Using functional genetic screens to understand and overcome PARP inhibitor resistance

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Chapter 2

General introduction: Understanding and overcoming resistance to PARP inhibitors in cancer therapy

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ABSTRACT

Developing novel targeted anticancer therapies is a major goal of current research. The use of poly (ADP-ribose) polymerase (PARP) inhibitors in patients with homologous recombination-deficient tumours provides one of the best examples of a targeted therapy that has been successfully translated into the clinic. The success of this approach has so far led to the approval of four different PARP inhibitors for the treatment of several types of cancers, and a total of seven different compounds are currently under clinical investigation for various indications. Clinical trials have demonstrated promising response rates among patients receiving PARP inhibitors, although the majority will inevitably develop resistance. Preclinical and clinical data have revealed multiple mechanisms of resistance and current efforts are focused on developing strategies to address this challenge. In this Review, we summarize the diverse processes underlying resistance to PARP inhibitors and discuss potential strategies that might overcome these mechanisms, such as combinations with chemotherapies, targeting the acquired vulnerabilities associated with resistance to PARP inhibitors or suppressing genomic instability.

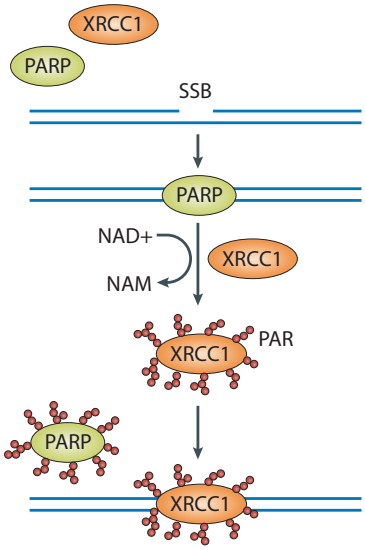
INTRODUCTION

Targeted killing of cancer cells while sparing surrounding nonmalignant tissues is a major goal of current treatment strategies. In 2005, two landmark studies demonstrated that inhibition of poly (ADP-ribose) polymerase 1 (PARP1) activity is specifically cytotoxic to cells lacking functional forms of the tumour suppressors BRCA1 or BRCA2[1,2]. This finding demonstrates that both PARP1 and BRCA1/2 are crucial for the efficient repair of DNA damage.

PARP1 is a nuclear enzyme that regulates multiple cellular processes through PARylation, including DNA damage signalling, chromatin remodelling, transcription, stabilization of replication forks, sensing of unligated Okazaki fragments during replication, inflammation and metabolism[3–5]. PARP1 has a crucial role in the timely and accurate repair of DNA damage. Upon DNA damage, PARP1 is rapidly recruited to single-strand breaks (SSBs) and double-strand breaks (DSBs), where, upon binding single-stranded DNA, it PARylates itself and other proteins resulting in the recruitment of downstream DNA repair factors[5] **(Box 1)**. BRCA1 and BRCA2 are then recruited further downstream to regulate one of the two major pathways for DSB repair during the S and G2 phases of the cell cycle, homologous recombination (HR). Unlike the other DSB repair pathways, HR repair is largely error free. BRCA1 is required for initiation of HR by promoting end-resection of the DSB and then acts further downstream together with BRCA2 and PALB2 to stimulate the recruitment of RAD51 to the resected single DNA strand[6]. HR then enables accurate repair of the DNA lesion using the newly replicated sister chromatid as a template[7].

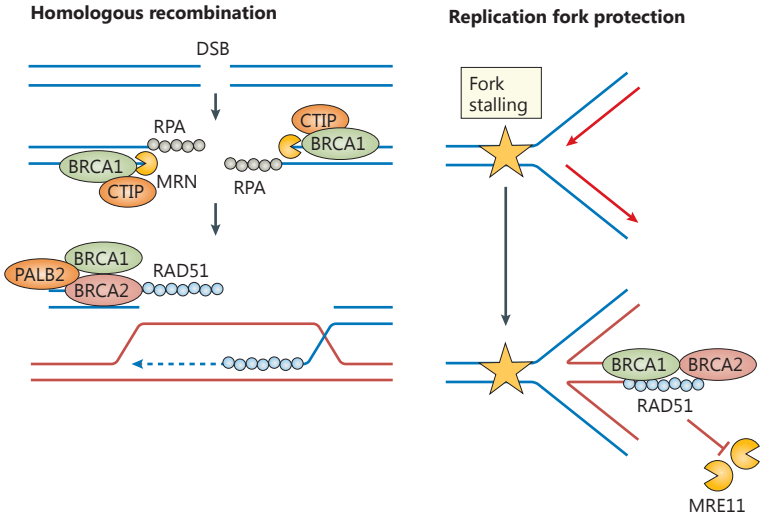
In addition to their role in HR, BRCA1 and BRCA2 are also crucial during S phase of the cell cycle, in which they protect stalled replication forks from degradation by nucleases, such as MRE11[8,9] **(Box 2)**. As a consequence of the above-mentioned roles of BRCA1 and BRCA2, heterozygous germline mutations in either of these genes confer a strong predisposition to breast[10], ovarian[11], prostate[12–14] and pancreatic cancers[15], which arise through loss of the remaining wild-type allele and are associated display with high levels of genomic instability owing to loss of HR. These HR-deficient *BRCA1/2*-mutant tumours are also dependent on compensatory DNA repair pathways. Pharmacological inhibition of key components of these pathways, such as PARP1, leads to DNA damage that, in the absence of BRCA1/2, triggers critical levels of genomic instability, mitotic catastrophe and cell death, ultimately resulting in a strong synthetic lethal relationship between BRCA1/2 and PARP[16]. BRCA1/2-deficient tumours often also have a pronounced level of sensitivity to other DNA-damaging agents, including platinum-based chemotherapies, topoisomerase (TOP) inhibitors and bifunctional alkylators, which likely generate classes of DNA lesions that are lethal to cells with deficient BRCA1/2 function[17–19].

The interaction between BRCA1/2 and PARP1 is the best studied synthetic lethal relationship to date and has been rapidly translated to the clinic through the development of small-molecule inhibitors of PARP enzymes. To date, several PARP inhibitors targeting the catalytic centre of the enzyme have been developed and approved for various clinical



Box 1 | PARP enzymes and PARylation. Poly (ADP-ribose) polymerase (PARP) 1 is the most abundant of a 17-member family of enzymes that share a common ADP-ribosyltransferase motif. By hydrolysing nicotinamide adenine dinucleotide (NAD+), PARP1 post-translationally modifies itself and/or other proteins with negatively charged poly (ADP-ribose) (PAR) moieties, a process known as PARylation[20]. Following DNA damage, PARP1 is rapidly recruited to DNA single-stranded DNA breaks, where it initiates a series of PARylation events, serving as a cellular sensor of DNA breaks and as a platform for the recruitment of downstream repair factors. In this process, PARP1 also autoPARylates, promoting its release from DNA[21]. In addition to PARP1, PARP2 and PARP3 are also activated by binding to DNA breaks[22–26]. PARP1 is responsible for more than 80% of PAR synthesis, while PARP2 accounts for the remainder[22,23]. Unlike PARP1 and PARP2, PARP3 modifies proteins primarily with mono (ADP-ribose)[24,25]. PARylation is a highly dynamic and reversible modification as its rapid turnover is mediated by PAR glycohydrolase (PARG), which degrades PAR[20,21]. ADP-ribosylhydrolase 3 (ARH3) is another PAR-degrading enzyme[27,28]. PARG has both endoglycosidase and exoglycosidase activities, while ARH3 seems to exert only exoglycosidase activity[27,29]. The eviction of PARP1 from sites of DNA damage is additionally regulated by the E3 ubiquitin ligase CHFR, which has been proposed to ubiquitinate the PARylated form of PARP1, resulting in PARP1 targeting for proteasomal degradation[30]. The removal of PARP1 from DNA is crucial for successful DNA repair and to prevent the collapse of replication forks owing to PARP1 trapping. Further studies attempting to understand which factors mediate the removal of PARylated PARP1 from DNA will be vital for optimizing the efficacy of PARP inhibitors and to uncover additional genetic vulnerabilities that could be exploited therapeutically. NAM, nicotinamide; SSB, single-strand break; XRCC1, X-Ray repair cross complementing 1.

indications[31], while numerous clinical trials that could further expand their use are currently ongoing. However, as also experienced with many other anticancer therapeutics, despite initial and often dramatic responses, patients receiving PARP inhibitors often ultimately develop treatment resistance. Therefore, the field is currently striving towards a better understanding of resistance to these agents and possible methods of overcoming this effect. In this Review, we summarize the various mechanisms of resistance to PARP inhibitors that have been reported and discuss several approaches that could be used to overcome or delay acquired resistance to these agents.



Box 2 | BRCA1/2 – similar names, different functions. BRCA1 and BRCA2 do not share homology and, although acting via a common pathway, also have several differing additional functions. Both factors are involved in homologous recombination (HR) and prevent replication-associated DNA damage, although BRCA1 is also known to regulate cell-cycle checkpoint activation as well as transcription[32]. BRCA1 and BRCA2 act at different levels during HR, resulting in functionally distinct mechanisms of resistance to PARP inhibitors. Resistance to PARP inhibitors via restoration of replication fork protection is observed in both BRCA1-deficient and BRCA2-deficient tumours, whereas reactivation of HR owing to loss of 53BP1 and its downstream factors is only reported in BRCA1-deficient tumours. The mutational signatures of BRCA1-deficient tumours and BRCA2-deficient tumours feature notable differences[10]. Small tandem duplications (<10 kb) are exclusively found in BRCA1-mutated tumours, although large deletions (beyond 100kb) are shared between tumours harbouring mutations in BRCA1 or BRCA2, thus further emphasizing the different implications of loss of BRCA1 versus BRCA2 function for DNA repair. BRCA1-deficient tumours and BRCA2-deficient tumours also differ on a pathological level. BRCA1-deficient tumours are usually of a basal-like or triple-negative subtype, while BRCA2-mutated cancers are not biased towards a specific subtype[33]. PALB2 has been reported to interact with BRCA2 and is required for its recruitment and DNA strand invasion during HR[34]. In line with its biological function, PALB2 mutations have been shown to confer an increased risk of developing breast cancer[35]. DSB, double-strand break; MRE11, meiotic recombination 11; MRN, MRE11–RAD50–NBS1 complex; PALB2, partner and localizer of BRCA2; RAD51, RAD51 recombinase; RPA1, replication protein A1.

TARGETING PARP IN CANCER

Mechanism of action

Four small-molecule PARP inhibitors are currently approved for clinical use (olaparib, rucaparib, niraparib and talazoparib) and a further three are being tested in phase III trials (veliparib, pamiparib and fluzoparib). In-depth discussions of these trials are provided elsewhere[36–38]. PARP inhibitors were thought to act by preventing the repair of SSBs, which accumulate during S phase of the cell cycle and pose a threat to replication fork progression[1,2]. However, genetic depletion or inhibition of PARP1 was soon discovered to not affect the number of SSBs occurring within a cell[39,40]. Additionally, depletion of XRCC1, a factor interacting with PARP1 during base-excision repair, failed to reveal any synthetic

lethal relationship with BRCA2 deficiency[41]. This led to the hypothesis that the antitumour activity of PARP inhibitors can be explained by trapping of PARP1 at DNA lesions and the formation of so-called DNA–protein crosslinks. These crosslinks then trigger the collapse of replication forks that encounter trapped PARP1, resulting in the accumulation of DSBs during S phase of the cell cycle. HR-deficient tumour cells are dependent on BRCA1/2-mediated repair to resolve DSBs in an error-free way; therefore, PARP inhibitors can induce DNA lesions that are lethal to such cells[1,2] **(Fig. 1)**.

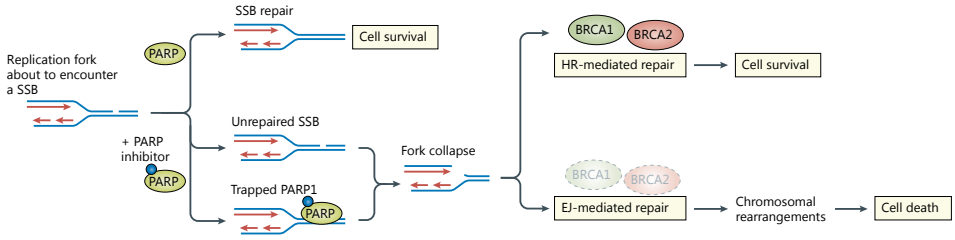


Figure 1 | PARP inhibitors and their mechanism of action. Poly (ADP-ribose) polymerase (PARP) inhibitors were initially thought to inhibit PARylation and thereby cause cytotoxicity; however, the main cause of tumour cell death was subsequently found to be trapping of PARP1 enzyme at DNA lesions. Single-strand breaks caused by DNA damage are faithfully repaired in the presence of PARP1; however, when trapped, PARP1 enzymes can cause a threat to replication forks during S-phase of the cell cycle, ultimately leading to collapse of the replication fork, resulting in double-strand breaks. In BRCA-proficient cells, homologous recombination (HR) enables the error-free repair of such breaks. By contrast, BRCA1/2-deficient cells are HR-deficient and are therefore reliant upon error-prone DNA end joining pathways such as classical nonhomologous end joining or alternative end joining to resolve the double strand breaks caused by replication fork collapse, triggering the accumulation of chromosomal aberrations and cell death by mitotic catastrophe.

The PARP1 trapping theory is currently supported by data from numerous studies. First, functional PARylation has been shown to be required for the release of PARP1 from DNA, indicating that trapped DNA–PARP complexes are indeed formed[42]. Second, the various PARP inhibitors have different levels of cytotoxicity, despite a generally similar capacity to inhibit the catalytic activity of PARP1[43] **(Table 1)**. As reported by Murai et al.[44], an apparent correlation exists between the relative ability of these compounds to trap PARP1 onto DNA and their cytotoxicity. The PARP inhibitor with the greatest PARP-trapping ability, talazoparib, is approximately 100 times more potent in trapping PARP1 than niraparib, which in turn is able to trap PARP1 more potently than olaparib and rucaparib[43,45]. Conversely, veliparib appears to have a limited ability to trap PARP1, despite its ability to inhibit PARylation, and fails to elicit the same level of synthetic lethality in preclinical models, compared with more effective PARP1 trappers[43,45,46]. PARP inhibitors not only differ in their ability to trap PARP1; they have also been shown to have differing allosteric effects. Release of PARP1 from the DNA is prevented by talazoparib and olaparib, but promoted by rucaparib,

niraparib and veliparib, which might further explain their differing *in vitro* potency[47]. As a result, the maximum-tolerated dose of PARP inhibitors decreases with increasing levels of PARP-trapping activity, and more potent PARP trappers often have to be administered at lower doses in the clinic[48–50]. Conversely, the main adverse effects observed in patients receiving these various agents (nausea, vomiting, fatigue, as well as anaemia) seem to largely overlap[51].

Data from the past few years suggest that PARP inhibitors might have a broader effect on cellular processes than previously anticipated. For example, PARP inhibitor-induced DNA–protein crosslinks have been found to occur not only in DNA lesions, but also at ssDNA during DNA replication and at genome-embedded ribonucleotides[4,52]. Additionally, cells that enter mitosis after sustained PARP inhibitor-induced damage during S phase often contain mitotic defects such as chromatin bridges and lagging chromosomes, which ultimately lead to cell death[53]. Data published in 2018 suggest that treatment with high doses of PARP inhibitors triggers an acceleration of replication fork elongation, which reduces the fidelity of DNA polymerases as well as promoting activation of the DNA damage response[54]. However, more research is needed to better understand how these novel mechanisms add to the cytotoxicity of PARP inhibitors, and whether their contribution is dependent on BRCA1/2-deficiencies. The broad range of DNA substrates and the various processes that PARP inhibitors are thought to target imply that PARP inhibitors might also reduce the survival of BRCA1/2-wild-type cells as well as other DNA repair-deficient cell lines, which is the rationale of current clinical trials testing PARP inhibitors in patients with cancers other than HR-deficient breast and ovarian cancers.

Clinically approved PARP inhibitors

In 2014, the first PARP inhibitor, olaparib, was approved for the treatment of women with *BRCA1/2*-mutated metastatic ovarian cancer who have received three or more prior lines of chemotherapy **(Supplementary table 1)**[68,69]; and for the maintenance treatment of women with *BRCA1/2*-mutated ovarian cancers who are in complete or partial remission after platinum-based chemotherapy[70]. In 2016, a second PARP inhibitor, rucaparib, was authorized for the treatment of women with advanced-stage ovarian cancers harbouring deleterious *BRCA1/2* mutations who have received two or more prior lines of chemotherapy[71–73]. In 2019, niraparib was approved for the treatment of patients with HR-deficient advanced-stage ovarian cancer who have received three or more prior chemotherapy regimens[74,75].

A retrospective analysis of the data from the clinical trials leading to the approval of olaparib in 2014 demonstrated a progression-free survival (PFS) benefit even for patients with *BRCA1/2*-wild-type ovarian cancer, suggesting that some patients with *BRCA1/2*-wild-type ovarian cancer might benefit from PARPi maintenance therapy[76]. Within three years, phase III trials testing either olaparib[76,77], niraparib[78] or rucaparib[79,80] demonstrated statistically significant improvements in the PFS of women with ovarian cancer harbouring either mutated or wild-type *BRCA1/2*. These observations led to the approval of these

Table 1 | Pharmacological characteristics of PARP inhibitor.

Drug	IC_{50} (nM)	Trapping ability [45,55]	P-glycoprotein substrate [55–59]	Targets [60]	CNS penetration [61–64]a small molecule inhibitor of poly (ADP-ribose	$T_{1/2}$ (hours) [55–59,65,66]recommended phase II dose (RP2D	Bioavailability [56–59,67]	Known off-target interactions
Olaparib	1	+	Yes	PARP1/2/3/4	+	11.9	55–60% in mice; 79% in dogs; <20% in rats	CYP3A4
Rucaparib	1	+	Yes	PARP1/2/3/4, TNKS1	+	25.9	36%	CYP enzymes
Niraparib	4	+	Weak	PARP1/2	++	48–51	75%	Dopamine, norepinephrine or serotonine transporters
Talazoparib	0.6	++	Weak	PARP1/2	-	90 ± 58 h	41%	None
Veliparib	2	-	No	PARP1/2/3	+	4.1–8.2	NA	NA
Pamiparib	0.83	+	No	PARP1/2	++	NA	NA	NA
Fluzoparib	1.5	NA	NA	PARP1/2	NA	?	35.8% in mice	NA

CNS, central nervous system; CYP, cytochrome P450; IC50, half maximal inhibitory concentration; NA, not available; PARP, poly(ADP- ribose) polymerase; TNKS1, tankyrase 1; –, poor; +, modest; ++, good.

three PARP inhibitors as maintenance therapies for patients with recurrent ovarian cancer, regardless of *BRCA1/2*-mutation status. This experience suggests that a subset of BRCA1/2-proficient cancers might nonetheless also harbour clinically relevant HR defects, either owing to mutations in other HR-related genes (such as *RAD51* paralogues) or other mechanisms.

PARP inhibitors have also been approved as first-line systemic therapies for women with ovarian cancer. In 2018, results from phase III trials demonstrated that maintenance therapy with olaparib provided a substantial PFS benefit for women with newly diagnosed *BRCA1/2*-mutant advanced-stage ovarian cancer, reducing the risk of disease progression or death by 70% compared with placebo[81]. This extension of PFS led to the approval of olaparib as a first-line maintenance therapy for women with *BRCA1/2*-mutated advanced-stage ovarian cancer[81]. In 2020, niraparib was approved for first-line maintenance therapy in patients with advanced-stage, platinum-sensitive ovarian cancer, regardless of *BRCA1/2* status[82].

Data from the OlympiAD trial led to the 2018 approval of olaparib as the first PARP inhibitor indicated for the treatment of patients with metastatic HER2-negative, *BRCA1/2*-mutant breast cancer who had previously received chemotherapy[83]. This decision was soon followed by the approval of talazoparib for similar indications[84]. In 2019, olaparib also became the first PARP inhibitor to be approved for the maintenance treatment of patients with *BRCA1/2*-mutated metastatic pancreatic adenocarcinoma[85]. In 2020, the use of PARP inhibitors was further expanded to include men with prostate cancer, with the approval of olaparib for patients with metastatic, castration-resistant prostate cancers (CRPC) harbouring mutations in HR-related genes after disease progression on enzalutamide, or abiraterone[86]. This approval is the first to permit the use of olaparib in patients with tumours harbouring mutations in genes other than *BRCA1/2*. Whether olaparib has any substantial efficacy in such tumours remains unclear due to the fact that PFS analysis in this study was performed in subgroups containing mutations in 15 DNA repair genes including *BRCA1* and *BRCA2*. This makes it difficult to determine the contribution of mutations in individual genes to the observed PFS benefit. Compared to patients with *BRCA1/2* mutations, patients with mutations in *ATM* and other DDR genes showed little PFS benefit from olaparib[86,87]. Following the 2020 approval of olaparib, rucaparib received accelerated approval for the treatment of men with *BRCA1/2*-mutant metastatic CRPC who have previously received androgen receptor-directed therapy and taxane-based chemotherapy[88].

Veliparib does not yet have an approved label and its use is being investigated mostly in combination with chemotherapy or targeted therapies in a range of solid tumours (such as NCT02032277, NCT02152982, NCT02163694, NCT02264990 and NCT02470585), likely owing to its reduced ability to trap PARP1 and, consequently, limited synthetic lethality and limited single-agent activity. The PARP inhibitors pamiparib and fluzoparib have been developed over the past few years and it remains to be determined whether they have advantages over currently approved agents. Pamiparib has shown a favourable safety profile

and preliminary antitumour activity in phase I testing[89,90], resulting in the initiation of a phase III trial with pamiparib versus placebo as maintenance therapy for women with platinum-sensitive advanced-stage ovarian cancer (NCT03519230). A phase III trial exploring the use of pamiparib in patients with inoperable locally advanced or metastatic gastric cancer with a previous response to platinum-based first-line chemotherapy has also been initiated (NCT03427814).

Data on the efficacy of fluzoparib remain limited, although preclinical data indicate a favourable safety profile and robust *in vivo* antitumour activity, with similar potency to that of olaparib[67]. Phase III studies investigating the use of fluzoparib as maintenance therapy in patients with platinum-sensitive recurrent ovarian cancer (NCT03863860) and as maintenance therapy in patients with *BRCA1/2* or *PALB2*-mutated pancreatic cancer that has not progressed on first-line platinum-based chemotherapy (NCT04300114) are currently ongoing.

PFS is currently the most widely used primary outcome in clinical trials testing PARP inhibitors. Since most studies have been initiated within the past few years, overall survival (OS) data remains limited. OS data have been published for olaparib in HER2-negative breast cancer and pancreatic cancer patients, but the results are mixed and the OS data from both studies might be underpowered since OS was not the primary end point[84,86]. In an analysis of OS data from a phase II trial, patients with recurrent platinum-sensitive advanced-stage *BRCA*-mutated ovarian cancer receiving olaparib maintenance monotherapy after platinum-based chemotherapy have both improved PFS and OS outcomes, although the OS benefit (29.8 months versus 27.8 months with placebo) did not reach statistical significance[91]. In November 2020, new data from the SOLO1 trial showed that maintenance treatment with olaparib in women with newly diagnosed advanced platinum-sensitive *BRCA1/2*-mutant ovarian cancer extended the median PFS by 42 months in comparison to placebo[92]. Strikingly, in patients with complete response at baseline, the risk of disease recurrence or death was reduced by 63%. Although OS data are not yet available in this setting, the significant increase in PFS may translate in an OS benefit. In March 2021, a preplanned OS analysis on the phase III SOLO2 trial demonstrated that maintenance treatment with olaparib extends the median OS of patients with relapsed platinum-sensitive advanced-stage *BRCA1/2*-mutant ovarian cancer by 12.9 months in comparison to placebo[93]. Importantly, this is the first report of improved OS with PARP inhibitors used as maintenance therapy. Nonetheless, OS benefit remains to be determined for other PARP inhibitors and for indications other than platinum-sensitive *BRCA1/2*-mutant ovarian cancer. Moreover, while emerging data show that PARP inhibitors delay disease recurrence and prolong patient survival, most patients receiving PARP inhibitors ultimately will eventually experience disease progression, thus indicating a need to better understand mechanisms of resistance and how to delay or overcome resistance.

Beyond *BRCA1/2* mutations

The benefits of PARP inhibitors seem to be greatest in patients with germline and/or somatic *BRCA1/2* mutations, although data from several clinical studies suggest that PARP inhibitors might also provide benefit for patients lacking these mutations, albeit to a lesser extent[78,80]. For example, a phase III trial of maintenance rucaparib in women with recurrent ovarian cancer with a response to platinum-based chemotherapy found an increase in median PFS from 5.4 months to 16.6 months in patients with *BRCA1/2*-deficient disease, while less pronounced but statistically significant effects were observed for those with *BRCA1/2*-wild-type but HR-deficient tumours (13.6 months) and the entire cohort (10.8 months)[80]. In line with these observations, several other trials have reported a statistically significant improvement in PFS in patients with *BRCA1/2*-wild-type ovarian cancers[82,94,95], and as a consequence, niraparib maintenance therapy was approved by the FDA for women with advanced-stage ovarian cancer regardless of HR status in April 2020.

These observations suggest that *BRCA1/2* mutations do not entirely account for the benefits derived from PARP inhibitors. Indeed, preclinical data have long suggested that deficiencies in other HR genes might also confer sensitivity to PARP inhibitors[96]. Several studies involving patients with ovarian[72,97] and prostate[98,99] cancers have demonstrated that those with *BRCA1/2*-wild-type tumours harbouring deleterious variants in other DNA repair genes, such as *PALB2*, various *RAD51* homologues, *ATM, CHEK2, CDK12, FANCA, RAD54L* and *BRIP1* might benefit from PARP inhibitors. Notably, data from an increasing number of studies indicate that mutations in *PALB2*, a factor acting together with BRCA1 and BRCA2 during HR **(Box 2)**, are a strong indicator of sensitivity to PARP inhibitors[35,100,101] and several others are currently ongoing (NCT02401347 and NCT03330847). Mutations in other core HR-related genes, such as *RAD51* paralogues, also likely confer sensitivity to PARP inhibitors[102]. It remains unclear whether mutations in other DNA damage response (DDR) related genes, such as *ATM* and *CHEK2,* impart a clinically relevant sensitivity to PARP inhibitors, and this possibility is currently being investigated in several ongoing clinical trials[100,103].

Apart from key DNA repair factors, mutations in chromatin regulators, such as *ARID1A*[104] and *BAP1*[105,106], have also been suggested to result in increased sensitivity to PARP inhibitors *in vitro*. Furthermore, indirect downregulation of HR-related proteins owing to mutations in genes involved in the Krebs cycle, such as *IDH1/2*, *FH* and succinate dehydrogenases, are reported to result in downregulation of HR-related proteins and increased sensitivity to PARP inhibitors in preclinical models[107,108]. Notably, certain cancers that lack mutations in HR-related genes (such as small-cell lung cancers) have demonstrated some sensitivity to PARP inhibitors[109,110], possibly owing to increased levels of replication stress arising from *RB1* mutations. Moving beyond a focus on mutations in *BRCA1/2* and on deficient HR repair and establishing a rationale for broadened use of PARP inhibitors for a wider range of cancers will therefore be important. Several diagnostic tools have been developed to

identify HR-deficient tumours irrespective of *BRCA1/2* mutation status, such as assays designed to determine *RAD51* status or assessments of mutational signatures and genomic instability associated with HR-deficiency, which might aid in identifying novel groups that derive benefit from PARP inhibitors[111]. However, differentiating between the general effects of PARP trapping — which might not provide a greater therapeutic window than conventional chemotherapies — from cancer-specific vulnerabilities to PARP inhibitors will be crucial.

RESISTANCE TO PARP INHIBITION

PARP inhibitors frequently elicit a good initial response, although most patients develop resistance to these agents, resulting in disease relapse. Acquired resistance to PARP inhibitors can develop via three general mechanisms: drug target-related effects, such as upregulation of drug efflux pumps or mutations in PARP or functionally related proteins; restoration of HR, owing to restoration of BRCA1/2 function; or loss of DNA end protection and/or restoration of replication fork stability **(Fig. 2)**.

Upregulation of drug efflux pumps

Upregulation of the drug efflux transporter ABCB1, also known as P-glycoprotein, was one of the first mechanisms proposed to trigger resistance to PARP inhibitors. ABCB1 belongs to a family of ATP-binding cassette (ABC) transporters, which are an established source of resistance to multiple chemotherapies and other agents by preventing their intracellular accumulation. ABCB1-induced resistance to PARP inhibitors was initially observed in BRCA1/2-deficient mouse models developing spontaneous mammary tumours. Long-term exposure of these models to olaparib resulted in the outgrowth of resistant, ABCB1-overexpressing tumours[112,113]. Importantly, resistance could be reversed by a combination of olaparib and the ABCB1 inhibitor tariquidar, proving that increased drug efflux is indeed the cause of resistance. Although, the clinical relevance of this mechanism is still unclear, upregulation of ABCB1 has been reported in chemotherapy-resistant ovarian cancers[11]. Even though all PARP inhibitors were designed to inhibit the same target domain, some inhibitors, such as veliparib and niraparib, are poor substrates for ABCB1, suggesting that these agents should circumvent ABCB1-induced resistance. Furthermore, it should be noted that ABCB1 overexpression frequently induces cross-resistance to chemotherapies, such as taxanes and doxorubicin; therefore, PARP inhibitors that are not transported by ABCB1 might be more effective in patients that have previously received chemotherapy[113,114].

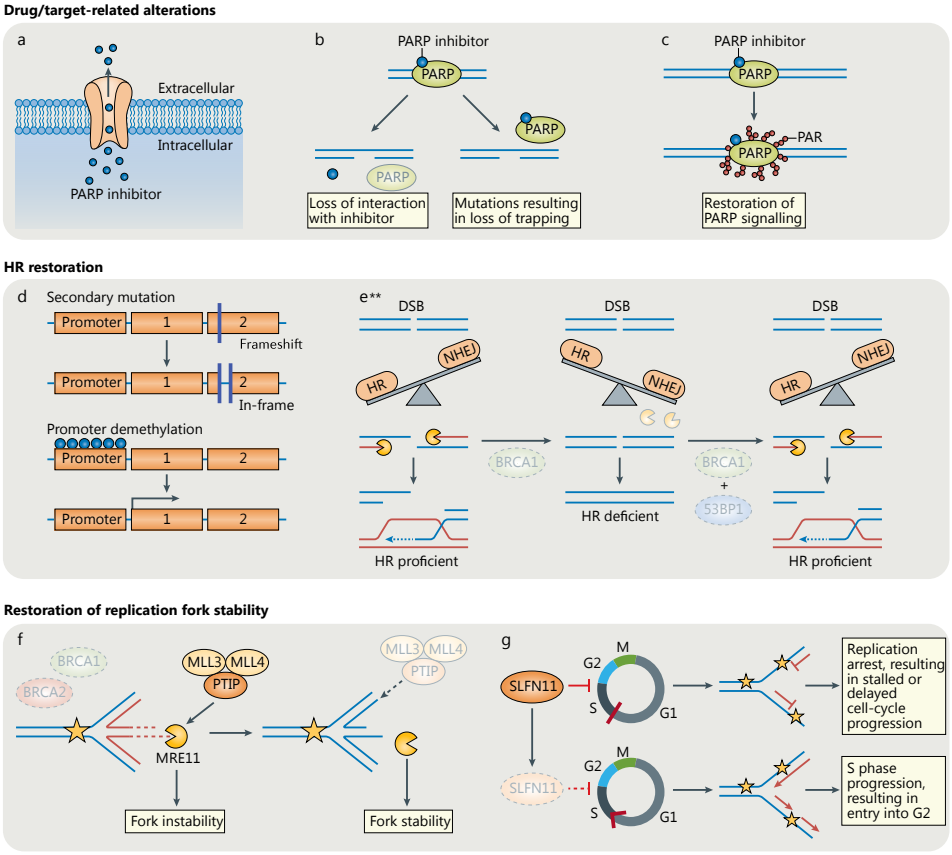


Figure 2 | Mechanisms of resistance to PARP inhibitors. Long- term follow- up data from clinical trials exploring the efficacy of various different poly(ADPribose) polymerase (PARP) inhibitors have demonstrated durable responses, although the majority of patients inevitably develop resistance. Clinical and preclinical studies indicate that such resistance occurs via one of three general mechanisms. Alterations related to the drug (or target) as observed with chemotherapies, such as upregulation of the efflux transporter P-glycoprotein (**a**), downregulation of or mutations in PARP1, which is restricted to cells expressing residual levels of BRCA1/2 (**b**) or loss of poly(ADP- ribose) glycohydrolase (PARG) (**c**). Restoration of homologous recombination (HR), which can occur either through reactivation of BRCA1/2 function (**d**) or loss of DNA end- protection (**e**), which is restricted to loss of BRCA1 and may occur via loss of the non- homologous end- joining (NHEJ) factor 53BP1. Restoration of replication fork stability via increased protection from fork degradation (**f**), for example, by loss of PTIP expression or loss of cell- cycle checkpoint arrest owing to loss of Schlafen 11 (SLFN11) (**g**). PAR, poly (ADP- ribose); DSB, double- strand break; MLL3, histonelysine N- methyltransferase 2C; MLL4, histone- lysine N- methyltransferase 2B; MRE11, Meiotic recombination 11; PTIP, Pax2 transactivation domain- interacting protein.

Target-related mechanisms of resistance

All current PARP inhibitors target the catalytic domain of PARP enzymes by competing with the cofactor NAD^+. Resistance might therefore arise from mutations in *PARP1* that either reduce its affinity to PARP inhibitors or preserve endogenous functions of the enzyme when

bound to PARP inhibitor[115]. Data from *in vitro* studies demonstrate that point mutations associated with resistance to PARP inhibitors are not exclusively found in the catalytic site of the enzyme, but also in domains necessary for trapping PARP1 onto DNA[115,116]. Corroborating these results, a mutation in *PARP1* that did not affect the recruitment of PARP1 to sites of DNA damage but nonetheless prevented efficient PARP1 trapping was identified in a PARP-inhibitor-resistant ovarian tumour[115]. Importantly, mutations in *PARP1* can only confer resistance in HR-proficient cells or in cells with hypomorphic *BRCA1* mutations and residual levels of BRCA1 activity, owing to the synthetic lethal effect of combined loss of PARP1 and BRCA1 function.

The enzyme that removes PAR chains from target proteins, poly (ADP-ribose) glycohydrolase (PARG), is another crucial factor in the development of resistance to PARP inhibitors both *in vitro* as well as *in vivo*. For example, loss of PARG confers resistance to PARP inhibitors in genetically engineered mouse models that develop BRCA1/2-deficient mammary tumours[117]. Interestingly, PARG depletion was able to partially rescue PARylation levels in cells exposed to PARP inhibitors in these models, suggesting that inhibition of PARP1 only reduces but does not fully inhibit PARylation. PARG-deficient cells exposed to PARP inhibitors are thought to retain sufficient PARylation of target proteins to induce the DNA damage signalling cascade and reduce the trapping of PARP1 onto DNA, owing to residual PARP activity [117]. Although clinical evidence remains limited, PARG-negative areas have been detected in tumours from a fraction of patients with triple-negative breast cancers (TNBCs) (76.8%) or ovarian carcinomas (78.4%), both of which are eligible for treatment with PARP inhibitors.

Restoration of BRCA1/2 function

The best clinically documented mechanism of resistance to PARP inhibitors occurs through reversion mutations or epigenetic alterations that induce the re-expression of a BRCA1 or BRCA2 wild-type protein or result in hypomorphic variants. Reversion of protein-truncating *BRCA1/2* mutations was originally described *in vitro* using *BRCA2*-mutated ovarian and pancreatic cancer cell lines following prolonged exposure to PARP inhibitors or cisplatin[118,119]. BRCA1/2-deficient cells have high levels of genomic instability, which are exacerbated by cisplatin and PARP inhibitors; therefore, the authors suggested that these cells accumulate further genetic alterations, resulting in the subsequent re-expression of novel BRCA2 isoforms. In line with the expected resistance to PARP inhibitors and cisplatin, cells of this revertant phenotype were again capable of recruiting RAD51 to sites of DNA damage and had reduced levels of genomic instability. Studies involving patient-derived xenograft (PDX) models of *BRCA1*-mutated and *BRCA1*-methylated TNBC revealed acquired resistance to PARP inhibitors driven by intragenic deletions that restored the reading frame of mutant *BRCA1*, as well as a loss of *BRCA1* promoter hypermethylation and de novo gene fusions causing re-expression of epigenetically silenced BRCA1[120]. Similarly, methylation of all *BRCA1* copies in PDX models of *BRCA1*-methylated ovarian cancer was associated with a response to PARP

inhibitors, whereas heterozygous methylation was associated with resistance[121]. In line with these preclinical findings, complete *BRCA1* methylation might predict clinical response to PARP inhibitors, and methylation loss can occur as a result of prior chemotherapy[121].

Over the past years, several other studies have reported genetic reversions of *BRCA1/2* as a mechanism of resistance to PARP inhibitors in patients with breast[122–124], ovarian[102,118,123–127], pancreatic[128] or prostate[129,130] cancers. An analysis of all reversion events in HR-related genes that have been previously associated with resistance to PARP inhibitors or platinum-containing chemotherapy was published in July 2020. Most reversions were found to be unique, although several positional hotspots could be identified across the coding sequence of *BRCA2*, suggesting that mutations in these positions might be more likely to lead to reversion, and thereby the development of resistance to PARP inhibitors, than others[131]. Importantly, reversions associated with resistance to PARP inhibitors are not exclusively found in *BRCA1/2* but have also been identified in other HR-related genes, such as *RAD51C*, *RAD51D* and *PALB2*[102,130]. The selection for reversion mutations during treatment with platinum-containing chemotherapies or PARP inhibitors also demonstrates that genomic instability induced by loss of BRCA1/2 function or that of other HR-related proteins is only required for initiation of tumorigenesis and dispensable for tumour maintenance. As such, therapy resistance induced by reversion mutations is an example of what can be called 'tumour suppressor tolerance', in which restoration of tumour suppressor gene function in an initially mutant cancer might actually increase fitness.

Owing to PARP inhibitors only being used clinically over the past few years and the complexities associated with the detection of reversion mutations, large-scale studies to estimate the frequency of BRCA1/2 reactivation in patients with PARP-inhibitor-resistant tumours are still unavailable. The fact that these agents were initially approved for second-line maintenance therapy, following first-line treatment with platinum-based chemotherapies, might bias the results of such investigations because reactivation of BRCA1/2 has been shown to be the main mechanism of platinum resistance in *BRCA1/2*-mutated tumours[123–126]. Future studies involving patients receiving PARP inhibitors as first-line therapies will enable a better understanding of shared mechanisms of resistance to platinum-based agents and PARP inhibitors, which might result in cross resistance to both classes of therapies, and to identify mechanisms of resistance specific to PARP inhibitors.

BRCA1-independent restoration of HR

BRCA1 and BRCA2 reversion events are found in a substantial proportion of patients with PARP inhibitor-refractory tumours, although they do not account for all cases of resistance[132], implying the existence of additional mechanisms. Indeed, data from preclinical studies indicate that restoration of HR can also be achieved by compensatory mutations that result in rewiring of the DDR **(Fig. 3)**. The first example of such a mechanism came from three landmark studies demonstrating that loss of the nonhomologous end joining (NHEJ) factor 53BP1 partially counteracts the effects of BRCA1 loss on HR and genomic instability[133–135]. Knockout of *53bp1*

in mice rescues embryonic lethality and attenuates tumorigenesis and chromosomal instability caused by BRCA1 deficiency[95]. In addition, data from *in vitro* studies demonstrate that loss of 53BP1 restores DNA end-resection in BRCA1-deficient cells and, as a result, rescues the HR defect and renders cells resistant to PARP inhibitors[133,134]. Importantly, 53BP1 loss does not restore HR in BRCA2-deficient cells, consistent with the different roles of BRCA1 and BRCA2 in HR **(BOX 2)**. Follow-up studies identified several proteins downstream of 53BP1, such as RIF1[136–140], REV7[141,142] and the shieldin complex[143–149], which are also thought to act as antagonists of end-resection and that confer resistance to PARP inhibitors in BRCA1-deficient cells and mouse mammary tumours upon inactivation. Additional evidence that loss of the 53BP1–RIF1–REV7–shieldin anti-resection signalling pathway mediates resistance to PARP inhibitors comes from *in vivo* studies in mouse models of BRCA1-deficient breast cancer. Prolonged exposure of these mice to PARP inhibitors resulted in acquired resistance, which was frequently associated with de novo mutations, DNA copy number aberrations and loss of *Trp53bp1*, *Rev7*, *Rif1 and Shld2* expression[150] (and our own unpublished data). Loss of 53BP1 and shieldin components has also been observed in PDX models with acquired resistance to PARP inhibitors[144,151]. Furthermore, several cases of resistance associated with BRCA-independent restoration of HR (owing to *MRE11* amplification or mutations in *TP53BP1*) have been reported in patients with *BRCA1*-associated breast cancer receiving platinum chemotherapy or a PARP inhibitor[152,153].

Apart from 53BP1–RIF1–REV7–shieldin signalling, several other factors have been reported to modulate end-resection, although clinical data on these factors are limited. The CTC1–STN1–TEN1 (CST) complex, located downstream of 53BP1–RIF1–REV7–shieldin, has been reported to prevent end-resection at DSBs, and loss of components of this complex leads to restoration of end-resection in the absence of BRCA1, resulting in resistance to PARP inhibitors[154,155]. Interestingly, loss of the CST complex seems to have a milder effect on resistance to PARP inhibitors than disruption of 53BP1–RIF1–REV7–shieldin signalling, indicating that additional mechanisms might protect DSBs from end-resection. HELB and DYNLL1 act downstream of 53BP1 to antagonize multiple components of the DNA end-resection machinery, and loss of these factors results in hyper-resected DNA ends and renders BRCA1-deficient tumour cells resistant to PARP inhibitors[156–158]. In addition to inhibition of end-resection factors, DYNLL1 might also promote NHEJ by stimulating 53BP1 oligomerization, thereby promoting recruitment and binding to DSBs[158]. Further upstream, loss of ERCC6L2, an accessory NHEJ factor, has also been shown to restore DNA end-resection, resulting in partial restoration of HR and resistance to PARP inhibitors in BRCA1-deficient cells[159,160]. Similarly, overexpression of factors promoting HR and suppression of NHEJ, such as TIRR[161], TRIP13[162] and miRNA-622[163] have also been shown to rescue HR and reduce the sensitivity of BRCA1-deficient cells to PARP inhibitors. Taken together, these data reinforce the notion that resistance to PARP inhibitors emerges via loss of DNA end-protection in cells lacking functional BRCA1. Moreover, the studies mentioned above demonstrate that, although BRCA1 is partially dispensable for the distal steps of RAD51-

mediated HR, BRCA2 is crucial for this pathway.

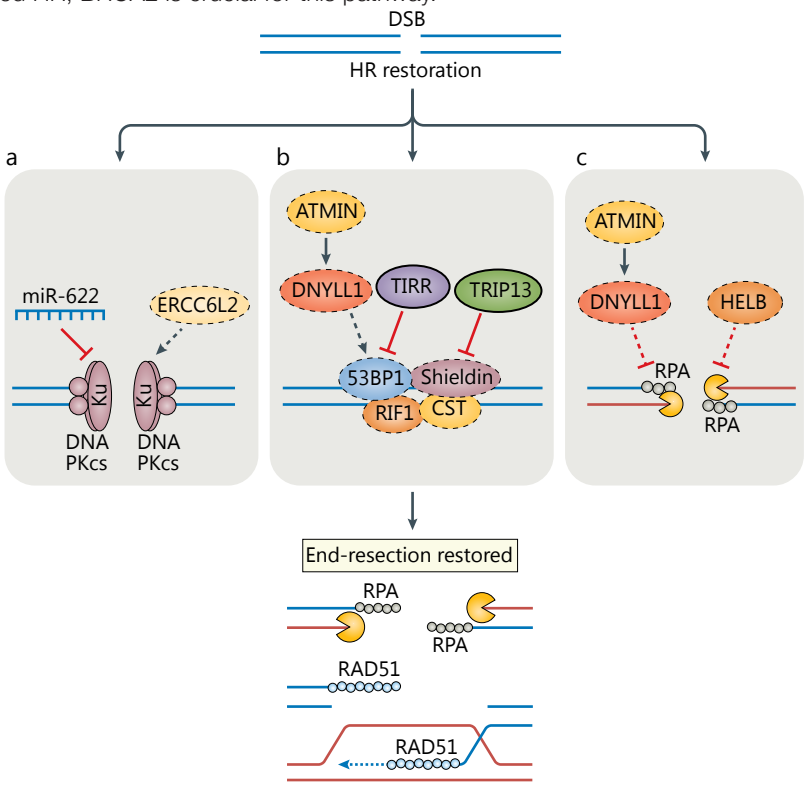


Figure 3| Mechanisms of BRCA1-independent restoration of HR. Various different mechanisms have been reported to enable the partial restoration of homologous recombination (HR) in BRCA1-deficient cells. **a |** Upstream of 53BP1, the DNA excision repair protein ERCC6L2 promotes nonhomologous end-joining (NHEJ), possibly by interacting with the Ku complex, and its loss leads to the restoration of DNA end-resection. Also upstream of 53BP1, overexpression of the microRNA miR-622 downregulates expression of the Ku complex, which in turn promotes HR. **b |** DYNLL1 stimulates 53BP1 oligomerization and promotes its recruitment and binding to DSBs. Consequently, loss of DYNLL1 or its transcriptional activator ATMIN results in restoration of HR. In addition, loss of any of the components of the 53BP1–RIF1–REV7–Shieldin pathway or of the CTC1–STN1–TEN1 (CST) complex results in loss of end-protection and, consequently, in restoration of end-resection. Accordingly, HR in BRCA1-deficient cells might be restored by overexpression of factors that suppress NHEJ, such as TIRR, which antagonizes 53BP1 localization to DSBs, or the E3 ubiquitin ligase TRIP13, which catalyses the dissociation of the REV7–Shieldin complex. **c |** HELB acts downstream of 53BP1 through interaction with the major single-stranded DNA binding protein RPA to prevent long-range end-resection during the G1 phase of the cell cycle. Consequently, loss of HELB results in hyper-resected DNA and restoration of HR. DYNLL1 also prevents multiple components of the DNA end-resection machinery, such as MRE11, from localizing to sites of DNA damage. Therefore, loss of DYNLL1 or of its transcriptional activator ATMIN promotes end-resection and enables HR to proceed in the absence of BRCA1.

Restoration of fork stability

Restoration of HR by perturbation of 53BP1 and downstream factors involved in end-resection and/or protection is restricted to BRCA1-deficient cells, whereas acquired resistance to PARP inhibitors arising from restoration of fork stability is a mechanism

common to cells deficient in either BRCA1 or BRCA2. As mentioned previously, BRCA1 and BRCA2 are not only required for HR; these proteins also govern the stability and protection of replication forks under replicative stress **(Box 2)**.

MRE11 and MUS81 are nucleases whose activity is required for the processing of stalled replication forks. In the absence of BRCA1/2, uncontrolled resection of unprotected, stalled forks by MRE11 leads to fork collapse and contributes to increased genomic instability[164–167]. In line with this observation, depletion of the MLL3/4 complex protein PTIP or the nucleosome remodeling factor CHD4 prevents MRE11 recruitment to stalled forks, resulting in fork protection and resistance to PARP inhibitors in BRCA1/2-deficient cells[164,168]. The chromatin-remodelling complex SMARCAL1 has also been shown to promote the MRE11-dependent degradation of nascent DNA in BRCA1/2-deficient cells[169,170]. In a manner similar to loss of PTIP, SMARCAL1 depletion decreases the sensitivity of BRCA1-deficient tumour cells to PARP inhibitors, although this effect seems to be cell type-specific[169].

RADX is another factor involved in replication fork protection; depletion of this factor in BRCA2-deficient cells also restores fork protection and alleviates the cytotoxic effects of PARP inhibitors[171]. Limiting the recruitment of MUS81 through inhibition of the methyltransferase EZH2 has also been shown to result in fork protection and partial resistance to PARP inhibitors, specifically in BRCA2-deficient cells[172]; however, conflicting data exist on the role of MUS81, including reports suggesting that this nuclease either protects[173,174] or disrupts[171,172] unprotected forks; thus how MUS81 affects the cytotoxicity of PARP inhibitors in BRCA1/2-deficient cells remains controversial[174].

Notably, depletion of either PTIP, EZH2 or RADX does not ameliorate HR function in BRCA1/2-deficient cells, suggesting that restoration of replication fork protection is a crucial component of resistance to PARP inhibitors[164,171,172]. Data from previous studies suggest that restoration of fork protection depends on the source of replication stress, the genetic context and the specific fork structures formed, which should all be considered in an attempt to better understand replication fork instability and how it can be exploited in cancer treatment.

Finally, and importantly, PARP1 is known to mediate the recruitment of MRE11 to stalled replication forks. PARP1 depletion results in synthetic lethality in BRCA1/2-deficient cells, whereas downregulation of PARP1 before BRCA1/2 loss restores the stability of stalled forks and promotes cell survival, likely by limiting the accumulation of MRE11 at replication forks[164,175]. Given the multifunctional role of PARP1 at replication forks, further studies are required to understand how it can potentially affect the results of combination therapies involving PARP inhibitors.

Schlafen 11 (SLFN11) is another factor implicated in replication stress. SLFN11 was originally identified by pharmacogenomic analyses of cancer cell databases as a strong determinant of response to multiple replication stress-inducing agents, including TOP I inhibitors, TOP II inhibitors, alkylating agents, DNA synthesis inhibitors and PARP inhibitors[176–180]. Studies conducted over the past few years suggest that, upon replicative

damage, cells undergo irreversible cell-cycle arrest at G1/S phase, mediated by the engagement of SLFN11 with the replication helicase complexes[180]. Binding of SLFN11 to stressed forks promotes chromatin relaxation and blocks cellular replication, which ultimately results in cell death[181]. Consequently, loss of SLFN11 impairs prolonged G1/S-phase arrest, thereby enabling cells to progress through S phase in the presence of replicative stress[181]. In line with this, loss of SLFN11 decreases the cytotoxicity of PARP inhibitors in both BRCA1/2-proficient and BRCA2-deficient cells[180]. Importantly, HR is functional in both SLFN11-proficient and SLFN11-deficient cells, indicating that this protein acts in parallel with HR[180].

STRATEGIES TO OVERCOME RESISTANCE

Further studies are required to develop therapeutic strategies that combat or delay the emergence of acquired resistance and to determine the extent of cross resistance between the various therapeutic options. Surgical debulking, a strategy pursued mostly in women with ovarian cancer, might reduce or even eliminate resistant clones and thus, theoretically, delay the onset of resistance. Other potential strategies to tackle resistance to PARP inhibitors include: combination therapies aimed at further amplifying the antitumour effects of PARP inhibitors; targeting the acquired vulnerabilities of PARP inhibitor-resistant cancers; and/or delaying the emergence of resistance through suppression of the mutator phenotype, which arises in *BRCA1/2*-mutated tumours. In this section, we will describe current developments and findings exploiting these three approaches **(Fig. 4 and 5)**.

Combination strategies

Suppression of alternative HR pathways

BRCA1-deficient cells are HR-deficient, nonetheless, DNA end-resection still takes place in these cells, albeit with delayed kinetics[182–185]. This observation suggests that BRCA1 has other roles in HR beyond promoting end-resection. Indeed, BRCA1 has been shown to recruit the PALB2–BRCA2 complex to ssDNA, thus promoting the BRCA2-mediated assembly of RAD51 nucleoprotein filaments (BOX 2). Data from the past few years indicate that PALB2 is recruited to ssDNA in an RNF168-dependent manner in BRCA1-deficient cells[186–189]. This finding implies that HR reactivation in BRCA1/53BP1-double-deficient cells is enabled by both RNF168-dependent recruitment of PALB2 and increased end-resection owing to loss of the 53BP1–RIF1–REV7–Shieldin axis[187,189–191]. The extent of HR restoration enabled by 53BP1 loss depends on the type of *BRCA1* mutation because the overall efficiency of RAD51 loading (and, consequently, the extent of resistance to PARP inhibitors) is enhanced by the presence of hypomorphic *BRCA1* alleles that retain the ability to associate with PALB2[186,189]. In line with its role in mediating PALB2 recruitment, loss of RNF168 compromises HR in *BRCA1* heterozygous cells and in BRCA1/53BP1-

double-deficient cells, thus rendering these cells sensitive to PARP inhibitors[189]. Therapeutic targeting of RNF168 might therefore be a useful method of inhibiting BRCA1-independent PALB2/BRCA2 recruitment and thus improving the efficacy of PARP inhibitors against *BRCA1*-mutant cancers with acquired resistance to PARP inhibitors via loss of the 53BP1–RIF1–Shieldin end-protection pathway.

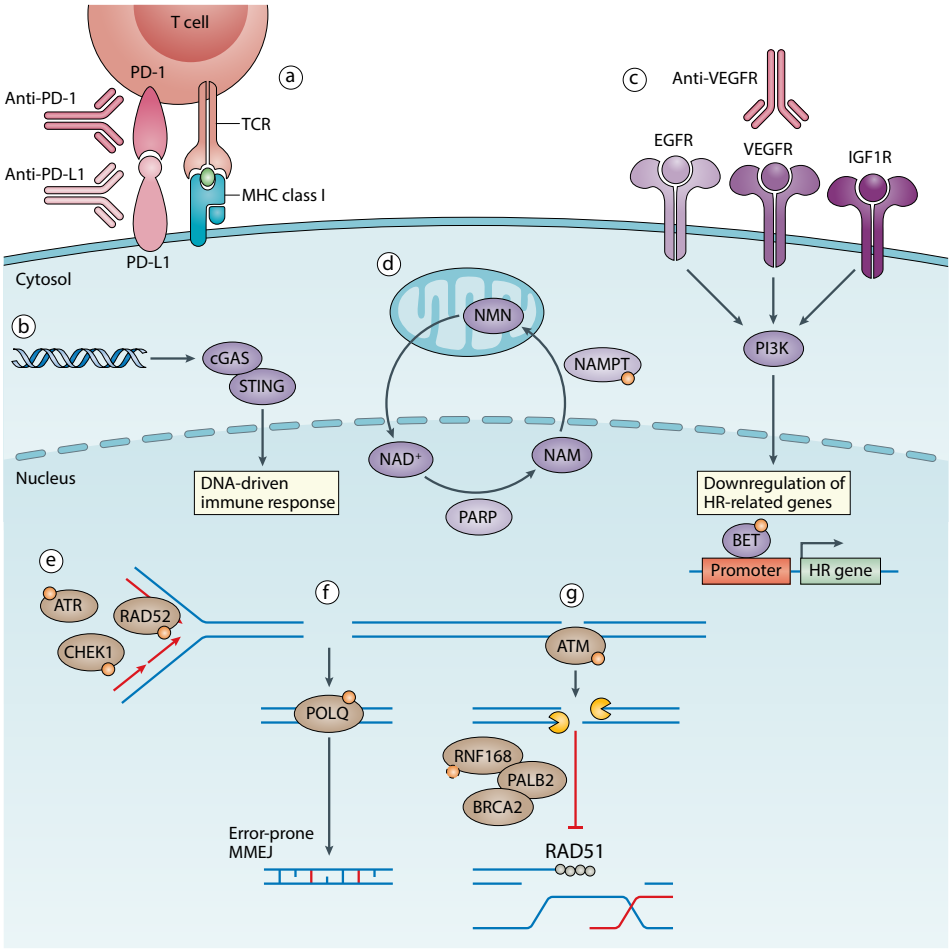


Figure 4 | Overcoming resistance to PARP inhibitors. Various combination strategies have been suggested to enhance the efficacy of poly(ADP- ribose) polymerase (PARP) inhibitors in treatment- resistant tumors. Immune- checkpoint inhibitors, such as anti- PD-1 or anti-CTLA4 antibodies, might be an alternative approach that is currently being assessed in the clinic given that homologous recombination (HR)- deficient tumours usually have high levels genomic instability and are thought to present an increased number of neoantigens on their surfaces (part **a**). Furthermore, PARP inhibitors have been shown to induce both PD- L1 expression as well as upregulation of cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) signaling, which might further boost the recruitment and/or activation of CD8+ T cells (part **b**). Reactivation of the HR pathway in tumors with acquired resistance to PARP inhibitors might be counteracted by treating patients with various tyrosine kinase inhibitors (such as VEGF- targeted therapies) or agents targeting epigenetic regulators of HR-related genes (such as bromodomain and extra- terminal domain (BET) inhibitors), or by targeting direct

mediators of HR, such as ATM or RNF168, which have been shown to promote HR in the absence of BRCA1 (parts **c, g**). NAD+ is the major substrate of PARP enzymes; therefore, inhibition of NAD+ synthesis might further enhance the cytotoxicity of PARP inhibitors through indirect inhibition of PARylation (part **d**). Multiple methods of suppressing restored replication fork protection in PARP inhibitor- resistant cells are currently being explored, such as inhibition of ATR or RAD52, which is thought to serve as a substitute for RAD51, as well as several cell cycle- related factors, such as CHEK1 (part **e**). Apart from combination therapy approaches, inhibition of microhomology-mediated end- joining (MMEJ) might be an alternative strategy because HR-deficient tumors are thought to depend on this pathway for DNA damage repair (part **f**); for example, inhibitors of the error- prone polymerase θ (POLQ) are currently being developed and are under consideration for use as single agents as well as in combination with PARP inhibitors. Inhibition of this mutagenic repair pathway might suppress or delay the onset of acquired resistance and reduce the extent of genomic instability of HR-deficient tumors.

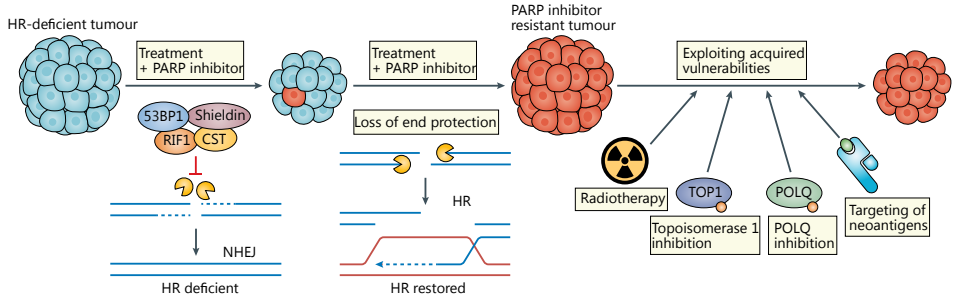


Figure 5 | Targeting acquired vulnerabilities of PARP inhibitor-resistant tumors. Acquired resistance to poly(ADPribose) polymerase (PARP) inhibitors often occurs through loss of additional DNA damage proteins, often resulting in the emergence of novel vulnerabilities that can be exploited therapeutically. For example, DNA- damaging agents such as radiotherapy or topoisomerase I inhibitors might be viable post- progression therapeutic strategies. Furthermore, inhibition of the error- prone DNA polymerase θ (POLQ) has been shown to be synthetic lethal with homologous recombination (HR) deficiency and might also delay the emergence of other resistance mechanisms. Depending on the type of resistance, tumors might also acquire and present tumour- specific neoantigens, which could potentially then be targeted using chimeric antigen receptor T cell therapies or immune-checkpoint inhibitors. CST, CTC1–STN1–TEN1; NHEJ, non- homologous end- joining.

Another factor that has become of increasing interest in the past years is the role of the DNA repair protein RAD52. Initial studies revealed only mild effects of RAD52 loss on viability *in vitro* as well as *in vivo*[192,193]; however, co-depletion of RAD52 and BRCA1/2 was later reported to confer synthetic lethality, suggesting that RAD52 might serve as a backup pathway enabling RAD51 to gain access to resected DNA ends in the absence of BRCA1/2[194,195]. Furthermore, evidence published over the past few years indicates that RAD52 might have a role in the repair of ssDNA at stalled replication forks as well as mediating fork reversal[196,197]. The observed synthetic lethality might, therefore, be a combined effect of both functions of RAD52 rather than a consequence of one function. RAD52 inhibitors have been developed in the past years (reviewed elsewhere[198]), and might provide an alternative method of targeting BRCA-deficient tumours. More research is needed to determine the *in vivo* efficacy of RAD52 inhibitors in both preclinical models and in patients, although results obtained thus far demonstrate a synergistic interaction between inhibitors of PARP and RAD52, leading to more potent cytotoxic effects on BRCA-deficient cells *in vitro* as well as *in vivo*[199].

Indirect inhibition of HR

To date, no direct inhibitors of proteins catalysing HR are available. An alternative strategy to inhibit HR restoration in patients receiving PARP inhibitors might be the use of drugs targeting actionable oncoproteins which, although not developed as HR inhibitors, interfere with gene expression, nuclear localization and/or the recruitment of HR factors, ultimately resulting in the indirect inhibition of HR (reviewed elsewhere[36]). For example, therapies targeting VEGF or the PI3K–AKT pathway, have been reported to impair HR[36]. In support of this notion, combining the VEGF antagonist bevacizumab with olaparib or niraparib improved the median PFS duration in two cohorts of women with ovarian cancer, even in those with HR-proficient tumours, relative to placebo or niraparib monotherapy[200,201]. Furthermore, a phase I trial combining the AKT inhibitor capivasertib with olaparib revealed durable responses in patients with advanced-stage solid tumours, irrespective of *BRCA1/2* status[202], and another phase I trial combining the MEK inhibitor selumetinib with olaparib is currently ongoing (NCT03162627)[203]. However, the activity of these inhibitors might reflect impaired cell-cycle progression rather than direct inhibition of HR, suggesting that the effects observed for combination treatment may be additive rather than synergistic.

Indirect inhibition of HR might also be induced via pharmacological targeting of epigenetic regulators. For example, the bromodomain and extra-terminal domain (BET) protein BRD4 promotes global transcription by RNA polymerase II, and BET and/or BRD4 inhibitors have been shown to suppress the transcription of key DDR genes, including *CTIP*, *BRCA1*, *RAD51*, *TOPBP1* and *WEE1*, resulting in abrogation of HR and synergy with PARP inhibitors in preclinical investigations[204–206]. Similarly, inhibition of histone deacetylases (HDACs) results in downregulation of HR and can be used to induce sensitivity to PARP inhibitors[207–210]. Efficient suppression of HR and sensitization to PARP inhibitors has also been observed upon inhibition of cyclin-dependent kinases (CDKs), such as CDK1, which phosphorylates BRCA1 and thus promotes HR repair[211], and CDK12, which is a transcriptional regulator of several HR genes, including *BRCA1*[212–214]. Furthermore, heat shock protein 90 (HSP90) promotes the stabilization of a subset of HR proteins, including RAD51, BRCA1 and BRCA2, and targeted inhibition of this protein induces HR deficiency and promotes sensitivity to PARP inhibitors[153,215,216]. The stability of these HR proteins can also be reduced by mild hyperthermia, which might be easily applicable in the clinic[217,218]. Lastly, hypoxia has been reported to induce long-term epigenetic silencing of *BRCA1*, thereby also bestowing a vulnerability to PARP inhibitors that could potentially be exploited therapeutically[219]. Importantly, all of these approaches can be used to enhance sensitivity to PARP inhibitors not only in tumours with restored HR, but also in those with hypomorphic or fully functional BRCA activity[153,212,216]. However, for these reasons, combination strategies might also lead to substantial toxicities in nonmalignant proliferating cells, such as haematopoietic progenitors in the bone marrow.

Abrogation of cell-cycle checkpoint signalling

Data from several studies suggest that abrogation of cell-cycle checkpoint signalling might alleviate resistance to PARP inhibitors. ATM and ATR are the two major kinases controlling cell-cycle checkpoint activation and the ensuing arrest of cells in response to DNA damage. Several processes that cause resistance to PARP inhibitors in *BRCA1*-mutant cells, such as BRCA1-independent HR and fork protection, are dependent on ATR, which controls both processes by promoting RAD51 loading onto DSBs and stalled forks[220]. Thus, combinations comprising PARP inhibitors and ATR inhibitors are currently being investigated as potential methods of overcoming resistance to PARP inhibitors in BRCA1-deficient tumours with restored HR function or restored fork protection[180,220–222].

The chromatin remodelling enzyme ARID1A has been shown to regulate the DNA damage checkpoint via interactions with ATR, and loss of ARID1A leads to impaired cell-cycle checkpoint activation and sensitization of cells to PARP inhibitors[104]. Similar to ATR, ATM kinase activity is required for the early stages of HR, and inhibition of ATM has been shown to resensitize cells with BRCA1 and 53BP1 or BRCA1 and REV7 deficiencies to PARP inhbitors[134,142].

PARP inhibitors have also been combined with WEE1 kinase inhibitors in preclinical models[223–225]. WEE1 kinase regulates G2/M progression by inhibiting CDK1 and CDK2, thereby activating the G2/M cell-cycle checkpoint, resulting in cell-cycle arrest and providing time for DNA damage repair. The combination of PARP and WEE1 inhibitors aims to abrogate G2 arrest and induce mitotic catastrophe. A study using ovarian cancer xenograft models demonstrated that sequential rather than concurrent inhibition of PARP and WEE1 improves the tolerability of these drug combinations while still preserving antitumour activity[225].

Multiple inhibitors of cell-cycle checkpoint kinases (such as ATR, ATM, CHK1 and WEE1) are being developed by pharmaceutical companies and are being tested in clinical trials designed to assess anticancer efficacy in combination with PARP inhibitors[36,226]. The clinical applicability of these combinations will greatly depend on whether they are effective in patients with acquired resistance to PARP inhibitors without excessive toxic effects on nonmalignant tissues.

Targeting NAD+ metabolism

PARP1 uses oxidized NAD (NAD^+) as a substrate for PARylation (BOX 1), which constitutes a major source of cellular NAD^+ catabolic activity, resulting in NAD^+ depletion to as low as 10–20% of its unstressed levels within minutes of induction of DNA damage[227]. Excessive PARP activation (for example, via oxidative stress or excessive DNA damage) effectively depletes the cellular pool of NAD^+, leading to a progressive decline in ATP levels, energy loss and cell death[228–230]. Thus, in order to maintain NAD^+ levels, cells are reliant on salvage pathways. Functional genetic screens have shown that depletion of nicotinamide phosphoribosyltransferase (NAMPT), a rate-liming enzyme in the NAD^+ salvage pathway, enhances the cytotoxicity of PARP inhibitors in TNBC cells. Moreover, combining the NAMPT

inhibitor FK866 with olaparib resulted in more potent inhibition of the growth of TNBC xenografts *in vivo* than either agent alone[231]. Neomorphic mutations in isocitrate dehydrogenase (IDH), which are common in gliomas and lead to decreased NAD^+ levels through downregulation of the NAD^+ salvage pathway enzyme nicotinate phosphoribosyltransferase (NAPRT1), render these tumours hypersensitive to NAD^+ depletion[232]. Importantly, mutant IDH also produces the oncometabolite D-2-hydroxyglutarate (D-2HG), which has been shown to inhibit HR and induce sensitivity to PARP inhibitors[233108,234]. Thus, neomorphic *IDH1/2* mutations might confer hypersensitivity to PARP inhibitors via two distinct mechanisms. Finally, the NAD^+ derivative $NADP^+$ can act as an endogenous PARP inhibitor that suppresses PARylation by competing for the NAD^+ binding site of PARP. Consequently, cancer cells with high $NADP^+$:NAD^+ ratios have increased sensitivity to chemical PARP inhibition, irrespective of their *BRCA* mutation status[235], which might reflect reduced PARylation rather than PARP trapping onto DNA.

Collectively, these studies suggest that the cytotoxicity of PARP inhibitors might be further enhanced by indirect inhibition of PARylation through targeted inhibition of NAD^+ metabolism. More research is required in order to provide a deeper understanding of the interplay between cancer metabolism and (deficiencies in) HR repair, and how these interactions affect the efficacy of PARP inhibitors in patients with cancer. Ultimately, this knowledge might uncover new metabolic vulnerabilities of cancer cells exposed to PARP inhibitors that can be targeted to improve the efficacy of these agents.

Immunotherapy in BRCA-deficient cancers

The combination of PARP inhibitors with immune-checkpoint inhibitors, such as anti-PD-1 antibodies, is another potential approach to the treatment of patients with *BRCA1/2*-mutant cancers. Several observations have sparked a rapidly growing interest in such combinations. First, HR-deficient cancers are reported to have an increased mutational burden, possibly resulting in increased availability of tumour-specific neoantigens, including antigens originating from large genomic rearrangements[236]. Second, HR deficiencies might lead to the cytosolic accumulation of unrepaired DNA fragments, which can activate cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) signalling[237]. The recognition of extranuclear double-stranded DNA by cGAS triggers activation of the IRF3–type I interferon signalling pathway, which is an important mediator of systemic immune responses that induces the activation of several immune cell types[238]. Genomic rearrangements in *BRCA1/2*-deficient tumours might also disrupt chromatin boundaries and lead to the expression of repetitive RNAs which can activate innate immune signalling[238,239]. Third, PARP inhibition induces both PD-L1 expression (via inactivation of GSK3β) and cGAS–STING signalling, leading to increased $CD8^+$ T cell infiltration and activation[240,241,242,243]. However, whether PARP inhibitor-mediated activation of cGAS–STING signalling is dependent on the *BRCA* mutation status of the tumour is still a matter of debate. Data from one study suggest that cGAS-STING signalling is activated regardless of *BRCA* mutation status, while others found that activation

of this pathway occurs either solely or more potently in BRCA-deficient tumours[240,241,191].

In line with these findings, data from multiple studies indicate that PARP inhibition enhances the antitumour effects of anti-PD-1 antibodies in mouse models of breast and ovarian cancer[240,241,242,243]. Several clinical trials are currently evaluating the effects of this combination in patients with these malignancies (reviewed elsewhere[244]). Initial clinical studies with PARP inhibitor–anti-PD-1 antibody combination have involved only small cohorts of patients and robust general conclusions therefore cannot be drawn, although certain interesting observations have already been reported. The combination of a PARP inhibitor with an anti-PD-1 antibody is generally well tolerated and seems to result in increased objective response rates when compared to monotherapies both in patients with ovarian cancer or breast cancer[245–247]. However, the results of a phase I/II clinical trial involving women with platinum-resistant ovarian cancer found no significant differences in the objective response rate when comparing subgroups of patients with *BRCA1/2* mutations to those with wild-type *BRCA1/2*[247]. Of note, not all patients enrolled in this trial were tested for the presence of *BRCA1/2* mutations. Moreover, the mutation status of other HR genes was also not assessed. As mentioned above, the combination of immune-checkpoint inhibitors with PARP inhibitors remains an area of active investigation; therefore, whether patients with acquired resistance to PARP inhibitors might benefit from treatment with this combination has yet to be determined.

Targeting acquired vulnerabilities

Drug resistance often comes with a fitness cost that leads to acquired vulnerabilities, which can theoretically be targeted to improve the efficacy of subsequent therapies[248] **(Fig. 5)**. Several loss-of-function mutations that cause resistance to PARP inhibitors have been found to result in increased sensitivity to ionizing irradiation. For example, PARG inactivation, although detrimental to the efficacy of PARP inhibitors, results in increased sensitivity to ionizing radiation[117,249]. In a similar fashion, loss of components of the 53BP1–RIF1–REV7–Shieldin or CST end-protection complexes, as well as PARP1 loss, have been demonstrated to result in hypersensitivity to ionizing radiation[139,144,250–254]. As a consequence, radiotherapy might be a viable option for patients with BRCA-deficient tumours with acquired resistance to PARP inhibitors owing to loss of PARG, PARP1 or DSB end-protection.

Beyond sensitization to ionizing radiation, cells lacking in PARP1 activity also have increased susceptibility to the TOP I inhibitor camptothecin, owing to the role of PARP1 in the repair of TOP I cleavage sites[255]. Furthermore, PARG downregulation has also been shown to result in metabolic depletion of NAD^+ and increased PARP1 trapping on chromatin, rendering such cells sensitive to the alkylating agent temozolomide[256,257] .

Apart from the increased sensitivity of tumours with acquired resistance to PARP inhibitors to ionizing radiation, camptothecin or temozolomide, an alternative approach suggested in 2020 involves targeting tumours with acquired resistance to PARP inhibitors owing to genetic reversion[131]. Genetic reversion often does not restore the complete original amino

acid sequence of the protein and, therefore, most revertant proteins contain stretches of amino acids, often at the reversion junction, that are not encoded in the wild-type gene and might thus be presented on the cell surface as neoantigens and subsequently recognized by the immune system. Tumours harbouring such alterations could then be targeted using anticancer vaccines, chimeric antigen receptor T cell therapies and/or immune-checkpoint inhibitors[131].

Despite the evidence described here, studies attempting to identify such acquired dependencies remain limited. A key approach to identify acquired vulnerabilities would be to perform pharmacological or genetic screens in different models of acquired resistance to PARP inhibitors. The identification of such dependencies might then enable specific targeting of PARP inhibitor-resistant tumours using therapies selected according to the underlying mechanism of resistance.

Preventing the emergence of resistance

Targeting drug-tolerant persisters

PARP inhibitors cause DNA damage specifically during the S and G2 phases of the cell cycle[258] and thereby specifically target proliferating cells. Consequently, cell populations in G_0 or early G_1 phase might be unaffected by PARP inhibitors because neither HR nor replication forks are active during these phases. Indeed, continuous exposure to PARP inhibitors has been shown to induce senescence in ovarian cancer cell lines, which is reversed upon PARP inhibitor withdrawal, suggesting that senescent persister cells might be capable of contributing to further tumour growth[259]. Moreover, several processes, such as epigenetic reprogramming, transcriptional regulation as well as interactions with the tumour microenvironment might all delay cell growth and thereby make cells less responsive to therapy (reviewed elsewhere[260]). The persistence of drug-tolerant tumour cells provides a rationale for the use of long-term maintenance therapy, which might be able to kill residual dormant cellular populations once they enter the cell cycle.

Suppression of the mutator phenotype

In order to repair DNA DSBs in the absence of HR, BRCA1/2-deficient cells are able to upregulate microhomology-mediated end joining (MMEJ) as a compensatory mechanism, which is thought to have only a minor role in HR-proficient cells[261]. MMEJ is an error-prone repair pathway, driven by the low-fidelity DNA polymerase θ (POLQ). POLQ joins two broken DNA strands based on short regions of sequence homology (>2 bp), which can be detected as an MMEJ-characteristic pattern of mutations in BRCA1/2-deficient tumours using whole-genome sequencing[262]. Given that DSB repair in HR-deficient cancers is dependent on POLQ-mediated MMEJ, suppressing this error-prone DNA repair pathway might be another way to target these tumours. In line with this hypothesis, two groups have reported a synthetic lethal interaction between POLQ depletion and proteins involved in HR[263,264]. Notably, POLQ inhibition suppresses the genomic instability arising from error-

prone MMEJ; therefore, this approach might be superior to PARP inhibition, which is thought to enhance genomic instability and thereby promote the emergence of HR-deficient cancers of a 'mutator phenotype'. Inhibition of POLQ might therefore be effective not only in tumours with acquired resistance to PARP inhibitors, but also in preventing or attenuating the emergence of treatment resistance in PARP-inhibitor-naive HR-deficient tumours.

The development of POLQ inhibitors is generating considerable research interest. The antibiotic novobiocin (NVB) is reported to inhibit the ATPase activity of POLQ[265]. In line with initial reports, inhibition of POLQ by NVB selectively kills HR-deficient tumours both *in vitro* as well as *in vivo*. Furthermore, NVB reduces tumour growth in a PDX model with combined loss of BRCA1 and 53BP1 function, suggesting that POLQ inhibition might also be a viable option for tumours with acquired resistance to PARP inhibitors owing to loss of DNA end-protection[265]. This study suggests that POLQ inhibition might be a promising strategy, either in combination with or as an alternative to PARP inhibitors. However, further research is needed to answer critical questions regarding the clinical use of POLQ inhibitors. For example, it remains to be determined (*i*) whether POLQ inhibition will be effective as monotherapy or only in combination with PARP inhibitors; (*ii*) whether all tumours that are resistant to PARP inhibitors will respond to POLQ inhibitors or whether sensitivity will be restricted to tumours that become resistant via certain mechanisms, such as the loss of DNA end-protection; (*iii*) whether tumours with genomic instability or mutations in HR-related genes other than *BRCA1* and *BRCA2* might be susceptible to inhibition of POLQ.

FUTURE DIRECTIONS

Over the past years, preclinical and clinical studies have substantially increased our knowledge of both the mechanism of action and possible sources of resistance to PARP inhibitors. PARP inhibitors are increasingly used clinically and their application is being expanded to indications beyond breast and ovarian cancer. Many mechanisms of resistance have been reported in cancer cell lines and mouse models, although the clinical relevance of most of these mechanisms remains unclear owing to the majority of the regulatory approvals of PARP inhibitors taking place in the past few years. Moreover, the mechanisms of resistance identified in preclinical studies might differ from those observed in patients given that most mature clinical data are from trials testing PARP inhibitors as second-line therapies. Tumour subclones in many of these patients might already have developed certain forms of resistance to previous treatments (such as taxanes or platinum-based chemotherapies) that confer cross-resistance to PARP inhibitors. Thus, determining whether improvements in PFS and, ultimately, in OS can be achieved by giving PARP inhibitors earlier in the course of treatment will be an important step. In comparison with second-line treatment regimens, first-line treatment with PARP inhibitors might not only further delay disease progression but could also postpone the onset of resistance and alter the underlying mechanisms of

resistance to these agents. For instance, PARP inhibitor resistance caused by genetic reversion mutations may result in cross resistance to second-line chemotherapy. In contrast, BRCA1-deficient tumours that have acquired resistance through loss of DNA end-protection may still respond to radiation therapy or POLQ inhibitors. It will therefore be important to determine how patients developed resistance to PARP inhibitors, so that cross resistance can be avoided in the second-line treatment.

Olaparib and niraparib have both been approved as first-line maintenance therapies and, with more patients likely to receive PARP inhibitors earlier in the course of disease, clinical trials of novel post-progression approaches are either planned or currently ongoing (for example NCT03106987). Molecular analysis of tumour biopsy samples and/or cell-free DNA samples **(Box 3)** obtained from these patients might provide more insight into the underlying mechanisms of resistance to PARP inhibitors as well as the mechanisms conferring cross-resistance between PARP inhibitors and other anticancer agents. Another key question is whether distinct or similar mechanisms drive disease recurrence after planned treatment cessation versus recurrence during maintenance therapy. Future studies will help to determine whether the recurring tumours might respond to re-challenge with PARP inhibitors.

Clinical attempts to determine which patients are most likely to benefit from PARP inhibitors and to identify the optimal treatment regimens are currently ongoing. Nonetheless resistance to PARP inhibitors might be an inevitable consequence of the genomic instability of these HR-deficient tumours. As mentioned above, targeted approaches designed to overcome resistance to PARP inhibitors remain limited. The systematic identification of the vulnerabilities of PARP inhibitor-resistant tumours will therefore be an important step. Data from *in vitro* as well as *in vivo* genetic screens will advance our understanding of the rewiring of treatment-resistant tumours, and pharmacological screens might also be used to identify compounds that specifically target these cancers. In order to study resistance to PARP inhibitors in settings more closely related to the clinic, genetically engineered mouse models that develop PARP inhibitor-resistant tumours might be of great value, as well as PDX models generated using samples from patients with acquired resistance. Conversely, in-depth analysis of tumours from patients with remarkably good responses to PARP inhibitors — so-called ‘exceptional responders’ — might be an alternative strategy to better identify novel molecular determinants of (hyper)sensitivity to these agents[266]. Finally, the establishment of single-cell omics technologies might facilitate more detailed investigations of patient-derived material, which is usually of limited availability.

Box 3| Detecting resistance in patients with cancer. Close monitoring of treatment response and the early detection of subclones that are likely to confer resistance to poly (ADP-ribose) polymerase (PARP) inhibitors are key to the success of a treatment regimen. However, standard tumor biopsy sampling is mostly invasive and thus often cannot be conducted on a regular basis. As a consequence, noninvasive methods of assessing tumor genomics using blood or plasma samples (liquid biopsies) are being intensively investigated as these can be performed serially and might provide a better indication of tumor heterogeneity. Several biomarkers can be detected within these biopsy samples, although circulating tumor DNA (ctDNA) is currently believed to be the most promising marker for the assessment of treatment response and the extent of residual disease. ctDNA is thought to be released from cells undergoing apoptosis or necrosis and has been suggested to provide a 'real-time' picture of disease status, owing to its short half-life, ranging from 16–150 minutes[263]. Initial evidence suggests that analysis of ctDNA might be a suitable method of detecting resistance to PARP inhibitors in patients. A subset of genetic reversion events leading to resistance to PARP inhibitors in patients with BRCA-mutated cancers can be readily detected in ctDNA[123,129,130,268]. Interestingly, the presence of polyclonal PARP inhibitor-resistant populations has also been described, some of which were already present before treatment initiation[123,268]. These studies suggest that ctDNA provides a simple method that not only enables the monitoring of treatment response but also might enable earlier switching of treatments as resistant clones begin to emerge. Certain genetic reversion events might well be detectable in ctDNA[106], although other mechanisms of resistance to PARP inhibitors, such as restoration of HR or replication fork protection might be more challenging because these processes are often caused by genomic alterations, such as large deletions or breakpoints in introns, that are less likely to be detected using current sequencing approaches. Although further research is required, ctDNA assays might provide a fast and cost-effective way of screening patients for common and/or patient-specific alterations associated with PARP inhibitor resistance. Currently ongoing clinical trials aimed at assessing the predictive value of ctDNA screening (e.g., NCT03182634) might shed more light on the potential use of this approach.

CONCLUSIONS

In conclusion, PARP inhibitors provide a promising treatment strategy that is potentially applicable to several stages of cancer progression. Multiple mechanisms of resistance to these agents have been identified and characterized over the past years and confirmation of the clinical relevance of these various mechanisms is urgently needed. Additionally, strategies designed to specifically target tumour cells with resistance to PARP inhibitors are still lacking. An improved understanding of the biology of HR-deficient cancers will facilitate the development of rational treatment strategies to prevent and/or delay the onset of resistance and will ultimately lead to improved long-term outcomes for patients.

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AUTHOR CONTRIBUTIONS

M.P.D. and S.C.M. made substantial contributions to researching data for this article and discussions of content. All authors contributed to writing the article and reviewing/editing of the manuscript before submission.

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Supplemetary table 1| Clinically approved PARP inhibitors.

Cancer type	Drug	Indication	Agency	Specific restrictions
Ovarian cancer[269]	Olaparib	First-line Maintenance: BRCA1/2-mutated, advanced-stage cancer, in CR or PR after first-line platinum-based chemotherapy	FDA, 2018	BRCA1/2 mutation detected using FDA-approved companion diagnostic [a]
			EMA, 2019	High-grade cancers only
		First-line Maintenance with Bevacizumab: advanced-stage cancer, in CR or PR after first-line platinum-based chemotherapy, associated with HRD-positive status defined by BRCA1/2 mutation and/or genomic instability	FDA, 2020	HRD status detected using FDA-approved companion diagnostic [a]
			EMA, 2020	High-grade cancers only, CR or PR after first-line platinum-based chemotherapy in combination with bevacizumab
		Second-line Maintenance: advanced-stage, recurrent cancer, in CR or PR to platinum-based chemotherapy	FDA, 2017	No specific restrictions
			EMA, 2018	High-grade cancers only, BRCA1/2 mutation
		Pre-treated Monotherapy: BRCA1/2-mutated, advanced-stage cancer, refractory to ≥3 prior lines of therapy	FDA, 2014	BRCA1/2 mutation detected using FDA-approved companion diagnostic [a]
	Rucaparib	Second-line Maintenance: advanced-stage, recurrent cancer, in CR or PR to platinum-based chemotherapy	FDA, 2018	No specific restrictions
			EMA, 2019	High-grade cancers only
		Pre-treated Monotherapy: BRCA1/2-mutated, advanced-stage cancer, refractory to ≥2 prior lines of therapy	FDA, 2016	BRCA1/2 mutation detected using FDA-approved companion diagnostic [a]
			EMA, 2018	High-grade, platinum-sensitive cancers only, unable to tolerate further platinum therapy
	Niraparib	First-line Maintenance: advanced-stage cancer, in CR or PR after first-line platinum-based chemotherapy	FDA, 2020	No specific restrictions
			EMA, 2020	High-grade cancers only
		Second-line Maintenance: advanced-stage, recurrent cancer, in CR or PR to platinum-based chemotherapy	FDA, 2017	No specific restrictions
			EMA, 2017	High-grade cancers only
		Pre-treated Monotherapy: advanced-stage cancer, refractory to ≥3 prior lines of therapy, associated with HRD-positive status defined by BRCA1/2 mutation and/or genomic instability	FDA, 2019	Progressed >6 months after response to the last platinum-based chemotherapy; HRD status detected using FDA-approved companion diagnostic [a]

Supplemetary table 1| Continued

Cancer type	Drug	Indication	Agency	Specific restrictions
Breast cancer[270]	Olaparib	Pre-treated Monotherapy: BRCA1/2-mutated, HER2-negative, locally advanced or metastatic cancer; patients with hormone receptor-positive cancer should also have progressed on or after prior endocrine therapy, or be considered unsuitable for endocrine therapy	FDA, 2018	Previously treated with chemotherapy in the (neo)adjuvant or metastatic setting; BRCA1/2 mutation detected using FDA-approved companion diagnostic [a]
			EMA, 2019	Previously treated with an anthracycline and a taxane in the (neo)adjuvant or metastatic setting, unless patients were not suitable for these treatments
	Talazoparib	Pre-treated Monotherapy: BRCA1/2-mutated, HER2-negative, locally advanced or metastatic cancer	FDA, 2018	BRCA1/2 mutation detected using FDA-approved companion diagnostic [a]
			EMA, 2019	Previously treated with an anthracycline and a taxane in the (neo)adjuvant or metastatic setting, unless patients were not suitable for these treatments; patients with hormone receptor-positive cancer should also have progressed on or after prior endocrine therapy, or be considered unsuitable for endocrine therapy
Prostate cancer	Olaparib	Pre-treated Monotherapy: Metastatic, castration-resistant prostate cancer	FDA, 2020	HR gene-mutated, progressed following prior treatment with enzalutamide, or abiraterone; HR gene mutation detected using FDA-approved companion diagnostic [a]
			EMA, 2020	BRCA1/2-mutated, progressed following prior therapy that included a new hormonal agent
	Rucaparib	Pre-treated Monotherapy: BRCA1/2-mutated, metastatic, castration-resistant prostate cancer, previously treated with androgen receptor-directed therapy and taxane-based chemotherapy	FDA, 2020 [b]	BRCA1/2 mutation detected using FDA-approved companion diagnostic [a]
Pancreatic adenocarcinoma	Olaparib	First-line Maintenance: BRCA1/2-mutated, metastatic cancer who have not progressed on ≥16 weeks of a first-line platinum-based chemotherapy regimen	FDA, 2019	BRCA1/2 mutation detected using FDA-approved companion diagnostic [a]
			EMA, 2020	No specific restrictions

FDA, food and drug administration; EMA, European medicines agency; CR, complete remission; PR, partial remission; HRD, homologous recombination deficiency; HER2, human epidermal growth factor receptor 2; HR, homologous recombination; a, See https://www.fda.gov/medical-devices/vitrodiagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools for details on FDA-approved companion diagnostics; b, accepted under accelerated approval.