Chapter 10
Classification of PARP Inhibitors Based on PARP Trapping and Catalytic Inhibition, and Rationale for Combinations with Topoisomerase I Inhibitors and Alkylating Agents

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Abstract All PARP inhibitors in clinical development (veliparib, olaparib, niraparib, rucaparib, talazoparib) are potent submicromolar competitive NAD^+ inhibitors for PARP1 and PARP2, thereby blocking PARylation reactions [i.e. formation of poly(ADPribose) polymers]. In addition, PARP trapping, which determines the anticancer activity of PARP inhibitors as single agents, is drug-specific, and PARP inhibitors can be ranked according to their PARP trapping potency: Talazoparib **>>** niraparib ≈ olaparib ≈ rucaparib > veliparib. The highly synergistic effects of PARP inhibitors in combination with alkylating agent (temozolomide or methyl methanesulfonate, MMS) and topoisomerase I (Top1) inhibitors (camptothecins and indenoisoquinolines) are well documented. Both classes of drugs induce DNA single-strand breaks sensed by PARP. Yet, the molecular mechanisms of synergy are different. For alkylating agents (temozolomide and MMS), both PARP trapping and PARylation inhibition account for the synergy, whereas for Top1 inhibitors, there is no involvement of PARP trapping and it is PARylation inhibition that deters the coupling of PARP with the repair enzyme, tyrosyl-DNA phosphodiesterase TDP1. In this chapter, we will review the differences between PARP inhibitors and the rationale for choosing among different PARP inhibitors in combination with alkylating agents or Top1 inhibitors.

Keywords PARP inhibitor · Topoisomerase · Camptothecin · PARP trapping · PARP-DNA complex · Synthetic lethal · Veliparib · Niraparib · Olaparib · Rucaparib · Talazoparib

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10.1 Introduction

10.1.1 PARP Inhibitors in Clinical Trials

The clinical PARP inhibitors, veliparib, olaparib, niraparib, rucaparib, and talazoparib (Fig. 10.1) were initially developed as catalytic inhibitors that compete with NAD^+ for the catalytic pockets of PARP1 and PARP2 and thereby inhibit PARylation [poly(ADPribose) polymer formation]. Since the discovery of the synthetic lethality of PARP inhibitors in BRCA-deficient (homologous recombination repair-deficient) cells [1, 2], the mechanism by which PARP inhibitors exert their cytotoxicity has been dominantly interpreted as an accumulation of unrepaired single-strand breaks (SSBs) resulting from catalytic inhibition of PARylation (Fig. 10.2a, top scheme). Therefore, until recently, PARP inhibitors were evaluated based on their ability to inhibit PARylation [3, 4] and on their selectivity toward different PARP family members (PARP1-PARP17) [5]. Although all PARP inhibitors are potent enough to effectively inhibit PARylation at low nanomolar concentrations, their differential cytotoxicity as single agent, which varies widely among the PARP inhibitors [6] is primarily based on the trapping of PARP-DNA complexes [7, 8] (Fig. 10.2a, bottom scheme; Table 10.1). Note that, in this review, we describe PARP1 and PARP2 as PARP for convenience, except in the case where PARP1 should be distinguished from PARP2.

10.1.2 Dual Mechanisms of Action of PARP Inhibitors: PARylation Inhibition and Trapping of PARP-DNA Complexes (PARP Trapping)

The trapping of PARP as a critical cytotoxic mechanism of PARP inhibitors was discovered by screening different PARP inhibitors for their cytotoxicity in a panel of DT40 lymphoma cells with defined inactivation of DNA repair genes [8]. Three experimental observations led to this conclusion: 1/Olaparib and niraparib were found to be much more cytotoxic than veliparib at drug concentrations that were equally effective at blocking PARylation; 2/the cytotoxicity of PARP inhibitors was totally abolished in PARP-deficient chicken DT40 cells (PARP1 knockout DT40 cells that are equivalent to PARP1/2 knockout cells due to the lack of PARP2 in avian genome), which could not be explained by catalytic inhibition; and 3/PARP1- and PARP2-DNA complexes could be detected in cells treated with PARP inhibitors, and the PARP-trapping potency of the drugs matched their cytotoxic activity. These results have recently been extended to talazoparib and rucaparib [9], against which PARP-deficient DT40 cells are totally immune even to the most cytotoxic agent talazoparib while PARP-proficient wild-type DT40 cells are killed by talazoparib in a dose-dependent manner at nanomolar concentrations [9]. These findings led to the conclusion that the cytotoxic mechanisms of PARP inhibitors are mediated by the presence of PARP proteins but not by catalytic inhibition of PARP.

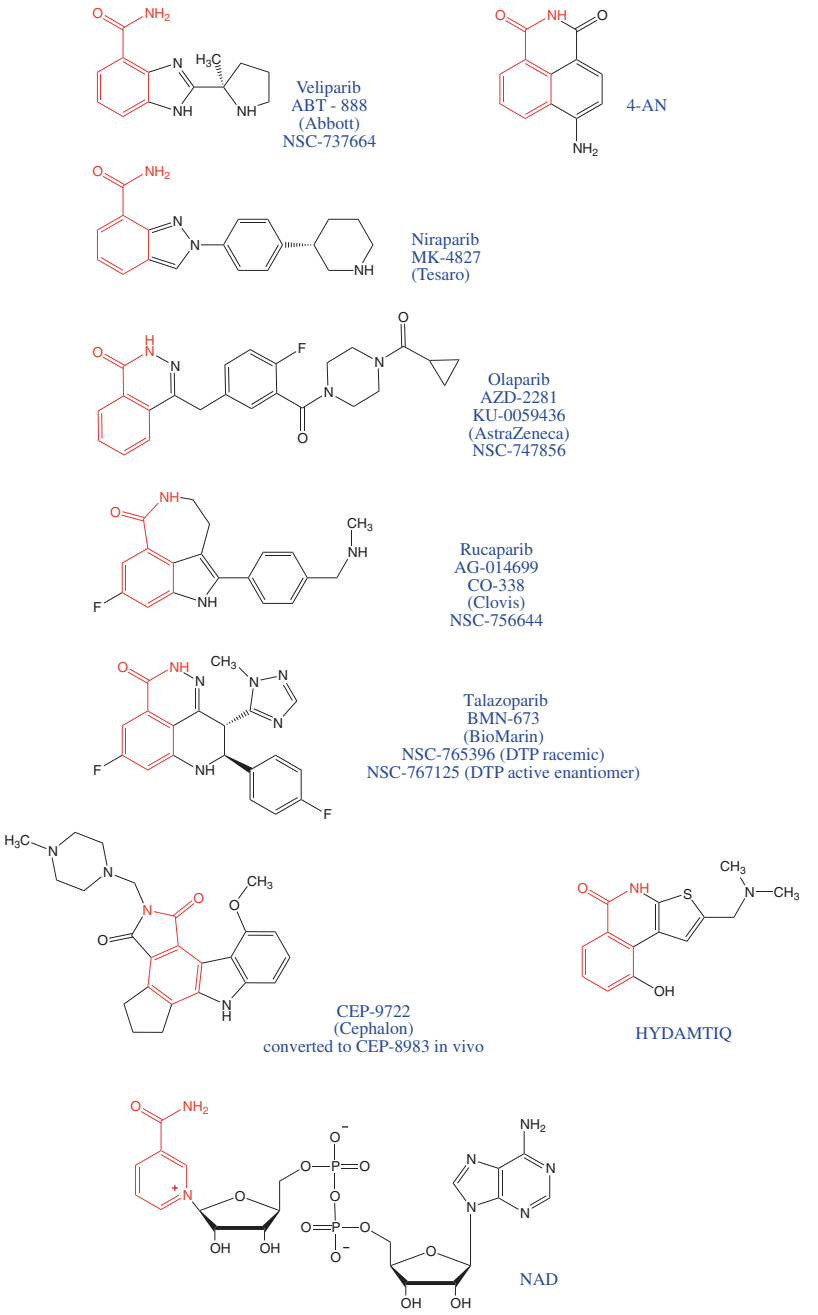

Fig. 10.1 Chemical structures of PARP inhibitors (veliparib, niraparib, olaparib, rucaparib, talazoparib, CEP-9722, 4-AN and HYDAMTIQ) and NAD. The nicotinamide moiety is outlined in *red*

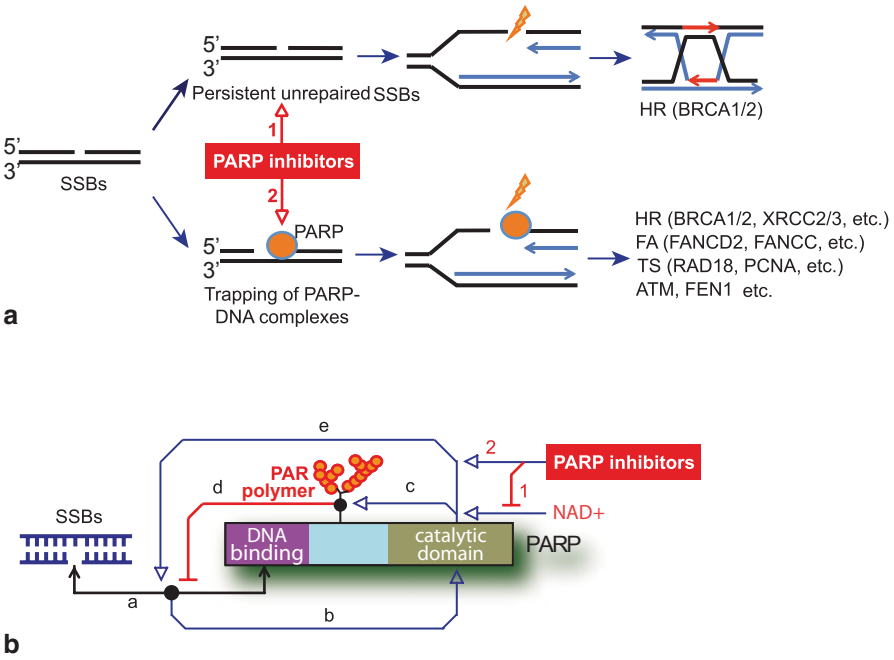

Fig. 10.2 Schematic representation of the two mechanisms of action of PARP inhibitors: *1*: catalytic inhibition; *2*: trapping of PARP-DNA complexes. **a** Dual cytotoxic mechanisms of action of PARP inhibitors. *1* (*upper* pathway): Catalytic inhibition interferes with the repair of single-strand breaks (SSBs) by PARP, leading to an accumulation of replication fork blocks that are repaired by homologous recombination repair (HRR). *2* (*lower* pathway): PARP is trapped at endogenous and alkylating agent-induced DNA lesions under the presence of PARP inhibitors. The trapped PARP-DNA complexes cause replication fork blocks accompanied with "dirty" 5′-DNA ends. Hence, the repair is not as simple as the *upper* pathway, and utilizes additional repair pathways including Fanconi anemia (FA), template switching (TS), ATM, FEN1 [8]. **b** Molecular interaction scheme showing the regulatory pathways for the formation and dissociation of PARP-DNA complexes. PARP binds to SSB (**a**), which induces conformational distortions that stimulate the catalytic domain (**b**). PARP inhibitors enhance the PARP-DNA complexes by two mechanisms. One is by inhibition of PARylation, which inhibits the dissociation of PARP from DNA (*1*, c and d). The other is drug binding to the catalytic pocket, which allosterically enhances the DNA binding of the PARP through the DNA binding domain (*2*, *e*). (Figures are modified from the reference [8])

Table 10.1 Comparison of clinical PARP inhibitors based on the three parameters evaluated using chicken DT40 cells, and classification based on catalytic inhibition and PARP trapping. (Data are modified from the references [8, 9])

	Catalytic inhibition (IC_{50} in wild-type DT40 cells) (nM)	Catalytic inhibition (IC_{90} in wild-type DT40 cells) (μM)	PARP-trapping potency (relative to olaparib)	Class
Veliparib	30	>50	<0.2	Type 1
Olaparib	6	4.5	1	Type 2
Rucaparib	21	3	1	Type 2
Niraparib	60	2.3	~2	Type 2
Talazoparib	4	0.04	~100	Type 2

As single agents, PARP inhibitors trap PARP1 and PARP2 at endogenous DNA lesions where PARP1 and PARP2 are recruited (base damage and strand breaks). The resulting PARP-DNA complexes are much more cytotoxic than merely unrepaired SSBs due to the absence of PARP, probably because the complexes strongly block DNA replication, leading to DNA double-strand breaks [7, 9] (Fig. 10.2a). The PARP-trapping mechanism explains the anticancer activity of PARP inhibitors as single agents. Hence, by analogy with topoisomerase inhibitors that kill cancer cells by trapping topoisomerase-DNA complexes [10, 11], PARP inhibitors have also been referred to as "PARP poisons". The potency of the PARP inhibitors to trap PARP on DNA is well correlated with the cytotoxicity of the PARP inhibitors. For the five clinical PARP inhibitors, their ranking for cytotoxicity is talazoparib ≫ niraparib ≅ olaparib ≅ rucaparib > veliparib, which corresponds to their PARP trapping potency, which is not simply correlated to their potencies as catalytic inhibitors (Table 10.1). Therefore, we propose to classify PARP inhibitors into two categories. Type 1 inhibitors correspond to relatively pure catalytic inhibitors, and type 2 to PARP trapping agents in addition to being catalytic inhibitors (each mechanism annotated as the "1" and "2" red symbols in Fig. 10.2). Although we classify veliparib as a type 1 inhibitor, it can also trap PARP at high concentration [8]. However, compared to talazoparib the strongest PARP trapping agent to date, veliparib has a 3–4 orders of magnitude lower potency for trapping PARP. Talazoparib and the other PARP inhibitors, niraparib, olaparib and rucaparib, can be classified as type 2 inhibitors because their cytotoxic and anticancer effects as single agents are primarily derived from PARP trapping at and below pharmacological concentrations (25 μM: the peak concentration of olaparib in clinical trials) [12].

The molecular mechanisms of differential PARP trapping by different PARP inhibitors are not fully elucidated. One theory proposed in 1992 is that catalytic PARP inhibition prevents dissociation of PARP from DNA and inhibits further repair [13]. However, PARP-trapping cannot be fully estimated from the catalytic inhibition potency of PARP inhibitors presented by conventional IC_{50} and IC_{90} (Table 10.1), as dose-dependent and drug-dependent cytotoxicities are observed above 1 μM where PARylation is almost completely inhibited [7, 9]. Thus, we speculate that the binding of PARP inhibitor to the catalytic pockets of PARP1 and PARP2 is transduced by an allosteric effect to the protein N-terminus, thereby tightening the binding of the DNA-binding domains of PARP (Fig. 10.2b, annotation "e"). Such a mechanism would be the reverse allosteric effect produced by the binding of PARP to DNA (Fig. 10.2b, annotation "b"), which induces conformational distortions that stimulate the catalytic domain [14].

10.1.3 Differential Combinations for Type 1 and Type 2 PARP Inhibitors

Combinations of PARP inhibitors with other anticancer drugs have been extensively studied, and various combinations are under clinical trials [15]. These combinations were recently reviewed by Curtin and Szabo [16]. Because PARPs have multiple

functions for DNA repair including base excision repair, alternative nonhomologous end joining, homologous recombination repair (see [17]); Chap. 3, it is logical that combinations of various DNA damaging agents confer synergistic effects with PARP inhibitors. However, the underlying molecular mechanisms of synergism remain to be fully determined. In light of the dual molecular effects of PARP inhibitors (PARP trapping and catalytic inhibition), it is necessary to revisit the molecular mechanism explaining the synergistic effects of PARP inhibitors (type 1 vs. type 2 inhibitors) with established chemotherapeutic agents. In the next section, we will focus on the effect of PARP inhibitors in combination with topoisomerase I inhibitors (camptothecins and indenoisoquinolines) and alkylating agents (temozolomide and methylmethane sulfonate MMS), which produce consistent and marked synergy [16]. Both topoisomerase I inhibitors and alkylating agents induce DNA single-strand breaks [18] with distinct involvement of PARP1 and PARP2 in the damages, which will be detailed below. It is also important to note that type 1 PARP inhibitors such as veliparib are likely to be better suited to treat neurological or cardiological diseases [16] where genome damaging is unwanted.

10.2 PARP Inhibitors with Top1 Inhibitors

The synergistic effects of Top1 inhibitors in combination with PARP inhibitors *in vitro* and *in vivo* are well documented in various cell lines including colon cancer cells that tend to be resistant to either single agent [19–24]. The mechanisms of synergy are likely to be multifactorial. First, we will introduce the molecular pharmacology of Top1 inhibitors. Following which, we will review the various mechanisms involving PARP1 for the repair of Top1-induced DNA lesion. In the end, we will discuss the mechanisms of synergy based on catalytic inhibition rather than PARP-trapping.

10.2.1 Top1 and Top1 Inhibitors

Top1 relaxes DNA supercoiling generated during replication and transcription. Supercoiling relaxation requires the production of transient Top1 cleavage complexes (Top1cc), which correspond to Top1-linked DNA single-strand breaks (SSBs) [25, 26]. Under normal conditions, the SSBs reverse quickly following the religation of the relaxed DNA and the release of Top1. Top1 inhibitors, camptothecins (CPT and its clinical derivatives irinotecan and topotecan) and indenoisoquinoline, selectively trap Top1cc, and prevent the religation of the SSBs which are eventually converted to DNA double-strand breaks (DSBs) by the collision of replication forks [25] and transcription complexes [18]. These DNA lesions account for cytotoxicity of Top1 inhibitors. A key enzyme for the repair of Top1cc is tyrosyl-DNA phosphodiesterase 1 (TDP1) [27, 28] (reviewed in [29]). TDP1 hydrolyzes the phosphodiester bond

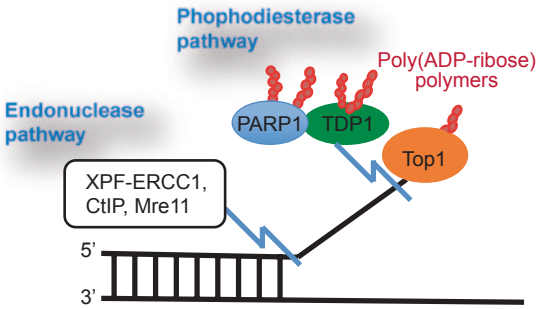

Fig. 10.3 Coupling of PARP1 and TDP1 and redundant pathways for the repair of Top1 cleavage complex (Top1cc) that can be induced by camptothecins (irinotecan and topotecan) and non-camptothecin Top1 inhibitors (indenoisoquinolines and ARC-111), and endogenous DNA lesions [29]. PARP1 coupling with TDP1 stimulates the excision of Top1cc by the phosphodiesterase activity of TDP1. The parallel pathways for the removal of Top1cc involve various endonucleases including XPF-ERCC1, CtIP and Mre11. (Figure is modified from [29, 30])

between the Top1 tyrosyl moiety and the DNA 3′-end. Alternative endonuclease pathways including XPF-ERCC1 [24], CtIP and Mre11 [31–33], can also repair Top1cc (Fig. 10.3). Base excision repair and homologous recombination are the following critical repair pathways after the removal of Top1cc for the SSB and DSB repair, respectively (reviewed in [29]).

10.2.2 Functions of PARP1 for the Repair of Top1-Induced Lesions

The involvement of PARP1 in the repair of Top1cc is based on several observations [30]. First, PARP1-deficient cells are hypersensitive to CPT [34–36] (Fig. 10.4a, b). Second, PARP inhibitors are highly synergistic with CPT. Third, PARylation is rapidly and markedly stimulated in CPT-treated cells [24, 37, 38], suggesting that catalytic PARP activation is necessary for the repair of Top1cc.

At least three molecular mechanisms account for the action of PARP enzymatic activity on the repair of Top1cc. First, PARP1 participates in the base excision repair by recruiting XRCC1 to the Top1cc site [39], which in turn recruits TDP1 for the removal of Top1cc [40] (reviewed in [16, 41]). Our recent studies revealed that a significant fraction of PARP1 and TDP1 are tightly bound to each other even in the absence of DNA damage, and that poly-ADP-ribosylation (PARylation) of TDP1 by PARP1 enhances TDP1 recruitment to Top1cc site while stabilizing TDP1, and that TDP1-PARP1 complexes, in turn recruit XRCC1. Hence, TDP1-PARP1 coupling is critical for the repair of Top1cc [30] (Fig. 10.3). Two additional mechanisms account for the resolution of Top1cc by PARP1. One is the direct PARylation of Top1 which reverses Top1cc [42]. The other is that PARylation stimulates replication fork reversal, which prevents replication fork collisions and DSB formation [43]. PARylation

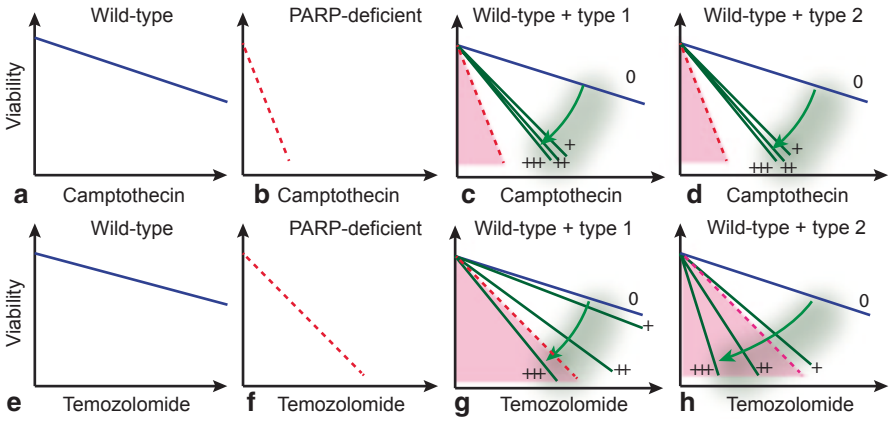

Fig. 10.4 Schematic representations of the differential effects of type 1 vs. type 2 PARP inhibitors, and the rationale for using type 1 and type 2 inhibitors in combination with Top1 inhibitors (camptothecin) and type 2 inhibitors in combination with temozolomide. Cell viability assays data [9] using chicken DT40 cells (wild-type and PARP-deficient cells) are schematically presented. Panels **a** & **e**: cytotoxicity of camptothecin or temozolomide in wild type cells; panels **b** & **f**: enhanced cytotoxicity of camptothecin or temozolomide in PARP deficient cells; panels **c** & **d**: similar potentiation of camptothecin by type 1 (catalytic PARylation) inhibitors and type 2 (PARP trappers and catalytic PARylation) inhibitors. The addition of type 1 or type 2 inhibitors with different concentration (+ < + + < + + +) synergistically increased the cytotoxicity in wild-type cells. The synergism is not dose-dependent very much since the PARylation inhibition is efficient enough with low concentration (+) of type 1 or type 2 inhibitors. Note that the potentiation in wild-type does not exceed the sensitivity of PARP-deficient cells, i.e. none of the drug concentration response curves are observed in the pink area. Panels **g** & **h**: Differential potentiation patterns by type 1 and type 2 inhibitors to temozolomide. The addition of type 1 or type 2 inhibitors with different concentration (+ < + + < + + +) synergistically increased the cytotoxicity in wild-type cells. The combination effects are much more pronounced with type 2 than type 1. The synergism is dose-dependent, and the combination potentiates temozolomide cytotoxicity beyond the effect of PARP-deficient cells because PARP-DNA complexes are more toxic than unrepaired SSBs (see also Fig. 10.2a). Note that we describe PARP1-deficient DT40 cells as PARP-deficient cells due to the lack of PARP2 in avian genome

of RECQ1, which inhibits its DNA helicase activity, has been proposed to play an essential role by inhibiting RECQ1-mediated fork restoration, thereby preventing premature restart of regressed forks [44]. According to these three mechanisms, catalytic PARP inhibition leads to replication fork collisions and replication run-off, leading to DNA double-strand ends [45] that cannot be repaired by TDP1 [30].

10.2.3 No PARP Trapping by CPT in Combination with PARP Inhibitors and Value of Class 1 Catalytic PARP Inhibitors

PARP-DNA complexes are detectable by conventional Western blotting, and also assessed by sensitivity assays [7, 9] (Fig. 10.4a–d). Our recent studies revealed that PARP-DNA complexes are undetectable even at highly cytotoxic concentration of

CPT (1 μM; ~100-fold higher than the cytotoxic concentrations) [46]. This explains why the sensitivity of wild-type cells to CPT in combination with PARP inhibitor (olaparib or veliparib) never exceeds the hypersensitivity of PARP-deficient DT40 cells to CPT (Fig. 10.4a–d). From these results and the mechanistic insights mentioned above, the contribution of PARP-DNA complexes needs to be viewed as minimal, if any, in the case of CPT and other Top1cc-targeted drugs. We will review later the DNA substrates for PARP-binding that explain the lack of PARP-trapping at CPT-induced lesion.

The mechanistic insights demonstrate why catalytic PARP inhibitors (type 1; Table 10.1), are rational in combination with CPT and other Top1 inhibitors, such as the indenoisoquinolines in clinical trials. In other words, both type 1 and type 2 agents are applicable for the combination with Top1 inhibitors (Fig. 10.4c, d). Yet, to avoid the additional cytotoxic effect and dose-limiting toxicity resulting from PARP-DNA complexes, the relatively pure catalytic PARP inhibitor veliparib (type 1), is a rational choice for combinations with Top1cc-targeted drugs such as topotecan, irinotecan and the non-camptothecin Top1 inhibitors (indenoisoquinolines and ARC-111 [25]) [46].

10.3 PARP Inhibitors with Alkylating Agents

10.3.1 Alkylating Agents and PARP

Temozolomide is a commonly used alkylating agent for cancer patients with glioblastoma multiforme (GBM). Mechanistically, temozolomide acts similarly to the methylating agent MMS (methyl methanesulfonate) commonly used as a biological reagent, as the active metabolite of temozolomide 3-methyl-(triazen-1-yl)imidazole-4-carboxamide (MTIC) methylates guanines at positions N7 and O6. It is those lesions that need to be repaired by base excision repair where PARP1 and PARP2 is involved (reviewed in Chaps. 3 and 22). Accordingly, PARP1- or PAPR2- or PARP1/2- deficient cells are hypersensitive to monofunctional alkylating agents (Fig. 10.4e, f) [8, 47]. It is important to note that monofunctional alkylating agents such as temozolomide and MMS are different from bifunctional DNA alkylating and crosslinking agents such as platinum derivatives, which are not synergized by PARP inhibitors in cancer cell line models [46].

10.3.2 Differential PARP Trapping by Different PARP Inhibitors Combined with Alkylating Agents

PARP trapping occurs at DNA damage sites that arise spontaneously and/or are produced by alkylating agents (temozolomide and MMS), which readily activate

PARylation [7, 9]. Synergistic effect of PARP inhibitors with temozolomide is observed with both types of PARP inhibitors in concentration-dependent manner. By contrast to the combinations with Top1 inhibitors, the sensitization can go beyond that of PARP-deficient cells (Fig. 10.4g, h), indicating that PARP-DNA complexes are more cytotoxic than merely unrepaired SSBs due to the absence of PARP. However, the potency of type 1 inhibitors for synergy is much weaker than that of type 2 inhibitors, and much higher doses of type 1 than type 2 inhibitors are required for the synergy (Fig. 10.4g, h). Hence, type 2 PARP inhibitors are likely to be preferable for the combination with temozolomide.

PARP trapping is efficient at killing cancer cells, but at the same time it can cause side effect due to damage on normal cells. Our *in vitro* study showed that PARP trapping induced by olaparib is reversible, and that PARP1 and PARP2 quickly dissociates from DNA after the removal of olaparib from culture medium [8]. However, little is known about the kinetics of PARP-DNA complexes *in vivo*, and further studies are warranted to establish methods to detect PARP-DNA complexes *in vivo* to monitor the kinetics of the complexes for the safe use of PARP inhibitors with temozolomide.

10.4 Differential Substrates for the Synergy of PARP Inhibitors with CPT or Temozolomide

Although both Top1 inhibitors (camptothecins and indenoisoquinoline) and alkylating agents (temozolomide and MMS) induce single-strand breaks (SSBs) sensed by PARP and repaired by TDP1 [7], the mechanisms and lesions that recruit PARP are different (Fig. 10.5). Recent biochemical studies have revealed that the binding of PARP1 depends on DNA substrates [48, 49], which most likely explain the different synergism between Top1 inhibitors and alkylating agents with PARP inhibitors. Alkylating agents induce base damage generating abasic sites that are cleaved by apurinic/apyrimidinic endonuclease 1, producing a 1-nucleotide gap with 3′-OH and 5′-deoxyribose phosphate (5′-dRP) groups at the ends of the breaks (reviewed in [50]). They can also induce abasic sites by base elimination leading to 3′-blocking lesions that are repaired by TDP1 [7, 51]. On the other hand, Top1 inhibitors induce 3′-DNA ends with covalently attached Top1 and 5′-DNA ends bearing a sugar hydroxyl [10, 26]. PARP1 preferentially binds directly to base excision repair-intermediates with a 5′-dRP rather than to 5′-phosphate ends [48, 52]. Thus, PARP-trapping preferentially occurs at 5′-dRP generated during base excision repair triggered by alkylating agents while it doesn't occur at Top1 induced-DNA break sites (Fig. 10.5). 5′-dRP is also generated during the repair of endogenous oxidative DNA damage, which can explain the differential cytotoxicity of clinical PARP inhibitors as single agent [7, 9]. Since any drugs that induce 5′-dRP can be favorable combination drugs with PARP inhibitors, it will be worthwhile searching such drugs as novel combinations for PARP inhibitors treatment.

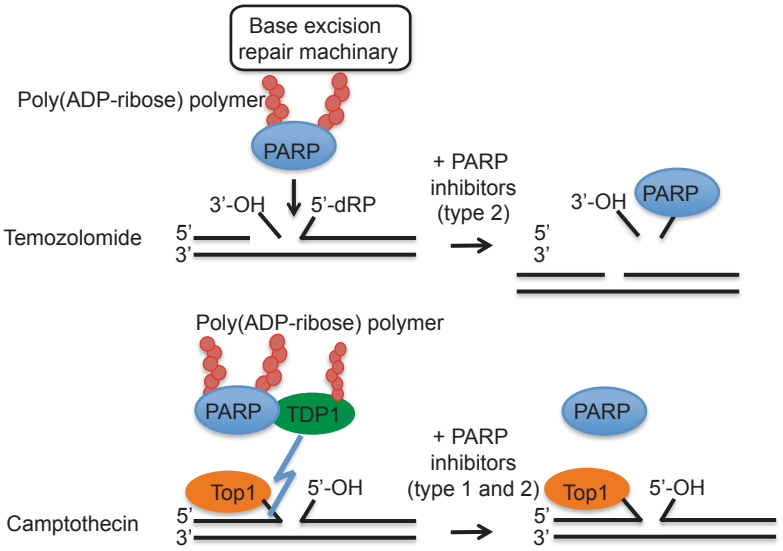

Fig. 10.5 Differential interactions of PARP with DNA damage sites induced by alkylating agents (temozolomide or MMS, top) and Top1 inhibitors (camptothecin, bottom). Temozolomide induces base damage sensed by PARP, and generates a 1-nucleotide gap with 3′-OH and 5′-deoxyribose phosphate (5′-dRP) groups at the ends of the breaks. PARP catalytically activates and recruits the base excision repair machinery. In the presence of PARP inhibitors (type 2), PARP binds 5′-dRP ends, generating PARP-DNA complexes. Camptothecin induces 3′-DNA ends with covalently attached Top1 and 5′-DNA ends bearing a sugar hydroxyl. Coupling of PARP and TDP1 mediated by catalytic PARP activity is required for the cleavage of Top1-DNA bound (see Fig. 10.3). In the presence of PARP inhibitors (type 1 and 2), PARP cannot work for the repair but not binds tightly to DNA ends, explaining the lack of detectable PARP trapping

10.5 Conclusion

Current clinical PARP inhibitors have dual functions: catalytic PARP inhibition, which is common and relatively similar across the different PARP inhibitors, and PARP trapping, which is markedly different for different PARP inhibitors with substantially diverse potency. Synergistic effect of PARP inhibitors in combination therapy can be derived from the different functions although it is not sharply distinguishable. Accordingly, it is important to take into account the differential property of each PARP inhibitor as well as the differential property of each drug combination.

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