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Critical Reviews and Perspectives

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Targeting DNA repair mechanisms in cancer therapy: the role of small molecule DNA repair inhibitors

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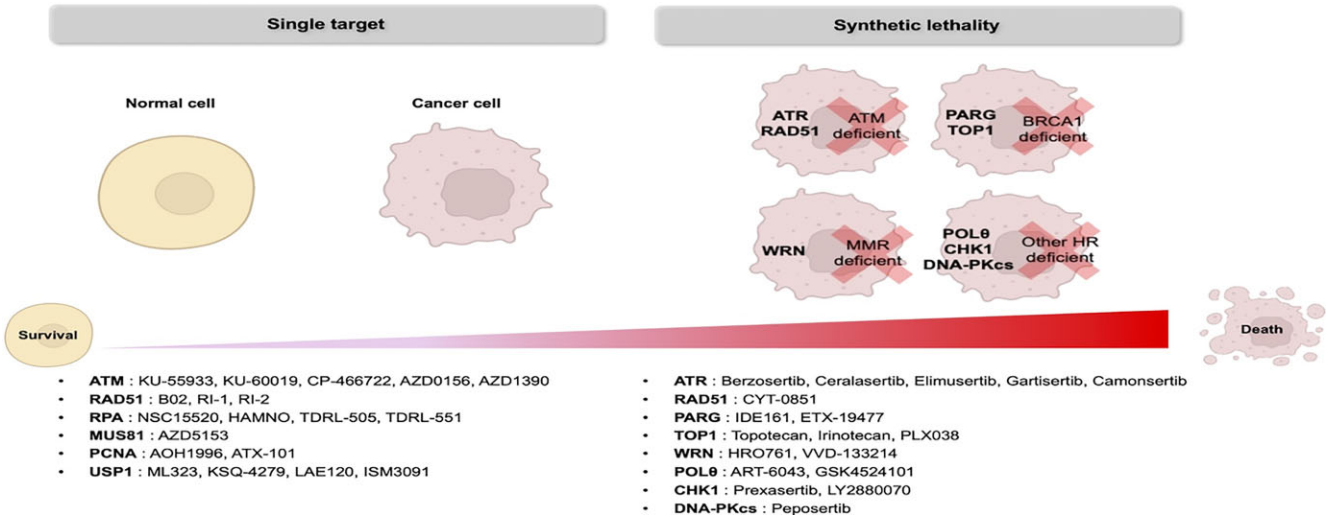
Abstract

Genomic instability and the accumulation of DNA damage are hallmarks of cancer, often resulting from defects in DNA repair pathways. While normal cells rely on highly coordinated DNA damage response (DDR) mechanisms to maintain genomic integrity, cancer cells exploit aberrant DDR regulation to sustain uncontrolled proliferation and survival. Despite significant advancements in chemotherapy, targeted therapy, and immunotherapy, the emergence of resistance remains a major challenge in cancer treatment. Small molecule inhibitors targeting key DDR proteins have emerged as promising therapeutic agents, not only as direct anticancer drugs but also as indispensable tools for dissecting the molecular intricacies of DNA repair. Recent therapeutic approaches leverage synthetic lethality and DDR pathway vulnerabilities to selectively eradicate tumor cells while minimizing damage to normal tissues. These inhibitors provide insights into mechanisms of tumor resistance, facilitating the rational design of combination therapies to enhance treatment efficacy. This review examines the latest advancements in DNA repair-targeted therapeutics, with a focus on small molecule inhibitors currently under clinical investigation. Additionally, we discuss emerging strategies for optimizing DDR-targeted interventions, including biomarker-driven patient selection and rational drug combinations. Understanding these molecular interactions will contribute to the development of novel, more effective treatment paradigms for cancer therapy.

Graphical abstract



Introduction

One of the hallmark features of cancer is genomic instability caused by defects in DNA damage repair mechanisms, which fundamentally drives uncontrolled cell division and proliferation. Normal cells maintain genomic stability through various DNA repair pathways, but cancer cells often exhibit defects in one or more repair pathways, leading to mutation accumulation and exacerbated genomic instability [1]. This instability not only contributes to cancer progression and metastasis but also serves as a major factor in treatment resistance.

Base excision repair (BER) is a fundamental pathway that resolves small base lesions and abasic sites to maintain genomic stability (Fig. 1). The BER process requires the coordinated activity of proteins such as poly (ADP-ribose) glycohydrolase (PARG), proliferating cell nuclear antigen (PCNA), and ubiquitin-specific protease 1 (USP1), which regulate repair efficiency and ensure proper coordination with DNA replication and chromatin remodeling. Defects in BER compromise cellular responses to endogenous and exogenous DNA damage and contribute to tumorigenesis,

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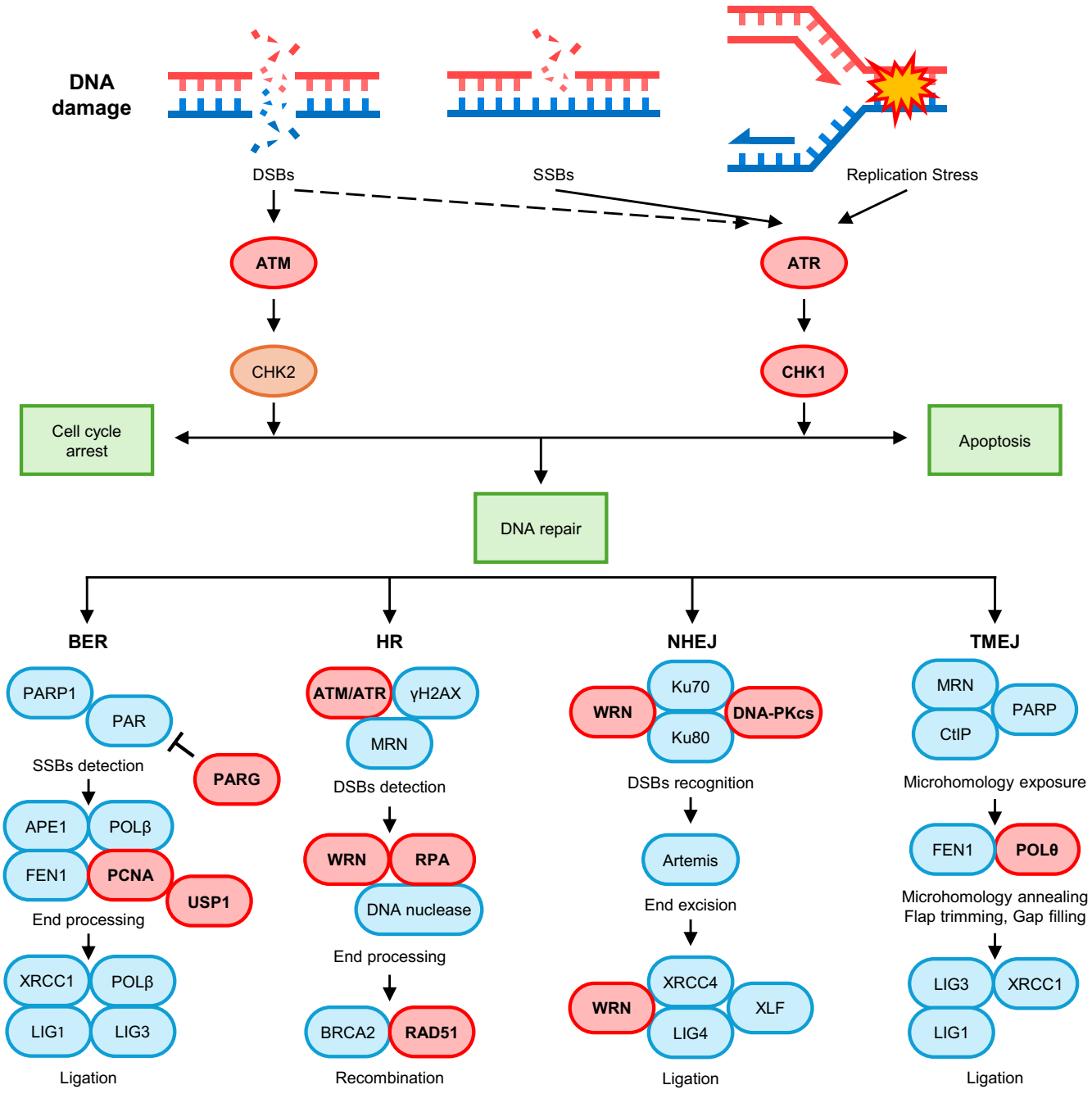

Figure 1. Overview of DDR and repair pathways DNA damage, including DSBs, SSBs, and replication stress, activates key sensor kinases such as ATM and ATR. ATM primarily responds to DSBs and signals through CHK2 to promote cell cycle arrest and facilitate DNA repair, while ATR responds to SSBs and replication stress via CHK1, leading either to repair or apoptosis depending on the extent of damage. DNA repair is orchestrated through four major pathways: BER, HR, NHEJ, and TMEJ. BER primarily addresses SSBs through PARP1 activation and subsequent recruitment of repair factors such as APE1, POLβ, and PCNA, with regulation by PARG. HR is a high-fidelity repair mechanism for DSBs involving ATM/ATR, γH2AX, MRN complex, RPA, WRN, and RAD51-mediated recombination. NHEJ repairs DSBs via Ku70/Ku80 heterodimer recognition, DNA-PKcs activation, and processing by Artemis, XRCC4, LIG4, and XLF, with WRN supporting intermediate processing. TMEJ, an alternative end-joining pathway mediated by POLθ and FEN1, repairs DSBs in the absence of classical HR or NHEJ. Proteins highlighted in red indicate key therapeutic targets currently under clinical trial for cancer treatment.

highlighting BER-associated proteins as potential therapeutic targets [2].

Homologous recombination (HR) is an essential pathway for accurately repairing DNA double-strand breaks (DSBs) by using a sister chromatid with identical sequence as a template [3] (Fig. 1). The HR process requires the coordinated action of proteins such as breast cancer type 1/2 (BRCA1/2) and RAD51 homolog 1 (RAD51). Dysfunction of HR results in increased genomic instability and altered sensitivity to certain therapies [4]. Consequently, strategies that directly inhibit HR or exploit HR deficiencies as therapeutic vulnerabilities have gained significant attention as anticancer therapies [5].

Nonhomologous end joining (NHEJ), on the other hand, rapidly repairs DSBs by directly ligating broken DNA ends

without requiring extensive sequence homology [6] (Fig. 1). NHEJ is particularly active during the G0/G1 phases of the cell cycle and serves as a primary DSB repair mechanism in situations requiring swift repair [7]. Cancer cells with defective HR often rely on alternative pathways like NHEJ for survival, making key NHEJ components attractive targets for selectively killing cancer cells [8].

Theta-mediated end joining (TMEJ), also known as polymerase TMEJ, serves as an alternative end-joining pathway that repairs DSBs when HR or classical NHEJ are unavailable (Fig. 1). This pathway is critically dependent on DNA polymerase theta (POLθ), which facilitates microhomology-mediated repair at resected DNA ends. Because cancer cells deficient in HR often rely on TMEJ for survival, POLθ has emerged as an attractive synthetic-lethal target in HR-deficient tumors [9].

The synthetic lethality is a promising concept in cancer biology that is being applied in clinical trials: loss of function in one gene alone does not significantly affect cell viability, but the simultaneous loss of two specific gene functions results in cell death [10]. In cancer therapy, targeting a specific defect in tumor cells (e.g. HR deficiency due to BRCA mutations) and pharmacologically inhibiting its compensatory pathway [e.g. poly-ADP ribose polymerase (PARP)-mediated opportunistic repair] can induce a "lethal vulnerability" absent in normal cells, selectively killing cancer cell [4, 11]. The remarkable efficacy of PARP inhibitors (PARPis) in BRCA-mutated cancers is a prime example, sparking extensive research and applications of synthetic lethal interactions among various DNA repair pathways [12].

This review summarizes key regulatory factors of HR and NHEJ pathways and anticancer strategies targeting them, while exploring the latest cancer treatment approaches based on synthetic lethality. Focusing on preclinical and clinical research trends of HR and NHEJ inhibitors, as well as clinical trial data for DNA damage repair targets, we examine the present and future of precision-targeted therapies for cancer patients with DNA repair deficiencies.

Targeting key regulators of HR and NHEJ pathways in cancer

ATM

Ataxia-telangiectasia mutated protein (ATM) is a key kinase that recognizes DSBs and phosphorylates various downstream proteins involved in DNA repair, cell cycle regulation, and apoptosis [13]. When DSBs occur due to radiation or other insults, ATM activation induces an effective DNA damage response (DDR), making it a critical factor in cancer cell resistance to radiation [14]. Thus, ATM inhibitors are actively studied to impair cancer cell DNA repair, disrupt cell cycle checkpoints, and enhance sensitivity to radiotherapy or chemotherapy [15].

Small molecule ATM inhibitors almost universally target the ATP-binding pocket of the kinase domain [16] (Table 1). The classic first-generation inhibitor KU-55933 was followed by second generation compounds that improved potency, selectivity, and solubility, most notably KU-60019 [17, 18]. KU-60019 radiosensitizes cells at low micromolar concentrations and suppresses prosurvival signaling in glioma models [19, 20]. While it is nontoxic *in vitro* and safe after direct intracranial injection, its poor blood–brain barrier (BBB) permeability limits systemic use [19].

CP-466722 was an early ATP-competitive probe that rapidly and reversibly inhibited ATM. Kinome profiling revealed substantial off-target activity (25 kinases, including ALK2), poor selectivity, and a short half-life, leading to its discontinuation from clinical development [21]. Nevertheless, it remains useful in preclinical settings for transient ATM blockade.

AZD0156 (AstraZeneca) is an oral, ATP–competitive ATM inhibitor that showed strong synergy with PARPi and topoisomerase (TOP)–I inhibitors in BRCA–mutated xenografts and sensitized TP53–deficient lung–cancer cell lines to radiation [22, 23]. However, a phase I trial (NCT02588105) was terminated after only 2 of 46 patients achieved partial responses and hemolytic toxicity was observed [24].

In response, AstraZeneca advanced AZD1390, a next–generation, BBB–penetrant ATM inhibitor engineered to evade P-glycoprotein (P-gp/*ABCB1*)–mediated efflux—the ATP-driven export of drugs and other exogenous compounds from cells. AZD1390 achieves ~6–fold higher brain exposure than AZD0156 and potently blocks ATM autophosphorylation and downstream signaling at 3 nM, inducing apoptosis when combined with radiation [25]. In orthotopic glioma models, AZD1390 prolongs survival, and PET imaging confirms its central nervous system (CNS) penetration [26]. Interim phase I data in recurrent glioblastoma (NCT03423628, NCT05182905, and NCT06894979) show a median overall survival of 12.7 months, suggesting clinical benefit [26–29]. Beyond radiosensitization, AZD1390 elicits immunomodulation by promoting cytosolic DNA accumulation, activating the cGAS–STING pathway, up–regulating PD–L1, and down–regulating galectin–9, thereby enhancing antitumor immunity [30]. Ongoing trials are testing AZD1390 in combination with radiotherapy, PARPi, or immunotherapies.

RAD51

RAD51 is a central protein in HR, forming filaments on single-stranded DNA (ssDNA) to facilitate strand exchange with homologous double-stranded DNA, enabling accurate DSB repair [31]. Overexpression of RAD51 in various cancers is associated with enhanced DNA repair capacity and resistance to radiation and chemotherapy [32]. RAD51 inhibition disrupts HR, forcing cells to rely on error-prone repair mechanisms and promoting genomic instability in replicating cancer cells [33].

Small–molecule inhibitors target RAD51 by blocking DNA binding, preventing oligomerization, or destabilizing filament assembly [34]. Unlike PARPi or ATM inhibitors that act upstream of the DDR, RAD51 inhibitors directly abrogate the core recombination step [31]. Tumors with elevated RAD51, high replication stress, or defective checkpoints are particularly vulnerable, whereas normal cells, which depend less on RAD51, may be spared—offering a potential therapeutic window [35].

B02 was identified from a high–throughput screen of >200 000 compounds [36] (Table 1). B02 binds the ATP–binding pocket of RAD51 and inhibits its ATP binding-dependent strand–exchange activity [37]. In cancer cells it suppresses RAD51 foci and sensitizes them to DNA–damaging agents such as cisplatin [38]. In xenograft studies, the combination of B02 and cisplatin markedly reduced tumor growth, demonstrating that RAD51 inhibition can potentiate chemotherapy–induced damage [39]. Although derivatives with improved affinity, pharmacokinetics, and activity against

Table 1. HR and NHEJ pathway inhibitors in clinical trials.

Target protein	Drug	NCT identifier	Type of cancer	Combination	Phase	References
ATM	KU-55933	Preclinical	–	–	–	[17]
	KU-60019	Preclinical	–	–	–	[17–20]
	CP-466722	Preclinical	–	–	–	[21]
	AZD0156	ClinicalTrials.gov, NCT02588105	Advanced cancer	Olaparib or Irinotecan, FOLFIRI	1	[23, 24]
	AZD1390	ClinicalTrials.gov, NCT03423628	Brain cancer	Radiotherapy	1	[26, 27]
		ClinicalTrials.gov, NCT05182905	Glioma	–	1	[28]
		ClinicalTrials.gov, NCT06894979	Glioma	Radiotherapy	1	[29]
RAD51	B02	Preclinical	–	–	–	[36–39]
	RI-1	Preclinical	–	–	–	[40–42]
	RI-2	Preclinical	–	–	–	[43]
RPA	NSC15520	Preclinical	–	–	–	[51, 52]
	HAMNO	Preclinical	–	–	–	[53, 54]
	TDRL-505	Preclinical	–	–	–	[56, 57]
	TDRL-551	Preclinical	–	–	–	[58]
MUS81	AZD5153	ClinicalTrials.gov, NCT03205176	Lymphoma and solid tumor	Olaparib	1, 2	[61, 62, 65, 66]
PCNA	AOH1996	ClinicalTrials.gov, NCT05227326	Advanced solid tumor	–	1	[69, 71–73]
	ATX-101	ClinicalTrials.gov, NCT04814875	Ovarian cancer	Carboplatin or Pegylated liposomal doxorubicin	1, 2	[74, 76, 77]
		ANZCTR, ACTRN 12618001070224	Non-Hodgkin lymphoma	Etoposide or Prednisone, Cyclophosphamide, Doxorubicin, Rituximab	1, 2	[74]
USP1	ML323	Preclinical	–	–	–	[82–84]
	KSQ-4279 (RO7623066)	ClinicalTrials.gov, NCT05240898	HRD and advanced solid tumor	Olaparib or Carboplatin	1	[81, 85–87]
	LAE120	–	Advanced solid tumor	Olaparib	1	[88]
	ISM3091 (XL309)	ClinicalTrials.gov, NCT05932862	Advanced solid tumor	Olaparib	1	[89]

triple–negative breast cancer (TNBC) that was resistant to olaparib have been generated, B02 remains limited by hydrophobicity, low target specificity, and poor *in vivo* stability, and it has not entered clinical trials.

RI–1 was identified in a 10 000–compound screen [40]. RI–1 covalently modifies cysteine 319 at the RAD51 oligomerization interface, disrupting filament assembly and HR [40]. It increases DNA damage and enhances radiosensitivity and PARPi efficacy [41, 42]. However, chemical instability and a short half–life preclude *in vivo* use [43]. Its non–covalent analog RI–2 was designed for better metabolic stability but shows reduced potency [43]. Neither compound has progressed to human studies, yet they validate RAD51 as a tractable target.

Furthermore, RAD51 inhibitors are linked to synthetic lethality strategies (see Section 2.1), with expectations of selective killing in tumors with increased RAD51 dependency due to ATM or CHK1 deficiencies.

RPA

Replication protein A (RPA) is a heterotrimeric complex that binds ssDNA with high affinity and plays a central role in DNA replication and repair [44]. By stabilizing ssDNA intermediates and coordinating the recruitment of diverse repair factors, RPA is essential for multiple DDR pathways, including HR, BER, and nucleotide excision repair (NER) [45]. Notably, RPA subunits are often overexpressed or hyperphosphorylated in cancers, correlating with enhanced replication stress tolerance and resistance to therapy [46]. Given its central role in preserving genome stability, RPA has emerged as an attractive target for cancer therapy, particularly in tumors exhibiting high replication stress or DNA repair deficiencies [47].

Several small molecule RPA inhibitors have been developed to either block RPA's DNA-binding activity or interfere with its protein–protein interaction domains, though most candidates remain in preclinical stages of development [48]. Unlike upstream DDR modulators such as PARP or ATM inhibitors that act on signaling pathways, RPA inhibitors directly cripple the core ssDNA scaffolding required for replication and repair [49]. Tumors experiencing heightened replication stress or lacking robust checkpoints (e.g. due to ATR or CHK1 deficiencies) are particularly vulnerable to RPA suppression, whereas normal cells with lower replication stress may better tolerate transient RPA inhibition, providing a potential therapeutic window [50].

NSC15520 (fumaropimaric acid) was one of the first RPA inhibitors identified via screening efforts [51] (Table 1). It binds to the N-terminal domain of the RPA1 subunit (RPA70N), a region responsible for recruiting ATR and other proteins [51]. In cancer cell models, NSC15520 treatment

destabilizes replication forks and sensitizes cells to genotoxic stress, effectively lowering the threshold for DNA damage-induced cell death [52]. However, NSC15520 is limited by suboptimal drug-like properties—including hydrophobicity, low specificity and poor metabolic stability—which have prevented its advancement beyond preclinical studies [52]. Derivatives with improved binding affinity and cellular uptake have been explored, but thus far none have progressed to clinical trials [52].

Another RPA70N-targeted inhibitor, HAMNO, similarly binds the RPA1 N-terminal domain and was shown to prevent ATR autophosphorylation [53]. While HAMNO increases DNA damage and can enhance the cytotoxicity of DNA crosslinking agents *in vitro*, its chemical instability and short half-life in cells have precluded *in vivo* use [54]. Like NSC15520, HAMNO remains a prototype tool compound, underscoring the challenges of targeting RPA's protein-interaction domain [55].

Efforts have focused on inhibiting RPA's DNA-binding activity. TDRL-505 was discovered through high-throughput screening as a small molecule that disrupts the binding of RPA to ssDNA [56]. TDRL-505 targets the central OB-fold domains of RPA1, preventing RPA from properly coating and protecting ssDNA. In cancer cells, this leads to unprotected replication forks, triggering replication stress, S-phase arrest, and apoptosis [56]. Importantly, TDRL-505 was found to synergize with DNA-damaging treatments: combining TDRL-505 with chemotherapeutic agents like cisplatin or etoposide markedly increases tumor cell killing compared to either agent alone [56, 57]. Structure–activity refinement of TDRL-505 yielded a second-generation analog, TDRL-551, with enhanced potency against RPA–ssDNA interactions [58]. TDRL-551 exhibits modest single-agent anticancer activity in preclinical models but shows strong synergy in combination with DNA-damaging chemotherapy, further validating the strategy of chemically targeting RPA's ssDNA-binding function [58].

MUS81

Crossover junction endonuclease MUS81 (MUS81) is a structure specific endonuclease that resolves stalled replication forks by cleaving complex DNA intermediates such as Holliday junctions [59]. Its activity is essential for replication restart and repair, especially in cancer cells where genomic instability drives heightened replication stress [60]. Elevated MUS81 correlates with aggressive tumor behavior and poor prognosis; in gastric cancer, for instance, overexpression associates with metastasis and adverse outcomes [61] (Table 1). Recent evidence also links MUS81 to the regulation of epithelial–to–mesenchymal transition (EMT) transcription factors, notably ZEB1, suggesting a dual role in DNA repair and metastatic progression [61, 62].

No direct MUS81 inhibitors have entered clinical use, but indirect strategies are being explored [63]. A promising avenue targets the epigenetic regulator bromodomain-containing protein 4 (BRD4) [61]. The bromodomain inhibitor AZD5153 downregulates MUS81 transcription in cancer cells [61]. Although AZD5153 is not a nuclease inhibitor, its broad modulation of chromatin architecture alters the expression of numerous genes, including MUS81 and the EMT factor ZEB1 [61, 62]. In xenograft models, AZD5153 markedly inhibited metastatic spread—a phenotype recapitulated by MUS81 knockout (KO), confirming MUS81 as a key mediator of the drug's activity [61]. Consequently, AZD5153 induces accumulation of unresolved DNA intermediates, enhances replication stress, and simultaneously suppresses cell motility and invasiveness [62]. UNI66, another inhibitor of BRD4, suppresses HR by inhibiting BRD4-mediated transcription of CtBP-interacting protein (CtIP) and RAD51, thereby inducing synthetic lethality in PARP1-deficient cells and enhancing tumor sensitivity to PARPis [64].

AZD5153 combined with olaparib is being evaluated as monotherapy in patients with solid tumors and hematologic malignancies (NCT03205176) [65]. It is orally bioavailable and has shown a favorable safety profile in early–phase trials. In hepatocellular carcinoma and other malignancies, AZD5153 downregulates oncogenic transcription programs, including c–Myc, producing pronounced antiproliferative and pro–apoptotic effects [66]. Clinical data confirm on–target engagement through suppression of BRD4–regulated genes [65]. Its impact on MUS81 and ZEB1 expression offers potential biomarker–guided therapeutic opportunities, particularly for tumors with high levels of these proteins [66].

PCNA

PCNA is a trimeric protein that acts as a clamp during DNA replication and repair, encircling the DNA double helix to recruit and anchor DNA polymerases and repair proteins for efficient function [67]. In rapidly dividing or treatment-resistant cancer cells, PCNA expression and activity are often elevated, reflecting high demands for DNA replication and damage repair [68]. Previously considered "undruggable" due to its essential role in normal cell survival, tumor–specific post–translational modifications generate a cancer–associated PCNA isoform with unique surface epitopes that can be selectively targeted [69, 70].

AOH1996 is a small–molecule inhibitor developed at City of Hope, named after a pediatric cancer patient [71] (Table 1). It binds at the trimer–trimer interface, locking PCNA in an inactive conformation and preventing interaction with client proteins [71]. Crystal structures reveal that three AOH1996 molecules occupy two PCNA trimers, effectively blocking function [71]. The resulting replication fork stalling, heightened replication stress, and transcription–replication conflicts produce DSBs and trigger apoptosis [71]. Preclinical data show activity in >70 solid–tumor cell lines (e.g. breast, lung, colorectal, prostate, etc.) with negligible effect on normal cells [72, 73]. AOH1996 is currently in a phase I trial for advanced solid tumors (NCT05227326).

ATX–101 is a cell–penetrating peptide from APIM Therapeutics that incorporates the natural PCNA–binding motif APIM (AlkB homolog 2 PCNA–interacting motif) [74, 75]. Normal cells, which rely primarily on PIP–box interactions under basal conditions, are less affected [76]. Preclinical studies demonstrate that ATX–101 induces apoptosis and markedly potentiates platinum drugs, gemcitabine, and radiotherapy by inhibiting DNA–repair pathways in tumor cells while sparing normal tissue [76, 77]. A phase I trial (ACTRN12618001070224) administered 20 terminal patients intravenously; 70 % achieved disease stabilization at 6 weeks, and one patient-maintained stability for >2 years [74]. No dose–limiting toxicities occurred up to 60 mg/m^2, with only mild infusion reactions. Phase II studies are underway (NCT04814875). Pharmacokinetics reveal a plasma half–life of <30 min, but weekly dosing sustains intracellular PCNA inhibition [74].

USP1

USP1 inhibitors are emerging precision anticancer agents targeting DDR pathways [78]. USP1 deubiquitinates PCNA and FANCD2—key mediators of replication-stress tolerance and interstrand crosslink (ICL) repair via the FANCI–FANCD2 axis [79]. At stalled forks, monoubiquitinated PCNA recruits translesion polymerases, while FANCD2-Ub localizes to damage sites to initiate ICL repair complex assembly; the USP1–UAF1 complex removes these ubiquitin marks to terminate signaling and resume replication/repair [80]. BRCA1-deficient tumors that depend on fork protection show synthetic lethal sensitivity, and high USP1 expression may further predict responsiveness to DDR-targeted strategies [81].

The preclinical inhibitor ML323 selectively targets USP1–UAF1, increases PCNA-Ub and FANCD2-Ub, amplifies replication stress, promotes apoptosis, and sensitizes tumor cells to cisplatin, suggesting a route to overcome platinum resistance [82–84]. KSQ-4279 (RO7623066), the first oral USP1 inhibitor to enter clinical trials, shows high selectivity and robust efficacy, including tumor regression in HR-deficient and PARPi-resistant models; combination with olaparib is synergistic (NCT05240898) [81, 85–87]. Ongoing phase I studies are evaluating monotherapy and combinations, with early readouts indicating favorable PK/PD and manageable, primarily hematologic, toxicity, supporting dose optimization and safety monitoring [86].

Additional agents include LAE120 (Laekna Inc.), an oral small molecule that has entered a first-in-human phase I study for advanced solid tumors in the U.S. The trial aims to evaluate safety, tolerability, pharmacokinetics, and preliminary efficacy, including potential combinations with PARPis [88]. As of 2025, the study has been initiated, although a ClinicalTrials.gov identifier has not yet been publicly listed. Another agent, ISM3091 (XL309), is being investigated in a U.S. phase I study (NCT05932862) for BRCA-mutated ovarian and breast cancers [89]. ISM3091 induces replication stress and DNA damage accumulation, and PARPi combinations are under active investigation [89]. Overall, USP1 inhibition offers a multifaceted strategy—synthetic lethality, fork destabilization, and reversal of PARPi resistance—and is poised to become a key component of future DDR-targeted combination therapies.

Synthetic lethality-based strategies in cancer therapy

ATR and RAD51 inhibition strategies in ATM-deficient cancer cells

ATM-deficient cancer cells cannot effectively signal DSBs, making them highly dependent on ataxia telangiectasia and Rad3-related protein (ATR) to manage replication stress and DNA damage [90]. ATR is the principal regulator of replication-stress responses: when forks stall during S phase, ATR stabilizes forks, activates checkpoint kinase 1 (CHK1) to pause the cell cycle, and promotes repair [91]. Because oncogene-driven tumors experience chronic replication stress, they often become dependent on ATR signaling, whereas normal cells tolerate partial ATR inhibition even though complete ATR loss is lethal [92]. Small molecule ATR inhibitors block the kinase domain and prevent phosphorylation of substrates such as CHK1, leading to unchecked origin firing, fork collapse, S/G2 checkpoint abrogation, and replication catastrophe with widespread DSBs and cell death [93]. Tumors with ATM loss, p53 loss, or ARID1A mutation are therefore hypersensitive, making ATR an attractive synthetic lethal target [90, 94].

Berzosertib (M6620, formerly VX-970) is the first ATR inhibitor to enter clinical trials and is an intravenous, selective ATP-competitive agent [95]. In preclinical models it potentiated cisplatin and irradiation, particularly in ATM-deficient settings, by preventing G2 arrest and repair and forcing lethal mitosis; combinations with cisplatin or topotecan produced deeper tumor regressions than chemotherapy alone [95–97]. Early trials using day-2/9 dosing every 21 days showed manageable toxicity and activity; with topotecan, a recommended phase II dose of 210–240 mg/m^2 yielded partial responses or durable stable disease in small cell lung and ovarian cancers (NCT02157792) [95, 96, 98]. A notable complete response occurred with monotherapy in ATM-mutant gastric cancer, illustrating synthetic lethality. Myelosuppression—especially neutropenia in chemotherapy combinations—is dose limiting; in platinum-resistant ovarian cancer, berzosertib plus gemcitabine produced responses in ∼17% but required dose reductions. A randomized study with topotecan in relapsed small cell lung cancer did not significantly improve progression-free survival (NCT03896503), underscoring the need for biomarker-driven combinations and patient selection [96, 99, 100].

Ceralasertib (AZD6738) is an orally bioavailable, selective ATR inhibitor optimized from AZ20 for potency and pharmacokinetics [101]. It enhances DNA damaging treatments such as carboplatin in xenografts and is advancing through phase I/II trials [102, 103]. Toxicities are mainly hematologic and gastrointestinal; cardiotoxicity seen at high mouse doses has not emerged as a dominant clinical signal [104]. Because ATR inhibition can increase cytosolic DNA and activate cGAS–STING, ceralasertib is being combined with immunotherapy [105]. Preclinical hepatocellular carcinoma models showed improved tumor control with anti-PD-L1 therapy, and in the ATLANTIS phase II study in PD-1–refractory melanoma, durvalumab plus ceralasertib achieved objective responses in a subset of patients (∼10%–15%, NCT03780608) [106–108]. A parallel gastric-cancer study is ongoing (NCT03682289), supporting ATR inhibitors as both chemo/radiation sensitizers and immune modulators [108].

Elimusertib (BAY 1895344) is a potent oral ATR inhibitor with strong target engagement in preclinical models and a favorable nonclinical safety profile [109–112]. Intermittent schedules (3 days on/4 days off; 40 mg twice daily) permit normal-tissue recovery [109]. Single-agent activity has been observed in ATM-deficient tumors, including lymphomas; in a first-in-human study, 30% of evaluable patients achieved ≥4-month stable disease and two patients with ATM-loss cancers achieved partial responses (NCT03188965) [109]. Ongoing studies focus on ATM-mutant chronic lymphocytic leukemia, lymphomas, and solid tumors [113].

Additional ATR inhibitors include gartisertib (M4344/VX-803), which is being combined with the PARPi niraparib to co-target replication stress and single-strand break repair (NCT04149145) [114], and camonsertib (RP-3500), a next generation oral agent developed via a synthetic lethality platform [115]. Early studies of camonsertib reported partial responses—particularly in ATM-loss and high replication stress tumors—using intermittent dosing with manageable anemia and thrombocytopenia (NCT04497116) [115]. Camonsertib is under evaluation alone and with PARPi such

as talazoparib to overcome PARPi resistance (NCT04972110) [116]; Roche licensed the program in 2023, and phase II cohorts emphasize biomarker-selected populations.

ATM-deficient cancer cells rely heavily on RAD51-mediated HR because DSB signaling is weakened, and CHK1-deficient cells similarly depend on RAD51 due to impaired checkpoints [117]. RAD51 inhibition can therefore drive selective lethality by amplifying unrepaired DNA damage in these backgrounds [42, 118].

CYT-0851 is the most advanced clinical candidate nominally targeting RAD51 and is being evaluated orally as monotherapy and with chemotherapies such as gemcitabine or capecitabine (NCT03997968) [119]. Preclinically, it reduces RAD51 foci, increases γH2AX, and synergizes with PARPis in BRCA-proficient TNBC [120]. In first-in-human studies, it showed good oral bioavailability and a manageable safety profile; among evaluable patients, partial responses and durable disease stabilization were observed. Mechanistic work suggests CYT-0851 may act primarily as an MCT1 inhibitor, causing metabolic stress that secondarily impairs HR; despite this, it remains a promising way to exploit RAD51 dependence [121].

Inhibiting RAD51 pathway function can broaden the utility of PARPi in BRCA-wild-type tumors by suppressing HR and creating synthetic lethality [122]. The concept of "RAD51 addiction" in high replication stress cancers further supports combining RAD51 pathway agents with chemotherapy or other DDR inhibitors [123].

POLθ, CHK1, and DNA-PKcs inhibition strategies in HRD cancers

HR-deficient (HRD) cancer cells, such as those with BRCA1/2 mutations, lose the ability to accurately repair DSBs and rely on error-prone repair pathways [124]. POLθ, encoded by *POLQ* gene, is a specialized polymerase–helicase that mediates TMEJ, an error-prone backup for DSB repair when HR is unavailable. POLθ functions as a last resort repair option for cells that cannot use HR or classical NHEJ, and BRCA1/2-mutant tumors become highly dependent on POLθ to repair replication-associated DSBs and rescue broken forks [124] (Table 2). POLθ overexpression correlates with genomic instability and poor outcomes, suggesting tumors exploit its error-prone repair [125]. The polymerase domain can insert across damaged bases and microhomologies, and the helicase-like N-terminal domain aligns DNA ends for joining [126]. POLθ inhibitors block polymerase activity to prevent TMEJ and selectively kill HR-defective cells while sparing HR-proficient normal cells [127].

In BRCA1/2-mutant cell lines, POLθ inhibition causes accumulation of unrepaired damage and apoptosis, mirroring POLQ KO [127, 128]. The tool compound ART558 inhibit POLθ's extension activity in the nanomolar range, indicating specificity for TMEJ [129]. Combining ART558 with olaparib increases replication stress and produces synergistic lethality [128]. POLθ inhibition can also overcome PARPi resistance caused by loss of the 53BP1/Shieldin complex, because such tumors remain dependent on POLθ for residual end joining [128]. In xenograft models of BRCA-mutant breast cancer, POLθ inhibitor monotherapy suppressed tumor growth, and in 53BP1-deficient, HR-restored tumors, POLθ inhibition resensitized tumors to PARP inhibition [128]. These findings position POLθ inhibitors as synthetic lethal monotherapies for HRD cancers and as agents to address PARPi resistance.

ART-6043 is the oral POLθ inhibitor to enter clinical trials developed by Artios. ART-6043 entered global first-in-human studies in 2021 as monotherapy and in combination with PARPi such as olaparib or niraparib (NCT05898399) in BRCA1/2-mutant breast, ovarian, prostate, and pancreatic cancers [124, 129]. Interim results indicate target engagement, an acceptable safety profile dominated by low grade fatigue and nausea, and disease stabilization in some patients; the combination with olaparib or niraparib has shown at least additive antitumor activity [124, 129]. Based on phase I signals, Artios initiated a phase II expansion in BRCA-mutant breast cancer to test whether POLθ inhibition can deepen responses or overcome PARPi resistance [124, 129].

GSK-4524101 is a POLθ inhibitor being developed by GlaxoSmithKline. While details are limited (structure not public), GSK disclosed the start of a Phase I trial (NCT06077877) of GSK-4524101 in late 2023, in patients with advanced solid tumors harboring HRD [130]. Likely, GSK's compound shares a similar mechanism—selective POL inhibition. Preclinically, GSK's POLθ inhibitor demonstrated synergy with niraparib and killed BRCA KO cancer cells [130].

HRD cancer cells also heavily rely on CHK1, a key downstream effector of ATR that stabilizes stressed replication forks and enforces the S and G2/M checkpoints by inactivating CDC25 phosphatases [131–133]. In HRD cells, CHK1 inhibition permits division with unrepaired lesions, leading to mitotic catastrophe and apoptosis, and this effect is amplified in p53-deficient tumors that have lost the G1/S checkpoint [134].

Prexasertib (LY2606368) is an intravenous, potent ATP-competitive CHK1 inhibitor (IC_{50} < 1 nM) with weaker activity against CHK2 [135]. By disabling checkpoint arrest after DNA damage, it forces entry into mitosis with broken DNA, producing γH2AX foci, fragmented chromosomes, and apoptosis [136]; as a single agent it can also trigger DSBs via unrestrained origin firing, reflected by increases in RAD51 and RPA foci [137–139]. Tumor cells with p53 disruption or cyclin-E overactivity are particularly sensitive [140]. Preclinically, prexasertib is active in squamous carcinomas, small cell lung cancer, and ovarian cancer, often in RB-deficient or high-E2F tumors [140–142]. In head and neck xenografts it induced regressions, and in combination it synergized with cisplatin, gemcitabine, and PARPi [143–145]; in BRCA-proficient TNBC, prexasertib plus PARPi produced *in vivo* efficacy, suggesting utility in PARPi resistance [140, 142].

Clinically, phase I/II trials show manageable safety dominated by transient, dose limiting neutropenia (105 mg/m^2 IV every 14 days), while nonhematologic events are generally mild [141, 142, 146]. Objective responses have been observed: in platinum-resistant ovarian cancer, prexasertib monotherapy achieved ~30% responses (including a complete response) irrespective of BRCA status, with some patients maintaining stable disease for 6–12 months (NCT03414047) [147]. Activity in recurrent BRCA-wild-type TNBC has been modest (NCT03057145 and NCT02124148), and a small study in small cell lung cancer combining prexasertib with an immune checkpoint inhibitor yielded responses in a subset (NCT02808650) [148–151].

LY2880070 is a second generation, orally bioavailable, selective ATP-competitive CHK1 inhibitor (IC_{50} ~1 nM) with minimal CHK2 activity [152]. As monotherapy it showed limited efficacy despite pharmacodynamic target engagement (re-

Table 2. Clinical trials employing synthetic lethality strategies.

Type of cancer	Target protein	Drug	NCT identifier	Combination	Phase	References
ATM-deficient cancer	ATR	Berzosertib (M6620, VX-970)	ClinicalTrials.gov, NCT02157792	Gemcitabine or Cisplatin, Etoposide, Carboplatin, Irinotecan	1	[95, 96]
			ClinicalTrials.gov, NCT03896503	Topotecan	2	[99]
		Ceralasertib (AZD6738)	ClinicalTrials.gov, NCT03780608	Durvalumab	2	[107, 108]
			ClinicalTrials.gov, NCT03682289	Monotherapy or Olaparib, Durvalumab	2	[108]
		Elimusertib (BAY1895344)	ClinicalTrials.gov, NCT03188965	–	1	[109]
		Gartisertib (M4344/VX-803)	ClinicalTrials.gov, NCT04149145	Niraparib	1	[114]
		Camonsertib (RP-3500)	ClinicalTrials.gov, NCT04497116	Monotherapy or Talazoparib, Gemcitabine	1, 2a	[115]
			ClinicalTrials.gov, NCT04972110	Niraparib or Olaparib	1b, 2	[116]
	RAD51	CYT-0851	ClinicalTrials.gov, NCT03997968	Gemcitabine or Capecitabine, Rituximab and bendamustine	1, 2	[119, 121]
HRD cancer	POLθ	ART-6043	ClinicalTrials.gov, NCT05898399	Olaparib or Niraparib	1, 2a	[124]
		GSK4524101	ClinicalTrials.gov, NCT06077877	Niraparib	1, 2	[130]
	CHK1	Prexasertib (LY2606368)	ClinicalTrials.gov, NCT03414047	–	2	[147]
			ClinicalTrials.gov, NCT03057145	Olaparib	1	[148]
			ClinicalTrials.gov, NCT02124148	Cisplatain or Cetuximab, G-CSF, Pemetrexed, Fluorouracil, Leucovorin, LY3023414	1	[149]
			ClinicalTrials.gov, NCT02808650	–	1	[150, 151]
		LY2880070	ClinicalTrials.gov, NCT02632448	Gemcitabine	1, 2	[153–155]
			ClinicalTrials.gov, NCT05275426	Gemcitabine	2	[155]
	DNA-PKcs	Peposertib (M3814)	ClinicalTrials.gov, NCT04533750	Cisplatin or Radiotherapy	1	[170]
			ClinicalTrials.gov, NCT03770689	Capecitabine or Radiotherapy	1, 2	[170, 171]
			ClinicalTrials.gov, NCT04092270	Pegylated liposomal doxorubicin	1	[161]
			ClinicalTrials.gov, NCT05687136	M1774 (ATR inhibitor)	1b	[172]
			ClinicalTrials.gov, NCT04068194	Avelumab	1, 2	[173]
BRCA-deficient cancer	PARG	IDE161	ClinicalTrials.gov, NCT05787587	Monotherapy or Pembrolizumab	1	[176, 181]
		ETX-19477	ClinicalTrials.gov, NCT06395519	–	1, 2	[176, 182]
	TOP1	Topotecan	ClinicalTrials.gov, NCT00484666	Docetaxel	1, 2	[188, 189]
			ClinicalTrials.gov, NCT00305942	Carboplatin	2	[190]
			ClinicalTrials.gov, NCT00276796	Paclitaxel or Cisplatin	2	[191]
			ClinicalTrials.gov, NCT03289910	Carboplatin or Veliparib	2	[197]
		Irinotecan/SN-38	ClinicalTrials.gov, NCT00311610	Liposomal SN-38	2	[192,193]
			ClinicalTrials.gov, NCT02292758	Cetuximab or Bevacizumab	2	[194]
			ClinicalTrials.gov, NCT00389870	Panitumumab or Cyclosporine	3	[195]

Table 2. Continued

Type of cancer	Target protein	Drug	NCT identifier	Combination	Phase	References
			ClinicalTrials.gov, NCT03290937	Utomilumab or Cetuximab	1	[196]
		PLX038 (PEGylated SN-38)	ClinicalTrials.gov, NCT05465941	–	2	[199]
			ClinicalTrials.gov, NCT04209595	Rucaparib or Ondansetron	1, 2	[198,200]
			ClinicalTrials.gov, NCT06337630	Tuvusertib (ATR inhibitor)	1	–
MMR-deficient cancer	WRN	HRO761	ClinicalTrials.gov, NCT05838768	Monotherapy or Pembrolizumab, Irinotecan	1	[205]
		VVD-133214	ClinicalTrials.gov, NCT06004245	Monotherapy or Pembrolizumab	1	[206]

duced phospho-CDC25C), possibly due to exposure or tumor heterogeneity.

In a phase I expansion in heavily pretreated pancreatic cancer, continuous oral LY2880070 combined with low dose weekly gemcitabine (~300 mg/m^2) was tolerable, with neutropenia and fatigue as expected, and produced signs of disease stabilization in several patients (NCT02632448) [153–155]. Separately, NCT05275426 is a Phase II clinical trial evaluating the safety and preliminary efficacy of the oral CHK1 inhibitor LY2880070 in combination with low-dose intravenous gemcitabine for patients with relapsed or refractory Ewing sarcoma, Ewing-like sarcoma, or desmoplastic small round cell tumor (NCT05275426) [155].

DNA-dependent protein kinase (DNA-PK) is the core enzyme of NHEJ, the pathway that repairs DSBs by ligating broken ends [156]. DNA-PK comprises the catalytic subunit DNA-PKcs and the DNA-binding Ku70/80 heterodimer [157]. After a DSB, Ku binds DNA ends, recruits DNA-PKcs, and promotes its autophosphorylation to coordinate end processing and ligation [158]. DNA-PK is critical for DSB repair in G1, when HR is unavailable, and for simple breaks induced by ionizing radiation or TOP poisons [159–161]. Inhibiting DNA-PK blocks NHEJ, leaving DSBs unrepaired or diverting them to slower, error-prone backups such as TMEJ [162]. Therapeutically, DNA-PK inhibitors act as radio- and chemosensitizers: by preventing rapid DSB repair, they enhance killing by radiotherapy and DSB-inducing drugs [163, 164]. Tumors often rely on NHEJ and can be more sensitive to its loss than normal tissues, which can use HR in S/G2 [160]. In tumors, DNA-PK inhibition increases mis-repair and genomic instability beyond viability [165]. Combinations with PARPi or ATR inhibitors can be lethal because NHEJ and HR compensate for each other, especially in p53-null settings [162, 166]. ATM-deficient tumors are particularly reliant on DNA-PK, making DNA-PK inhibition a synthetic lethal partner for ATM loss [167].

Peposertib (M3814) is an orally bioavailable, highly selective DNA-PKcs inhibitor from Merck KGaA that binds the ATP active site with low nanomolar affinity [168]. It robustly sensitizes tumor cells and xenografts to radiation and chemotherapy; in colorectal and head and neck models, adding peposertib to fractionated radiation significantly delayed tumor growth versus radiation alone [168]. As expected for a nondamaging agent, monotherapy produced only modest delays, but combinations yielded persistent γH2AX, fragmented nuclei, and mitotic catastrophe [164, 168, 169].

In early clinical studies, peposertib has been combined with radiotherapy, chemoradiotherapy, and systemic chemotherapy. In locally advanced head and neck cancer, 5-day-per-week dosing with cisplatin–radiation was tolerated up to 150 mg/day, whereas some patients developed mucositis at 200 mg; complete responses were observed in certain HPV-negative patients (NCT04533750) [170]. Short course radiotherapy plus peposertib for rectal cancer improved tumor necrosis and pathologic responses, and another rectal cancer study using peposertib up to 150 mg/day with full dose capecitabine–radiation showed no unexpected toxicities, tumor downstaging in over half of patients, and some complete pathologic responses, albeit with slightly higher acute grade-3 lymphopenia (NCT03770689) [170, 171]. In recurrent ovarian cancer, peposertib (up to 250 mg once daily or 100 mg twice daily) with pegylated liposomal doxorubicin produced partial responses with manageable hematologic toxicity (NCT04092270) [161].

Rational combinations include ATR and DNA-PK inhibitors—such as peposertib with Merck's ATR inhibitor M1774 in phase Ib (NCT05687136)—in which ATR blockade forces reliance on NHEJ that is then disabled by DNA-PK inhibition, and DNA-PK inhibitors with immunotherapy, because unrepaired DSBs can activate cGAS–STING and increase immunogenicity [172]. Trials of peposertib plus avelumab in solid tumors have been feasible with doses up to 400 mg twice daily and without increased immune-related adverse events (NCT04068194); early efficacy signals include prolonged stable disease in a microsatellite-stable (MSS) colorectal cancer patient [173].

PARG and TOP1 inhibition strategies in BRCA-deficient cancers

PARG is the primary enzyme that cleaves and removes poly (ADP-ribose) (PAR) chains from proteins, effectively reversing PARP activity [174]. After DNA damage, PARP1 adds PAR chains to itself and other proteins to signal repair, and PARG hydrolyzes these chains to reset repair complexes and recycle PARP [175]. Inhibiting PARG causes PAR to accumulate on chromatin and traps PARP–DNA complexes, which stalls repair; persistent PARylation can block factor turnover [176]. PARG inhibition also hyperactivates PARP and depletes NAD$^+$, compounding repair deficits [177]. The net effect resembles PARP inhibition but via excess PAR rather than its absence [177]. Tumors, which often exhibit heightened PARP activity, rely on timely PAR turnover, so cancers with replication stress or HR defects may be vulnerable to

PARG inhibitors, as PAR accumulation at forks can precipitate fork collapse [178]. PARG inhibition also creates synthetic lethal interactions distinct from PARPi; in some HR-proficient but replication-stressed settings PARG can be essential [179, 180]. Although PARG was long considered difficult to drug because its regulatory macrodomain binds PAR rather than presenting a classic small molecule pocket, recent advances have yielded compounds that occupy the ADP-ribose–binding groove [175].

In preclinical models of HRD cancers, including BRCA2-null patient-derived xenografts, IDE161 monotherapy produced marked growth inhibition or regression and retained efficacy in PARPi-resistant tumors [177]. By preventing PAR degradation, IDE161 induces replication stress with fork slowing, ssDNA gap accumulation, and transcription–replication conflicts that can progress to fork collapse and cell death [181]. Normal cells with intact checkpoints tolerate transient PAR accumulation, and IDE161 showed selective cytotoxicity toward DDR-defective cancer cells while sparing normal cells *in vitro* [181]. IDE161 is in phase I trials for BRCA1/2-mutated and HRD solid tumors (NCT05787587) [181].

ETX-19477 from 858 Therapeutics is a small molecule PARG inhibitor in phase I (NCT06395519) [176, 182]. ETX-19477 has low nanomolar cellular potency, induces PAR accumulation at damage sites, and shows broad antiproliferative activity—particularly in HRD or high replication stress models such as $ER^+/HER2^-$ breast, serous ovarian, lung, and gastric cancers [182]. It is orally bioavailable with favorable pharmacokinetics and was well tolerated in animals, achieving plasma levels that drove tumor PAR accumulation [182].

Another promising approach in BRCA-deficient tumors is increasing DNA replication stress through TOP1 inhibition [183]. TOP1 inhibitors stabilize the transient TOP1–DNA cleavage complex during replication, and collisions with advancing forks convert these single-strand lesions into DSBs that HRD cells cannot resolve, triggering apoptosis [184, 185]. The camptothecin derivatives topotecan and irinotecan (via the active metabolite SN-38) exemplify this class [186]. Topotecan, engineered with a water solubilizing dimethylaminomethyl side chain for intravenous and oral use, binds the TOP1–DNA complex, intercalates at the nick, and interacts with catalytic tyrosine 723 to lock the complex, producing fork-collision–induced DSBs [187]. Clinically, topotecan is used in ovarian cancer (NCT00484666) [188, 189], small cell lung cancer (~20% response as second line, NCT00305942) [190], and cervical cancer (NCT00276796) [191], whereas irinotecan/SN-38 is a backbone drug in colorectal (NCT00311610 and NCT02292758) [192–194] and other solid tumors (NCT00389870 and NCT03290937) [195, 196]. Resistance mechanisms include increased drug efflux and TOP1 alterations that reduce binding or enhance religation. Ongoing trials (NCT03289910) continue to evaluate these agents and combinations in HRD [197].

PLX038, a PEGylated SN-38 formulation designed to improve pharmacokinetics and tumor targeting, enhances therapeutic efficacy in HRD, ATM- or BRCA-mutant tumors [198]. In preclinical models it produced tumor regressions and growth delays, and early phase I studies in solid tumors indicate that PLX038 can be combined with PARPi such as rucaparib with manageable toxicity, leveraging continuous DNA damage alongside HR blockade [198]. PLX038 is also being tested as monotherapy in platinum-resistant ovarian cancer (Phase II, NCT05465941) [199]. Combination trials with PARPi (NCT04209595) aim to maximize DNA-damage accumulation and achieve synthetic lethality [198, 200], and a reported case of ATM-mutated breast cancer treated with PLX038 plus a PARPi achieved sustained complete remission (NCT06337630).

WRN inhibition strategies in MMR-deficient cancers

Mismatch repair (MMR)–deficient tumors with high microsatellite instability (MSI-H) carry heavy mutational burdens and genomic instability, respond well to immunotherapy, and show strong dependence on Werner syndrome helicase (WRN) for coping with replication-associated DNA damage [201]. Because MMR defects increase replication stress, WRN, a RecQ-family enzyme with both helicase and exonuclease, becomes critical for maintaining genome stability, creating a synthetic lethal requirement in MSI-H cancers while remaining largely dispensable in MSS contexts [202]. In MSI-H settings, WRN loss triggers fork collapse, DSBs, mitotic catastrophe, and cell death, which makes WRN an attractive therapeutic target [201, 203, 204].

WRN inhibition is therefore being pursued as a selective synthetic lethality strategy for MSI-H tumors, with clinical evaluation of small molecule inhibitors such as HRO761 and VVD-133214 [205, 206]. HRO761, discovered by Novartis, is a first-in-class allosteric helicase inhibitor that binds a pocket at the D1–D2 interface, locks WRN in a closed, non-unwinding conformation, and does not compete with ATP [205]. It is highly potent and selective *in vitro* and, in cells, recapitulates WRN KO: MSI-H cancer cells accumulate γH2AX-marked breaks and undergo p53-independent cell death, while MSS cells are spared [205]. Notably, HRO761 induces WRN protein degradation specifically in MSI-H cells, likely reflecting ubiquitin–proteasome turnover of trapped, inactive WRN; this provides a useful pharmacodynamic marker of target engagement [205].

HRO761 is being tested in a Phase I/Ib trial (NCT05838768) in MSI-H advanced solid tumors as monotherapy and in combinations, including with the PD-1 antibody tislelizumab and with irinotecan [205, 207]. The drug is given orally on 28-day cycles. Early findings show pharmacodynamic activation and tumor regressions in some MSI-H patients, little activity in MSS tumors, and no dose limiting toxicities at initial exposure levels, which has enabled full dose combinations.

Roche/Genentech and Vividion have developed VVD-133214, a covalent allosteric inhibitor that targets cysteine 727 in the WRN helicase domain [206]. Binding is cooperative with nucleotide, allowing the compound to lock WRN in an inactive conformation through an irreversible bond [206]. Preclinical data show selective lethality in MSI-H cells, proteasomal degradation of bound WRN, and robust tumor regressions in multiple MSI-H xenograft and PDX models, with minimal effects in MSS controls [206]. Combination with irinotecan further enhanced tumor regressions, consistent with synergy between induced replication stress and WRN blockade [208]. As of late 2023, a first-in-human study had entered startup for MSI-H cancers, with oral dosing anticipated and safety monitoring focused on off-target covalent interactions; chemoproteomic discovery was used to maximize selectivity (NCT06004245) [206, 207].

These clinical programs represent the first translation of WRN synthetic lethality to the clinic and are expected to clarify the therapeutic value of WRN targeting in MSI-H cancers, either alongside or after immunotherapy.

Conclusion

Defects in DDR are a defining vulnerability of many cancers and continue to reshape therapeutic strategy. By exploiting pathway interdependencies—most notably HR, NHEJ, and replication-stress signaling—agents such as PARP, ATR, POLθ, CHK1, PARG, TOP1, and WRN inhibitors are moving from mechanism to medicine. As reviewed here, these drugs can function as monotherapies in biomarker-selected tumors or deliver potent synergy with radiotherapy and cytotoxic chemotherapy by converting reparable lesions into lethal DNA damage. Beyond classic DDR targets, modality-expanding approaches are emerging: 2-chloro-*N*,*N*-diethylethanamine hydrochloride (CDEAH), a guanine-alkylating agent, preferentially kills PARP1-deficient cells by forcing dependence on BER and NER and shows combination potential with PARP inhibition [209]; and cancer-specific INDEL attacker (CINDELA) leverages CRISPR–Cas9 to inflict multi-site DSBs specifically at mutated sequences, offering a blueprint for truly personalized, genotype-directed cytotoxicity [210].

A central challenge is widening the therapeutic index. While cancer cells are often hyper-dependent on HR mediators such as RPA and RAD51 or replication checkpoints (e.g. ATR/CHK1) owing to oncogene-driven stress and checkpoint erosion, normal proliferating tissues still require baseline DDR, making myelosuppression and gastrointestinal toxicity recurring on-target effects. Strategies to enhance selectivity include (i) biomarker-based enrichment (e.g. BRCA/HRD, ATM loss, MSI-H for WRN inhibition); (ii) synthetic-lethality pairing to reduce dose intensity (e.g. ATR+PARP, POLθ+PARP, DNA-PKcs+radiation); (iii) intermittent/scheduled dosing to exploit tumor repair liabilities while allowing normal-tissue recovery; (iv) tumor-directed delivery and CNS-penetrant design where needed; and (v) immune-oncology combinations, as persistent DNA damage can activate cGAS–STING, increasing immunogenicity but also necessitating vigilance for immune-related adverse events. Long-term monitoring for selection of resistant clones, genomic instability in survivors, and secondary malignancies remains imperative as DDR-targeted regimens move earlier in care.

Looking forward, three forces will accelerate clinical impact: (i) Precision patient selection using composite biomarkers (genotype, functional HRD, replication-stress signatures, and dynamic pharmacodynamic readouts); (ii) Rational combinations that collapse compensatory repair (ATR with DNA-PKcs or POLθ; PARP with PARG or TOP1 payloads) while mitigating overlapping toxicities; and (ii) Next-generation modalities that expand beyond occupancy-based inhibition—such as targeted degraders, genome-editing approaches like CINDELA, and small molecules like CDEAH that exploit context-specific repair dependence. As clinical datasets mature, DDR-targeted therapies are poised to integrate into standard care pathways, improving survival and quality of life while advancing the promise of mechanism-guided, minimally toxic precision oncology.

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Conflict of interest

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