

REVIEW

Drugging the Cancers Addicted to DNA Repair

Jac A. Nickoloff, Dennie Jones, Suk-Hee Lee, Elizabeth A. Williamson, Robert Hromas

Affiliations of authors: Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO (JAN); Department of Medicine and the Cancer Center, University of Florida Health, Gainesville, FL (DJ, EAW, RH); Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN (SHL).

Correspondence to: Jac A. Nickoloff, PhD., Department of Environmental and Radiological Health Sciences, 1681 Campus Delivery, Colorado State University, Fort Collins, CO 80523 (e-mail: j.nickoloff@colostate.edu); or Robert Hromas, MD, FACP, Department of Medicine, University of Florida, PO Box 100277, Gainesville, FL 32610 (e-mail: rhromas@ufl.edu).

Abstract

Defects in DNA repair can result in oncogenic genomic instability. Cancers occurring from DNA repair defects were once thought to be limited to rare inherited mutations (such as BRCA1 or 2). It now appears that a clinically significant fraction of cancers have acquired DNA repair defects. DNA repair pathways operate in related networks, and cancers arising from loss of one DNA repair component typically become addicted to other repair pathways to survive and proliferate. Drug inhibition of the rescue repair pathway prevents the repair-deficient cancer cell from replicating, causing apoptosis (termed synthetic lethality). However, the selective pressure of inhibiting the rescue repair pathway can generate further mutations that confer resistance to the synthetic lethal drugs. Many such drugs currently in clinical use inhibit PARP1, a repair component to which cancers arising from inherited BRCA1 or 2 mutations become addicted. It is now clear that drugs inducing synthetic lethality may also be therapeutic in cancers with acquired DNA repair defects, which would markedly broaden their applicability beyond treatment of cancers with inherited DNA repair defects. Here we review how each DNA repair pathway can be attacked therapeutically and evaluate DNA repair components as potential drug targets to induce synthetic lethality. Clinical use of drugs targeting DNA repair will markedly increase when functional and genetic loss of repair components are consistently identified. In addition, future therapies will exploit artificial synthetic lethality, where complementary DNA repair pathways are targeted simultaneously in cancers without DNA repair defects.

Our DNA is not contained pristine in the nucleus, but rather is subject to assault by endogenous and exogenous genotoxins. Exogenous insults to DNA include hypoxia, lack of nutrients, radiation, dietary carcinogens, and medications (1–3). Endogenous insults include oxygen-free radicals from metabolism, aberrant incision of DNA by immune or repair nucleases, and collision of replication forks with messenger RNA transcription or noncanonical DNA structures (1–4). Almost every element of the DNA structure can be damaged, from base damage to breaks in phosphodiester bonds. Given the precarious existence of DNA and the need to maintain genome stability to prevent cell death or neoplastic transformation, DNA repair is a critical function for all cells.

Defects in DNA repair can lead to an increase in genomic instability, which is one mechanism of oncogenic transformation (5–8). Genomic instability produces the mutations that dysregulate growth and promote tumor cell invasion and metastasis

(5,9,10). However, DNA repair defects can be exploited in cancer therapy because excessive genomic instability itself can have lethal consequences by inducing deadly mutations, mitotic catastrophe, or chromothripsis (11,12). The same defects in DNA repair that produced oncogenesis in the first place make replication more stressful for that cell because the continuous DNA replication a cancer cell undergoes requires many DNA repair components (13,14). The cancer cell must find replacements for the original oncogenic loss of the DNA repair component to continue replicating. These replacement DNA repair components can be targeted to prevent the repair and restart of stressed replication forks (15,16).

There are four major types of DNA repair pathways, some with multiple subpathways (Figure 1) (17–19). These repair pathways operate within the DNA damage response (DDR), a complex network of checkpoint signaling and DNA repair pathways that

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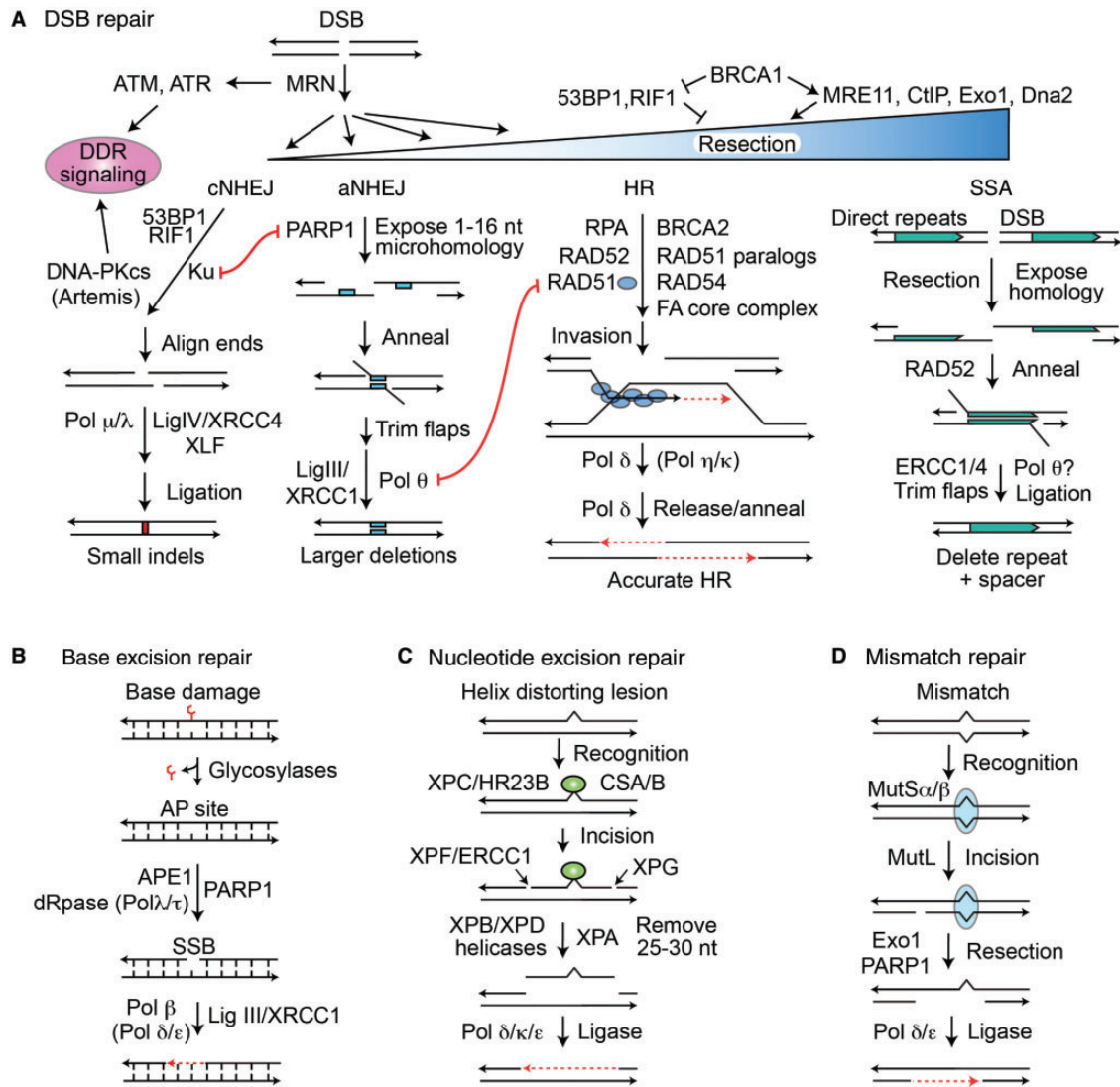


Figure 1. DNA repair pathways in mammalian cells. **A)** Double-strand breaks (DSBs) activate DNA damage response signaling including checkpoint arrest through ATM, ATR, and DNA-PKcs. DSB repair pathway choice is determined by the amount of 5' end resection at the DSB, inhibited by 53BP1/RIF1, promoted by BRCA1/CtIP. MRE11 initiates limited end resection, and this is followed by Exo1/EEP1 and Dna2 nucleases for extensive resection. 53BP1/RIF1 and Ku protect DSB ends from resection, promoting classical nonhomologous end joining (cNHEJ). PARP1 competes with Ku and promotes limited end resection for alternative nonhomologous end joining (aNHEJ). RAD51 catalyzes invasion by the resected 3' end into the sister or other homologous sequences, and Pol θ catalyzes repair synthesis across the DSB. The amount of 3' end resection regulates DSB pathway choice. cNHEJ requires little or no end resection, aNHEJ requires limited resection, and homologous recombination (HR) and single-strand annealing (SSA) require extensive resection. DNA polymerase θ (Pol θ) promotes a microhomology search by the opposing 3' single strands after short resection in aNHEJ. The HR subpathway SSA requires extensive end resection to expose large homologous regions, usually direct repeats, with RAD52 promoting annealing, producing large deletions between direct repeats. **B)** Base excision repair repairs minor lesions (eg, oxidized bases), promoted by PARP1. Repair intermediates include an abasic site and nicking of the DNA backbone, with short gap filling by Pol β . **C)** Nucleotide excision repair (NER) repairs large helix-distorting lesions and involves excision of about 15 nucleotides on either side of the lesion, followed by gap filling. GG-NER and TC-NER differ in lesion recognition by XPA or CSA/B, respectively. **D)** Mismatch repair involves recognition of the mismatched nucleotide by MutS α/β , followed by strand nicking with MutL, extensive resection with Exo1, and repair synthesis. DNA ligases catalyze religation, the final step in all pathways. aNHEJ = alternative nonhomologous end joining; cNHEJ = classical nonhomologous end joining; DDR = DNA damage response; DSB = double-strand break; HR = homologous recombination; SSA = single-strand annealing.

promote cell survival and genome stability or trigger programmed cell death when damage is excessive (20–23). Defects in DDR components predispose to cancer, determine tumor response to chemo- and radiotherapy, and underlie several congenital conditions including multiple types of Seckel syndrome, primordial dwarfism, and premature aging syndromes (24–26). DDR components are often defective in cancer, but the DDR comprises interacting/crosstalking pathways, and defects in one can be compensated by alternative pathways. Such compensatory pathways are formidable obstacles to successful cancer treatment.

Among the most dangerous DNA lesions are double-strand breaks (DSBs), which can trigger apoptosis or lead to oncogenic translocations (1–3,17–19,27). There are two major DSB repair pathways, nonhomologous end joining (NHEJ) and homologous recombination (HR), each with two subpathways (Figure 1A) (17–19,27). In NHEJ, DSB ends are trimmed and ligated, and NHEJ is therefore error prone, while HR uses a homologous sequence (typically the sister chromatid) as a repair template and is generally accurate (27,28).

NHEJ includes classical (cNHEJ) and alternative (aNHEJ) pathways (Figure 1A). The choice between these pathways is

regulated by 53BP1/RIF1, which promotes cNHEJ, the dominant pathway (29), and PARP1, which promotes aNHEJ (30). Similar to HR, aNHEJ involves 5' strand end resection, which reveals microhomologies for annealing. By contrast, cNHEJ directly ligates free DSB ends (29,31). aNHEJ serves as the backup repair pathway for two important HR functions. If HR is not functional, aNHEJ can rescue resected DSBs (31,32), such as might occur in G0/G1 of the cell cycle or abnormally in BRCA1/2-mutant cancers. aNHEJ also provides a backup mechanism to repair broken replication forks when HR is deficient at the cost of genome stability (2,31,32).

HR has accurate (conservative) and inaccurate subpathways that require extensive 5' end resection to produce 3' SS DNA tails, a process that initiates when BRCA1/CtIP out-competes 53BP1/RIF1 for DSBs (Figure 1A) (28,29). In the accurate HR subpathway, 3' SS DNA coated with RAD51 invades a homologous sequence to copy genetic information to effect repair. Inaccurate HR, termed single-strand annealing (SSA), is RAD52 dependent and deletes one repeat and DNA between linked repeats, or it can cause translocations when two DSBs occur in or near repetitive elements on different chromosomes (33,34). RAD51-mediated HR is the dominant pathway for restarting stalled or broken replication forks, which if processed improperly by NHEJ cause genome instability and neoplastic transformation (2,27,28).

Three pathways repair single-strand damage: base excision repair (BER), mismatch repair (MMR), and nucleotide excision repair (NER) (35–37). In these pathways, the undamaged, complementary strand serves as repair template. BER is initiated by glycosylases that remove the damaged base, followed by strand nicking, PARP1-promoted DNA synthesis across the lesion site and strand religation (Figure 1B) (35). NER repairs bulky DNA lesions by excising approximately 30 nt containing the lesion, followed by DNA synthesis and ligation (Figure 1C) (37). MMR recognizes and excises mismatched nucleotides introduced during DNA replication (and HR heteroduplex intermediates), and repair is completed by DNA synthesis and ligation (Figure 1D) (36).

Like DSBs, DNA interstrand crosslinks (ICLs) are dangerous because they present an absolute block to replication. ICL repair in the G1/0 phase involves dual incisions flanking the ICL, excision via NER, and DNA synthesis to fill the gap. ICL repair in the S phase is similar but involves HR to provide an accurate template for repair synthesis across the excised lesion (Figure 2) (38). When replication forks converge on an ICL, BRCA1 contributes to replisome dissociation, and the consequent SS DNA is protected with RAD51. A host of Fanconi anemia proteins activate ATR and recruit XPF and MUS81-EME1 nucleases that make two incisions on one strand flanking the ICL. This creates a substrate for translesion synthesis in one duplex (39) and a DSB on the other. NER then excises the lesion, and HR completes repair (Figure 2).

Defects in any of these repair pathways can lead to malignant transformation, and any pathway can also be subverted to assist the cancer cell in resisting therapy. There is considerable crosstalk among the single- and double-strand lesion repair pathways and replication fork restart pathways. This crosstalk reflects the many mechanistic commonalities in the pathways: lesion recognition, SS DNA binding, structure-specific endo- and exonuclease cleavage, strand annealing, polymerase gap filling, and ligation. Repair pathways display several types of crosstalk. There is signaling crosstalk between the HR and cNHEJ pathways through ATR, ATM, and DNA-PK (40,41). There is functional crosstalk, shown by several examples in which overexpression of a DNA repair component in one pathway compensates for a repair defect in another, conferring

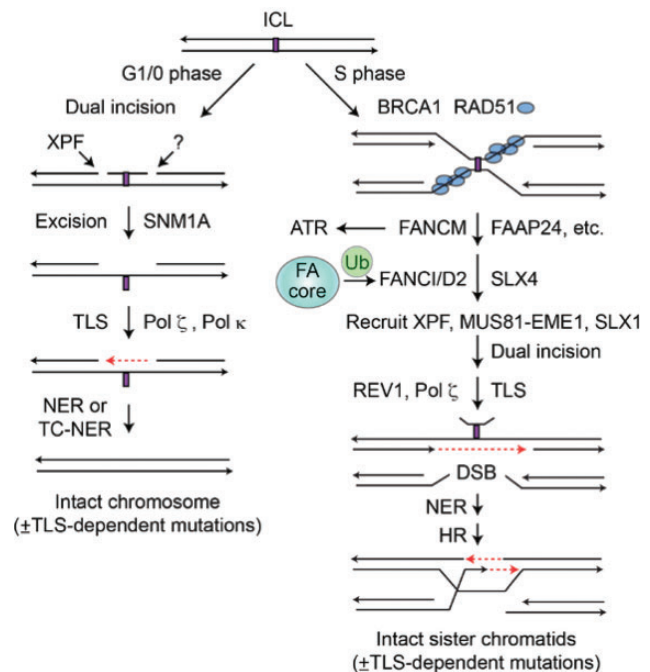


Figure 2. Interstrand crosslinks can be repaired by replication-independent (left) and replication-dependent (right) mechanisms. Both repair pathways involve dual incisions, translesion synthesis across the crosslinked segment, and nucleotide excision repair (NER) to remove the incised crosslink. Replication-dependent interstrand crosslink (ICL) repair also involves homologous recombination (HR). When forks converge on an ICL, BRCA1 and RAD51 protect the stalled fork, and the Fanconi anemia (FA) repair pathway repairs the crosslink. FA repair is initiated by the ubiquitination of FANCD2, which recruits nucleases XPF, MUS81/EME1, and SLX1 to incise the crosslink, followed by translesion synthesis across the lesion, NER to remove the lesion, and HR to repair the replication fork. Both mechanisms are error free, except for mutations that may be introduced by translesion synthesis polymerases. DSB = double-strand break; FA = Fanconi anemia; HR = homologous recombination; ICL = interstrand crosslink; NER = nucleotide excision repair; TC-NER = transcription-coupled NER.

therapeutic resistance (42). Finally, there is direct crosstalk when specific components are shared among pathways, for example, PARP1 functions in BER and in aNHEJ (Figure 1) (30). Although PARP1 is not required for HR repair of frank DSBs (43), PARP1 promotes MRE11 recruitment to collapsed replication forks prior to HR repair (Figure 3). PARP1 may also promote HR by increasing repair factor accessibility to damage by modifying chromatin (44,45). Also, when PARP1-dependent BER is blocked, unrepaired lesions cause fork collapse, which requires HR for proper restart (13–15,46). Thus, PARP1 plays similar roles in repairing different types of DNA lesions (27,30,35). This promiscuous functionality makes PARP1 a common crutch for malignancies that arise due to defects in DNA repair, and thus an attractive synthetic lethal target. Other shared DNA repair components may similarly prove useful as synthetic lethal targets, including RPA, DNA polymerases, and structure-specific nucleases.

The Concept of Synthetic Lethality

Original oncogenic events are mutations that promote uncontrolled cell replication, and these will occur more frequently in cells with inherited or acquired DNA repair defects (7,8,20). Cell replication requires several of the DNA repair pathways to be

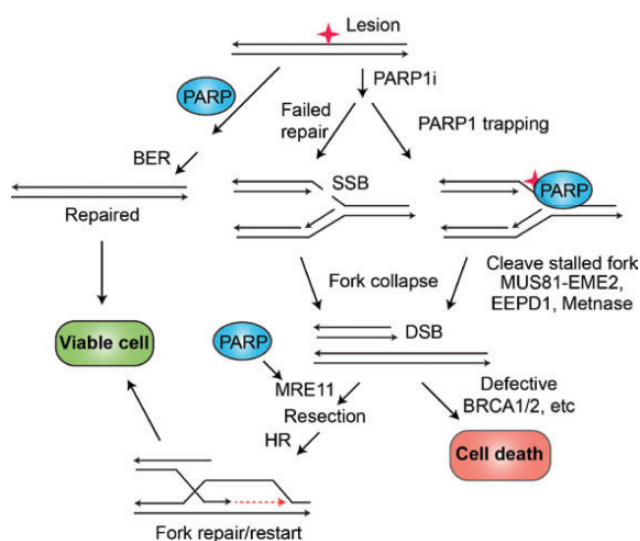


Figure 3. PARP1 at intersecting repair pathways. PARP1 promotes base excision repair (BER) and cell survival. PARP1 inhibitors (PARP1i) cause unrepaired intermediates such as SS nicks to accumulate. They also trap PARP1 on chromatin, causing replication forks to stall. Double-strand breaks are either from the collision of the fork with a BER SS nick repair intermediate or from nuclease cleavage of a stalled replication fork. Collapsed replication forks are repaired and restarted by homologous recombination (HR) promoted by PARP1 modification of MRE11, which initiates 5' end resection. In HR-deficient cells (eg, BRCA1/2 mutants), HR repair of stalled forks cannot occur, accounting for the synthetic lethality of PARP1i in HR-deficient cancers. BER = base excision repair; DSB = double-strand break; HR = homologous recombination; SSB = single-strand break.

functional (15,16,18,22,27). When there is a systemic and persistent defect in DNA repair in the transformed cell, then that cell becomes dependent on another repair pathway that can back up the defective pathway for replication (27,28,34). Synthetic lethality occurs when the backup pathway is chemically inhibited and the cancer cell can no longer replicate (13–15). Such stalled replication induces apoptosis.

A well-known example of this is the inherited mutations in the HR components BRCA1 and 2 in breast and ovarian cancer (13,14). All the patient's cells have one mutated allele of BRCA1 or 2, but the cancer has both alleles mutated, and the cancer cell has an unstable genome because it has lost HR capability. Because HR is the most important pathway for repairing and restarting stalled replication forks, these cancers become dependent on PARP1-mediated SSB repair and alternative nonhomologous end joining to repair and restart their replication forks (12–15). When PARP1 is inhibited by a drug, then these cancers cannot repair and restart replication forks (Figure 3) (13,14,19,42,44). The stalled forks collapse and can aberrantly ligate together, and distinct chromosomes can fuse, resulting in mitotic catastrophe and subsequent apoptosis (45,46). Because the nonmalignant cells have normal HR as they retain one normal BRCA1 and 2 allele, only the cancer cells are sensitive to PARP1 inhibition (42–46).

Subversion of Synthetic Lethality: Synthetic Rescue

Synthetic lethality is an important tool in cancer treatment, but it has limitations. For example, PARP1 inhibitors are very effective against BRCA1-defective cancers, but treatment can lead to

development of resistance because of second-site mutations in other checkpoint signaling or DNA repair proteins, such as 53BP1, Rif1, PTIP, SFLM11, JMJD1C, and REV7 (47–52). At least three PARP1 inhibitor resistance mechanisms have been identified: restoration of HR, for example, by mutation or downregulation of 53BP1; loss of PARP1 itself; and upregulation of the PgP drug transporter (53). PARP1 inhibitor resistance by 53BP1 loss appears specific to BRCA1-defective tumors, reflecting the interplay between BRCA1, 53BP1, and RIF1 (Figure 1A); in BRCA2-defective tumors, resistance to PARP1 inhibition develops because of secondary BRCA mutations (53).

These findings have clinical relevance. For example, 53BP1 expression is reduced in a fraction of sporadic triple-negative and BRCA1-defective breast cancers (47). Generally, breast cancer patients respond well to PARP1 inhibitors, but eventually resistance develops and disease progresses (54). This has stimulated efforts to identify targets to prevent or overcome resistance to PARP1 inhibition and to identify markers of resistance (55–57).

Drugging Base Excision Repair

The most important rationale for targeting BER is that cancer cells have a higher oxidative status than normal cells and therefore suffer more oxidative DNA damage (4,35,58). Oxidized nucleotides can result in two forms of crosslinks that block replication; the abasic deoxyribose can react with an adenine on the opposing strand or covalently link with DNA polymerase β (Pol β), forming a DNA-protein adduct (59–61). In addition, BER repairs alkylation damage, and thus can mediate chemotherapy resistance (35,62). The rate-limiting step in BER is APE1 phosphodiester cleavage 5' to an abasic site, following glycosylase removal of the oxidized base (35). Not surprisingly, APE1 is overexpressed in many cancer types, and there have been multiple attempts to target APE1 for cancer therapy (42,63–65). However, APE1 inhibitors have not had much clinical impact because their biochemical activity did not translate well to cell and animal models (63–65).

We and others have described compounds that inhibit Pol β (42,66,67), but these have not been pursued pharmaceutically because of their relatively modest activity. In addition, specificity for Pol β compared with other DNA polymerases has not been defined, so these compounds may have in vivo toxicity that would limit their usefulness (66,67). However, Pol β remains an attractive target in BRCA1/2-defective tumors. Inhibiting Pol β generates the same cleaved, unrepaired SS BER intermediate that PARP1 inhibition does, and also causes replication fork DSBs (35,66–68). Thus, Pol β inhibitors should also be effective in treating HR-deficient cancers, similar to PARP1 inhibitors.

PARP1 is an essential component for BER, and its inhibition in HR-defective cancers has been by far the most effective means of targeting of DNA repair for cancer therapy to date (Figure 3) (69,70). Two groups demonstrated that BRCA1/2-mutant tumors, defective in HR, were sensitive to PARP1 inhibitors (13,14). DSBs generated at replication forks are largely repaired by HR (2,27,28), and HR requires BRCA1/2 (27,28). PARP1 is required for BER, and catalytic inactivation causes accumulation of SSBs. Thus, replication fork collision with SSBs causes fork collapse to DSBs, and repair occurs predominantly (and most accurately) by HR (Figure 3, middle). In BRCA1/2 mutants, or other HR-defective cancers, such forks cannot be repaired appropriately (13,14,69). Replication-associated DSBs are highly

toxic DNA lesions that cause mitotic catastrophe and apoptosis if improperly repaired or unrepaired (13,14,69,70). Apart from SSB generation, PARP1 inhibition kills BRCA1/2-mutant cancer in at least two other ways, trapping PARP1 on DNA at the lesion site (71) and increasing ribonucleotide-adenine and ribonucleotide-Pol β adducts (59–61). Trapped PARP1 and DNA adducts stall replication forks, which are cleaved by structure-specific nucleases (MUS81-EME2, EEPD1, Metnase) (72–75), causing fork collapse (Figure 3), again requiring HR for restart and lacking in BRCA1/2-mutated malignancies (66–68). Thus, the remarkable efficacy of PARP1 inhibitors in HR-deficient malignancies is due in part to PARP1's multiple roles in DNA repair.

Drugging Mismatch Repair

During DNA replication, incorrect nucleotides are occasionally incorporated into the daughter DNA strand, creating mismatched base pairs corrected by MMR (36); mismatches also form in heteroduplex DNA during HR (76). MMR comprises four key steps: mismatch recognition, excision of the lesion, DNA synthesis across the SS gap, and ligation (Figure 1D) (36,77). Two heterodimeric proteins recognize the lesion: MutS α (MSH2/6 complex) recognizes short mismatches, and MutS β (MSH2/3 complex) recognizes longer insertion-deletion loops (36,77,78). Binding of either heterodimer recruits the heterodimer MutL (MLH1 and PMS2 complex). MutL recruits Exo1, which excises the mismatched DNA (79) in a reaction enhanced by PARP1 (80). Pol δ fills in the gap, and DNA ligase I seals the nick (81).

Hereditary nonpolyposis colorectal cancer (HPNCC or Lynch syndrome) is an inherited autosomal-dominant disease resulting from defects in MMR proteins, with the majority of mutations affecting MLH1, MSH2, and MSH6 (82). Silencing by somatic methylation of MMR gene promoters also decreases MMR (83,84) and confers resistance to platinum-based chemotherapy (85). DNA demethylating agents such as 5-azacytidine induce re-expression of MMR components in these cancers, restoring sensitivity to cisplatin or carboplatin (86,87).

Cancers with MLH1, MSH2, or MSH6 defects display synthetic lethality with therapeutic potential, but it is important to identify the specific MMR deficiency in the tumor as they differ in therapeutic response. For example, MSH2-mutant cancers are sensitive to methotrexate, an antimetabolite that inhibits DNA synthesis, and psoralen, a DNA crosslinking agent, but MLH1-mutant cancers are resistant to both treatments (88,89).

Unrepaired oxidized nucleotides accumulate upon BER repression (eg, Pol β or PINK1 inhibition) or methotrexate treatment, which increases mismatch formation during DNA synthesis, increasing the burden on MMR, and mutagenesis. These effects are strongly exacerbated in MMR-defective cancers, a dynamic that presents synthetic lethal opportunities. For example, Pol β inhibition is synthetically lethal in MSH2- or MLH1-deficient tumors (90) (Figure 4A). Thus, Pol β inhibitors are promising agents for treatment of MMR-deficient cancers, as well as the aforementioned BRCA1/2-mutant cancers (42,66).

Some MMR-deficient cancers behave like BRCA1/2-mutant cancers, with defects in stressed replication fork repair. MSH3 is critical for loading RAD51 during HR repair, and thus MSH3-deficient cancers are sensitive to PARP1 inhibition (Figure 4B) (91,92). Clinical trials with PARP1 inhibitors in MSH3-mutant colon cancer are warranted, especially in conjunction with an immune checkpoint inhibitor because genomic instability associated with MMR-deficient colon cancer increases neoantigen

production and thus increases the chance of immune recognition (93).

Drugging Nucleotide Excision Repair

NER processes DNA lesions resulting from exposure to UV light, environmental toxins, and some chemotherapeutic drugs (37,94,95). NER has two subpathways: global genome NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER repairs lesions across the whole genome, whereas TC-NER repairs lesions in transcribed DNA, initiated by RNA polymerase II stalling at DNA lesions. These pathways differ in only two respects: how the DNA lesion is recognized and kinetically (TC-NER is faster than GG-NER) (37).

In GG-NER DNA, damage is recognized by XPC-RAD23B, which binds to the undamaged DNA strand opposite the lesion, recruiting downstream NER components. Helicase XPB unwinds the DNA, and XPD recruits the RPA/XPA/XPG complex. This complex recruits the nuclease ERCC1-XPF, which incises 5' to the lesion, and initiates DNA synthesis across the gap by Pol δ and Pol κ or Pol ϵ , followed by 3' incision by XPG to remove the damaged DNA and ligation by DNA ligase III/XRCC1 or DNA ligase I (Figure 1C) (37,94,95). In TC-NER lesions that stall RNA polymerase II are recognized by WD repeat protein CSA, SWI/SNF family member CSB, and XAB2. This complex is exchanged with the TFIIH complex, and repair proceeds as above (96).

Three known autosomal recessive inherited diseases are associated with defects in the NER pathway: xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD). These inherited mutations result in either an extreme predisposition to cancer (XP) or neurodevelopmental defects associated with rapid aging but without cancer predisposition (CS and TTD) (97).

NER deficiency confers sensitivity to crosslinking agents such as cisplatin, reflecting reduced crosslink repair (98,99). The ERCC1-XPF nuclease complex is essential for repair of platinum-DNA crosslinks, as well as trimming flaps during DSB repair by SSA (98) and aNHEJ (100). Unrepaired crosslinks cause replication stress and eventually apoptosis (2,17,18). Importantly, lower expression of ERCC1 correlated with increased sensitivity to platinum agents in several tumor types (98,99,101). Low expression of ERCC1 is a biomarker for response to cisplatin in non-small cell lung cancers (NSCLCs), and PARP1 inhibitors are synthetically lethal in cancers with low ERCC1 (102), implying that PARP1 inhibitors may be effective in this fraction of NSCLC patients, especially when combined with cisplatin (103). Inhibitors targeting the ERCC1-XPF active site are in development as adjuncts to platinum-based chemotherapy (104,105).

Protein-DNA interactions that mediate NER have also been targeted, and small molecules have been identified that block DNA interaction with RPA and XPA (106,107). RPA is important for both NER and HR, and RPA inhibition causes cell cycle arrest, cell death, and enhances sensitivity to cisplatin and etoposide (106,108). This suggests that RPA inhibitors could be combined with PARP1 inhibitors to mediate synthetic lethality in cancers with nonmutated BRCA1/2.

Perturbation of NER components may be synthetically lethal with PARP1 inhibition. For example, PARP1 inhibition in combination with DDB1 or XAB2 deficiency is synthetically lethal in non-BRCA mutant cells (109), and combined inhibition of PARP1 and topoisomerase I (with camptothecin) has greater cytotoxicity in cancer cells depleted of XPF-ERCC1 (Figure 5) (110).

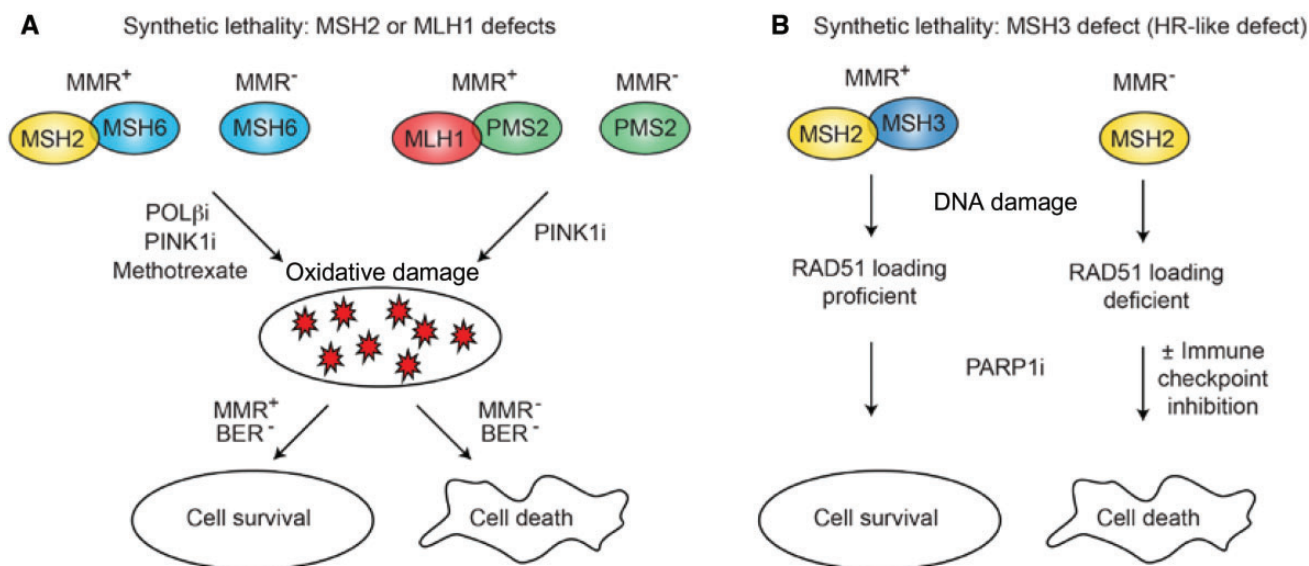


Figure 4. Synthetic lethality with mismatch repair (MMR) defects. **A)** MSH2 or MLH1 defects are synthetically lethal, with increased oxidative damage caused by inhibition of Pol β or PINK1, or by treatment with methotrexate. PINK1 increases oxidation of nucleotides, burdening base excision repair (BER), and Pol β inhibition decreases BER repair of oxidized nucleotides, which induce DNA mismatches during DNA synthesis. **B)** MSH3 assists in loading RAD51 onto end-resected SS DNA during homologous recombination (HR) repair. MSH3 defects can behave similarly to HR defects and are synthetically lethal when BER is blocked by inhibition of PARP1. MSH3 deficiency could also increase neoantigen generation within the tumor, which would result in synergy between the PARPi and immune checkpoint inhibitors in these cancers. BER = base excision repair; HR = homologous recombination; i = inhibition; MMR = mismatch repair.

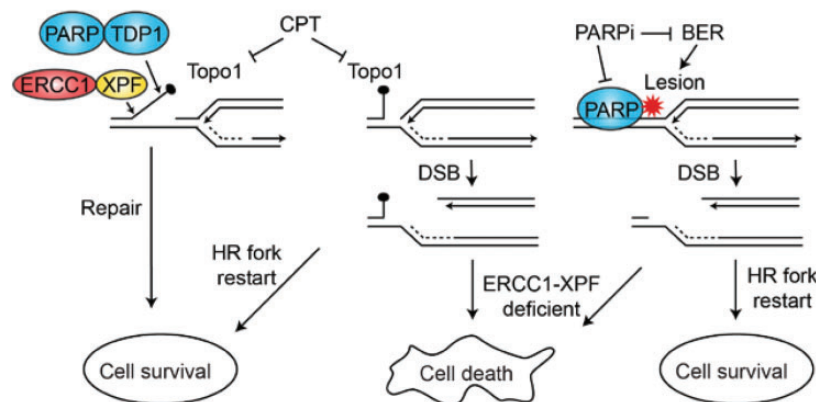


Figure 5. Synthetic lethal targeting with PARP1 and ERCC1-XPF deficiencies. Camptothecin traps Topo1 covalently onto DNA, blocking replication. Repair can proceed via a PARP-TDP1 and ERCC1-XPF1 pathway (left) or by fork repair and restart via homologous recombination; PARP1 inhibition, coupled with ERCC1-XPF deficiency, is synthetically lethal (middle). PARP1 inhibition also blocks base excision repair of single-strand lesions that block replication; these lesions are similarly lethal with ERCC1-XPF deficiency (right). BER = base excision repair; CPT = camptothecin; DSB = double-strand break; HR = homologous recombination.

ATR inhibition in ERCC1-depleted cancer cells is synthetically lethal (111). ERCC1 depletion not only increases DNA mismatches, but also single-strand lesions, which stall replication forks. Repair of stalled forks requires ATR, accounting for the synthetic lethality of ATR inhibition and ERCC1 depletion; this effect of ATR inhibition is specific to ERCC1 as no other NER deficiencies are sensitive to ATR inhibition, consistent with ERCC1-XPF functioning in other DNA repair pathways (99). The combination of an ERCC1 inhibitor with a PARP1 or ATR inhibitor may create artificial synthetic lethality, where one DNA repair inhibitor induces dependency on another pathway blocked by a second drug. An example of this, mentioned above, is combining RPA and PARP1 inhibitors, which could generate artificial synthetic lethality in cancers that do not harbor an HR defect. This principle could be widely applied to many clinical scenarios (66–68).

Drugging HR and CrossLink Repair

In addition to defects in BRCA1 or 2, cancer genome sequencing revealed mutations in many other HR pathway components that promote oncogenesis, including PALB2 (112), BRCA1-interacting protein 1 (BRIP1; also termed FANCD1 and BACH1) (113), BARD1 (114), BAP1 (115) and RAD51C (116). This expansion of HR driver mutations should broaden the clinical application of PARP1 inhibitors (117). To achieve this goal, oncologists need reliable methods to identify patients carrying mutations in any HR components. Exome sequencing is currently the most common method and is accepted by regulatory bodies that approve PARP1 inhibitor indications (118,119). However, such sequencing misses a fraction of tumors that are functionally deficient in HR but lack mutations in known HR

genes. One solution to this problem is to use DNA sequencing to measure genomic abnormalities that are due to functional HR loss, such as loss of heterozygosity, telomeric allelic imbalance, and chromosomal translocations (118,120,121). In this way, tumors with functional HR deficiency but without mutations in known HR genes can be identified and treated using synthetic lethal approaches. Such an approach has been used with great success in recent trials with PARP1 inhibitors (118,120).

Several upstream modulators of HR have been identified as synthetic lethal targets that can sensitize cancer cells to PARP1 inhibitors (Figure 6). For example, several cyclin-dependent kinases (CDKs) are upstream HR regulators, and a recent study showed that the pan-CDK inhibitor dinaciclib impairs HR repair and sensitizes cancer cells to the PARP1 inhibitor veliparib (122). Dinaciclib probably acts by blocking required phosphorylation of the HR components Exo1 and BRCA1 (17,28,29,122). ATR is another upstream HR regulator that promotes restart/repair of stalled replication forks. Not surprisingly, PARP1 inhibition synergizes with ATR blockade (123).

Interestingly, PARP1 is also essential for the survival of malignancies with isocitrate dehydrogenase 1 and 2 mutations (*IDH1* or 2, mainly glioblastomas and acute myeloid leukemias). These *IDH1/2* mutations generate the oncometabolite 2-hydroxyglutarate (2-HG). 2-HG inhibits the dioxygenase class of enzymes, such as histone demethylases, and inhibits HR repair. These *IDH1/2*-mutant cancers are exquisitely sensitive to PARP1 inhibition with olaparib or BMN-673 (124). Adding exogenous 2-HG to non-*IDH1/2*-mutant cancers produced PARP1 sensitivity (124). Thus, PARP1 inhibitors may be effective to treat these malignancies, and 2-HG itself may be another tool to induce artificial synthetic lethality.

The tumor suppressor phosphatase and tensin homolog (*PTEN*) promotes HR repair during replication stress (125). *PTEN* is mutated in a fraction of malignancies; this mutation sensitizes cancer cells to PARP1 inhibitors (Figure 6) (125). These results suggest that clinical trials might be warranted to test PARP1 inhibitor effects on cancers with *PTEN* mutations, including breast cancer and glioma (126). *PTEN* mutations are also responsible for *PTEN* hamartomatous syndromes (such as Cowden's syndrome), and while not true malignancies, these syndromes are debilitating and disfiguring and can progress to cancer. The relatively low toxicities of PARP1 inhibitors suggest these syndromes as attractive targets for such intervention (127).

The clinical success of PARP1 inhibitors stimulated efforts to identify other proteins whose inactivation in cancer might make those cancers responsive to PARP1 inhibition. RNAi screening approaches have identified the deubiquitinating enzyme *USP11* (128), *CDK12* (129), and cohesins (130) that when silenced or mutated cause synthetic lethality with PARP1 inhibitors (Figure 6). Interestingly, acute myeloid leukemia driven by aberrant transcription factors is also highly sensitive to PARP1 inhibition (131), suggesting that PARP1 inhibition may be synthetically lethal in many types of cancers with defects in various DNA repair genes.

HR is important for the resolution of DNA crosslinks by the Fanconi anemia (FA) DNA repair pathway (Figure 2) (38,132), and inherited mutations in FA components cause cancer predisposition syndromes (133). Although autosomal recessive biallelic mutations of FA genes contribute to leukemogenesis, targeting FA components may be exploited for therapeutic gain in malignancies in patients without FA. For example, cancer cells depleted of FA components are extremely sensitive to crosslinking agents such as cisplatin (134,135), and FA pathway inhibition confers sensitivity to PARP1 inhibition (136). Thus,

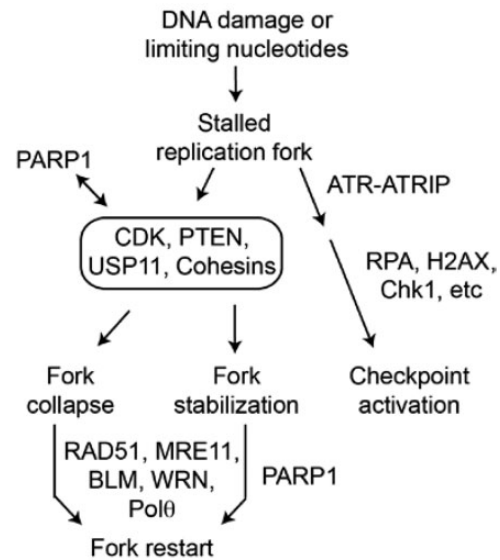


Figure 6. Repression of or mutation in several upstream regulators of homologous recombination (HR) can be synthetically lethal with PARP1 inhibition because they can cause a variety of HR repair defects that prevent fork restart. These upstream regulators include several CDKs, *PTEN*, *USP11*, and the cohesins. Inherited or acquired mutations in downstream HR components such as *RAD51*, *MRE11*, *BLM*, and *WRN* can lead to cancer. Such cancers also demonstrate synthetic lethality with PARP1 inhibition, although there could be biologically significant normal tissue toxicity when the mutation is an inherited autosomal recessive. When HR is defective in any of these cases, *Pol θ* inhibition would also be synthetically lethal because *Pol θ* is also required for the alternative nonhomologous end joining backup replication fork repair pathway.

combining FA and PARP1 inhibitors might expand the use of PARP1 inhibitors beyond *BRCA1/2*-mutant cancers.

The FA component *FANCD2* must be mono-ubiquitinated to activate FA pathway crosslink repair (Figure 2), and most therapeutic efforts have targeted this event (136). For example, proteasome inhibition with bortezomib was reported to decrease *FANCD2* mono-ubiquitination and block crosslink repair (137). A PARP1 inhibitor may enhance bortezomib efficacy in mantle cell lymphoma or myeloma (136,137). A small molecule inhibitor of *NEDD8* activation, *MLN4924*, decreases *FANCD2* activation, which would sensitize cancer cells to crosslink damage (138). Small molecule inhibitors of *USP1*-*UAF*-mediated deubiquitination of *FANCD2* prevent *FANCD2* recycling and ultimately decrease FA pathway activity (134,139). These selective *USP1*/*UAF1* deubiquitinase inhibitors also enhance sensitivity of cancer cells to crosslinkers such as cisplatin (134–136,139).

RAD52 mediates SSA (Figure 1A) in humans and also serves as a backup for *BRCA2* to load *RAD51* onto SS DNA during HR (140). *BRCA1/2*-mutant cancer cells are forced to rely on *RAD52* to repair replication forks (140,141). Thus, depleting *RAD52* in *BRCA1/2*-mutant cancer presents another synthetic lethal approach distinct from PARP1 inhibition (141,142). Several groups, including ours, have generated small molecule inhibitors of *RAD52* that are cytotoxic to cancer cells with *BRCA1/2* defects (143–145). *RAD52* and PARP1 inhibitors could be combined to treat HR-deficient cancers to increase the duration or depth of response (66–68).

Drugging NHEJ

There is substantial crosstalk among DSB repair pathways (15,17,28,29), and this presents many opportunities to exploit

synthetic lethal interactions to improve cancer therapy (68). HR, cNHEJ, and aNHEJ share the MRE11/RAD50/NBS1 (MRN) complex, an early DSB sensor important for activation of ATM-dependent DNA damage checkpoint signaling (15,28,29,146). HR and aNHEJ share 5' end resection, which is regulated by 53BP1/RIF1, DNA-PK, and BRCA1/CtIP (28,29,32,146) and mediated by several nucleases (MRE11, Dna2, Exo1, EEPD1) (17,75,147). As discussed above, the extent of 5' end resection regulates DSB repair pathway choice (Figure 1A) (28,29,147).

In cNHEJ, broken ends are initially bound by the Ku heterodimer that inhibits end resection and recruits DNA-PKcs, which then recruits the end-ligation complex XRCC4/ligase 4/XLF (31,148). PARP1 initiates aNHEJ by out-competing Ku for DSB ends (Figure 1A) (30,148). Thus, Ku and PARP1 competition regulates cNHEJ vs aNHEJ choice, and it is likely that cNHEJ is favored approximately 10:1 because Ku is more abundant and has high affinity for DNA ends (30,148–150).

In aNHEJ, Mre11/CtIP promote limited 5' end resection, and Pol θ mediates micro-homology-mediated alignment between 3' SS DNA tails at each end of the break (151,152). This creates 3' flaps that are trimmed by one or more structure-specific endonucleases such as FEN1; repair is completed by ligase III/XRCC1 (148,150,153). Although cNHEJ is inaccurate, producing small insertions and deletions at the repair junction, inherited defects in cNHEJ confer genome instability and predispose to cancer (18,27,28,148,150), probably because the aNHEJ backup pathway is even more inaccurate, generating larger deletions (148,153,154) and mediating most chromosomal translocations (34,69,155,156).

Targeting cNHEJ to enhance cancer treatment was originally studied in radiation therapy (42,157,158). Radiation therapy typically uses x-rays or protons, whose most deleterious lesions are DSBs repaired by cNHEJ (157). Carbon ion radiotherapy uses high-mass/high-charge particles to create dense ionization tracks that produce clustered DNA damage that is effective against radioresistant cancers (159). While cNHEJ is the dominant repair pathway for sparsely ionizing x-rays and protons, HR appears to play a more important role in repair of clustered DSBs (160–163). HSP90 inhibitors can block HR by downregulating RAD51, and these agents sensitize cancer cells to carbon ions (163–166).

Other HR inhibitors such as the CDK inhibitor dinaciclib, or ATR inhibitors, may also potentiate the lethal effects of heavy ion DNA damage, but these agents may have limited use in x-ray or proton therapy, where cNHEJ dominates. Thus, it is critical to have a clear understanding of the relevant repair pathways when attempting to augment particular radiotherapy modalities. This concept also extends to chemotherapy. For example, repressing cNHEJ sensitizes tumor cells to etoposide, a TopoII α inhibitor that generates DSBs, but the same cNHEJ defect confers resistance to camptothecin, a TopoI inhibitor that produces SSBs (167).

PARP1 functions in multiple DNA repair pathways (Figures 1, 3, and 6), and synthetic lethal strategies that exploit this fact are promising (13,14,68–70,93,94). For example, nearly 60% of prostate cancer patients carry a TMPRSS2-ERG translocation event, and this fusion protein interferes with cNHEJ (168). PARP1 functions in aNHEJ and BER, suggesting that TMPRSS2-ERG prostate cancer cells treated with a PARP1 inhibitor would be deficient in BER, cNHEJ, and aNHEJ. This would increase unrepaired DNA damage with lethal consequences (168). Indeed, the PARP1 inhibitor olaparib was effective in relapsed prostate cancers carrying BRCA1/2 mutations, although these patients were not stratified by TMPRSS2-ERG (69,119).

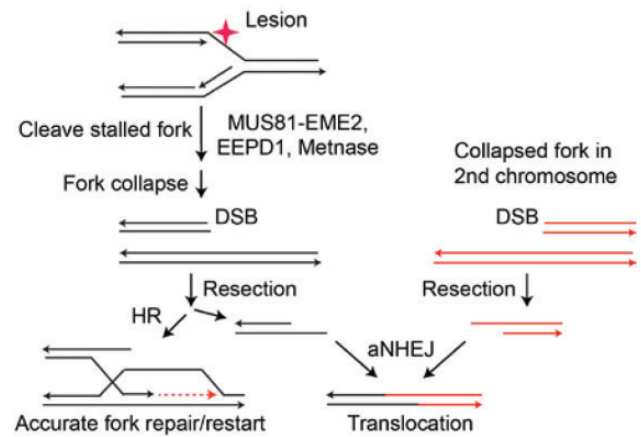


Figure 7. Collapsed forks are normally repaired by homologous recombination (HR). If HR fails, double-strand breaks on different chromosomes may be repaired by the alternative nonhomologous end joining (aNHEJ) backup pathway. The aNHEJ pathway can mediate chromosomal translocations, however, and thus is a riskier repair mechanism for the cell. aNHEJ = alternative nonhomologous end joining; DSB = double-strand break; HR = homologous recombination.

KRAS mutations are common in acute leukemias, and it was recently found that these mutations correlate with overexpression of the aNHEJ factors PARP1, ligase III, and XRCC1 (169). Overexpression of these aNHEJ components produced an increased reliance on aNHEJ to repair DNA damage in these malignancies, and this could mediate treatment resistance. Combining PARP1 inhibitors with chemotherapeutics that induce replication stress may overcome resistance of KRAS-mutant leukemias and improve outcomes (169).

Histone deacetylases (HDACs) play important regulatory roles in chromatin function, and HDAC inhibitors have been developed as antineoplastic agents and radiosensitizing agents (170,171). HDACs are not restricted to histone targets; they are more properly described as protein deacetylases. Acetylation is important for activation of certain NHEJ components. Consistent with this, the pan-HDAC inhibitor trichostatin A enhances acetylation of the critical cNHEJ factor Ku and the aNHEJ initiator PARP1, which inhibits cNHEJ and traps PARP1 on chromatin. This blocks both NHEJ pathways and is synthetically lethal in leukemia cells (172).

Recent evidence implicates the aNHEJ component Pol θ as a key target for cancer therapy. High Pol θ levels are associated with poor breast cancer patient survival (173); Pol θ is overexpressed in greater than 80% of NSCLCs, and expression levels correlate with poor outcomes (174). One reason that cancer cells are addicted to DNA repair is that they are programmed to proliferate, regardless of whether their environment is impoverished or the extent of genome damage. Many (but not all) cancers must divide to survive, and replication fork arrest is fatal (15,16,32,75). The common mechanism of action of many chemotherapeutic agents is replication stress (2,19,28). Thus, cancers with HR deficiency become addicted to aNHEJ, which is the backup repair pathway for stalled replication forks (Figure 7) (31,32). It is not surprising then that there appears to be a synthetic lethal relationship between aNHEJ and HR mediated by Pol θ . HR-deficient cancers need all the components of aNHEJ, not just PARP1, to continue replication in the face of an oxidized genome. Consistent with this model, Pol θ depletion in BRCA1/2-deficient ovarian cancer cells was synthetically lethal (175,176). Thus, Pol θ is a very exciting target for HR-deficient cancers (177).

Clinical Effectiveness of Targeting DNA Repair

The most effective clinical drugging of the addiction of cancer to DNA repair has been with PARP1 inhibitors (13,14,68–70,118–121). Many of these compounds also inhibit PARP2 to some extent, although their activity is thought to be due to their PARP1 inhibition (13,14,68–70). The first PARP1 inhibitor to complete clinical evaluation was olaparib (AZD-2281, Lynparza, AstraZeneca). The first phase I trial examined single-agent olaparib in relapsed breast, ovarian, and prostate cancer (178). In that trial, 60 patients were treated with various doses, but objective anti-tumor activity was observed only in eight ovarian cancer patients who had documented BRCA1/2 mutations. Based on a phase II trial in BRCA1/2-mutant ovarian cancer showing a 34% objective response rate (95% confidence interval = 26% to 42%) and a median response duration of 7.9 months, olaparib was granted accelerated approval by the US Food and Drug Administration (179–181). Olaparib was similarly effective against BRCA1/2-mutant ovarian cancers that were sensitive or resistant to platinum-based chemotherapy. Randomized and nonrandomized phase II trials in patients with previously treated ovarian cancer confirmed that approximately one-third of patients with BRCA1/2-mutant ovarian cancer experience objective tumor regression with olaparib monotherapy (182,183).

Modest gastrointestinal toxicity and hematopoietic toxicity seem to be the most common side effects of olaparib, and these are manageable (180,181,183). More worrisome is the 2% rate of myelodysplasia and acute myeloid leukemia seen in olaparib-treated patients (182,183). However, these are known risks of chemotherapeutics used in ovarian cancer, and it is possible that these adverse events were not due to olaparib but rather to prior chemotherapy. Olaparib does not appear to produce objective responses in tumors lacking BRCA1/2 function, although there was some benefit in terms of progression-free survival (182,183).

Ovarian cancers with BRCA1/2 mutations are not the only HR-deficient cancers that respond to olaparib. A recent phase II trial of olaparib in relapsed and refractory metastatic prostate cancer found that 16 of 49 patients had an objective response, and 14 of the responders had mutations in HR components such as BRCA1/2 or ATM (119). This indicates that a clinically significant fraction of prostate cancers have HR defects treatable with PARP1 inhibitors, perhaps as frontline agents to attack metastatic disease given their minimal toxicity.

Responses to olaparib in other BRCA1/2-mutant cancers have not been as clinically significant as in ovarian cancer. BRCA1/2-mutant breast cancers often have a worse outcome than comparable nonmutated breast cancer, with a response rate of 12.9% in one trial (179). In addition, BRCA1/2-mutant pancreatic cancers did not respond as well to olaparib as a single agent as did the ovarian cancers (179). Multiple studies are exploring whether combining olaparib with crosslinking agents such as carboplatin or cisplatin in triple-negative breast cancer will enhance responses in these cancers. However, a randomized study of adding the PARP1 inhibitor veliparib to cyclophosphamide in relapsed triple-negative breast cancer did not show any benefit compared with veliparib alone (184). Veliparib did show single-agent activity in ovarian cancer though, with a 26% response rate (185).

Niraparib is a highly selective PARP1/2 inhibitor soon to be approved in the United States and Europe. Similar to olaparib, niraparib was evaluated in patients with platinum-sensitive ovarian cancer in maintenance therapy. This trial was a randomized, placebo-controlled, phase III study that enrolled

553 patients over 35 months (120). Patients were not required to have BRCA1/2-mutated cancer but were stratified into BRCA1/2-mutated and wild-type cohorts. Patients with wild-type BRCA1/2 were analyzed to determine if tumors were functionally HR deficient by assaying for increased genomic and telomeric rearrangements (120,121). Importantly, niraparib monotherapy showed statistically significant improvement in the primary end point, progression-free survival (PFS), in all three groups. PFS improved from 5.5 to 21.0 months in the BRCA1/2-mutant cohort, from 3.9 to 12.9 months in the BRCA1/2 wild-type/HR-deficient cohort, and from 3.8 to 6.9 months in the BRCA1/2 wild-type/HR-proficient cohort (120). The improvement in PFS with niraparib in the HR-proficient cohort was surprising and could be due to several factors. It could indicate that many HR-proficient ovarian cancers depend on active PARP1 for survival, perhaps to repair oxidative DNA damage. It is also possible that the responsive BRCA1/2 wild-type tumors could have expressed ERCC1 at low levels (102). As PARP1 inhibitors with improved specific activity are developed, they may prove effective against a far broader range of malignancies than originally considered, similar to niraparib.

At least 16 additional PARP1 inhibitors are under clinical development (70). PARP1 inhibitors are being evaluated in combination regimens with DNA-damaging agents, such as platinum analogs or ionizing radiation, or with agents that interfere with other steps in DNA repair or replication. For example, olaparib potentiates the activity of the TopoI inhibitor SN-38 (the active metabolite of the chemotherapeutic agent irinotecan) by blocking RAD51-dependent DNA repair (186). However, a phase I trial combining olaparib with the TopoI inhibitor topotecan was terminated early because of hematologic toxicity at doses below the known effective single-agent dosing levels of each drug (187). Notably, adequate doses of veliparib and topotecan were safely administered together in another phase I study, supporting additional studies of this combination in cervical cancers, where activity was seen in patients with cancers having low PARP1 expression at baseline (188). Veliparib was also examined in multiple types of relapsed hematologic malignancies in combination with carboplatin and topotecan, with some activity seen in chronic myelomonocytic leukemia; interestingly, leukemias with FA pathway deficiency showed the best responses (189).

Summary

There is not a one-to-one correspondence between loss of a repair process and a druggable addiction, and not every cancer has a systemic defect in a DNA repair pathway. While all cancers have many DNA mutations, this is by no means equivalent to the systemic loss of a DNA repair pathway. Such mutations could be random events, with all DNA repair pathways intact. However, the systemic loss of a DNA repair pathway is more common than previously appreciated, and therefore offers a wealth of opportunities to exploit in therapy.

Given the complexity of DSB repair pathways and crosstalk with DNA damage signaling networks, many more synthetic lethal strategies are likely to be revealed through continued study of the regulators of cellular responses to genotoxic cancer therapies. As more data are reported on targeting DNA repair for cancer therapy, several principles are evident that will extend DNA repair targeting beyond those cancers with BRCA1 or 2 defects. First, cancer cells must overcome more endogenous DNA damage than normal cells because of increased oxidative damage

and replication stress from forced cell division (2,28,29,58,190). Because specific DNA repair pathways are backed up by other repair pathways, synthetic lethality only occurs when the primary pathway is defective and the backup rescue pathway is repressed (66–68).

Interestingly, it appears that cancer cell death is less due to the lack of repair, but rather the persistence of toxic repair intermediates (75). Incomplete repair can be more toxic than if a repair pathway is never initiated (191). Therefore, defining repair pathway relationships will reveal additional synthetic lethal targets. For example, the understanding that aNHEJ backs up HR in replication fork restart (32) leads to the concept that inhibiting other aNHEJ components besides PARP1, such as Pol θ , will also confer synthetic lethality in HR-defective cancers (177). Such investigations will increase the number of DNA repair components that can be targeted.

A second principle is that cancers can be responsive to drugs that target DNA repair even if they do not carry mutations in known repair proteins such as BRCA1 or 2. Thus, cancers may be functionally defective in repair but not genetically deficient in any known repair pathway (120,121). It will be important to reach a consensus on methods for identifying cancers that are functionally defective in specific repair pathways, such as the genomic instability assay used in the niraparib trial (120), because this will expand the patient base that could benefit from DNA repair-targeted therapies.

Finally, many cancers do not have an identifiable functional or genetic deficiency in a DNA repair pathway that would lend itself to synthetic lethality. Such cancers may be best treated with drug combinations that induce artificial synthetic lethality by blocking primary and backup repair pathways. In these cases, the therapeutic index is not due to differential repair capacity in normal vs cancer cells, but rather to the heavier load of endogenous DNA damage characteristic of many cancers. Such approaches would permit DNA repair targeting to be much more widely applied in cancer therapy. It is also important to identify synthetic rescue pathways and develop strategies to block these before therapeutic resistance develops. It is clear that while drugging DNA repair is still in its infancy, there is enormous potential to this approach because it will be applicable to many other malignancies besides those with BRCA1 or 2 mutations.

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