

# BRCAness, DNA gaps, and gain and loss of PARP inhibitor–induced synthetic lethality

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Mutations in the tumor-suppressor genes *BRCA1* and *BRCA2* resulting in *BRCA1/2* deficiency are frequently identified in breast, ovarian, prostate, pancreatic, and other cancers. Poly(ADP-ribose) polymerase (PARP) inhibitors (PARPis) selectively kill *BRCA1/2*-deficient cancer cells by inducing synthetic lethality, providing an effective biomarker-guided strategy for targeted cancer therapy. However, a substantial fraction of cancer patients carrying *BRCA1/2* mutations do not respond to PARPis, and most patients develop resistance to PARPis over time, highlighting a major obstacle to PARPi therapy in the clinic. Recent studies have revealed that changes of specific functional defects of *BRCA1/2*-deficient cells, particularly their defects in suppressing and protecting single-stranded DNA gaps, contribute to the gain or loss of PARPi-induced synthetic lethality. These findings not only shed light on the mechanism of action of PARPis, but also lead to revised models that explain how PARPis selectively kill *BRCA*-deficient cancer cells. Furthermore, new mechanistic principles of PARPi sensitivity and resistance have emerged from these studies, generating potentially useful guidelines for predicting the PARPi response and design therapies for overcoming PARPi resistance. In this Review, we will discuss these recent studies and put them in context with the classic views of PARPi-induced synthetic lethality, aiming to stimulate the development of new therapeutic strategies to overcome PARPi resistance and improve PARPi therapy.

## The concepts of synthetic lethality and BRCAness

The concept of synthetic lethality was first described by the fly geneticist Calvin Bridges in 1922 (1, 2) as the combination of two genetic events that results in cell death or death of an organism. The term “synthetic lethality” is commonly used to describe a specific type of genetic interaction whereby simultaneous loss of two nonessential functions is incompatible with cell or organismal viability. Since its conception, synthetic lethality has been widely used by genetic studies in different model organisms to help with understanding the functional relationships between different genes and pathways. During the last two decades, the concept of synthetic lethality has been successfully applied to cancer therapy, allowing us to exploit various vulnerabilities of cancer cells (3). The basic rationale for this approach is that the oncogenic events in cancer cells give rise to distinct vulnerabilities, rendering cancer cells more dependent on certain genes and pathways for survival than normal cells. When the functions of these genes and pathways are disrupted, selective or preferential killing of cancer cells can be achieved. Thus, the synthetic lethality of cancer cells in a therapeutic setting is not an effect of two incompatible genetic events, but a result of a targeted drug exploiting a liability in a specific oncogenic context. A classic example of the use of synthetic lethality in cancer therapy

is the development of poly(ADP-ribose) polymerase (PARP) inhibitors for the treatment of cancer patients carrying mutations in *BRCA1/2* tumor-suppressor genes (3). As we will discuss below, the loss of *BRCA1/2* functions in cancer cells creates a dependency upon proper PARP functions and cycling for cell survival.

*BRCA1* and *BRCA2* are two tumor-suppressor genes frequently mutated in breast, ovarian, prostate, pancreatic, and other cancers. Familial mutations in *BRCA1/2* genes significantly increase the lifetime risk of breast (up to 85%) and ovarian (15%–56%) cancers in carriers (4). The best-known functions of *BRCA1* and *BRCA2* proteins are in homologous recombination (HR), a DNA repair pathway that accurately repairs DNA double-stranded breaks (DSBs) (5, 6). Loss of *BRCA1/2* in cancer cells results in HR deficiency (HRD) and increased levels of DSBs in the genome, which likely fuels tumorigenesis (7). Mutations in other HR genes, such as *PALB2*, *RAD51C*, *RBBP8* (also known as *CtIP*), and *BRIPI* (also known as *FANCI/BACH1*), are also found in cancers (8–11). More recent studies have revealed additional roles of *BRCA1/2* in genome protection, including their functions in protecting stalled DNA replication forks (12, 13), preventing accumulation of DNA gaps (14–16), and suppressing R-loops, a 3-stranded nucleotide structure formed by stable RNA-DNA hybrids (17–19). Defects in these *BRCA1/2* functions may also contribute to genomic instability in cancer cells. In addition to loss-of-function mutations in *BRCA1/2* and other HR genes, expression of these genes can also be lost in cancers through DNA hypermethylation and other mechanisms (20). Loss of *BRCA1/2* and related functions in cancer is often described as “BRCAness,” which refers to both the functional defects in cancer cells and the consequent vulnerabilities (21). Levels of HRD in tumors can be scored by measuring

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loss of heterozygosity, telomeric allelic imbalance, and large-scale transitions, providing a biomarker for BRCAness (22). More recent approaches that combine chromosomal rearrangement signatures and base substitution signatures associated with BRCA1/2 or HR loss may provide a more effective way to predict BRCAness in tumors (23, 24). The concepts of synthetic lethality and BRCAness have established a framework for targeting BRCA1/2 mutant tumors and other HR-deficient tumors in cancer therapy.

## Development and advancement of PARP inhibitors

The genomic instability associated with BRCAness offers an opportunity to selectively induce synthetic lethality in cancer cells (25). PARP1 was the first member of the family of PARP enzymes shown to function in DNA repair (26). By recognizing DNA nicks and ends and synthesizing poly(ADP-ribose) (PAR) chains at sites of DNA damage, PARP1 plays an important role in base excision repair (BER) and the repair of single-stranded breaks (SSBs) (27). The first PARP inhibitor (PARPi) was developed in 1975 and found to enhance the cytotoxicity of the DNA-damaging agent dimethyl sulfate (28). This observation motivated the subsequent development of additional PARPis to augment the effectiveness of conventional radiotherapy and chemotherapy. In 2005, two landmark studies by the Ashworth and Helleday labs demonstrated that BRCA1/2-deficient cancer cells are highly sensitive to PARP inhibition (29, 30). Four years later, a clinical trial demonstrated the antitumor activity of the PARPi olaparib (AZD2281) in BRCA mutation carriers (31). The success of these early studies inspired the development of additional PARPis by various pharmaceutical companies and clinical trials in different patient cohorts.

To date, at least eight different PARPis have been or are being tested in clinical trials (Table 1). These PARPis include olaparib (KuDOS/AstraZeneca) (31), veliparib (Abbvie) (32), rucaparib (Pfizer/Clovis) (33), niraparib (Merck/Tesaro) (34), talazoparib (Lead/Biomarin/Medivation/Pfizer) (35), pamiparib (BeiGene) (36), and venadaparib (Idience) (37). All PARPis listed inhibit both PARP1 and PARP2 proteins (38, 39), but they exhibit different potency, selectivity, and efficacy in patients. In addition, a PARP1-selective PARPi (AZD5305) developed by AstraZeneca (40) is being tested in clinical trials.

Olaparib (Lynparza; AstraZeneca) was the first clinically applied PARPi, receiving approval from the USA FDA and European Medicines Agency (EMA) for advanced ovarian cancer patients with deleterious or suspected deleterious germline BRCA (*gBRCA*) mutations in 2014 (41, 42). Olaparib was subsequently approved by the FDA for *gBRCA*-mutated HER2-negative metastatic breast cancer in 2018 (ClinicalTrials.gov NCT01844986), *gBRCA*-mutated pancreatic cancer in 2019 (NCT02184195), and adjuvant treatment of high-risk early breast cancer in 2022 (NCT02032823). The latest follow-up studies in the SOLO1 ovarian cancer maintenance trial showed that olaparib significantly improved the overall survival (OS) of advanced ovarian cancer patients carrying BRCA1/2 mutations after seven years, marking it the first PARPi shown to provide an OS benefit (43). Olaparib also extended median progression-free survival (PFS) in HER2-negative breast cancer patients carrying *gBRCA* mutations in the OlympiAD trial, but the OS benefit was less pronounced than that in the ovarian cancer trial (44).

Rucaparib was approved by the FDA for the maintenance of advanced ovarian cancer patients with deleterious BRCA mutations. It is currently being tested in patients with metastatic castration-resistant prostate cancer with deleterious BRCA mutations (NCT02952534) and multiple recurrent cancer maintenance therapies (NCT01968213). A clinical trial of niraparib resulted in significantly longer PFS in ovarian cancer patients (NCT02655016), leading to the FDA approval of using niraparib in first-line maintenance therapy, regardless of HRD status (45). Talazoparib (BMN 673/Talzenna; Pfizer) was approved by the FDA for advanced breast cancer patients with *gBRCA* mutations (NCT01945775) and patients with metastatic castration-resistant prostate cancer with mutations in HR genes (NCT03395197). Veliparib is generally considered to be a weak PARPi and is primarily used in combination with platinum-based chemotherapy or radiation therapy (46). Pamiparib displayed antitumor activity in advanced ovarian cancer patients with *gBRCA* mutations (36). A trial of AZD5305 is being conducted in patients with advanced solid malignancies (NCT04644068). AZD5305 is 500-fold more selective for PARP1 than PARP2 (47). In preclinical models, AZD5305 selectively killed BRCA2-deficient cells and displayed much less hematologic toxicity than olaparib (40). The minimal side effects of AZD5305 compared with PARP1/2 dual inhibitors are likely due to the loss of PARP2 trapping-associated toxicity (see below). In the ongoing trial, AZD5305 exhibited a promising safety profile and compelling clinical activity (48). In addition to the monotherapy trials using various PARPis, several PARPis are also being tested in combination with radiotherapy, chemotherapy, and immunotherapy (recently reviewed in ref. 49). These trials may offer new opportunities to broaden the utility of PARPis and enhance their efficacy in cancer therapy.

## Mechanisms of action

**Classic models.** Several models have been proposed to explain why PARP inhibition selectively induces synthetic lethality in BRCA1/2- or HR-deficient cancer cells (Figure 1A). The first model focused on the SSB repair function of PARP1 (29, 30). In this model, inhibition of PARP activity results in accumulation of unrepaired SSBs in the genome, giving rise to high levels of DSBs when SSBs and replication forks collide (Figure 1A). Because BRCA1/2-deficient cancer cells are HR defective, they are forced to use error-prone repair pathways (e.g., nonhomologous end joining [NHEJ]) to repair DSBs at collapsed replication forks, leading to toxic repair products and selective killing by PARPi. Thus, loss of HR and SSB repair results in synthetic lethality. Consistent with this model, an SSB repair-defective *XRCC1* mutant cell line displays high levels of spontaneous DSBs and RAD51 foci, indicating that HR is used to repair SSB-derived DSBs in HR-proficient cells (29). Furthermore, BRCA1/2-deficient cells, but not BRCA1/2-proficient cells, display higher levels of DSBs and chromosomal aberrations upon PARPi treatment, showing the ability of PARPi to selectively induce DSBs and toxic repair products in BRCA1/2-deficient cells.

A second model to explain the effects of PARPi in BRCA1/2-deficient cells was proposed following the observation that PARPis trap PARP1/2 on chromatin (38). Notably, the potency of various clinical PARPis in trapping PARP1/2 correlates with their efficacy

**Table 1. Selected ongoing clinical trials with PARPis**

Agents	Alternative name	PARP1-trapping ability (relative to olaparib (135))	Targets (37, 40, 68, 136–138)	Producer	First FDA approval
Olaparib	AZD-2281, MK-7339	1	PARP1/2/3/4	Lynparza, AstraZeneca	2014
Veliparib	ABT-888	<0.2	PARP1/2/3	AbbVie	Not approved by FDA as a monotherapy
Rucaparib	AG014699	1	PARP1/2/3/4	Rubraca, Clovis	2018
Niraparib	MK-4827		PARP1/2	Zejula, Tesaro Inc.	2017
Talazoparib	BMN-673	100	PARP1/2	Talzenna, Pfizer	2018
Pamiparib	BGB-290-102	NA	PARP1/2/3	BeiGene	NA (approved in China in 2021)
Venadaparib	IDX-1197	NA	PARP1/2	Idience	NA
AZD5305	NA	NA	PARP1	AstraZeneca	NA

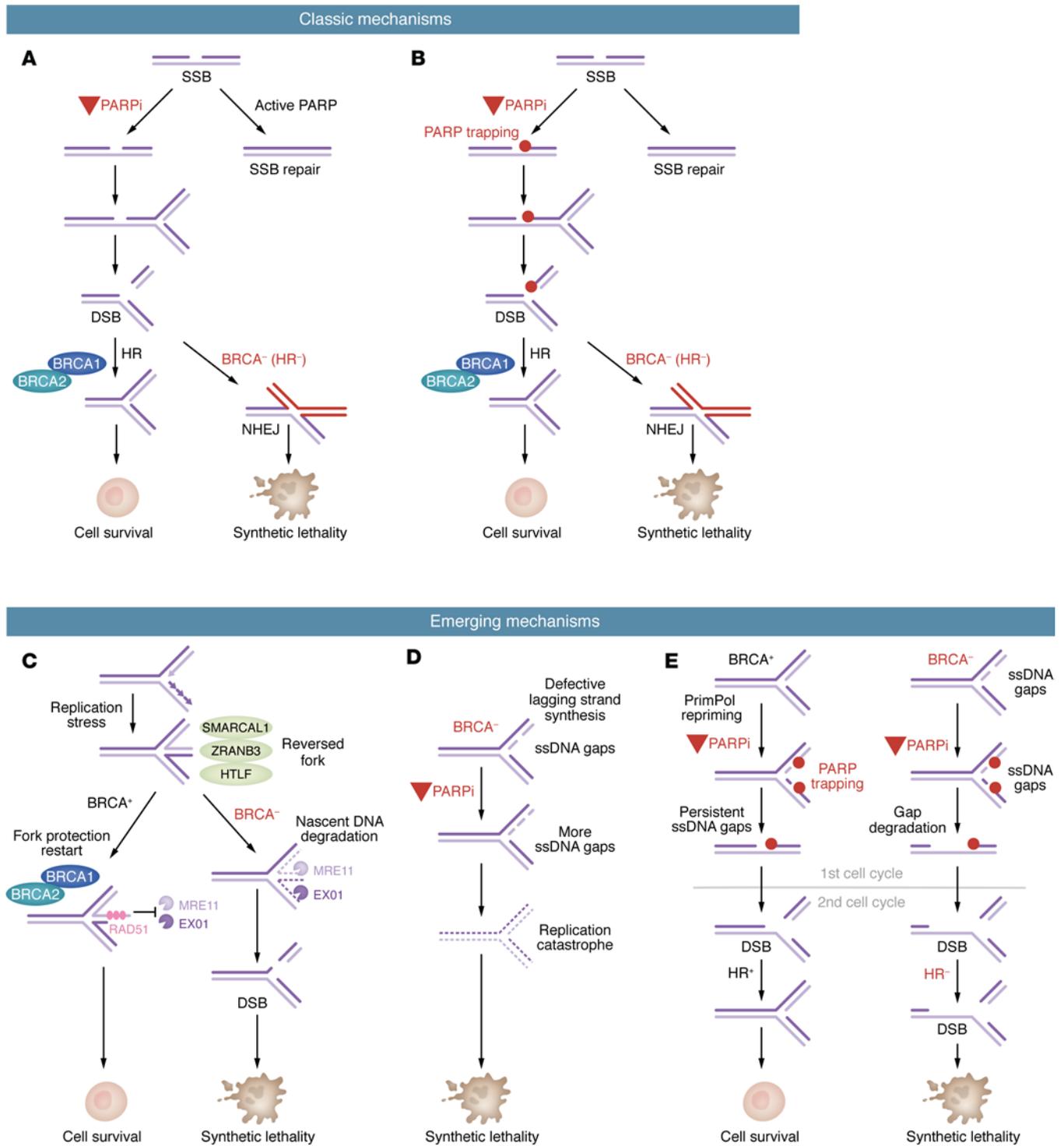
in killing BRCA1/2-deficient cancer cells, suggesting that PARPi induces DNA damage by trapping PARP1/2 on DNA (Figure 1B). In this model, PARPi-mediated trapping of PARP1/2 at SSBs creates barriers for replication forks, increasing DSBs in both BRCA1/2-deficient and -proficient cells. BRCA1/2-deficient cells are selectively killed by PARPi because they cannot repair DSBs properly and efficiently, supporting a synthetic lethal relationship between HR loss and PARP trapping (Figure 1B). Indeed, *PARP1* mutations in BRCA1/2-deficient cells were shown to confer PARPi resistance (39), suggesting that PARP1 is the key target for trapping to kill BRCA1/2-deficient cells. Nonetheless, a recent study challenged the concept that PARP trapping by PARPi drives the killing of BRCA1/2-deficient cells (50). This study suggests that PARP functions with TIMELESS and TIPIN to alleviate transcription-replication conflicts, and the loss of this PARP function results in synthetic lethality of BRCA1/2-deficient cells.

**Emerging models.** While the roles of BRCA1/2 in HR are most extensively studied, their functions at replication forks are increasingly appreciated. Both BRCA1 and BRCA2 are important for preventing the nucleolytic degradation of nascent DNA at stalled replication forks (12, 13). The degradation of nascent DNA in BRCA-deficient cells involves MRE11 and EXO1 nucleases and requires SMARCA1, ZRANB3, and HLF, which convert stalled replication forks into 4-way structures known as reversed forks (51–54), suggesting that BRCA1/2 protect reversed forks from degradation by MRE11 and EXO1. Notably, when BRCA1-deficient cells acquire resistance to PARPi, they regain fork protection (55). In BRCA2-deficient cells, loss of the MLL3/4 complex protein PTIP allows cells to regain fork protection and confer PARPi resistance (56). These results suggest a model in which increased degradation of nascent DNA at reversed forks in BRCA-deficient cells exacerbates fork collapse and impairs proper fork recovery, driving genomic instability and cell death in PARP inhibition (Figure 1C).

Although BRCA1/2 protect the replication forks reversed under stress, PARP inhibition reduces the accumulation of reversed forks (57), suggesting that the defect of BRCA-deficient cells in protecting reversed forks may not be directly relevant to their sensitivity to PARPi. A number of recent studies showed that BRCA-deficient cells accumulate elevated levels of single-stranded DNA (ssDNA) or ssDNA gaps (14, 15, 58–61). Some of these studies suggested that the ssDNA gaps in BRCA-deficient

cells are generated by PrimPol-mediated repriming (14, 15, 60), whereas others linked the ssDNA gaps to defective lagging strand synthesis and alterations in PCNA cycling, which interfere with chromatin assembly (16, 61). In particular, the Caldecott lab showed that PARP1 was activated by defective Okazaki fragment processing and PARP inhibition impeded lagging strand maturation during DNA replication, leading to an increase in postreplicative single-strand nicks or gaps (62). BRCA-deficient cells are also defective for the postreplicative repair of ssDNA gaps (63). Interestingly, PARP inhibition was shown to induce the accumulation of ssDNA gaps in nascent DNA in both BRCA1-proficient and -deficient cells (15). Both PrimPol-mediated repriming and defects in lagging strand maturation may contribute to the formation of PARPi-induced ssDNA gaps (15, 62). Two different models were proposed to explain how PARP inhibition may exacerbate the ssDNA gaps in BRCA-deficient cells to kill them selectively. In the first model, PARP inhibition increases ssDNA gaps in BRCA-deficient cells, which kill cells through replication catastrophe, a process triggered by extremely high levels of ssDNA (59) (Figure 1D). In the second model, PARP inhibition does not drastically increase ssDNA gaps in BRCA-deficient cells, but rather makes ssDNA gaps more persistent by trapping PARP1 (15). Of note, BRCA-deficient cells are defective for protecting ssDNA gaps from degradation by MRE11 (63). Inhibition of MRE11 in BRCA-deficient cells restores gap filling, suggesting that MRE11-mediated DNA degradation from gaps interferes with gap repair (63). In addition, PARP inhibition preferentially increases the gap-initiated nascent DNA degradation in BRCA-deficient cells (64). When ssDNA gaps with trapped PARP1 persist into mitosis and the following S phase, DSBs are formed (15, 65) (Figure 1E). BRCA-deficient cells cannot repair gap-derived DSBs efficiently and fail to activate the replication checkpoint to slow down the cell cycle; therefore, they accumulate more DSBs than BRCA-proficient cells over multiple cell cycles (15). Thus, the PARPi-induced persistent ssDNA gaps are incompatible with the HR and checkpoint defects of BRCA-deficient cells, resulting in an alternative form of synthetic lethality. This model may explain the progressive killing of BRCA-deficient cells by PARPi over time.

It should be noted that the different models above may not be mutually exclusive. Some of these models may be linked by a common underlying mechanism, and multiple mechanisms may contribute to the killing of BRCA-deficient cells by PARPi.



**Figure 1. Models of PARPi-induced synthetic lethality: classic and emerging mechanisms.** (A) A model showing how increasing SSBs in HR-defective cells leads to error-prone NHEJ, which results in cell death. (B) A model showing how trapping of PARP by PARPis on DNA interferes with replication. (C) A model showing how nascent DNA is degraded from reversed replication forks in BRCA-deficient cells. (D) A model showing how PARPis induce ssDNA gaps and trigger replication catastrophe in BRCA-deficient cells. (E) A model showing how PARPis induce persistent ssDNA gaps and collisions between gaps and replication forks in the trans-cell-cycle manner. BRCA-deficient cells fail to repair fork collapsed at gaps due to their HR defects.

For example, the defects of BRCA-deficient cells in gap suppression may increase the chance for PARPi to trap PARP at gaps and generate more DSBs, whereas the HR defects of BRCA-deficient cells may prevent the repair of these DSBs. This model may explain why HR defect, gap-suppression defect, and PARP trap-

ping all contribute to the PARPi sensitivity of BRCA-deficient cells. It will be important to test and compare the above models further in future preclinical and clinical studies. The relevance of the emerging models in the PARPi resistance of BRCA-deficient cells will be discussed below.

## Potential side effects of PARPis

While PARPi-induced trapping of PARP1 may be the key driver for the killing of BRCA-deficient cells, most PARPis inhibit and trap both PARP1 and PARP2. The PARP family has 18 members (26). Among all PARP family proteins, PARP1, PARP2, and PARP3 have the most extensively studied roles in DNA repair (66). The conserved regions common to these three proteins include the DNA damage-sensing Trp-Gly-Arg-rich (WGR) region, the helical domain (HD) region, which regulates catalytic activity, and the ADP-ribosyl transferase (ART) domain. Most PARPis share a similar mode of action that leads to the selective killing of BRCA-deficient cancer cells by targeting PARP1; however, their primary targets and adverse drug reactions vary (67). The side effects of PARPis could be caused by common or unique molecular features of these compounds. Cumulating evidence suggests that PARPis also target other PARP family proteins (68, 69), potentially explaining some side effects caused by PARPis. Loss of PARP2 activity is believed to be responsible for certain hematological side effects observed in PARPi-treated patients (70). A recent study showed that talazoparib, niraparib, and rucaparib exert an allosteric effect on PARP2 that retains PARP2 at DNA breaks, suggesting that PARP2 trapping may be responsible for the side effects of these PARPis (71). Surprisingly, none of the current clinical PARPis exert the same allosteric effect on PARP1. In the future, generating PARPis capable of exerting this allosteric effect on PARP1 is likely to enhance efficacy and reduce side effects.

## Mechanisms of PARPi resistance

Despite the ability of PARPi to induce synthetic lethality in BRCA-deficient cancer cells, only half of cancer patients carrying *BRCA1/2* mutations respond to PARPi therapy (72). Furthermore, most of the patients treated with PARPi developed PARPi resistance over time. Thus, the preexisting and acquired resistance to PARPi represents a major obstacle to PARPi therapy in the clinic. In this section, we will discuss the three main classes of PARPi resistance mechanisms, with an emphasis on the recently discovered mechanisms associated with the restoration of fork protection and gap suppression.

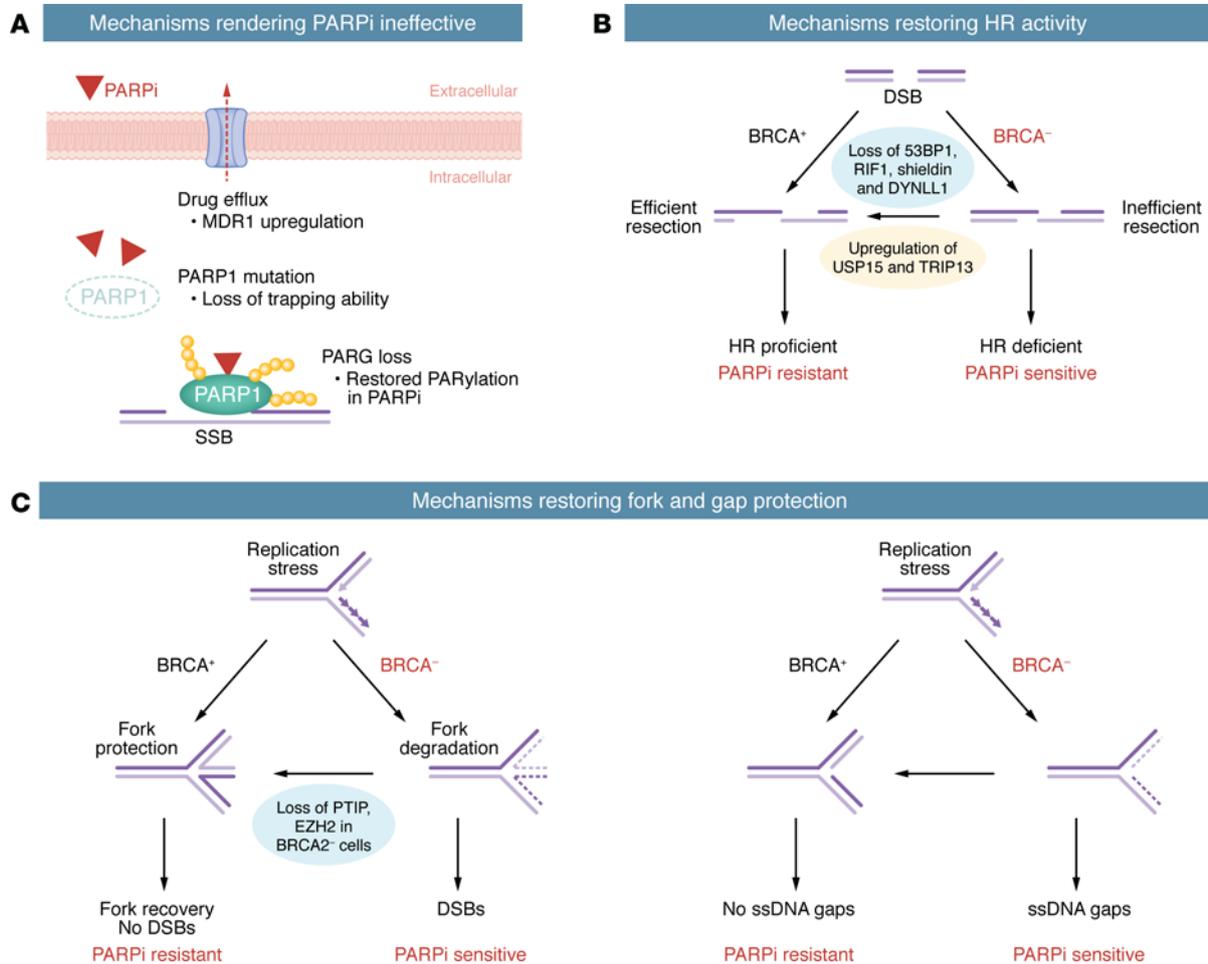
**Mechanisms rendering PARPis ineffective.** BRCA-deficient cancer cells can acquire PARPi resistance through several mechanisms that render PARPis ineffective (Figure 2A). For example, upregulation of drug efflux pumps can remove PARPi from cells and cause PARPi resistance in mouse tumor models (73). Several mutations in PARP1, the key target of PARPis, are shown to confer PARPi resistance in BRCA-deficient cell lines, possibly by altering the DNA-binding properties of PARP1 and reducing PARP1 trapping (39). PARP enzymes catalyze the addition of PAR chains onto themselves and their substrates (a process known as PARYlation), whereas PAR glycohydrolase (PARG) removes PAR chains and antagonizes PARP activity. Loss of PARG protein restores PARYlation in PARPi-treated cells and counters PARPi-mediated synthetic lethality (74). Interestingly, the contributions of PARG loss to PARPi resistance are different in BRCA1- and BRCA2-deficient cells (75). While PARG loss is a major mechanism for PARPi resistance in BRCA2-deficient cells, the resistance of BRCA1-deficient cells is mainly caused by restoration of HR and protection of gaps

and forks (see sections below). Collectively, the resistance mechanisms above reduce the effectiveness of PARPis, but they don't necessarily alter the DNA repair in BRCA-deficient cells.

**Mechanisms restoring HR activity.** BRCA-deficient cancer cells can also acquire PARPi resistance by restoring HR activity (Figure 2B). Secondary "revertant" mutations in *BRCA1/2* genes, which restore the open reading frames after frameshift mutations, increase HR activity and confer PARPi resistance in vitro and are found in PARPi-resistant patients (76, 77). Additionally, another prevalent resistance mechanism, observed particularly in *BRCA1* mutant sporadic triple-negative breast tumors, is *BRCA1* promoter demethylation that restores *BRCA1* expression (78). Moreover, BRCA1-deficient cells can restore HR by increasing DNA end resection through the loss of resection inhibitors, such as 53BP1 (79, 80), REV7 (81), and dynein light chain 1 protein (DYNLL1) (82), or by upregulating resection enhancers, such as the ATPase TRIP13 (83) and ubiquitin carboxyl terminal hydrolase 15 (USP15) (84). Loss of the 53BP1 interacting protein RIF1 and the shieldin complex, which comprises REV7, SHLD1, SHLD2, and SHLD3, also increases HR in BRCA1-deficient cells by increasing end resection (85).

**Mechanisms restoring fork and gap protection.** As discussed above, both BRCA1 and BRCA2 are important for protecting reversed replication forks from nucleolytic degradation. A BRCA2 point mutant in the C terminus (S3291A) is unable to stabilize RAD51 filaments and fails to protect replication forks, suggesting that BRCA2 functions through RAD51 in this process (12). Cells expressing a RAD51 mutant (T131P) defective for fork protection but proficient for HR are modestly sensitive to PARP inhibition (53, 54, 86). Interestingly, in a panel of PARPi-resistant cell lines derived from a BRCA1-deficient cell line, protection of replication forks is uniformly restored (55). In BRCA2-deficient cells, loss of PTIP restores fork protection and renders cells resistant to PARPi (56). Furthermore, loss of the histone methyltransferase EZH2 also prevents fork degradation in BRCA2-deficient cells and confers PARPi resistance (87). These results suggest that degradation of reversed replication forks in BRCA-deficient cells may be a key determinant for PARPi sensitivity (Figure 2C). Consistent with this hypothesis, in organoids derived from PARPi-resistant, HR-defective ovarian cancer patients, fork protection is restored (88). Despite the strong correlation between fork degradation and PARPi sensitivity, whether reversed forks are the structure triggering nascent DNA degradation in PARP inhibition remains unclear. Notably, the standard fork degradation assay is done in the presence of hydroxyurea (HU), a replication inhibitor, but not in the presence of PARPi (12). In addition, PARP inhibition prevents the accumulation of reversed forks by allowing the RECQ1 helicase to resolve them (57), suggesting that reversed forks are unlikely to be the structure degraded in BRCA-deficient cells. A recent study showed that depletion of RADX, an antagonist of RAD51, partially suppressed ssDNA formation in cells expressing the RAD51 T131P mutant, but did not alter PARPi sensitivity (59), arguing that fork degradation is not always correlated with PARPi sensitivity.

In addition to protecting reversed replication forks, BRCA1 and BRCA2 also suppress the accumulation of ssDNA gaps during replication (14, 15, 58–61). Several studies suggested that ssDNA gaps in BRCA-deficient cells are the key determinants of PARPi

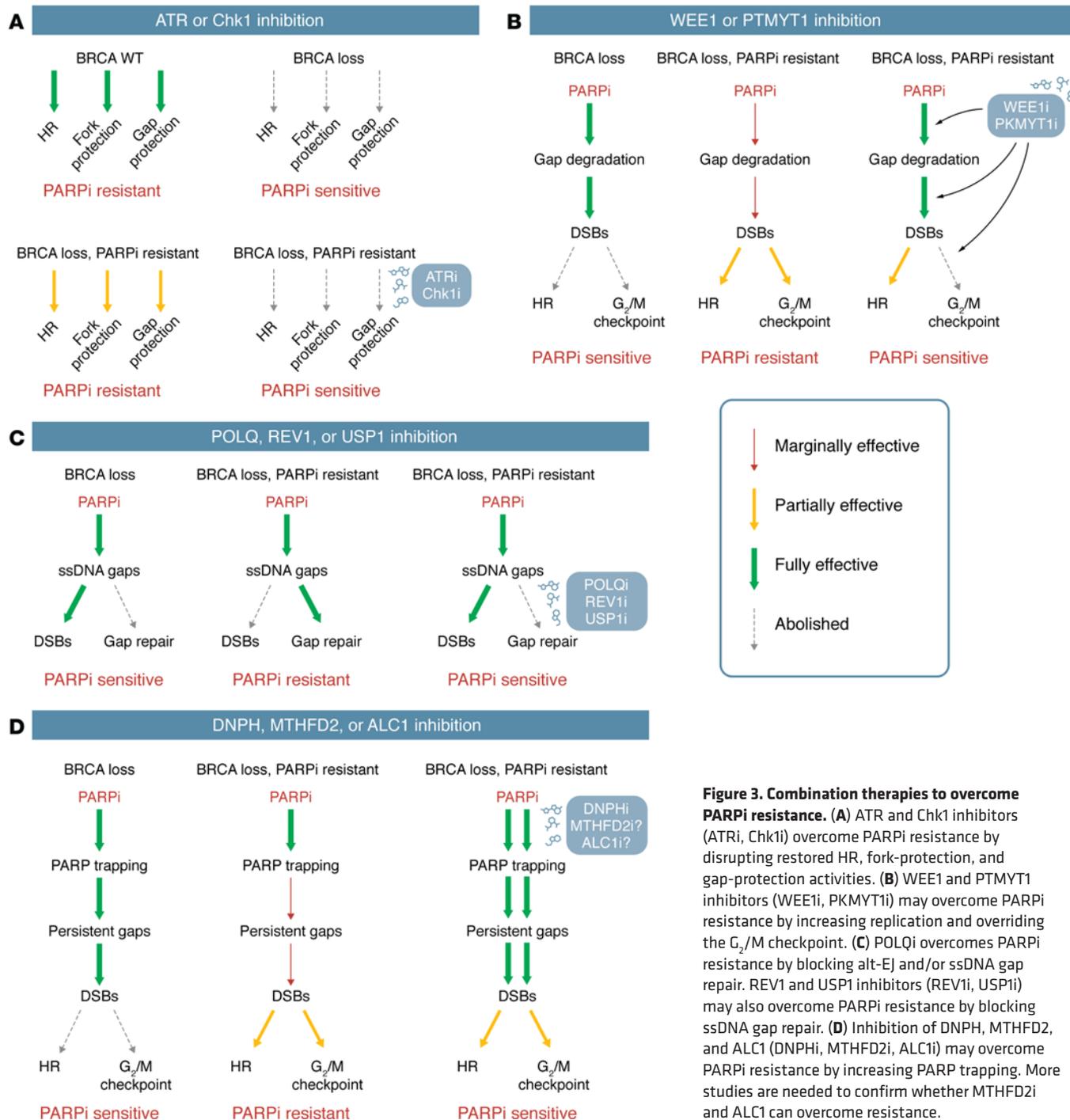


**Figure 2. Mechanisms of PARPi resistance.** An overview of the three classes of PARPi resistance mechanisms, with subcategories and examples described. (A) Mechanisms rendering PARPi ineffective. (B) Mechanisms restoring HR activity. (C) Mechanisms restoring fork and gap protection.

sensitivity, though different models were proposed to explain PARPi sensitivity in this context (14, 15, 59, 61). LIG3 loss promotes formation of MRE11-mediated postreplicative ssDNA gaps in BRCA1-deficient cells exposed to PARPi and increases PARPi sensitivity in BRCA-deficient cells and tumors (89), suggesting that the DNA degradation at gaps is important for PARPi-induced cell death. Notably, PARPi preferentially induced gap-initiated nascent DNA degradation in BRCA-deficient cells (64), suggesting that PARPi may impose synthetic lethality to BRCA1-deficient cells through gap-derived DNA damage (Figure 2C). In PARPi-resistant cell lines derived from multiple BRCA1-deficient cell lines, the suppression of ssDNA or gaps is restored in the presence of PARPi (59, 64). Because PARP inhibition induces ssDNA gaps and renders gaps persistent (15), ssDNA gaps, rather than reversed forks, are likely the relevant structure triggering nascent DNA degradation in BRCA-deficient cells after PARPi treatment. When analyzed in patient-derived cells and organoids, PARPi-induced and gap-initiated nascent DNA degradation may serve as a useful biomarker for predicting PARPi sensitivity in patients.

It is worth noting that the protection of replication forks and ssDNA gaps by BRCA1/2 may be mechanistically linked. In the absence of BRCA1/2, both reversed forks and ssDNA gaps are resected by MRE11 (12, 51, 63). It is possible that BRCA1/2

enable RAD51 loading/stabilization at reversed forks and ssDNA gaps to protect them from MRE11 (Figure 2C) (15, 54, 55, 63, 64), which would explain why fork protection in HU and gap protection in PARPi are largely correlated. A recent study showed that a BRCA2 mutant defective for HR, gap suppression, and fork protection failed to suppress genomic instability in cells and tumor formation in mice, but another BRCA2 mutant defective for only gap suppression and fork protection remained largely functional for suppressing genomic instability and tumor formation (90). These results suggest that loss of gap suppression and fork protection is insufficient for tumorigenesis, and loss of HR is an additional necessary step. Thus, the BRCA-deficient cells in tumors are likely defective for all three functions and both the defects in HR and protection of gaps and forks may contribute to PARPi sensitivity. Finally, it should be mentioned that different assays have been used to analyze the ssDNA exposure or gap formation at or behind replication forks (14, 15, 59, 63, 64). While the DNA fiber assay coupled with S1 nuclease digestion specifically analyzes ssDNA gaps, the exposure of BrdU/CldU-labeled ssDNA under non-denaturing conditions measures ssDNA exposure through multiple mechanisms. Whether ssDNA exposure is driven by gaps in various contexts needs to be examined carefully.



**Figure 3. Combination therapies to overcome PARPi resistance.** (A) ATR and Chk1 inhibitors (ATRi, Chk1i) overcome PARPi resistance by disrupting restored HR, fork-protection, and gap-protection activities. (B) WEE1 and PTMYT1 inhibitors (WEE1i, PKMYT1i) may overcome PARPi resistance by increasing replication and overriding the G<sub>2</sub>/M checkpoint. (C) POLQ<sub>i</sub> overcomes PARPi resistance by blocking alt-EJ and/or ssDNA gap repair. REV1 and USP1 inhibitors (REV1i, USP1i) may also overcome PARPi resistance by blocking ssDNA gap repair. (D) Inhibition of DNPH, MTHFD2, and ALC1 (DNPHi, MTHFD2i, ALC1i) may overcome PARPi resistance by increasing PARP trapping. More studies are needed to confirm whether MTHFD2i and ALC1i can overcome resistance.

### Combination therapies to overcome PARPi resistance

When BRCA-deficient cells become PARPi resistant, their defects in HR, fork protection, and gap protection are often partially or fully reverted, suggesting that these restored activities may contribute to the resistance. Additionally, BRCA-deficient but PARPi-resistant cells may be increasingly dependent on alternative DSB repair pathways for survival. These possibilities have fueled the development of strategies to overcome PARPi resistance by combining PARPi with other DNA damage response (DDR) drugs.

*Combinations of PARPi with ATR/Chk1 inhibitors.* The ataxia telangiectasia and Rad3-related (ATR) kinase, a master regulator of the replication stress response, plays a crucial role in stabilizing replication forks (64, 91). In addition to inducing fork instability, ATR inhibition also blocks HR at DSBs by preventing RAD51 recruitment (92). Thus, ATR inhibitors (ATRi) may be the ideal drugs to increase replication fork collapse and simultaneously prevent the repair of resulting DSBs.

In a panel of PARPi-resistant cell lines derived from a BRCA1-deficient cell line, ATRi effectively suppressed the restored HR and fork protection activities, resensitizing the resistant cells

to PARPi (55). In addition, BRCA1-deficient, PARPi-resistant cells also displayed restored abilities to prevent gap-initiated nascent DNA degradation and to activate the ATR checkpoint upon PARPi-induced fork collapse (15, 64). Again, these restored activities in the resistant cells are suppressed by ATRi. These results raise the possibility that a common underlying mechanism may be responsible for the various phenotypes associated with PARPi resistance and that this mechanism is disrupted by ATR inhibition. The exact mechanism that drives PARPi resistance in BRCA-deficient cells remains unclear, but restored RAD51 loading to ssDNA gaps and collapsed forks would alleviate PARPi-induced DNA damage. Indeed, RAD51 foci were observed in the cancer cells from *BRCA1/2* mutant patients that acquired PARPi resistance (93). Importantly, ATR inhibition abolishes the restored RAD51 foci and the loading of RAD51 to replication forks in BRCA1-deficient, PARPi-resistant cancer cells (55), supporting the idea that ATR inhibition disrupts a common RAD51-mediated mechanism driving PARPi resistance (Figure 3A).

The ability of ATR inhibition to overcome the PARPi resistance of BRCA1/2-deficient cells is seen not only in cell lines, but also in patient-derived xenografts (PDXs) and organoids of BRCA-deficient tumors (88, 94, 95). Inhibitors of Chk1, the effector kinase of ATR, have similar effects in PARPi-resistant cell lines and PDXs (Figure 3A) (94, 96). In a clinical trial, the combination of ATRi and PARPi showed efficacy in HR-deficient, PARPi-resistant, high-grade serous ovarian cancer (HGSOC) patients (97). Several ATRis are being tested in clinical trials (98), providing opportunities to overcome PARPi resistance in the near future.

**Combinations of PARPi with *WEE1/PKMYT1* inhibitors.** The tyrosine kinase *WEE1* inhibits both CDK1 and CDK2 by phosphorylating the tyrosine 15 of these two kinases (99). *WEE1* inhibitor (*WEE1i*) induces hyperactivation of CDK1/2 and overrides the G<sub>2</sub>/M DNA damage checkpoint, leading to excessive replication origin firing, replication catastrophe in S phase, and mitotic catastrophe in mitosis (99, 100). The combination of *WEE1i* and PARPi overcomes PARPi resistance in breast and ovarian cancer models (101). *WEE1i* and PARPi also display a synergy in pre-clinical models of BRCA wild-type triple-negative breast cancer (TNBC) by activating antitumor immune responses (102). *WEE1* inhibition also increases PARPi sensitivity in BRCA wild-type pancreatic cancer cells (103) and HGSOC cells (104), suggesting a utility of *WEE1i* as a PARPi sensitizer. A recent study suggested that *PKMYT1*, another member of the *WEE* kinase family regulating the G<sub>2</sub>/M transition, is a promising therapeutic target in cancer cells overexpressing cyclin E1, which are under high replication stress (105). *PKMYT1* inhibitor (*PKMYT1i*) induces unscheduled activation of CDK1 in S phase, driving cells into mitosis before completion of DNA replication. It is interesting to note that PARPi-resistant, BRCA1-deficient cells regain the abilities to suppress ssDNA and activate the checkpoint response (15, 59, 64) and that the abilities of *WEE1i* and *PTMYT1i* to increase replication and override the checkpoint may enable them to revert the changes in PARPi-resistant cells and overcome PARPi resistance (Figure 3B).

**Combinations of PARPi with inhibitors of gap repair.** DNA polymerase  $\theta$  (*POL $\theta$*  or *POLQ*) plays a crucial role in repairing gDSBs through the alternative end-joining (alt-EJ) pathway

(also known as the microhomology-mediated end-joining or MMEJ pathway). The expression of *POLQ* is upregulated in HR-defective epithelial ovarian cancers, suggesting that alt-EJ functions as a backup DSB-repair pathway to compensate for the loss of HR (106). *POLQ* is also shown to fill in ssDNA gaps in BRCA-deficient cancer cells (107). The antibiotic novobiocin (*NVB*) inhibits *POLQ* by binding to its ATPase domain and overcomes the PARPi-resistance of BRCA-deficient cells and PDXs (108). *ART558*, which inhibits the polymerase activity of *POLQ*, also overcomes the PARPi resistance of BRCA-deficient tumors (109). Notably, *POLQ* inhibitors (*POLQis*) induce high levels of ssDNA in PARPi-resistant, BRCA-deficient cells, suggesting that they prevent the repair of resected DSB ends and/or ssDNA gaps. Like *POLQ*, the translesion synthesis (TLS) pathway is also implicated in the repair of ssDNA gaps in BRCA1-deficient cells (14). TLS is initiated by PCNA monoubiquitylation at stalled forks or gaps, which allows the recruitment of *REV1* and several TLS DNA polymerases to bypass various DNA lesions. *JH-RE-06*, a TLS inhibitor that disrupts the interaction between *REV1* and *POL $\zeta$* , preferentially kills BRCA1 mutant cells and overcomes their PARPi resistance (14). Inhibition of *USP1*, a deubiquitylase of PCNA, leads to persistent PCNA mono- and polyubiquitylation and fork instability, preferentially killing BRCA-deficient cells (110). Recent studies showed that *USP1* inhibitors (*USP1is*) increase ssDNA gaps in BRCA1-deficient cells, synergize with PARPi in killing BRCA-deficient cells, and overcome PARPi resistance in BRCA1-deficient cells and PDXs (111, 112). Together, these results suggest that combining PARPi with inhibitors of ssDNA gap repair is a promising strategy for overcoming the PARPi resistance of BRCA-deficient cells (Figure 3C).

**Combinations of PARPi with drugs increasing PARP trapping.** *DNPH1* (2'-deoxynucleoside 5'-monophosphate *N*-glycosidase, also known as *RCL*) is an enzyme that eliminates the cytotoxic hydro5-hydroxymethyl-deoxyuridine (hmdU) monophosphate. Inhibition of *DNPH1* leads to increased hmdU misincorporation, PARP trapping, and fork collapse in BRCA1-deficient cells (113). Consequently, *DNPH1* inhibitor (*DNPHi*) preferentially kills BRCA1-deficient cancer cells and overcomes their PARPi resistance. Notably, the effects of *DNPH1* are dependent on the *SMUG* glycosylase, suggesting that DNA nicks or gaps are involved. Somewhat analogously, inhibition of the folate metabolism enzyme methylenetetrahydrofolate dehydrogenase/cyclohydrolase (*MTHFD2*) leads to an imbalanced dUTP:dTTP pool, increased replication stress, and preferential killing of acute myeloid leukemia (AML) cells (114). It would be interesting to determine whether *MTHFD2* inhibitor synergizes with PARPi in BRCA-deficient cells to overcome their PARPi resistance. The trapping of PARP by PARPi is also stimulated by the loss of PAR-binding chromatin remodeling factor *ALC1* (*CHD1L*) (115–117), likely due to the decrease of chromatin accessibility at DNA damage sites and reduced recruitment of repair proteins. Loss of *ALC1* drastically increases the PARPi sensitivity of BRCA-deficient cells and overcomes their PARPi resistance, making *ALC1* an attractive therapeutic target (Figure 3D). The studies on *ALC1* suggest that nucleosome remodeling, by influencing the repair of DNA nicks or gaps, is a key determinant of PARP trapping and the PARPi sensitivity of HR-deficient cells.

It is worth noting that recent CRISPR/Cas9 loss-of-function screens have served as a powerful and unbiased tool to explore synthetic lethal interactions with PARPis in BRCA-proficient and -deficient cells (118). In addition to ALC1 (115), RNase H2 was identified as a strong synthetic lethal screen hit with olaparib (119). Loss of RNase H2 renders cells hypersensitive to PARPi and also selectively kills BRCA1/2-deficient cells. CRISPR screens in prostate cancer cells revealed that loss of MMS22L drastically increases PARPi sensitivity (120). On the other hand, CRISPR screens also revealed mechanisms of PARPi resistance, such as point mutations in PARP1 (39), loss of CHK2 (120), and loss of ARH3 (121).

It is also important to note that, while combinations of PARPis with other targeted drugs may overcome PARPi resistance, these drug combinations may also increase general cellular toxicity and side effects, including hematological toxicity. Optimization of drug scheduling and dosing is likely important for achieving the maximal efficacy of these combination therapies. It remains unclear whether the selectivity of these combinations toward BRCA-deficient cells are reduced or enhanced compared with PARPis, which is an important question to address in clinical trials.

### Expanding application of PARPis beyond BRCA1/2-mutated cancers

While mutations in *BRCA1/2* and other HR genes are useful biomarkers for PARPi therapy, it has become increasingly clear that many other common oncogenic events can also alter the PARPi sensitivity of cancer cells. Some of the oncogenic events may induce BRCAness by compromising HR or increasing replication stress indirectly, whereas others may promote HR to allow cancer cells to survive genomic instability. Both types of oncogenic events may provide opportunities for PARPi therapy. For example, mutations in the *PTEN* tumor suppressor lead to defective HR and increased PARPi sensitivity (122). Oncogenic mutants of isocitrate dehydrogenase 1 and 2 (*IDH1/2*) induce accumulation of the oncometabolite 2-hydroxyglutarate (2-HG), which impairs HR and generates heterochromatin-dependent DNA replication stress, making *IDH1/2* mutant cancer cells hypersensitive to PARPi (123, 124). Ewing's sarcomas expressing the *EWS-FLI1* or *EWS-ERG* fusion oncogene are also sensitive to PARP inhibition, possibly due to R-loops and HR defects (125, 126). In castration-resistant prostate cancer, inhibition of androgen receptor (AR) induces HR defects and confers PARPi sensitivity (127, 128), suggesting that AR signaling promotes cancer cell survival by enhancing HR. Given the relevance of ssDNA gaps for the PARPi sensitivity of BRCA-deficient cells, it would be important to test to determine whether the oncogenic events above alter gap levels. Of note, *IDH1/2* mutants were recently shown to induce ssDNA gaps (125). Expression of *APOBEC3A*,

a driver of mutagenesis in multiple tumor types, also induces ssDNA gaps and confers PARPi sensitivity (129).

### Conclusions and perspectives

As discussed above, recent studies have revealed that the PARPi-induced synthetic lethality in BRCA-deficient cells can be gained or lost under various conditions, shedding light on the mechanism underlying this phenomenon. While the HR defect of BRCA-deficient cancer cells is clearly relevant to their sensitivity to PARPi (130, 131), the accumulation of ssDNA gaps in BRCA-deficient cells and the trapping of PARP at gaps are also determinants of PARPi sensitivity (15, 58, 59, 90). Given that loss of the HR activity of BRCA2 is critical for tumorigenesis (90), most if not all BRCA-deficient tumors are likely defective for both HR and gap suppression. We propose that both the HR and gap-protection defects of BRCA-deficient cells contribute to their PARPi sensitivity. The gap-protection defect of BRCA-deficient cells increases PARP trapping and DSB formation, whereas the HR defect prevents the repair of these DSBs. When gap suppression is compromised but HR remains largely proficient (e.g., in BRCA2 S3219A and RAD51 T131P mutant cells), PARPi-induced synthetic lethality is profoundly weaker than that in double-defective cells. Thus, PARPi-induced synthetic lethality is not a switch of cell death, but a quantitative dial of cell fitness. This model helps explain why quantitative changes in ssDNA gaps and PARP trapping may affect the PARPi sensitivity of BRCA-deficient cells. According to this model, reductions in ssDNA gaps and PARP trapping or increases in DSB repair through HR or alt-EJ are the main mechanisms dialing up PARPi resistance. On the other hand, strategies to increase ssDNA gaps and PARP trapping or inhibit HR and alt-EJ would dial down PARPi resistance. In addition, the levels of ssDNA gaps in cancer cells may quantitatively reflect the replication stress that drives PARPi sensitivity. It should be noted that the effects of PARPi on BRCA-deficient tumors in vivo also involve antitumor immunity (132, 133). This finding raises the possibility that the immunosuppression in the tumor microenvironments may contribute to PARPi resistance (134), whereas enhancing antitumor immunity may help overcome PARPi resistance. Future studies testing the models and possibilities above in preclinical and clinical settings would likely improve the efficacy of PARPi therapy in the clinic.

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