently, a number of clinical trials investigating the utility of PARPi in Ewing's sarcoma are underway (132, 133).

CHALLENGES AND FUTURE DIRECTIONS FOR CLINICAL DEVELOPMENT IN CANCERS OTHER THAN BREAST AND OVARIAN

There is considerable interest in the clinical development of PARPi for use in solid tumors other than breast and ovarian cancers. The optimal dose, scheduling, and sequencing of PARPi, and combination with other cytotoxic or biologic agents need to be evaluated in carefully designed clinical trials. The incorporation of predictive biomarkers into studies of gBRCAm and BRCA-like cancers presents challenges. First is the development of a mechanism with which to identify patients who are most likely to benefit from PARPi therapy. Predictive biomarkers applied to readily available bioresources, such as archival tissue or non-tumor tissue, have been proposed. Changes in or baseline PAR incorporation into PBMC DNA was suggested and evaluated as a putative early on-treatment pharmacodynamic measure; while present, there was no relationship to clinical outcome (134). BRCA1/2 somatic mutation or promoter methylation, ATM mutation, MRE11-dominant negative mutations in MMR-deficient cancers, FANCF promotor methylation and PTEN deficiency are all potential biomarkers of sensitivity to PARPi (51). Importantly, not all patients with deficiencies in BRCA1 or 2 are responsive to PARP inhibition (135). Therefore, identification and validation of predictive biomarkers of those gBRCAm who will respond to PARPi is also an important area of ongoing research.

The second challenge is dissecting and defining mechanisms of development of resistance to PARPi, and whether they portend potential collateral resistance to other DNA-damaging agents. Acquisition of a secondary mutation in BRCA1/2 that allows BRCA1/2 gene read-through and yields a functional protein has been demonstrated in cell lines and some patients; this was correlated with loss of susceptibility to PARPi treatment (136). Other potential mechanisms of clinical resistance have been proposed based on preclinical models, including loss of 53BP1, or increased activity of RAD51 (137, 138). Whether these findings can be used as selective or predictive biomarker is yet to be determined. Ang et al. recently reported that gBRCAm-associated ovarian cancer patients retain the potential to respond to subsequent chemotherapy, including platinum-based agents, after progression on PARPi (139). This observation has implications for chemotherapy sequencing. Further studies are needed to evaluate outcomes to subsequent chemotherapies or another PARPi in other solid tumor patients who have a BRCA-like phenotype. Understanding the mechanism(s) of resistance to PARPi will lead to optimal application and sequencing of PARPi and other DNA-damaging agents.

CONCLUSION

PARPi are a class of agents with mechanisms of action beyond their documented role in BER pathway. They potentially have a broader application in the treatment of cancer patients, both within the confines of gBRCAm and BRCA-like disease, but also extending to a wide range of aberrations in DNA damage repair pathways. Ongoing research will aim to identify optimal predictive biomarkers in order to improve patient selection and thus, clinical responses to treatment. It is anticipated that novel clinical trial

design strategies will help minimize toxicity and maximize therapeutic efficacy. Other pertinent questions relate to the duration of treatment and long-term effects of treatment, which need to be carefully investigated (20). Future directions for PARPi will include clinical trials directed at patient subsets that are most likely to respond to treatment, based on their molecular characteristics and predictive biomarkers. This may ultimately result in practice-changing treatments in malignancies such as pancreatic cancer, prostate cancer, and Ewing's sarcoma. The results of trials of PARPi, either as single-agents or in combination with chemotherapy, RT, or biological agents in other solid tumors are eagerly awaited.

AUTHOR CONTRIBUTIONS

All authors substantially contributed to the concept of the manuscript, drafted and revised, and approved final version.

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Successes and challenges of PARP inhibitors in cancer therapy

Tiffany K. Ricks, Haw-Jyh Chiu, Gwynn Ison, Geoffrey Kim, Amy E. McKee, Paul Kluetz and Richard Pazdur*

Office of Hematology and Oncology Products (OHOP), Office of New Drugs (OND), Center for Drug Evaluation and Research (CDER), U.S. Food and Drug Administration, Silver Spring, MD, USA

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INTRODUCTION

Poly (ADP-ribose) polymerases (PARPs) are a family of enzymes involved in cellular homeostasis, including DNA transcription, cell-cycle regulation, and DNA repair (1, 2). PARPs can detect DNA damage and bind to DNA single strand breaks (SSBs) through their N-terminal zinc finger domains. DNA binding activates the C-terminal catalytic domain, which hydrolyzes NAD^+ to attach poly ADP-ribose (PAR) polymers covalently to nuclear proteins, including PARP itself. Negatively charged PAR polymers promote recruitment of DNA repair proteins, and auto-PARylation causes dissociation of PARPs from DNA, allowing completion of DNA repair. In the absence of PARP activity, unrepaired SSBs can lead to more deleterious double strand breaks (DSBs), which require high fidelity, homologous recombination (HR) or low fidelity, non-homologous end joining (NHEJ) for repair.

In vitro and *in vivo* studies have demonstrated that tumor cells harboring defects in DNA repair are highly sensitive to PARP inhibitors, leading to genomic instability and cell death. Two publications demonstrated the concept of synthetic lethality in BRCA-deficient cells treated with PARP inhibitors (3, 4). Cells lacking functional alleles of *BRCA* are defective in HR repair and have an increased susceptibility to cause tumor development. Loss of BRCA or inhibition of PARP alone has little effect on *in vitro* and *in vivo* tumor growth; however, loss of function of both proteins enhances anti-tumor activity. Restoring BRCA expression blocks the cytotoxic effects of PARP inhibitor treatment.

Several clinical PARP inhibitors are under investigation in Phase 2 and Phase 3 clinical trials as monotherapy in cancers with DNA repair defects or in combination with radiation, chemotherapy, or other targeted agents (**Table 1**). Progress in PARP inhibitor development has led to the recent accelerated approval of Lynparza (olaparib) by the U.S. Food and Drug Administration (5). Lynparza is currently indicated as monotherapy for patients with advanced germline *BRCA*-mutated ovarian cancer who have received three or more prior lines of chemotherapy. Lynparza was approved with a companion diagnostic test to select patients with deleterious or suspected deleterious *BRCA* mutations. PARP inhibitors are anticipated to have a much broader clinical application in additional tumor types, particularly those with DNA repair defects and in combination with chemotherapy and other targeted agents. In light of renewed interest in PARP inhibitors and the recent approval of Lynparza, this review will highlight data of PARP inhibitors in *in vitro* and *in vivo* cancer models and explore some of the clinical applications and challenges of PARP inhibitor therapy.

MECHANISMS OF ANTI-TUMOR EFFECT OF PARP INHIBITORS

Poly (ADP-ribose) polymerase inhibitors are structurally similar in that they contain a nicotinamide moiety and mimic the NAD^+ substrate. PARP inhibitors competitively bind to the catalytic domain of PARPs and inhibit PAR synthesis with half-maximal inhibitory concentration (IC_{50}) values in

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Christina Annunziata, National Cancer Institute, USA

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Lawrence Panasci, Jewish General Hospital, Canada
Shiv K. Gupta, Mayo Clinic, USA
Joyce Liu, Dana-Farber Cancer Institute, USA

****Correspondence:***
Tiffany K. Ricks
tiffany.ricks@fda.hhs.gov

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TABLE 1 | PARP inhibitors in Phase 2 and Phase 3 clinical development[a].

PARP inhibitor	Clinical trial	Therapy	Tumor type
Olaparib	Phase 2	Monotherapy	Ovarian, peritoneal, fallopian tube, breast, colorectal, lung, Ewing's sarcoma, prostate, pancreatic, advanced tumors
		Combination	Breast, ovarian, peritoneal, fallopian tube, endometrial, gastric, prostate, lung, pancreatic
	Phase 3	Monotherapy	Breast, ovarian
		Combination	Ovarian, peritoneal, fallopian tube, gastric
		Maintenance	Ovarian, pancreatic
Veliparib	Phase 2	Monotherapy	Ovarian, fallopian tube, peritoneal, breast
		Combination	Breast, ovarian, peritoneal, fallopian tube, colorectal, lung, cervical, prostate, liver, glioblastoma, melanoma, pancreatic, advanced tumors
	Phase 3	Combination	Breast, lung, glioblastoma
Rucaparib	Phase 2	Monotherapy	Breast, ovarian, fallopian, peritoneal, pancreatic
	Phase 3	Combination	Breast
		Maintenance	Ovarian, fallopian tube, peritoneal
Niraparib	Phase 2	Monotherapy	Ovarian
	Phase 3	Monotherapy	Breast
		Maintenance	Ovarian
Talazoparib	Phase 2	Monotherapy	Breast, ovarian, endometrial, advanced tumors
	Phase 3	Monotherapy	Breast
E7016	Phase 2	Combination	Melanoma

[a]*Completed and active clinical trials obtained from www.clinicaltrials.gov, data accessed August 2015.*

the low nanomolar range (6–8). PARP inhibitors were developed to block the enzymatic activity of PARPs and prevent SSB repair by inhibiting the base excision repair (BER) pathway, and initial clinical development focused on potentiating the effects of chemotherapy and radiation (6, 9, 10). Subsequent studies demonstrated that PARP inhibitors alone were cytotoxic in HR-deficient cells (3, 4, 11). Based on these findings, a model was proposed in which PARP inhibition causes unrepaired SSBs, which are subsequently converted to DSBs, leading to synthetic lethality in HR-deficient cells (4). However, knockdown of XRCC1, the protein immediately downstream of PARP in the BER pathway did not lead to synthetic lethality (12), suggesting that loss of PARP activity is critical for synthetic lethality, but the loss of BER is not.

Poly (ADP-ribose) polymerases function in other aspects of DNA repair, and emerging data suggest other mechanisms of action for the anti-tumor activity of PARP inhibitors in HR-deficient cells (13, 14). One potential mechanism proposes that PARP inhibition activates NHEJ in HR-deficient cells, leading to genomic instability and cell death (12). *In vitro* studies have demonstrated that PARPs can regulate components of the NHEJ machinery, including DNA-dependent protein kinase (DNA-PK), Ku70, and Ku80 (15–18). In HR-deficient cells, PARP inhibitor treatment induced the activation of DNA-PK and phosphorylation of downstream substrates and increased NHEJ of a reporter plasmid containing a DSB (12). Pharmacological blockade or loss of NHEJ proteins reduced chromosomal aberrations and the cytotoxic effects of PARP inhibition, indicating a role for NHEJ in PARP inhibitor activity.

In vitro studies have demonstrated that the activity of PARP inhibitors may also involve formation of deleterious PARP–DNA complexes, which hinder DNA replication and repair (19–21). Avian cells lacking PARP1 and PARP2 were resistant to olaparib treatment and remained viable at concentrations greater than 10 μM (19). In contrast, olaparib caused significant cytotoxicity in wild type cells and increased levels of γ-H2AX, a marker of DNA damage. PAR polymers were undetectable by ELISA in both olaparib-treated wild type cells and PARP-deficient cells, suggesting that PARP inhibition is distinct from genetic deletion of PARP.

A comparison of PARP inhibitors demonstrated comparable inhibition of PAR synthesis by Western blot and ELISA (19, 20). In contrast, each PARP inhibitor showed varying ability to induce PARP–DNA complexes in the presence of alkylating agent. In the absence of PARP inhibitor, PARP1 was detected in the nuclear soluble fraction by Western blot and accumulated in the chromatin-bound fraction following PARP inhibitor treatment. In tumor cells, BMN 673 (talazoparib) induced greater accumulation of PARP1 and PARP2 in the chromatin-bound fraction compared to olaparib and rucaparib. Niraparib induced greater PARP–DNA binding than olaparib, and veliparib was the least effective enhancer of PARP–DNA binding at concentrations that maximally inhibited PARP enzymatic activity. PARP–DNA binding was detected at pharmacologically relevant concentrations and correlated with the cytotoxicity of each agent *in vitro*. *In vivo*, enhanced PARP–DNA binding did not correlate with better anti-tumor activity but resulted in increased toxicity (22). The significance of differential PARP–DNA binding on efficacy and tolerability requires further investigation in the context of different tumor types and different PARP inhibitor and chemotherapy regimens. The complex role of PARPs in cellular homeostasis, including DNA repair, highlights the need to evaluate PARP inhibitors for modulating other biological functions of PARPs.

FUTURE CHALLENGES OF PARP INHIBITOR DEVELOPMENT

Clinical evaluation of the pharmacodynamic (PD) activity of PARP inhibitors has focused primarily on measuring inhibition of *ex vivo* enzymatic activity or PAR incorporation in tumor tissues and peripheral blood mononuclear cells (PBMCs). In a Phase 0 clinical trial, the National Cancer Institute and Abbott Laboratories validated a sandwich immunoassay to evaluate the PD response of veliparib during clinical development (23–25). The immunoassay measured changes in PARylated substrates collected from peripheral blood and tumor biopsy samples. While PD evaluations have demonstrated target engagement by veliparib and other PARP inhibitors, it is currently unclear what level of PARP inhibition is required to translate into a clinical response. In the case of olaparib, patients with BRCA-deficient ovarian or breast cancer demonstrated maximal PARP inhibition in PBMCs at doses greater than 60 mg BID olaparib capsules; however, dose-dependent anti-tumor activity was observed at higher doses of 100 and 400 mg BID olaparib capsules (26–28).

Several factors may contribute to the lack of a clear relationship between PARP inhibition and clinical activity. Exploratory analysis of olaparib pharmacokinetic (PK)/PD data suggested that sustaining unbound steady-state trough concentrations above the IC_{90} for PARP inhibition affords better clinical efficacy.[1] These results correlated with *in vivo* PK/PD modeling of mouse tumor xenograft data that demonstrated a marked increase in DNA SSBs when PAR levels were decreased by more than 90%, and exceeding this threshold improved the anti-tumor activity of olaparib in BRCA-deficient tumors. A simulation of unbound steady-state trough concentrations in patients receiving 100, 200, and 400 mg BID olaparib capsules indicated that patients receiving 400 mg BID achieved steady-state trough concentrations exceeding the IC_{90} value for PARP inhibition. Other potential reasons for lack of a PK/PD relationship include off-target effects of PARP inhibitors or variability in PK data. Another possibility is that the cytotoxicity of PARP inhibitors may involve other mechanisms of action.

To date, investigation of the mechanisms of resistance to PARP inhibitor anti-tumor effects has been limited (29, 30). Potential mechanisms of resistance to PARP inhibitors may involve restoration of HR or modulation of PARP itself. One potential mechanism was demonstrated in the Capan-1 human metastatic pancreatic adenocarcinoma cell line, which lacks a wild type copy of *BRCA* while harboring a 6174delT mutant *BRCA* allele. This mutation causes a frameshift in the normal open reading frame (ORF), resulting in expression of truncated BRCA protein and a deficiency in HR (31, 32). Analysis of Capan-1 clones resistant to PARP inhibitors showed that additional mutations (i.e., deletion, insertion, or deletion/insertion) within *BRCA* in these cells rectified the 6174delT frameshift mutation and restored *BRCA2* normal ORF and BRCA function. Additional evidence that at least a partial restoration of HR can lead to resistance to PARP inhibitors include secondary mutations in the *BRCA* gene, restoring expression of wild type BRCA protein in patients (33) and somatic mutation of *TP53BP1* (34, 35).

In addition to restoration of HR, studies have also correlated resistance to PARP inhibitors with PARP itself and PD markers such as γ–H2AX (36, 37). In an *in vivo* study, responsiveness of mice bearing TC-71 Ewing sarcoma tumors to a combination of talazoparib and temozolamide was correlated with decreased levels of total or cleaved PARP and increases in γ–H2AX; however, tumors that were resistant to the combination treatment were shown to have some cleaved PARP but no decrease in total or cleaved PARP, or increases in γ–H2AX (38). Although the status of the genes involved in HR was not evaluated in tumors tested in this study, these results suggest that another potential mechanism of resistance to anti-tumor effects of PARP inhibitors may involve regulation of PARP itself.

The most concerning potential adverse reactions associated with PARP inhibition are myelodysplastic syndrome and acute myeloid leukemia (MDS/AML), especially in patients harboring a germline *BRCA* mutation. BRCA1 is critically involved with the Fanconi anemia proteins in repairing DNA damage, whereas BRCA2 is itself a Fanconi anemia protein. Biallelic mutations of *BRCA2* are linked to Fanconi's anemia, a genetic disorder characterized by congenital abnormalities and a profound increase in cancer predisposition, namely AML (39, 40). The U.S. Package Insert for Lynparza (olaparib) contains the following warning for the development of MDS/AML: MDS/AML have been confirmed in 6 out of 298 (2%) patients enrolled in a single arm trial of Lynparza monotherapy, in patients with deleterious or suspected deleterious germline *BRCA*-mutated advanced cancers[2]. In a randomized placebo controlled trial, MDS/AML occurred in 3 out of 136 (2%) patients with advanced ovarian cancer treated with Lynparza. Overall, MDS/AML were reported in 22 of 2,618 (<1%) patients treated with Lynparza. The majority of MDS/AML cases (17 of 22 cases) were fatal, and the duration of therapy with Lynparza in patients who developed secondary MDS/cancer-therapy related AML varied from <6 months to >2 years. All patients had previous chemotherapy with platinum agents and/or other DNA damaging agents. The addition of further DNA damage induced by chemotherapy or other environmental factors, coupled with enhanced impairment of a compensatory repair pathway by means of PARP inhibition, may prime patients with germline DNA repair deficiencies for the development of MDS/AML. Monitoring of complete blood counts and perhaps PBMCs for micronuclei is warranted for patients receiving PARP inhibitors, and further investigations should be performed for prolonged hematologic toxicity (see text footnote 2).

CONCLUSION

Notwithstanding the current knowledge regarding the biological role of PARP and its demonstrated clinical benefit in cancers with germline *BRCA* mutations, future studies are needed to improve

[1]Pharmacology/Toxicology NDA review: olaparib 2014. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/206162Orig1s000PharmR.pdf

[2]LYNPARZA™(olaparib) label: Available from http://www.accessdata.fda.gov/scripts/cder/drugsatfda/

the therapeutic potential of PARP inhibitors. For example, better understanding of the contribution of the various mechanisms of action *in vivo*, in the context of different PARP inhibitors and different tumor types, together with better understanding of mechanisms of resistance will aid in improving the therapeutic potential of this class of drugs by optimizing patient selection (e.g., based on baseline or PARP inhibitor-mediated changes in HRD profile) or optimizing selection of therapeutic agents in combination clinical trials by targeting separate mechanisms of drug resistance. Additionally, studies are needed to identify predictive biomarkers and to develop validated, diagnostic tests to extend the therapeutic landscape of PARP inhibitors beyond *BRCA*-mutated tumors (41–43).

AUTHOR NOTE

This article reflects the views of the authors and should not be construed to represent FDA's views and policies.

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