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-