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Poly(ADP-ribose) polymerase inhibitors as cancer therapy

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1. ABSTRACT

Poly(ADP-ribose) polymerase (PARP) inhibitors are pharmacologic agents which primarily inhibit the PARP-1 and PARP-2 enzymes within the cell. Inhibition of PARP results in failure of base-excision repair (BER) to correct single-stranded breaks in DNA. This failure results in double-stranded breaks that are subsequently repaired either by homologous recombination (HR) repair, which is error-free, or by non-homologous end joining (NHEJ), which is an error-prone process. Clinically, PARP inhibitors demonstrate activity in tumors which lack a functional HR system (i.e. BRCA1 and BRCA2 mutations) by forcing NHEJ repair. Known as synthetic lethality, the use of NHEJ in these tumors generates genomic instability and eventual cell death due to rapid development of non-viable genetic errors. In addition due their BER effects, PARP inhibitors are being developed as chemotherapy and radiation sensitizers in a number of tumor types. This review will examine the role of the PARP enzymes in DNA repair, PARP inhibitors in HR-deficient tumors, current results of clinical studies of PARP inhibitors and research efforts to expand the clinical activity of PARP inhibitors beyond HR-deficient tumors.

2. INTRODUCTION

The enzyme poly(ADP-ribose) polymerase (PARP) plays a critical role in the detection and repair of single-stranded breaks (SSBs) in DNA and its inhibition may represent a significant therapeutic advance in medical oncology. Although much of the focus has been on tumors with non-functional BRCA1 or BRCA2 proteins, preclinical data is suggestive that PARP inhibitors also may be active in any tumor with non-functional homologous recombination (HR) repair. In addition, due to their role in DNA repair, PARP inhibitors are also being evaluated as a potential chemotherapy/radiation therapy sensitizer. In addressing this topic, this review will primarily focus on: 1) the role of PARP in Base-excision DNA repair (BER); 2) mechanisms of compensation when PARP is inhibited; 3) the preclinical evidence supporting PARP inhibitors as therapy for human cancer; 4) clinical trials of PARP inhibitors in HR-deficient tumors and as chemotherapy/radiation sensitizers; and, 5) mechanisms of resistance and efforts to expand the clinical utility of PARP inhibitors beyond HR-deficient tumors.

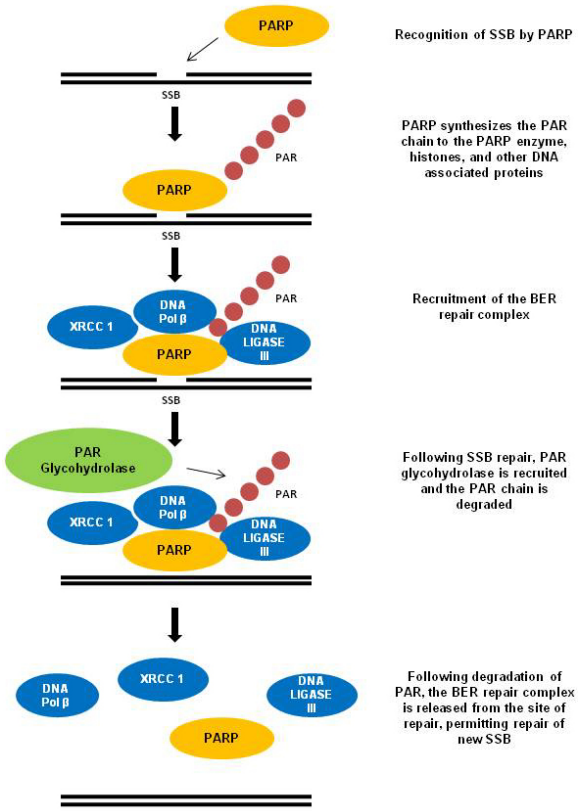

Figure 1. The role of PARP in BER repair. Detection of SSBs requires PARP activity which binds to the identified lesion. Upon binding, PARP synthesizes a PAR chain to itself and components of the chromatin. These chains recruit the other components of the BER system and repair of the lesion is completed. Upon completing repair, PAR glucohydrolase degrades the synthesized PAR chain, allowing the BER repair complex to disengage the site of repair and be available to repair the next site of damage.

3. BASE-EXCISION REPAIR, HOMOLOGOUS RECOMBINATION REPAIR AND THE EFFECT OF PARP INHIBITION

3.1. Overview of Base-Excision and Homologous recombination repair

In all human cells, DNA is subjected to frequent damage secondary to environmental insults, toxic metabolites and DNA replication errors. Single-stranded breaks (SSBs) are one of the most frequent mechanisms of damage and can occur at a rate as high as 10 000 per day (1). A SSB is defined as a loss on continuity in the deoxyribose sugar backbone in one strand of the DNA double helix and can be potentially accompanied by the loss of the nucleotide base at the site of the break (2). The most common causes of SSB are: 1) reactive oxygen species generated by cellular metabolism (2); 2) failure of DNA base-excision repair (BER) to completely repair damaged or absent nucleotide bases (3); and, 3) failure of DNA topoisomerase I to resolve cleavage complexes generated during gene transcription and DNA replication, leaving a break in the deoxyribose sugar backbone that was initially generated by the creation of the complex (4).

Detection of SSBs, and subsequent activation of BER, is thought to require the activity of PARP enzymes. Of the 17 known family members, PARP-1 has been the most extensively evaluated and appears to play the most important role. Figure 1 provides an overview of the role that PARP is presumed to play in BER. Found in the nucleus, PARP-1 recognizes SSBs in DNA via two zinc-fingers which are structurally homologous to the DNA binding sites in DNA ligase III and 3' DNA phosphoesterase, two other DNA repair enzymes (5). Upon binding to a SSB, PARP-1 undergoes a conformation shift, activating its catalytic capabilities, leading to the synthesis of a poly (ADP-ribose) (PAR) polymer to itself, histones and other nuclear proteins using nicotinamide adenine dinucleotide as the substrate. The PAR polymer then serves as a signal to recruit other key enzymes in the BER repair process, such as DNA ligase III, DNA polymerase beta (Pol-beta) and X-ray cross-complementing gene 1 (XRCC1) (6). In particular, recruitment of XRCC1 appears to be critical as it acts as the primary scaffold protein upon which the BER complex is assembled (7). In addition, the attachment of PAR to histones H1 and H2B relaxes the chromatin structure, facilitating the repair process (8). PARP-2 has been shown to interact with PARP-1 and independently contribute to the BER complex recruitment process (9). Once the PAR polymer has been synthesized and repair completed, PARP-1 dissociates from DNA and the PAR chains are degraded by poly(ADP-ribose) glycohydrolase (PARG), resetting PARP-1 to its inactive conformation and restoring the chromatin structure (10).

Failure to repair SSBs results in DNA double-stranded breaks (DSBs) as replication forks encountering SSBs either stall or collapse (11). The two major DSB repair pathways within the cell are HR and non-homologous end-joining (NHEJ). HR represents a largely error-free mechanism to repair DSBs as homologous duplex DNA is used as a template for repair DNA synthesis; in contrast, NHEJ promotes the direct ligation of DSB ends, potentially resulting in insertions, deletions, base-substitutions and translocations if different components of the genome are brought together (12). Because NHEJ is significantly error-prone, DSBs represent a significant risk to overall genomic integrity and a significant threat to cellular viability (13).

A simplified schematic of DSB repair is presented in Figure 2. NHEJ is active throughout the cell cycle and is facilitated by 53BP1. 53BP1 interferes with end-resection activity of MRE11/RAD50/NBS1 (MRN) complex, directing the cell towards NHEJ repair of a DSB (12). In contrast, HR activity needs to be restricted to the S/G2 phase of the cell cycle as an intact sister chromatid is required to serve as a template for repair. In human cells, restriction of HR to S/G2 occurs through three primary mechanisms. First, BRCA1 expression is tightly regulated and begins to increase in late G1 and S phase with peak levels detected during G2 (14). Second, protein levels of

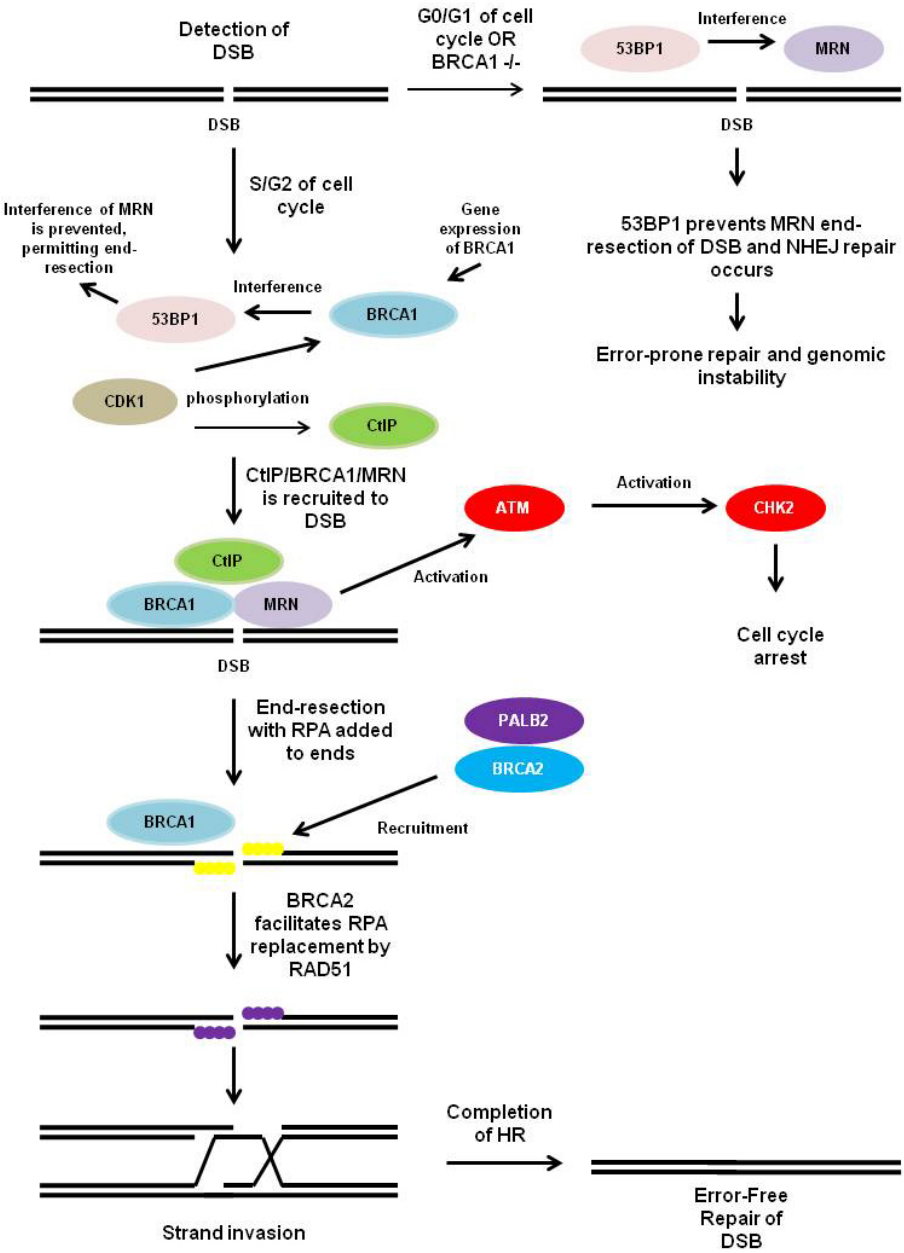

Figure 2. Simplified schematic of DSB repair. This figure represents a simplified schematic of HR repair. For cells in G0/G1 or BRCA1-/-, the absence of BRCA1 protein results in unfettered 53BP1 activity. 53BP1 is then capable of interfering with the MRN complex, preventing end-resection and promoting NHEJ repair of the DSB. For cells in S/G2, CDK1 activity is increased and BRCA1 protein is now expressed. CDK1 phosphorylates both BRCA1 and CtIP, activating both proteins. BRCA1 then interferes with 53BP1 activity, allowing the MRN complex to commence with end-section at the DSB with the newly generated ssDNA protected from forming secondary structures by RPA. ATM is activated by the MRN/CtIP/BRCA1 complex, leading to cell cycle arrest. BRCA1 then facilitates the recruitment of BRCA2 and PALB2 to the repair site, with BRCA2 then facilitating RAD51 loading onto DNA. Strand invasion then occurs, with the sister chromatid serving as the template for repair. Following completion of repair, the HR complex disassembles, resulting in error-free repair of the DSB.

CtIP, an important activator of HR, remain low due to proteasome-mediated degradation until S/G2; at this point in time, this degradation becomes inhibited, allowing for CtIP accumulation within the nucleus (15). Third, cyclin-dependent kinases (CDK) 1 and 2, which are most active during S/G2, phosphorylates CtIP and BRCA1, resulting in their activation (15-17). Activation of BRCA1 prevents 53BP1 from interfering with MRN, permitting end-resection and creating the single-stranded sequences (ssDNA) at the DSB necessary to initiate HR repair (18).

Replication Protein A (RPA) is then added to the ssDNA to prevent the formation of secondary structures (19). Following end-resection, BRCA2 facilitates RAD51 loading which leads to strand-invasion and DNA repair in an error-free process (12, 20-23).

Cell cycle arrest is another crucial aspect of the HR repair process. Without arrest, cell cycle progression will lead to mitosis and loss of the sister chromatids necessary for HR repair. The Ataxia Telangiectasia-Mutated (ATM) protein, in conjunction with MRN complex, is recruited and activated in response to DSBs (24). Activated ATM interacts and phosphorylates multiple proteins involved in initiating repair and checkpoint arrest, including Checkpoint kinase 2 (Chk2), NBS1, BRCA1 and MDC1 (25). Activation of these proteins leads to cell cycle arrest through actions on p53, Cdc25, BRCA1, FOXM1 and E2F1 (26). Loss of the ATM-Chk2 pathway has been shown to lead to genomic instability within cells as loss of cell cycle arrest limits HR repair of DNA damage, leading to accumulation of mutations and genomic instability as NHEJ assumes responsibility for repair.

3.2. The effect of PARP inhibition on DNA repair

As cells which have PARP-1 inactivated show an increase in RAD51 foci, it is thought that HR serves as the primary repair mechanism in situations where PARP-1 is inactivated (27). Because of this redundancy, loss of PARP-1 activity alone does not appear to represent a lethal event; for example, PARP-1 deficient mice embryos are viable and survive gestation despite increased sensitivity to alkylating agents and radiation (28-30). In contrast, in cells with non-functional HR-repair, loss of PARP function represents a lethal event as this redundancy has been lost. This concept was clearly demonstrated in two separate reports exploring the effects of PARP inhibition in cells with inactive HR due to loss of key components of the HR complex. Bryant and colleagues demonstrated that cells deficient in HR due to loss of XRCC2 and XRCC3, two proteins associated with the RAD51 complex, showed decreasing viability when exposed to increasing concentrations of the PARP inhibitors NU1025 and AG14361; this sensitivity was reversed when XRCC2 and XRCC3 activity was restored, confirming their hypothesis that HR-deficient cells are unable to compensate for BER loss. Bryant and colleagues then demonstrated *in vitro* and *in vivo* that BRCA2 deficient tumors, either by mutation or siRNA, showed similar reduced viability with loss of PARP enzymatic activity (31). Farmer and colleagues showed that BRCA1 and BRCA2-deficient cells demonstrated loss of viability when exposed to the PARP inhibitor KU0058684 in both *in vitro* and *in vivo* models. Farmer also showed that PARP inhibition in BRCA1 and BRCA2-deficient cells resulted in significant genomic instability with complex chromatid rearrangements, suggestive that HR-deficient cells respond to loss of HR activity by repairing DSBs via NHEJ. Due to the loss of genetic integrity from NHEJ, cellular viability is rapidly loss due to accumulation of genetic mutations in critical genes, leading to either G2/M cell cycle arrest or apoptosis (32). PARP inhibition in HR-deficient cells is representative of a concept known as synthetic lethality, which is defined as the situation where the combination of loss of activity in two different genes results in cell death while the loss of either activity does not affect the viability of the cell (Figure 3) (33).

Although it is thought that PARP inhibitors act by preventing the initiation of BER at SSBs, this is not universally agreed upon as some data suggests that PARP-1 activity may not essential for BER repair to take place. First, mouse knockouts of BER repair proteins APE1, Pol-beta, and XRCC1 are not viable while PARP-1 knockouts survive, suggesting that BER is still functional despite the loss of PARP-1 (34). Second, the alkylating agent dimethylsulfate resulted in accumulation of SSBs in cells exposed to PARP inhibitors but not in cells exposed to PARP-1 siRNA; this is unexpected as PARP inhibitors and loss of PARP-1 through siRNA should result in similar, not different, phenotypes (35). Third, the steady state levels of SSBs do not appear to increase in either wild type or BRCA2 defective cells exposed to PARP inhibitors (36). Overall, these observations are suggesting that PARP-1 plays a non-essential role in BER. One caveat is that PARP inhibitors inhibit both PARP-1 and PARP-2 while PARP-1 siRNA does not. Since PARP-1 and PARP-2 knockout mice are not viable (37), it is possible that the failure for PARP-1 siRNA to produce the same effects as a PARP inhibitor may be secondary to PARP-2 compensation.

Based on the above observations, alternative mechanisms of action for PARP inhibitors have been proposed. These proposals, as well as the evidence supporting them, are discussed in detail by Helleday and the reader is referred to his manuscript if greater detail on this topic is desired (34). These alternative models for the mechanism of action for PARP inhibitors are illustrated in Figure 4. First, it has been observed that overall BER kinetics can be significantly reduced in the presence of activated PARP-1 enzyme (38); therefore, one model is that PARP inhibitors work by preventing the release of PARP-1 from the SSB intermediate, trapping the complex at the site of repair. Failure to release PARP-1 from the SSB repair intermediate physically prevents resolution of the lesion, resulting in replication fork arrest which can only be repaired by HR mechanisms (39). A second possibility is that stalled replication forks can be repaired by either DSB-repair HR or a PARP-1-dependent HR-repair mechanism. It has been observed that PARP depletion results in reduced recruitment of MRN, RPA and RAD51 to collapsed replication forks but not to HR-mediated DSB repair (18); this data suggests that stalled replication forks can either be repaired by a PARP-dependent HR process or the DSB-mediated HR repair process. In cells which are HR-deficient, inhibition of the PARP activity prevents PARP-mediated restart of stalled replication forks, resulting in replication failure and synthetic lethality (34).

4. PARP INHIBITORS AS A THERAPY FOR HUMAN CANCER

Following the reports demonstrating that BRCA-deficient cells have increased sensitivity to PARP inhibitors, there has been a great deal of interest in testing

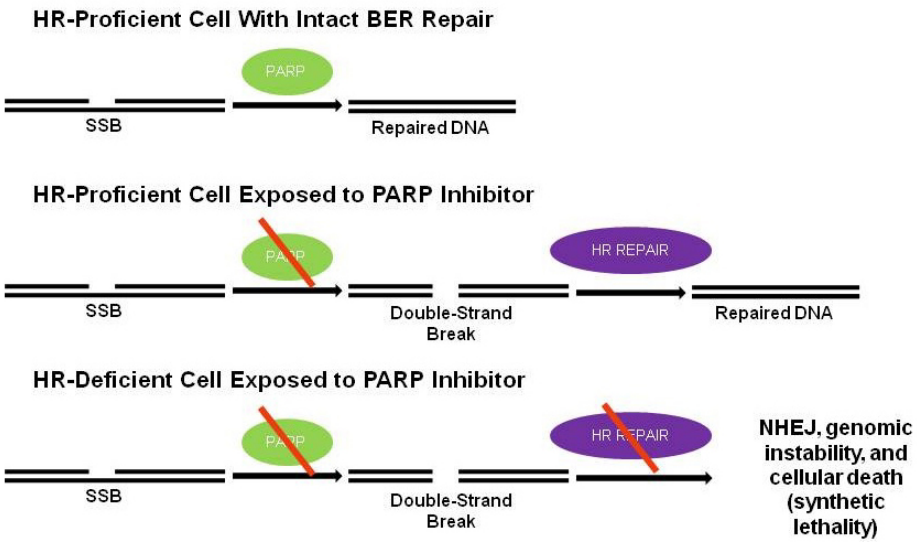

Figure 3. PARP inhibitors induce synthetic lethality in HR-deficient tumors. In cells where HR is non-functional due to loss of one or more of the proteins that make up, activate or facilitate the HR repair complex (such as BRCA1, BRCA2, PTEN, ATM, and CHK2), HR is incapable of compensating for loss of BER activity. As a result, cells are forced to repair the resulting DSBs caused by loss of BER activity through NHEJ, which is a highly error-prone process. Repeated activation of NHEJ causes the genome of the cell to become highly unstable, eventually resulting in cell death due to the accumulation of non-viable genetic errors. Loss of BER and HR is a situation known as synthetic lethality, which is defined as the situation where the loss of activity in two different genes results in cell death.

PARP inhibitors in patients with known BRCA-deficient cancers. In this population, PARP inhibitors represent an opportunity to directly target tumor cells with therapy while sparing normal tissues, thus avoiding the systemic toxicities observed with standard chemotherapy and radiation therapy. Although many efforts have been made to specifically test these agents in this cohort of patients, theoretically, any tumor with HR-deficiency, such as those with defects in RAD51, RAD54, DSS1, RPA1, NBS1, FANCD2, FANCA, or FANCC, should demonstrate increased sensitivity to PARP inhibition (40). Loss of cell cycle checkpoint control may also be predictive of sensitivity to PARP inhibition. Experiments in cells with disrupted ATM activity show sensitivity to PARP inhibition (40-43). In addition, mantle cell lymphoma cell lines deficient in both ATM and p53, which are involved in checkpoint control, showed greater sensitivity to the PARP inhibitor olaparib than cell lines which are deficient in ATM activity alone, highlighting the importance of initiating checkpoint arrest in order to facilitate HR repair (44). PTEN knockout cells also have increased chromosomal instability due to roles in controlling the expression of RAD51 and as well as cell cycle checkpoint function (45, 46). In both *in vitro* and *in vivo* preclinical models, PTEN-deficient tumors were found be more sensitive to PARP inhibitor exposure compared to PTEN-functional tumors (47). Given the potential for a number of tumors to be sensitive to PARP inhibitors beyond those with BRCA1/2 germline mutations, identifying sporadic tumors with HR-defects has increased in importance; this phenotype referred to as "BRCAness" in the literature (48). Konstantinopoulos and colleagues have reported on preliminary efforts to design a gene expression profile to identify HR-deficient tumors (49). Further efforts like this will be critical for the full clinical potential of PARP inhibitors to be realized.

Given that PARP-deficient tumors have demonstrated increased sensitivity to chemotherapy agents and radiation, PARP inhibitors are also being evaluated as potential chemotherapy and radiation sensitizers. Preclinical models have shown that PARP inhibitors increase the cytotoxic effects of alkylating agents, topoisomerase inhibitors, platinum agents and γ-radiation in a number of tumor types (50-53). Based on these results, a number of clinical trials have been initiated assessing the safety and activity PARP inhibitors in combination with chemotherapy or radiation independent of any "BRCAness" phenotype.

4.1. PARP inhibitors currently in clinical development

A number of pharmaceutical companies have designed PARP inhibitors; however, despite the high interest in this class of compounds, development of a number of them has stalled. Currently, the three main PARP inhibitors under active study are rucaparib, olaparib, and veliparib. These three compounds will be the focus for the remaining discussion. This section will review the preclinical evaluations and single agent dose escalation results for each of these compounds. Subsequent sections will discuss these agents in: 1) breast and ovarian cancer patients with germline BRCA1/2 mutations; and 2) in combination with other treatment modalities.

Rucaparib (AG-014699) is a potent inhibitor (K_i=1.4 nM) of PARP-1 and PARP-2 with preclinical

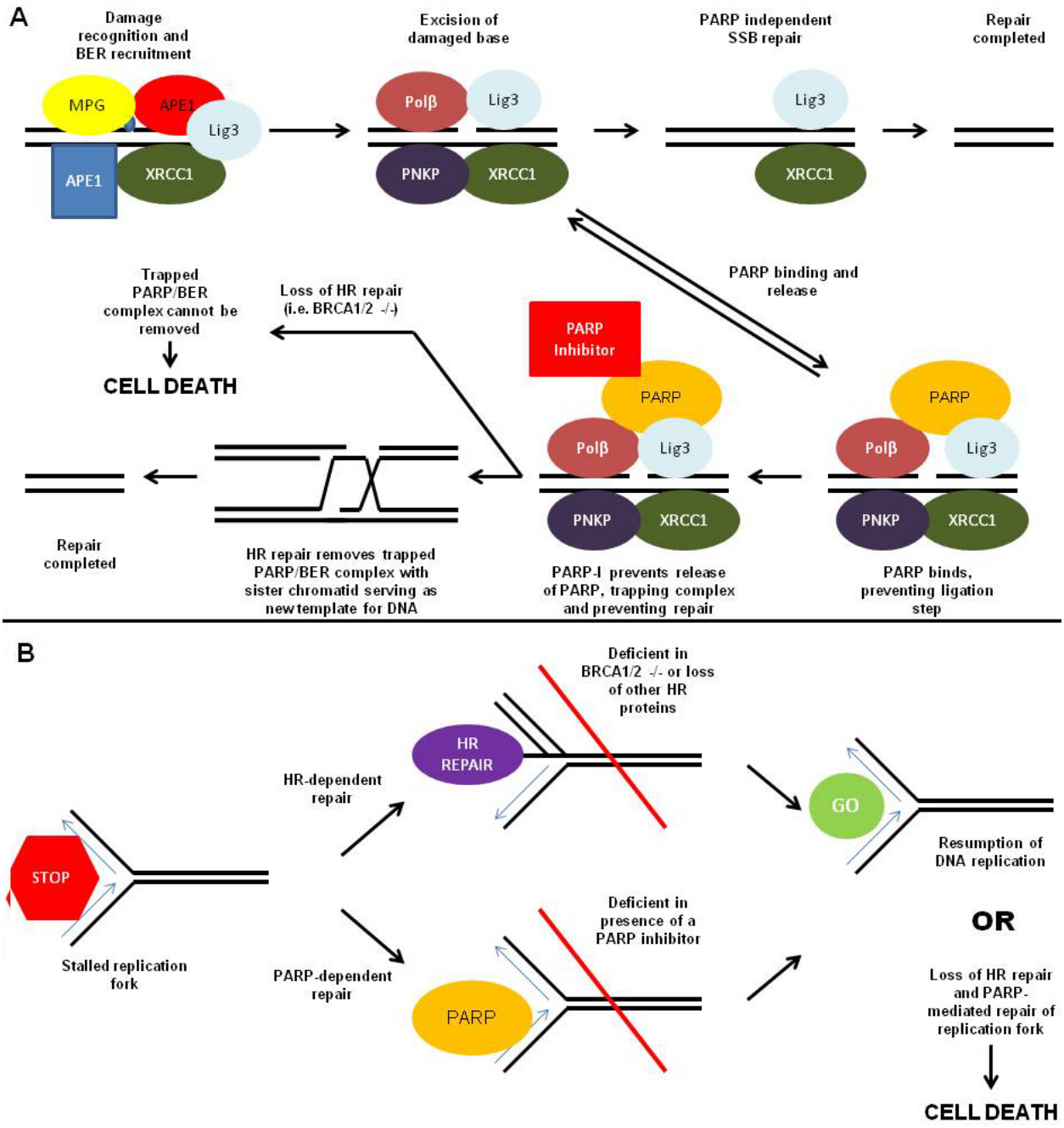

Figure 4. Potential alternative mechanisms of action for PARP inhibitors. This figure presents two alternative models for PARP inhibitor activity. A) PARP inhibitors trap the BER complex at the site of DNA damage. In this model, BER can complete repair without interaction with the PARP enzyme. In cases where PARP interacts with the BER complex, PAR synthesis is required in order to complete the repair process. In the presence of a PARP inhibitor, PAR chains cannot be synthesized, trapping the complex at the site of repair. This trapped complex can only be removed by HR. In HR-deficient cells, the HR complex cannot be removed, resulting in cell death. B) Stalled replication forks are restarted by either DSB HR repair process or a PARP-dependent HR repair process. In the presence of a PARP inhibitor, cells become dependent on the DSB HR repair process, which cannot be activated in HR-deficient tumors, resulting in cell death.

modeling demonstrating that combining rucaparib with either the alkylating agent temozolomide or the topoisomerase inhibitor irinotecan improved overall clinical efficacy of the cytotoxic agents (54). As the first PARP inhibitor to be evaluated in human studies, rucaparib was initially only formulated for intravenous use and was

tested in combination with temozolomide. Single-agent testing of the agent occurred only after the combination of it with temozolomide proved too toxic. As a single agent administered to 38 evaluable BRCA1/2 deficient breast and ovarian cancer patients, a 5% partial response rate (PR) was observed with 26% of participants achieving SD ≥ 4 months with no significant toxicities reported (55). In order to facilitate continuous dosing schedules, rucaparib recently has been converted to an oral formulation and is being reevaluated in phase I dose escalation (NCT01482715). This trial is currently ongoing. Clinical trials of rucaparib in combination with temozolomide are discussed in the section below.

Olaparib (AZD-2281) is a potent, orally administered PARP inhibitor (K_i=5 nM and 1 nM for PARP-1 and 2 respectively) with preclinical evidence of *in vitro* and *in vivo* activity in HR-deficient cell lines and in combination with alkylating agents (56). In phase I dose escalation, the maximally tolerated dose was 400 mg twice a day, with responses only seen in BRCA1/2 deficient tumors. In a cohort separate from the dose escalation, 19 BRCA deficient tumors were evaluated; in this cohort, 47% obtained a PR and 63% experienced clinical benefit (defined as either PR or SD ≥ 4 months), suggesting potent single-agent activity in this population (57).

Veliparib (ABT-888) is an orally administered PARP inhibitor with highly potent inhibitory activity against both PARP-1 and PARP-2 (K_i=5.2 nM and 2.9 nM for PARP-1 and 2 respectively). Preclinical models have suggested that veliparib increased the activity of temozolomide, cisplatin, carboplatin and cyclophosphamide. In addition, veliparib appeared to increase the cytotoxic efficacy of radiation therapy (58, 59). In phase 0 testing, veliparib was well tolerated with the dose of 50 mg twice a day; in tumor biopsies, PARP activity was inhibited by 95% at this dose (60).

Initially thought to be a PARP inhibitor, early phase clinical trials of iniparib (BSI-201) in estrogen receptor, progesterone receptor and HER2 negative breast cancers, known as the triple-negative phenotype (TNBC), suggested that this agent was beneficial when administered in combination with carboplatin and gemcitabine (61, 62). Based on these results, a randomized phase III trial was initiated; however, the trial did not meet the pre-specified criteria for significance in terms of progression free survival (PFS) and overall survival (OS). Further work into the mechanisms of iniparib has indicated that the drug is not a functional PARP inhibitor but instead works synergistically with chemotherapy to increase DNA damage (63). For this reason, iniparib will not be considered further in this review.

4.2. PARP inhibitors for the treatment of HR-deficient breast and ovarian cancer

Based on the preclinical observations by Bryant and Farmer demonstrating that BRCA1 or BRCA2 deficient cell lines were sensitive to PARP inhibition (31, 32), there has been a great deal of interest in testing PARP inhibitors in these populations of patients. Following the impressive phase I results showing single agent activity of olaparib in BRCA1/2 deficient tumors, a phase II study was initiated evaluating olaparib in patients with advanced ovarian cancer with known or suspected BRCA1 or BRCA2 mutations. Overall, 40% of these participants obtained at least a PR and 46% in total obtained clinical benefit (defined as either response or SD ≥ 4 months). In addition, olaparib appeared to offer more clinical benefit in patients defined as platinum sensitive compared to those who were platinum resistant or refractory (69% vs. 45% vs. 23% respectively) (64). This has lead to the hypothesis that platinum sensitivity in ovarian cancer may be mechanistically related in some way to loss of HR within the tumor cell and that platinum sensitivity may be a predictive factor for clinical benefit from PARP inhibitors.

Olaparib was then definitively evaluated as a single agent in BRCA1 and BRCA2 deficient breast and ovarian cancer, with participants requiring a confirmed, rather than suspected, germline mutation in order to participate. In the breast cancer study, patients with confirmed germline BRCA1 or BRCA2 mutations were enrolled to either the RP2D of olaparib (400 mg twice daily) or a lower dose with evidence of PARP inhibitory activity (100 mg twice daily). This trial suggested that single agent olaparib in active in this breast cancer population, with an overall response rate (ORR) of 41% in those 400 mg twice a day cohort. Clinical activity, although less robust, was also observed in the lower dose cohort (ORR=11%) (65). Using an identical design to the breast cancer study, evaluation of olaparib in BRCA1 or BRCA2 deficient ovarian cancer showed that 400 mg twice a day cohort appeared to have superior clinical activity compared to 100 mg twice a day, with an ORR of 33% compared to 13% respectively (66). In both studies, although the higher dose cohort had a higher rate of grade 3 or greater toxicities compared to the lower dose cohort, overall toxicities were manageable; therefore, the data strongly suggests that, given the apparent therapeutic advantage of 400 mg twice a day, this dose should be used in future trials.

Given that TNBC and high grade ovarian cancer share a number of pathologic features to breast and ovarian tumors with confirmed germline BRCA1 and BRCA2 mutations, it has been hypothesized that these sporadic cancers may have acquired deficiencies in HR repair and may also be sensitive to single agent PARP inhibitors (67). To evaluate this hypothesis, Gelmon and colleagues enrolled patients with TNBC or high grade serous ovarian cancer to receive olaparib 400 mg twice a day. Following enrollment, patients were then stratified based on the presence or absence of BRCA1/2 mutations. In the study, a total of 91 patients were enrolled (65 ovarian, 26 breast). In patients with confirmed BRCA1/2 mutations, an ORR of 41% was observed. Surprisingly, an ORR of 24% was observed in patients without BRCA1/2 mutations. This finding is the first result in clinical trials demonstrating single agent PARP activity in non-BRCA mutation carriers and offers the first evidence that a "BRCAness" phenotype may be relevant clinically (68). Gelmon and colleagues did not identify any responses in the breast cancer patients

enrolled, regardless of mutation status. Given that there was a total of 26 patients TNBC patients enrolled with only 10 possessing confirmed BRCA1/2 mutations, this unexpected observation is more likely due a combination of low enrollment numbers and heavy pretreatment (3 median prior lines of treatment) than PARP inhibitor inactivity in BRCA1/2 mutated breast cancer.

As normal tissues are spared the toxicity of PARP inhibition due to functional HR activity, PARP inhibitors may represent a better tolerated and efficacious treatment that standard chemotherapy options in patients with germline BRCA1/2 mutations. To assess this question, 97 patients with ovarian cancer with confirmed BRCA1/2 mutations were randomized in a 1:1:1 fashion to receive either olaparib 400 mg twice a day, olaparib 200 mg twice a day or liposomal doxorubicin at its standard dose of 50 mg/m^2 every 28 days. The overall response rate was 31%, 25% and 18% in each of the three arms respectively. Unfortunately, olaparib failed to demonstrate a progression free survival (PFS) benefit compared to liposomal doxorubicin (HR=0.88; 95% CI: 0.51-1.56; p=0.66) (69). In their editorial discussing the study, Konstantinopoulos and Cannistra commented that the failure to see a PFS benefit may have been due to selecting doxorubicin as the standard agent. As a topoisomerase II inhibitor, doxorubicin induces DSBs in DNA; theoretically, these types of agents are potentially more efficacious in the BRCA1/2 population than what might be predicted in a non-selected ovarian cancer population (70). A retrospective review of doxorubicin in the BRCA1/2 population offers further support for this hypothesis (71). This study illustrates some of the challenges that investigators face in designing future trials evaluating PARP inhibitors. First, because of the biology and chemotherapy responsiveness of BRCA1/2-deficient tumors, particularly early on in treatment, it will be challenging to demonstrate a PFS benefit for PARP inhibitors compared to standard chemotherapy agents. One could address this issue by choosing an equivalency rather than a superiority endpoint; however, one drawback to this approach is that equivalency trials require a much larger number of candidates compared to superiority trials to reach the necessary statistical power, resulting in a higher cost and length of time to obtain a result. Second, because of the large number of identified standard agents for both breast and ovarian cancer, identifying an OS benefit will be difficult, if even possible, and will take a long follow up period to detect.

Due to their toxicity profiles, PARP inhibitors may represent a potential maintenance therapy for BRCA1/2-deficient ovarian cancer. In addition, given that high grade serous ovarian cancers may also have a "BRCAness" phenotype due to sporadic loss of HR repair (67, 68), it is possible that these agents may be effective in an unselected ovarian cancer population. To asses this hypothesis, 265 patients with high grade serous ovarian cancer were randomized to receive either olaparib 400 mg twice a day or placebo. To enroll in the study, patients were required to have platinum sensitive disease as previous work had correlated platinum sensitivity with increased PARP inhibitor efficacy (64). Although patients were stratified based on ethnicity as some populations are more frequent carriers of germline BRCA1/2 mutations, the study did not specifically stratify based on BRCA mutation status. This study demonstrated that although olaparib improved progression-free survival (8.4 months vs. 4.8 months; HR=0.35; 95% CI: 0.25-0.49; p=<0.001), at the time of publication, no overall survival benefit was identified (HR=0.94; 95% CI: 0.63-1.39; p=0.75). A subgroup analysis performed by the study team suggests that although patients with germline BRCA1/2 mutations received more benefit from olaparib maintenance compared to patients without mutations, benefit was observed regardless of mutation status (72). Given that patient with ovarian cancer patients have a number of treatment options available, it is not surprising that no overall survival benefit has been observed at this point in time. Maintenance olaparib in combination with carboplatin is currently being evaluated; reported preliminary results indicated that the combination of olaparib and carboplatin resulted in an improved PFS compared to carboplatin alone (12.2 months vs. 9.6 months; HR=0.51; 95% CI: 0.34-0.77; p=0.0012) (73).

4.3. PARP inhibitors in combination with chemotherapy or radiotherapy

Because of preclinical findings showing activity in combination with a large number of chemotherapies, veliparib has been the most extensively evaluated in combination with either chemotherapy or radiation. Veliparib and temozolomide has been investigated in metastatic breast cancer, melanoma and colorectal cancer. Although preclinical modeling suggested that HR-deficiency was not necessary to obtain benefit from the combination, in the breast cancer study, the activity of the combination was limited to patients with confirmed BRCA1 or BRCA2 mutations (74). The combination of veliparib and temozolomide in melanoma was disappointing; after randomizing participants to either veliparib with temozolomide compared to temozolomide plus placebo, no overall survival benefit was observed (75). In patients with advanced colorectal cancer who were heavily pretreated, the combination of temozolomide plus veliparib resulted in 2 PRs, but the median time to progression was short (11 weeks) (76). The combination of veliparib plus irinotecan was also evaluated in 32 patients with advanced solid tumors with unknown BRCA mutation status. At the RP2D, PARP activity was reduced in tumor specimens and the clinical benefit rate was 61% with 5 PRs identified (77). The combination of veliparib, doxorubicin and cyclophosphamide has also been tested. Pharmacodynamic analysis has confirmed that veliparib inhibits PARP activity in peripheral blood mononuclear cells at the RP2D. Reported clinical activity at this time has only seen in BRCA mutation carriers and the trial continues to enroll breast cancer patients to an expansion cohort (78). Multiple trials of veliparib in combination with either chemotherapy or radiation remain open to accrual. These trials are being conducted in a number of different tumor types, including prostate, hepatocellular, pancreatic, cervical, lymphoma and myeloma.

Due to preclinical work showing increased efficacy, rucaparib was first evaluated in combination with temozolomide. In a total of 32 patients evaluated, the combination of rucaparib and temolomide demonstrated preliminary clinical activity, with 3 responses (1 complete response (CR)) and 7 patients achieving stable disease (SD) ≥ 6 months. In addition, tumor biopsies confirmed that rucaparib inhibited PARP enzymatic activity in the tumor (79). Based on the phase I result, the combination was evaluated in phase II for patients with metastatic melanoma; although clinical activity was observed, significant toxicity, including death and hospitalizations from myelosuppression, preventing further clinical development (80).

Olaparib has been tested in combination with paclitaxel, cisplatin and gemcitabine and carboplatin in BRCA1/2 mutation carriers. Similar to the rucaparib experience, these combinations have resulted in significant myelosuppression, limiting the dose of olaparib that can be administered (81-83). Further clinical trials of olaparib in combination with other chemotherapy agents as well as radiation therapy are ongoing.

At the current time, it is difficult to draw conclusions regarding the future of PARP inhibitors in combination with other anti-cancer modalities. Of the studies that have reported, it does appear difficult to administer PARP inhibitors with chemotherapies at single-agent dose levels since toxicity rates, particularly myelosuppression, unacceptably increase. In addition, the question of whether the combination offers any distinct treatment advantage over chemotherapy alone in tumors which have functional HR has not been directly answered. Hopefully, with many of the active studies reporting results over the next few years, it will be possible to address these issues and determine the role of PARP combinations, if any, for the treatment of human cancer.

5. RESISTANCE TO PARP INHIBITORS

As with all anti-cancer therapies, eventual resistance to PARP inhibitors occurs and a search for mechanisms of resistance is ongoing. One confirmed resistance mechanism is restored BRCA1/2 function through the acquisition of reversion mutations. Edwards (84), Sakai (85) and Swisher (86) identified reversion mutations as an explanation for PARP inhibitor resistance. Subsequent work by Norquist and colleagues evaluating germline BRCA1/2 ovarian tumors after acquiring platinum resistance suggests that reversion mutations may happen relatively frequently and may be the cause of this phenotype (87). These results also explain why Fong and colleagues observed that platinum sensitive patients experience greater clinical efficacy from PARP inhibitors compared to platinum resistant patients (64).

Another potential mechanism of resistance is tumor loss of 53BP1. In general, homozygous BRCA1 mice knockouts are not viable and those homozygous for mutations which produce BRCA protein but with significantly reduced function ($BRCA^{\Delta 11/\Delta 11}$) have premature aging and high malignancy rates. Cao and colleagues demonstrated that mice embryos homozygous for $BRCA^{\Delta 11/\Delta 11}$ mutation could be rescued from premature aging and malignancy by knocking out 53BP1 function (23). Bunting and colleagues subsequently showed that elimination of 53BP1 activity in BRCA1 deficient cells restores RAD51 foci. As BRCA1 prevents 53BP1 from interfering with MRN-mediated end-resection in HR-proficient cells, it is likely that by removing 53BP1 function in BRCA1 deficient cells MRN-mediated end-resection is no longer prevented, facilitating HR repair (22). In addition, Bouwman and colleagues demonstrated that loss of 53BP1 could restore the viability of embryonic stem cells following acute loss of BRCA1 function (88). These observations suggest that 53BP1 loss in tumor cells may represent a mechanism of resistance to PARP inhibitors by restoring end-resection activity in BRCA1-mutated cancers. One remaining issue is what compensation mechanisms exist to overcome the role of BRCA1 in other aspects of HR repair following 53BP1 loss; this question is under current study.

6. IS IT POSSIBLE TO INDUCE HR-DEFICIENCY IN TUMOR CELLS?

Although PARP inhibitors represent an exciting potential treatment option for HR-deficient tumors, it should be recognized that this only represents a small proportion of all cancers. Given that most evaluations of germline BRCA1 and BRCA2 mutations has occurred in patients with strong family histories, it is uncertain what the true frequency is for HR-deficiency in the general population. One study of 977 ovarian cancer patients in Ontario Canada found an overall BRCA deficiency rate of 13.2% (89). Given this, it is unlikely that PARP inhibitors will represent a broad treatment option as most tumors will have functional HR repair; therefore, any mechanism which could inhibit HR function in cancer cells represents an intriguing treatment strategy as it would allow for PARP inhibitors to be used in tumors where initially no benefit would be predicted.

Preclinical work by Johnson and colleagues has suggested that inducing HR-deficiency in cancer cells may be possible. Johnson demonstrated that cyclin-dependent kinase-1 (CDK1) phosphorylates BRCA1 at serine 1189, 1191 and 1497 and this action is necessary for BRCA1 to efficiently form repair foci at sites of DNA damage and induce the necessary checkpoint arrest (17). This observation suggests that inhibition of CDK1 could induce HR-deficiency by limiting overall BRCA1 activity. Johnson subsequently demonstrated that the administration of a pharmacologic CDK1 inhibitor could stop the formation of RAD51 foci in HR-proficient tumors *in vitro* and *in vivo*, confirming that a CDK1 inhibitor could inactivate HR repair. As expected from these findings, Johnson confirmed that CDK1-treated tumors demonstrated PARP sensitivity *in vitro* and *in vivo*. Johnson also demonstrated that non-transformed cells are not sensitized to PARP inhibitors when CDK1 is inhibited, suggesting that this combination will be tumor-specific if brought forward for clinical development (90). Based on this

preclinical data, the National Cancer Institute has sponsored a phase I clinical trial evaluating the combination of veliparib with the CDK1 inhibitor SCH727965 in patients with advanced solid tumors which are HR-proficient (NCT01434316). This trial is in dose escalation and results are not yet available.

7. CONCLUSIONS

PARP inhibitor use in BRCA-deficient populations represents a potential significant advance in cancer therapeutics and further efforts to evaluate PARP inhibitors as a single agent in any cancer with confirmed HR-deficiency are likely to demonstrate clinical benefit. Significant efforts are ongoing in order to identify these patients, with the gene array described by Konstantinopoulos and colleagues representing some of the preliminary work in this area (49). In addition, with recent preclinical work suggesting that it may be possible to render HR-proficient tumors HR-deficient through CDK1 inhibition, it is possible that the spectrum of tumors treated with PARP inhibitors may greatly increase, potentially expanding the number of people that may be helped by these agents. PARP inhibitors are still being investigated as potential chemotherapy/radiation therapy sensitizers; with toxicity becoming a significant issue, it is likely that further work in terms of dose scheduling will be required if these combinations are to be brought forward. It is likely over the next few years, the populations of patients that will benefit from PARP inhibitors will become further defined and the overall role for these agents in the treatment of cancer will become more established.

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