

Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer

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ABSTRACT

Approximately 50% of epithelial ovarian cancers (EOC) exhibit defective DNA repair via homologous recombination (HR) due to genetic and epigenetic alterations of HR pathway genes. Defective HR is an important therapeutic target in EOC as exemplified by the efficacy of platinum analogues in this disease, as well as the advent of PARP inhibitors, which exhibit synthetic lethality when applied to HR-deficient cells. Here, we describe the genotypic and phenotypic characteristics of HR-deficient EOCs, discuss current and emerging approaches for targeting these tumors, and present challenges associated with these approaches, focusing on development and overcoming resistance.

Significance: Defective DNA repair via HR is a pivotal vulnerability of EOC, particularly of the high-grade serous histologic subtype. Targeting defective HR offers the unique opportunity of exploiting molecular differences between tumor and normal cells, thereby inducing cancer-specific synthetic lethality; the promise and challenges of these approaches in ovarian cancer are discussed in this review. *Cancer Discov*; 5(11); 1137-54. ©2015 AACR.

INTRODUCTION

Epithelial ovarian cancer (EOC) remains the most lethal gynecologic malignancy and the fifth most frequent cause of cancer-related mortality in women in the United States (1). Approximately 75% of EOC patients are diagnosed with advanced disease, which is curable in only a minority of the cases, resulting in a modest 5-year overall survival rate of 20% to 30% (2, 3). The standard-of-care management of EOC consists of primary surgical cytoreduction followed by platinum-based chemotherapy (3, 4). Platinum analogues have been used to treat ovarian cancer since the late 1970s, when clinical trials demonstrated that cisplatin was capable

of achieving almost double the overall response rates and number of complete responses compared with nonplatinum agents (5, 6). Since then, platinum agents (initially cisplatin, then carboplatin, which is better tolerated but equally effective; ref. 7) have constituted the backbone of chemotherapy used in EOC and have defined the comparison arms for the majority of the clinical trials conducted in this disease. However, despite important advancements in the efficacy of platinum chemotherapy achieved by incorporation of taxanes (8) in the 1990s and by administration of chemotherapy via the i.p. route (9) in early 2000, the plateau of the survival curve has not changed appreciably (3, 8, 10–12), suggesting that alternative approaches are urgently needed.

Platinum analogues induce intrastrand and interstrand cross-links (ICL) between purine bases of the DNA. ICLs are extremely deleterious lesions that covalently tether both duplex DNA strands and pose formidable blocks to DNA repair (13). Repair of ICLs is dependent on both Fanconi anemia (FA) and BRCA proteins, which act in a common DNA repair pathway (also referred to as the FA–BRCA pathway) that involves homologous recombination (HR; refs. 14, 15; Fig. 1). The striking platinum sensitivity of EOC tumors is thought to be related to an underlying defect in HR-mediated DNA repair, particularly in those with high-grade serous histology (approximately 70% of all EOCs). In this regard, a plethora of genetic studies, and most recently The Cancer Genome Atlas (TCGA) project, have consistently shown that high-grade serous ovarian cancers (HGSO) are characterized

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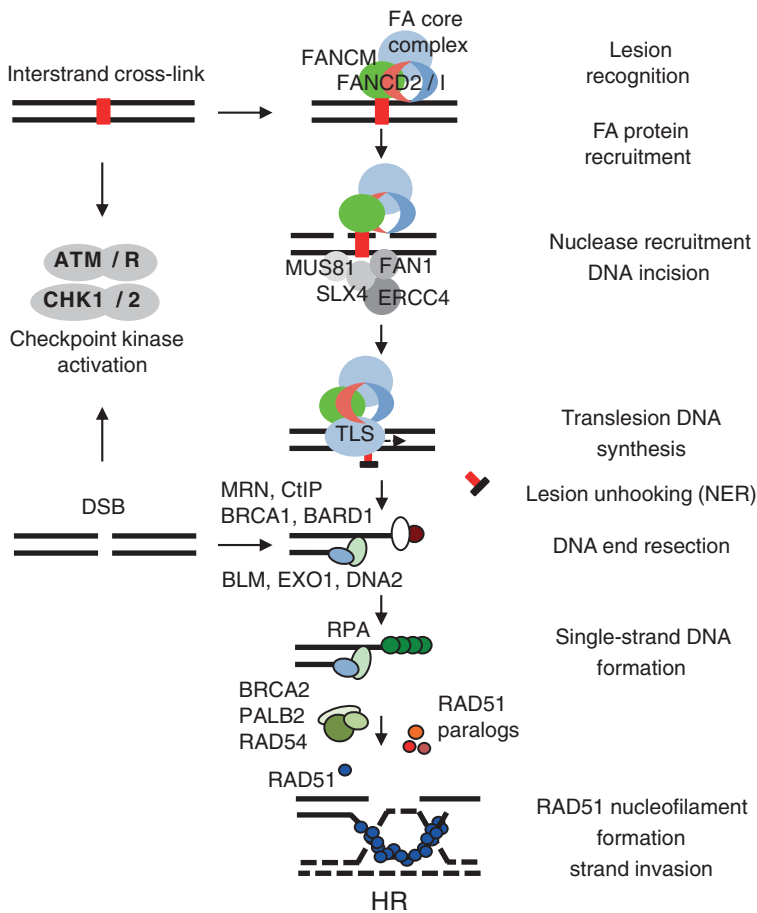


Figure 1. Cooperation of the FA and BRCA1/2 proteins in a common ICL repair pathway. Stalling of replication forks on DNA ICLs induces lesion recognition by the FANCM–FAAP24–MHF1/2 complex and subsequent recruitment of the FA core complex, which, in turn, recruits the mono-ubiquitinated FANCD2–FANCI to the ICL region. FANCM also initiates checkpoint response, which phosphorylates multiple FA proteins. Ubiquitinated FANCD2 acts as a landing pad for recruiting several nucleases to coordinate nucleolytic incisions. Unhooking the DNA leaves the cross-linked nucleotides tethered to the complementary strand, which are bypassed by translesion synthesis (TLS) polymerases. DNA incisions create a DSB, which is then repaired by HR. Downstream FA proteins, such as BRCA1, BRCA2, and PALB2, promote RAD51-dependent strand invasion and resolution of recombinant intermediates.

by frequent genetic and epigenetic alterations of HR pathway genes, most commonly the *BRCA1* and *BRCA2* genes (16, 17). Defective HR is an important therapeutic target in EOC, as exemplified by the central role of platinum agents in the management of this disease as well as the advent of PARP inhibitors (PARPi), a novel class of anticancer agents that exhibit synthetic lethal effects when applied to cells with defective HR (18–21). In this review, we discuss the molecular alterations and clinical phenotype of HR-deficient EOCs, describe current and emerging approaches for targeting HR-deficient ovarian cancers, and present the challenges associated with these approaches focusing on development and overcoming drug resistance.

HR PATHWAY ALTERATIONS IN EOC

Approximately 50% of HGSOCs exhibit genetic or epigenetic alterations in the FA–BRCA pathway (Fig. 2; ref. 16). Although these alterations are most commonly encountered in high-grade serous histology, nonserous histologies, including clear-cell, endometrioid, and carcinosarcomas, have also been shown to harbor such alterations (22). Germline *BRCA1* and *BRCA2* mutations are the most common alterations and are present in 14% to 15% of all EOCs (23, 24) and as high as 22.6% of HGSOCs (16, 23, 24), whereas somatic *BRCA1* and *BRCA2* mutations have been identified in 6% to 7% of high-grade serous EOCs (16, 25). Although in the TCGA

dataset, there was a similar incidence of germline and somatic *BRCA1* and *BRCA2* mutations, *BRCA1* mutations are more commonly observed (60% of all *BRCA* mutations) in other datasets (23, 24). Importantly, 81% of *BRCA1* and 72% of *BRCA2* mutations are accompanied by heterozygous loss (16), indicating that both alleles are inactivated, as predicted by Knudson’s two-hit hypothesis. The majority of germline and somatic *BRCA1/2* mutations are frameshift insertions or deletions, whereas missense mutations are rare; mutations have been identified in all functional domains of *BRCA1* [RING, coiled-coil, and BRCA C-terminal (BRCT) domains] and *BRCA2* (BRC, DNA binding, oligonucleotide-binding folds, and tower domains) genes (26).

Epigenetic silencing via promoter hypermethylation occurs for *BRCA1*, but not *BRCA2*, in EOC. *BRCA1* promoter hypermethylation has been reported in approximately 10% to 20% of HGSOCs and is mutually exclusive of *BRCA1/2* mutations, suggesting that there is strong selective pressure to inactivate *BRCA* via either mutation or epigenetic silencing in this disease (16, 27, 28). Other HR pathway alterations include mutations in several FA genes (mainly *PALB2*, *FANCA*, *FANCI*, *FANCL*, and *FANCC*), in core HR RAD genes, such as *RAD50*, *RAD51*, *RAD51C*, and *RAD54L*, and in DNA damage response genes involved in HR, such as *ATM*, *ATR*, *CHEK1*, and *CHEK2* (Fig. 2). Interestingly, pathogenic germline *RAD51C* and *RAD51D* mutations have been identified in families with both breast and ovarian tumors but not in families with

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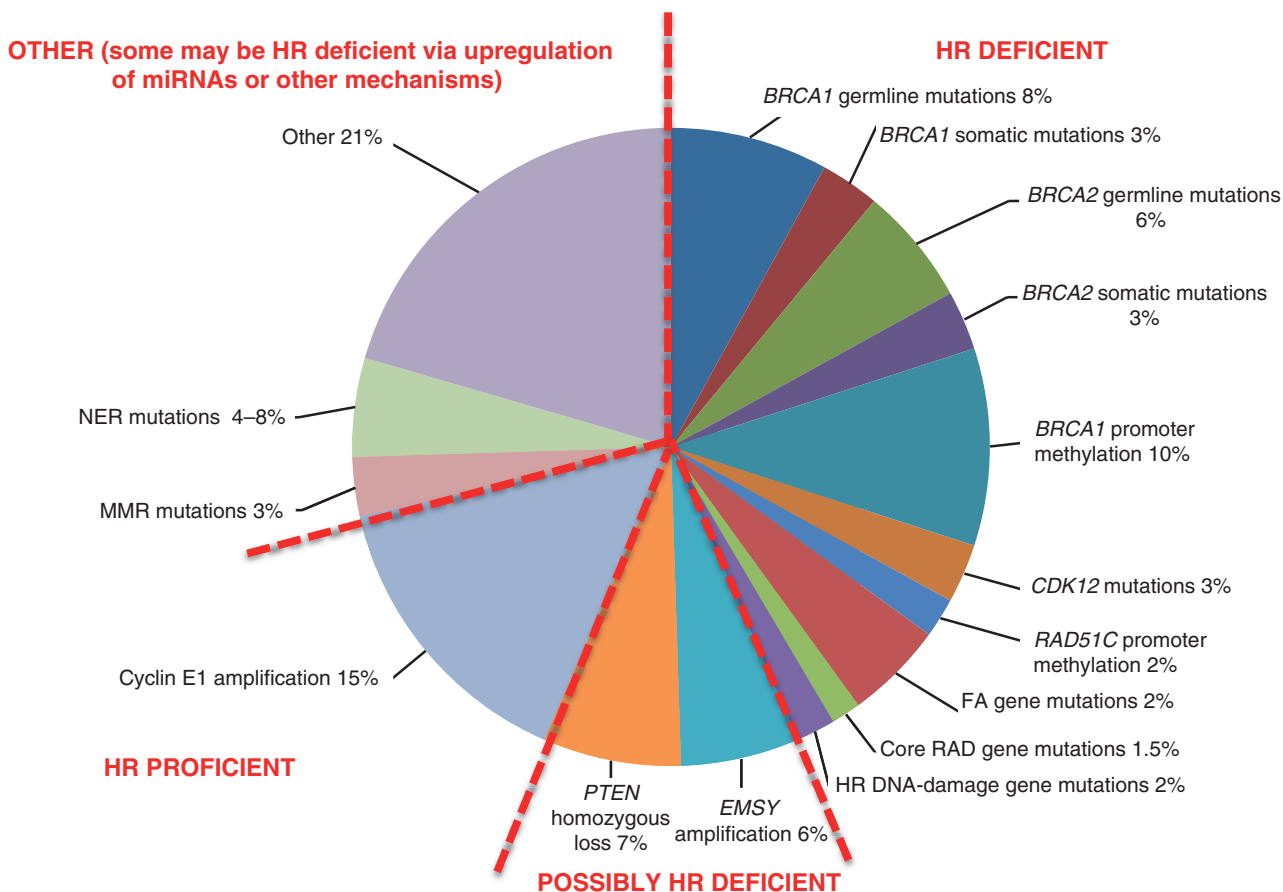


Figure 2. Approximately 50% of high-grade serous EOCs have alterations in HR repair genes. Frequency of genetic and epigenetic changes involving HR pathway genes or non-HR pathway genes that modulate HR pathway. FA/BRCA pathway alterations have been experimentally found to be associated with HR deficiency (HR-deficient tumors on the right). *PTEN* deletion and *EMSY* amplification have been reported to confer HR deficiency, but data are evolving (possibly HR-deficient tumors on the bottom). Tumors with cyclin E1 (*CCNE1*) amplification are enriched for HR proficiency (HR-proficient tumors on the left) and are associated with inferior outcome and response to platinum-based chemotherapy. Among the remaining tumors, some may be HR deficient via miRNA upregulation or other unknown mechanisms.

only breast cancer (29, 30). *RAD51C* was also epigenetically silenced via promoter hypermethylation in about 2% of the cases in the TCGA dataset.

Defective HR in EOC may also occur via alterations in non-*bona fide* HR genes that are known to modulate the HR pathway and indirectly cause HR deficiency. *PTEN* deficiency has been reported to be synthetically lethal with PARP inhibition, and one of the proposed mechanisms is transcriptional downregulation of *RAD51* (31, 32). A focal deletion region at 10q23.31 that includes only *PTEN* has been found in approximately 7% of high-grade serous EOCs; these tumors exhibit homozygous *PTEN* deletion, which is also associated with downregulation of *PTEN* at the mRNA level (16). Furthermore, several studies have reported both overexpression and amplification of *EMSY* as another mechanism of HR deficiency in as high as 17% of high-grade sporadic EOC (33). *EMSY* was identified in a yeast two-hybrid screen to interact with the transactivation domain of *BRCA2*, leading to inhibition of its transcriptional activity (33). *EMSY* also colocalizes with *BRCA2* at DNA-damage sites and interacts

with several chromatin-remodeling proteins. However, *EMSY* is located at 11q13, a region known to be amplified in multiple cancers that contains multiple different oncogenes, including *LRR32* (*GARP*) and *PAK112* (34). In addition, *EMSY* amplification has been associated with worse outcome (34), a finding that would be inconsistent if it caused HR deficiency. For these reasons, although *EMSY* alterations are commonly cited as a mechanism underlying deficient HR, its role remains controversial. Unlike *PTEN* and *EMSY*, the association between inactivating mutations of *CDK12* and HR deficiency is clearly established (35, 36). *CDK12* is one of the only 9 significantly mutated genes in ovarian cancer (3% of cases in the TCGA dataset) and is known to promote the transcription of several HR pathway genes, including *BRCA1*. Inactivation of *CDK12* leads to suppression of HR via reduced expression of *BRCA1* and other HR genes, and confers PARPi sensitivity.

It is important to underscore that there may be additional mechanisms underlying defective HR in EOC. Overexpression of specific miRNAs that induce HR deficiency has been

identified in breast cancer (such as mir-182, which targets *BRCA1*; ref. 37), and analogous miRNAs have also been identified in ovarian cancer (such as miR-1255b, miR-148b*, and miR-193b*, which target *BRCA1*, *BRCA2*, and *RAD51*; ref. 38). Finally, alterations in other DNA repair pathways, such as nucleotide excision repair (NER) and mismatch repair (MMR), have been reported in up to 8% and 3% of high-grade serous EOCs, respectively (Fig. 2; ref. 39).

CLINICAL PHENOTYPE OF HR-DEFICIENT EOC

Several studies have highlighted a distinct clinical phenotype associated with HR-deficient cancers, especially those with *BRCA1/2* mutations. Patients with germline *BRCA1/2* mutations are associated with the hereditary breast/ovarian cancer syndrome, which is characterized by familial clustering of breast and ovarian tumors (40). This syndrome has also been linked to germline mutations in other HR genes, such as *BARD1*, *BRIP1*, *MRE11A*, *NBN*, *RAD50*, *CHEK2*, *ATM*, *PALB2*, *RAD51C*, and *RAD51D*, although the exact penetrance of these genes in terms of breast and/or ovarian cancer remains unknown (22). Large studies have also consistently demonstrated that patients with *BRCA1/2*-mutated ovarian cancers exhibit significantly improved overall survival compared with patients with non-*BRCA*-mutated tumors; this effect is more pronounced for *BRCA2* mutation carriers who exhibit even longer survival compared with *BRCA1* carriers (16, 41, 42). Interestingly, the survival advantage of *BRCA1* carriers was shown to be dependent on the location of the mutation; worse survival was observed as the mutation site moved from 5' to 3' end of the *BRCA1* gene (41). Unlike *BRCA1/2* mutations, EOCs with epigenetic silencing of *BRCA1* through promoter hypermethylation appear not to respond as favorably to platinum and not to exhibit improved survival, suggesting that different mechanisms of HR deficiency may confer distinct clinical phenotypes (16, 43). The survival advantage of *BRCA*-mutated tumors is at least partly related to their enhanced responsiveness to platinum-based chemotherapy, although a more indolent natural history due to intrinsic biologic differences compared with non-*BRCA*-mutated tumors may also play a role. In this regard, although available data suggest that HR deficiency may be both a predictive factor of response to first-line platinum chemotherapy and a prognostic factor in EOC, it is unclear whether the prognostic significance of HR deficiency in EOC is solely due to its association with increased sensitivity to chemotherapy or due to other independent factors. For example, several lines of evidence indicate that *BRCA1/2*-mutated tumors may harbor more tumor-infiltrating lymphocytes and thus be more immunogenic compared with HR-proficient EOCs, which may relate to a greater number of mutations observed in these tumors (44, 45). In this regard, the increased immunogenicity of HR-deficient tumors may explain their prognostic significance independent of their predictive association with response to first-line chemotherapy. Whether additional mechanisms may explain the improved survival of HR-deficient tumors independently of their enhanced sensitivity to chemotherapy remains to be determined.

BRCA1/2-mutated tumors are also associated with higher-grade (grade 2 or grade 3), poorly differentiated, or undiffer-

entiated tumors, higher stage (stage III or IV) at presentation, and serous histology (as opposed to endometrioid, clear cell, or mucinous histologies; refs. 23, 24, 41, 46). Finally, in terms of pattern of recurrence, *BRCA1/2*-mutated tumors are more likely to develop visceral metastases (parenchymal liver, lung, adrenal, spleen, and brain metastases), and this effect appears more prominent for *BRCA1*-mutated tumors (23, 47). These clinical features may be at least partly related to the high degree of genomic instability that is characteristic of *BRCA1/2*-mutated tumors.

BIOMARKERS OF HR DEFICIENCY

Development of a robust biomarker that adequately captures the diverse genetic and epigenetic mechanisms of HR deficiency and is compatible with formalin-fixed and paraffin-embedded (FFPE) specimens remains elusive. Several approaches have been proposed, including application of gene expression profiles of BRCAness (48) or DNA repair (49), evaluating *BRCA1* protein expression by immunohistochemistry (50), and assessing the wider tumor genome nucleotide sequences and mutational spectrums, or “sequence scars,” that may be characteristic of defective DNA repair via HR (51). Targeted mutational profiling of HR genes using next-generation sequencing has also been evaluated. BROCA is a targeted capture and massively parallel sequencing assay that accurately identifies all types of mutations of key HR genes, including single-base substitutions, small insertions and deletions, and large gene rearrangements (22, 52). Identification of HR gene mutations by BROCA is highly predictive of improved primary response to platinum chemotherapy and longer overall survival in EOC (22). Alternative multi-gene, next-generation sequencing assays are also offered in several cancer centers in the United States and routinely include assessment of core HR genes (53).

HR-deficient tumors exhibit large (>15 Mb) subchromosomal deletions and harbor allelic imbalance extending to the telomeric end of the chromosomes with or without changes in overall DNA copy number. Recently, three quantitative metrics of these structural chromosomal aberrations have been developed using SNP array data. These include (i) the whole-genome tumor LOH score (54), (ii) the telomeric allelic imbalance (TAI) score (55), and (iii) the large-scale state transitions (LST) score, which quantifies chromosomal breaks between adjacent regions of at least 10 Mb (56). All three scores are highly correlated with alterations in *BRCA1/2* and other HR pathway genes in ovarian cancer, and with sensitivity to platinum and PARPis (57). These scores have been implemented either alone or in combination with targeted sequencing approaches such as the BROCA assay to achieve better sensitivity in capturing HR deficiency (57). Of note, genomic LOH was recently shown to correlate well with response to the PARPi rucaparib in a phase II clinical trial in EOC (ARIEL2). Specifically, among the women without the *BRCA1/2* mutations, those with high genomic LOH had an overall response rate of between 32% and 40%, whereas those without LOH had an overall response rate of just 8% (58).

A major limitation of these assays is that they are largely insensitive to reversion of HR deficiency, which may occur upon development of resistance to platinum and PARPis.

When reversion of HR deficiency to HR proficiency occurs, the cumulative defects that had occurred in the cancer genome as the result of the original HR deficiency do not reverse; therefore, these assays still interpret these HR-proficient tumors as HR deficient. This phenomenon has been observed in *BRCA1/2*-mutated cell lines with *BRCA1/2* reversion mutations, which restore *BRCA1/2* and HR function; these lines are still interpreted as HR deficient by the aforementioned assays (54). One way to overcome this problem is by development of dynamic, functional biomarkers of HR deficiency, whereby the HR pathway is mechanistically evaluated by directly assessing RAD51 foci formation via immunofluorescence or by assessing other DNA repair complexes via immunohistochemistry (59–61). The challenge of functional biomarkers of HR deficiency is that they require the cancer specimen to be exposed to some form of DNA damage (i.e., radiation or chemotherapy) *ex vivo* before the RAD51 foci or other DNA repair complexes can be evaluated. This requirement precludes use of FFPE specimens, increases the technical complexity, and limits the reproducibility of these assays. Overall, there is currently no prospectively validated biomarker of HR deficiency that has been incorporated in clinical practice, and this remains an active area of investigation.

TARGETING HR-DEFICIENT TUMORS

Conventional Chemotherapy

A number of conventional chemotherapy agents that are used routinely in the management of EOC exhibit significant cytotoxicity against HR-deficient tumors. Platinum analogues, which have formed the backbone of first-line chemotherapy of EOC for more than 30 years, induce ICLs that are highly lethal against tumors with defective HR (Fig. 1). The integral role of platinum-based chemotherapy in the clinical management of EOC is further evident by the fact that management of relapsed disease is stratified based on the platinum-free interval (PFI; i.e., the time between completion of platinum-based treatment and the detection of relapse; PFI \geq 6 months is assigned as platinum-sensitive and PFI $<$ 6 months as platinum-resistant disease). Clinically, patients with *BRCA1/2*-mutated tumors are associated with significantly higher response rates and prolonged progression-free survival (PFS) after platinum-based chemotherapy (16, 41, 62). These patients commonly exhibit good responses after retreatment with platinum upon development of recurrence, and many of them end up receiving multiple lines of platinum chemotherapy. Of note, the enhanced sensitivity of *BRCA*-mutated tumors to platinum agents challenges the traditional clinical definition of platinum resistance because many of these patients respond well to platinum rechallenge even within 6 months from the end of first-line platinum therapy (23).

The high correlation between HR deficiency and response to platinum chemotherapy is also highlighted by the fact that development of platinum resistance is commonly related to restoration of proficient HR via various mechanisms (discussed in more detail below). Platinum sensitivity has been used as a clinical surrogate of HR deficiency, and clinical trials of PARPis have used platinum sensitivity as an eligi-

bility criterion for selecting patients who are enriched for HR-deficient tumors that would respond to PARPis (63, 64). However, it is important to underscore that platinum sensitivity may also result from defective NER, and in that case, it does not necessarily translate into PARPi sensitivity (39).

Nonplatinum cytotoxic agents that induce double-strand breaks (DSB) have also been shown to be active against HR-deficient tumors. Topotecan, a semisynthetic water-soluble camptothecin analogue, is FDA approved for recurrent ovarian cancer and has demonstrated response rates ranging from 13% to 33% in phase II trials depending on platinum sensitivity (65, 66). In a phase III trial, topotecan demonstrated an overall objective response rate (complete response plus partial response) of 17.0% (28.8% in platinum-sensitive and 6.5% in the platinum-resistant/refractory disease; ref. 65). Topotecan inhibits the religation step of the breakage/reunion reaction of topoisomerase I (TopI), resulting in accumulation of topotecan–TopI–DNA covalent complexes that are converted to DNA DSBs when replication forks encounter the single-strand breaks (SSB). Studies in yeast have demonstrated that the DSBs induced by TopI inhibitors are repaired by HR during S phase (67); the nonhomologous end joining (NHEJ) pathway is significantly less involved in repair of TopI inhibitor–induced DSBs (68).

Similar to TopI inhibitors, topoisomerase II (TopII) inhibitors, such as doxorubicin and etoposide, are also more active in HR-deficient cells and are routinely used in the management of relapsed EOC [pegylated liposomal doxorubicin (PLD) is also FDA-approved for this indication; refs. 65, 66, 69]. TopII (Top2a and b) cleaves both strands of one DNA duplex simultaneously and forms transient tyrosyl–DNA cleavage complex intermediates to allow another duplex to pass through the TopII-linked DSB; TopII generates DSBs in cycling cells especially during mitosis phase, in which both HR and NHEJ are available for repair (68). Etoposide and doxorubicin are TopII poisons which inhibit the religation step of the breakage/reunion reaction of TopII and trap the TopII cleavage complex intermediates. Etoposide is a nonintercalating drug that acts mainly as a TopII trap, whereas doxorubicin is an intercalator that not only traps TopII but also kills cells by intercalation and generation of oxygen radicals (70). Although etoposide and doxorubicin are more active in HR-deficient cells, one striking difference from TopI inhibitors is that the NHEJ pathway is significantly more involved in repair of DSBs induced by TopII than TopI inhibitors. Consistent with their enhanced activity in HR-deficient cells, the activity of both etoposide and doxorubicin is much higher in the platinum-sensitive compared with the platinum-resistant setting (65, 69). Furthermore, treatment of *BRCA*-associated EOC patients with PLD has been shown to result in higher response rates, longer time to treatment failure, and improved overall survival compared with non-*BRCA*-mutated patients (71).

Finally, HR-deficient cells are also sensitive to antimetabolites that induce base lesions and/or replication fork stalling, such as gemcitabine. Gemcitabine is a nucleoside analogue whose metabolites (diphosphate and triphosphate nucleosides) facilitate incorporation of gemcitabine nucleotide into DNA, which blocks further extension of the nascent strand and causes stalling of replication forks. Furthermore, gemcitabine irreversibly inhibits the ribonucleotide reductase enzyme,

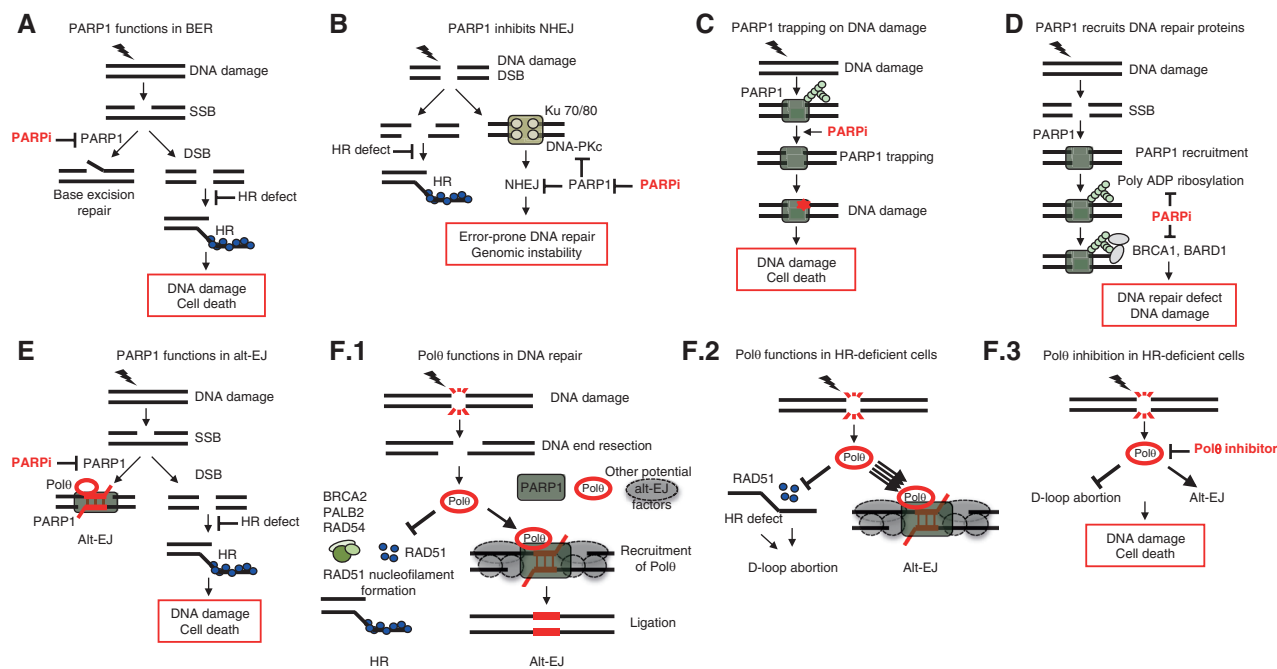


Figure 3. Mechanisms of synthetic lethality between PARP1 or POLθ inhibition and HR deficiency. Inhibition of PARP1 activity in base excision repair (BER; **A**) and classic NHEJ (C-NHEJ; **B**) is toxic in HR-deficient cells and explains the observed PARPi–HR synthetic lethality. **C**, PARPis induce PARP1 trapping on DNA lesions, which is highly toxic in HR-deficient cells. **D**, PAR-mediated recruitment of the BARD1–BRCA1 complex is impaired by PARPi, resulting in the persistence of DNA lesions that are toxic to HR-deficient cells. **E**, inhibition of PARP1/POLθ-mediated alternative end joining (alt-EJ) is toxic in HR-deficient cells. **F.1**, under physiologic conditions, Polθ expression is low and its impact on the repair of DNA DSBs is limited. Polθ limits RAD51–ssDNA filament assembly and subsequent HR activity; at the same time, it promotes alt-EJ through its polymerase domain. **F.2**, upon an HR defect, Polθ expression increases substantially and channels DSB repair into alt-EJ. **F.3**, in the case of an HR defect, inhibition of Polθ causes cell death through the persistence of toxic RAD51 intermediates and inhibition of alt-EJ.

leading to cells’ inability to produce the deoxyribonucleotides required for DNA replication and repair, and thus inducing apoptosis. Gemcitabine is currently FDA approved in combination with carboplatin for the treatment of EOC that has relapsed at least 6 months after completion of platinum-based therapy (i.e., in platinum-sensitive disease; ref. 72). However, gemcitabine has also been studied and is one of the standard treatment options as a single agent in platinum-resistant disease, although the response is less in that setting (73).

PARP Inhibition as a Synthetic Lethal Strategy against HR-Deficient Cancers

Mechanism of Action of PARPis

HR-deficient cells have been shown to be extremely sensitive to PARPis (18, 74, 75). Different aspects of PARP1 biology have been proposed to explain the synthetic lethal interaction between PARPi and HR deficiency, but the mechanistic basis is incompletely understood (76).

Because PARP1 was originally shown to be essential for base excision repair (BER; refs. 77, 78), preventing the repair of DNA SSBs by PARPis (normally repaired by BER) would convert them into the more cytotoxic DSBs that are normally repaired by HR (79). In that scenario, an HR-proficient cell will repair these DSBs by HR, whereas these lesions will remain unrepaired and cause cytotoxicity in HR-deficient cells (Fig. 3A; refs. 80, 81). However, some findings do not

fit with this model; for instance, knockdown of XRCC1, a downstream effector of PARP1 in BER, does not affect the survival of HR-deficient cells, suggesting that BER activity is not critical for HR-deficient cell survival (82).

Another proposed explanation for the PARPi–HR synthetic lethality pertains to the role of PARP1 in limiting classic NHEJ (C-NHEJ) repair activity (82, 83). C-NHEJ is error-prone and induces genomic instability, which is believed to be particularly deleterious for HR-deficient cells (Fig. 3B). Thus, PARPi-mediated inhibition of PARP1 would promote C-NHEJ and genome instability. Experimental evidence supporting this model is found in studies showing that the genomic instability induced by PARPi treatment in HR-deficient cells is reduced by concomitant inhibition of DNA-PK, a critical factor of C-NHEJ (18, 82). Several C-NHEJ proteins, such as KU70, KU80, and DNA-PKcs, bind to poly(ADP ribose) polymers that are generated by PARP enzymes, and these interactions are critical for suppressing C-NHEJ (84–88); furthermore, PARP1 and KU80 compete for DNA ends *in vitro* (82). Even though the role of PARP1 in limiting C-NHEJ is now well established, the link between PARPi-mediated C-NHEJ activation and the HR-deficient/PARPi synthetic lethal interaction remains to be fully elucidated.

It has also been suggested that the extreme sensitivity of HR-deficient cells to PARPi might result from the trapping of PARP1 at sites of endogenous damage (Fig. 3C; ref. 89). When DNA damage activates PARP1 (90), the PAR-dependent

recruitment of additional repair proteins (91–93) simultaneously reduces PARP1 affinity for DNA (94), thereby ensuring tight control of the repair process. Because mutant PARP1 that is unable to synthesize poly(ADP ribose) polymers has been shown to be trapped on DNA and to inhibit DNA repair (94), PARPi-mediated inactivation of PARP1 may likewise induce PARP1 trapping and inhibition of DNA repair. Accordingly, recent studies have demonstrated that PARPi-mediated trapping of PARP1–DNA complexes showed higher cytotoxicity than unrepaired SSBs caused by knockdown of PARP1 (89). This mechanism may explain the cytotoxic effect of certain drug combinations, such as PARPi and temozolomide or topotecan (95–97), and is believed to account for the observation that PARP1 knockdown selectively kills HR-deficient cells (18, 75, 82), as these PARP-trapping-induced DNA lesions are thought to be mostly toxic in an HR-deficient setting.

An alternative model might provide an explanation for the cytotoxicity of PARPis in certain BRCA1-deficient cells. DNA damage activates PARP1 (90), which in turn poly(ADP-ribosyl)ates or PARYlates many proteins at DNA break sites to orchestrate repair (92). During the HR process, BRCA1 is recruited to damage sites by the PAR- and BRCA1-binding protein BARD1 (98). Indeed, the BARD1 PAR-binding domain is critical for BRCA1 localization to DNA-damage sites, particularly when the additional mode of BRCA1 recruitment through γ H2AX binding is impaired (for instance in certain BRCA1-mutated tumors). Although this model (Fig. 3D) may explain the PARPi hypersensitivity of certain BRCA1-mutated tumors, it cannot be expanded to other HR-deficient contexts to serve as a global mechanism for PARPi sensitivity.

A clue in this regard is the recent finding that HR-deficient cells are dependent on the alternative end joining (alt-EJ) DSB repair pathway for survival. Inhibition of proteins functioning in alt-EJ, such as PARP1 or the polymerase Pol θ , is synthetically lethal with defective HR. Thus, the HR-deficiency/PARPi synthetic lethality likely stems from the simultaneous loss of HR and alt-EJ (Fig. 3E, also discussed below; refs. 99, 100). Finally, some PARPis likely inhibit all PARP family members, and the observed synthetic lethality could arise from a compound effect, not solely that of PARP1 inhibition.

PARPis in the Clinical Management of HR-Deficient Ovarian Cancers

PARPis, including olaparib (AZD2281), rucaparib (CO338, AG014699, and PF01367338), veliparib (ABT888), and niraparib (MK4827), have been extensively studied in EOC (101). Niraparib was also initially evaluated, but it is now clear that it exhibits very low PARP inhibition *in vitro*, and its mechanism of action *in vivo* remains to be elucidated. All aforementioned PARPis inhibit PARP1 and PARP2 *in vitro* at nanomolar concentrations but differ in their ability to trap PARP1 and PARP2 on the DNA SSB sites; niraparib and the newer PARPi BMN673 exhibit higher potency in trapping PARPs than olaparib and rucaparib, whereas veliparib is the least potent of all PARPis in terms of its PARP-trapping ability (89). In December 2014, olaparib was granted accelerated approval by the FDA for use in EOC patients with germline BRCA1/2 mutations who have received three or more chemotherapy

regimens, based on the results of an international single-arm trial that demonstrated an objective response rate (ORR) of 34% and a median duration of response of 7.9 months (19, 102–104). Olaparib was previously approved in Europe (in October 2014) by the European Medicines Agency (EMA) for a different indication, i.e., for use in the maintenance treatment of patients with platinum-sensitive relapsed BRCA-mutated (germline and/or somatic) high-grade serous EOC who had a complete or partial response to platinum-based chemotherapy. This approval by the EMA was based on a randomized, double-blind, phase II clinical trial that showed that olaparib maintenance therapy significantly prolonged PFS, compared with placebo, in patients with BRCA-mutated (germline or somatic) ovarian cancer with an HR of 0.18 (64, 105). Strikingly, 40% of patients with BRCA-mutated tumors that were treated with olaparib derived long-term benefit, without developing progressive disease for at least 3 years after randomization. Furthermore, exposure to olaparib did not decrease subsequent sensitivity to platinum or other chemotherapies in BRCA1/2-mutated tumors; upon development of PARPi resistance, subsequent response to platinum-based chemotherapy has been reported to be as high as 40% by RECIST (106). Randomized phase III trials of maintenance niraparib, rucaparib, and olaparib are currently ongoing in patients with high-grade serous ovarian cancer who had demonstrated a response and platinum sensitivity for both the ultimate and the penultimate platinum regimens (Table 1).

PARPis have also demonstrated activity in non-BRCA-mutated EOC patients, although they are not approved for these patients in any setting, either in the United States or Europe. This is consistent with the fact that HR deficiency may occur in EOC via multiple mechanisms in the absence of BRCA1/2 mutations (Fig. 2). In the aforementioned phase II study of olaparib maintenance, patients whose tumors lacked a BRCA1/2 mutation also derived a benefit from olaparib with an HR for PFS of 0.53 (64, 105). Furthermore, in a phase II study of high-grade serous EOC with unknown/nonmutated BRCA status, olaparib was associated with a 24% ORR and a 30% combined RECIST or CA125 response rate (104). Olaparib sensitivity was higher in platinum-sensitive compared with platinum-resistant non-BRCA-mutated tumors; 50% of non-BRCA-mutated platinum-sensitive tumors responded to olaparib as compared with only 4% of non-BRCA-mutated platinum-resistant tumors, suggesting that platinum sensitivity may be a good surrogate of HR deficiency and PARPi response among non-BRCA-mutated EOCs. A similar correlation between olaparib and platinum sensitivity has also been found for BRCA-mutated tumors, but the difference is less pronounced (ref. 104; 60% among platinum-sensitive vs. 33% among platinum-resistant BRCA-mutated tumors), suggesting that platinum resistance cannot be used as an exclusion criterion for PARPi therapy in BRCA-mutated cancers because these tumors may be PARPi sensitive even if they are platinum resistant. PARPi studies investigating candidate biomarkers of PARPi response are currently being performed in non-BRCA-mutated EOCs.

Finally, combinations of PARPis with conventional chemotherapy, such as platinum compounds and topoisomerase inhibitors, have been explored in BRCA-mutated EOCs (107–109).

Table 1. Important previous and ongoing phase II/III studies of PARPis in EOC

Setting	Study identifier	Agents	Design	Patients/accrual	Primary endpoint	Results/status
First-line/maintenance	NCT01844986	Olaparib vs. placebo	Phase III, randomized, double-blind, placebo controlled	BRCAm high-grade serous or high-grade endometrioid ovarian cancer N = 397	PFS	Ongoing
Recurrent/maintenance	NCT00753545	Olaparib vs. placebo	Phase II, randomized, double-blind, placebo controlled	Recurrent platinum-sensitive high-grade serous N = 265	PFS	BRCAm PFS: 11.2 vs. 4.3 months; HR, 0.18 Non-BRCAm PFS: 7.4 vs. 5.5 months; HR, 0.54
Recurrent/maintenance	NCT01847274	Niraparib vs. placebo	Phase III, randomized, double-blind, placebo controlled	Platinum-sensitive recurrent high-grade serous endometrioid/ BRCA stratified N = 490	PFS	Ongoing
Recurrent/maintenance	NCT01874353	Olaparib vs. placebo	Phase III, randomized, double-blind, placebo controlled	Recurrent platinum-sensitive BRCAm high-grade serous or endometrioid EOC N = 297	PFS	Ongoing
Recurrent/maintenance	NCT01968213	Rucaparib vs. placebo	Phase III, randomized, double-blind, placebo controlled	Platinum-sensitive recurrent high-grade serous endometrioid/ BRCA stratified N = 540	PFS	Ongoing
Recurrent/maintenance	NCT01081951	Olaparib/Carbo/Taxol vs. Carbo/Taxol	Randomized phase II, open-label	Platinum-sensitive recurrent HGSOE (both BRCAm and non-BRCAm) N = 173	PFS	ALL: 12.2 vs. 9.6 months; HR, 0.51 BRCAm: HR, 0.21
Recurrent	NCT01116648	Olaparib/cediraniib vs. olaparib	Randomized phase II, open-label	Platinum-sensitive recurrent HGSOE (both BRCAm and non-BRCAm) N = 90	PFS	ALL: 17.7 vs. 9.0 months; HR, 0.42 BRCAm: 19.4 vs. 16.5 months; HR, 0.55 non-BRCAm: 16.5 vs. 5.7 months; HR, 0.32

NOTE: ALL refers to all patients in the study, both BRCA-mutated and non-BRCA-mutated.

Abbreviation: BRCAm, BRCA-mutated.

Given that PARPis inhibit BER, which is partly responsible for repair of the damage caused by these chemotherapy agents, addition of PARPis may potentiate the action of these agents. However, when PARPis are combined with chemotherapy, achievement of full-dose chemotherapy has been challenging because of the overlapping myelosuppression of

PARPis and chemotherapy (110). In a recently reported randomized, open-label, phase II study, in patients with platinum-sensitive, recurrent, high-grade serous ovarian cancer who had received up to three previous courses of platinum-based chemotherapy, olaparib plus paclitaxel and carboplatin (at lower than standard doses) followed by maintenance olaparib

monotherapy significantly improved PFS versus paclitaxel plus carboplatin alone (given at their standard doses), with the greatest clinical benefit in *BRCA*-mutated tumors (PFS HR, 0.22), and had an acceptable and manageable tolerability profile (108). PARPis are currently not part of the initial standard-of-care chemotherapy regimen for *BRCA*-mutated EOC (which still remains a platinum and taxane doublet), although clinical trials are exploring their incorporation into first-line chemotherapy (Table 1).

Inhibition of the Pol θ -Dependent Alt-EJ Pathway as a Synthetic Lethal Strategy against HR-Deficient Cancers

Recent observations indicate that PARP1 functions in a pathway required for the repair of DNA DSBs, referred to as error-prone alt-EJ or microhomology-mediated end joining (MMEJ; ref. 111). Furthermore, recent studies have shown that HR-deficient ovarian and breast tumors have a compensatory increase in the Pol θ /PARP1-mediated alt-EJ pathway that appears to occupy a key role for their survival and proliferation (99, 100). The importance of this pathway in addition to classical NHEJ (C-NHEJ) is now increasingly appreciated (112).

Early evidence for alt-EJ came from studies in yeast and mammalian cells deficient in C-NHEJ that were still able to repair DSBs via end joining (113, 114) and by the observation that mice deficient in C-NHEJ still exhibited chromosomal translocations and V(D)J recombination (115, 116). Molecular characterization of alt-EJ revealed that the XRCC1/DNA ligase III complex and PARP1 were involved (84, 117, 118). Initially, alt-EJ was considered merely a backup repair pathway for C-NHEJ for end joining of chromosomal DSBs (118–120), but subsequent studies have demonstrated that alt-EJ might have a role in repairing chromosomal DSBs, depending on the biologic context, such as HR deficiency (99, 100). However, the use of alt-EJ for repairing DSB poses a particular threat to genomic stability because of its predilection for joining DNA breaks on different chromosomes, generating chromosomal translocations (121–123). Indeed, fill-in synthesis in alt-EJ is likely mediated by the Pol θ polymerase, which is error-prone and likely produces point mutations, as well as random insertions and deletions (indels; refs. 124, 125). Indeed, upregulation of budding yeast Pol θ appears to generate random deletions or insertions of 20 to 200 base pairs (126, 127). Thus, the use of alt-EJ, which could be indicative of an HR defect, is likely to leave a mutational signature comprising indels at sites of microhomology. Characterization of such a mutational signature may ultimately define a biomarker of HR deficiency (126).

The alt-EJ genetic signature likely hinges upon Pol θ , which has two distinct functions in DNA repair. First, Pol θ prevents RAD51 assembly on ssDNA and, thus, toxicity in HR-deficient cells. This function is mediated by the RAD51-binding domain and is distinct from the polymerase domain. Second, Pol θ mediates PARP1-dependent alt-EJ replication rescue through its polymerase domain. Cells expressing a mutant Pol θ polymerase exhibit reduced survival when *BRCA1* is knocked down. Given the synthetic lethal interaction between HR deficiency and inhibition of Pol θ (39, 100), it is important to determine which Pol θ functions (RAD51 binding vs. polymerase) should be targeted to efficiently impair the

survival of HR-deficient cells (Fig. 3F). Although both the RAD51-binding motifs and the polymerase domain of Pol θ contribute to the survival of HR-deficient cells, the exact relative contribution of each domain remains to be elucidated in order to induce selective killing of HR-deficient tumors.

Cell-Cycle and DNA-Damage Checkpoint Inhibitors against HR-Deficient Tumors

Checkpoint signaling facilitates the coordination between DNA-damage response and cell-cycle control to allow ample time for repair and prevent permanent DNA damage produced by replication and mitosis. Two of the PI3K-related protein kinases, Ataxia-telangiectasia mutated (ATM) and Ataxia-telangiectasia and RAD3-related (ATR), occupy a central role in signaling DNA damage to cell-cycle checkpoints and DNA repair pathways (128). The ATM–CHK2 pathway primarily responds to DSBs to induce G₁ arrest via phosphorylating and activating CHK2 and p53, whereas the ATR–CHK1 pathway triggers S and G₂ phase arrest. ATM promotes HR by recruiting *BRCA1* to DSBs, but can also antagonize *BRCA1* and promote NHEJ by recruiting p53-binding protein 1 (53BP1), and these antagonistic functions are cell-cycle regulated. ATR is activated by DNA single strand–double strand junctions that arise as intermediates in NER, by replication stress, which is defined as the slowing or stalling of replication fork progression, and at resected DSBs. ATR triggers the intra-S phase and the G₂ checkpoints via phosphorylation of CHK1 at Ser345 and Ser317, leading to its activation (129). Activated CHK1, in turn, phosphorylates WEE1 (which activates this kinase) and cell division cycle 25 (CDC25A and CDC25C) phosphatases (which inhibits them) to inhibit cell-cycle progression through the coordinated suppression of cyclin-dependent kinase activity (130). ATR and CHK1 also phosphorylate a number of proteins involved in HR and ICL repair, including *BRCA2*, *RAD51*, *FANCD2*, and *FANCE*. Importantly, there is significant crosstalk between the ATM/CHK2 and ATR/CHK1 pathways, and they share many substrates (130).

Abrogation of cell-cycle checkpoints leads to accumulation of DNA damage and cellular death, and this approach has shown significant promise as an anticancer strategy. HR-deficient EOCs are p53 mutated (which is also the case for almost all high-grade serous cancers) and have lost G₁ checkpoint control, which makes them hyper-dependent on the S and G₂ checkpoints to prevent DNA damage triggering cell death (16, 131). In this regard, targeting the S and G₂ checkpoints by inactivation of the ATR–CHK1–WEE1 pathway will inhibit the DNA-damage-induced G₂ checkpoint arrest, leading to mitotic catastrophe and cell death (131). HR-deficient tumors are even more sensitive to combinations of checkpoint inhibitors with DNA-damaging chemotherapy drugs because they are both deficient in repairing the DNA damage caused by chemotherapy and susceptible to abrogation of S and G₂ checkpoints.

Importantly, even in the absence of cytotoxic chemotherapy, unrepaired endogenous DNA damage in HR-deficient EOC cells may sensitize them to checkpoint inhibition (131). In this regard, it has been shown that FA-deficient tumor cells are hypersensitive to inhibition of CHK1, which is more pronounced when combined with platinum therapy (132). DNA repair through the FA pathway occurs primarily during

the S phase of the cell cycle, and FA tumor cells acquire extensive DNA damage in S phase. These lesions persist throughout the remainder of the S and G₂ phases, ultimately activating the G₂-M checkpoint; increased accumulation of cells in the G₂ phase of the cell cycle is a useful diagnostic feature of FA cells and correlates with the hyperactivation of the G₂-M checkpoint (133). FA pathway-deficient cancer cells have a greater requirement for CHK1 function than DNA repair-proficient cells, thereby supporting the presence of a therapeutic window that could be exploited in treating DNA repair-deficient cancers with CHK1 inhibitors, while sparing toxicity in normal, DNA repair-proficient cells (132). Although FA-deficient cells are hypersensitive to cisplatin, addition of a CHK1 inhibitor further increases cytotoxicity to a significant degree. Besides abrogation of G₂ and S checkpoints, it has been shown that FA-deficient cells are hypersensitive to ATM inhibition, suggesting that ATM and FA pathways also function in a compensatory manner to maintain genome integrity (134). As with CHK1 inhibition, the selectivity of ATM inhibition alone for FA-deficient cells is modest, but the effect of combining ATM inhibition with platinum is significantly augmented in FA-deficient cells (134). Importantly, a synthetic lethal interaction also exists between the ATM and ATR signaling pathways, i.e., ATR inhibitors exhibit significant antitumor activity in ATM-deficient, but not ATM-proficient, backgrounds (135). Taken together, between the three pathways, i.e., the ATM, ATR, and FA pathways, synthetic lethal interactions exist between all individual pairs, i.e., all FA/ATM, FA/ATR, and ATR/ATM combinations are synthetically lethal.

Several approaches to inhibit the ATR-CHK1-WEE1 pathway, including ATR inhibitors (such as VX-970 and AZD6738), WEE1 inhibitors (such as AZD1775), and CHK1 inhibitors (GDC-0425 and LY2606368), are currently in early clinical trial evaluation in EOC. Of note, AZD1775 has already shown clinical activity as monotherapy in BRCA-mutated tumors (136). In these trials, these agents are combined with chemotherapy, primarily drugs that cause replication stress, such as antimetabolites (particularly nucleoside analogues that cause replication arrest, such as gemcitabine), topoisomerase I poisons, and DNA cross-linking agents, such as platinum agents. However, although cell-cycle checkpoint inhibition offers the advantage of selective cytotoxicity by exploiting molecular alterations (p53 mutations, HR defects) that are present only in tumor cells, there is always concern for toxicity, especially when they are combined with chemotherapy. In this regard, phase I trials of combinations of these agents with chemotherapeutic agents have started at lower doses of chemotherapy, which are being escalated to assess for safety. Overall, abrogation of the S and G₂ checkpoint via inhibitors of the ATR-CHK1-WEE1 pathway in combination with chemotherapy appears to exert a synthetic lethal interaction with HR-deficient EOCs and may thus be an attractive therapeutic strategy against these tumors.

MECHANISMS OF RESISTANCE IN HR-DEFICIENT EOCs

In BRCA1/2-mutated tumors, the most common acquired mechanism of resistance to cisplatin or PARPis is second-

ary intragenic mutations restoring the BRCA1 or BRCA2 protein functionality (Fig. 4; refs. 137, 138). Restoration of BRCA1/2 functionality occurs either by genetic events that cancel the frameshift caused by the original mutation and restore the open reading frame (ORF), leading to expression of a functional nearly full-length protein, or by genetic reversion of the inherited mutation, which also restores full-length wild-type protein. These genetic events were originally observed in BRCA2- and BRCA1-mutated cancer cells under selective pressure due to exposure to cisplatin or PARPis, and were associated with secondary genetic changes on the mutated allele that restored a functional protein and conferred platinum and PARPi resistance (Fig. 4; refs. 139-142). This mechanism of resistance is highly clinically relevant for patients with BRCA-mutated EOC who are treated with platinum-based therapy; 46% of platinum-resistant BRCA-mutated EOCs exhibit tumor-specific secondary mutations that restore the ORF of either BRCA1 or BRCA2 (143). Similar observations have been made in biopsies from olaparib-resistant tumors in which acquisition of secondary BRCA2 mutations restored a functional BRCA2 protein (144). Of note, multiple reversion events in BRCA1/2 genes have also been reported as a mechanism of platinum resistance in a recent study of whole-genome characterization of chemoresistant ovarian cancer (145). Strikingly, in 1 patient with BRCA2-mutated EOC, 12 independent BRCA2 reversion events were identified, with multiple reversion events occurring even in individual tumor deposits. Furthermore, in the same study (145), reversal of BRCA1 promoter methylation has also been reported in 1 patient as a mechanism of platinum resistance. In that case, the primary sensitive sample showed extensive promoter methylation and low BRCA1 expression, whereas the sample from the relapsed disease had lost BRCA1 methylation, and the BRCA1 gene was expressed at comparable levels to HR-proficient tumors. Of note, a specific rather than generalized pattern of altered methylation was noted at relapse in this patient. Even though BRCA mutations remain the strongest predictor for sensitivity to PARPis, not every mutation will result in the same functional defect and response to these agents. Analysis of BRCA1 missense mutations suggests that the conserved N- and C-terminal domains are most important for the response to HR-deficiency-targeted therapies (146). Specifically, tumors carrying the BRCA1^{C61G} mutation, which disrupts the N-terminal RING domain, responded poorly to platinum drugs and PARPis, and rapidly developed resistance (147). Similarly, *Brca1*^{Δ11/Δ11}; *p53*^{-/-} mouse mammary tumors, which express only the BRCA1^{Δ11} isoform (generated by the exon 11 splicing), can acquire resistance to cisplatin (148, 149), showing that some hypomorphic BRCA alleles, although unable to prevent tumor development, can affect response to therapy. Interestingly, mutations in the BRCT domain of BRCA1 commonly create protein products that are subject to protease-mediated degradation as they are unable to fold. HSP90 may stabilize the BRCT domain of these mutant BRCA1 proteins under PARPi selection pressure (150); the HSP90-stabilized mutant BRCA1 proteins can efficiently interact with PALB2-BRCA2-RAD51, form RAD51 foci, and confer PARPi and cisplatin resistance. Treatment of resistant cells with the HSP90 inhibitor

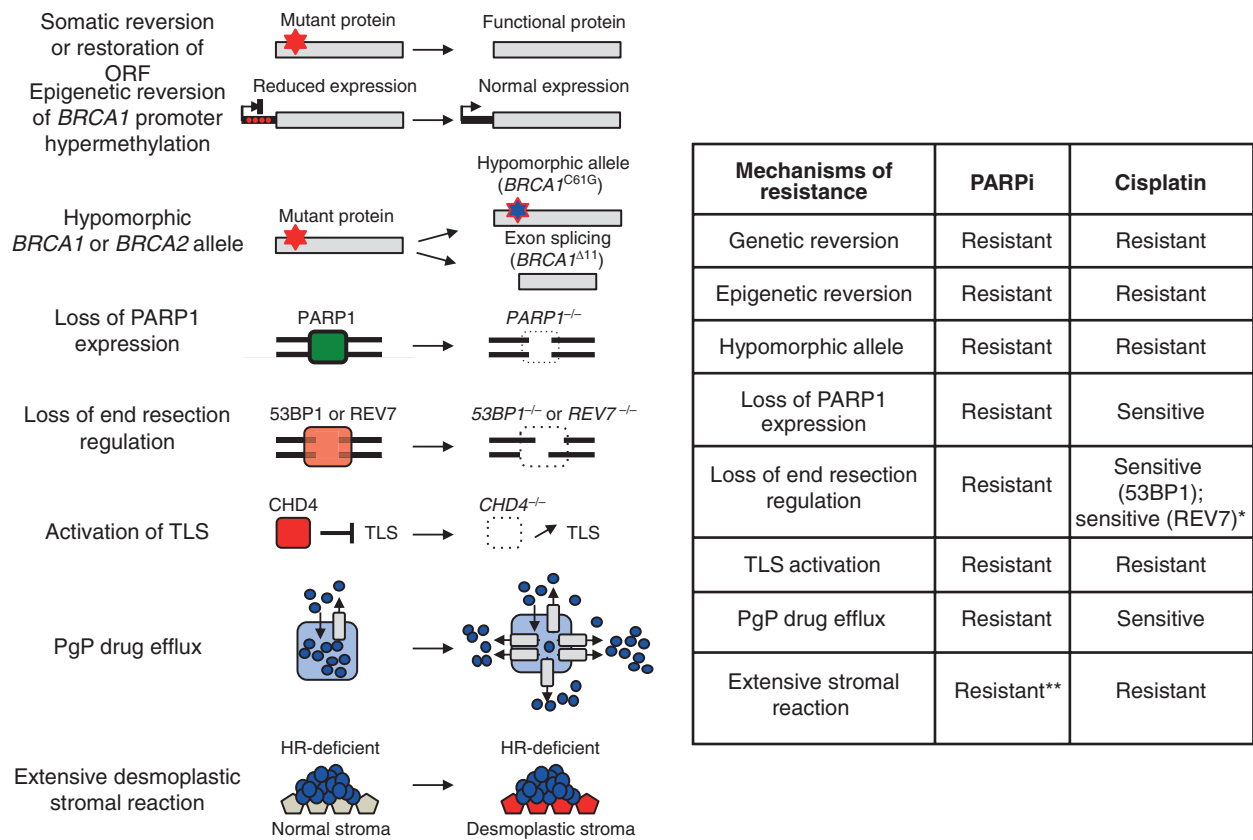


Figure 4. Mechanisms of PARPi resistance in HR-deficient cells. Known mechanisms conferring PARPi resistance in tumor cells and cross-resistance to cisplatin are indicated. An acquired genetic reversion of the original truncating mutations restores functional protein expression, inducing PARPi resistance. Alternatively, acquired epigenetic reversion of *BRCA1* promoter hypermethylation can restore normal *BRCA1* protein expression levels, conferring PARPi resistance. Hypomorphic alleles, such as *BRCA1*^{C61G} or *BRCA1*^{A11}, are unable to prevent tumor development but confer resistance to PARPi. Tumor cells may also become PARPi resistant through loss of PARP1 expression. Rescue of DNA end resection in *BRCA1*-deficient tumors through loss of 53BP1 or REV7 increases HR capacity and confers resistance to PARPi. Loss of CHD4, a negative regulator of translesion synthesis (TLS), enhances DNA-damage tolerance and induces PARPi resistance. Increased in P-glycoprotein (PgP)-mediated efflux, notably through ABCB1 upregulation (via fusion with SLC25A40), reduces intracellular PARPi concentrations, inducing resistance. Desmoplastic stromal reaction is associated with reduced drug uptake conferring chemoresistance. *, personal communication from Dr. Rottenberg and Dr. Guotai; **, although this mechanism has been described for cisplatin, it might also apply for PARPi.

17-dimethylaminoethylamino-17-demethoxygeldanamycin reduced mutant *BRCA1* protein levels and restored their sensitivity to PARP inhibition (150).

Because PARPis function by blocking the enzymatic action of PARP enzymes, another possible mechanism of PARPi resistance may be decreased expression of PARP enzymes (Fig. 4). This mechanism of resistance may be particularly relevant to the PARP-trapping mechanism of action of PARPis. A mutagenesis screen designed to identify mechanisms of resistance to PARPis revealed PARP1 loss of function as a potent mechanism of resistance to olaparib in mouse embryonic stem cells and in human tumor cells (151). Accordingly, PARP1 levels have also been shown to be low in human cancer cell lines that have acquired resistance to the PARPi veliparib (95). Taken together, these observations suggest the possibility that tumor-specific mutation or inhibition of PARP1 (for instance, by epigenetic silencing or increased turnover of the protein) would result in resistance and disease progression, a hypothesis that has not yet been validated in patients.

Several mechanisms of resistance involving reacquisition of DNA end resection capacities have also been described. Discovery of these mechanisms came from the observation that the requirement of *BRCA1* for HR can be alleviated by concomitant loss of 53BP1. 53BP1 blocks CtIP-mediated DNA end resection via downstream effectors like RIF1 and PTIP (152–157) and thus commits DNA repair to C-NHEJ (158, 159). Loss of 53BP1 partially restores the HR defect of *BRCA1*-deleted mouse embryonic stem cells and reverts their hypersensitivity to DNA-damaging agents (158, 159). However, a deficiency in Ligase IV, another component of the C-NHEJ pathway, does not rescue cell proliferation or the HR defect in *BRCA1*-deficient cells. The authors showed that loss of 53BP1 but not Ligase IV was able to promote ssDNA formation competent for RPA phosphorylation. These data suggest that loss of 53BP1 but not Ligase IV promotes activation of DNA end resection. This discordance might explain why combined deficiencies in *BRCA1*/53BP1 but not *BRCA1*/Ligase IV reverse the HR defect in *BRCA1*-deficient cells (158). Recently, an shRNA screen for hairpins promoting

survival of BRCA1-deficient mouse mammary tumors to PARPi identified REV7 and 53BP1 as the top hits (160). REV7 was shown to promote C-NHEJ by inhibiting DNA end resection downstream of RIF1. Loss of REV7 in BRCA1-deficient cells induces CtIP-dependent end resection, leading to HR restoration and PARPi resistance (160, 161). Even though there is little evidence of such resistance mechanisms in human EOCs, a mouse model of BRCA1-associated breast cancer demonstrated low 53BP1 expression in a few olaparib-resistant BRCA1-deficient mouse tumors, suggesting that an acquired change in 53BP1 expression could occur *in vivo* as a resistance mechanism (162). In BRCA1-mutant cells, loss of 53BP1 confers resistance to PARPi. However, whether loss of 53BP1 confers cross-resistance to cisplatin is still elusive to date. In BRCA1-deficient cell lines, shRNA-mediated loss of 53BP1 fully abolished the cisplatin sensitivity (159). However, in the olaparib-resistant BRCA1-deficient mouse tumors, the HR restoration conferred by 53BP1 loss is only partial (measured by RAD51 foci formation) and may explain the lack of cross-resistance to cisplatin (162).

Similar response was observed in the BRCA1- and REV7-deficient tumors, where the tumors still respond to cisplatin treatment (no refractory disease), even though tumors tended to relapse earlier (personal communication from Dr. Rotenberg and Dr. Guotai). Further studies are necessary to fully address to what extent 53BP1 or REV7 loss *in vivo* can also confer resistance to platinum therapy and whether these resection-dependent resistance mechanisms (described only in BRCA1-deficient cells so far) may also be relevant to other HR-deficient settings such as BRCA2-mutated cells.

Apart from the mechanisms of resistance intrinsic to the DNA-damage response, pharmacologic effects that alter the cellular response to PARPis may also be relevant. Several studies have shown that PARPi responses may be modified by ATP-binding cassette (ABC) transporters (163). Increased expression in tumor cells of ABC transporters, such as the P-glycoprotein (PgP) efflux pump [also known as multidrug resistance protein 1 (MDR1) or ATP-binding cassette subfamily B member 1 (ABCB1)] has been implicated in reducing the efficacy of many compounds by enhancing their extracellular translocation (Fig. 4). In a genetically engineered mouse model for *Brca1*-mutated breast cancer, PARPi resistance was mediated via upregulation of the *Abcb1a* and *Abcb1b* genes encoding PgP pumps. Of note, upregulation of the *ABCB1* gene through promoter fusion and translocation involving the 5' region of the gene (most frequently with *SLC25A40* gene) was found in approximately 8% of HGSOc recurrence samples in another study (145). Although not relevant to platinum analogues, this mechanism of resistance may be relevant to PARPi resistance and other drugs, such as etoposide, paclitaxel, and doxorubicin. Furthermore, resistance to PARPi could be reversed by coadministration of PgP inhibitors, arguing that upregulation of PgP may be a clinically relevant and drug-gable acquired mechanism of PARPi resistance (164, 165). Of note, PgP can be inhibited in the clinic (such as with tariquidar; ref. 164); furthermore, novel PARPis (such as AZD2461) have been developed that have lower affinity to PgP, thereby circumventing this mechanism of PARPi resistance (162, 166).

Extensive tumor desmoplasia has also been suggested as a mechanism of resistance in a BRCA1-mutated tumor without

BRCA1 reversion (145). In that case, an extensive desmoplastic stromal reaction was observed at autopsy; tumor desmoplasia has been associated with chemoresistance and suboptimal drug uptake in pancreatic cancer and may have accounted for resistance despite persistence of HR deficiency due to the *BRCA1* mutation. In another study, loss of the nucleosome remodeling factor CHD4 was found to be associated with cisplatin resistance in BRCA2-mutated tumors (167). Restoration of cisplatin resistance was independent of HR but correlated with restored cell-cycle progression, reduced chromosomal aberrations, and enhanced DNA-damage tolerance. Of note, BRCA2-mutant ovarian cancers with reduced CHD4 expression significantly correlated with shorter PFS and shorter overall survival (167).

Interestingly, in a genetically engineered mouse model for BRCA1-deficient tumors, in which genetic reversion was made impossible by the large intragenic *Brca1* deletion, no acquired platinum drug resistance was observed (168). This raises the question of whether mechanisms other than genetic BRCA1/2 reversions can result in resistance to platinum-based chemotherapy in patients (169).

Finally, besides the aforementioned resistance mechanisms, an important question relates to the nature of resistance of residual tumor cells that may respond again to platinum drugs (i.e., recurrence of disease that responds again to platinum chemotherapy). Several approaches have been attempted to target these residual cells that are not killed by first-line chemotherapy. First, maintenance chemotherapy, i.e., continuing chemotherapy after achievement of complete clinical remission to first-line chemotherapy, has been widely studied, but no chemotherapy regimen has been associated with an improved overall survival or cure rate in that setting (170). Second, increasing dose-intensity approaches have also been attempted, including increasing the dose of platinum, combining platinum agents, increasing the number of cycles of chemotherapy, or using high-dose chemotherapy also incorporating alternative DNA cross-linking agents, such as melphalan and cyclophosphamide, in combination with bone marrow transplantation or with peripheral blood stem cell support; all these approaches failed to improve outcome compared with standard chemotherapy (171). The only dose-intensity approach that has been associated with improved survival was administration of chemotherapy via the intraperitoneal route, which is capable of achieving high local concentrations of chemotherapy with acceptable systemic side effects (10). However, definitive data regarding comparison of i.p. chemotherapy versus i.v. dose-dense chemotherapy (GOG252 study) are still pending.

In conclusion, understanding the mechanisms of resistance to PARPis in HR-deficient EOCs is critical in order to identify approaches that may overcome resistance and/or minimize the emergence of secondary resistant clones.

OVERCOMING DE NOVO AND ACQUIRED HR PROFICIENCY

The promise of platinum agents and PARPis in the management of ovarian cancers is tempered by the fact that HR-proficient tumors do not respond to these agents, suggesting that as many as 50% of ovarian patients (i.e., those with tumors that are *de novo* HR proficient) do not benefit from these

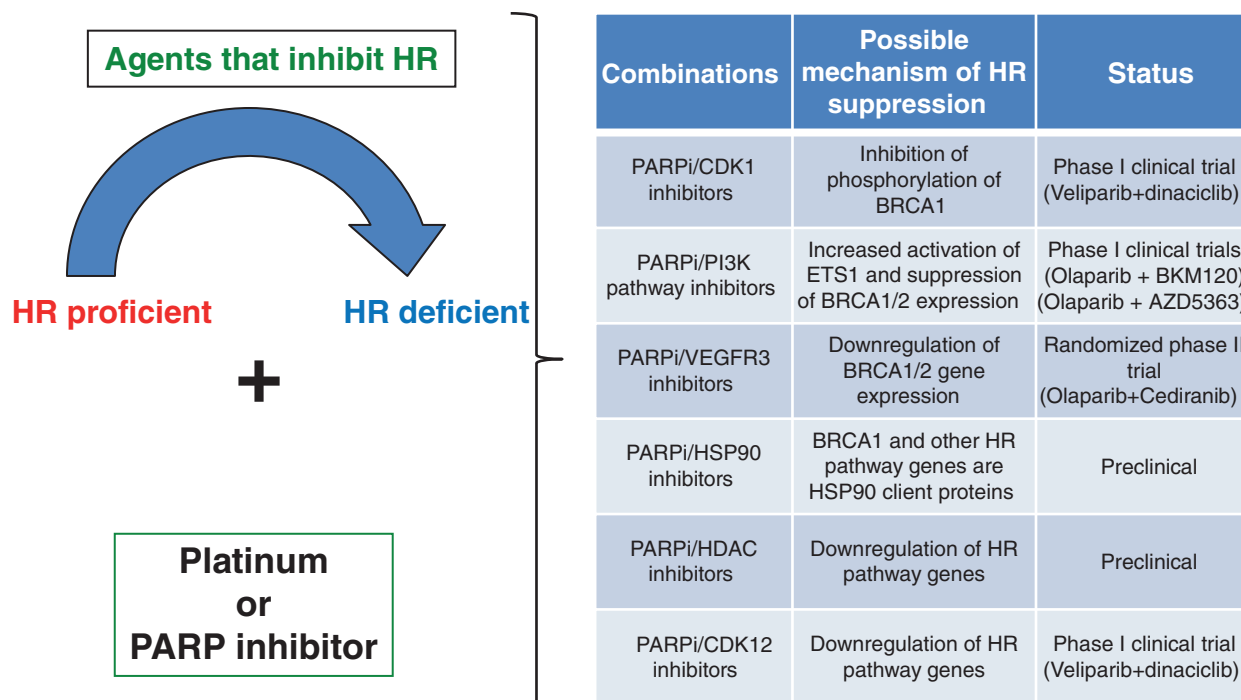


Figure 5. PARPi combinations against HR-proficient tumors. Rationale behind use of specific PARPi combinations as a strategy against HR-proficient tumors. Specifically, use of agents that inhibit HR, such as CDK1 or HSP90 inhibitors may render HR-proficient tumors into HR-deficient tumors and thus sensitize them to platinum or PARPis. The proposed mechanism of HR suppression and the clinical status of these PARPi combinations are presented in the right panel.

drugs. Furthermore, even the 50% of EOCs which are initially HR-deficient eventually become HR-proficient as a result of development of resistance to platinum or PARPis. Combination of platinum or PARPis with agents that inhibit HR may therefore represent an effective strategy to sensitize HR-proficient tumors to platinum and PARPis, and thus potentially expand use of these agents into EOCs with *de novo* or acquired HR proficiency. Multiple strategies designed to selectively disrupt HR in cancer cells and sensitize them to PARPis or platinum have been evaluated both preclinically and in early clinical trials in EOC (Fig. 5). Such strategies include combinations of platinum or PARPis with (i) CDK1 inhibitors (inhibition of CDK1 induces HR deficiency via inhibition of phosphorylation of BRCA1 by CDK1; refs. 172, 173), (ii) with PI3K or AKT inhibitors (inhibition of the PI3K pathway leads to ERK activation/phosphorylation, increased activation of ETS1, and suppression of BRCA1/2 expression and of HR; refs. 174, 175), (iii) CDK12 inhibitors (abrogation of CDK12 leads to downregulation of HR genes as discussed above), (iv) HDAC inhibitors (which induce coordinated downregulation of HR pathway genes; ref. 176), and (v) HSP90 inhibitors [which induce HR deficiency because multiple HR proteins, including BRCA1, are HSP90 clients (177), and they may also overcome HSP90-mediated stabilization of BRCA1-mutant proteins as a mechanism of PARPi resistance (150)]. Preclinical evaluation has demonstrated that CDK1, CDK12, PI3K, AKT, HDAC, and HSP90 inhibitors are able to inhibit HR and sensitize HR-proficient cells to PARPis and/or platinum. Of

note, phase I clinical evaluation of olaparib with PI3K inhibitors (BYL719 or BKM120) or the AKT inhibitor (AZD5363) in ovarian and breast cancers provided evidence of response in patients who were expected to have HR-proficient tumors, thereby providing proof of principle for this approach.

Interestingly, in a randomized, open-label, phase II study, the combination of olaparib plus cediranib (which is a VEGFR1, VEGFR2, and VEGFR3 inhibitor) significantly improved PFS in recurrent platinum-sensitive EOC compared with olaparib alone, and the greatest benefit was observed among patients without *BRCA1/2* mutations (63). Although a number of mechanisms may explain this result, this finding may also indicate that there is greater synergism between these two drugs in the setting of HR-proficient tumors. In this regard, VEGFR3 inhibition has been shown to downregulate *BRCA* gene expression, reverse chemotherapy resistance, and restore chemosensitivity in resistant cell lines in which a *BRCA2* mutation had reverted to wild-type (178). It is therefore possible that cediranib may be enhancing the response to olaparib in HR-proficient tumors via inhibition of HR (due to VEGFR3 inhibition).

An important challenge for the clinical development of these combinations of HR inhibitors with PARPis is the potentially low therapeutic window between normal and cancer cells and thus the risk of enhanced toxicity. Therefore, careful phase I evaluation of these combinations will be required, with increased focus on proof of mechanism pharmacodynamic studies. Nonetheless, thus far, the clinical

trials of olaparib combinations with PI3K pathway inhibitors have not shown any alarming signals besides the expected nonoverlapping toxicities of these agents.

CONCLUSIONS

Approximately 50% of EOCs exhibit defective DNA repair via HR and represent a distinct EOC subtype with unique clinical characteristics that have important implications for management. Germline and somatic *BRCA1/2* mutations are the most common mechanisms of HR deficiency, but multiple alternative mechanisms also contribute to this phenomenon in EOC. HR deficiency explains the enhanced sensitivity of EOC to platinum-based chemotherapy and is an important therapeutic strategy in this disease. The striking activity of PARPis in HR-deficient EOCs highlights the potential of synthetic lethality as anticancer strategy and is the first molecular-targeted therapy approved in this disease. Additional, potentially non-cross-resistant, synthetic lethal approaches, such as inhibition of Alt-EJ pathway and cell-cycle checkpoint inhibition, are exciting novel approaches against HR-deficient cancers.

Although PARPis are now FDA approved in patients with *BRCA1/2*-mutated EOCs, patients with HR-deficient/non-*BRCA*-mutated tumors do not have access to these agents outside a clinical trial. This highlights the importance of development of a robust and prospectively validated biomarker of HR deficiency that is capable to identify non-*BRCA*-mutated patients who may benefit from these agents. Another challenge is *de novo* and acquired resistance, which are often encountered in the clinic and have tempered the enthusiasm for the potential of PARPis in HR-deficient EOCs. Understanding the mechanisms of PARPi resistance and their relation to platinum resistance may aid in the development of novel non-cross-resistant therapies and may help optimize the sequence of how these agents are incorporated in the clinical management of HR-deficient EOC. Finally, combinations of PARPis with agents that inhibit HR are exciting strategies to sensitize HR-proficient tumors to platinum and PARPis, and thus potentially expand use of these agents into EOCs with *de novo* or acquired HR proficiency. Initial reports from the clinical evaluation of these combinations provide clinical proof of principle for this approach without prohibitive toxicities.

Disclosure of Potential Conflicts of Interest

P.A. Konstantinopoulos is a consultant/advisory board member for Vertex. G.I. Shapiro reports receiving commercial research support from Clovis Oncology (to Dana-Farber for conduct of phase I clinical trial) and Vertex Pharmaceuticals (to Dana-Farber for conduct of phase I clinical trial), and is a consultant/advisory board member for G1 Therapeutics, Lilly, and Vertex Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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