Role of Biomarkers in the Development of PARP Inhibitors

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Supplementary Issue: Biomarkers and their Essential Role in the Development of Personalised Therapies

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ABSTRACT: Defects in DNA repair lead to genomic instability and play a critical role in cancer development. Understanding the process by which DNA damage repair is altered or bypassed in cancer may identify novel therapeutic targets and lead to improved patient outcomes. Poly(adenosine diphosphate-ribose) polymerase 1 (PARP1) has an important role in DNA repair, and novel therapeutics targeting PARP1 have been developed to treat cancers with defective DNA repair pathways. Despite treatment successes with PARP inhibitors (PARPi), intrinsic and acquired resistances have been observed. Preclinical studies and clinical trials in cancer suggest that combination therapy using PARPi and platinating agents is more effective than monotherapy in circumventing drug resistance mechanisms. Additionally, identification of biomarkers in response to PARPi will lead to improved patient selection for targeted cancer treatment. Recent technological advances have provided the necessary tools to examine many potential avenues to develop such biomarkers. This review examines the mechanistic rationale of PARP inhibition and potential biomarkers in their development for personalized therapy.

KEYWORDS: PARP1, biomarkers, personalized therapy, PARP inhibitors

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Introduction

Genomic instability plays a pivotal role in both cancer initiation and progression. DNA repair is a cellular mechanism that critically maintains a balance between genomic integrity and cell survival.[1] Low-fidelity repair processes or escape of DNA damage surveillance and repair may result in survival of cells with genomic abnormalities. In the former case, cells employ the DNA damage tolerance (DDT) pathway, which recruits a specialized low-fidelity translesion synthesis polymerase to bypass lesions for repair at a later time point. Thus, DDT is not a repair pathway per se, but it provides a mechanism to tolerate DNA lesions during replication. Paradoxically, DDT is also associated with increased mutagenesis. As such, DDT function represents a double-edged sword. In addition to tumor promotion, ineffective or deficient repair processes in cancer cells may lead to generation of tumor subclones with unique mutations and/or phenotypes including differential growth rates, metastatic potential, and response to therapy. The resulting genomic heterogeneity may provide cancer cells a selective advantage against drug therapies but may also offer an avenue for therapeutic intervention.[2] Understanding the process by which DNA damage repair is altered or bypassed in cancer cells may identify novel therapeutic targets and use of genomically guided therapeutics to overcome resistance and lead to improved patient outcomes.

Links between genomic instability and cancer were validated upon the discovery that breast cancer 1 (*BRCA1*) or breast cancer 2 (*BRCA2*) tumor suppressor genes play a critical role in DNA repair.[3] Further, somatic mutations and altered expression of DNA repair genes have been shown to occur in cancer cells as well. In general, DNA repair can be divided into two pathways, those that repair damage affecting one strand of DNA and those that repair damage affecting both strands. Homologous recombination (HR) and nonhomologous end joining (NHEJ) are repair mechanisms for double-strand breaks (DSBs), while nucleotide excision repair, base excision repair (BER), and mismatch repair (MMR) are repair mechanisms for DNA single-strand breaks (SSBs).[4] Extensive research has shown that BRCA1/2 proteins help maintain genome integrity as critical factors in HR and transcriptional regulation in response to DNA damage. More

recently, a number of germline alterations in other DNA repair genes, for example, RAD51 recombinase (*RAD51*), partner and localizer of BRCA2 (*PALB2*; Fanconi anemia complementation group N [*FANCN*]), BRCA1-interacting protein 1 (*BRIP1*; Fanconi anemia complementation group J [*FANCJ*]), ataxia telangiectasia-mutated (*ATM*), and ataxia telangiectasia and Rad3-related protein (*ATR*) have also been shown to predispose to hereditary cancers.[5] In the setting of defects in DNA damage, the physiologic milieu uses poly(adenosine diphosphate-ribose) polymerase (PARP) enzymes to repair DNA damage. In the absence of DNA repair through PARP enzyme function, cells have reduced viability. Therefore, the inhibition of PARP is a promising strategy for targeting cancers with defects in DNA damage repair, including *BRCA1*- and *BRCA2*-mutated cancers. Several PARP inhibitors (PARPi) are currently being evaluated in clinical trials for the treatment of subsets of ovary, breast, pancreas, lung, and colon cancers and one Food and Drug Administration (FDA)-approved inhibitor for the treatment of *BRCA1/2*-related ovarian cancer. Hence, this review provides a discussion of the role of PARP in DNA repair, the mechanistic rationale of PARPi alone or in combination with platinating agents, and potential biomarkers in the development of PARPi for personalized therapy.

PARP is a Key Player in DNA Repair

The PARP family of proteins includes over 17 proteins. Each family member contains a highly conserved catalytic domain. PARP has many roles in the cell, including DNA repair, replication fork arrest, transcription, metabolism, chromatin dynamics, apoptosis, and protein degradation. While a number of these proteins have been studied, PARP1 is the most extensively studied, and it is the most abundant and catalytically active member of this family of enzymes. PARP1 catalyzes the synthesis of poly(ADP ribose) (PAR) polymers that become covalently attached to histones, other proteins, or PARP itself in response to a variety of signals but especially DNA damage.

The role of PARP1 in DNA repair mechanisms was initially described in BER.[6,7] The damage of DNA bases and SSBs induced by reactive oxygen species, radiation, alkylating agents, and other DNA-damaging agents activates the BER process for DNA repair (Fig. 1). While PARP1 null or PARP1-deficient cells were shown to have defective BER, other studies suggest that PARP1 is not necessary for efficient BER.[8,9] Although the exact role of PARP1 in BER is still under investigation, the most widely accepted function is that PARP1 produces accumulation of PAR chains which then recruits proteins involved in BER such as X-ray repair cross-complementing protein 1.[10]

Catalytic activation of PARP1 is triggered upon sensing DNA breaks. Activated PARP1 then mediates PARylation of substrate proteins. While a variety of PARP substrates have been identified in vitro, the major in vivo substrates are histones and PARP itself.[11] A number of

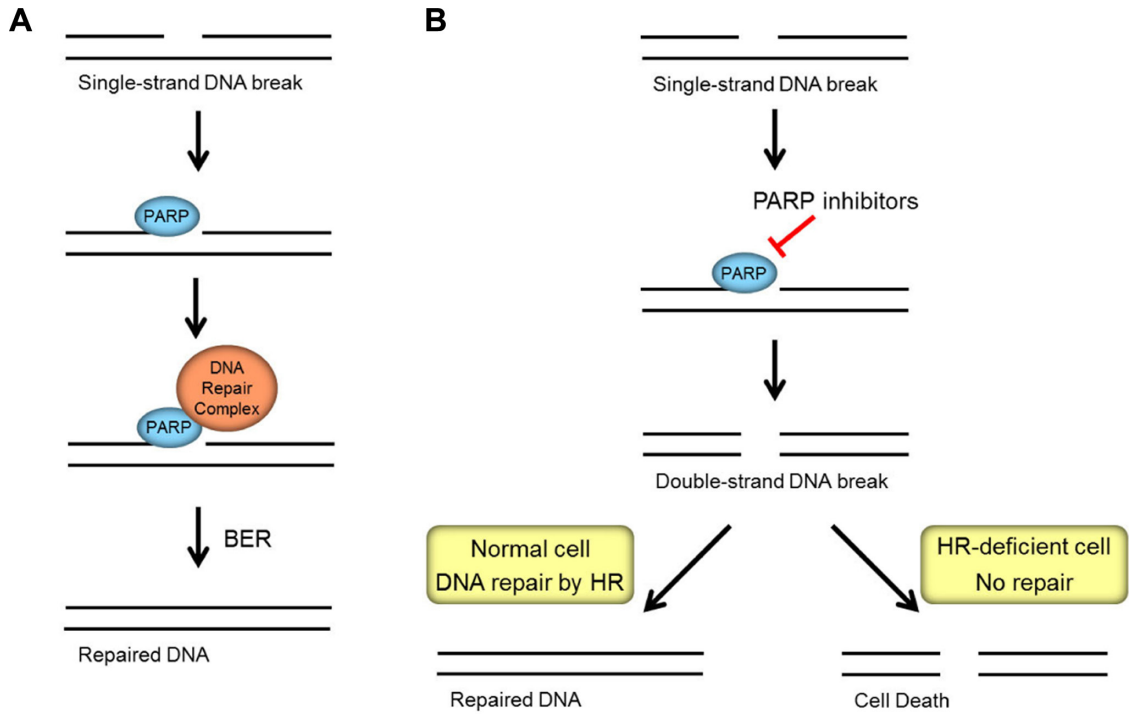

Figure 1. Role of PARP in DNA damage repair. (**A**) In response to single-strand breaks (SSBs), PARP binds to DNA and recruits the DNA repair complex of scaffold proteins and enzymes to the site of DNA damage. The SSB is repaired mainly by BER and to a lesser extent by nucleotide excision repair and mismatch repair (MMR). (**B**) In the presence of PARPi, SSBs remain unrepaired resulting in the formation of double strand breaks (DSBs). In cells without defects in DNA damage repair, the DNA is repaired by homologous recombination (HR). However, in HR-deficient cells, for example, *BRCA1/2*-mutated cells, the DNA damage is unable to be repaired. This leads to the accumulation of DSBs and ultimately cell death.

downstream proteins, for example, amplified in liver cancer 1 (ALC1), aprataxin (APTX), and polynucleotide kinase 3'-phosphatase (PNKP)-like factor, recognize PARylated PARP1 and are recruited to the sites of DNA breaks.[12–15] These proteins act to remodel chromatin and/or recruit other proteins, which in turn facilitate DNA repair. Concomitant to the initiation of this signaling cascade, PARylation, by virtue of the repulsion caused by its negative charge, is also believed to relax the local chromatin structure to facilitate repair.[16] At these sites, PARP1 can add PAR to histones to relax the chromatin structure, facilitating access to the damaged DNA by the repair proteins and PARylate the DNA repair proteins themselves.[12,17,18] PARP1 activity also leads to the recruitment of tripartite motif-containing 33 (TRIM33) to the sites of DNA damage. The E3 ligase activity of TRIM33 regulates the PARP-dependent systematic sequence of repair proteins recruited to damaged chromatin,[19] including the two important helicases, known as ALC1[15] and chromodomain-helicase-DNA-binding protein 4 (CHD4).[20] While these changes in the local chromatin landscape have been initiated and downstream signaling proteins recruited, PARP1 is subsequently dislodged from the DNA due to its accumulating negative charge. PARP1 thus loses its catalytic activity and stalls any further auto-PARylation. Simultaneously, PAR glycohydrolase acts to degrade the PARylation-restoring PARP1 to its unmodified form.

More recent work has implicated PARP1 in the repair of DNA DSBs by HR and non-homologous end joining (NHEJ).[21,22] During HR-mediated repair, PARP1 binds to DSBs and initiates PARylation at these sites, which recruits protein complexes involved in DNA DSB repair by HR. PARP1 has been shown to physically interact with proteins important for NHEJ. However, the exact mechanism of PARP1 in NHEJ is still unclear. PARP1 has also been associated with a role in binding and restoring stalled replication forks.[23]

Therapeutic Relevance of PARP Inhibition

Early preclinical studies with PARPi demonstrated that they induce synthetic lethality in cancer cells with the loss-of-function mutations in *BRCA1* and *BRCA2*.[24,25] As a result of PARP inhibition, persistence of SSBs leads to stalled replication forks and DSBs. Cells with the loss-of-function mutations in *BRCA1* or *BRCA2* are deficient in HR, and the resultant accumulation of unrepaired DSBs leads to cell death (Fig. 1). However, recent data suggest that trapping of PARP on DNA may be more important for cytotoxicity than catalytic inhibition of PARP activity. Thus, the critical DNA lesion is not repaired due to the trapped PARP1–DNA–inhibitor intermediate complex, which leads to obstruction in replication fork progression. Although BRCA1/2-dependent HR repairs this lesion, BRCA1/2-deficient cells are unable to repair this damage. Another model suggests that PARP is directly involved in catalyzing the replication repair.[26] Despite the unresolved mechanism of action of PARPi, the synthetic lethal effect of PARPi in tumors with a defective HR pathway has been successfully exploited in the clinical setting for the treatment of breast, ovarian, and other cancers.

Several PARPi have been developed for investigation in preclinical and clinical studies. Iniparib was one of the first PARPi to enter late-phase clinical trials. However, clinical trial results were very disappointing and at first suggested that the inhibition of PARP may not be an effective cancer therapy. However, more recent data show that iniparib does not inhibit PARP activity, suggesting that it is not a true PARPi. This led to the reemergence of other PARPi as targeted anticancer therapy.[27] Early phase I clinical trials were conducted to determine the safety and tolerability of olaparib (an oral PARPi) following chemotherapy in patients with germline mutations in *BRCA1/2*-mutated cancers. These studies established the maximum tolerated dose with mild toxicity when compared with the standard of care and showed promising response rates.[26,28] This prompted phase II trials to evaluate the efficacy of olaparib in patients with *BRCA1/2*-mutated breast and ovarian cancers.[29,30] These initial phase II trials demonstrated the effectiveness of PARPi monotherapy for patients with this genetic background. Moreover, another phase II trial compared olaparib with placebo control in platinum-sensitive, relapsed, serous ovarian cancer, and the results show that patients treated with olaparib had an increase in progression-free survival, especially in the subset of patients with *BRCA1/2* mutations.[31,32]

A number of other PARPi, including niraparib, rucaparib, veliparib, and talazoparib, are being investigated as single-agent treatment in phase II and phase III clinical trials to determine the efficacy in *BRCA1/2*-mutated breast and ovarian cancers, triple-negative breast cancers, and a wide range of other cancer types (Table 1).

In preclinical studies, the mechanism of PARP inhibition by niraparib was shown to be dependent on the conversion of sublethal SSBs into lethal DSBs as a consequence of BER inhibition. In translational studies of tumor cells in primary culture, germline *BRCA1/2* mutations or other deficiencies in HR were associated with sensitivity to niraparib.[33] Another small molecule PARPi, rucaparib, is currently in phase III studies for patients with high-grade serous ovarian cancer, which is not related to germline *BRCA1/2* mutations. Preliminary results show that the overall response rate is significantly higher in *BRCA1/2*-mutated ovarian cancer when compared with *BRCA1/2* wild-type cancers.[34] Rucaparib is in phase II study for *BRCA1/2*-related, metastatic pancreatic cancer that has progressed after one or two lines of therapy. It is also in phase I trials for patients with advanced solid tumors (Table 1). Additionally, another potent anticancer drug namely veliparib (ABT-888) causes PARP inhibition by preventing auto-PARylation, which is known to promote the dissociation of PARP from DNA. It is under investigation in phase III trials in combination with chemotherapy for patients with

Table 1. Representative clinical trials of PARP inhibitors alone or in combination for the treatment of cancer.

TUMOR TYPE	PARP INHIBITOR	COMBINATION*	EXAMPLES OF BIOMARKERS ASSESSED	PERTINENT PRELIMINARY RESULTS
Ovarian	Olaparib	None[a,b,c]		Greater PFS benefit in *BRCA1/2* mt vs. *BRCA1/2* wt[78]
		Cediranib maleate[b,c]	*BRCA1/2* mutation status (germline or somatic); BRCA1/2-HR assay	
	Veliparib	None[a,b,c]		26% response rate. Median PFS: 8.18 months[79]
		Carboplatin, paclitaxel and bevacizumab[f]	*BRCA1/2* mutation status (germline or somatic); changes in PARP inhibition in PBMCs	
		Pegylated liposomal doxorubicin hydrochloride, carboplatin, and bevacizumab[c]	Germline mutations, alterations and/or rearrangements in *BRCA1* or *BRCA2*	
	Niraparib	None[c]	*BRCA1/2* mutation status (germline); HRD status	
		Bevacizumab[c]	*BRCA1/2* mutation status	
	Rucaparib	None[c]	HRD status (based on amount of genomic 'scarring' measured by extent of tumor genomic LOH)	ORR: 69% *BRCA1/2* mt, 39% *BRCA1/2* wt/LOH high, and 11% *BRCA1/2* wt/LOH low patients[34]
Breast	Olaparib	None	*BRCA1/2* mutation status	
	Veliparib	Carboplatin[a,g]	PARP1 activity	
		Cyclophosphamide	PARP1 expression	
	Talazoparib	None[a,b]	*BRCA1/2* mutation status	
Breast/Ovarian	Olaparib	Carboplatin		RR and median PFS of 36% and 3.5 months in platinum-sensitive, 6% and 4 months in platinum-resistant disease[80]
		PI3K inhibitor		Evidence of clinical benefit at all dose levels[81]
Prostate	Olaparib	None[d]	*BRCA1/2* mutation status (germline or somatic), *ATM*, *FANCA*, *CHEK2*, *PALB2*, *HDAC2*, *RAD51*, *MLH3*, *ERCC3*, *MRE11*, *NBN* mutation status; IHC levels of PAR, γ-H2AX, pH2A(s129), Rad51 foci, FancD2 foci and ATM/ATR/CHK1/CHK2	Antitumor activity of olaparib is significantly associated with DNA repair defects in the tumor[69]
	Veliparib	Temozolomide		Well tolerated with some anti-tumor activity[82]
		Abiraterone acetate and prednisone	PAR expression	97% concordance of ETS status between primary and metastatic site;[83] Molecular profiling results[65]
CNS	Olaparib	Temozolomide	HRD status (by Rad51 foci); MSI status; MGMT methylation status; MMR, PTEN, γ-H2AX expression; PARP inhibition	Intratumoral levels of olaparib in recurrent GBM are therapeutic[84]
	Veliparib	Temozolomide[e,f]	Genetic or epigenetic alterations in *PARP1*, *MGMT*, and DNA repair or replication genes; NHEJ activity; MGMT methylation status	Combination well tolerated[85]
		Radiation and temozolomide[f]	PARP or NHEJ activity in PBMCs; γ-H2AX levels in PBMCs	
Hematologic	Veliparib	Temozolomide	RAD51, γ-H2AX foci, PAR levels, DSB repair, NHEJ repair	
		Topotecan +/− carboplatin	PAR levels; mutation and/or expression of genes in select DNA repair pathways (Fanconi complementation groups A-F, Blooms, and ataxia-telangiectasia)	

Pancreatic	Veliparib	+/– Gemcitabine and cisplatin	*BRCA1*/*BRCA2*/*PALB2* mutation, genetic reversions of *BRCA1/2* mutations, PAR levels	
Gastro-intestinal	Olaparib	None	MSI status	
	Veliparib	Capecitabine and radiation[f]		Combination well tolerated, promising preliminary antitumor activity[86]
Mixed Tumors	Olaparib	None[a,b,c]		Antitumor activity in germline *BRCA1/2* mutation carriers with advanced ovarian cancer, including heavily pretreated, platinum-resistant cancers.[87] Resistance to platinum decreases sensitivity to olaparib[28]
		AKT inhibitor	pERK, RAD51, BRCA1/2 and PARP expression	
		Cediranib maleate[a]		Combination extended PFS and ORR, 44% ORR in ovarian cancer patients[88]
		mTORC1/2 or AKT inhibitor	Presence or absence of aberrations in PI3K/AKT/mTOR and HR defect pathway	
	Veliparib	None[a,b]	BRCA1/2 expression, γ-H2AX, PAR expression and levels	Antitumor activity with veliparib in BRCA1/2-expressing tumors compared to *BRCA1/2* wt TNBC[89]; Well-tolerated, antitumor activity in both BRCA1/2-expressing and *BRCA1/2* wt tumors[90]
		Metronomic cyclophosphamide		Well tolerated combination; PAR significantly decreased in PBMCs and tumor; γ-H2AX levels increased[91]
		Topotecan[b,c]	ADP-ribose polymer formation, *BRCA1/2* mutation status, levels of topoisomerase I, PARP, BRCA1, BRCA2, XRCC1, TDP1, P-glycoprotein and BCRP	Reduction in PAR levels in the tumor and PBMCs; increase in γ-H2AX levels in PBMCs[92]
		+/– Carboplatin and paclitaxel[a], or FOLFIRI	DNA repair defects; γ-H2AX and PAR levels; BRCA levels by IHC	Well tolerated combination, promising antitumor activity[93]
		+/– Mitomycin C[h]	*BRCA1/BRCA2* mutations, FancD2 foci formation, γ-H2AX foci	
		Gemcitabine[a]	ATM levels in PBMCs	
		Radiation	ERCC1, XRCC1, BRCA1, BRCA2, and PAR by IHC	Well tolerated combination, disease stability[94]
	Talazoparib	None[a]	*BRCA1/2* mutation status (germline or somatic); *BRCA1/2* somatic deletions; *BRCA1/2* pathway gene alterations (*ATM*, *PALB2*, *NBS1*, Fanconi Anemia genes); *PTEN* deletion/mutation; HRD status (by LOH, TAI, and LST-Myriad test)	Antitumor activity in advanced previously treated SCLC and significant activity in patients with germline *BRCA1/2* mutant ovarian and breast cancer[95]
	Rucaparib	None[a]		Well tolerated with promising clinical benefit in ovarian, breast, and pancreatic cancer[96]
	CEP-9722	+/– Temozolomide		Well tolerated combination[97]
Ewing Sarcoma	Olaparib	None		Safe and well tolerated[98]
Endometrial	Talazoparib	None	MSI; *PTEN* and *MRE11* mutation status	

Notes: *Treatment for advanced, metastatic tumors unless otherwise noted. For at least one trial with this treatment regimen: [a]*BRCA1/2* mutations are an eligibility requirement, [b]platinum resistant, [c]platinum sensitive, [d]neoadjuvant treatment, [e]adjuvant treatment, [f]treatment naïve, [g]HER2 negative, [h]Fanconi anemia pathway deficient.

Abbreviations: DSB, double-strand break; GBM, glioblastoma multiforme; HR, homologous recombination; HRD, homologous recombination deficiency; IHC, immunohistochemistry; LOH, loss of heterozygosity; LST, large-scale state transitions; MSI, microsatellite instability; MT, mutant; NHEJ, nonhomologous end joining; ORR, overall response rate; OS, overall survival; PBMC, peripheral blood mononuclear cell; PFS, progression-free survival; RR, response rate; SCLC, small cell lung cancer; TAI, telomeric allelic imbalance; TNBC, triple negative breast cancer; WT, wild type; +/-, with or without.

germline *BRCA1/2* mutations with metastatic or advanced human epidermal receptor 2-negative breast cancer. Phase I and/or II study is being conducted to evaluate veliparib in combination with chemotherapy for patients with metastatic pancreatic cancer and in combination with chemotherapy and radiotherapy for patients with non-small cell lung cancer (Table 1). Talazoparib is another potent PARPi under the study for *BRCA1/2*-mutated solid tumors. It is active through both inhibiting PARP catalytic activity and tightly trapping PARP to DNA at sites of SSB.[35] Talazoparib is currently in randomized phase III clinical trials for *BRCA1/2*-related, metastatic breast cancer and in phase II trials for tumors with somatic alterations in *BRCA1/2*, phosphatase and tensin homolog (*PTEN*) mutation or loss, defects in HR, mutations/deletions in other DNA repair pathway genes, and germline *BRCA1/2*-related non-breast, non-ovarian cancers (Table 1).

PARPi continue to be developed as targeted therapy for tumors harboring loss of function *BRCA1/2* mutations. Emerging evidence supports the use of PARPi for tumors with defects in HR or other DNA repair pathways independent of *BRCA1/2* mutations. Some preclinical and clinical studies suggest that *PTEN* mutated cells or *PTEN* loss may induce sensitivity to PARPi.[36,37] However, the exact mechanism of *PTEN* loss and its relationship with DNA repair deficiency has not yet been elucidated. Furthermore, INPP4B loss results in increased sensitivity to olaparib in preclinical studies possibly due to the loss of protein stability of critical components of DNA damage response including BRCA1, ATM, ATR,[38] and PALB2.[39] These findings extend the clinical utility of PARPi to patients with cancers harboring HR repair deficiency beyond the loss-of-function mutations in *BRCA1/2*.

PARPi have proven to be effective in other *BRCA1/2*-related cancers such as prostate and pancreatic cancers.[40,41] Preliminary data from an open-label, single-arm study using olaparib as monotherapy in germline *BRCA1/2*-associated cancers suggest that olaparib has broad activity regardless of tumor type.[32] Additional investigation of the potential spectrum of olaparib activity is ongoing for a number of ovarian, breast, prostate, lung, central nervous system, pancreatic, gastrointestinal, and a variety of other tumor types (Table 1). Recently, a case report of an exceptional response to velaparib in combination with cytotoxic chemotherapy in a patient with metastatic castration-resistant prostate cancer underscores the potential clinical relevance of PARPi across a range of tumor types.[40]

Resistance to PARPi is Mediated Through Multiple Mechanisms

Preclinical studies and clinical trials using PARPi as monotherapy or in combination therapy for some cancers have shown encouraging results.[42] The use of PARPi therapy for cancer treatment is predicted to increase in the future based on these results and recent approval of olaparib for the treatment of advanced ovarian cancer with *BRCA1/2* mutations. Despite these clinical successes, the development of resistance to PARP inhibition has been documented. Understanding mechanisms of resistance would open avenues for the development of subsequent therapies in PARPi-resistant tumors. There are at least four potential mechanisms of resistance to PARPi. First, tumor cells display increased capacity for HR that can be achieved by reversion mutations in *BRCA1/2* which restore BRCA1/2 function or by compensating mutations in 53 binding protein 1 (*53BP1*) that can partially restore HR function in BRCA1-deficient cells, or increased activity of *RAD51* (Fig. 2). A second mechanism of resistance involves an altered NHEJ capacity. Upregulation of the error-prone NHEJ pathway, normally suppressed by PARP1, is one of the causes of synthetic lethality of PARPi in HR-deficient cells. A decrease in NHEJ capacity in these cells would increase their resistance to PARPi.[43] Alternatively, decreased levels or activity of PARP1 may lead to resistance. Even though PARP1 is present in abundance and binds to DNA strand breaks, it will not have the active form of PAR to facilitate DNA repair breaks. Finally, decreased intracellular bioavailability of PARPi may occur due to the upregulation of permeability glycoprotein (PgP) pumps, which leads to an increased PARPi efflux out of the cell and decreased intracellular drug concentrations.[42,44]

In an attempt to screen for novel drugs that selectively kill BRCA2-deficient cells, 6-thioguanine (6TG) was identified.[45] This drug has similar efficacy as PARPi in killing BRCA2-deficient tumors. When 6TG induces DNA DSBs, cells that are deficient in HR cannot repair the DNA damage, which leads to cell death. The importance of this drug is that cells that have acquired resistance to PARPi or cisplatin through genetic reversion of the *BRCA2* remain sensitive to 6TG. Although HR is reactivated in cells with a *BRCA2* reversion mutation, it is not fully restored for the repair of 6TG-induced lesions.

Platinum Agents as Alternatives to PARP Inhibition

Since the discovery of cisplatin >50 years ago, other platinum drugs have been developed. Besides cisplatin, only oxaliplatin and carboplatin have been approved by the FDA for clinical use in the USA[46,47] and are believed to function in a manner similar to that of cisplatin. The mechanism of action of platinum salts has identified their potential benefit in tumors with defects in DNA repair. Activated platinum complexes can react with nucleophilic centers on purine bases of DNA. The two labile coordination sites on the platinum center permit cross-linking of adjacent guanine bases. The platinum center can coordinate with guanine bases from different DNA strands to form interstrand cross-links. The major intrastrand dGpG cross-link induces a significant distortion in the DNA double helix.

Despite the clinical benefit of cisplatin, significant side effects including nephrotoxicity, fatigue, emesis, ototoxicity, peripheral neuropathy, and myelosuppression, can limit its use.[48,49] Carboplatin has been shown to have reduced toxicity when compared with cisplatin. Also, two new platinum

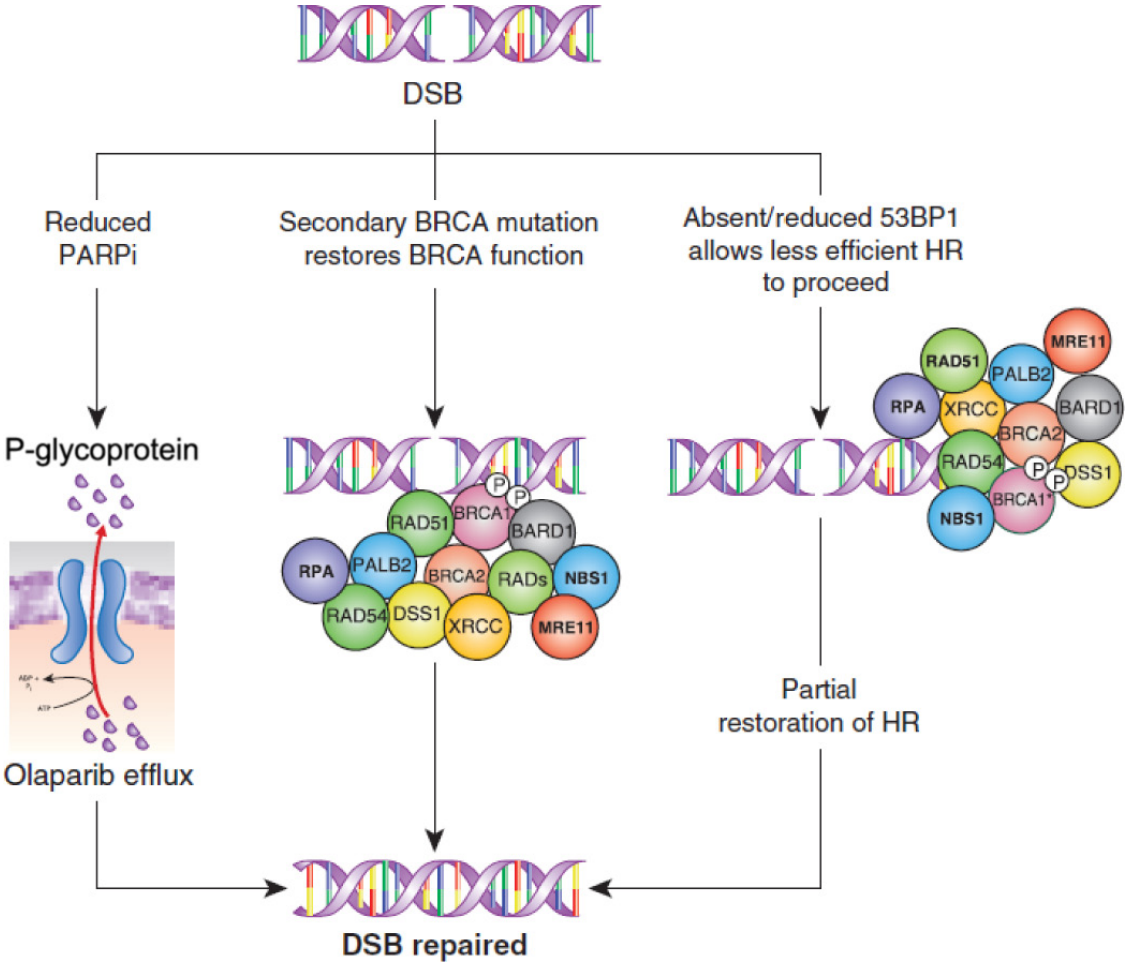

Figure 2. Currently known mechanisms of resistance to PARPi include secondary *BRCA1/2* mutations that restore BRCA1/2 function, increased drug efflux mediated by P-glycoprotein and reduced/absent 53BP1 expression resulting in partial restoration of HR (Reprinted by permission from the American Association for Cancer Research: Fojo T, Bates S. Mechanisms of resistance to PARP inhibitors—three and counting. *Cancer Discov.* 2013;3:20–23. doi: 10.1158/2159-8290).

drugs, that is, LA-12 and dicycloplatin (improved prodrug formulations of platinum agents), have entered clinical trials. These new drugs have been designed using encapsulation with macrocycles. This unique structure provides high affinity and selectivity for protein targets and prevents degradation by other proteins, allowing them to be more potent platinum analogs.[46,47] Clinical use of platins has been successful in the treatment of patients with different types of cancers including small cell lung cancer, ovarian cancer, cancers of soft tissue, bones, muscles, blood lymphomas, bladder cancer, and cervical cancer. This success had led to the development of different metal-based antineoplastic compounds until severe side effects were encountered.[50] Carboplatin is also used to treat a varied spectrum of cancers and in preparation for a stem cell or bone marrow transplant. Oxaliplatin has its activity in a broad spectrum of tumors that are resistant to cisplatin and carboplatin[51,52] and has more limited use in the treatment of gastrointestinal malignancies. It is notable that MMR deficiency, which is common in colon cancer, may confer resistance to cisplatin and carboplatin and not to oxaliplatin.[53] Oxaliplatin is only effective when combined with 5-fluorouracil (5FU). The mechanism is believed to downregulate or inhibit dihydropyrimidine dehydrogenase, slowing the catabolism of 5FU, not the creation of DNA adducts as in the case of carboplatin or cisplatin.

As might be predicted, *BRCA1/2*-mutated ovarian carcinomas are initially sensitive to platinum compounds, a standard of care drug for all types of ovarian cancer.[54,55] However, *BRCA1/2*-mutated ovarian cancer eventually develops platinum resistance. In the cisplatin-resistant *BRCA2*-mutated breast cancer (HCC1428) and pancreatic cancer (SW1990/GZ) cell lines, the development of a *BRCA2* reversion mutation rescues BRCA2 function.[55] Reversion mutations have been observed in vivo and are believed to arise under selective pressure. Therefore, a second mutation in *BRCA2* can restore the wild-type reading frame and can be a major clinical mediator of acquired resistance to platinum-based chemotherapy.[47] Tumors with reversion mutations would also be resistant to PARPi due to partial blockage of the HR pathway.

In the absence of germline *BRCA1/2* mutations, ovarian and other cancers may exhibit a phenotype designated

as "*BRCA*ness".[56,57] "*BRCA*ness" is defined by an HR DNA repair defect in the absence of germline *BRCA1/2* mutation that occurs in up to 50% of ovarian cancers. This phenotype has become the basis for the design of clinical trials that include *BRCA1/2* wild-type tumors and includes all cases with somatic mutations in *BRCA1/2* as well as other genes such as *PALB2*, *ATM*, BRCA1-associated really interesting new gene (RING) domain 1 (*BARD1*), *BRIP1*, checkpoint kinase 1 (*CHEK1*), checkpoint kinase 2 (*CHEK2*), family with sequence similarity 175, member A (*FAM175A*), meiotic recombination 11 homolog A (*MRE11A*), Nijmegen breakage syndrome (*NBS*), *RAD51C*, and *RAD51D*. Both germline and somatic mutations in HR genes were recently shown to predict not only platinum response but also survival rates in ovarian, fallopian tube, and peritoneal carcinomas.[58] Although platinum-resistance confers PARPi resistance, PARPi-resistant tumors may still retain sensitivity to platinum drugs. It is believed that there is a high prevalence of alterations in the HR pathway, which result in resistance to PARPi, but not affect sensitivity to platin drugs. The results suggest that platinum sensitivity may be used as a surrogate marker for HR deficiency.[59,60]

Combinatorial Therapy with Platinating Agents and PARPi

PARPi, such as cisplatin and carboplatin, have proven to be promising in both preclinical and early clinical studies as therapy for subsets of cancer. However, not all BRCA1/2-deficient tumors respond favorably to either PARPi or platinum as monotherapy.[26] Moreover, despite their toxicity and the development of resistance,[24,25] PARPi and platinating agents are used as combination therapy in multiple clinical trials for a variety of tumor subtypes (Table 1). The major benefit of combination treatment with PARPi and platinum drugs is the achievement of a greater efficiency while limiting toxicity, thereby increasing the therapeutic index.

Recent studies using a PARPi concurrent with platinum drug in BRCA1- or BRCA2-deficient tumor mouse models support the use of this combination.[61–63] The rationale underlying these studies is that PARPi and platinum drugs have shown synergistic interactions and specificities because of the lack of HR repair only in BRCA1/2-deficient tumor cells. The treatment of xenografts using a PARPi/carboplatin combination has been conducted using only BRCA1/2-deficient models and have shown promising results with enhanced tumor growth delay when compared with either of the drugs alone.

The combination of PARPi and platinum drugs has also been tested in clinical trials for prostate and *BRCA1/2* mutated solid tumors.[40,64–66] In clinical trials, the combination therapy of PARPi, gemcitabine, and cisplatin have shown synergistic effect in basal-like breast cancers but not in luminal breast cancers.[27] Clinical studies strongly suggest that the combination of cytotoxins with PARPi would have clinical benefit in this breast cancer subtype.

Biomarkers Used in PARPi Trials

Predictive biomarkers have increased our potential to improve cancer treatments by identifying a priori the subset of patients most likely to derive benefit from a specific targeted drug, including PARPi. It is worthy to note that, currently, the only validated biomarker of cancer sensitivity to PARPi is the presence of germline mutation in either *BRCA1* or *BRCA2*. In general, biomarkers for PARPi can be classified into several subcategories, including markers of DNA repair deficiency, transcription regulation, and cell cycle control. In the case of DNA repair, *BRCA1/2* loss-of-function mutation impairs HR repair and induces PARP hyperactivation, which is reflected in an increased abundance of PAR. A number of clinical trials using *BRCA1/2* mutation as a biomarker in response to PARPi are in progress (Table 1). HR damage may occur without *BRCA1/2* mutation in the context of "*BRCA*ness". The E26 transformation-specific (ETS) fusion genes are overexpressed in prostate cancer and induce DNA damage. This damage is potentiated by PARP1 inhibition in a manner similar to that of BRCA1/2 deficiency. The ETS fusions are currently under investigation as potential biomarkers in metastatic castration-resistant prostate cancer, as preclinical work suggests that prostate cancers harboring the ETS fusion have increased sensitivity to PARPi[65] (Table 1). As the main effectors of the HR pathway, 53BP1, PARP-binding protein, MRE11 from the MRN protein complex (MRE11, RAD50 and NBS1), and RAD51[67,68] are also being evaluated as biomarkers in clinical trials with olaparib, veliparib, and talazoparib (Table 1). In metastatic prostate cancer, the association of olaparib with DNA repair defects has been evaluated in a cohort of 49 patients. In this cohort, functional mutations or genomic alterations in DNA repair genes including *BRCA1/2*, *ATM*, *CHEK2*, and *PALB2* were identified by next-generation sequencing in 16 of 49 tumors[69] While validation in larger cohorts is needed, these mutations are significantly associated with clinical response to olaparib suggesting that DNA repair genes may be valuable biomarkers in response to PARPi.

In transcriptional regulation and cell cycle control, aurora A kinase is an essential player of mitosis and cell cycle regulation and is often overexpressed in tumors. The overexpression of aurora A kinase inhibits RAD51 recruitment, which is a necessary step in the function of the DNA damage response (DDR) pathway. Apart from HR repair deficiencies, these two main players (ie aurora A kinase and RAD51) of the final steps of the BER pathway may be of interest as biomarkers and are being evaluated for this purpose. This specific approach of identifying and validating single-gene biomarkers for PARPi sensitivity is ongoing (Table 1) and high-throughput systems are being developed to systematically and effectively identify these markers.[67,70]

As a preponderance of studies has been carried out in the setting of *BRCA1* or *BRCA2* mutations, germline *BRCA1/2* status is a major biomarker for PARPi treatment in clinical trials. Somatic *BRCA1/2* mutations and *BRCA1* promoter

methylation also account for a sizable fraction of "*BRCA*ness" in cancers that could be targeted with PARPi. Assays are being optimized to identify somatic *BRCA1/2* mutations in cancer.[71,72] These assays, if validated, could be clinically implemented to increase the identification of patients with *BRCA1/2*-associated tumors that could be eligible to receive and are predicted to have greater benefit from PARPi. *BRCA1* promoter methylation has also been proposed as a marker for HR deficiency, as functional methylation of the *BRCA1* promoter leads to repression of *BRCA1* mRNA expression.[70,73] Specific assays to detect *BRCA1* promoter methylation in clinical samples have been developed and are being validated in clinical trials. In addition, a functional assay for RAD51 foci[68] formation by ionizing radiation has been investigated as a tool to identify patients with HR-deficient tumors. This assay has been evaluated using breast tumors and shows promise as an alternative method to select patients, whose tumors may be sensitive to PARPi in the absence of a known loss-of-function *BRCA1/2* mutation.[68]

Recent studies have shown that the risk of breast cancer in females with germline *PALB2* mutations is significantly higher when compared with the general population. Moreover, breast cancer risk for *PALB2* mutation carriers may overlap with that of *BRCA2* mutation carriers.[39,74,75] PALB2-deficient cells (EUFA1341), similar to germline *BRCA2* mutated cells, are sensitive to PARPi. As a result of their deficiency in HR, collapsed replication forks may not be efficiently repaired in PALB2-deficient cells. These preclinical studies establish PALB2 as a critical mediator of HR in human cells, which is similar to that observed in BRCA2-deficient cells. The fundamental understanding of the function of PALB2 may be beneficial for its use as a biomarker in cancer treatments using PARPi.[75]

Additional studies have also been used to explore the role of biomarkers in PARPi trials. One such method is MyChoice™ HR deficiency (HRD) companion diagnostic test (myriad®). This test is the first and only companion diagnostic to measure three modes of HRD, including loss of heterozygosity, telomeric allelic imbalance, and large-scale state transitions in cancer cells. The HRD score is used to indicate the inability of cancer cells to repair DNA damage and as such may reflect tumor sensitivity to DNA-damaging agents such as PARPi and platinating agents. Validation in the setting of clinical trials is ongoing, but these biomarkers may help select patients most likely to derive a therapeutic response to DNA-damaging agents based on the biology of their tumor.[76]

Current Challenges and Future Directions

There are a number of challenges that researchers are currently facing in further clinical development of PARPi. First, PARPi will likely be more effective in a subset of cancers with underlying defects in HR-mediated DNA repair opposed to an unselected patient population. However, the selection of the relevant tumor subtypes based on preclinical data, rational design, and biomarkers will increase the likelihood of identifying the population likely to achieve the greatest benefit. Second, more preclinical and clinical studies are needed to determine the most effective approach for the incorporation of PARPi, either as monotherapy or in combination therapy, tumor subtype, and timing for maximal effectiveness, for example, low tumor burden or post-platinum as maintenance therapy. Additionally, there is a major need to determine an effective schedule of administration of drugs, that is, continuous versus intermittent dosing with chemotherapy, to effect the best patient outcomes. Moreover, it is not clear at present whether PARPi will be better than optimally dosed platinum agents. Finally, the development of new primary malignancies is a key concern with drugs that inhibit DNA damage repair. This is known to occur with the treatment of other cancers with DNA-damaging agents, for example, anthracyclines and platinum. Some cases of myelodysplastic syndrome and acute myeloid leukemia have been reported in PARPi studies with a frequency of <1%, warranting a high level of attention when developing PARPi therapy.[77]

Alternative strategies targeting DNA repair either directly with PARPi or indirectly through DNA-binding (eg, trabectedin), damage potentiation (eg, iniparib), or proliferation signaling (eg, receptor tyrosine kinase inhibitors [TKIs] and mammalian target of rapamycin [mTOR] inhibitors) are currently being evaluated. Finally, approaches to treating PARPi-resistant tumors will become more relevant as their use as standard of care therapies or in clinical trials becomes more universal. Examples such as 6TG, which is already in clinical trial for PARP-resistant tumors, demonstrate that alternative approaches may be within reach.

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Author Contributions

Wrote the first draft of the manuscript: BG and SCD. Contributed to the writing of the manuscript: BG, SCD, SG, and KMH. Agree with manuscript results and conclusions: BG, SCD, LR-R, SG, and KMH. Jointly developed the structure and arguments for the paper: BG, SCD, and KMH. Made critical revisions and approved final version: BG, SCD, LR-R, SG, and KMH. All authors reviewed and approved of the final manuscript.

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