

Molecular Pathways: How Can BRCA-Mutated Tumors Become Resistant to PARP Inhibitors?

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Abstract

PARP inhibition is synthetic lethal with defective DNA repair via homologous recombination. Phase I and II clinical trials show that PARP inhibitors are effective at well-tolerated doses and have antitumor activity for *BRCA1*- and *BRCA2*-associated cancers. However, not all patients respond equally well and tumors may eventually become resistant. Thus far, the only resistance mechanism that has been found in human tumors is genetic reversion that corrects or bypasses the original *BRCA1*- or *BRCA2*-inactivating mutation. However, data from fundamental and preclinical research suggest that resistance to PARP inhibitors may be induced by additional mechanisms involving hypomorphic activity of mutant *BRCA1* alleles, upregulation of drug efflux pumps, and rewiring of the DNA damage response. Preclinical models will be instrumental to develop methods for adequate patient stratification, as well as treatment strategies that prevent or counteract resistance to PARP inhibitors. *Clin Cancer Res*; 20(3); 540–7. ©2013 AACR.

Background

Treatment of Homologous Recombination-deficient tumors

Soon after the identification of *BRCA1* and *BRCA2* as hereditary breast and ovarian cancer genes, their importance for DNA double-strand break (DSB) repair by homologous recombination became clear. Although homologous recombination deficiency leads to error-prone DSB repair and genomic instability, it also forms an Achilles' heel that can be exploited therapeutically (1). Loss of homologous recombination in *BRCA1*- or *BRCA2*-deficient tumors results in hypersensitivity to DNA-damaging therapy, which is commonly applied to treat patients with cancer. Indeed, high-dose chemotherapy markedly improves overall survival of patients with advanced *BRCA1*-mutated or *BRCA1*-like breast cancer (2). Despite the increased therapeutic window in this group of patients, DNA-damaging therapies cause severe side effects because they are also toxic to normal cells. In contrast, phase I clinical trials have shown that treatment with PARP inhibitors does not result in major side effects (3, 4), whereas it is known to be synthetic lethal with *BRCA* deficiency. Phase II trials with the PARP inhibitor olaparib did not show a significant overall survival benefit in heavily pretreated and nonselected breast and ovarian cancer patients, but progression-free survival was improved (3, 5–8). The positive effect of olaparib on pro-

gression-free survival will be further evaluated in phase III clinical trials in platinum-sensitive *BRCA*-associated patients with ovarian cancer.

Although better patient stratification is likely to improve therapeutic outcome, there is evidence that also PARP inhibitor-sensitive tumors may become resistant to therapy. More insight in PARP inhibitor resistance mechanisms will therefore be instrumental to develop the most optimal treatment strategies for this novel class of targeted compounds. Before we discuss the different resistance mechanisms that have been identified to date, we will briefly summarize the current knowledge of the role of PARP1 in the DNA damage response (DDR) and the synthetic lethality between PARP inhibition and *BRCA* deficiency.

PARP1 in the DNA damage response

PARP1 is a highly abundant DNA binding protein that plays an important role in chromatin modification, transcription, and DNA repair (9). The 1104 aa PARP1 protein contains 3 DNA binding zinc finger motifs, a BRCT auto-modification domain, and a catalytic domain that transfers ADP-ribose from the nicotinamide adenine dinucleotide substrate to proteins. Although there are several other ADP-ribosyltransferases, PARP1 is the most abundant. Together with PARP2, PARP1 is thought to be responsible for most of the PARylation in the DDR. DNA single-strand breaks (SSB) are recognized by the PARP1/2 zinc fingers and binding of PARP1/2 to the damaged DNA induces rapid autoPARylation and recruitment of DDR proteins such as XRCC1 and MRE11A (Fig. 1). Subsequently, PARP1 can parylate XRCC1 and other substrates, leading to the formation of a protein complex that can repair the DNA breaks. PARP1 can also directly bind to other proteins via its BRCT domain and PARylation can both enhance and inhibit protein–protein interactions. PARylation at SSBs is a rapidly induced and

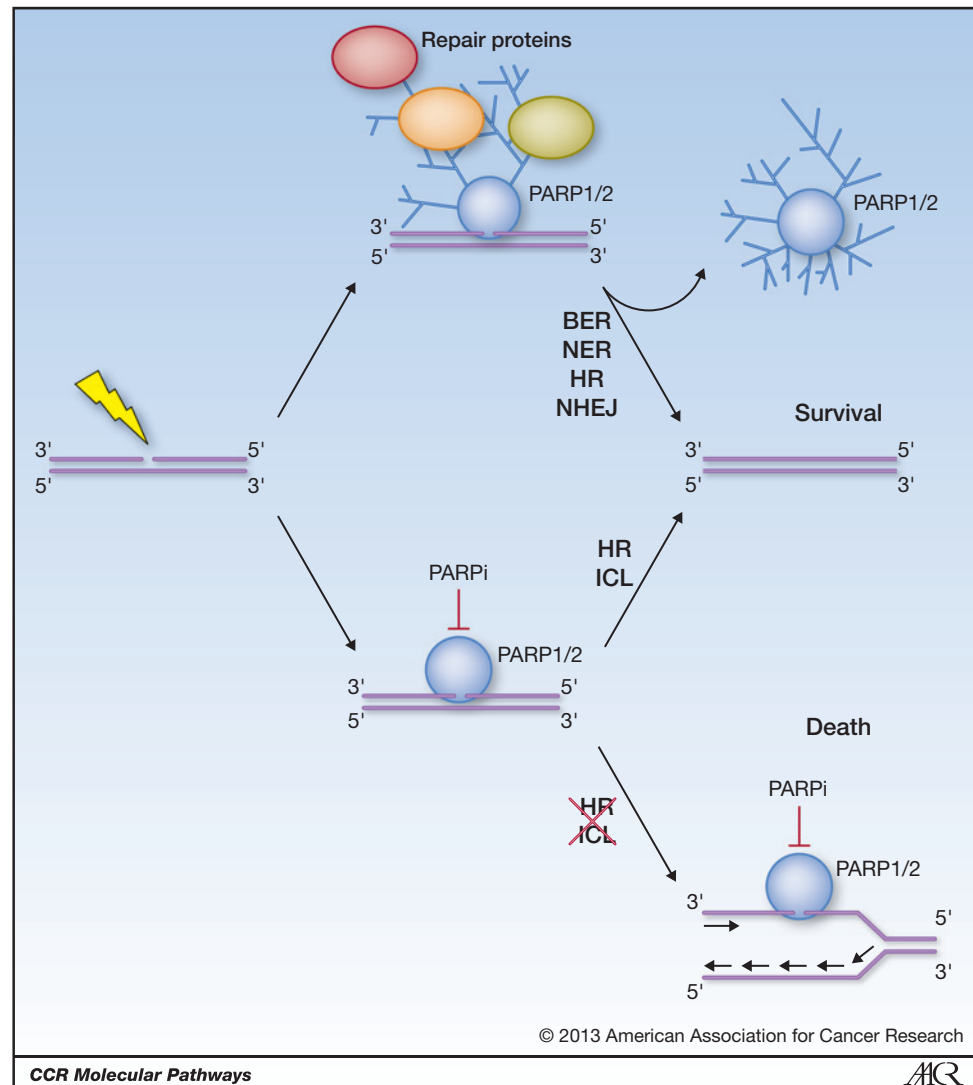
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Figure 1. Effects of PARP inhibition on the DNA damage response. Early in the DNA damage response, PARP1 and PARP2 enzymes are recruited to damaged DNA. The catalytic domains of PARP1 and PARP2 use the ADP ribose units from nicotinamide adenine dinucleotide for autopolylation and the recruitment and polylation of DNA repair proteins. Excessive autopolylation results in dissociation of PARP enzymes from the DNA, followed by DNA repair via base excision repair (BER), nucleotide excision repair (NER), homologous recombination (HR), or non-homologous end-joining (NHEJ). In the presence of PARP inhibitors, the enzymatic activity of the PARP enzymes is blocked and their interaction with the DNA is strengthened. When homologous recombination or DNA interstrand crosslink repair (ICL) are impaired, PARP enzymes trapped on the DNA cannot be removed and result in stalled replication forks and cell death.



transient process, which can be controlled via the extent of autoPARylation of PARP1 because the accumulation of negatively charged ADP-ribose molecules results in dissociation of PARP1 from the DNA. Despite the importance of PARylation in the DDR, PARP1 knockout mice are viable and only display subtle defects in genome stability such as increased sister chromatid exchange (10). In part, this may be explained by functional redundancy as concomitant deletion of PARP2 does result in embryonic lethality (11). However, it is clear that PARP1 and PARP2 also have unique functions in the maintenance of genomic integrity (12). Despite its interaction with XRCC1, PARP1 is not required for base excision repair and it has in fact been shown that recruitment of XRCC1 and OGG1 is not blocked by PARP inhibition (13). The current view is that PARP1 plays an important role in multiple DNA repair pathways including the alternative non-homologous end-joining (NHEJ) pathway (12, 14–16) and prevents excessive homologous recombination. The increase in sister chromatid exchange in PARP1 knockout mice and the accumula-

tion of RAD51 foci in PARP1-deficient or PARP inhibitor-treated cells (17) are consistent with this model.

Synthetic lethality of BRCA deficiency with PARP inhibition

In addition to the hyper-recombination phenotypes described earlier, PARP1-deficient cells also show defective SSB repair (18), suggesting an increased dependence on homologous recombination to prevent genomic instability. This led to the hypothesis that homologous recombination-deficient cells would be especially sensitive to PARP inhibition. In 2005, 2 seminal articles showed that PARP1/2 inhibition is synthetic lethal to BRCA-deficient cells (19, 20). The encouraging results in cell lines were confirmed by subsequent studies in genetically engineered mouse models (21), and in phase I (3, 4) and phase II (5–7) clinical trials. All PARP1/2 inhibitors that are currently used in clinical trials (Table 1) are based on the natural inhibitor nicotinamide and target the catalytic sites of the enzymes (22). In addition, it has recently been shown that more potent PARP

Table 1. PARP inhibitors in clinical trials

PARP inhibitor	Company	PARP1 IC ₅₀ ^a	Clinical trials ^b	Combination therapies ^b
Olaparib (AZD-2281)	AstraZeneca	5 nmol/L	Phase I–III	Bevacizumab, carboplatin, cediranib, cisplatin, dacarbazine, doxorubicin, gefitinib, gemcitabine, irinotecan, mitomycin C, paclitaxel, radiation, temozolomide, topotecan
Veliparib (ABT-888)	Abbott	5.2 nmol/L	Phase I–II	Abiraterone acetate, bendamustine, bevacizumab, bortezomib, capecitabine, carboplatin, cisplatin, cyclophosphamide, dexamethasone, dinaciclib, docetaxel, doxorubicin, etoposide, floxuridine, fluorouracil, gemcitabine, irinotecan, leucovorin, mitomycin C, oxaliplatin, paclitaxel, prednisone, radiation, rituximab, temozolomide, topotecan, vinorelbine ditartrate
AZD-2461	AstraZeneca	Not published	Phase I	NA
BMN-673	BioMarin	0.57 nmol/L	Phase I	NA
CEP-9722	Teva	20 nmol/L	Phase I–II	Cisplatin, gemcitabine, temozolomide
E-7016	Eisai	Not published	Phase II	Temozolomide
Niraparib (MK-4827)	Tesaro	3.8 nmol/L	Phase I	Temozolomide
Rucaparib (CO-338)	Clovis	1.7 nmol/L	Phase I–II	Carboplatin, cisplatin

^aPARP1 IC₅₀ values determined by biochemical assays (22).
^b<http://clinicaltrials.gov/>.

inhibitors, such as olaparib or niraparib, function through a large extent by trapping PARP1/2 on the DNA (13, 23), which explains why these PARP inhibitors are much more toxic to homologous recombination–deficient cells than genetic depletion or deletion of these enzymes. PARP inhibitors that do not or weakly trap PARP–DNA complexes (such as veliparib) are also much less toxic to homologous recombination–deficient cells (23). Hence, even though several PARP inhibitors are already in clinical development, there may still be room for further improvement. This is highlighted by recently published preclinical data for the novel, more potent PARP1/2 inhibitor BMN-673 (24).

Clinical–Translational Advances

To date, a number of possible resistance mechanisms that may counteract PARP inhibitors have been identified (Fig. 2). Because PARP inhibitors are relatively new compounds that have only recently been introduced in clinical trials, most evidence for these mechanisms comes from preclinical *in vitro* and *in vivo* model systems.

Intrinsic or adaptive resistance because of partial homologous recombination deficiency









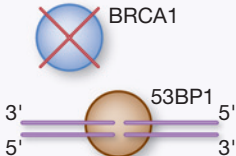

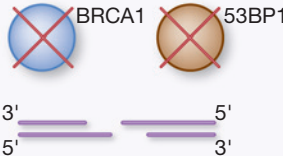

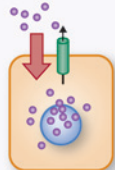
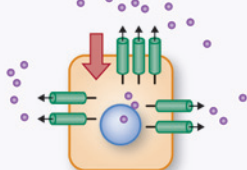
Not every homologous recombination–deficient tumor shows the same level of homologous recombination impairment. This may seem obvious but it is an important issue that needs to be taken into account when patients are selected for treatment with homologous recombination–deficiency targeting therapeutics, such as PARP inhibitors. The level of homologous recombination deficiency—and consequently the degree of synthetic sickness with PARP

inhibition—may depend on which homologous recombination–associated gene is affected and how it is affected.


Following the discovery that homologous recombination deficiency is a major determinant for sensitivity to PARP inhibition, there has been an active search for homologous recombination–inactivating mutations in sporadic homologous recombination–deficient tumors, as this would expand the applicability of PARP inhibitors as therapeutic agents. Although loss of BRCA1 has also been observed in sporadic tumors (25, 26), it is thus far mainly associated with hereditary breast and ovarian cancer. In contrast, loss of the tumor suppressor PTEN occurs in many sporadic tumors and has been associated with defective homologous recombination and increased PARP inhibitor sensitivity in colorectal (27) and endometrial (28) cancer cell lines. However, the effect of PTEN loss on sensitivity to PARP inhibition is considerably less pronounced than for BRCA1 and BRCA2 (27, 28). It is also unclear if PTEN loss can be used as a marker for PARP inhibitor sensitivity as no correlation was found in prostate cancer cell lines (29). Besides PTEN loss, also inhibition of PI3-kinase may in some cases render tumors homologous recombination deficient by downregulating BRCA1/2 expression (30).

Thus far, BRCA mutations remain the strongest genetic indications for sensitivity to PARP inhibition. However, not every mutation in BRCA1 or BRCA2 will result in the same functional defect. Analysis of BRCA1 missense mutations suggests that the conserved N- and C-terminal domains are most important for the response to homologous recombination–deficiency targeted therapy (31). The E3-ubiquitin ligase activity of the N-terminal RING domain may not be as important for homologous recombination as the

Figure 2. PARP inhibitor resistance mechanisms. Based on results from preclinical models, 4 different mechanisms of PARP inhibitor resistance have been suggested for BRCA1- or BRCA2-deficient tumors. The first 3 mechanisms depicted restore initially severe (red) or mild (orange) homologous recombination (HR) defects, either completely (dark green) or partially (light green). Genetic reversion of truncating mutations resulting in (near) wild-type BRCA2 protein has also recently been observed in 2 patients treated with the PARP inhibitor olaparib. Alternative mechanisms that (partially) restore homologous recombination include increased activity of BRCA1 or BRCA2 variants encoded by hypomorphic alleles (e.g., BRCA1-C61G) and rescue of DNA end-resection in BRCA1-deficient tumors through loss of 53BP1. Tumors may also become resistant because of increased P-glycoprotein (P-gp)-mediated drug efflux.

Resistance mechanism	PARPi sensitive	PARPi resistant
Genetic reversion of truncating mutation in BRCA1 or BRCA2 gene	BRCA1-truncated  HR: 	BRCA1-revertant  HR: 
Hypomorphic BRCA1 or BRCA2 activity	BRCA1-C61G  HR: 	BRCA1-C61G  HR: 
DDR rewiring	 HR: 	 HR: 
Drug transport by P-gp		

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phosphoprotein binding capacity of C-terminal BRCT domains (32), although the level of genomic instability of mouse tumors carrying the BRCA1-C61G RING inactivating mutation is identical to that of *Brca1*-null tumors (33). BRCA1-C61G tumor cells are able to form RAD51 foci upon irradiation, albeit not at the same level as BRCA1 wild-type cells, and rapidly develop resistance to PARP inhibitors (33). It has been suggested that an intact RING domain is important for rapid recruitment of BRCA1 to damaged DNA (34), which has recently been confirmed by a thorough analysis of BRCA1 repair kinetics (35). Moreover, the BRCA1/BARD1 heterodimer seems to bind to parylated proteins at sites of damage, allowing BRCA1 to play a role in the early phase of DNA repair. In contrast, the BRCT domain is important for retention of BRCA1 at DSBs and

inactivating mutations in the BRCT domain lead to a higher sensitivity for the combination of PARP inhibitors and irradiation than mutations in the RING domain (35). It still needs to be determined if these data can be translated to the *in vivo* tumor response to homologous recombination-deficiency targeted therapy. Of note, recent data suggest that even BRCT mutant BRCA1 with abolished CtIP binding may support homologous recombination and confer PARP inhibitor resistance if present at sufficiently high levels (36).

As illustrated by the data from the BRCA1-C61G mouse model, before treatment it may be difficult to distinguish tumors with hypomorphic *BRCA1* activity from *BRCA1*-null tumors based on their genomic profile. Whether this also applies to other markers of homologous recombination activity, such as RAD51 foci formation (37–39) or the

presence of specific types of genomic lesions (40), remains to be determined.

Resistance caused by limited drug uptake or increased drug export

Clearly, reduction of the concentration of chemotherapeutics in tumor cells will have profound consequences for treatment response. PARP inhibitors are cell permeable and it has been shown that the PARP inhibitor olaparib can accumulate in sufficiently high concentrations in mouse (21, 41) and human tumors (3), suggesting that drug uptake is normally not limiting. However, data from a preclinical mouse model shows that olaparib is readily extruded from tumor cells by the P-glycoprotein drug efflux transporter (21). Whether P-glycoprotein is also an important mediator of olaparib resistance in human tumors is not known. However, P-glycoprotein may be inhibited (e.g., by tariquidar; ref. 21) and there are already efficient PARP inhibitors available, which are poor P-glycoprotein substrates (e.g., Veliparib; ref. 42 and AZD-2461; ref. 43), indicating that resistance because of drug export may be circumvented.

Resistance caused by rewiring of the DNA damage response

Perhaps more challenging mechanisms of resistance are those that revive homologous recombination and thereby prevent synthetic lethality with PARP inhibition. Surprisingly, the requirement of BRCA1 for homologous recombination appeared to be alleviated by concomitant loss of another mediator of the DDR, p53-binding protein 1 (53BP1) (44, 45). In the absence of both proteins, sensitivity to homologous recombination–deficiency targeted therapy returned to normal levels in mouse embryonic stem cells (45), although other cell types required additional suppression of NHEJ (46). Loss of 53BP1 was found to be associated with BRCA-mutation status and poorer metastasis-free survival of breast cancer patients (45), although the opposite has been suggested for ovarian cancer (47). Mechanistically, 53BP1 seems to block CtIP-mediated DSB end-resection (44) via its downstream effectors RIF1 (48–52) and PTIP (PAXIP1) (53). Although it is not known if the requirement of BRCA2 for homologous recombination can also be alleviated by DDR rewiring, depletion of 53BP1 does not rescue BRCA2-deficient mouse embryonic fibroblasts (45).

It will be important to determine if functional impairment of 53BP1, RIF1, and PTIP may also play a role in PARP inhibitor resistance of human tumors. In the case of 53BP1, there is already preclinical *in vivo* evidence from a mouse model of BRCA1-associated breast cancer in which P-glycoprotein was inactivated (43). Loss of 53BP1 expression was found in 3 of 11 olaparib-resistant BRCA1-deficient mouse mammary tumors, suggesting that this may indeed be a relevant PARP inhibitor resistance mechanism.

Resistance caused by genetic reversion of the BRCA mutation

To date, the only resistance mechanism for homologous recombination–deficiency targeted therapy that has

been found in human tumors is genetic reversion of the original BRCA1 or BRCA2 mutation. Secondary mutations can correct or bypass the original lesion and thereby render tumors (partially) homologous recombination proficient and resistant to therapy. Genetic reversion of BRCA genes is not unprecedented as it has previously been observed in acute myelogenous leukemia cells from a Fanconi anemia patient with biallelic mutations in BRCA2 who was treated with chemotherapy (54). Secondary mutations restoring the BRCA2 open reading frame seemed to be a dominant mechanism of resistance to platinum compounds and PARP inhibitors in breast and pancreatic cancer cell lines with the BRCA2 6174delT frameshift mutation (55, 56). These findings were extended to cell lines derived from a patient with platinum-resistant ovarian cancer (57) and to platinum-resistant BRCA1- and BRCA2-associated ovarian tumors (58, 59). Whether genetic reversion is also a dominant mechanism for clinical resistance to PARP inhibition remains to be determined. Although genetic reversion events have recently been identified in 2 patients treated with olaparib (60), these were not found in 6 other patients with olaparib refractory BRCA1/2 associated cancer (61).

Conclusions and Future Prospects

Although treatment of certain types of tumors has considerably improved over the last few decades, most patients with cancer still undergo highly standardized therapies that do not take into account the unique characteristics of each individual tumor. With the advance of high-throughput sequencing technology it is hoped that patients with cancer will soon benefit from detailed knowledge of the aberrancies that are unique for their specific tumor. At present, one of the best-known examples of targeted therapy that is specifically toxic to certain types of tumors is inhibition of PARP enzymes in tumors with defective DNA repair via homologous recombination. Because PARP inhibitors do not show major side effects, they would be ideal for long-term treatments that could turn cancer from a lethal into a chronic disease. However, as with any other therapeutic strategy, tumors can eventually become resistant to PARP inhibition. To maximize benefit from PARP inhibition, it will be important to perform accurate patient stratification based on molecular markers of homologous recombination deficiency such as RAD51 foci formation (37), as well as functional analysis of the underlying mutations. Currently, inactivating germ-line mutations in BRCA1 or BRCA2 are the best predictors of sensitivity to PARP inhibitors. Data from preclinical studies in mouse models suggest that not all deleterious mutations confer the same homologous recombination defect and it will be important to investigate this further by gathering mutation-specific information on PARP inhibitor response. With respect to acquired resistance, increased drug efflux through P-glycoprotein might play a role although it is not yet known if this is a relevant resistance mechanism in human tumors. However, this type of resistance may be blocked by P-glycoprotein inhibition

or prevented because not all efficient PARP inhibitors are substrates for P-glycoprotein. In case of partial restoration of homologous recombination because of increased hypomorphic BRCA1 or BRCA2 activity, pathway rewiring or secondary mutations, resistant tumors may still be responsive to other targeted therapy. Proof of principle comes from experiments with cells that developed PARP inhibitor resistance because of loss of 53BP1 but were still sensitive to platinum drugs or combined inhibition of PARP and ATM (43, 44, 46). In line with these preclinical data, a recently published retrospective study shows that olaparib-refractory BRCA mutant ovarian cancer may still respond to platinum drugs (61). Ideally, PARP inhibitor resistance is prevented by using drug combinations that completely eradicate tumors (1). To this end, established chemotherapeutic agents may be used, although these may also induce more severe side effects in the presence of PARP inhibitors (62, 63). Alternatively, combination with other targeted therapeutics such as inhibitors for PI3 kinase (30, 64), cell-cycle checkpoints (65) or other nicotinamide adenine dinucleotide metabolizing enzymes (66) may sensitize tumors to PARP inhibition.

Information from basic and preclinical research will be important to prevent or antagonize resistance to PARP inhibitors. Together with the results from clinical trials, this

should allow the design of optimal treatment strategies for patients with homologous recombination-deficient cancers. In addition, insights from preclinical studies may yield improved therapies based on combinations of PARP inhibitors and compounds that target other tumor-specific defects.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: P. Bouwman, J. Jonkers

Writing, review, and/or revision of the manuscript: P. Bouwman, J. Jonkers

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