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Review

REVIEW

Recent advancements in PARP inhibitors-based targeted cancer therapy

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Abstract

Poly(ADP-ribose) polymerase inhibitors (PARPi) are a new class of agents with unparalleled clinical achievement for driving synthetic lethality in BRCA-deficient cancers. Recent FDA approval of PARPi has motivated clinical trials centered around the optimization of PARPi-associated therapies in a variety of BRCA-deficient cancers. This review highlights recent advancements in understanding the molecular mechanisms of PARP 'trapping' and synthetic lethality. Particular attention is placed on the potential extension of PARPi therapies from BRCA-deficient patients to populations with other homologous recombination-deficient backgrounds, and common characteristics of PARPi and non-homologous end-joining have been elucidated. The synergistic antitumor effect of combining PARPi with various immune checkpoint blockades has been explored to evaluate the potential of combination therapy in attaining greater therapeutic outcome. This has shed light onto the differing classifications of PARPi as well as the factors that result in altered PARPi activity. Lastly, acquired chemoresistance is a crucial issue for clinical application of PARPi. The molecular mechanisms underlying PARPi resistance and potential overcoming strategies are discussed.

Key words: PARP1; classifications of PARP inhibitors; BRCA1/2; synthetic lethality; acquired chemoresistance to PARP inhibitors; immunotherapy

Introduction

Poly(ADP-ribose) polymerase (PARP) family proteins have attracted attention in the last decade due to the clinical success of PARP inhibitors (PARPi) in cancer treatment. Among the seventeen family members, PARP1 and PARP2 are two key enzymes that mediate DNA damage response (DDR) by serving as DNA damage sensors and signal transducers.[1,2] To respond DNA damage such as nicks and double-strand breaks (DSB), PARP1 is rapidly recruited to the sites of damaged DNA, and its catalytic activity increases 10- to 500-fold through an allosteric activation mechanism. This results in the synthesis of protein-conjugated poly(ADP-ribose) (PAR) chains using NAD^+ as a critical substrate.[3,4] The negatively charged

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PAR functions as a high-density protein-binding scaffold and recruits components of the DNA damage repair machinery.[1,5] PARP2, the less abundant homologous protein of PARP1, probably has a less dominant role due to the absence of N-terminal zinc-finger (Zn) domains for DNA binding.[6–8] PARP1 is thus an attractive target for cancer therapy, with four PARPi being licensed in clinic to date: olaparib, talazoparib, niraparib, and rucaparib. All these PARPi compounds share a nicotinamide moiety competing with NAD^+ for binding to PARP1, and thus inhibit the catalytic activity of PARP1. Hundreds of clinical trials are currently ongoing to optimize the use of each PARPi and to test effective combination treatment strategies aiming to improve response, overcome resistance, or minimize overlapping toxicity. For example, administration of PARPi in combination with immune checkpoint inhibitors represents a novel therapeutic strategy. In this review, we will discuss the molecular mechanisms of PARPi-based targeted therapy and classification of PARPi, acquired PARPi resistance, and potential biomarkers of PARPi response.

PARPi-associated synthetic lethality

PARPi are developed in breast and ovarian cancers with *BRCA* gene mutations using synthetic lethal screening.[9,10] *BRCA1* and *BRCA2* are two human tumor suppressor genes that play an important role in DNA repair, and their mutations play a critical role in the development of several cancers including breast and ovarian cancer. Byrant *et al.*[9] and Farmer *et al.*[10] have first demonstrated synthetic lethality of *BRCA1*- and *BRCA2*-deficient tumor cells by PARP inhibition. Two models are proposed to explain underlying mechanisms. The first model stands on the notion that PARP1, the major target of PARPi, is primarily associated with base excision repair (BER) and single stranded break (SSB) repair by recruiting DNA repair effectors such as XRCC1, DNA polymerase-β and DNA ligase III.[10] When PARP1 is inhibited, SSBs cannot be repaired and are switched to DSBs during DNA replication. This forces defective homologous recombination (HR) and cytotoxicity in the absence of BRCA1/2.[11,12] In contrast, the second model describes a process by inhibiting PARP1 autoPARylation[13] and inducing allosteric changes in the PARP1 structure. The cytotoxicity of PARPi relies on sufficient trapping of PARP1 on DNA lesions.[14,15] The trapped PARP1 forms a toxic lesion and stalls the progress of replication forks. This can be repaired by HR in a BRCA1- and BRCA2-dependent manner.[16,17] However, the replication fork collapses in *BRCA1/2*-deficient cancer cells that is paired with an increased genomic instability, ultimately resulting in cell death. The second model is currently favored as the preferred mechanism of synthetic lethality for two reasons. 1) PARP inhibition by inhibitors is more cytotoxic than the genetic deletion of PARP;[18] and 2) The cytotoxicity of PARPi is correlated with its ability to trap PARP on DNA rather than its catalytic inhibitory properties. This is evident upon comparison of three PARPis: talazoparib, olaparib, and rucaparib. These PARPis are comparable in their catalytic PARP inhibition. However, talazoparib results in higher levels of cytotoxicity compared to olaparib and rucaparib due to its superior PARP-trapping potency.[19] This logic is further supported by recent evidence suggesting that PARPi modulates PARP-1 trapping through effects on a critical allosteric regulatory domain of PARP-1.[14] In addition to synthetic lethality in cells with the *BRCA1/2*-defect, PARPi also shows similar mechanistic pathologies when reactive oxidative species (ROS) accumulate, which cause oxidative DNA damage and are likely to increase replication stress. Alantolactone, a ROS inducer, is shown to synergize with olaparib and induce lethality irrespective of HR status.[20]

Classification of PARPi based on their allosteric effects on PARP1

PARPi exist in clinical and non-clinical varieties. There are currently five clinical inhibitors. Four of them, namely olaparib, talazoparib, niraparib, and rucaparib have been approved for treating *BRCA1/2*-deficient ovarian and breast cancers (Table 1).[21,22] Two recent trials (NCT02184195 and NCT02987543) have shown that olaparib also benefits patients with either metastatic pancreatic cancer or metastatic castration-resistant prostate cancer.[23,24] Notably, niraparib is the first drug granted approval irrespective of the *BRCA1/2* status. The fifth clinical inhibitor, veliparib, is still in Phase III clinical trials after two failed Phase III attempts.[25] Benzamide adenine dinucleotide (BAD) and EB-47 are two inhibitors in preclinical studies. BAD is an analog of the PARP1 substrate nicotinamide adenine dinucleotide (NAD^+),[26] and EB-47 can mimic NAD^+ binding with an affinity comparable to that of clinical PARPi.[27,28] Although all different PARPi compounds bind at the catalytic center to block binding of NAD^+ and prevent PAR production, they exhibit different potencies in inducing cancer cell death. This is presumably due to their varying capabilities to induce catalytic inhibition and trap PARP1 on DNA breaks.

The establishment of a classification system would be useful to predict new PARPi activity and to promote PARPi development. According to recent X-ray structure analysis,[14] PARP inhibitors affect a critical allosteric regulatory domain of PARP1 as well as in the helical domain (HD).[15,29] Based on the varying impact on PARP1 allostery, PARPi molecules are categorized into three types: type I, allosteric pro-retention on DNA; type II, non-allosteric pro-retention on DNA; and type III, allosteric pro-release from DNA (Table 2).

BAD and EB-47 are designated as type-I inhibitors that contact the helix αF of the HD and have a strong reverse allosteric effect by destabilizing the HD. HD instability results in functional allosteric changes, increases PARP1 affinity for DNA and retains PARP1 on DNA breaks.[14] Laboratory results clearly show downstream effects of

Table 1. The list of approved PARP inhibitors and prescribing information*.

Drug	Approval date	Single/Combination	Recommended dose	Based clinical trials	Indications
Olaparib (LYNPARZA, AstraZeneca)	May 19, 2020	single	300 mg orally twice daily	NCT02987543	adult patients with deleterious or suspected deleterious germline or somatic HRR[1] gene-mutated mCRPC[2], who have progressed following prior treatment with enzalutamide or abiraterone
	May 8, 2020	in combination with bevacizumab	olaparib, 300 mg orally twice daily; bevacizumab, 15 mg/kg intravenously every three weeks	NCT03737643	adult patients with advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in complete or partial response to first-line platinum-based chemotherapy and whose cancer is associated with HRD[3]-positive status
	Dec 27, 2019	single	300 mg orally twice daily	NCT02184195	adult patients with deleterious or suspected deleterious gBRCAm[4] metastatic pancreatic adenocarcinoma whose disease has not progressed on at least 16 weeks of a first-line platinum-based chemotherapy regimen
	Dec 19, 2018	single	300 mg orally twice daily	NCT01844986	adult patients with deleterious or suspected deleterious gBRCAm[4] or sBRCAm[5] advanced epithelial ovarian, fallopian tube or primary peritoneal cancer who are in complete or partial response to first-line platinum-based chemotherapy
	Jan 12, 2018	single	300 mg orally twice daily	NCT02000622	patients with deleterious or suspected deleterious gBRCAm[4], HER2-negative metastatic breast cancer who have been treated with chemotherapy either in the neoadjuvant, adjuvant, or metastatic setting
	Aug 17, 2017	single	300 mg orally twice daily	NCT01874353 NCT00753545	adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy
	Dec 19, 2014	single	400 mg (capsules) orally twice daily	NCT00494442	patients with deleterious or suspected deleterious gBRCAm[4] advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy

Table 1. Continued

Drug	Approval date	Single/Combination	Recommended dose	Based clinical trials	Indications
Talazoparib (TALZENNA, Pfizer)	Oct 16, 2018	single	1 mg orally once daily	NCT01945775	patients with deleterious or suspected deleterious gBRCAm[4], HER2negative locally advanced or metastatic breast cancer
Rucaparib (RUBRACA, Clovis Oncology)	May 15, 2020	single	600 mg orally twice daily	NCT02952534	patients with deleterious gBRCAm[4] or sBRCAm[5]-associated mCRPC[2] who have been treated with androgen receptor-directed therapy and a taxane-based chemotherapy
	Apr 6, 2018	single	600 mg orally twice daily	NCT01968213	patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy
	Dec 19, 2016	single	600 mg orally twice daily	NCT00664781	patients with deleterious gBRCAm[4] or sBRCAm[5]-associated advanced ovarian cancer who have been treated with two or more chemotherapies
Niraparib (ZEJULA, GlaxoSmithKline)	Apr 29, 2020	single	200 or 300 mg orally once daily[6]	NCT02655016	adult patients with advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to first-line platinum-based chemotherapy
	Oct 23, 2019	single	300 mg orally once daily	NCT02354586	patients with advanced ovarian, fallopian tube, or primary peritoneal cancer treated with three or more prior chemotherapy regimens and whose cancer is associated with HRD[3]-positive status
	Mar 27, 2017	single	300 mg orally once daily	NOVA	adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in complete or partial response to platinum-based chemotherapy

*All the information is obtained from the Drug Approvals and Databases of FDA.
[1]HRR, homologous recombination repair.
[2]mCRPC, metastatic castration-resistant prostate cancer.
[3]HRD, homologous recombination deficiency.
[4]gBRCAm, germline *BRCA*-mutated.
[5]sBRCAm, somatic *BRCA*-mutated.
[6]For patients weighing less than 77 kg or with a platelet count of less than 150,000/μL, the recommended dose is 200 mg taken orally once daily. For patients weighing greater than or equal to 77 kg and who have a platelet count greater than or equal to 150,000/μL, the recommended dose is 300 mg taken orally once daily.

Table 2. Distinct properties among three types of PARP inhibitors.

Item	Type I	Type II	Type III
Inhibitors	EB-47, BAD	olaparib, talazoparib	rucaparib, niraparib, veliparib
PARP-1 allostery	allosteric	non-allosteric	allosteric
HD conformation	destabilization	neutral	more folded
PARP-1 affinity for DNA	large increase	small increase	decrease
Trapping potency	pro-retention	pro-retention	pro-release

BAD and EB-47 on trapping PARP1 on DNA breaks. However, they have not been developed as clinical inhibitors, possibly due to BAD's non-selective nature and EB-47's impermeability in cell lines.[27] A cell permeable version of EB-47 may be worth testing as it could have clinical potential as a potent regulator of PARP1 binding. Type II inhibitors (including olaparib and talazoparib) are relatively neutral toward PARP1 allostery and produce minimal increases in affinity for a DNA break. Olaparib does not contact the HD, and thus has no effect on interdomain communication or DNA binding domains in PARP1. Talazoparib is very efficient at trapping PARP1 on DNA breaks, and it does not exert a pronounced reverse allosteric effect on PARP1. Talazoparib is extremely potent at catalytic inhibition of PARP1 and further limits PARP1 autoPARylation which is required for rapid release of PARP1 from the site of DNA damage.[19,30] Even subtle differences in PARPi potencies affecting auto-PARylation levels could yield significant differences in PARP1 release from DNA.[31] Talazoparib's high trapping efficiency can possibly be attributed to its inhibitory potency combined with its slow dissociation half-life[32] and neutral effect on the HD. Type III inhibitors include rucaparib, niraparib, and veliparib. As allosteric pro-release inhibitors, they promote highly folded conformation of the HD by stabilizing αB/αF helices and decreasing PARP1 affinity for DNA. Thus, they act opposite in function to type I inhibitors. Both niraparib and rucaparib boast a strong catalytic inhibition that can limit the auto-PARylation-dependent release from DNA. Combined with their long half-life, this allows both niraparib and rucaparib to compensate for their pro-release allosteric effects.[18] Rucaparib and talazoparib exhibit the similar inhibitory potency and binding properties to PARP1, but they have remarkable differences in trapping abilities.[33] Rucaparib's propensity to promote stabilization of the HD decreases PARP1 affinity for DNA.[14] In contrast, veliparib is considered a poor trapper, probably due to its pro-release allosteric effect, relatively low catalytic inhibition potency, and rapid clearance rates in vivo.[34]

HD reverse allostery has proven vital to define the potency of an inhibitor to trap PARP1 on DNA breaks.[29,35,36] Mutagenesis breaking interdomain communication in PARP1 or disrupting the inhibitor-HD interaction can abolish the reverse allosteric effect of a type I inhibitor and transform them into type II inhibitors. In contrast, inducing HD contact that generates PARP1 reverse allostery transforms type III inhibitors into type I inhibitors.[14] Of note, several inhibitors, such as A-966 492,[37] 3-Aminobenzamide,[38] and AG-14 361,[39] have been omitted from this classification system because of a lack of data supporting their influence of PARP1 allostery. To develop new PARPi derivatives in the future, studies should emphasize the outstanding drug-like properties, high potency of catalytic inhibition, and allosteric pro-retention of inhibitors on DNA breaks.

PARPi sensitivity and 'HRDness'

Growing evidence demonstrates that multiple factors can affect PARPi sensitivity in addition to *BRCA* gene mutations. Homologous recombination defectiveness, or 'HRDness', indicates a non-*BRCA*-related mechanism of PARPi sensitivity.[40] Extending the application of PARP inhibition from tumors with BRCA-defects to other HR-deficient backgrounds, such as PTEN- and ATM-mutations,[41,42] allow a greater population of patients to benefit from therapy. To this end, silencing critical mediators of DDR potentially enhances cellular sensitivity to PARPi. For example, the histone lysine demethylase PHF2 was recently identified as a novel regulator of DDR. PHF2-depleted cells display increased sensitivity to PARPi. PHF2 deficiency mechanistically results in impaired HR by affecting CtIP-dependent resection of DSBs and decreasing BRCA1 protein levels.[43] Similarly, NFBD1 loss could disrupt HR by decreasing the formation of BRCA1, BRCA2 and RAD51 foci and thereby sensitize nasopharyngeal tumor cells to PARPi.[44] The HR repressor ZPET (zinc finger protein proximal to RAD18) may play a role in inhibiting MRE11 binding to chromatin and stalling replication forks, and loss of this protein confers PARPi resistance.[45] In addition, cellular sensitivity to PARPi can potentially be increased by silencing some important mediators of DDR, such as RPL6,[46] which regulates the recruitment of MDC1 and RNF16827, and VRK1 chromatin kinase, which controls the configuration of locally altered chromatin induced by DNA damage[47]. Cellular sensitivity was also found to increase upon inhibition of the microtubule-depolymerizing kinesin Kif2C that regulates the mobility of DSBs and the generation of DNA damage foci.[48] Alternatively, pharmacological inhibition of key mediators in HR also synergistically enhances PARPi sensitivity in HR-proficient or PARPi-resistant cells. Recent studies indicate that the WEE1/PLK1 dual inhibitor AZD1775, the FOXM1 inhibitor thiostrepton, the DNMT inhibitor 5-azacytidine, or the CDK1/2 inhibitor dinaciclib may induce a HRDness phenotype and thereby sensitize tumor cells to

PARPi by downregulating HR-related genes, blocking BRCA1 phosphorylation, and reducing RAD51 foci formation.[49–53] Therefore, the identification of the potential inhibitors of the HRDness phenotype will promote the optimal usage of PARPi combined with other inhibitors in clinic.

PARPi and non-homologous end-joining (NHEJ)

Although most studies have focused on HR, DSBs are repaired primarily by non-homologous end-joining (NHEJ), likely due to the fact that HR relies on the presence of a sister chromatid while NHEJ can repair DSBs at any cell cycle stage. There are two major types of NHEJ: the classical pathway initiated by the Ku heterodimer (C-NHEJ), and the alternative pathway initiated by PARP1 (Alt-NHEJ). [54–56] Albeit error prone, C-NHEJ is a critical repair pathway for DSBs and directly joins broken ends of DNA with little to no regard for sequence homology. Therefore, C-NHEJ deficiency might be expected to show synthetic lethality with PARPi. Paradoxically, the inhibition of C-NHEJ by depletion of Ku80 or DNA-PK inhibitors reduced the genomic instability and lethality of PARPi in HR-deficient cells rather than aggravating it.[57] Similarly, cancer cell cultures with NHEJ competence and HR were sensitive to rucaparib while NHEJ-defective cell cultures were resistant to rucaparib,[58] thus indicating that the C-NHEJ repair pathway is required for the sensitivity to PARPi. Furthermore, impaired C-NHEJ induced by deletion of DYNLL1 or ASCIZ (an organizer of the 53BP1 complex) confers BRCA1-deficient tumor cells resistant to PARPi.[59] Thus, evidence conclusively supports that the genomic instability resultant from NHEJ is an important promoter of PARP inhibition. While alt-NHEJ mediated by PARP1 and DNA polymerase θ (Pol θ) using microhomology is a crucial backup repair pathway for blunt DSBs and stressed replication forks in absence of HR, it comes at a cost of promoting chromosomal translocations and genomic instability.[54,60,61] Cancer cells deficient in BRCA1 (or its obligate partner BAP1) often repress miR223–3p and allow repairing stressed replication forks through Alt-NHEJ.[62] PARP1 initiates the Alt-NHEJ response by displacing the Ku complex from DSBs and is the rate-limiting initial step of Alt-NHEJ. Therefore, PARP1 inhibition with olaparib or rucaparib could suppress Alt-NHEJ and decrease chromosomal translocations,[54] indicating that PARPi are negative regulators of the Alt-NHEJ repair pathway.

PARPi and immunotherapy

Immunotherapy using immune checkpoint blockades has obtained a great success in treating human cancers. Unfortunately, the response rate is relatively low. Accumulating evidence suggests that PARPi might induce the anti-tumor immune response and collaborate with checkpoint blockade to inhibit tumor growth.[63–71] PARP inhibition by olaparib or talazoparib may promote cytotoxic T lymphocyte infiltration through BRCA1/2-independent mechanisms in several *in vivo* tumor models, including small cell lung cancer (SCLC), ovarian cancer, and breast cancer.[64–67] PARP inhibition has also been shown to upregulate PD-L1, and this may be mediated by Chk1 phosphorylation, IRF3 expression, or through recruitment of myeloid cells into tumor sites, thereby potentiating tumor responsiveness to PD-1 or PD-L1 blockade therapy.[63–66] The mechanistic studies suggest that PARP inhibition promotes the accumulation of cytosolic DNA fragments from unrepaired DNA lesions and activates the DNA-sensing cyclic guanosine monophosphate–adenosine monophosphate (cGAMP)-cyclic GMP-AMP synthase (c-GAS) cascade. Subsequently, cGAMP binding to stimulator of interferon genes (STING) induces the phosphorylation of IRF3 through Tank-binding kinase-1 (TBK1). Phospho-IRF3 then translocates to the nucleus and induces the expression of downstream effector genes, such as type I interferon, various chemokines and PD-L1.[63,66,67] This is consistent with the mechanisms that account for activation of type I interferon signaling and PD-L1 upregulation in response to DSBs.[68,69] Based on these interesting findings, the PARPi and anti–PD-L1 blockade represents a rational therapeutic combination,[70,71] and has been widely tested in clinical trials. The combination of PARPi, olaparib, and the PD-L1 inhibitor durvalumab has been the most common strategy to yield desired clinical success rates in patients with metastatic castrate-resistant prostate cancer or recurrent ovarian cancer.[72–74] In addition, niraparib in combination with pembrolizumab, a PD-1 inhibitor, has also exhibited promising antitumor activity and tolerance in metastatic triple-negative breast cancer and recurrent ovarian cancer patients.[75,76] Ongoing trials combining PARPi and immune checkpoint blockades demonstrate the synergistic antitumor effect and will benefit more patients regardless of platinum-resistance, *BRCA* mutations, or prior treatments. In addition, PARP1 was reported to repress the expression of NKG2DLs and therefore mediate immune evasion of leukemic stem cells (LSCs) in acute myeloid leukemia. PARP1 inhibition could restore the expression of NKG2DLs on the LSCs surface and promote their clearance by NK cells.[77] Thus, the multifactorial functions of PARP1 in immunomodulation and immune escape of cancer stem cells may pave the way for novel therapeutic strategies in clinic.

PARPi resistance and overcoming strategies

PARPis have boasted unprecedented clinical success for cancer patients with HR-defects. At the same time, acquired resistance to PARPi treatments impedes optimal clinical outcome. Defining both molecular features

and elucidating underlying mechanisms of PARPi resistance stand at the foundation of overcoming resistance to PARP treatments. While activation of epithelial-mesenchymal transition (EMT)[78,79] and induction of P-glycoprotein expression[12,80] have been observed in PARPi-resistant biopsies, they are not sufficient to confer resistance.[78] Recent research supports the notion that the primary mechanisms for PARPi resistance are HR restoration and replication fork protection.[81] PARP mutations that affect DNA or inhibitor binding have also been explored as a potential mechanism. In addition, combination therapy of PARPi and tyrosine kinase inhibitors might represent a possible avenue to circumvent acquired resistance. This information is summarized in Fig. 1.

HR restoration

HR restoration describes a process in which HR-deficient patients become HR-proficient post-resistance to PARPi; this occurs in about 50% of PARPi-resistant patients with ovarian cancers.[81,82] The most common mechanism of HR restoration, genomic reversion of *BRCA1/2*, has been observed in a subset of post-resistant patients with ovarian cancer,[82–84] pancreatic cancer,[85] prostate cancer,[86] and in about 50% of metastatic breast cancer patients.[87] Mechanisms that describe this restoration involve either 1) secondary intragenic mutations; or 2) upregulation of the remaining functional allele; or 3) the loss of *BRCA1* promoter methylation. First, secondary intragenic mutations in carcinomas lead to the expression of functional BRCA1/2 proteins by either restoring the open reading frames (ORF) or the inherited mutation.[82–86,88,89] Amplification of the mutant *BRCA2* allele that expresses the truncated BRCA2 protein[90] or the expression of a BRCA1 hypomorphic protein[91] also results in resistance. These pathways have been verified in PARPi-resistant PDXs and patients.[82,91] Epigenetic silencing of BRCA1 or RAD51C by the promoter hypermethylation can sensitize tumor cells to PARPi. In contrast, the promoter demethylation or *de novo* gene fusions placing *BRCA1* under the transcriptional control of a heterologous promoter will lead to the re-expression of BRCA1 and result in subsequent PARPi resistance.[92,93] Heterogeneous tumors treated with PARPi could eliminate tumor cells with *BRCA1* methylation, leading to the positive selection of BRCA1-expressing tumor cells.[81] Likewise, *de novo* rearrangements at the *BRCA1* locus that skip the *BRCA1* promoter hypermethylation could also restore BRCA1 expression.[93] In addition, mutant BRCA1 proteins with a loss of the RING domain (185delAG) or that otherwise carry a mutation (C61G) in the RING domain were reported to develop rapid resistance to PARPi.[94,95] Loss of 53BP1 has also been shown to confer PARPi-resistance,[91] probably due to a shift in DNA repair mechanism from NHEJ to HR.[40,80] This might also notably serve as a biomarker to test for PARPi-resistance. Recent patient genomic data enrichment supports the notion that a loss of ARID1A or GPBP1 could drive PARPi-resistance by upregulating the genes involved in HR in ovarian cancer patients.[96]

Inhibitors targeting DDR or DDR-relative pathway components can pharmacologically induce a HRDness phenotype, known as "chemical HRDness".[40] Any compounds that induces chemical HRDness may overcome PARPi-resistance; common examples can include inhibitors of FOXM1, DNMT, CDK1/2, and WEE1/PLK1. In addition, mTOR, PI3K/AKT, MEK, VEGFR, EGFR, androgen receptors, and BET inhibitors that indirectly regulate DDR might induce a similar HRDness phenotype.[40,97,98] Most of these inhibitors have been well discussed in recent reviews[40,81,99] and are not covered here. Several studies have reported that receptor tyrosine kinases (RTK) are involved in resistance to PARPi. Inhibition of FLT3, (a member of Class III RTK) by AC220 or AIU2001 could suppress DDR genes (such as *BRCA1*, *BRCA2*, *PALB2*, *RAD51*, and *LIG4*) and subsequently result in inhibition of DSB repair pathways. This results in synthetic lethality with PARPi.[100,101] In addition, c-MET and EGFR, both similar RTKs, were also found to be hyperactivated in TNBC cells with acquired resistance to PARPi. This is presumably because EGFR and MET heterodimers interact with and phosphorylate the Tyr907 residue of PARP1. While PARP1's enzymatic activity subsequently increases, PARPi binding slowly decreases.[102,103] Combined c-MET and EGFR inhibition by small molecules reversed the acquired resistance of PARPi in TNBC cells.[104] Taken together, inhibitors that induce chemical HRDness can contribute to overcoming PARPi-resistance and potentially extend PARPi application to HR-proficient cancer patients.

Replication fork protection

An important alternative mechanism for PARPi resistance is replication fork protection. By trapping PARP1 on chromatin, PARPi forces collapse of the replication fork and increases overall replication stress to induce cell death. Furthermore, BRCA1/2 deficient tumor cells can acquire PARPi resistance through several molecular mechanisms that are independent of BRCA1/2 restoration, including a loss of PTIP or EZH2, which ultimately serves to safeguard their replication forks. More specifically, PTIP deficiency protects replication forks from degradation by impairing the recruitment of the MRE11 nuclease to stalled replication forks, driving PARPi resistance in BRCA1/2-deficient cells.[105] Likewise, EZH2 localizes at stalled forks and mediates H3K27 trimethylation and subsequent recruitment of the MUS81 nuclease. A loss of EZH2 confers PARPi resistance by blocking MUS81 recruitment to stalled forks and allowing greater fork stabilization.[106]

PARPi resistance may also be obtained through increased expression or activity of replication fork stabilizers. Hyperactivation of tousled-like kinases reduces PARPi sensitivity in addition to contributing to chromatin assembly and maintaining replication fork integrity.[107] In PARPi-resistant cancer cells, the ATR/CHK1 pathway was

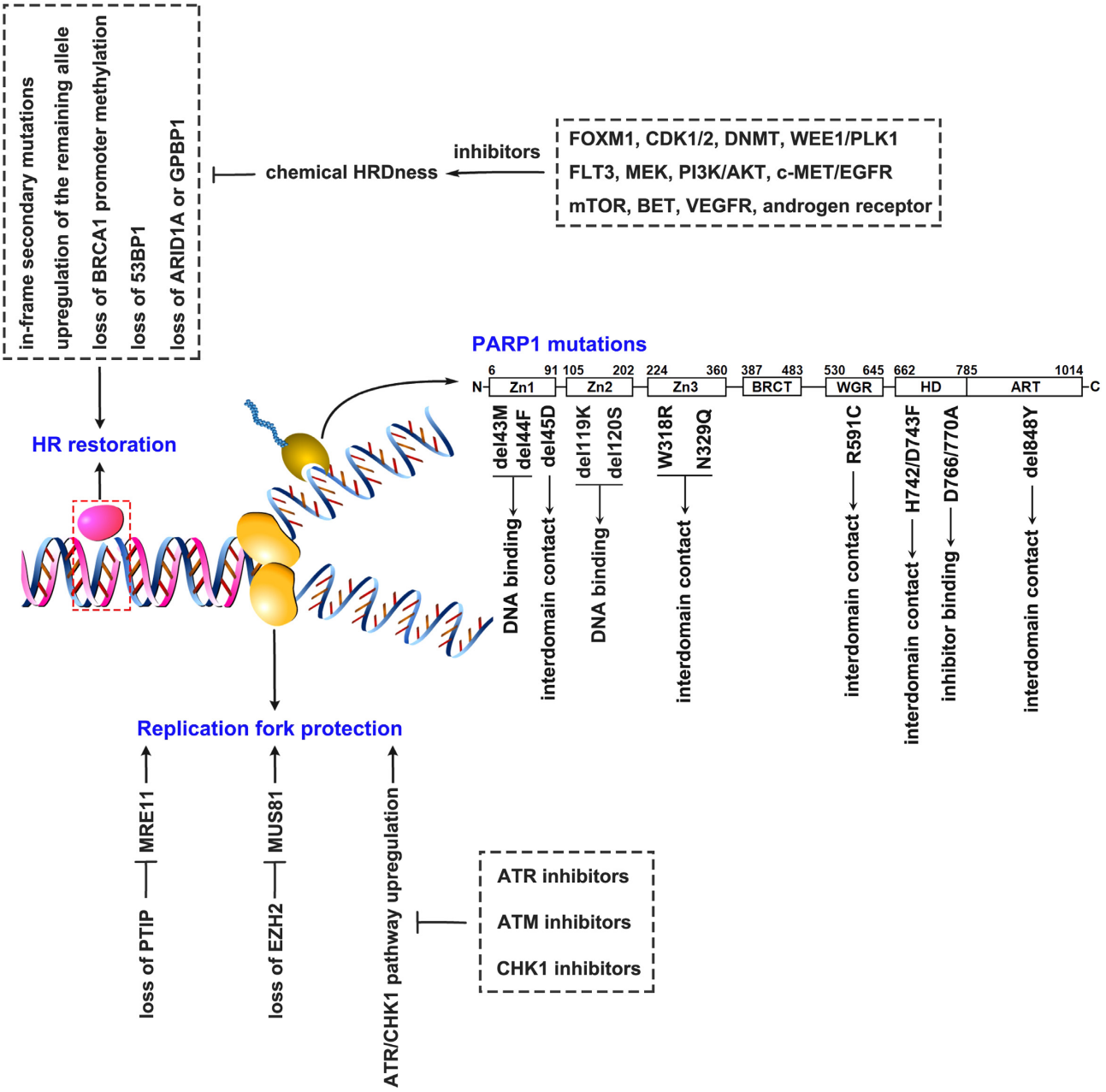

Figure 1. Schematic representation of the mechanisms contributing to PARP inhibitors (PARPi)-resistance and potential overcoming strategies. Homologous recombination (HR) restoration, replication fork protection and PARP1 mutations are three categories of mechanisms underlying acquired PARPi-resistance. HR restoration is usually induced by in-frame secondary mutations restoring BRCA1/2 function, upregulation of the remaining functional allele, loss of BRCA1 promoter methylation, and loss of 53BP1, ARID1A or GPBP1, and can be overcome via combining PARPi with a series of inhibitors that can result in chemical HRDness. Replication fork protection is mediated by loss of PTIP or EZH2 that impairs the recruitment of the MRE11 or MUS81 nuclease to stalled replication forks, and upregulation of the ATR/CHK1 pathway that can be potentially resolved via combining PARPi with inhibitors of ATR, ATM or CHK1. Mutations in PARP1 that can affect interdomain contacts, DNA binding potency or inhibitor binding also have a potential to lead to PARPi-resistance, which might have to be resolved by developing new inhibitors.

often upregulated, thereby inducing the phosphorylation of multiple proteins that stabilize the replication fork.[81] Therefore, combination treatment of PARPi with ATR or CHK inhibitors can potentially overcome acquired PARPi-resistance.[108–110] Similar to ATR, ATM is another key mediator that resolves DNA replication stress and maintains genomic stability.[111,112] ATM inhibition potentially enhances PARPi sensitivity or reverses acquired PARPi-resistance by preventing replication fork protection or by enhancing replication stress. Combination treatment therapy of olaparib and AZD0156, an ATM inhibitor, is currently being tested in patients with advanced-stage solid cancers (NCT02588105).[40]

PARP mutations

Most clinical PARPis generate cytotoxic lesions by trapping PARP1 at the site of damaged DNA. Therefore,

PARPi resistance can be caused through PARP1 mutations that affect trapping potency. The WGR domain-mediated interdomain interactions between regulatory HD and DNA binding domains link DNA binding to catalytic PARP1 activation. These mechanisms are central to PARP1 trapping procedures.[29,35] Notably, *de novo* resistance to olaparib was found to result from the R591C mutation (1771C > T) in the WGR domain and was recently identified in an ovarian cancer patient. More specifically, the R591C mutation affects PARP1 trapping by disrupting the WGR domain at the contact region with the HD and the DNA-binding Zn1 domain.[113] Mutations of other residues involved the Zn1-WGR-HD axis, including D45 in the Zn1 domain and H742/D743 in the HD, might also result in PARPi resistance. This has been partially supported in H742/D743F breast cancer cells and p.45delD mouse ES cells in response to talazoparib treatment.[113] Similarly, the presence of a W318R mutation in the Zn3 domain that disrupts Zn3/HD interaction also eliminates PARPi pro-retention functions.[14] Other mutations that affect interdomain communication, such as N329Q and 848delY, also promote talazoparib resistance through partial PARP1 trapping defects.[113]

Mutations that directly regulate DNA-binding within the PARP1 Zn domains can also affect PARP1 trapping. More specifically, residues M43 and F44 in the Zn1 domain are conserved and involved in base stacking interactions;[114] mutation or deletion of these residues causes talazoparib resistance in *BRCA1*-deficient SUM149 cells and in mouse ES cells by impairing PARP1 trapping.[113] Deletion of residues K119 and S120, both DNA-contacting residues in the Zn2 domain,[114] potentially eliminates microirradiation-induced PARP1 recruitment to the sites of DNA damage. This suggests that mutations in K119 and S120 residues result in PARPi resistance by impairing PARP1 DNA-binding functions.[113] In addition, mutations affecting PARPi binding to PARP1 can also cause resistance. For example, mutation in the residues that define molecular interaction between EB-47 and HD (D766/770A) truncates EB-47 ability to influence PARP1 allostery and retention on DNA breaks.[14]

It must be noted that a large number of mutations that affect residues involved in either interdomain communication or direct DNA binding have been identified in cellular or mouse models, but have not been confirmed in PARPi-resistant patients. However, the preliminary data strongly suggest that such mutations can develop in patients that could potentially promote PARPi resistance. Recent progresses in the genomic profiling of from PARPi-resistant tumors[86,115] could be used to identify the mutant PARP residues associated with PARPi resistance.

Predictors of clinical response to PARPi treatment

PARPi sensitivity can be predicted utilizing germline *BRCA1/2* mutations, somatic *BRCA1/2* mutations, or a genomic instability score. However, even with these methods available, identification of predictive biomarkers would allow for treatment regimens to be more effectively tailored to each individual patient. The homologous recombination deficiency loss of heterozygosity (HRD-LOH) assay also serves as an important factor that helps the detection of HRD irrespective of etiology. It is measured by levels of genomic LOH.[116,117] Although HRD score or HR gene mutation assessment can be used to augment for PARPi responders, neither test accurately predicts PARPi response. More specifically, objective response rates to these compounds in *BRCA1/BRCA2*-mutant relapsed platinum sensitive ovarian cancers vary from 30% to 80%.[118–120] Attempts to identify predictive biomarkers are ongoing. Potential clinical biomarkers of interest should serve as an indicator of a tumor's HR-defectiveness, PARPi resistance, or 'PARPness', which refers to the phenomenon of PARPi benefit despite a lack of HR mutations in the patient.

RAD51 loss as a predictor of HR-defectiveness

Two eukaryotic recombinases, RAD51 and DMC1, mediate homologous DNA pairing and strand exchange during HR. While DMC1 is only expressed in meiosis, RAD51 functions in both mitotic HR and meiotic HR events.[121] After recruitment to DNA breaks by BRCA1/2, RAD51 mediates the formation of DNA joints that links homologous DNA molecules.[122–124] RAD51 nuclear foci serve as a critical biomarker of intact HR. The low expression of RAD51 has been found to be coupled with an objective response to PARPi. In a recent clinical trial, the presence of RAD51 foci was tested by immunohistochemistry and was detected in all patients with acquired PARPi resistance probably due to reconstitution of HR.[87] Interestingly, RAD51 nuclear foci were the common characteristic in PDXs and patient samples with primary or acquired PARPi resistance, regardless of BRCA2 restoration, expression of BRCA1 hypomorphic proteins, or 53BP1 loss. This largely resolves a practical distinction between PARPi sensitivity and resistance which genomic testing alone may not be sufficient to clarify.[91,125] Thus, immunostaining of RAD51 foci offers real-time analysis of a tumor's HR proficiency irrespective of mechanism. In addition, results are more rapidly available and economically favorable than in exome sequencing. Taken together, these features favor the use of RAD51 as a biomarker to predict PARPi sensitivity in clinic. The RAD51 score could be used not only to identify PARPi-sensitive cancer patients, but also to expand the optimal patient demographic for PARPi therapies beyond the standard *BRCA1/2*-mutations.

53BP1 loss as a predictor of PARPi resistance

Recent reports have extensively linked 53BP1 loss to PARPi resistance,[126,127] presumably as loss of 53BP1 partially reinstates HR in BRCA1-deficient cells.[128] This

restoration is made possible because HR and NHEJ contest to repair DNA breaks during DNA replication.[129] After DYNLL1 recruits necessary proteins to the damaged site,[59] 53BP1 protects DNA ends from excessive resection through a shieldin complex;[130] the specifics depend on PTIP3 and RIF1 interactions.[131] In addition, 53BP1 facilitates NHEJ-related repair of intrachromosomal breaks, immunoglobulin class-switch recombination and fusion of unshielded telomeres.[132] 53BP1 deletion partially restores the formation of RAD51 filaments at resected DSBs in a PALB2- and BRCA2-dependent manner, and promotes ATM-dependent processing of broken DNA ends to facilitate repair by HR in BRCA1-deficient cells. Moreover, PALB2 chromatin recruitment is facilitated by an interaction between its chromatin associated motif and the nucleosome acidic patch region which is bound by 53BP1's ubiquitin-directed recruitment domain in 53BP1-proficient cells.[133] 53BP1 loss attenuates hypersensitivity of BRCA-mutant cells to PARPi and reinstates error-free repair by HR. This suggests that 53BP1 loss may be a predictor of PARPi resistance in patients. In a Phase I open-label clinical trial of PARPi ABT-767 among ovarian cancer patients, a decreased 53BP1 immunostaining score was correlated to a decreased antitumor efficacy of ABT-767 in the HR-deficient subset.[120] These results are consistent with those obtained from TNBC patients,[134] xenografts,[135] and BRCA1-deficient mouse mammary tumors.[80] These results unanimously support the use of 53BP1 loss as a predictor of PARPi resistance in clinic.

SMAD4 loss in head and neck squamous cell carcinoma (HNSCC)

A major contributor to HNSCC initiation and progression is a mutation or deletion in the *SMAD4* gene and occurs in 35% of primary HNSCC specimens and 41.3% of PDXs.[136] This is significant because SMAD4 loss could reduce BRCA1 and RAD51 protein levels, and thus result in genomic instability. In a Phase I clinical trial testing combination treatments of olaparib with radiotherapy and cetuximab for advanced HNSCC, it was found that five of six patients with SMAD4 loss showed prolonged progression-free survival (PFS), while four of eight SMAD4-positive patients exhibited effective response.[137] While the sample size leaves much to be desired, this study indicates that SMAD4 loss can possibly sensitize HNSCCs to olaparib, and potentially serve as a biomarker of therapeutic response to PARPi in HNSCC patients. Similarly, *SMAD4* FISH assays may function as a new platform for clinical diagnosis of SMAD4 chromosomal loss,[136] and promote the application of the biomarker in clinic.

MYC serves as a biomarker of PARPness

A substantial number of patients lacking of HR mutations may still benefit from PARP inhibitors,[138] and can be classified on a spectrum of relative 'PARPness' to specify their responsiveness to PARPi.[40] Usually, PARP1 is the initiator of Alt-NHEJ pathway of DNA repair[54] besides for regulating base-excision repair (BER). However, this is not the case in Multiple Myeloma (MM),[139] TK-activated leukemia,[140] MYC–positive Burkitt lymphoma,[141] and neuroendocrine prostate cancer (NEPC).[142] A recent analysis of a MM patient dataset (GSE24080) showed significant positive correlation between PARP1 and MYC expression,[139] an important oncogene[143] and a driver transcription factor hyper-activated in a majority of MM patients.[144] MYC directly binds to the PARP1 promoter and mediates transcription; high MYC expression correlates to PARPi sensitivity in MM,[139] Burkitt lymphoma[140] and neuroblastoma[145]. Likewise, albeit BRCA-proficient, glioblastoma cells with MYC or MYCN amplification exhibit sensitivity to PARPi, likely due to the repression of CDK18 that facilitates ATR activation.[146] In addition, PARPi induced cytotoxic effects are detectable among most MYC-dependent cancer types. All these results strongly suggest that MYC can serve as a potential response biomarker for PARPi-containing treatment protocols irrespective of HR status.

Conclusions and future perspectives

Great strides have been made in PARPi, with several selective and potent inhibitors emerging in the clinic. At present 432 trials of five clinical PARP inhibitors (olaparib, talazoparib, veliparib, niraparib, and rucaparib) are ongoing either alone or in combination with other antitumor agents. These trials aim to extend the license for use beyond BRAC-deficient tumors, expand indications beyond breast and ovarian cancers, and test the efficacy of PARPi combination therapy. To improve predictive accuracy of tumor response to PARPi or PARPi-involved therapies, there remains a need to identify more effective biomarkers. In addition, an emphasis has been placed on understanding underlying mechanisms in preclinical models of acquired PARPi-resistance. These mechanisms are being verified in patients. Moreover, efforts to reveal the relationship between structure and activity and to optimize the chemical structure of PARPi are still in progress. This process involves the development of derivatives of current PARPi as well as the identification of new PARPi compounds. These compounds should ideally contain outstanding drug-like properties and have high potency of both catalytic inhibition and allosteric pro-retention on DNA breaks. Through incremental improvements, PARPi therapeutics can revolutionize patient care by transforming non-responders into responders, and generate optimal outcomes with minimal side effects.

Author contributions

P.Z., J.W., and D.M. wrote the manuscript. P.Z. and C.Y.W. chose the targeted areas. C.Y.W provided critical revision of the manuscript.

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Conflict of interest

All authors declared that they had no conflict of interest.

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