Trends in Cancer

CellPress OPEN ACCESS

Review

# PARPi, BRCA, and gaps: controversies and future research

Diego Dibitetto [1,2,3,*], Carmen A. Widmer [1,2], and Sven Rottenberg [1,2,4,*]

**In recent years, various poly(ADP-ribose) polymerase (PARP) inhibitors (PARPis) have been approved for the treatment of several cancers to target the vulnerability of homologous recombination (HR) deficiency (e.g., due to BRCA1/2 dysfunction). In this review we analyze the ongoing debates and recent breakthroughs in the use of PARPis for BRCA1/2-deficient cancers, juxtaposing the 'double-strand break (DSB)' and 'single-stranded DNA (ssDNA) gap' models of synthetic lethality induced by PARPis. We spotlight the complexity of this interaction, highlighting emerging research on the role of DNA polymerase theta (POLθ) and ssDNA gaps in shaping therapy responses. We scrutinize the clinical ramifications of these findings, especially concerning PARPi efficacy and resistance mechanisms, underscoring the heterogeneity of *BRCA*-mutated tumors and the urgent need for advanced research to bridge the gap between laboratory models and patient outcomes.**

## Highlights

Poly(ADP-ribose) polymerase (PARP) inhibitors (PARPis) are approved drugs used in neoadjuvant, adjuvant, or maintenance therapies for the treatment of breast, ovarian, pancreatic, and prostate cancers carrying pathogenic mutations in *BRCA1/2*.

PARP inhibition leads to synthetic lethality in BRCA-deficient cancers. PARPis induce a wide spectrum of DNA lesions, including DNA double-strand breaks (DSBs), single-stranded DNA (ssDNA) gaps, and stalled/degraded replication forks.

PARP trapping onto DNA and PARP catalytic inhibition are two main drivers of synthetic lethality with BRCA loss, but the precise mechanism of toxicity induced by PARPis in BRCA-deficient tumors is still a subject for debate.

The implications of recent findings on the efficacy and resistance mechanisms of PARPis underscore the clinical need for advanced research that bridges the gap between laboratory models and patient outcomes.

ssDNA gaps have distinct molecular outcomes depending on their spatial distribution and on the presence of trapped proteins.

## Synthetic lethality: THE knowledge 'gap'

In the realm of oncology, the pursuit of targeted therapies that exploit the unique vulnerabilities of cancer cells has led to significant advancements in treatment strategies. Among these, PARPis have emerged in a paradigm-shifting approach to the management of cancers harboring *BRCA1* and *BRCA2* mutations [1–3]. These mutations impair the cell's ability to repair DNA DSBs through HR, a critical pathway for maintaining genomic integrity [4]. The inception of PARP inhibition was based on the principle of synthetic lethality, aiming to selectively target BRCA-deficient tumors, thereby inducing cancer cell death while sparing normal cells. This therapeutic strategy led to the clinical approval of four different PARPis (niraparib, rucaparib, olaparib, and talazoparib), and demonstrated efficacy across a spectrum of cancer types, including breast, ovarian, prostate, and pancreatic cancers. However, the mechanisms underlying the synthetic lethality induced by PARPis and the emergence of resistance remain areas of intense investigation and debate. In this context, it is crucial to consider the distinct roles of PARP family members, such as PARP1, PARP2, and PARP3 [5]. Of these, PARP1 is the most studied and is primarily responsible for detecting DNA single-strand breaks (SSBs) and facilitating their repair. PARP2, although less abundant, also participates in DNA repair and has also been shown to play a role in maintaining genomic stability and supporting DNA replication. PARP3, however, is considered to be a mono(ADP-ribosyl)transferase, and it appears to have a more specialized role in the repair of DNA DSBs through non-homologous end-joining (NHEJ). The differential effects of inhibiting these PARP family members are significant. Recent studies have highlighted that while PARP1 trapping is a critical determinant of PARPi efficacy, the inhibition of PARP2 and PARP3 also contributes to the therapeutic effects, albeit through different mechanisms [5,6]. While PARP1 inhibition remains a primary mechanism for the effectiveness of PARPis in HR-deficient cancers, as highlighted by the efficacy of the PARP1-specific inhibitor saruparib [7], the precise contributions of PARP2 and PARP3 require further investigation. The differential roles of these PARP family members in

[1]Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland
[2]Bern Center for Precision Medicine and Cancer Therapy Research Cluster, Department for Biomedical Research, University of Bern, 3012 Bern, Switzerland
[3]Molecular Oncology and DNA Damage Response Laboratory, Department of Experimental Oncology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Via Mario Negri 2, 20156 Milan, Italy

DNA repair processes underscore the complexity of PARPi action and highlight the potential for further development of specific inhibitors that target individual PARP family members. As the landscape of cancer therapy continues to evolve, understanding these mechanisms not only holds the key to optimizing the use of PARPis but also opens new avenues for the development of combination therapies and overcoming resistance. This review delves into the current state of knowledge regarding PARPis, the controversies surrounding their mechanisms of action, and the future directions in research that may pave the way for improved outcomes for patients with *BRCA*-mutated cancers.

[4]Division of Molecular Pathology, The Netherlands Cancer Institute, 1066CX Amsterdam, The Netherlands

*Correspondence:
diego.dibitetto@marionegri.it
(D. Dibitetto) and
sven.rottenberg@unibe.ch
(S. Rottenberg).

## The DSB model: falling into the trap

In 2014, a groundbreaking study revealed that PARPis act by trapping PARP1/2 proteins onto DNA [8]. In contrast to the covalent crosslinking observed with topoisomerases treated with topoisomerase poisons, biochemical and molecular studies identified two main mechanisms contributing to this unique molecular property: (i) the prevention of self-conjugation of negatively charged PAR molecules necessary for PARP's dissociation from DNA (Figure 1A, Key figure), and (ii) allosteric modifications of PARP's structure, reinforcing its interaction with DNA, independent of enzymatic activity inhibition [9,10]. With these studies, the scientific community came to the conclusion that collisions between trapped PARP–DNA complexes and the replication machinery trigger *de novo* DNA DSB formation, necessitating BRCA1/2-mediated HR for repair (Figure 1A). This DSB model has received initial support from molecular data showing that PARPis with increased trapping capacity (i.e., talazoparib and niraparib) exhibit a higher cellular toxicity [8,10]. Another substantial piece of evidence supporting the importance of PARP trapping came from previous work showing that the cellular sensitivity to talazoparib, the clinically approved PARPi with the highest trapping capacity, is alleviated in PARP1-depleted cells or in cells expressing PARP1 allosteric mutations in the ZnF1 and WGR domains [11,12]. Mutations in these regions were shown to reduce PARP1's DNA-binding capacity or to reduce the trapping effect enforced by PARPis [11]. Based on these data, protein trapping has been considered a critical determinant of PARPi-induced cellular toxicity. Consistent with the mechanism of PARP trapping, which creates bulky lesions on replicating DNA templates, an increased sensitivity of HR-deficient tumors to DNA topoisomerase 1 (TOP1) inhibitors (e.g., topotecan, which forms covalent TOP1-DNA complexes) and DNA crosslinking agents (e.g., cisplatin and carboplatin) has been observed [13,14]. However, recent data challenge this understanding by demonstrating that PARPis induce transcription–replication conflicts (TRCs) in S phase, leading to DNA damage that is not primarily due to PARP trapping but is rather due to an inability to resolve these conflicts (Figure 1A) [15]. This mechanism suggests that the efficacy of PARPis in HR-deficient cells is due primarily to the enzymatic inhibition of PARP, making the PARP1 trapping capacity less relevant. In line with the key role of enzymatic PARP inhibition, another mechanism was put forward [16,17]: replication gaps caused by PARPis are the key determinant of PARPi synthetic lethality, not even necessitating BRCA function in HR (Figure 1B). These controversial mechanisms of action are intriguing, and elucidating which one is key to fully understanding synthetic lethality and learning from this for new approaches. It is also crucial to link these molecular findings to clinical outcomes. For example, if it is not the trapping, how are we to explain that the PARPi veliparib, which shares a similar degree of catalytic inhibition with olaparib but a much lower trapping capacity, does not seem to have a significant clinical impact on patient survival [18]? In this context, critical insights came from the use of *BRCA*-mutated patient-derived xenograft (PDX) models of ovarian cancer [19]. Indeed, it was recently shown that olaparib treatment in three *BRCA1*-mutated PDX models had no effect on tumor proliferation despite the complete loss of intratumoral poly(ADP-ribose) signal [20]. These results highlight that inhibition of PARP catalytic activity does not sufficiently explain the synthetic lethality with BRCA loss. Conversely, in the same study, the authors showed that these tumor models positively responded to the PARPi saruparib,

CellPress
OPEN ACCESS

## Key figure

### The source of genomic instability in BRCA-deficient cells

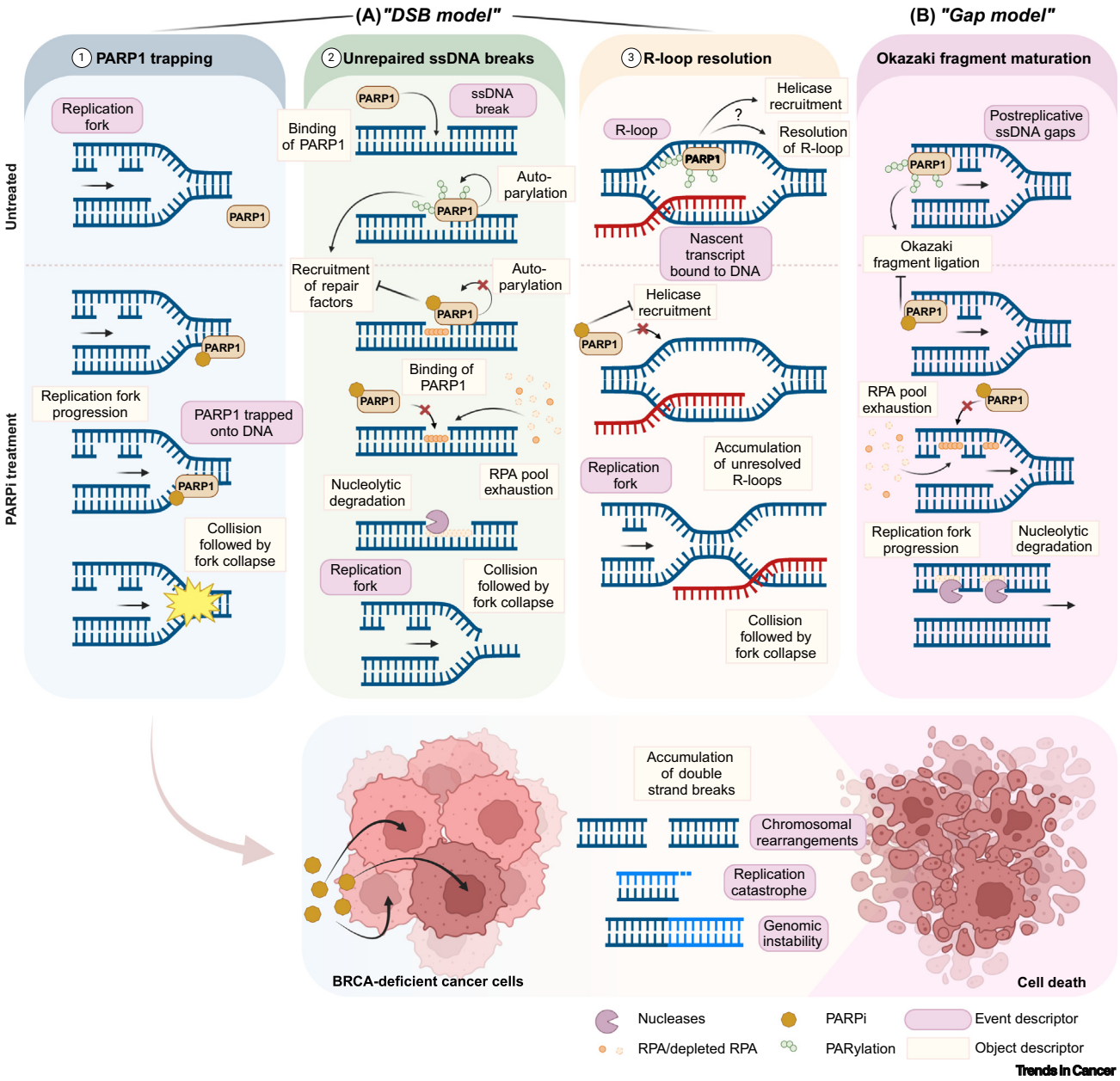


Figure 1. DNA double-strand breaks (DSBs) can accumulate by various mechanisms in BRCA-deficient tumor cells following poly(ADP-ribose) (PARP) inhibition. Currently two models of synthetic lethality are described. (A) The accumulation of DSBs by PARP1 trapping ① and subsequent collision with an approaching replication fork, unrepaired single-strand DNA (ssDNA) breaks ② due to the inhibition of PARP enzymatic activity or the inhibition of auto-PARylation, and the inhibition of R-loop resolution ③ are summarized under the 'DSB model'. (B) The 'gap model' describes the newly found role of PARP1 in suppressing post-replicative ssDNA gaps. Here, PARP1 trapping or inhibition of enzymatic activity upon PARP inhibitor (PARPi) treatment may lead to the extension of long ssDNA stretches and subsequent accumulation of DSBs, leading to cell death. Abbreviation: RPA, replication protein A. Figure created with Biorender.

a newly developed inhibitor with strong trapping activity [7], providing strong evidence to support the importance of PARP trapping in killing BRCA-deficient tumors.

Mechanistically, an important aspect of the DSB model is the presence of a DNA lesion (trapped PARP1/2, SSB, etc.) or the transcription machinery in front of an incoming replication fork (RF) prior to DSB generation (Figure 1A). By contrast, recent data showing a prominent role for PARP1 in the processing of Okazaki fragments (OFs) during lagging-strand maturation behind the RF [21] have challenged this model (Figure 1A). These new findings have raised the question of how PARP1 trapping or unligated gaps behind an RF can induce a DSB that is synthetically lethal with BRCA deficiency? This puzzle can still be explained by the DSB model, depending on whether the lesion persists until the next S phase. This would be consistent with the observation that BRCA-deficient tumors usually have a lag phase before they respond to PARPi therapy [22], suggesting the necessity for a few cycles of DNA replication before the synthetic lethality manifests. Hence, regarding the DSB model, a future challenge is the tracking of the DNA lesions triggered by PARPis through the cell cycle and the measurement of PARPi-induced genomic instability threshold, above which toxic DSB occurs.

## The gap model: another 'chicken and egg' paradox?

For many years the exclusive requirement of BRCA1/2 for homology-directed repair formed the cornerstone of our understanding of PARPis' synthetic lethality with BRCA deficiency. Yet, a shift in perspective came with the suggestion of another alternative, the gap model [16,17]. This model pivots on the role of PARP1 in mending unligated OFs during lagging-strand maturation (Figure 1B) [21,23]. In this context, BRCA proteins have been theorized to shield these nascent fragments from premature degradation, thereby preventing the exposure of long ssDNA gaps behind the RF (Figure 1B) [16,17,24]. Although the gap-induced toxicity has been suggested to be independent of the HR defect in BRCA-deficient cells, there may still be a dynamic interplay with the DSB model. For instance, ssDNA gaps may increase genomic instability by reducing the pool of the recombination factors replication protein A (RPA) and RAD51, leading to subsequent DSB formation at unprotected ssDNA gaps (Figure 1B). Conversely, DSB repair pathways may also lead to ssDNA gap formation in the form of resected tracts or repair-synthesis intermediates. This interdependence frames a quandary reminiscent of the 'chicken and egg' paradox: are BRCA-deficient cells compromised by PARPis due to initial ssDNA gap formation, or do these gaps serve as molecular precursors for subsequent DSBs? Recent efforts aimed to dissect this dilemma by using *BRCA2* separation-of-function mutants and define the role of gap suppression in therapy response and tumorigenesis [25,26]. It was shown that a *BRCA2* point mutant lacking its gap suppression function, but retaining its HR activity, showed no PARPi sensitivity or increased tumor incidence in mice. These data undoubtedly support the idea whereby the BRCA2 HR function, and not its function in gap suppression, is required for therapy resistance. However, this does not diminish the potential hierarchical significance of these mechanisms in genomic integrity, positing that the crucial function of BRCA2 in HR may only manifest following ssDNA gap accumulation. To fully prove this model, specific mutants that selectively impair HR without affecting gap accumulation would be useful, if they exist. Such insights would prompt a deeper insight into the nuanced roles of gap suppression and recombination, potentially unraveling the intricate 'chicken and egg' dynamics at play.

## ssDNA gaps: the new frontier of synthetic lethality?

The concept of leveraging ssDNA gaps as a target in cancer therapy has marked a significant evolution in our approach to synthetic lethality. Initially, the gap model spurred a paradigm shift, directing attention towards the mechanisms governing ssDNA gap formation and repair. This novel perspective has kindled interest in pharmacologically manipulating these gaps to refine

treatment strategies for BRCA-deficient tumors. A notable development in this field is the discovery of the synthetic lethality between *BRCA1/2* and the polymerase POLθ [27,28]. In this context, we would like to note the high correlation in the expression levels of *POLQ* and *PARP1* or *PARP2* that can be observed in the Cancer Cell Line Encyclopedia (Broad/DepMap) and Genomics of Drug Sensitivity in Cancer databases and visualized using CellMinerCDB (https://discover.nci.nih.gov/). This correlation could imply a coordinated regulation or functional partnership between POLθ (the protein product of the *POLQ* gene) and PARP1/2 in maintaining genomic stability, particularly under conditions of replication stress or DNA damage. Early research showing an essential role for POLθ in *BRCA*-mutated cells linked this dependency to POLθ's function in theta-mediated end-joining (TMEJ) [27,29,30], an essential DSB repair pathway in HR-deficient cells [31]. More recently, different groups additionally identified a role for POLθ in the fill-in of post-replicative gaps (Figure 2) [32–34], leading to the hypothesis that the accumulation of post-replicative gaps, in addition to the inhibition of TMEJ repair [27,35–37], may explain the synthetic lethality between *BRCA1/2* and *POLQ*. It is interesting to note that the role of POLθ in post-replicative gap-filling was observed in the absence of additional DNA damaging agents [33,34], although it remains unclear which lesions are processed by POLθ during unchallenged replication. POLθ was shown to be primarily involved in the extension of stochastically stalled OFs (Figure 2A) [34]. Another study has proposed a role for POLθ at both lagging and leading strands in the bypass of endogenous lesions (e.g., DNA adducts formed by aldehyde metabolism or due to the misincorporation of toxic nucleotides) (Figure 2B) [33]. Despite the robust *BRCA–POLQ* genetic interaction, the clinical translation of this basic finding is still uncertain. Recent preclinical works studying the effects of POLθ inhibitors (POLθis) as monotherapy agents in BRCA-deficient tumors have shown variable results. In many studies, POLθis exhibited low to modest toxicity in different BRCA-deficient cellular or organoid models [32,33,38,39], and in some cases, non-physiological POLθi concentrations were used to achieve a satisfactory cellular response (ranging between 10 and 25 μM). Based on these data, it seems likely that POLθ and PARP inhibition act in BRCA-deficient cancers through different molecular mechanisms.

Despite these advances, the precise role and implications of ssDNA gaps in the context of cancer therapy remain elusive. Various sources of ssDNA gaps, from repair failures to the normal function of replicative enzymes, manifest distinctly within cells, challenging our understanding of their impact. For instance, ssDNA gaps accumulate not only due to the failure in repair/filling mechanisms (i.e., following PARP or POLθ inhibition), but also due to the physiological activity of the primase/polymerase PRIMPOL (Figure 3) [40]. Due to its exquisite ability to reprime DNA synthesis downstream of bulky adducts, PRIMPOL generates post-replicative ssDNA gaps, facilitating lesion bypass on the leading strand (Figure 3) [41]. Notably, the cellular fate of PRIMPOL-induced gaps seems to be substantially different from that of POLθi- or PARPi-induced gaps. Indeed, hyperactivation of PRIMPOL activity has been proposed to rescue genomic instability in BRCA-deficient cells increasing DNA damage tolerance and chemotherapy resistance [40], indicating that PRIMPOL-induced gaps are not toxic. This discrepancy raises critical questions about the nature of ssDNA gaps and their differential effects on cellular viability, hinting at complex underlying mechanisms that govern their toxicity. PRIMPOL-induced gaps have been shown to be promptly filled by the REV1-POLζ complex [42], maintaining genome stability in HR-deficient cells (Figure 3). It is interesting to note that this complex does not seem to act on POLθi-induced gaps. Since PRIMPOL has been primarily shown to promote repriming on the leading strand [43], while PARPi-induced gaps accumulate on the lagging strand [23], there may be DNA strand-specific effects contributing to the cytotoxicity of these intermediates. Moreover, the distinct spatial distribution of the gaps, which accumulate far behind the fork following PARP inhibition and right behind the fork after PRIMPOL-mediated repriming, could have an influence on this phenomenon (Figure 3). Exonucleases may have a higher selectivity towards regions distal to the

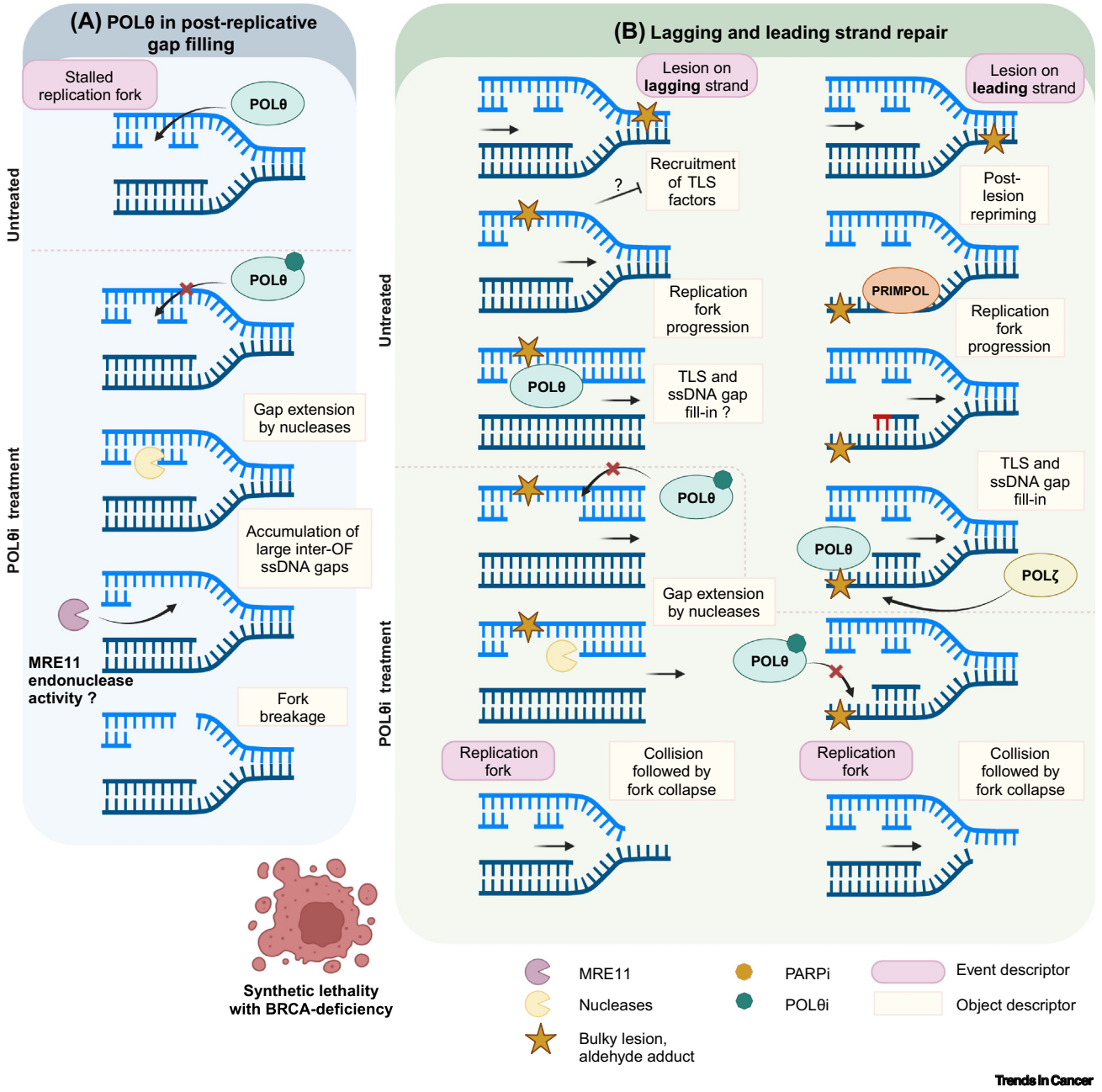


Figure 2. The role of DNA polymerase theta (POLθ) in single-stranded DNA (ssDNA) gap repair. (A) A novel mechanism of POLθ in the fill-in of post-replicative gaps in unchallenged replication has been described. (B) A role for POLθ has also been found in the bypassing of bulky endogenous lesions, such as aldehyde adducts. These newly found roles might explain, in addition to the inhibition of theta-mediated end-joining (TMEJ), the synthetic lethality between BRCA1/2 and POLθ. Abbreviations: OF, Okazaki fragment; PARPi, poly(ADP-ribose) polymerase (PARP) inhibitor; POLθi, POLθ inhibitor; PRIMPOL, primase/polymerase; TLS, translesion synthesis. Figure created with Biorender.

RF, and/or lesions more proximal to the RF may be more protected. Another explanation is that the accumulation of ssDNA gaps may not be sufficient *per se* to explain the synthetic lethality without additional lesions or trapping of replication/repair factors onto these intermediates. In

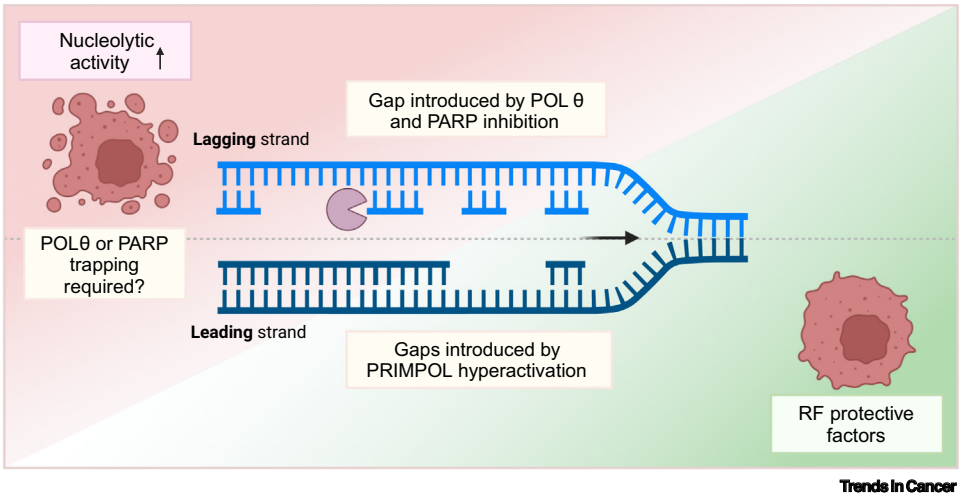


Figure 3. Spatial dependence of single-stranded DNA (ssDNA) lesion toxicity. Primase/polymerase PRIMPOL-induced ssDNA gaps accumulate mainly on the leading strand directly behind the replication fork (RF). Polymerase θ inhibitor (POLθi)- and poly(ADP-ribose) polymerase (PARP) inhibitor (PARPi)-induced gaps, however, accumulate on the lagging strand and last far behind the RF, where protective factors surrounding the RF may be less active. Figure created with Biorender.

this regard, a recent work has developed a new class of POLθ small-molecule inhibitors with the ability to trap POLθ onto DNA [44]. Future research will elucidate whether these drugs have the same therapeutic outcome as PARPis in several BRCA-deficient cellular and animal models. As research progresses, unravelling the mysteries of ssDNA gap formation, repair, and its role in synthetic lethality holds the promise of unlocking new therapeutic approaches to target BRCA-deficient cancers.

## Deciphering therapy resistance: a pathway to molecular insights

A useful approach to get critical insights into the mechanisms driving synthetic lethality with PARPis is the study of therapy resistance in BRCA-deficient tumors. It is reasonable to assume that crucial factors involved in facilitating synthetic lethality are altered in at least some of the resistant tumors. Obviously, BRCA1 and BRCA2 are affected, and secondary mutations that restore BRCA1/2 function are indeed frequently found in patients [45–47] and PDX models [48]. As this usually correlates with the restoration of RAD51 foci formation [48,49], it should be assumed that HR restoration is a major mechanism of PARPi resistance. Since *BRCA1/2* reversion mutations do not explain all mechanisms of resistance [45,47], the *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ (KB1P) [22] and *K14cre;Brca2*$^{F/F}$*;p53*$^{F/F}$ (KB2P) [50] genetically engineered mouse models (GEMMs) were used in the context of *BRCA1/2*-mutated breast cancer. The advantage of these models is that tumors cannot restore BRCA1/2 function due to large intragenic deletions of *Brca1* or *Brca2* in mammary tumors, offering insights into BRCA1/2-independent resistance pathways. HR restoration, marked by RAD51 foci formation, was shown to be prevalent in KB1P tumors that acquired PARPi resistance *in vivo* [51]. As the underlying mechanism of HR restoration, the 53BP1-RIF1-REV7-SHLD-CST pathway was frequently inactivated [51–54], which counteracts the end resection of DSBs by filling in ssDNA overhangs [55–57]. If this pathway is inactivated, resected DNA ends appear to be sufficient to trigger HR, even in the absence of BRCA1. The frequent presence of HR restoration in the PARPi-resistant tumors from patients as well as PDX and KB1P models confirms the notion that the cellular HR status is a critical determinant for the synthetic lethality of PARPis. Given that homology-directed repair is a downstream pathway required for the repair of a plethora of DNA lesions, it is difficult to extrapolate from these genetic data which specific

intermediates precede the DSB. In this context, data derived from the KB2P model are of interest. In contrast to the KB1P tumors, the restoration of RAD51 foci formation was not found in any of the PARPi-resistant KB2P models [51,58]. Apparently, in BRCA2-deficient tumors, cells cannot easily restore HR if not through the restoration of BRCA2 activity. Loss of PARP1/2 expression or inactivating *Parp1/2* mutations were not observed in these PARPi-resistant tumors, as this would also be synthetic lethal in the BRCA-deficient tumors. Instead, a main hit in these tumors, explaining at least a third of the resistant cases, is the loss of poly(ADP-ribose) glycohydrolase (PARG), a main counterplayer of PARP1/2 activity [51,58]. PARG depletion restores PAR chains and partially rescues PARP signaling, highlighting the contribution of endogenous PARG activity to synthetic lethality. Analyzing the essential gene set for tumor cells incapable of restoring HR (due to the irreversible BRCA2 deficiency) and PARP signaling (due to irreversible *Parg* loss) led to the conclusion that the *Parg*$^{-/-}$ KB2P tumors depend on EXO1 and FEN1 and are much more sensitive to EXO1/FEN1 inhibition compared with HR-proficient *Parg*$^{-/-}$ KP tumors [59]. Both EXO1 and FEN1 encode for 5′ flap endonucleases and 5′-to 3′-exonucleases, which are important during replication, SSB repair, and OF processing [60,61]. Indeed, compromised RF progression, SSB, and OF processing was reported in the PARG;BRCA2;p53-deficient cells upon EXO1/FEN1 inhibition [59]. Of note, an increased dependence of the PARG;BRCA2;p53-deficient cells on *Timeless* and its interactors *Clspn* and *Mcm2* was also observed [59], suggesting that these cells are also more dependent on the resolution of TRC. Another interesting result came from the analysis of the PARPi-resistant KB2P tumors that still have functional PARG. In many of these, a significant downregulation of *H2AFX* gene expression was found, which is consistent with the enrichment of gRNAs targeting *H2AFX* in multiple CRISPR/Cas9 screens using BRCA1/2-deficient mouse and human cell lines [62]. Mechanistically, it was shown that H2AX contributes to the synthetic lethality in BRCA1/2-deficient cells by orchestrating drug-induced RF degradation. Interestingly, H2AX loss restores RF stability and increases chemoresistance in BRCA1/2-deficient tumor cells without restoring HR [62]. In summary, the data from the drug resistance studies do not pinpoint a single mechanism in the DSB or gap models to be responsible for the synthetic lethality of PARPis in BRCA1/2-deficient cells. Instead, the data suggest a complex interplay of factors beyond the simple dichotomy of DSBs and gaps, implicating trapped PARP1, unresolved SSBs, and gaps resulting from unligated OFs to cause replication stress when hit by the RF. This stress, which may be exacerbated by TRCs, culminates in H2AX-mediated RF degradation and DSB formation. Moreover, it underscores the lethal impact of HR deficiency in *BRCA1/2*-mutated cells following PARPi treatment.

## PARPi resistance: from bench to bedside

Despite the significant advances in this area, our mechanistic understanding of the mechanisms of PARPi resistance in the clinic is still scarce. To date, the restoration of BRCA activity by secondary mutations is the primary mechanism of resistance manifesting in clinical settings. The frequency of this phenomenon changes across tumor types, highlighting the heterogeneity of *BRCA*-mutated tumors due to their profound genomic instability [63,64]. For instance, restoration of *BRCA* function occurs in approximately 15–25% of ovarian cancers that progressed on olaparib treatment [45,65], while it increases up to 60% in patients with *BRCA*-mutated metastatic breast cancer [47]. Genetic reversion of somatic and germline *BRCA2* mutations was also observed in two parallel studies analyzing patients with metastatic castration-resistant prostate cancer following progression after olaparib treatment, albeit the cohort was significantly smaller in size [66,67]. While these studies are still in their infancy, a potential limitation in the general assessment of the frequency of *BRCA* genetic reversion may come from the use of distinct PARPis in different diseases and the inclusion of patients with different stratification criteria. For example, it is possible that talazoparib, a PARPi with high trapping activity now used to treat metastatic breast cancer in patients with germline *BRCA* mutations [68], may impose a higher selective

pressure towards the restoration of BRCA function compared with olaparib in ovarian cancer. It is also worth mentioning that the different use of PARPis in the clinical practice of breast and ovarian cancer (i.e., as adjuvant therapy in breast cancer [68,69] and as a maintenance therapy in ovarian cancer [70–72]) could underpin the different rate of *BRCA* genetic reversion between these tumor types.

Although the restoration of BRCA function seems to account for a significant fraction of all the resistant cases, this mechanism alone does not sufficiently explain all the resistance cases observed in the clinic. In the past 15 years, several molecular studies have elucidated mechanisms of PARPi resistance independent of *BRCA* genetic reversion [73], leading to various biomarkers that may be useful to monitor in clinical settings. With the growing accessibility of clinical data from patients with *BRCA* mutations treated with PARPis, the expression and the mutational status of these biomarkers should be carefully assessed in prospective large-scale trials. Innovative studies have detected loss-of-function mutations in crucial DNA repair genes through circulating tumor DNA (ctDNA) sequencing [47]. Loss-of-function mutations in *TP53BP1* (gene encoding 53BP1), *RIF1*, and *PAXIP1* (gene encoding PTIP) and copy number loss of *SHLD2* were observed in a cohort of patients with metastatic breast cancer with *BRCA* mutations. Notably, these alterations occurred at a moderate allelic frequency after disease progression, and subclonally occurred in primary or secondary sites where novel *BRCA* mutations were not detected [47]. This strongly suggests that different mechanisms of resistance can either be selected in different areas of the primary tumor as a result of its heterogeneity, or distal metastatic niches might be more prone to acquire therapy resistance through mechanisms other than genetic reversion.

The selective pressure on BRCA-deficient tumors to restore HR capacity – either by restoration of BRCA activity, or by inactivation of the 53BP1-RIF1-REV7-SHLD-CST pathway in BRCA1-deficient tumors – points to the complex interplay of resistance mechanisms that may include fork protection and gap suppression. These functions become particularly crucial under conditions of oncogene-induced stress, indicating that the battle against resistance in the clinic is multifaceted, involving both direct and indirect alterations in DNA repair pathways. As we look forward, it becomes clear that addressing PARPi resistance in *BRCA*-mutated cancers will require a multifaceted approach, incorporating spatial omics studies to explore the tumor microenvironment's influence and a deeper investigation into the role of ssDNA gap suppression and other BRCA functions in therapy resistance. The path forward is not through the study of a single mechanism but through an understanding of the mosaic interactions that define cancer's resilience and adaptability to targeted therapies.

## Concluding remarks and future perspectives

Defining the molecular basis of the PARPi-BRCAness synthetic lethality is paramount for the design of novel combination therapies and the comprehension of therapy resistance mechanisms (Figure 4). Despite the growing use of PARPis in the clinic, the precise molecular consequences of PARP inhibition in BRCA-deficient cancers are still poorly understood (see Outstanding questions). In this review we have compared two molecular models, the DSB and the gap models. Although there are conceptual differences, there appears to be a complex interplay between the different mechanisms. Based on the frequent occurrence of HR restoration in PARPi-resistant tumors and the recent work using BRCA2 separation-of-function mutants [25], we believe that it is unlikely that gaps on their own, without compromised HR, fully explain the synthetic lethality with PARP inhibition. Instead, synthetic lethality appears to be elicited by distinct molecular defects with the lack of HR being a common downstream trait. A major source of variation comes from the presence of hypomorphic *BRCA1/2* mutations in tumors, and we lack a full understanding of their precise consequences. Such hypomorphic mutations are also frequently present

### Outstanding questions

What is the dominant mechanism by which PARPis induce toxicity in BRCA-deficient tumors? Is it through DNA damage caused by trapped PARP1, enzymatic PARP inhibition in the context of OF ligation, TRCs, or compromised SSB repair in general?

How persistent is PARP trapping, and in which cell cycle phase are trapped PARP-DNA complexes resolved?

Can we isolate an HR-deficient *BRCA2* mutant that retains its gap suppression function?

Why do POLθi- and PRIMPOL-induced gaps have distinct cellular toxicities?

What are the molecular traits that make an ssDNA gap toxic in BRCA-deficient cells?

What are the molecular mechanisms that lead to secondary resistance against PARPis in clinical settings, particularly in cases where there is no *BRCA* reversion mutation?

Why do different tumor types (e.g., ovarian and breast cancers) have different frequencies of *BRCA* genetic reversion?

What is the contribution of the tumor microenvironment to selecting different PARPi resistance mechanisms?

Can more effective biomarkers be developed to predict the responsiveness to PARPis based on the complex biology of *BRCA* mutations and PARPi mechanisms?

What combination therapies could potentially enhance the efficacy of PARPis in treating BRCA-mutated cancers? How can these combinations be optimized based on the underlying mechanisms of action and resistance?

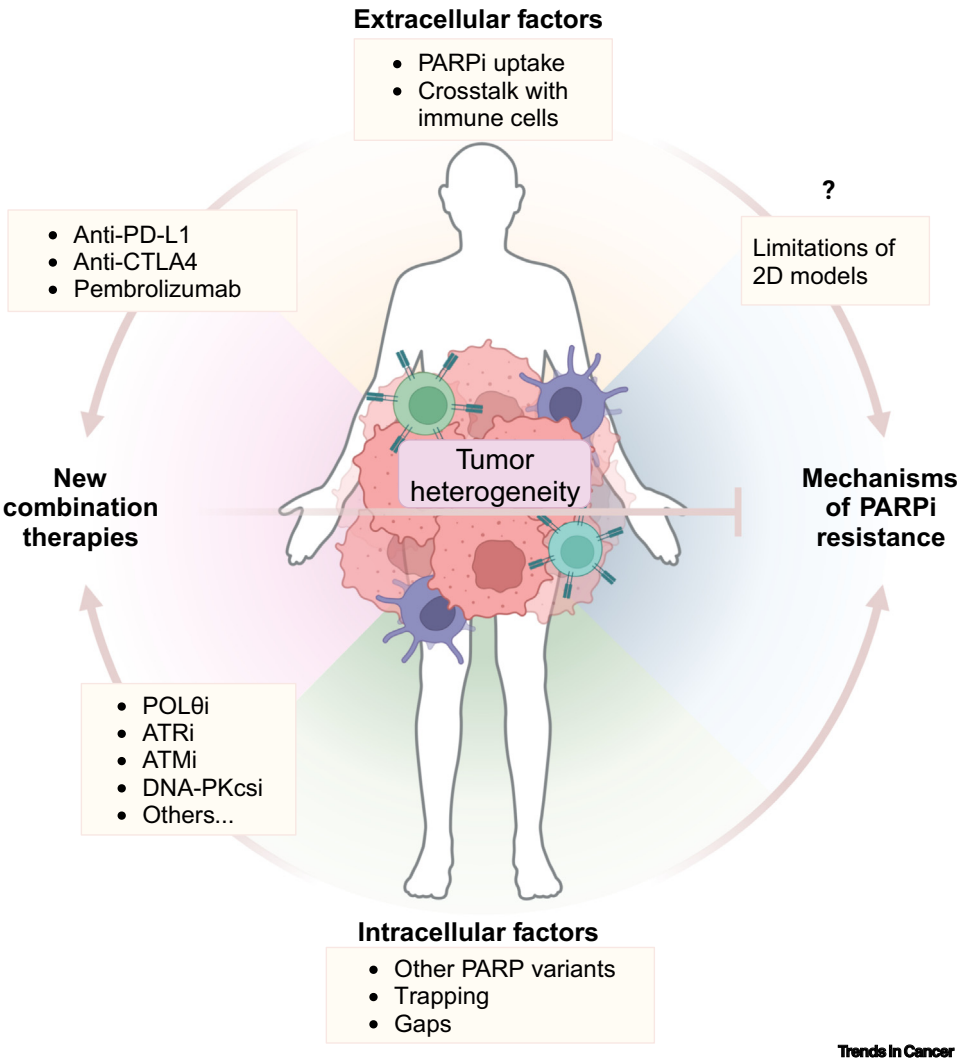


Figure 4. Novel poly(ADP-ribose) polymerase (PARP) inhibitor (PARPi) combination therapies: from bench to bedside. he efficacy of a PARPi is dictated by several intracellular and extracellular factors. The study of how intratumor heterogeneity shapes PARPi responses contributes to the optimization of novel combination therapies and to a better understanding of the PARPi resistance mechanisms in BRCA-deficient tumors. Abbreviations: ATMi, ataxia telangiectasia mutated kinase inhibitor; ATRi, ataxia telangiectasia Rad3-related kinase inhibitor; CTLA4, cytotoxic T lymphocyte-associated protein 4; DNA-PKcsi, DNA-dependent protein kinase catalytic subunit inhibitor; PD-L1, programmed death ligand 1. Figure created with Biorender.

in most of the currently available human *BRCA1/2*-mutated cell lines [74]. In the future, it would be useful to have more human BRCA1/2-deficient cancer-derived model systems available that recapitulate the high PARPi sensitivity in the low nanomolar range. Possibly, the derivation of matched 3D tumoroids from patients with highly PARPi-sensitive tumors and the subsequent second biopsy after the tumor has acquired drug resistance may be useful.

Another future challenge represents the study of intratumoral heterogeneity, in particular when it comes to understanding PARPi resistance. While in this review we have focused mostly on mechanisms that involve HR restoration, RF stabilization, and loss of PARG, other tumor-cell intrinsic

mechanisms have been described, including the overexpression of drug efflux transporters, lack of SLFN11 expression, and inactivation of the apoptotic pathway [75]. In the coming years it will be interesting to see whether specific combinations can be identified to overcome PARPi resistance. Although one can envision the targeted use of specific combinations for the treatment of relapsing tumors, we think that the overall goal should be to identify drug combinations that eradicate tumors upfront and supersede the treatment of tumor recurrence.

In addition to tumor-cell-intrinsic mechanisms, drug resistance also involves complex interactions with the tumor microenvironment. These can affect PARPi response by multiple means, including, for instance, the intratumoral drug disposition or the crosstalk with the immune system (Figure 4). This latter aspect has been shown to be relevant in PARPi response: for example, the recruitment of $CD8^+$ T cells by intratumoral stimulator of interferon genes (STING) pathway activation [76]. Consequently, PARPis are now being administered to patients in combination with the immune checkpoint inhibitors anti-CTLA4 and anti-PD-L1 [77–80]. Surprisingly, the use of pembrolizumab (anti-PD-1) hardly had an effect in recurrent high-grade serous ovarian cancer [81], the tumor subtype in which PARPis have a major impact. Further studies will shed light on how to fully unleash the immune response in this aggressive cancer. In this regard, the use of genetically engineered animals or the use of PDX models with a humanized immune system may be useful to mitigate these current limitations and will allow us to better understand the complete picture of the synthetic lethality between PARPis and BRCA loss.

## Acknowledgments

We thank Giovanna Damia, Paola Francica, Naguleswaran Arunasalam, and Lea Lingg for critical reading of the manuscript. The work leading to this manuscript was supported by the AIRC – Italian Association for Cancer Research ETS – Start-Up 2023 grant #29029 to D.D., the Swiss National Science Foundation (320030M_219453 to S.R.), the European Union (ERC-2019-AdG-883877 to S.R.), the Swiss Cancer League (KFS-5519-02-2022 to S.R.), the Department of Defense (W81XWH-22-1-0557 to S.R.), the Basser Center for BRCA, and the ISREC foundation.

## Declaration of interests

The authors declare no competing interests.

## References

1. Farmer, H. *et al.* (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434, 917–921
2. Bryant, H.E. *et al.* (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434, 913–917
3. Lord, C.J. and Ashworth, A. (2017) PARP inhibitors: synthetic lethality in the clinic. *Science* 1158, 1152–1158
4. Prakash, R. *et al.* (2015) Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. *Cold Spring Harb. Perspect. Biol.* 7, a016600
5. Curtin, N.J. and Szabo, C. (2020) Poly(ADP-ribose)polymerase inhibition: past, present and future. *Nat. Rev. Drug Discov.* 19, 711–736
6. Huang, D. and Kraus, W.L. (2022) The expanding universe of PARP1-mediated molecular and therapeutic mechanisms. *Mol. Cell* 82, 2315–2334
7. Illuzzi, G. *et al.* (2022) Preclinical characterization of AZD5305, a next-generation, highly selective PARP1 inhibitor and trapper. *Clin. Cancer Res.* 28, 4724–4736
8. Murai, J. *et al.* (2014) Differential trapping of PARP1 and PARP2 by clinical PARP inhibitors. *Cancer Res.* 72, 5588–5599
9. Langelier, M.F. *et al.* (2023) Clinical PARP inhibitors allosterically induce PARP2 retention on DNA. *Sci. Adv.* 9, eadf7175
10. Murai, J. *et al.* (2014) Rationale for poly(ADP-ribose) polymerase (PARP) inhibitors in combination therapy with camptothecins or temozolomide based on PARP trapping versus catalytic inhibition. *J. Pharmacol. Exp. Ther.* 349, 408–416
11. Pettitt, S.J. *et al.* (2018) Genome-wide and high-density CRISPR-Cas9 screens identify point mutations in PARP1 causing PARP inhibitor resistance. *Nat. Commun.* 9, 1849
12. Krastev, D.B. *et al.* (2022) The ubiquitin-dependent ATPase p97 removes cytotoxic trapped PARP1 from chromatin. *Nat. Cell Biol.* 24, 62–73
13. Zander, S.A.L. *et al.* (2010) Sensitivity and acquired resistance of BRCA1;p53-deficient mouse mammary tumors to the topoisomerase I inhibitor topotecan. *Cancer Res.* 70, 1700–1710
14. Pajic, M. *et al.* (2017) Selected alkylating agents can overcome drug tolerance of $G_0$-like tumor cells and eradicate BRCA1-deficient mammary tumors in mice. *Clin. Cancer Res.* 23, 7020–7033
15. Petropoulos, M. *et al.* (2024) Transcription–replication conflicts underlie sensitivity to PARP inhibitors. *Nature* 628, 433–441
16. Cong, K. *et al.* (2021) Replication gaps are a key determinant of PARP inhibitor synthetic lethality with BRCA deficiency. *Mol. Cell* 81, 3128–3144
17. Dias, M.P. *et al.* (2021) Loss of nuclear DNA ligase III reverts PARP inhibitor resistance in BRCA1 / 53BP1 double-deficient cells by exposing ssDNA gaps. *Mol. Cell* 81, 4692–4708
18. Diéras, V. *et al.* (2024) Veliparib with carboplatin and paclitaxel in BRCA-mutated advanced breast cancer (BROCADE3): final overall survival results from a randomized phase 3 trial. *Eur. J. Cancer* 200, 113580
19. Ricci, F. *et al.* (2014) Patient-derived ovarian tumor xenografts recapitulate human clinicopathology and genetic alterations. *Cancer Res.* 74, 6980–6990

20. Dellavedova, G. *et al.* (2022) The PARP1 inhibitor AZD5305 impairs ovarian adenocarcinoma progression and visceral metastases in patient-derived xenografts alone and in combination with carboplatin. *Cancer Res. Commun.* 3, 489–500
21. Hanzlikova, H. *et al.* (2018) The Importance of poly(ADP-ribose) polymerase as a sensor of unligated Okazaki fragments during DNA replication. *Mol. Cell* 71, 319–331
22. Rottenberg, S. *et al.* (2008) High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. *Proc. Natl. Acad. Sci. U. S. A.* 105, 17079–17084
23. Vaitsiankova, A. *et al.* (2022) PARP inhibition impedes the maturation of nascent DNA strands during DNA replication. *Nat. Struct. Mol. Biol.* 29, 329–338
24. Panzarino, N.J. *et al.* (2021) Replication gaps underlie BRCA-deficiency and therapy response. *Cancer Res.* 81, 1388–1397
25. Lim, P.X. *et al.* (2024) BRCA2 promotes genomic integrity and therapy resistance primarily through its role in homology-directed repair. *Mol. Cell* 84, 447–462
26. Dibitetto, D. and Rottenberg, S. (2024) DNA repair, gap suppression, or fork protection: BRCA2 needs a break! *Mol. Cell* 84, 409–410
27. Ceccaldi, R. *et al.* (2015) Homologous-recombination-deficient tumours are dependent on PolQ-mediated repair. *Nature* 518, 258–262
28. Mengwasser, K.E. *et al.* (2019) Genetic screens reveal FEN1 and APEX2 as BRCA2 synthetic lethal targets. *Mol. Cell* 73, 885–899
29. Zhou, J. *et al.* (2021) A first-in-class polymerase theta inhibitor selectively targets homologous-recombination-deficient tumors. *Nat. Cancer* 2, 598–610
30. Wyatt, D.W. *et al.* (2016) Essential roles for polymerase θ-mediated end joining in the repair of chromosome breaks. *Mol. Cell* 63, 662–673
31. Ramsden, D.A. *et al.* (2022) Mechanism, cellular functions and cancer roles of polymerase-theta-mediated DNA end joining. *Nat. Rev. Mol. Cell Biol.* 23, 125–140
32. Schrempf, A. *et al.* (2022) POLQ processes ssDNA gaps and promotes replication fork progression in BRCA1-deficient cells. *Cell Rep.* 41, 111716
33. Belan, O. *et al.* (2022) POLQ seals post-replicative ssDNA gaps to maintain genome stability in BRCA-deficient cancer cells. *Mol. Cell* 82, 4664–4680
34. Mann, A. *et al.* (2022) POLQ prevents MRE11–NBS1–CtIP-dependent fork breakage in the absence of BRCA2/RAD51 by filling lagging-strand gaps. *Mol. Cell* 82, 4218–4231
35. Mateos-Gomez, P.A. *et al.* (2015) Mammalian polymerase θ promotes alternative NHEJ and suppresses recombination. *Nature* 518, 254–257
36. Brambati, A. *et al.* (2023) RHINO directs MMEJ to repair DNA breaks in mitosis. *Science* 381, 653–660
37. Gelot, C. *et al.* (2023) Polθ is phosphorylated by PLK1 to repair double-strand breaks in mitosis. *Nature* 621, 415–422
38. Oh, G. *et al.* (2023) POLQ inhibition elicits an immune response in homologous recombination-deficient pancreatic adenocarcinoma via cGAS/STING signaling. *J. Clin. Invest.* 133, e165934
39. Zatreanu, D. *et al.* (2021) Polθ inhibitors elicit BRCA-gene synthetic lethality and target PARP inhibitor resistance. *Nat. Commun.* 12, 3636
40. Quinet, A. *et al.* (2020) PRIMPOL-mediated adaptive response suppresses replication fork reversal in BRCA-deficient cells. *Mol. Cell* 77, 461–474
41. García-Gómez, S. *et al.* (2013) PrimPol, an archaic primase/polymerase operating in human cells. *Mol. Cell* 52, 541–553
42. Taglialatela, A. *et al.* (2021) REV1-Polζ maintains the viability of homologous recombination-deficient cancer cells through mutagenic repair of PRIMPOL-dependent ssDNA gaps. *Mol. Cell* 81, 4008–4025
43. Mehta, K.P.M. *et al.* (2022) CHK1 phosphorylates PRIMPOL to promote replication stress tolerance. *Sci. Adv.* 8, eabm0314
44. Fried, W. *et al.* (2024) Discovery of a small-molecule inhibitor that traps Polθ on DNA and synergizes with PARP inhibitors. *Nat. Commun.* 15, 2862
45. Tobalina, L. *et al.* (2021) A meta-analysis of reversion mutations in BRCA genes identifies signatures of DNA end-joining repair mechanisms driving therapy resistance. *Ann. Oncol.* 32, 103–112
46. Pettitt, S.J. *et al.* (2020) Clinical BRCA1/2 reversion analysis identifies hotspot mutations and predicted neoantigens associated with therapy resistance. *Cancer Discov.* 10, 1475–1488
47. Harvey-Jones, E. *et al.* (2024) Longitudinal profiling identifies co-occurring BRCA1/2 reversions, TP53BP1, RIF1 and PAXIP1 mutations in PARP inhibitor-resistant advanced breast cancer. *Ann. Oncol.* 35, 364–380
48. Cruz, C. *et al.* (2018) RAD51 foci as a functional biomarker of homologous recombination repair and PARP inhibitor resistance in germline BRCA-mutated breast cancer. *Ann. Oncol.* 29, 1203–1210
49. Pellegrino, B. *et al.* (2022) Preclinical *in vivo* validation of the RAD51 test for identification of homologous recombination-deficient tumors and patient stratification. *Cancer Res.* 82, 1646–1657
50. Jonkers, J. *et al.* (2001) Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat. Genet.* 29, 418–425
51. Bhin, J. *et al.* (2023) Multi-omics analysis reveals distinct non-reversion mechanisms of PARPi resistance in BRCA1- versus BRCA2-deficient mammary tumors. *Cell Rep.* 42, 112538
52. Jaspers, J.E. *et al.* (2013) Loss of 53BP1 causes PARP inhibitor resistance in BRCA1-mutated mouse mammary tumors. *Cancer Discov.* 3, 68–81
53. Duarte, A.A. *et al.* (2018) BRCA-deficient mouse mammary tumor organoids to study cancer-drug resistance. *Nat. Methods* 15, 134–140
54. Xu, G. *et al.* (2015) REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature* 521, 541–544
55. Barazas, M. *et al.* (2018) The CST complex mediates end protection at double-strand breaks and promotes PARP inhibitor sensitivity in BRCA1-deficient cells. *Cell Rep.* 23, 2107–2118
56. Mirman, Z. *et al.* (2018) 53BP1–RIF1–shieldin counteracts DSB resection through CST- and Polα-dependent fill-in. *Nature* 560, 112–116
57. Mirman, Z. *et al.* (2022) 53BP1–shieldin-dependent DSB processing in BRCA1-deficient cells requires CST–Polα–primase fill-in synthesis. *Nat. Cell Biol.* 24, 51–61
58. Gogola, E. *et al.* (2018) Selective loss of PARG restores PARylation and counteracts PARP inhibitor-mediated synthetic lethality. *Cancer Cell* 33, 1078–1093
59. Andronikou, C. *et al.* (2024) PARG-deficient tumor cells have an increased dependence on EXO1/FEN1-mediated DNA repair. *EMBO J.* 43, 1015–1042
60. Keijzers, G. *et al.* (2015) Human exonuclease 1 (EXO1) activity characterization and its function on flap structures. *Biosci. Rep.* 35, e00206
61. Balakrishnan, L. and Bambara, R.A. (2013) Flap endonuclease 1. *Annu. Rev. Biochem.* 82, 119–138
62. Dibitetto, D. *et al.* (2024) H2AX promotes replication fork degradation and chemosensitivity in BRCA-deficient tumors. *Nat. Commun.* 15, 4430
63. Nik-Zainal, S. *et al.* (2012) Mutational processes molding the genomes of 21 breast cancers. *Cell* 149, 979–993
64. Sokol, E.S. *et al.* (2020) Pan-cancer analysis of BRCA1 and BRCA2 genomic alterations and their association with genomic instability as measured by genome-wide loss of heterozygosity. *JCO Precis Oncol.* 4, 442–465
65. Lin, K.K. *et al.* (2019) BRCA reversion mutations in circulating tumor DNA predict primary and acquired resistance to the PARP inhibitor rucaparib in high-grade ovarian carcinoma. *Cancer Discov.* 9, 210–219
66. Quigley, D. *et al.* (2017) Analysis of circulating cell-free DNA identifies multiclonal heterogeneity of BRCA2 reversion mutations associated with resistance to PARP inhibitors. *Cancer Discov.* 7, 999–1005
67. Goodall, J. *et al.* (2017) Circulating cell-free DNA to guide prostate cancer treatment with PARP inhibition. *Cancer Discov.* 7, 1006–1017
68. Litton, J.K. *et al.* (2018) Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. *N. Engl. J. Med.* 379, 753–763

69. Tutt, A.N.J. *et al.* (2021) Adjuvant olaparib for patients with BRCA1- or BRCA2-mutated breast cancer. *N. Engl. J. Med.* 384, 2394–2405
70. Ray-Coquard, I. *et al.* (2019) Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *N. Engl. J. Med.* 381, 2416–2428
71. Moore, K. *et al.* (2018) Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N. Engl. J. Med.* 379, 2495–2505
72. Ledermann, J. *et al.* (2012) Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N. Engl. J. Med.* 366, 1382–1392
73. Gogola, E. *et al.* (2019) Resistance to PARP inhibitors: lessons from preclinical models of BRCA-associated cancer. *Annu. Rev. Cancer Biol.* 3, 235–254
74. Nacson, J. *et al.* (2018) BRCA1 mutation-specific responses to 53BP1 loss-induced homologous recombination and PARP inhibitor resistance. *Cell Rep.* 24, 3513–3527.e7
75. Dias, M.P. *et al.* (2021) Understanding and overcoming resistance to PARP inhibitors in cancer therapy. *Nat. Rev. Clin. Oncol.* 18, 773–791
76. Pantelidou, C. *et al.* (2019) PARP inhibitor efficacy depends on $CD8^+$ T-cell recruitment via intratumoral STING pathway activation in BRCA-deficient models of triple-negative breast cancer. *Cancer Discov.* 9, 722–737
77. Domchek, S.M. *et al.* (2020) Olaparib and durvalumab in patients with germline BRCA-mutated metastatic breast cancer (MEDIOLA): an open-label, multicentre, phase 1/2, basket study. *Lancet Oncol.* 21, 1155–1164
78. Drew, Y. *et al.* (2024) Olaparib plus durvalumab, with or without bevacizumab, as treatment in PARP inhibitor-naïve platinum-sensitive relapsed ovarian cancer: a phase II multi-cohort study. *Clin. Cancer Res.* 30, 50–62
79. Tan, T.J. *et al.* (2024) Phase II DORA study of olaparib with or without durvalumab as a chemotherapy-free maintenance strategy in platinum-pretreated advanced triple-negative breast cancer. *Clin. Cancer Res.* 30, 1240–1247
80. Freyer, G. *et al.* (2024) Bevacizumab, olaparib, and durvalumab in patients with relapsed ovarian cancer: a phase II clinical trial from the GINECO group. *Nat. Commun.* 15, 1985
81. Matulonis, U.A. *et al.* (2019) Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study. *Ann. Oncol.* 30, 1080–1087