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REVIEW ARTICLE

Reconsidering the mechanisms of action of PARP inhibitors based on clinical outcomes

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Abstract

PARP inhibitors (PARPis) were initially developed as DNA repair inhibitors that inhibit the catalytic activity of PARP1 and PARP2 and are expected to induce synthetic lethality in *BRCA*- or homologous recombination (HR)-deficient tumors. However, the clinical indications for PARPis are not necessarily limited to *BRCA* mutations or HR deficiency; *BRCA* wild-type and HR-proficient cancers can also derive some benefit from PARPis. These facts are interpretable by an additional primary antitumor mechanism of PARPis named PARP trapping, resulting from the stabilization of PARP-DNA complexes. Favorable response to platinum derivatives (cisplatin and carboplatin) in preceding treatment is used as a clinical biomarker for some PARPis, implying that sensitivity factors for platinum derivatives and PARPis are mainly common. Such common sensitivity factors include not only HR defects (HRD) but also additional factors. One of them is *Schlafen 11* (*SLFN11*), a putative DNA/RNA helicase, that sensitizes cancer cells to a broad type of DNA-damaging agents, including platinum and topoisomerase inhibitors. Mechanistically, SLFN11 induces a lethal replication block in response to replication stress (ie, DNA damage). As SLFN11 acts upon replication stress, trapping PARPis can activate SLFN11. Preclinical models show the importance of SLFN11 in PARPi sensitivity. However, the relevance of SLFN11 in PARPi response is less evident in clinical data compared with the significance of SLFN11 for platinum sensitivity. In this review, we consider the reasons for variable indications of PARPis resulting from clinical outcomes and review the mechanisms of action for PARPis as anticancer agents.

KEYWORDS
chemotherapy, DNA damage, PARP inhibitor, replication stress, SLFN11

Abbreviations: ALC1, amplified in liver cancer 1; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; BAP1, BRCA1-associated protein 1; BRAD1, BRCA1-associated RING domain protein 1; BRCA, breast cancer susceptibility gene; BRIP1, BRCA1-interacting protein 1; CDT1, chromatin-licensing and DNA replication factor 1; CHEK1, checkpoint kinase 1; CPT, camptothecin; CtIP, C-terminal–binding protein-interacting protein; DPC, DNA-protein crosslink; DSB, DNA double-strand break; EXO1, exonuclease 1; FAM111A, family with sequence similarity 111 member A; FANCA, Fanconi anemia complementation group A; GDSC, Genomics of Drug Sensitivity in Cancer; gLOH, genome-wide loss of heterozygosity; HR, homologous recombination; HRD, homologous recombination deficiency/defect; MRE11, meiotic recombination 11; NBN, nibrin; NCI, National Cancer Institute; PALB2, partner and localizer of BRCA2; PARP, poly ADP-ribose polymerase; PARylation, poly ADP-ribosylation; PCNA, proliferating cell nuclear antigen; PIAS4, protein inhibitor of activated STAT 4; RNF4, ring finger protein 4; RPA, replication protein A; SLFN11, schlafen11; SPRTN, Spartan; SUMO, small ubiquitin-related modifier; TDP, tyrosyl-DNA phosphodiesterase; TOP, topoisomerase; VEGF, vascular endothelial growth factor; XRCC1, X-ray repair cross-complementing protein 1.

1 | INTRODUCTION

Among the PARP family members (*PARP1-PARP17*), PARP1 and PARP2 act as DNA repair enzymes for DNA single-strand breaks. Hence, catalytic PARP inhibition by PARP inhibitors (PARPis) prevents the repair of DNA single-strand breaks, and PARPis act as DNA repair inhibitors. Since the discovery of synthetic lethality of PARPis in *BRCA* mutant cells that impair the repair of DNA double-strand breaks (DSBs),[1,2] clinical PARPis with comparably high catalytic inhibition potency (olaparib, niraparib, talazoparib, rucaparib, and veliparib) have been developed. According to the original concept, PARPis should be selectively toxic to *BRCA* mutation or HRD cancer cells. However, clinical trials revealed the significant benefit of PARPis in *BRCA* wild-type or HR-proficient cancers, while *BRCA* mutation or HR-deficient cancers received superior benefit.[3-6] Hence, current indications of PARPis have been expanded and are not restricted to cancers with *BRCA* mutations (Table 1). Former favorable response to platinum derivatives (cisplatin and carboplatin) is used as a clinical biomarker for PARPi sensitivity regardless of *BRCA* status. These facts are not interpretable by the initial synthetic lethal model of PARPis.

A decade ago, we reported an additional primary mechanism of action of PARPis, named PARP trapping.[7,8] In the presence of PARPis, PARP1 and PARP2 (hereafter, which we described as PARP if not explicitly mentioned) bind the 5′-deoxyribose phosphate group–containing DNA ends noncovalently,[9] generating highly toxic PARP-DNA complexes (Figure 1). As PARPis turn the PARP protein toxic, they act as "PARP poisons," which explains that the antitumor effects of PARPis completely disappear in PARP-deficient cells.[8] PARP-DNA complexes strongly block DNA replication, leading to DSBs with bulky PARP proteins at one strand of 5′-DNA ends. Cancer cells employ multiple repair factors beyond BRCA to manage PARP trapping. Hence, PARP-trapping lesions can also damage HR-proficient cells (see our review for detailed information[10]). The potency of PARP trapping is widely different among PARPis, talazoparib being the strongest and veliparib the weakest (Table 1). Structural studies revealed an allosteric folding change of a helical domain of PARP1, leading to different retention potency of PARP1 on single-strand breaks.[11] Overall, these differences are reflected in the drug dosing; for example, the daily dose of talazoparib is 1 mg, while the daily dose of other PARPis is hundreds mg, indicating that PARP-trapping potency is the limiting factor to decide the clinical dose. The variety of indications and usages of PARPis based on the results of clinical trials deepen the understanding of PARPis and let us reconsider the most relevant mechanisms of action of PARPis in the human body. In this review, we first summarize recent topics about PARP trapping and then consider the reasons for variable indications of PARPis resulting from clinical outcomes. Next, we introduce *Schlafen 11* (*SLFN11*) as a cause of cross-sensitivity with platinum derivatives and propose the "hyper synthetic lethal strategy" using SLFN11 protein expression and *BRCA* mutation as biomarkers for PARPis.

2 | HOW DO CANCER CELLS PROCESS PARP-TRAPPING LESIONS?

Replication is often challenged by proteins covalently bound to DNA, also known as DNA-protein crosslinks (DPCs). DPCs originate when proteins become crosslinked to DNA after exposure to UV light or aldehydes or due to faulty enzymatic reactions.[12] A representative example of enzymatic DPC is a topoisomerase 1 (TOP1)-DNA cleavage complex (TOP1cc) generated through the TOP1-mediated covalent bond between 3'-DNA ends and the catalytic tyrosyl residue of TOP1.[13] Failure in the self-resealing of TOP1ccs results in stabilized TOP1-DPCs, which are trapped by TOP1 poisons, such as camptothecin (CPT), and its clinical derivatives irinotecan and topotecan.[14] Because TOP1-DPCs are products of a physiological reaction, eukaryote cells possess multiple pathways to dissolve the TOP1-DPCs by excising and ligating the associated breaks. Tyrosyl-DNA phosphodiesterase 1 (TDP1) cleaves the tyrosyl-DNA bonds, whereas a structure-specific endonuclease MRE11 removes the TOP1-DPC along with the adjacent DNA segment.[14] A metalloprotease Spartan (SPRTN) debulks TOP1-DPCs to make the peptide-DNA bonds accessible to the repair factors.[14] Similar repair pathways exist for TOP2-DPC with TDP2 and MRE11 for their excision.[15]

Getting back to the subject of PARP trapping, the PARP-DNA complex, a noncovalent bond at the 5'-DNA ends, is an unnatural product that is uniquely formed in the presence of PARPis. One possible exception can be the case happening in *XRCC1*-deficient condition, where PARP1 occupies DNA ends and blocks base excision repair.[16] However, *XRCC1*-deficient cell is not found at the transcription level and in mutation status in the NCI-60 and Genomics of Drug Sensitivity in Cancer (GDSC) database (https://discover.nci.nih.gov/cellminercdb/).[17] Then, it is questionable how human cells process the unnatural PARP-trapping lesions. The 5'-DNA ends should be clean to access exonucleases (MRE11, CtIP, EXO1, and DNA2) to initiate HR. Recently, several factors have been reported to process trapped PARP1 or PARP2 (Figure 1).

The metalloprotease SPRTN involved in the debulking of TOP1-DPCs is recruited to trapped PARP1 in S-phase to assist in the excision and replication bypass of PARP1-DNA complexes.[18] Hence, *SPRTN*-deficient cells are hypersensitive to talazoparib and olaparib but not to veliparib.[18] The serine protease FAM111A, a PCNA-interacting protein, also plays a vital role in mitigating the effects of protein obstacles on replication forks. FAM111A protects replication forks from stalling at PARP1-trapping lesions, thereby promoting cell survival after PARPi treatment.[19] Amplified in liver cancer 1 (ALC1), a chromatin-remodeling enzyme, can remove inactive PARP1 indirectly through binding to PARylated chromatin.[20] Consequently, *ALC1* deficiency enhances PARP1 trapping, conferring PARPi sensitivity, while *ALC1* overexpression reduces the sensitivity of *BRCA*-deficient cells to PARPis.[20] Moreover, ALC1 appears strictly required for PARP2 release, and catalytic inactivation of ALC1 quantitatively traps PARP2 but not PARP1, enhancing PARPi-induced cancer cell killing.[21] Mass spectrometry–based

TABLE 1 Summary of indications, usage, and features for each PARP inhibitor (March/2022)

PARP inhibitor	Indications in FDA			Requirement of BRCA mutation or HR-deficiency	Requirement of prior response to platinum-based chemotherapy	Dose (mg)	Time/day	Half-life (h)[d]	Relative PARP trapping potency	The clinical trial on which the indication is based
	Cancer type	First-line/recurrent	Treatment type							
Olaparib	Recurrent ovarian ca.	Recurrent	Maintenance	No	Yes	300	2	5-11	1	SOLO-2,[64] study19[4]
	Advanced ovarian ca.	First-line	Maintenance	Yes	Yes					SOLO-1[65]
			Maintenance/with bevacizumab	Yes	Yes					PAOLA-1[25]
	Advanced ovarian ca.	Treatment after 3 or more chemotherapy regimens		Yes	No					study42[66]
	HER2-negative metastatic breast ca.	Treatment after chemotherapy in the neoadjuvant, adjuvant, or metastatic setting		Yes	No					OlympiAD[67]
	Metastatic castration-resistant prostate ca.	Treatment after progression following enzalutamide or abiraterone		Yes	No					PROFOUND[68]
	Pancreatic ca.	First-line	Maintenance	Yes	Yes					POLO[69]
Niraparib	Recurrent ovarian ca.	Recurrent	Maintenance	No	Yes	300	1	36	2	NOVA[5]
	Advanced ovarian ca.	First-line	Maintenance	No	Yes	200 or 300[b]				PRIMA[3]
	Advanced ovarian ca.	Treatment after three or more chemotherapy regimens		Yes	No	300				QUADRA[70]
Talazoparib	HER2-negative locally advanced or metastatic breast ca.	Treatment		Yes	No	1	1	50	100	EMBRCA[28]
Rucaparib	Recurrent ovarian ca.	Recurrent	Maintenance	No	Yes	600	2	17-19	1	ARIEL3[6]
	Advanced ovarian ca.	Treatment after two or more chemotherapy regimens		Yes	No					Study10,[71] ARIEL2[72]
	Prostate ca.	Treatment after androgen receptor-directed therapy and a taxane-based chemotherapy		Yes	No					TRITON2[73]
Veliparib[a]	Advanced ovarian ca.	First-line	Maintenance	Yes	No	300-400[c]	2	6	<0.2	VELIA[26]

Abbreviations: ca., cancer; FDA, Food and Drug Administration.

[a]Veliparib is not yet approved by FDA.

[b]200 mg (<77 kg) or 300 mg (≥77 kg).

[c]300 mg (first 2 weeks) and 400 mg for maintenance.

[d]Data obtained from https://www.fda.gov or reference 74.

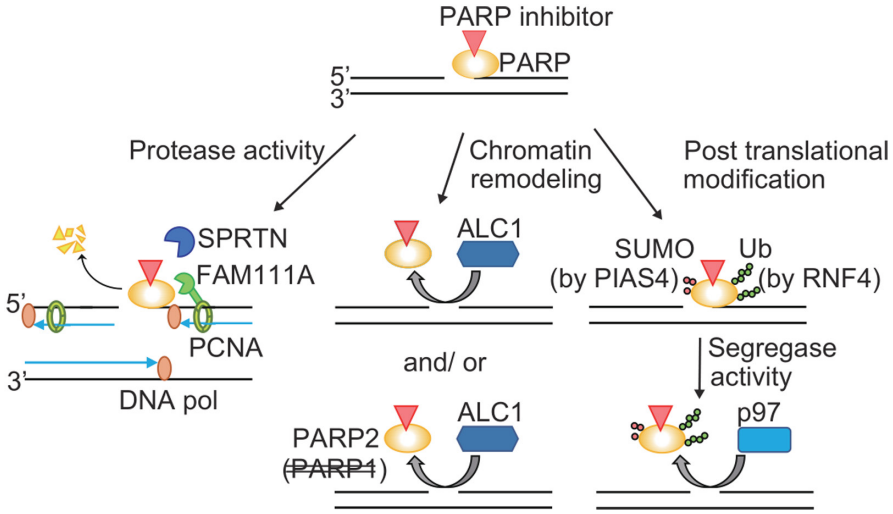

FIGURE 1 Schematic representation of the multiple pathways that resolve the PARP-DNA complex. A metalloprotease Spartan (SPRTN) or a PCNA-interacting serine protease FAM111A degrade trapped PARP1 (left). A chromatin-remodeling enzyme ALC1 removes PARP1 and/or PARP2. The model by Blessing et al. specifies that not PARP1 but PARP2 is the target of ALC1-mediated PARP removal (middle). SUMOylation on PARP1 by PIAS4 followed by ubiquitination on PARP1 by the E3 ligase RNF4 promotes the recruitment of segregase p97 (right)

interactomes identified an interaction between trapped PARP1 and the ubiquitin-regulated p97 ATPase/segregase.[22] Trapped PARP1 has been shown to be SUMOylated by PIAS4 and subsequently ubiquitylated by the SUMO-targeted E3 ubiquitin ligase RNF4, promoting the recruitment of p97 and removal of trapped PARP1 from chromatin (Figure 1).[22] Notably, this pathway appears rather general as it is also involved in the repair of trapped TOP cleavage complexes.[23] Thus, cells use redundant pathways to efficiently remove PARP-DNA complexes trapped by PARPis. Inhibitors for the resolving factors of trapped PARP are expected to synergize with the trapping-PARPis.

3 | DIFFERENCES BETWEEN PARPIS AND CONVENTIONAL DNA-DAMAGING ANTICANCER DRUGS

PARPis generate lesions leading to replication-dependent DSBs with trapped PARP. In terms of expectation of replication-dependent cell death, platinum drugs and TOP inhibitors, which are conventional DNA-damaging anticancer agents, also have similar mechanisms of action in that they ultimately induce DSBs.[24] Hence, HR genes are common critical repair factors for PARPis and DNA-damaging agents.

Here, we point out the differences between PARPis and the conventional DNA-damaging agents that generate bulky DNA adducts. Platinum drugs covalently crosslink DNA. TOP inhibitors trap covalent TOP1- and TOP2-DPC. Therefore, even if the drug concentration is reduced, DNA lesions, once generated, will not be restored unless they are repaired. In contrast, the PARP-DNA complex is not a covalent bond, so the trapped PARP can be quickly released from DNA when the concentration of PARPis becomes lower (Figure 2).[8] We previously showed that when the PARPi was removed from the cell culture medium, PARP-DNA complexes began to be released after 5 minutes and were wholly released after 30 minutes with the recovery of PARylation.[8] Once trapped PARP is released, the remaining single-strand breaks can be rapidly repaired by the reactivated PARP regardless of HR status (Figure 2). However, if the PARP-DNA complex has already generated collisions with replication forks, DSBs with clean (ie, protein-unbound) DNA ends remain. The clean DSBs can be repaired in HR-proficient cells, while still highly toxic in HR-deficient cells, which is attributed to the original synthetic lethality model (Figure 2).

We assume that these points can partly explain the expected clinical benefit among PARPis for HR-deficient cancers, as well as the inconsistent clinical benefit among PARPis for HR-proficient cancers (Table 1). Niraparib is a unique PARPi in terms that it is approved for first-line maintenance in patients with advanced ovarian cancer after initial response to platinum-based chemotherapy, regardless of *BRCA* status.[3] Although the study design is different, olaparib did not benefit HR-proficient cancers in the first-line maintenance setting in the PAOLA-1 study that combined olaparib and bevacizumab (VEGF inhibitor).[25] Veliparib was tested in combination with first-line chemotherapy (cisplatin and paclitaxel) and as maintenance therapy as a single agent in ovarian cancer in the VELIA study. The VELIA study revealed significant benefits of veliparib in *BRCA* mutated or HR-deficient (myChoice assay HRD score ≥ 33) cancers, but not in *BRCA* wild-type or non-HRD cancers.[26]

According to our model, transient PARP trapping is toxic enough for HR-deficient cancers if sufficient levels of DSBs are generated by replication block (Figure 2). By contrast, maintaining PARP trapping should be a key for anticancer acting in HR-proficient cancer cells (Figure 2). The long elimination half-life (time required for the blood concentration of a drug component to decrease by half) of niraparib (~36 h) and relatively high cell membrane permeability and volume of distribution[27] possibly enable niraparib to maintain PARP trapping and be effective in HR-proficient cancers. On the other hand, the relatively short elimination half-life of olaparib (5-11 h) or veliparib (~6 h), which accounts for their twice-daily administration protocols (Table 1), may explain the lack of efficacy of these drugs in HR-proficient cancers. Talazoparib, which has an extended half-life (~58 hours) and most potent PARP-trapping power, is supposed to have the potency to benefit HR-proficient cancers. However, clinical

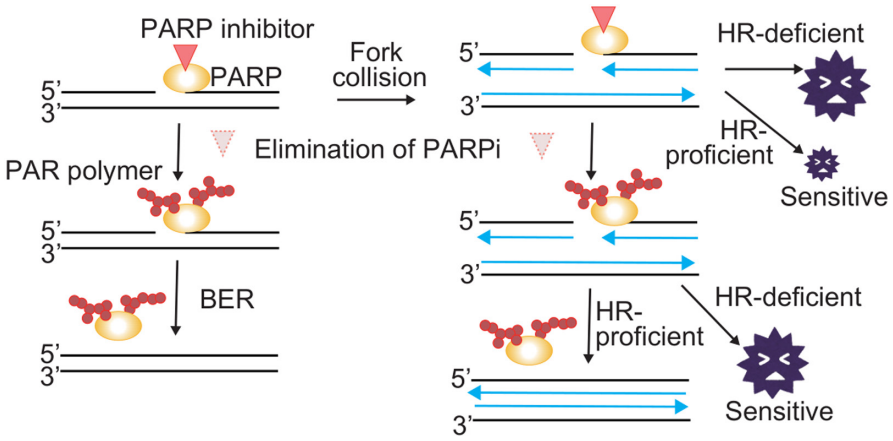

FIGURE 2 Schematic representation of repair pathways for PARP-trapping lesion following the removal of PARP inhibitors. Elimination of PARPi reactivates poly ADP-ribosylation (PARylation) and releases the noncovalently bound PARP from DNA. The remaining single-strand DNA breaks are repaired by the base excision repair (BER) pathway, while the remaining double-strand breaks (DSBs) after replication fork collision need homologous recombination (HR). "Clean" DSBs with the ends free of protein crosslinks sensitize HR-deficient cancers, while HR-proficient cancers can repair those "clean" DSBs. Persistent PARP trapping is toxic to HR-proficient cells but more to HR-deficient cells (reflected in the size of cartoons)

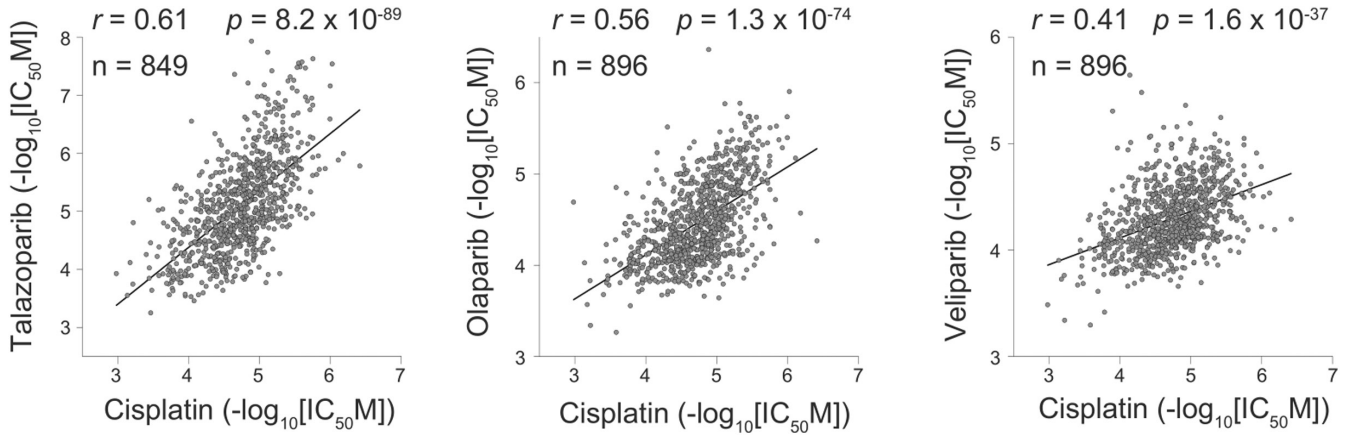

FIGURE 3 Highly significant correlation of drug response in the Genomics of Drug Sensitivity in Cancer (GDSC) database between cisplatin and PARP inhibitors (talazoparib, olaparib, and veliparib). The data were obtained using the public resource CellMinerCDB[17] (https://discover.nci.nih.gov/cellminercdb/). Pearson's correlation (*r*), *P* value (*p*), number of the samples (*n*), and regression line (black line) are shown

trials have been selectively performed in breast cancer patients with *BRCA* mutations, and the benefit of talazoparib in *BRCA* wild-type cancers has not been evaluated.[28,29] In the setting of recurrent tumors, olaparib, niraparib, and rucaparib benefit HR-proficient cancers to a significant extent but are less effective compared with HR-deficient cancers[30] (Table 1).

The VELIA study examined the effect of veliparib in combination with first-line chemotherapy (cisplatin and paclitaxel) by comparing the combination-only and chemotherapy-only groups. The study revealed little additional effect of veliparib combination.[26] These results are interpretable because PARP is not a primary repair factor of the cisplatin-induced lesions (ie, *PARP* deficiency does not confer hypersensitivity to cisplatin treatment)[31] and because veliparib is a relatively weak PARP trapper. It is assumed that the toxicity of cisplatin and paclitaxel is dominant in the combination setting where PARP inhibition has little impact on the toxicity.

4 | WHICH GENES CONTRIBUTE TO THE CROSS-SENSITIVITY BETWEEN PARPIS AND PLATINUM DERIVATIVES?

Although some repair factors are uniquely crucial for platinum derivatives or PARPis,[10] the utility of platinum sensitivity as a clinical biomarker for PARPis implies that PARPis and platinum agents share similar sensitivity and resistance factors. The clinical outcomes are readily recapitulated across ~900 cell lines, revealing the extremely high sensitivity correlation between PARPis (talazoparib, olaparib, and veliparib) and cisplatin regardless of tumor types (Figure 3). Notably, the *P* value of each PARPi correlates with their PARP-trapping potency (Figure 3 and Table 1), indicating that such common sensitivity factors are involved in the cellular responses to PARP-trapping lesions as well as DNA-crosslinking lesions. Because both lesions eventually induce replication-coupled DSBs repaired by

HR, such common factors are assumed to be HR-associated genes. A recent report revealed associations between genome-wide loss of heterozygosity (gLOH) and alternation of HR-associated genes in 160,790 tumors.[32] Known/likely deleterious alterations in HR-associated genes were found in 18.9% of cases. HR-associated genes include *BRCA1*, *BRCA2*, *PALB2*, *BRAD1*, *ATR*, *ATRX*, *ATM*, *BAP1*, *RAD51B*, *RAD51C*, *RAD51D*, *BRIP1*, *NBN*, *CHEK1*, *CHEK2*, *FANCA*, *MRE11*, and others. Hence, the ~20% of cases showing high sensitivity to both drugs can be explained by altered HR-associated gene mutations. However, it is important to point out that cancer cells can be sensitive to PARPis or cisplatin without such repair gene mutations. In the NCI-60 cell lines, about 30 cell lines are susceptible to talazoparib, while the rest are resistant.[33] According to the mutation analysis of repair genes,[34] 15 cell lines are sensitive to talazoparib without apparent DNA repair mutations in the list (data not shown). However, we may miss unlisted repair factors involved in the cross-sensitivity. Hence, we need to find a way to identify the patients who do not carry HR gene mutations but are yet sensitive to PARPis and platinum agents.

5 | *SLFN11* IS A COMMON SENSITIZER TO PLATINUM DERIVATIVES AND PARPIS IN CANCER MODELS

We raise the issue of why each cell line dot in Figure 3 is along the regression line but does not exhibit bipolar distribution. HR deficiency drastically sensitizes cancer cells to PARPis with orders of magnitude in IC_{50} values, possibly leading to bipolar distribution if the cross-sensitivity is majorly attributed to HR deficiency. The linear distribution implies the presence of determining factors controlled by expression level. One such factor is most likely *SLFN11*, a member of the *SLFN* family,[35] with a DNA/RNA helicase domain and a nuclear localization signal. In 2012, using different cell line databases, two independent laboratories identified that *SLFN11* expression is highly correlated with sensitivity of the TOP1 inhibitor topotecan.[36,37] The high correlation also applies to cisplatin and DNA replication inhibitors such as cytarabine.[36] Although the initial actions are different, these drugs commonly induce replication blocks, activate S-phase checkpoint, and generate abnormal (stressed) replication forks. Replication stress[38] activates the kinase ataxia telangiectasia and Rad3-related (ATR) that mediates S-phase checkpoint to support cell survival by reducing replication speed and transiently inhibiting origin firing. Our recent studies demonstrated that SLFN11 blocks the elongation of stressed replicons in parallel to ATR and induces cell death contrary to the effect of ATR.[39] Recent studies have revealed more actions for SLFN11, including tRNA cleavage leading to insufficient ATR synthesis,[40–42] chromatin opening,[43] degradation of the replication initiation factor CDT1,[44] degradation of reversed replication forks[45] and protection from proteotoxic stress.[46] These actions do not seem cell type–dependent but rather general activities of SLFN11 regardless of tissues of origin. While the mechanisms of SLFN11-mediated cell killing are not fully understood, the importance of SLFN11 in drug sensitivity is validated in various settings[47–50] (see recent reviews[38,51]). Patient data are also accumulating with a broad type of cancers including breast cancer,[50,52] ovarian cancer,[53] gastric cancer,[54] bladder cancer,[55] small cell lung cancer,[56] esophageal cancer,[57] and prostate cancer.[58] Notably, most of these data have been obtained without considering the presence or absence of *BRCA* or HR deficiency. Thus, several independent clinical protocols are being initiated to validate whether SLFN11 confers high sensitivity to DNA-damaging agents independently of HR status in cancer patients.

The correlation between *SLFN11* expression and drug sensitivity is also applicable to PARPis. We previously showed that *SLFN11* expression is significantly correlated with sensitivity to talazoparib in the NCI-60.[59] The significant correlation is also validated in the GDSC database for talazoparib, olaparib, and veliparib (Figure 4). Again, the *P* values were correlated with the PARP-trapping potency. We showed that SLFN11 enhances sensitivity to olaparib

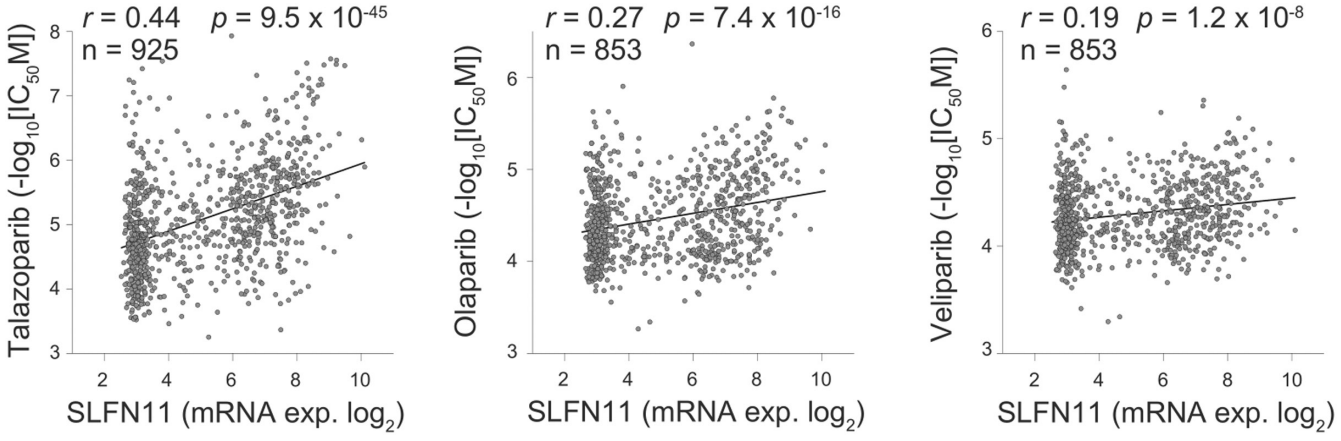

FIGURE 4 Highly significant correlation of drug response in the Genomics of Drug Sensitivity in Cancer (GDSC) database between SLFN11 expression and PARP inhibitors (talazoparib, olaparib, and veliparib). The data were obtained using the public resource CellMinerCDB[17] (https://discover.nci.nih.gov/cellminercdb/). Pearson's correlation (*r*), *P* value (*p*), number of the samples (*n*), and regression line (black line) are shown

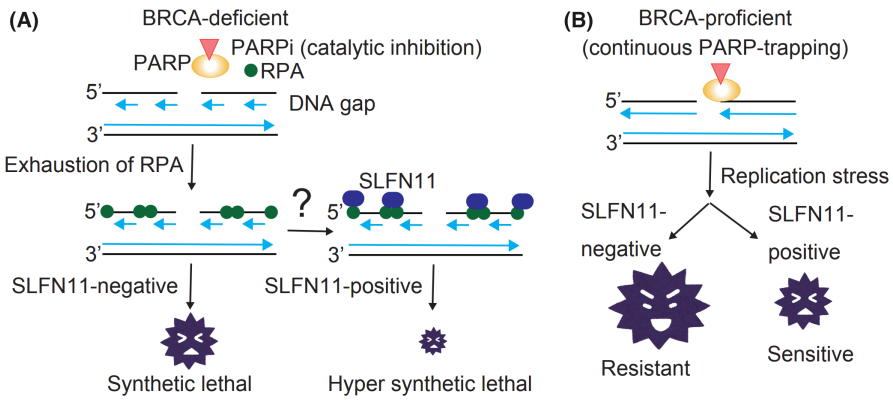

FIGURE 5 Schematic representation to interpret the better clinical outcomes of olaparib in SLFN11-positive and *BRCA*-deficient cancers than in SLFN11-negative and *BRCA*-deficient ones. (A) *BRCA*-deficient cells harbor replication gaps (single-strand DNA gap) under the treatment of sub-micromolar concentration of olaparib. The single-strand binding protein replication protein A (RPA) protects the replication gaps, but exhaustion of RPA results in genome instability, leading to synthetic lethality[62] (left). In SLFN11-positive cells, SLFN11 likely binds the DNA-bound RPA, possibly leading to SLFN11-mediated cell death named "hyper synthetic lethal." (right, the smaller cartoon indicates more shrunk cancer). Note that the sub-micromolar concentration of olaparib is not supposed to generate a PARP-DNA complex based on our previous report but is enough to inhibit the catalytic activity of PARP.[8] (B) Continuous PARP trapping is likely a key for the sensitivity of SLFN11-positive cancer cells to PARP trappers. Continuous PARP trapping induces replication stress that activates SLFN11 toward cell death

and talazoparib using genetically modified isogenic cell lines and that SLFN11 and *BRCA* deficiency independently contribute to the PARPi sensitivity.[59] Two independent groups revealed the relevance of SLFN11 for PARPi sensitivity in patient-derived xenograft (PDX) models.[60,61] These facts make sense because PARP-DNA complexes cause replication stress, activating SLFN11 toward cell death. Together, the observations and conclusions listed above explain why SLFN11 can be a primary common sensitivity factor for platinum drugs and PARPis.

6 | DOES SLFN11 NEED *BRCA* MUTATION TO ENHANCE OLAPARIB SENSITIVITY IN THE CLINICAL SETTING?

Recently, the group of AstraZeneca examined the effect of SLFN11 on olaparib sensitivity.[56] They first showed that patients with high-SLFN11 tumors had significantly longer overall survival when treated with first-line platinum and etoposide in small cell lung cancer. Although the role of SLFN11 was uncertain in their cohort of high-grade serous ovarian cancers treated with paclitaxel-platinum, they found that high levels of SLFN11 were associated with improved clinical outcomes with olaparib treatment. Intriguingly, subgroup analyses revealed that only the patients with *BRCA* mutation benefited from the high SLFN11 expression under the olaparib treatment, which is inconsistent with the series of in vitro data where SLFN11 sensitizes cancer cells regardless of *BRCA* status. Yet, a timely report by Cong[62] may provide an answer. Accordingly, *BRCA*-deficient cells harbor excess single-strand DNA gaps in response to sub-micromolar concentrations of olaparib due to an Okazaki fragment–processing defect (Figure 5A). RPA protects the excess single-strand DNA gaps, but the exhaustion of RPA increases unprotected single-strand DNA, leading to genome instability[63] (Figure 5A). Because SLFN11 is recruited on chromatin via RPA binding, SLFN11 can enhance cell death in *BRCA*-deficient cancer at the sub-micromolar olaparib treatment condition (Figure 5A). HR-proficient cells should be intact from SLFN11-mediated cell death without the DNA gaps and PARP trapping under the sub-micromolar concentration of olaparib.[8] This scenario may explain the clinical outcome with olaparib being more effective in *BRCA* mutant and high-SLFN11 cells and may provide a "hyper synthetic lethal strategy" (Figure 5A). As SLFN11 induces cell death under replication stress, niraparib and talazoparib that can maintain PARP trapping possibly activate SLFN11 also in *BRCA*-proficient cancers (Figure 5B). Hence, results of the analyses of clinical data of niraparib and talazoparib are warranted.

7 | CONCLUSION

Eight years have passed since olaparib was first approved as a clinically available PARPi, followed by other PARPis. Once the clinical indications are set, the mechanisms of action are usually not discussed anymore. However, the variety of clinical outcomes from different PARPis give us chances to reconsider the therapeutically relevant molecular mechanisms of action of PARPis in cancer patients and reconnect them to the basic research.

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CONFLICTS OF INTEREST

Hiroshi Onji has no conflict of interest to disclose. Junko Murai received lecture fees from Takeda Pharmaceutical Co., Ltd. and AstraZeneca plc, and research fees from Taiho Pharmaceutical Co., Ltd., and Modulus Discovery Inc.

ETHICAL APPROVAL

Approval of the research protocol by an Institutional Reviewer Board.

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