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# Clinical Utility of Poly (ADP-Ribose) Polymerase Inhibitors in Ovarian Cancer

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## Abstract

Inhibitors of the enzyme poly (ADP-ribose) polymerase (PARP) are the most promising class of targeted therapies in ovarian cancers in recent times. Approximately 30% to 50% of these cancers are characterized by aberrant DNA repair pathways due to mutations in tumor suppressor genes: *BRCA1* or *BRCA2*. Therapeutic approach with PARP inhibitors in *BRCA* deficient tumors exploits a synthetic lethal strategy targeting the deficiency in homologous recombination DNA repair pathway. This short review provides an overview of *BRCA*ness in maintaining genomic integrity, the role of PARP in DNA repair, utility of PARP inhibitors as monotherapy or in combination with other therapies, mechanisms of resistance to PARP inhibitors and biomarkers for clinical utility of PARP inhibitors in management of ovarian cancer.

**Keywords:** DNA repair; Homologous recombination; PARP; *BRCA1*; *BRCA2*

## Introduction

Ovarian cancer rates as the second most common gynecological cancer and is the fifth leading cause of cancer-related deaths among women, with an estimate of 22,440 new cases and 14,080 deaths in the US alone in 2017 [1]. Surgery followed by platin or taxane chemotherapy remains the standard of care for patients with stage I-IVA epithelial ovarian cancer (EOC) [2]. EOC is characterized as a heterogeneous disease with five major histological types: high-grade serous carcinoma (70%), clear cell (10%), endometrioid (10%), mucinous (3%), and low-grade serous carcinomas (7%) [3]. The disease shows poor prognosis as tumor progression or recurrence is observed in 80% of treated cases after an initial response to chemotherapy [4]. Higher incidence of chemo resistance, recurrence and mortality rate classifies ovarian cancer as a highly unmet medical need and emphasizes the call for improved treatment strategies for the management of ovarian cancer.

A high percentage (30% to 50%) of ovarian cancer patients show alterations in the homologous recombination (HR) DNA repair pathway and exhibit HR deficiency due to *BRCA* mutations [5,6]. HR pathway is largely involved in the repair of DNA lesions that stall DNA replication forks and/or cause DNA double-strand breaks (DSBs). It is considered to be a conservative form of DNA repair that restores the DNA sequence to its original form. Tumor suppressors *BRCA1* and *BRCA2* act as guardians of the genome and are crucial for the process of DNA repair by HR. Cells deficient in conventional HR rely on error prone alternative repair pathways such as non-homologous end joining (NHEJ) that are known to introduce mutations especially DNA deletions. Hence DNA repair pathways provide a rational therapeutic target for *BRCA* mutated cancers [5,6].

A poly (ADP-ribose) polymerase (PARP) catalysis the transfer of ADP- ribose to single strand -DNA and occupies a central position in controlling DNA damage and repair of both DSBs and single strand breaks (SSBs). PARP inhibitors (PARPi) as monotherapy are one of the most promising new classes of targeted agents for use in ovarian cancer and essentially work through inhibition of alternate DNA repair pathways exhibiting synthetic lethality in *BRCA* deficient tumors. Recent studies have also indicated the utility of PARP inhibitors in HR deficient non *BRCA* mutated subset of ovarian cancers further extending its utility in *BRCA* proficient tumors. The present review describes utility of PARPi in ovarian cancer treatment and management with a briefing on the mechanism of action of PARP enzyme in DNA repair, pharmacological development of PARP inhibitors based on synthetic lethal approach in *HRR* deficient cancers, molecular mechanisms of resistance to PARPi, clinical biomarkers and effectiveness of PARPi as monotherapy or combination therapy with existing treatment modalities in ovarian cancers.

## Literature Review

### Mechanisms of DNA damage repair

Cells are constantly subjected to DNA damaging insults that result from exogenous agents such as exposure to ionizing radiation (IR), UV radiations and mechanical stress or endogenous events such as free radicals generated during metabolic processes and genotoxic chemicals. To counter these assaults, cells are equipped with well co-ordinated machinery of DNA repair, cell cycle checkpoints, and cell death pathways that help to maintain genomic integrity following DNA damage. Major DNA repair pathways that constantly work to maintain DNA throughout different stages of the cell cycle include base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), and nonhomologous end joining (NHEJ) [7,8]. Whilst BER and NER are major pathways for SSBs repair, DSBs repair mainly occurs by HR and NHEJ. A few specific DNA damage lesions can also be corrected by direct chemical reversal and Interstrand Crosslink (ICL) repair whereas small subsets of DNA lesions (eg. UV photo-lesions, alkylated

bases) can be simply reversed in an error-free manner during DNA replication [7,8]. DNA Damage repair pathways for SSBs and DSBs involve multiple steps and PARP participation is required in repairing both of these damages. A schematic representation of mechanisms associated with DNA strand break repair with PARP involvement is given in Figure 1.

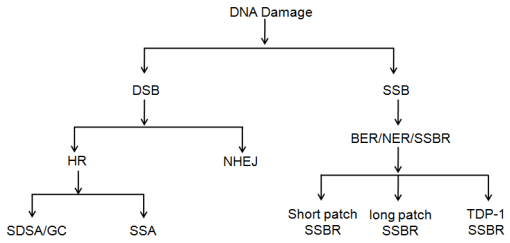


**Figure 1:** Schematic representation of DNA damage repair pathways following Double strand break (DSB) or Single strand break (SSB). Note: HR-Homologous Recombination, NHEJ-Non-Homologous End Joining, SDSA-Synthesis Dependent Strand Annealing, GC-Gene Conversion, SSA- Single Strand Annealing, BER-Base Excision Repair, NER-Nucleotide Excision Repair, SSBR-Single Strand Break Repair, TDP-1- Tyrosyl-DNA Phosphodiesterase 1.

SSBs are often generated from oxidative damage to the DNA, from abasic sites, or from erroneous activity of the DNA topoisomerase 1 (TOP1) enzyme [9]. Unresolved SSBs often collapse DNA replication, stall ongoing transcription, and effect PARP1 activation, which releases cellular NAD1, ATP, and apoptosis inducing factor in cells [10]. SSB Repair (SSBR) can either occur through long patch SSBR pathway or short patch SSBR (Figure 2).

In the long patch SSBR pathway, SSBs are transiently detected by PARP1, which undergoes poly-ADP-ribosyl (PAR)-ation (PARylation) and dissociates to detect the next SSB [11]. After this, the ends undergo end processing by the apurinic-apyrimidic endonuclease 1 (APE1) or DNA glycosylase, PNKP (polynucleotide kinase 3' -phosphate), and aprataxin (APTX) followed by removal of the damaged 5' DNA termini by a multiprotein complex comprising of FEN1, PARP1 and PCNA, leaving behind a single stranded DNA gap. Subsequently the gap is filled by POL β, in combination with POL δ/ε. The final step of ligation is carried out by the Ligase1, which is dependent on the presence of PCNA and X-ray repair cross-complementing 1 (XRCC1) proteins [10,11].

In the short patch SSBR pathway, SSBs generated are recognized by APE1 followed by a similar end processing pathway as the long patch repair. The gap filling step, however, is carried out only by POL β enzyme, followed by Ligase3-catalyzed ligation [11]. Another DNA damage repair pathway that is involved in repairing SSBs in the cells is the Topoisomerase-1 (TOP-1) induced DNA damage repair pathway. This pathway is a variant of the PARP1-dependent long patch SSB repair in which the end-processing is carried out by the TDP1 (tyrosyl-DNA phosphodiesterase 1) [11,12]. TOP1 relaxes super helical tension in DNA during replication and generates a reversible and transient intermediate known as the TOP-1 cleavage complex (TOP1cc). TDP1 is a key repair enzyme for trapped TOP1cc that mediates the hydrolysis of the phosphodiester bond between the DNA 3′-end and the TOP1 tyrosyl moiety.

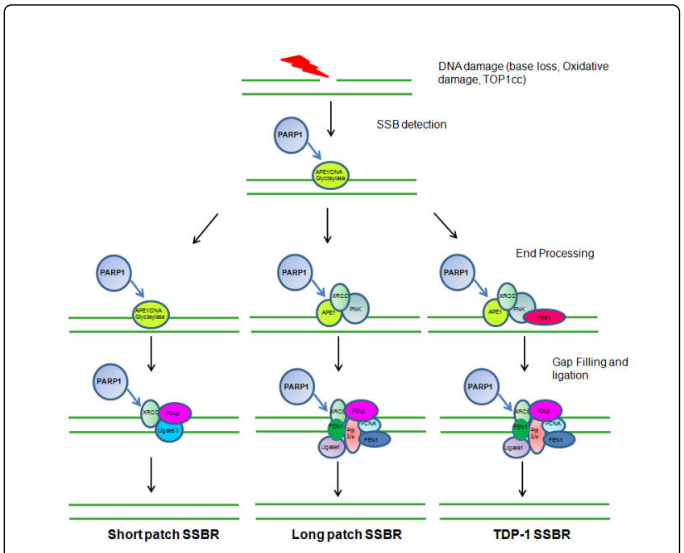


**Figure 2:** Mechanism of single strand break repair (SSBR) following DNA damage. SSBs can arise through enzymatic incision of an abasic site (AP) by APE1, or action of DNA glycosylase or oxidative damage or TOP1 cleavage complex. PARP binds to an SSB and is activated, following which the cells undergo DNA damage repair through short patch or long patch or TDP-1 mediated repair pathways.

PARP activation is essential for the recruitment of TDP1 at DNA damage sites and repair of trapped Top1cc by TDP1 through PARylation. Unrepaired TOP-1cc further gets converted into irreversible DNA - DSBs by collision with a replication fork, or DNA - SSBs by collision with the transcriptional machinery or SSB by proximity to some types of DNA lesions following which the cells either undergo DSBR or SSBR (Figure 3) [11-13].

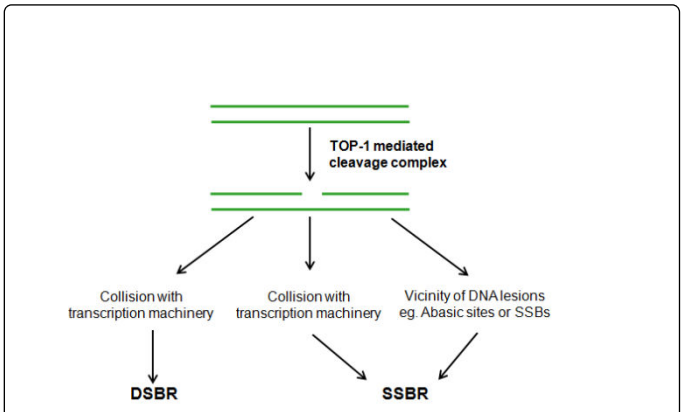


**Figure 3:** TOP-1 cleavage complex repair.

In comparison to SSBs, DSBs pose a higher challenge for repair as a template complementary strand is not available for replication [14,15]. HR and NHEJ are the major repair mechanisms for DSB repair. HR

repair (*HRR*) is a conservative form of DNA repair and relies on sequence homology for restoring DNA to its original form. It is mediated through the *Rad52* family of proteins. *Rad52* interacts and co-localizes with *Rad51*, induces *Rad51* activity, binds preferentially to DSBs and protects them from exonuclease activity.

*HRR* can occur *via* two mechanisms: the conserved synthesis dependent strand annealing pathway (SDSA) or Gene conversion and single strand annealing (SSA) also referred to as alternate-End joining repair pathway (Figure 4) [16]. SDSA uses a homologous sequence as a template to re-synthesize the sequence surrounding the DSB. This pathway is generally thought to result in the accurate repair of the DSBs (Figure 4). During SDSA *HRR*, the two broken DNA ends are brought together by the meiotic recombination11 homologue A (MRE11A)–Nijmegen breakage syndrome 1 (NBS1)–*RAD50* (MRN) complex that detects and binds the broken ends. This is followed by recruitment of ataxia telangiectasia mutated (ATM) and activation of DSB repair. Resection of 5′ DNA, a *BRCA1*-dependent process leads to the exposure of two regions of single-stranded DNA on either side of the DSB. The resulting flanking regions then get coated by replication protein A (RPA), which prevents the formation of secondary structures. *BRCA2* then localizes the DNA recombinase *RAD51* to the exposed SSDNA regions, in a process dependent on partner and localizer of *BRCA2* (*PALB2*). *RAD51* catalysis the looping of the homologous sequence on the sister chromatid, which is then used as a template for accurately repairing the broken DSB ends by DNA polymerase. Following polymerization, DNA ligases and endonucleases resolve the complex DNA completing the DSB repair [16]. Single strand annealing is *RAD51* independent and involves the annealing of DNA strands formed after resection at the DSB without any strand invasion (Figure 4). Initially, DNA ends are resected by an exonuclease, most likely the MRN complex, to yield long single-strand overhangs that RPA and *RAD52* may bind to. Once homology is exposed in the overhangs, they are annealed, and the protruding ends are trimmed by the *ERCC1/XPF* nuclease and the gap is filled by DNA polymerase. This pathway is error-prone as it results in the retention of only one of the homologous sequences and deletion of the intervening sequence [17]. NHEJ is an error prone repair mechanism that utilizes no or little sequence homology for DNA repair. During NHEJ repair, the broken DNA ends are annealed without using a homologous DNA sequence to guide repair and is thought to be dependent on the MRN complex (Figure 4). DNA sequence at the break site change frequently due to lack of a homologous guiding sequence. Furthermore, these undefined breaks lead to joining of previously unlinked DNA molecules, resulting in gross chromosomal rearrangements [18]. NHEJ repair is initiated through the binding of Ku70/Ku80 heterodimer to broken DNA ends followed by recruitment of DNA-dependent protein kinase (*DNA-PKs*). Along with the DNA-PK substrate Artemis, DNA-PKs then acts as an endonuclease which processes the DSB ends. Further repair occurs through ligation by XRCC4-Ligase IV [17,18]. NHEJ plays a significant role in tumor progression as it can introduce mutations in crucial cancer driver genes leading to enhanced tumorigenesis.18 Whether homologous recombination or NHEJ is used to repair double-strand breaks is largely determined by the phase of cell cycle. HR repairs DNA before the cell enters mitosis (M phase). It occurs during and shortly after DNA replication, in the S and G2 phases of the cell cycle, when sister chromatids are more easily available whereas, NHEJ is predominant in the G1 phase of the cell cycle, when the cell is growing but not yet ready to divide [19].

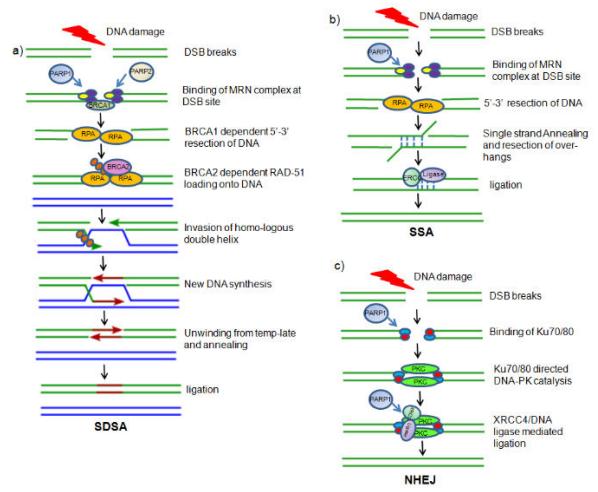


**Figure 4:** Mechanisms of double strand break repair (DSBR) following DNA damage. (**a**) synthesis dependent strand annealing (SDSA) (**b**) single strand annealing (SSA) (**c**) Non-homologous end joining (NHEJ). - MRN complex, - RAD51, - Ku70/Ku80.

## Role of BRCA in maintenance of genomic integrity

*BRCA* gene products play a crucial role in maintaining genomic integrity and deciding cellular fate by directing the DNA damaged cells to undergo HR repair (*HRR*) mechanism (Figure 4). *BRCA1* and *BRCA2* gene products are the major effectors of the cellular response to DSB break repair through *HRR*. In the canonical *HRR*, *BRCA1/2* binds directly to the DNA recombinase *RAD51* and then localizes it to damaged DNA as described earlier. In the absence of functional *BRCA1/2*, *HRR* is impaired [15]. Furthermore, the role of *BRCA1* in DNA damage responses includes control of the signal transduction processes involved in *HRR*, as well as determining whether DSBs are resected before *RAD51* nucleoprotein formation. Additional functions of *BRCA1* such as in chromatin remodelling and transcriptional control might also contribute to tumour suppression [20-22].

The *BRCA1* gene is located on chromosome 17q21, while *BRCA2* is located on chromosome 13q12 [20,23]. Germline mutations in *BRCA1* and *BRCA2* account for the majority of inherited ovarian cancers. Patients harboring *BRCA* mutations have up to 60% lifetime risk of developing ovarian cancer [24]. In addition to germline mutations, somatic mutations of *BRCA1* and *BRCA2* as well as epigenetic silencing of *BRCA1* may yield tumors that behave like *BRCA*-deficient tumors despite their normal germline *BRCA* genes. *BRCA1*- or *BRCA2*-mutant tumor cells exhibit a high level of sensitivity to DSBs inducing agents e.g. platinum salts (cisplatin or carboplatin), mitomycin C, Camptothecin, etc. [25-28]. A high level of genomic instability with a very high frequency of DNA deletions and reordered chromosomes (indels) is observed in familial *BRCA1*- or *BRCA2*-mutant ovarian tumors. Additionally, TP53 mutation and amplification of the MYC proto-oncogene is also a common feature of familial *BRCA1/2*-mutant tumors [26-28].

Cells with nonfunctional or deficient *BRCA1/2* proteins are *HRR* deficient. When *HRR* fails, cells fail to repair DNA lesions efficiently. Subsequently, these cells use alternative, error-prone DNA repair

mechanisms, such as NHEJ, leading to accumulation of DNA damage, genetic instability, and subsequent tumorigenesis or cell death secondary to excessive DNA damage (Figure 5) [26-30].

## Role of enzyme PARP in DNA repair

The PARPs are a family of enzymes that catalyze poly ADP ribosylation that is the active transfer of ADP-ribose to target proteins [31]. There are at least 18 members of the PARP family encoded by different genes that share a homology in the conserved catalytic domain [31] PARP occupies a central position in controlling DNA damage and repair of both DSBs and SSBs through activation of DNA repair proteins that act as PARP substrates and undergo PARylation [32,33]. These substrates include XRCC1, histone H1, DNA PK, Ku70, Ku80, ATM, MRE11, Topoisomerase 1 that are involved in DNA repair and maintenance of genomic stability. Functional consequences of PARylation of these proteins is given in Tables 1 and 2 [34].

| PARP Substrate Protein | Functional consequence of PARylation |
|---|---|
| XRCC1 | Recruitment of XRCC1 and Ligase3 to SSBs and repair by BER |
| Histone H1 | Altered chromatin binding during DNA damage and transcription |
| DNA PK, Ku70, Ku80 | DSB repair by NHEJ |
| ATM | DSB repair by HR and checkpoint activation |
| MRE11 | HR and restarting of collapsed replication forks |
| Topoisomerase 1 | Genomic maintenance |

**Table 1:** List of PARP substrates involved in DNA repair [34].

| Drug | Study title | NCT | No. of patients | Status | Phase |
|---|---|---|---|---|---|
| BMN673 (Talazoparib) | POSITION: A pilot study of induction PARP inhibition in ovarian cancer | NCT02316834 | 30 | Active, not recruiting | Early Phase 1 |
| Talazoparib Tosylate | Study of the PARP inhibitor BMN 673 in advanced cancer patients with somatic alterations in BRCA1/2, mutations/deletions in PTEN or PTEN Loss, a homologous recombination defect, mutations/deletions in other BRCA pathway genes and germline mutation in BRCA1/2 (Not breast or ovarian cancer) | NCT02286687 | 150 | Recruiting | Phase 2 |
| Drug: ZL-2306 (niraparib) | The clinical trial to evaluate the pharmacokinetics, safety and tolerability of ZL-2306 (Niraparib) in patients with ovarian cancer | NCT03551171 | 42 | Active, not recruiting | Phase 1 |
| KU-0059436 (AZD2281) | A study to assess the safety and pharmacokinetics of an inhibitor of Poly ADP-ribose Polymerase-1 (PARP) | NCT00516373 | 93 | Active, not recruiting | Phase 1 |
| Fluzoparib | A study of fluzoparib（SHR-3162）in BRCA1/2-mutant relapsed ovarian cancer | NCT03509636 | 112 | Recruiting | Phase 2 |
| Olaparib | Olaparib after response to trabectedin-pegylated liposomal doxorubicin in recurrent ovarian carcinoma | NCT03470805 | 66 | Recruiting | Phase 2 |
| Olaparib | Olaparib tablets maintenance monotherapy ovarian cancer patients after complete or partial response to platinum chemotherapy (L-MOCA) | NCT03534453 | 300 | Recruiting | Phase 3 |
| BMN673 (Talazoparib) | Pilot trial of BMN 673, an oral parp inhibitor, in patients with advanced solid tumors and deleterious brca mutations | NCT01989546 | 24 | Recruiting | Phase 1&2 |
| IMP4297 | The safety and pharmacokinetics of IMP4297 in patients with advanced solid tumors | NCT03507543 | 30 | Recruiting | Phase 1 |
| Niraparib | A study of niraparib maintenance treatment in patients with advanced ovarian cancer following response on front-line platinum-based chemotherapy | NCT02655016 | 620 | Active, not recruiting | Phase 3 |
| Olaparib | A study of long-term responders on Olaparib (OLALA) | NCT02489058 | 100 | Recruiting | |
| Rucaparib | ARIEL4: A study of rucaparib *versus* chemotherapy BRCA mutant ovarian, fallopian tube, or primary peritoneal cancer patients | NCT02855944 | 345 | Recruiting | Phase 3 |

| | | | | | |
|---|---|---|---|---|---|
| Rucaparib | A study of oral rucaparib in patients with a solid tumor (Phase I) or with gBRCA mutation ovarian cancer (Phase II) | NCT01482715 | 136 | Active, not recruiting | Phase 1&2 |
| Olaparib | Olaparib maintenance monotherapy in patients with BRCA mutated ovarian cancer following first line platinum-based chemotherapy. (SOLO-1) | NCT01844986 | 450 | Active, not recruiting | Phase 3 |
| Fluzoparib | A Phase I study of fluzoparib in patient with advanced solid malignancies | ClinicalTrials.gov Identifier: NCT02575651 | 42 | Recruiting | Phase 1 |

**Table 2:** Ongoing clinical trials evaluating PARP inhibitors as single agents in ovarian cancer.

Within the PARP family, PARP1 and PARP2 appear to play a significant role in DNA damage repair. PARP1 detects and signals the presence of an SSB by binding to DNA adjacent to the damage *via* its DNA binding domain. Once bound, PARP1 catalyzes the cleavage of the coenzyme nicotinamide adenine dinucleotide (NAD+) into nicotinamide and ADP ribose to produce highly negatively charged branched chains of poly (ADP-ribose) (PAR).

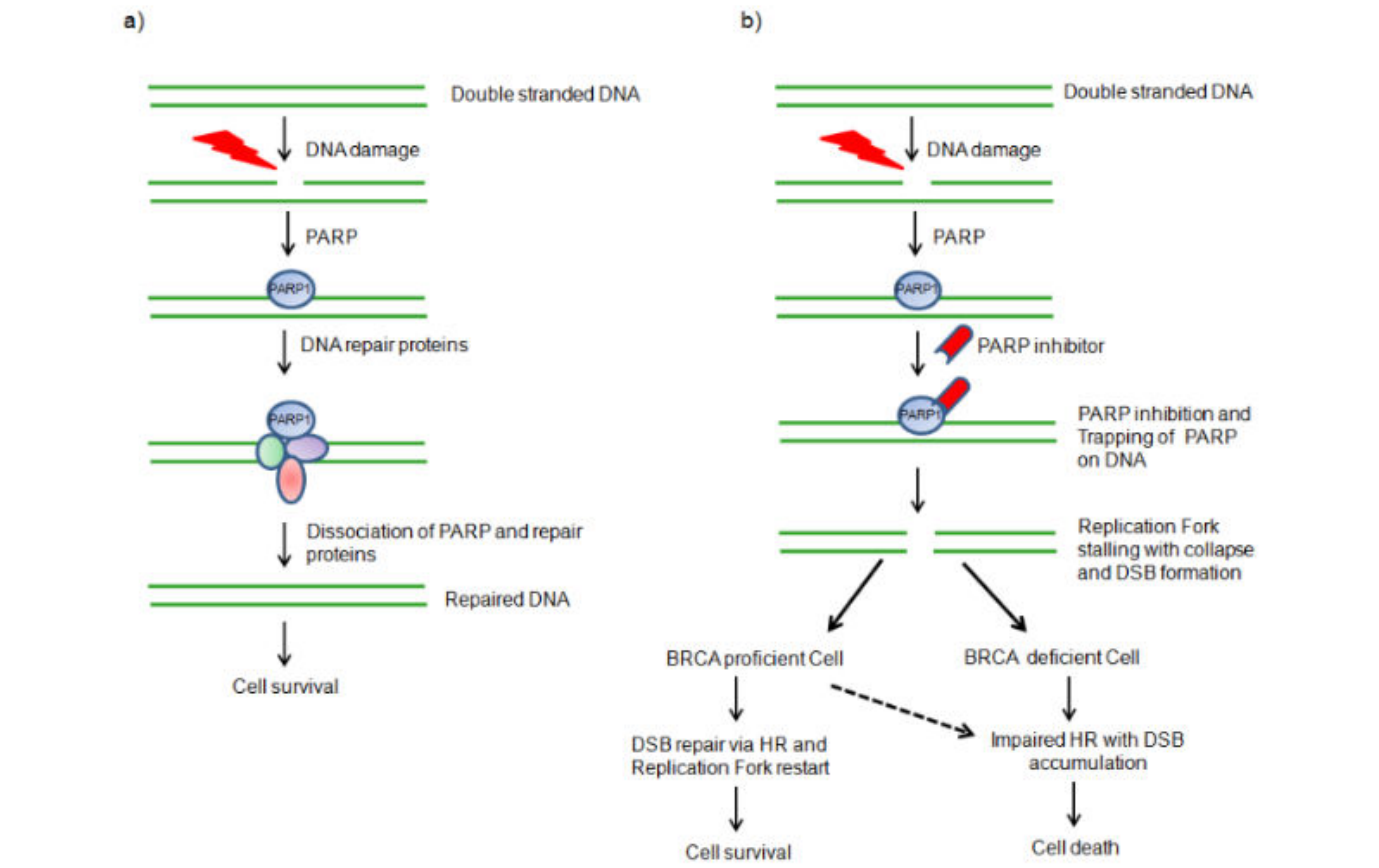


**Figure 5:** Role of PARP and PARP inhibitors in DNA damage and repair (**a**) Mechanism of PARP action. When single-strand break (SSB) is detected, PARP recruitment and activation leads to SSB repair through PARYlation by PARP, and recruitment of PARP-dependent DNA repair proteins. Repaired DNA can undergo replication determining cell survival. (**b**) Outcome of PARP inhibition in BRCA deficient and BRCA proficient tumors. In the presence of PARP inhibitors, PARPs recruited to DNA-damage sites are no longer able to activate PARP-dependent repair systems due to catalytic activity inhibition and/or direct trapping of PARP on DNA leading to replication fork stalling and formation of double strand breaks (DSBs). In BRCA proficient tumors, DSBs can be repaired by homologous recombination (HR) and replication may restart, leading to cell survival. In BRCA-deficient cells or HR deficient BRCA proficient cells, HR is impaired, leading to accumulation of DSBs and cell death.

A multi-protein repair complex is then formed including repair enzymes, DNA ligase III, the DNA polymerase, and scaffolding proteins such as XRCC1. Following ADP-ribosylation, owing to the dense negative charge of PAR, PARP1 loses affinity for DNA, and is released allowing the recruitment of repair proteins by PAR to the damaged DNA. After repair, the PAR polymers are degraded *via* poly (ADP-ribose) glycohydrolase (PARG) (Figure 5) [34].

Similarly, PARP2 can be activated by DNA damage but contributes to a small proportion (5% to 10%) of the repair activity [35,36]. A suppression of PARP1/2 activity leads to the accumulation of unrepaired SSBs and subsequently DSBs (Figure 5). This results in stalling of replication forks and its subsequent degradation *via* Mre11. In cancer cells, PARP1 with *BRCA* is known to protect the stalled replication forks from Mre11 degradation thus allowing HR bypass and replication to proceed resulting in cell survival. Inhibition of *PARP* in HR deficient *BRCA1/2* mutant cells leads to accumulation of DSBs resulting in genomic instability and eventually cell death (Figure 5) [34,37,38]. Hence HRR deficient tumors are most likely to be sensitive to PARP inhibition. The activity of PARPs and response to PARP inhibition is dictated by the extent of DNA damage and cellular NAD+ levels (Table 3).

| Combination | Study title | NCT | No of patients | Status | Phase |
|---|---|---|---|---|---|
| AZD2281+ Carboplatin | AZD2281 plus carboplatin to treat breast and ovarian cancer | NCT01445418 | 103 | Completed | I |
| BKM120+ Olaparib<br>And BYL719+ Olaparib | Oral PI3kinase inhibitor BKM120 or BYL719 and the Oral PARP Inhibitor Olaparib in patients with recurrent triple negative breast cancer or high grade serous ovarian cancer | NCT01623349 | 118 | Active, not recruiting | I |
| Niraparib+ bevacizumab | niraparib *versus* niraparib-bevacizumab combination in women with platinum-sensitive epithelial ovarian cancer (AVANOVA) | NCT02354131 | 108 | Recruiting | I&II |
| Cediranib+ Olaparib | A study of Cediranib and Olaparib at disease worsening in ovarian cancer | NCT02681237 | 30 | Recruiting | |
| Carboplatin+ Olaparib | Olaparib in combination with carboplatin for refractory or recurrent women's cancers | NCT01237067 | 77 | Completed | I |
| AZD2281+ Carboplatin+ Paclitaxel | Study to assess the safety and tolerability of a PARP inhibitor in combination with Carboplatin and/or Paclitaxel | NCT00516724 | 188 | Active, not recruiting | I |
| Olaparib+ Tremelimumab | PARP-inhibition and CTLA-4 Blockade in BRCA-deficient Ovarian Cancer | NCT02571725 | 50 | Recruiting | I&II |
| Fluzoparib+ Apatinib | A study of Fluzoparib given in combination with Apatinib in ovarian or breast cancer patients | NCT03075462 | 76 | Recruiting | I |
| Niraparib+ pembrolizumab | Niraparib in combination with Pembrolizumab in patients with Triple-negative breast cancer or ovarian cancer (TOPACIO) | NCT02657889 | 114 | Recruiting | I&II |
| Veliparib+ Floxuridine | Veliparib and floxuridine in treating patients with metastatic epithelial ovarian, primary peritoneal cavity, or fallopian tube cancer | NCT01749397 | 102 | Recruiting | I |
| Olaparib+ paclitaxel+ carboplatin | Study to compare the efficacy and safety of Olaparib when given in combination with Carboplatin and Paclitaxel, compared with Carboplatin and Paclitaxel in patients with advanced ovarian cancer | NCT01081951 | 162 | Active, not recruiting | II |
| ABT-888 (veliparib)+ Cyclophospha-mide | Phase II ABT-888 With Cyclophosphamide | NCT01306032 | 124 | completed | II |
| Niraparib+ Bevacizumab | Phase 2, a study of niraparib combined with bevacizumab maintenance treatment in patients with advanced ovarian cancer following response on front-line platinum-based chemotherapy | NCT03326193 | 92 | Recruiting | II |
| Olaparib+ Cediranib | Study evaluating the efficacy of maintenance Olaparib and Cediranib or Olaparib alone in ovarian cancer patients (ICON9) | NCT03278717 | 618 | Not yet recruiting | III |
| Carboplatin+ Eribulin+ Veliparib | Combination of Carboplatin, Eribulin and Veliparib in stage IV Cancer patients | NCT03032614 | 0 | Withdrawn (Lack of funding) | II |

| | | | | | |
|---|---|---|---|---|---|
| Olaparib+ Cediranib+ MEDI4736 | Phase I/II study of the anti-programmed death ligand-1 antibody MEDI4736 in combination with Olaparib and/or Cediranib for advanced solid tumors and advanced or recurrent ovarian, triple negative breast, lung, prostate and colorectal cancers | NCT02484404 | 421 | Recruiting | I&II |
| Veliparib+ Carboplatin+ Paclitaxel+ FOLFIRI | A study evaluating veliparib as a single agent or in combination with chemotherapy in subjects with solid tumors | NCT02033551 | 47 | Completed | I |
| Olaparib+ MEDI4736 | A Phase I/II study of MEDI4736 in combination with Olaparib in patients with advanced solid tumors. (MEDIOLA) | NCT02734004 | 148 | Active, not recruiting | I&II |
| Cediranib Maleate+ Olaparib | Cediranib maleate and Olaparib in treating patients with recurrent ovarian, fallopian tube, or peritoneal cancer or recurrent triple-negative breast cancer | NCT01116648 | 162 | Active, not recruiting | I&II |
| ABT-888+ Temozolomide | A Phase I study of ABT-888 in combination with Temozolomide in cancer patients | NCT00526617 | 41 | Completed | I |
| Olaparib+ AZD2014 or AZD5363 | A Phase Ib study of the oral PARP inhibitor Olaparib with the oral mTORC1/2 inhibitor AZD2014 or the oral AKT inhibitor AZD5363 for recurrent endometrial, triple negative breast, and ovarian, primary peritoneal, or fallopian tube cancer | NCT02208375 | 159 | Active, not recruiting | I&II |
| Durvalumab+ Olaparib+ Tremelimumab | Olaparib, Durvalumab, and Tremelimumab in treating patients with recurrent or refractory ovarian, fallopian tube or primary peritoneal cancer with BRCA1 or BRCA2 mutation | NCT02953457 | 39 | Recruiting | I&II |
| Avelumab+ Talazoparib | Javelin Parp Medley: Avelumab plus talazoparib in locally advanced or metastatic solid tumors | NCT03330405 | 296 | Recruiting | I&II |
| Cediranib Maleate+ Olaparib<br>Chemotherapy: Paclitaxel, Pegylated Liposomal Doxorubicin, Topotecan Hydrochloride | Cediranib Maleate and Olaparib or standard chemotherapy in treating patients with recurrent platinum-resistant or -refractory ovarian, fallopian tube, or primary peritoneal cancer | NCT02502266 | 680 | Suspended - scheduled interim monitoring | II&III |
| Veliparib+ Carboplatin+ Paclitaxel | Veliparib with carboplatin and paclitaxel and as continuation maintenance therapy in subjects with newly diagnosed stage iii or iv, high-grade serous, epithelial ovarian, fallopian tube, or primary peritoneal cancer | NCT02470585 | 1100 | Active, not recruiting | III |
| Radiation: Radiation Therapy + Veliparib | Veliparib and radiation therapy in treating patients with advanced solid malignancies with peritoneal carcinomatosis, epithelial ovarian, fallopian, or primary peritoneal cancer | NCT01264432 | 34 | Completed | I |
| Cediranib+ olaparib | Efficacy and safety study of cediranib in combination with olaparib in patients with recurrent platinum-resistant ovarian cancer (CONCERTO) | NCT02889900 | 100 | Recruiting | II |

**Table 3:** Clinical trials evaluating PARP inhibitor combinations.

## Pharmacological development of PARP inhibitors for management of ovarian cancer

PARP inhibitors act by blocking the enzyme PARP. Pharmacological development of PARP inhibitors is based on two approaches: the role of PARP in DNA damage repair with a potential chemo-sensitization role [38] and synthetic lethality of PARP inhibition with deficient HR pathway resulting from *BRCA* aberrations [39]. Three oral PARP inhibitors have been granted FDA-approval for specific indications in ovarian cancer: Lynparza (olaparib) in December 2014, Rubraca (rucaparib) in December 2016, and Zejula (niraparib) in March 2017. Other PARP inhibitors in development include veliparib, talazoparib, fluzoparib [24,40].

Lynparza (olaparib) or AZD2281, the first approved PARP inhibitor, is the most extensively studied and is indicated for women with germline *BRCA*-mutated advanced ovarian cancer who have had three or more lines of chemotherapy [41-43]. A Phase I study in patients with familial *BRCA1*- or *BRCA2*-mutant breast or ovarian cancers with olaparib provided considerable clinical proof of concept for the synthetic lethal approach [44]. Subsequently, two Phase II clinical trials established that olaparib could elicit profound and sustained antitumor

responses in familial *BRCA1*- or *BRCA2*-mutant breast and ovarian cancers [45,46]. On the basis of these Phase II studies, olaparib was approved for use in December 2014 by both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) as a monotherapy for the maintenance treatment of women with *BRCA1*- or *BRCA2*-mutant high grade serous ovarian cancer (*HGS-OvCa*), or fallopian tube or primary peritoneal cancer, who have had either a complete or partial response to platinum-based chemotherapy [47]. In the EMA approval, the inclusion of both *BRCA1* or *BRCA2* germline and somatically mutated tumours makes olaparib the first targeted treatment for an inherited cancer disorder (namely, familial, *BRCA1*-mutant or *BRCA2*-mutant *HGS-OvCa*) as well as the first *BRCA*ness-targeted therapy. In addition, Lynparza's new tablet formulation received FDA approval in August 2017, as maintenance treatment for women with platinum-sensitive recurrent ovarian cancer regardless of *BRCA*-mutation status (https://www.astrazeneca.com/media-centre/press-releases/2017).

Rubraca (Rucaparib) has been approved by the FDA for single-agent treatment of women with ovarian cancer who have either a germline or somatic *BRCA* mutation and who have received a minimum of two prior lines of chemotherapy. The phase II trial data supporting this indication revealed an objective response rate (ORR) of approximately 80% in patients with a *BRCA* mutation [48]. As per ESMO 2017 Press Release (http://www.esmo.org/ Conferences/Past-Conferences/ESMO-2017), Rucaparib maintenance therapy (ARIEL3 trial) resulted in increased progression-free survival in *BRCA* mutant recurrent ovarian cancer by 77% [49].

Zejula (niraparib) is prescribed as maintenance therapy following a partial or complete response to platinum chemotherapy, and it can be used in women who do not harbor *BRCA* mutations. A phase III trial (NCT01847274) with progression-free survival as the end point concluded that among patients with platinum-sensitive, recurrent ovarian cancer, the median duration of progression-free survival was significantly longer among those receiving niraparib than among those receiving placebo, regardless of the presence or absence of *BRCA* mutations or HR deficiency status, with moderate bone marrow toxicity [50].

In addition to the approved indications, a list of ongoing clinical trials evaluating PARP inhibitors as single agents in ovarian cancer is included as Table 2 (source: clinicaltrials.gov).

## Safety and tolerability of PARP inhibitors

PARP inhibitors are relatively well tolerated either as single agents or in combination with other cytotoxic or biologic agents in the treatment of ovarian cancer. Their main toxicities are gastrointestinal, fatigue, and hematological, and these toxicities are common to all PARP inhibitors. Myelodysplastic syndromes are seen in up to 2% of patients treated with these medications, and patients should be appropriately counseled regarding this adverse effect [44,51].

## Development of resistance to PARP inhibitors

Inhibitor resistance may develop on continued therapy and acquired resistance is an acknowledged clinical problem with PARP inhibitors. Numerous mechanisms of resistance to PARP inhibitors have been identified. Since pharmacological development of PARP inhibitors was mainly based on synthetic lethal approach in *HRR* deficient cancers, the major mechanism of resistance to PARP inhibitors is restoration of *HRR* in *BRCA1/2*-mutant cells.51 Restoration of *HRR* can occur *via* several mechanisms. Firstly, inactivation of proteins involved in controlling DNA resection at DSBs namely p53-binding protein 1 (53BP1) [52] or mitotic arrest deficient 2-like protein 2 (MAD2L2) [53] may restore *HRR*. Hong et al. observed that 53BP1 might be a predictor of PARP inhibitor resistance in patients with ATM-deficient tumors on the basis of their study findings that indicated an improved overall survival in triple-negative breast cancer patients with lower levels of phospho- ATM and in patients with negative 53BP1 [54]. Secondly secondary mutations in *BRCA1/2* (for eg intragenic deletion of the c.6174delT) restore the open reading frame of mutant *BRCA* alleles restoring *BRCA* protein function and *HRR*, therefore inducing resistance to PARP inhibitors [55-58]. Platinum therapy is implicated in selection of secondary *BRCA* mutations as demonstrated by Norquist [58] In this study 28.3% (13 of 46) of recurrent ovarian carcinomas that emerged after platinum therapy had secondary *BRCA* mutations, compared with a secondary mutation frequency of 3.1% (2 of 64) in primary tumors. Moreover, 46.2% (12 of 26) of platinum-resistant recurrences in 26 patients with ovarian cancer had secondary mutations restoring *BRCA1* or *BRCA2* function, compared with 5.3% (1 of 19) of platinum-sensitive recurrences indicating the role of platinum therapy. Resistance may also result from germline or secondary mutations of *HRR* component genes. Sequencing of *HRR* pathway genes in tumor samples from ARIEL2 trial revealed that secondary somatic mutations in *RAD51C* and *RAD51D* are associated with Rucaparib resistance in *HGS-OvCa* [59]. Germline mutations in *RAD51C* and *RAD51D* are rare and associated with an increased risk of ovarian cancer, whereas germline PALB2 mutations are associated with an increased risk of breast and pancreatic cancers. The pathogenic mutations in *RAD51C*, *RAD51D*, and PALB2 are synthetically lethal with PARP inhibitors [58,59]. In addition to these, enhanced enzymatic recombinase activity of *RAD51*, may restore HR function rendering cells insensitive to PARP inhibition [60]. Other mechanisms that contribute to PARP inhibition include down-regulation of NHEJ pathway or reduction in PARP activity. Reduction of PARP activity can occur either due to reduction in PARP expression, trapping potential of PARP inhibitors or loss of PARP catalytic activity [61]. Lastly, an augmentation of Abcb1a/b genes encoding P-glycoprotein efflux pumps leading to reduction in intracellular concentrations of PARP inhibitors due to increased efflux rate may cause resistance to PARP inhibitors [62].

In addition to the mechanisms described above using data from clinical studies and patient samples, preclinical studies have indicated role of micro RNA regulation, epigenetic re-expression of *BRCA1*, phosphorylation of PARP by c-Met and mTOR pathways and overexpression of HOX family members as some of the probable mechanism of development of resistance to PARP inhibition [62-66].

## Biomarkers for PARP inhibitor sensitivity in monotherapy

Biomarkers for PARP inhibitor sensitivity would include scrutiny of *BRCA* status (*BRCA1/2* mutation), Silenced or mutated *BRCA* related genes, PARP protein levels, PARP activity, RNA/DNA signatures correlating with *BRCA* status and Functional homologous recombination pathways [e.g. RAD51 foci, Fanconi anaemia (FA)]. Germline mutations in *BRCA1/2* predispose a cell naturally to HR deficiency and serve as the first biomarker to select a patient population that would respond to PARP inhibition. In addition, *BRCA*ness (pheno-copy of *BRCA1* or *BRCA2* mutation) that describes the situation in which an *HRR* defect exists in a tumour in the absence of a germline *BRCA1* or *BRCA2* mutation needs to be evaluated [67]. To develop a *BRCA*ness signature associated with platinum and PARP-

inhibitor responsiveness, Konstantinopoulos mined publicly available gene expression data from *BRCA1*-mutant, *BRCA2*-mutant or *BRCA*-wild-type *HGS-OvCa*. The *in vitro* validated gene signature in this study when assessed clinically in 70 patients revealed that patients whose tumours had a high level of expression of the *BRCA*ness profile had improved disease-free survival (34 months *versus* 15 months; P=0.013) and improved overall Survival (72 months *versus* 41 months; P=0.006) compared with those with the *BRCA*-wild-type profile. This gene signature comprised predominantly platinum resistance or DNA repair signature genes such as *APEX1*, *MGST3*, and *PMS1*confirming the role of platinum sensitivity to predict sensitivity to PARP inhibitors. In this study, *BRCA*ness profile was found to be independent of other clinical prognostic factors such as the age of the patient at diagnosis, and the tumour stage, grade and histology [68]. Other experimental biomarkers of *BRCA*ness in *HGS-OvCa* include the presence of genetic or epigenetic alterations in genes that control *HRR* including secondary mutations in *BRCA* and transcriptomic signatures associated with *HRR* gene defects. Both somatic mutations described earlier and epigenetic loss of *BRCA* function through *BRCA1* promoter methylation or overexpression of the *BRCA2* transcription suppressor EMSY can lead to *BRCA*ness [68,69]. Defects in DNA repair pathway genes that modulate *HRR* and thus *BRCA*ness include ataxia telangiectasia mutated (ATM), ataxia telangiectasia and *Rad3*-related (ATR), CHEK1 and CHEK2, deleted in split hand/split foot protein 1 (*DSS1*), *RAD51*, *PALB2*, *NBS1*, excision repair cross-complementation group 1 (ERCC1) and the Fanconi anaemia complementation gene family (*FANC*) [67]. Additionally, mutations in cyclin dependent kinase 12 (*CDK12*), a key regulator of transcription elongation can also alter the response to platinum therapy and PARP inhibitors [70-74]. Key HRR genes that are regulated through *CDK12* include *BRCA1*, *ATR*, *FANCI* and *FANCD2* [74].

Functional approaches aimed at identifying *BRCA*ness include assays that can detect tumors unable to perform *HRR* efficiently. One approach is detection of *RAD51* nuclear localization by immunohistochemical (IHC) staining. A drawback to this testing is that DNA damage being a highly dynamic response, *RAD 51* detection may lead to false interpretations as the basal level of their activity might not always indicate *HRR* defect [75,76]. For example, in *in-vitro* tissue cultured *BRCA2* null and *BRCA2* wild type cells, nuclear *RAD51* foci are almost absent in the basal state and detected only when the cells are exposed to DNA damage resulting from exposure to ionizing radiation or PARP inhibition [75]. This drawback can be corrected by using an *ex vivo* approach with fresh tumour biopsies wherein following an *ex-vivo* DNA damage exposure, *RAD51* is measured [75-77].

Other promising biomarkers for sensitivity to PARP inhibitors is a measurement of PARP protein level, its catalytic activity (detection of PAR levels) and ability of PARP inhibitors to trap PARP1 and PARP2 enzymes [78]. Likewise, measurement of 53BP1 or MAD2L2 expression in cancer cells can detect its sensitivity or resistance to PARP inhibitor treatment [52,53]. Synthetic lethality has been observed with Myc and PARP inhibition. Thus, reducing MYC oncogene addiction can leverage cancer cell sensitivity to PARPi, facilitating the clinical use of c-myc as a predictive biomarker with PARP inhibitor treatment [79].

Clinical development of PARP inhibitors faces a major roadblock in availability of a suitable pharmacodynamic (PD) marker. Although rational for suitability of several biomarkers as PD markers is described above, currently the only clinically validated PD assay available for testing PARP inhibitors is an ELISA based method that quantifies basal PAR levels both in peripheral blood mononuclear cells (PBMCs) and tumour cells [80,81]. Preclinically, reduction in PAR chain formation has been a useful PD biomarker to confirm target engagement for PARP inhibitors. However, in clinic several studies have demonstrated lack of correlation between this PD read-out and clinical anti-tumour activity as exemplified by rucaparib and Olaparib trials [82,83]. In rucaparib study, a near complete inhibition of PARP enzymatic activity was demonstrated even at sub-therapeutic doses where there is no appreciable relationship between PK and PD studies. A possible reason for this discordance may be related to the PARP trapping to DNA mechanism of PARP inhibitors with the most potent PARP inhibition appears to be those that bind DNA most strongly. Another limitation with this assay is sensitivity as low basal levels of PAR are not detectable and hence the assay is applicable only to a limited set of patients that exhibit sufficiently high PAR levels. Furthermore, high day-to-day PAR level variation within the same individual and inter assay variability makes the quantification and subsequent comparison of PARP activity between samples difficult. Hence the PAR level detection methods should be complemented with other biomarkers such as *RAD51* and *γH2AX* in early phase clinical trials to build confidence that robust PD activity was achieved.

## Combination therapy with PARP inhibitors

Combination strategies of existing treatment modalities with PARPi are an area of intense research. Designing of clinically relevant drug combinations to maximize synergistic effects with PARP inhibitors will be based on retention of DNA damage with induction of *HRR* deficiency. Currently, multiple clinical trials are underway examining the antitumor activity of PARP inhibitor combination therapy. A brief list of ongoing combination trials in ovarian cancers resourced through PubMed, ClinicalTrials.gov and data from proceedings from scientific conferences pertaining to clinical combination of PARP inhibitors in ovarian cancer is presented as Table 2. Combinations include the addition of a PARP agent to standard of care platinum-based cytotoxic therapy, or in combination with bevacizumab (standard of care in ovarian malignancy). In updated findings from a small phase 3 trial studying the combination that was reported at the annual meeting of the American Society of Clinical Oncology in June 2017, adding cediranib (antiangiogenic) to Lynparza showed a superior median progression-free survival of 23.7 months, compared with 5.7 months with single-agent Lynparza in 23 women with recurrent platinum-sensitive ovarian cancer, but without a known *BRCA* germline mutation. The 24 women in the study with a *BRCA* mutation did not derive the same benefit. "In this trial, patients who were non–*BRCA*-mutated actually did better than those who were" [84] further emphasizing the requirement for a deeper understanding of mechanistic effects of drugs under study. Another completed study (NCT01306032) that evaluated single-agent, low-dose cyclophosphamide in *HGS-OvCa*, peritoneal, fallopian tube, and *BRCA*-mutant ovarian cancers, combination of veliparib with cyclophosphamide was well tolerated. However, the addition of veliparib at 60 mg daily did not improve either the response rate or the median progression-free survival [85]. PARP-immunotherapy combinations are also being explored with two phase 1/2 studies recruiting patients that is examining combination of Lynparza with the monoclonal antibody tremelimumab (NCT02571725) and tremelimumab and Durvalumab, (NCT02953457) in *BRCA 1* and *BRCA 2* carriers with recurrent ovarian cancer. Newer combination strategies being evaluated to leverage the DNA damage response of

PARP inhibitors include the addition of cell cycle inhibitors and other DNA repair-targeting agents (ASCO, 2017). Furthermore, at the Preclinical level, combination of Rucaparib with *MDM2* inhibitors, Nutlin-3 and RG7388, was found to be synergistic in ovarian cancer [86].

Combination designs of PARP inhibitors with chemotherapy should also aim at minimizing toxicity commonly observed with PARPi and chemotherapy. Overlapping myelosuppression is commonly observed with PARP inhibitor and chemotherapy combinations [51]. In a recent study Oral rucaparib combination with a clinically relevant dose of carboplatin studied in 85 patients with advanced solid tumours (15/85 ovarian) (NCT01009190) was found to be safe [87]. Another study ABT-888 plus low-dose fractionated whole abdominal radiation therapy (LDFWAR) in patients with advanced solid malignancies and peritoneal carcinomatosis with a dose escalation in ovarian and fallopian tube cancers indicated gastrointestinal symptoms, fatigue and myelosuppression as the most common toxicities [88].

Strategizing use of clinically relevant combination trials with PARP inhibitors would involve critically analyzing efficacy, safety, tolerance and an in-depth knowledge of DNA repair mechanisms and mode of action of combination compounds.

### Utility of PARP inhibitors in BRCA-proficient cancers

The designing of PARP inhibitors was based on synthetic lethality in *BRCA* deficient tumors (Figure 5b). However, in recent times, understanding of DNA repair pathways and biological role of PARP has raised the possibility of utilizing PARP inhibitors as a treatment modality in *BRCA* proficient but HR deficient tumors. The recent FDA approval of lynpraza as a maintenance treatment for women with platinum-sensitive recurrent ovarian cancer regardless of *BRCA*-mutation status indicates that the key property for predicting a favorable response to PARP inhibitors is HR deficiency (https://www.astrazeneca.com/media-centre/press-releases/2017). Thus, PARP inhibition in ovarian cancer might have utility extending beyond those cases associated with BRCA mutations. Key work supporting this line of thought initially comes from the cancer genome atlas (TCGA) network (2011) wherein the authors observed that within the CGA framework up to 50% of cases of high-grade serous ovarian cancer might be candidates for PARP inhibition, based on a range of genetic defects in addition to *BRCA 1/2* germline and somatic mutations [5]. The clinical relevance of the observations was assessed in a clinical trial published in 2011 by Gelmon, which demonstrated efficacy of Olaparib in patients with sporadic, *BRCA* wild-type ovarian cancer, albeit at a slightly lower level (24%) and confined mainly to patients with platinum-sensitive disease [89]. In this study, Olaparib induced sustained responses in non-*BRCA* mutant *HGS-OvCa*. Responses to Olaparib were also observed in ovarian cancer patients with wild type or unknown *BRCA* status in a study of maintenance therapy after platinum-based chemotherapy [90] and in a study of Olaparib plus cediranib [91].

Identification of the *BRCA* proficient but HR deficient subgroup of patients however, is a challenge as efficient HR deficiency assays are not available clinically. Furthermore, understanding of DNA repair systems has provided some insights to exploit PARP inhibition in *BRCA* proficient tumors using combinatorial strategies and a number of preclinical and clinical studies are reported in the literature. A combination of PARP and CDK inhibition was observed to be synergistic in mouse model of lung adenocarcinoma wherein, reduced Cdk1 activity, impaired *BRCA1* function and consequently, repair by HR, Inhibition of Cdk1 along with PARP thus represents a clinically viable strategy for *BRCA*-proficient cancers [92] similarly reduced PAK1 activity impaired *FA/BRCA* function and inhibition of this kinase in *PAK1* amplified and/or overexpressing breast cancer cells represents a plausible strategy for expanding the utility of PARP inhibitors to *BRCA*-proficient cancers [93]. Additionally, synergistic combinations of PARP inhibitors is also observed with *PI3K/mTor* inhibitors in *BRCA* proficient tumors [94,95].

*BRCA* proficiency but HR deficiency due to epigenetic modulation of *BRCA* is reported in up to 20% of *HGS-OvCa* [96]. In these tumors the loss of *BRCA1* or *BRCA2* function occurs through epigenetic events [96]. The molecular profiling of this subgroup implies that these patients expressing an HR-deficient phenotype in the absence of somatic/germline *BRCA* mutation will benefit from PARP inhibition. Accordingly, several preclinical studies have demonstrated synergistic combinations of PARPi with epigenetic modulators. In a preclinical study, BET bromodomain inhibitor JQ1 was found to synergistically act with olaparib in *BRCA*-proficient ovarian cancers [97]. The mechanistic in-view of this synergistic combination suggests that synegism is due to an increase in DNA damage and checkpoint defects (WEE1, TOPBP1), that allowed the cells to enter mitosis despite the accumulation of DNA damage, ultimately causing mitotic catastrophe [97]. Similarly, DNA methyl transferase (DNMT) inhibitors with PARPi synergistically inhibited tumor growth in acute myeloid leukemia (AML) and breast cancer cells, by binding into DNA and increasing PARP trapping into chromatin. Thus, a combination of PARPi with epigenetic modulators offers immense potential to be tried in clinical setting for *BRCA* proficient tumors.

## Discussion and Conclusion

PARP Inhibitors are emerging as one of the most active and promising therapies for the treatment of ovarian cancers. Physicians find these drugs to be extremely attractive because of their efficacy, oral bioavailability, safety, tolerability and convenient dosing schedule. Maximal benefit of PARP inhibitor monotherapy has been observed in ovarian cancer with deficient HR DNA repair system due to *BRCA* mutations working on the principle of synthetic lethality. Accordingly, identification of target patient population to maximize PARPi utility should involve incorporation of *BRCA* as well as *BRCA*ness diagnostic tests into clinical practice. Additionally, ovarian cancer patients with HRR deficiency but without *BRCA* mutations may also be susceptible to PARP inhibition and a robust diagnostic HR Deficiency (HRD) assay in clinical practice is required to identify this patient subgroup as candidates for PARPi treatment. Major challenges in optimization of using PARP inhibitors clinically include identification of predictive biomarkers, avoidance of creating conditions for new Drug limiting toxicities (DLTs) and combinatorial strategies with available treatment modalities to improve efficacy, overcome resistance and expand the utility of PARPi to *BRCA* proficient patients. PARP inhibitors are thus poised to be a critical therapeutic component in clinical management of ovarian cancer patients.

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