Mechanisms of PARP inhibitor resistance in ovarian cancer

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Purpose of review
To summarize recently discovered PARP inhibitor resistance mechanisms and highlight the clinical relevance of these findings to date.

Recent findings
A predominant mechanism of acquired PARP inhibitor resistance in homologous recombination-deficient cancers is the acquisition of homologous recombination proficiency as a consequence of secondary genetic or epigenetic events, such as secondary mutations in BRCA1 or BRCA2, or reversal of BRCA1 promoter methylation that restores homologous recombination and leads to PARP inhibitor resistance. Multiple other potential mechanisms of acquired resistance to PARP inhibitors including loss of DNA end resection inhibition (53BP1/REV7/RIF1/Sheldin) or DNA replication fork protection (PTIP/EZH2), but also increased drug efflux or induction of a reversible senescent or mesenchymal cell state have been described in ovarian cancer models. However, only few of these mechanisms have been identified in clinical samples.

Summary
Multiple adaptive responses following PARP inhibitor treatment have been identified. Further research is needed to better understand what role these mechanisms play for clinical PARP inhibitor resistance and how these mechanisms may render ovarian cancer cells susceptible to subsequent novel combination therapies.

Keywords
homologous recombination deficiency, ovarian cancer, polyadenosine diphosphate ribose polymerase inhibitors, resistance mechanisms

INTRODUCTION

Polyadenosine diphosphate (ADP) ribose polymerase (PARP) inhibitors have been approved by the US Food and Drug Administration (FDA) for the treatment of patients diagnosed with recurrent ovarian, primary peritoneal, and fallopian tube cancer who have received previous lines of chemotherapy and as maintenance therapy for patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who achieved complete or partial response to platinum-based chemotherapy. In 2019, olaparib also received FDA approval for use as a maintenance therapy after front-line chemotherapy for patients with a BRCA1 or BRCA2 mutations. In October 2019, new data were reported at the European Society for Medical Oncology (ESMO) meeting on three clinical trials that may lead to approval of additional ovarian cancer front-line treatment strategies. The PRIMA study evaluated platinum-based chemotherapy followed by niraparib maintenance, regardless of BRCA mutation status [1▪▪]. The VELIA study evaluated platinum-based chemotherapy with veliparib followed by veliparib maintenance, regardless of BRCA mutation status [2▪▪]. The PAOLA-1 study evaluated platinum-based chemotherapy with bevacizumab followed by bevacizumab with olaparib maintenance [3▪▪]. All three studies demonstrated very promising results that may lead to approval of additional strategies in front-line therapy of ovarian cancer including use of niraparib, veliparib and bevacizumab with olaparib as maintenance therapy.

However, despite these successes, the potential of PARP inhibitors in the management of all ovarian cancer patients is mitigated by the fact that ovarian

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Curr Opin Obstet Gynecol 2019, 31:000–000
DOI:10.1097/GCO.0000000000000600

KEY POINTS

- The successes of PARP inhibitors are mitigated by the fact that nearly all patients with initial sensitivity to PARP inhibition develop resistance over time.
- Acquisition of homologous recombination proficiency as a consequence of secondary genetic or epigenetic events that restores homologous recombination leads to PARP inhibitor resistance.
- Multiple other mechanisms of acquired resistance including loss of DNA end resection inhibition, DNA replication fork protection, increased drug efflux or induction of senescence or EMT have been described.
- Further research is needed to better understand what role these mechanisms play for clinical PARP inhibitor resistance.

cancers with intrinsic homologous recombination DNA repair proficiency do not respond as well as homologous recombination-deficient cancers [1▪▪–3▪▪]. Additionally, responses to PARP inhibitors are all too frequently transient in homologous recombination-deficient cancers and ultimately nearly all patients with initial sensitivity to PARP inhibition develop resistance to PARP inhibition over time [1▪▪–3▪▪].

The current review summarizes recently discovered resistance mechanisms and highlights the clinical relevance of these findings to date.

REVERSION MUTATIONS

PARP is an enzyme family that posttranslationally modifies its target proteins by conjugating polymeric chains of ADP-ribose (PARylation) during a number of cellular processes including DNA repair of single-stranded DNA breaks. PARP inhibitors demonstrate synthetic lethality in ovarian cancer cells with homologous recombination deficiency and clinical efficacy has been shown for ovarian cancers harboring deleterious germline or somatic BRCA mutations as well as in those that display a BRCA-like phenotype [1▪▪,2▪▪,3▪▪].

A key resistance mechanism to platinum-based chemotherapies and PARP inhibitors in *BRCA*-mutant cancers is the acquisition of *BRCA* reversion mutations that restore protein function. Reversion mutations are somatic base substitutions or insertions/deletions that are typically close to the primary protein-truncating mutation and restore the open reading frame of the gene and functional protein, switching the neoplastic cell from homologous recombination-deficient to proficient [4–6]. Reversion mutations in multiple homologous recombination pathway genes, including *BRCA1, BRCA2, RAD51C, RAD51D*, and *PALB2*, have been reported in ovarian, prostate, and breast carcinomas as a mechanism of acquired resistance to platinum-based chemotherapies and PARP inhibitors [7–12]. Recent studies have identified somatic *BRCA* reversion mutations in circulating cell-free DNA (cfDNA) in germline *BRCA* mutation carriers with ovarian cancer [13,14]. Moreover, a recent study also identified reversion mutations in cfDNA patients harboring somatic BRCA mutations [15▪]. Interestingly, these studies have shown polyclonality of multiple reversion mutations in a single patient illustrating the profound selection pressure of these tumors to restore BRCA protein activity and overcome PARP inhibitor sensitivity.

HYPOMORPHIC BRCA PROTEINS AND BRCA ALTERNATIVE SPLICING ISOFORMS

It is unclear whether all pathogenic *BRCA1* mutations have similar effects on the response to therapy. For example, mutations in the BRCT domains of BRCA1 often prevent proper protein folding, and misfolded proteins are subject to protease-mediated degradation [16–18]. Under PARP inhibitor selection pressure, the HSP90 protein interacts with and stabilizes mutant BRCA1 proteins. The stabilized C-terminal-truncated BRCA1 protein is semifunctional and retains the protein domains necessary to mediate interactions with PALB2–BRCA2–RAD51 [19,20]. Importantly, the mutant BRCA1 protein is capable of promoting RAD51 loading onto DNA following DNA damage and maintains a partial BRCA1 function under PARP inhibitor selection pressure [21].

Further, genetically engineered mouse models mimicking the two most common *BRCA1* founder mutations, *BRCA1 (185delAG)* and *BRCA1 (5382insC)*, suggest that some N-terminal BRCA1 mutations may have some residual activity in DNA damage response. These studies in mice show that both mutations predisposed animals to mammary tumors. However, *BRCA1 (185delAG)* tumors responded significantly worse to homologous recombination-targeted therapy than the *BRCA1 (5382insC)* tumors. *BRCA1 (185delAG)* tumor cells produce a RING-less BRCA1 protein. In preclinical experiments this RING-less structure led to PARP inhibitor resistance through its residual activity in the DNA damage response pathway by activating RAD51 [22]. Nevertheless, further validation of these findings in clinical samples will be required to further substantiate the clinical significance of RING-less BRCA1 proteins and the development of PARP inhibitor resistance.

Additionally, BRCA1 mRNA isoforms generated by alternative splicing that lack specific exons may

generate hypomorphic proteins with residual function. Recent studies provide preclinical evidence that BRCA1 splice isoforms lacking exon 11 are capable of producing truncated but hypomorphic proteins that have residual BRCA1 function. Importantly, ovarian cancer cells with BRCA1 splice isoforms lacking exon 11 may have a clonal selection and survival advantage under selection pressure of PARP inhibitor treatment. Intriguingly, analysis of clinical ovarian cancer samples indicate that exon 11 mutation carriers had worse overall survival when compared with nonexon 11 mutation carriers [23]. These findings suggest that exon 11 mutation carriers may be less sensitive to platinum-based chemotherapy because of residual BRCA1 function of the hypomorphic protein. Further correlative work is required to better understand the role of BRCA alternative splicing in clinical resistance to PARP inhibitor treatment.

LOSS OF RESECTION INHIBITION

Under normal circumstances 53BP1, a protein involved in nonhomologous end-joining (NHEJ), blocks homologous recombination by limiting DNA end resection, a process that generates single-stranded DNA at DNA double-stranded breaks. Notably, in its physiological function BRCA1 inhibits 53BP1, which is an important initial step to allow double-strand break repair to occur. Loss of BRCA1 prevents the release of 53BP1 from DNA ends and secures arrested DNA repair. However, loss of BRCA1 can be bypassed by concomitant loss of 53BP1 or loss of associated factors, such as RIF1, REV7, and Shieldin (SHLD). Recent studies suggest that a protein complex constituted of REV7, SHLD1, SHLD2, and SHLD3, is recruited to double-stranded breaks via SHLD3 in a 53BP1 and RIF1-dependent manner. Theoretically, loss of expression in any of these proteins blocking double-stranded break repair may promote homologous recombination even in the absence of a functional BRCA1 protein and confer PARP inhibitor resistance [24–34]. However, clinical validations of these findings beyond observations stemming from patient-derived xenograft models are still needed.

REPLICATION FORK PROTECTION

Upon replication stress (slowing or stalling of the replication fork), cells arrest, allowing time for repair. If the repair is successful, the cell reenters the cell cycle. However, in the case of insurmountable damage, cells undergo apoptosis. In addition to their role in homologous recombination, BRCA1 and BRCA2 are required for the protection of stalled replication forks [35]. In the absence of BRCA1/2, nucleases, such as MRE11 and MUS81 attack stalled replication forks, leading to fork collapse and chromosomal aberrations [36,37]. EZH2 and PTIP are involved in recruiting MUS81 and MRE11 to the stalled replication fork, respectively, and loss of EZH2 or PTIP may lead to decreased attack of stalled replication forks by MRE11 and MUS81, and thus to fork head protection in the absence of BRCA1/BRCA2 [38].

PARP inhibitors induce fork degradation of unprotected replication forks in BRCA1/BRCA2-mutated cells. In turn, protected replication forks may lead to PARP inhibitor resistance. In addition, the chromatin-remodeling factors SMARCAL1, ZRANB3, and HTLF induce fork reversal. Replication fork reversal is a key protective mechanism that allows forks to reverse their course when they encounter DNA lesions and resume DNA synthesis without chromosomal breakage. Fork remodeling by the chromatin-remodelers SMARCAL1, ZRANB3, and HLTF has been shown to be required for MRE11-dependent degradation of replication forks, and depletion of these factors leads to fork head protection and to PARP inhibitor resistance [39].

MUTATIONS IN POLYADENOSINE DIPHOSPHATE RIBOSE POLYMERASE AND POLYADENOSINE DIPHOSPHATE RIBOSE GLYCOHYDROLASE

Using CRISPR–Cas9 genome-wide mutagenesis screens, Pettitt *et al.* recently discovered that mutations both within and outside of the PARP1 DNA binding zinc finger domains cause PARP inhibitor resistance and alter PARP1 trapping. PARP trapping is a function very distinct from its other role in sensing single-stranded DNA breaks and mediating the recruitment of substrate proteins involved in DNA damage repair. Trapping of PARP1 on the damaged DNA leads to stalled replication forks. A PARP1 mutation observed in a tumor from a PARP inhibitor-resistant patient prevented PARP trapping, suggesting that PARP1 mutations that impair trapping could contribute to clinical PARP inhibitor resistance. Further studies will be necessary to validate the broader clinical relevance of these findings [40].

PARylation is the reversible posttranslational modification of proteins via the covalent addition of poly(ADP-ribose) (PAR) chains. PARylation is catalyzed by PAR polymerase (PARP) proteins and reversed by PAR glycohydrolase (PARG). In that respect, PARG works in the same direction as a PARP inhibitor by preventing PAR accumulation. Genetic screens in murine models identified loss of PARG as

a cause for PARP inhibitor resistance [41]. Loss of PARG partially restored PARylation in PARP inhibitor-treated cells. This restoration of PARylation diminished PARP1 trapping on the DNA and partially rescued PARP1-dependent DNA damage signaling. Further studies will be necessary to validate the clinical relevance of these findings.

POLYADENOSINE DIPHOSPHATE RIBOSE POLYMERASE INHIBITOR DRUG EFFLUX

Overexpression of P-glycoprotein efflux pumps is a common mechanism of resistance. In a murine model of BRCA1-mutated breast cancer, the majority of tumors that developed resistance to PARP inhibition showed increased cellular drug efflux caused by up-regulation of *Abcb1a/b* genes encoding P-glycoprotein efflux pumps [42]. Moreover, overexpression of P-glycoprotein efflux pumps has also been observed in a PARP inhibitor-resistant human ovarian cancer cell line. Interestingly, resistance was reversed by co-treatment with the P-glycoprotein inhibitors verapamil and elacridar [43]. Furthermore, recent evidence suggests that overexpression of P-glycoprotein efflux pumps are commonly seen in chemotherapy-treated ovarian and breast cancers because of chromosomal translocations involving the *Abcb1a/b* genes [44]. Nevertheless, the association between increased expression of P glycoprotein efflux pumps and resistance to PARP inhibitors has not yet been validated in clinical trial populations. Therefore, it remains to be seen whether co-administration of P-glycoprotein inhibitors with PARP inhibitor treatment may be a useful strategy to prevent PARP inhibitor resistance.

EPITHELIAL MESENCHYMAL TRANSITION

Preclinical studies using immunohistochemical analysis of epithelial to mesenchymal transition (EMT)-associated transcription factors, such as ZEB1, ZEB2, TWIST, and SNAIL suggest that resistance to PARP inhibition may be associated with epithelial to mesenchymal transition. However, further clinical studies are necessary to confirm the clinical relevance of these findings [45].

RE-EXPRESSION OF NORMAL OR MUTATED BRCA

Recent studies suggest regained BRCA expression as a potential mechanism of resistance to PARP inhibitor therapy whereby previously PARP inhibitor responsive tumors restore homologous recombination through regained BRCA expression driven by copy number-gain and/or upregulation of the remaining allele [46]. Studies with clinical specimens obtained at progression showed either regained BRCA1 expression as the result of a single-copy gain of the remaining allele (resulting in copy-neutral LOH of 17q) or restoration of BRCA expression through marked upregulation from the remaining single wild-type allele. In addition, a recent preclinical study found that PARP inhibitor-resistant cell line clones harbored amplification of a mutant BRCA2 allele that lead to increased expression of the truncated protein. Importantly, these changes led to rescued homologous recombination-mediated DNA repair [47]. However, as with many of the proposed resistance mechanisms, further clinical studies will be necessary to fully understand the clinical relevance of regained BRCA expression in PARP inhibitor resistance.

REVERSIBLE SENESCENCE

Senescence is a tumor suppression mechanism defined by stable proliferation arrest. Recent studies suggest that PARP inhibition and DNA repair triggers p53-independent ovarian cancer cell senescence defined by senescence-associated phenotypic hallmarks including DNA-SCARS, inflammatory secretome, Bcl-XL-mediated apoptosis resistance, and proliferation restriction via Chk2 and p21 (CDKN1A). The concept of senescence as irreversible remains controversial but recent preclinical studies suggest that that PARP inhibitor senescent cells reinitiate proliferation upon drug withdrawal, potentially explaining the requirement for sustained PARP inhibitor therapy in the clinic [48,49].

CONCLUSION

Ovarian cancer tumors likely exhibit increased clonal diversity and branching at progression and selection pressure under PARP inhibitor treatment facilitates the outgrowth of resistant clones. This article and other reviews [50▪] summarize recent discoveries on PARP inhibitor resistance mechanisms and suggest that multiple adaptive responses may exist in a tumor following PARP inhibitor treatment. Importantly, however, additional studies in large patient cohorts will be needed to clarify the clinical relevance of these different PARP inhibitor resistance mechanisms. Furthermore, obtaining tissue biopsies upon progression on PARP inhibitor therapy may provide valuable information to better understand, which of the many aforementioned potential resistance mechanisms play a predominant role in the evolution of clinical PARP inhibitor resistance. Moreover, assays aiming to understand PARP inhibitor resistance will need to assess allele-

specific mutations and copy number changes, as well as the expression of all genes critically involved in homologous recombination to help us understand how best to treat those patients that have failed PARP inhibition. A better understanding of PARP inhibitor resistance will allow researchers and clinicians to exploit therapeutic liabilities engendered by these adaptive responses and develop rational combination strategies that specifically target or reverse these compensatory signaling pathways.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
- ■■ of outstanding interest

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