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DNA repair inhibitors to enhance radiotherapy: progresses and limitations

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Les inhibiteurs de la réparation de l'ADN au secours de la radiothérapie : progrès et limites

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Abstract

Radiotherapy is one of the most common form of treatment in oncology care. Indeed, radiotherapy proved to be very effective in treating a wide range of malignancies. Nevertheless, certain tumours are intrinsically radioresistant or may evolve to become radioresistant. Resistance to radiotherapy is often associated with dysregulated DNA damage response and repair. Recently, a number of strategies have been developed to improve radiotherapy efficacy by targeting the DNA damage response and repair pathways. Ongoing clinical trials showed the potential of some of these approaches in enhancing radiotherapy, but also highlighted the possible limitations. Here, we will describe (i) the main mechanisms involved in double-strand break repair; (ii) available strategies that target these DNA repair processes to improve radiotherapy and (iii) the clinical outcomes and challenges that have emerged so far.

Résumé

La radiothérapie est l'une des formes de traitement la plus couramment utilisée dans la prise en charge des cancers. En effet, la radiothérapie s'est révélée très efficace dans le traitement d'un large éventail de tumeurs malignes. Néanmoins, certaines tumeurs sont intrinsèquement radiorésistantes ou peuvent évoluer pour le devenir. La résistance à la radiothérapie est souvent associée à une dérégulation de la réponse aux dommages de l'ADN et de leur réparation. Récemment, plusieurs stratégies ont été développées pour améliorer l'efficacité de la radiothérapie en ciblant ces voies. Les essais cliniques en cours ont démontré le potentiel de certaines de ces approches pour améliorer les résultats de la radiothérapie, mais ont également mis en évidence les limites possibles. Nous décrivons ici (i) les principaux mécanismes impliqués dans la réparation des ruptures double-brin; (ii) les stratégies disponibles qui ciblent les processus de réparation de l'ADN afin d'améliorer la radiothérapie et (iii) les résultats cliniques et les défis qui ont émergé jusqu'à présent.

Keywords

Radiotherapy, radiosensitizer, DNA repair inhibitors, radioresistance, radiotherapy enhancement, chemoradiation

Mots clé

Radiothérapie ; radiosensible ; ADN ; réparation ; inhibiteur ; radiorésistance ; chimioradiothérapie

Conflict of interests

MD: cofounder of DNA Therapeutics, consultant for Onxeo.

1. Introduction

DNA integrity is constantly challenged by various types of genotoxic insults (1). An uncorrupted DNA damage response is essential to preserve genomic integrity. Dysregulation of DNA damage signalling and/or DNA damage repair pathways are a known hallmark of cancer (2, 3). The DNA damage response of eukaryotic cells has evolved to repair a diversity of DNA damage types, by many independent repair pathways. Part of these pathways are functionally redundant allowing for a fail-safe mechanism whereby dysfunction of one pathway will be compensated by the hyperactivation of another pathway (4-7). These compensatory mechanisms in tumour cell context can result in increased cancer resistance to DNA damaging agents including radiotherapy, affecting cell response and

outcomes to genotoxic therapies (8, 9). On the other hand, it may create a vulnerability specific to the tumor as its cells are reliant on alternative pathways for survival (10).

Half of cancer patients are treated with radiation as a primary line of treatment (11, 12).

Radiotherapy's cytotoxic effect is mainly exerted by inducing DNA double-strand breaks (13). DNA double-strand breaks are the most deleterious type of DNA lesion and are also one of the most difficult to repair, making radiotherapy one of the most effective forms of cancer therapy (14,15). The major pathways involved in the repair of DNA double-strand breaks are: non-homologous end joining, homologous recombination and microhomology end joining. Due to their genetic instability and active proliferation, tumour cells are in general more radiosensitive than normal cells (16-18). Nevertheless, efficacy of radiotherapy greatly depends on the delivered dose. However, the maximal radiation doses that can be administered are limited by the sensitivity of the tissues and organs surrounding the tumour and the acceptable levels of morbidity (12, 19, 20).

Enhancement of tumour response to radiotherapy through DNA repair inhibition has been in the foresight of translational radiotherapy research for decades (21). The use of DNA repair inhibitors may help tackling three of the major clinical challenges associated to cancer treatment: (i) widening the therapeutic index (11), (ii) prevent or reverse resistance to treatments by impeding the cell's recovery (18); and/or (iii) kill cells that rely on compensatory DDR mechanisms through synthetic lethality (10). The research and development of drugs affecting DNA damage signalling and repair has been the object of extensive research in the preclinical and clinical realm with so far, a moderate success. Limitation associated to the use of these inhibitors are often lined to excessive toxicity at effective doses and or resistance to the treatments through compensatory pathways that limit their efficiency. Many DNA repair inhibitors are already being tested in combination with radiotherapy. In this review we will focus on the main three pathways involved on the repair induced by radiotherapy, non-homologous end joining, homologous recombination and microhomology end joining, and on the therapeutic value and clinical outcomes of inhibiting them.

2. Inhibitors of DNA damage signalling

An approach to hamper DNA double-strand breaks repair is through dysregulation of its signalling. The detection and signalling of DNA double-strand breaks are the very first steps of DNA repair pathways. The DNA damage response pathway transduces the presence of DNA damage to effector protein that will act in a choreographed manner to repair the insult. The most effective sensors of DNA double-strand breaks are the Mre11, Rad50 and NBS1 (MRN) complex and Ku heterodimer (22-24). These sensors will recruit signal transducers from the phosphatidylinositol 3-kinase-related kinases (PIKKs), a family of serine/threonine-protein kinases including: ataxia–telangiectasia mutated (ATM), ataxia telangiectasia and Rad3 related (ATR) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs), mammalian target of rapamycin (mTOR), nonsense mediated mRNA decay associated

PI3K-related kinase (SMG1), and transformation/transcription domain-associated protein (TRRAP) (25-28). These family of DNA damage signal transducers will in turn modify a large set of proteins involved in DNA repair, transcription regulation, cell cycle and apoptosis. Inhibition of the PIKKs through their catalytic site has shown to affect the cell's response to DNA damage and genotoxic insults, making them interesting targets to enhance radiotherapy efficacy. The DNA damage response is mostly regulated by ATM, ATR and DNA-PK, justifying the fact that inhibitors of these three PIKKs are the most exploited and advanced in the clinic. ATM and DNA-PKcs are activated upon DNA double-strand breaks sensing, while ATR is activated by single-strand breaks and replication stress. Inhibition of signal transduction of DNA double-strand breaks will seriously hamper all downstream DNA repair as effector proteins cannot be recruited to the damage site without it. Combination of DNA damage signalling inhibitors with genotoxic therapies, such as radiotherapy, should enhance the efficacy of those therapies by preventing the repair of the damage induced by the treatment.

Inhibition of DNA-PK will be covered in “the inhibitors of non-homologous end joining repair pathway” section as DNA-PK is an important mediator of such pathway. In this section we will focus on the inhibition of ATM and ATR combined to radiotherapy. The grounds to use such inhibitors are based on their two main functions: (i) signal transduction of DNA double- and single-strand breaks and (ii) their role on cell cycle control through modification of CHK2 and CHK1 checkpoint kinases. Failing to transduce DNA damage signalling will hinder recruitment of DNA repair effectors affecting all downstream pathway. While at the same time, the cell will not undergo cell cycle arrest and take the necessary time to repair and recover from the damage induced by radiotherapy.

ATM inhibitor AZD1390 has shown very interesting results in combination with radiotherapy in preclinical brain tumour models (29). It is currently being tested in a phase I clinical trial for safety and tolerability in combination with radiotherapy, as another ATM inhibitor M3541 (NCT03225105). Other ATM inhibitor, AZD0156, is being tested in the clinic for phase I safety trial in association with various chemotherapies including other DNA damage response inhibitors (NCT02588105).

ATR inhibitors are as well an interesting target to be associated with radiotherapy, as they are signal transducers of single-strand breaks. Most of the DNA damage induced by radiation is by single-strand breaks generation. Inhibition of ATR upon radiotherapy will prevent the repair of single-strand breaks and the phosphorylation of checkpoint kinase 1 (CHK1). Unrepaired single-strand breaks may become DNA double-strand breaks at replication or transcription steps and lead to cell death if left unrepaired. ATR inhibitor M6620 is being tested in combination with radiotherapy in brain metastasis (NCT02589522) and with other chemoradiotherapy protocols (NCT03641547). As well, AZD6738 ATR inhibitor is being tested with RT (NCT02223923) and other DDR inhibitors too

(NCT03682289). All these trials are still in early stages, aiming to determine whether ATR inhibitors are tolerable and if their combination with radiotherapy may be beneficial to patients.

Challenging the conventional targeted approach of inhibition, an innovative concept has been proposed through Dbait/AsiDNA™ molecules that work by priming the hyperactivation of DNA damage response systems instead of inhibiting it. Dbait molecules are made of a double-strand oligonucleotide mimicking a DNA double-strand breaks (30). Main DNA repair proteins as DNA-PK and PARP recognize and bind to it, being thereby activated (31,32). Their activation triggers an hypertransduction of DNA damage signal in the absence of any actual chromosomal damage (30, 33). This signal “blinds” the cell from the damage created by genotoxic therapies, such as radiotherapy, hindering and reducing the cell’s ability to recruit repair effectors enzymes to the real damage site induced by radiation. The proteins involved in single- or DNA double-strand breaks repair will not be able to form repair complexes and overall DNA repair is ineffective. Major repair pathways, such as non-homologous end joining and homologous recombination for DNA double-strand breaks and PARP-dependent single-strand breaks repair are hampered with a single agent. It was recently shown that the ability of Dbait to deregulate DNA damage response as a whole, instead of a single enzyme, reduces the cell’s ability to evolve resistance to the treatments (34). This broad spectrum of inhibition has also allowed for the use of AsiDNA™ combined to other DNA repair inhibitors as PARP inhibitors in order to create a drug-driven synthetic lethality, bypassing the requirement for tumour with a homologous recombination deficiency (35).

A phase I/II clinical trial combining AsiDNA™ with radiotherapy for melanoma skin metastasis has showed promising results on the effectiveness of the treatment and notably, without dose-limiting toxicity (36). As a matter of fact, the maximum-tolerated dose was not reached or found confirming the preclinical results of which irreversible toxicity was never found (30, 33, 35, 37, 38). The ongoing clinical trial (NCT03579628), using a systemic delivery, will be vital to understand whether its systemic administration is safe, so that it can be further explored in various types of cancers in combination to radiotherapy.

3. Inhibitors of non-homologous end joining repair pathway

In humans, non-homologous end joining plays the largest role in DNA double-strand breaks repair (39). It is estimated that non-homologous end joining is accountable for the repair of 85% of the DNA double-strand breaks generated by radiation (40). Non-homologous end joining is then a very attractive target as an anticancer therapy to exacerbate the cytotoxic effect of DNA double-strand breaks induced by radiation. Non-homologous end joining mediates the direct end processing of the broken DNA without the need for a homologous template as it is the case for homologous recombination. non-homologous end joining-mediated repair can be accurate unless small processing at the DNA double-strand break ends is needed leading to small alterations as insertions or deletions.

Basically, the sequential repair steps of non-homologous end joining are the (i) recognition of the DNA break end by the MRN complex that will activate ATM (27, 41-43). ATM will in turn phosphorylate further ATMs and the H2AX flanking the break forming γ H2AX; (ii) Ku heterodimer will assemble at the DNA double-strand breaks within seconds, to protect it from degradation and recruit DNA-PKcs leading to its activation (44-46). Once activated, DNA-PKcs will phosphorylate itself and Artemis (46, 47). It is the phosphorylation of DNA-PKcs that will favour DNA double-strand breaks repair through non-homologous end joining, inhibiting homologous recombination (48, 49) Artemis will help process break ends, that otherwise would be non-ligatable, like 5' or 3' overhangs or flaps (50, 51); (3) The DNA-PK complex will bridge the two physically proximal DNA ends working as a scaffold. (4) Finally, recruitment of XRCC4-XLF and DNA ligase IV will ligate both ends terminating repair with the non-homologous end joining complexes disassembly (4, 52-55) (Fig.1). Non-homologous end joining can be active in all phases of the cell cycle, yet more predominantly in G0 and G1 phase (56).

Though being a promising strategy for radio-sensitization, **non-homologous end joining** inhibition has faced two main obstacles: (i) high abundance of the involved proteins in tumor cells making effective inhibition of their activity a challenge and (ii) **non-homologous end joining** activity inhibition is easily toxic as it is key for healthy tissues maintenance. For instances, in clinical trials, the DNA-PK inhibitor M3814, despite being well tolerated as monotherapy, was found to enhance normal tissues reactivity to radiotherapy in some patients (57) (Fig.1). Ongoing clinical trials with M3814 (NCT03724890, NCT03770689 and NCT02516813) will tell whether it can be both efficient and tolerable in combination with radiotherapy. Other DNA-PK inhibitor, MSC2490484A, is currently being tested for tolerability and efficacy in chemoradiotherapy protocols (cisplatin) (NCT02516813). The CC-115 dual-inhibitor of mTOR and DNA-PKcs, has shown to be better tolerated and with observable antitumour activity (58) (Fig.1). However, it has not yet been tested in combination to radiotherapy. Inhibitors of Ku70/80 (59) or DNA ligase IV (60) to be combined to radiotherapy are being developed and explored in preclinical studies but have not yet reached the clinical trial step.

Other inhibitors of non-homologous end joining, more specifically those targeting its regulation, are as well being tested. A sideway to inhibit non-homologous end joining is through the inhibition of epidermal growth factor receptor (EGFR) pathway. Ionizing radiation activates EGFR pathway, translocating EGFR to the nucleus to be associated to DNA-PKcs (61). Dysregulation of EGFR pathway confers cells radioresistant (62, 63) and is associated with poorer prognosis in cancers (64, 65). Inhibition of EGFR activation through monoclonal antibodies, cetuximab and panitumumab, combined with radiotherapy or platinum-based agents is presently used in the clinic and patients are presenting improved overall survival (66-68) (Fig.1).

Another interesting approach of non-homologous end joining inhibition is through the androgen-deprivation therapy, commonly used in the treatment of prostate cancer since the 1980s (69-71). It was not long ago, that the mechanisms of synergy were unravelled. The androgen receptor and DNA-PKcs comodulate each other in a positive feedback loop manner. While DNA-PKcs will stimulate the transcription of androgen receptor, androgen receptor signalling stimulates DNA double-strand breaks repair through DNA-PKcs and its gene expression (72, 73). Pharmacological androgen-deprivation therapy can be achieved by different drugs with different mechanism of action: luteinizing hormone-releasing hormone (LHRH) agonists (e.g. goserelin), LHRH antagonist (e.g. degarelix), CYP17 inhibitor (e.g. abiraterone), and antiandrogens (e.g. flutamide). Pharmacological or surgical androgen-deprivation therapy synergizes with radiotherapy, increasing tumour radiosensitivity through an inefficient repair of the DNA double-strand breaks induced by radiation (71, 74-76) (Fig.1).

4. Inhibitors of homologous recombination repair pathway

DNA double-strand breaks repair by homologous recombination is an extremely complex process in which multiple proteins are tightly orchestrated to lead to an error-free repair (77). Even though it repairs a smaller number of the DNA double-strand breaks, its high-fidelity repair makes it essential to preserve genome stability. Homologous recombination will use the sister chromatid as a template for the accurate resynthesis of the DNA, thereby, it is essentially restricted to S-G2 phase of the cell cycle (78). This property makes homologous recombination inhibition an interesting strategy specially when considering that one important feature of tumour cells is their active proliferation.

The sequential steps for homologous recombination repair are: (i) sensing of dsDB by MRN complex, and signalling by ATM kinase (41-43); (ii) 5' to 3' DNA end resection by CtIP, RPA binding to 5' single-strand DNA to prevent formation of secondary structures or promiscuous annealing, later replaced by Rad51 monomers (79-82); (iii) recruitment of BRCA1, BRCA2, Rad52 and XCC2/3 and Rad51 complex to the 3' strand (83,84); (iv) homology search mediated by Rad51 and D-loop strand formation; (v) resynthesis of the DNA by DNA polymerase δ formation and ligation of the Holliday junctions by DNA ligase I and finally resolution with or without crossover (85,86). (Fig.2)

Like DNA-PKcs for the NHEJ, Rad51 plays a central role in homologous recombination-mediated repair. Few direct inhibitors of Rad51 have been tested in preclinical and clinical trials. Rad51 inhibitor CYT-0851, is currently being tested as monotherapy in a phase I trial (NCT03997968) (Fig.2). An alternative way to for inhibiting Rad51, is to target its regulation through the c-Alb tyrosine kinase; c-Alb undergoes a strong activation upon radiotherapy and modulates Rad51 response to DNA damage (87,88). Imatinib, a c-Alb inhibitor known to impact Rad51, has been already tested in combination with radiotherapy in a phase I trial (Fig.2). Issues in safety-toxicity were raised due to the rate of spontaneous adverse effects associated to the nature of the paediatric brainstem malignant gliomas (89). Final results of that trial will dictate whether it can be further

explored with radiotherapy, and if it is of interest to test it in other tumours with less spontaneous adverse effects.

Because of low potency and/or selectivity many of the MRN complex inhibitors developed in preclinical studies, such as mirin and derivatives, have not reached yet the clinic (90).

5. Inhibitors of microhomology end joining

Early studies of cells deficient in non-homologous end joining have identified alternative error-prone mechanism of end joining repair. This alternative mechanism, also known as alternative non-homologous end joining, will hereafter be named as microhomology end joining. Contrary to classical non-homologous end joining that uses minimal processing of the DNA double-strand breaks and is relatively accurate, microhomology end joining involves larger segments of DNA at the break site with alignments of microhomologous sequences (as few as one nucleotide in mammalian cells) and often results in flank deletions of the DNA double-strand breaks at the repair junction (55, 91-96). Microhomology end joining is an unfaithful and mutagenic repair pathway of DNA double-strand breaks that has been associated to carcinogenesis (91, 94, 97). Microhomology end joining is often seen as an alternative backup of non-homologous end joining, but both can also coexist in certain settings (98).

The chronology of microhomology end joining repair is the following: (i) DNA double-strand breaks will be sensed by MRN complex, which will recruit and activate PARP1 in the absence or in competition of Ku proteins (Ku70 and Ku80) (93, 99, 100). PARP will produce poly(ADP-ribose) and end resection will be initiated by the MRN complex or CtIP endonuclease (101-104); (ii) microhomologies will anneal (105, 106); and (iii) resection of the heterologous 3' flaps (8 to 9 nucleotides) will be executed by Fen1 endonuclease (98); (iv) fill-in synthesis will be done by DNA polymerase θ , that may create artificial homologies throughout DNA synthesis (107, 108); and finally (v) DNA ligation by DNA ligase III, scaffolded by XRCC1 (99, 109) (Fig.3). Increased levels of cyclin-dependent kinases throughout S/G2 cell cycle phases limit resection by MRN-CtIP and decrease Ku levels and its binding to DNA double-strand breaks concomitantly, enabling homologous recombination and alternative non-homologous end joining to act. Like homologous recombination, microhomology end joining activity is generally observed to S/G2 phases (110-112) (79) though, the choice between homologous recombination and microhomology end joining is not fully clear except for the presence or absence of certain dominant factors belonging to each pathways (107, 113-115).

Following the rationale of synthetic lethality for anticancer treatment, inhibition of alternative non-homologous end joining may be beneficial for tumours that rely on such pathway as a compensatory pathway. It is known that microhomology end joining not only may serve as a backup in non-homologous end joining-deficient cells but also in homologous recombination-deficient (107, 115,

116). While conventional non-homologous end joining depends critically on DNA-PK, and homologous recombination on BRCA1 or Rad51, microhomology end joining depends mainly on the initial detection of the DNA double-strand breaks by PARP1. Given the role of PARP on microhomology end joining and on single-strand breaks repair (in base excision repair pathway), pharmacological inhibition of PARP in combination with radiotherapy may be of interest. PARP inhibitors in combination with radiotherapy have been already tested and continue as well to be tested in different tumours in clinic (inaparib, NCT0068776 and NCT01551680, and veliparib, NCT01589419 and NCT01477489) (Fig.3). Treatments were well tolerated but only a patient with tumour bearing a BRCA mutation showed a significant response to the treatment (117, 118). The lack of success of these clinical trials demonstrates a need for better understanding of microhomology end joining pathway and its role on radiation-mediated DNA damage in order to better stratify the potential patients benefiting from such treatments.

6. Conclusions

Our understanding of the molecular events involved in the repair of DNA damage has greatly improved during these past years. It has made possible the development of inhibitors to target such processes for anticancer purposes. Nonetheless, as integrity of the genome is a life issue, organism systems have evolved to be highly robust and redundant, making the targeting of DNA repair pathways a challenge. Various independent pathways must be inactive at once to lead to effective tumour cell death. To overcome its robustness and redundancy, it is of importance to further understand what DNA repair pathways or proteins are dysregulated in what kind of cancers to understand their vulnerability. This way, optimizing the use of certain DNA repair inhibitors and ensure successful clinical translation and outcomes.

The various preclinical and clinical studies have demonstrated that DNA repair inhibitors can effectively sensitize cancer cells to radiation. However, not all patients or cancers types seem to benefit equally. This may be due to a suboptimal inactivation, either by compensatory mechanism of the cancer cells or inappropriate therapeutic targeting for certain tumours. Better stratification could avoid such, and as well help identify which patients could see their effective dosage being reduced, like radiotherapy de-escalation.

The targeting of the master regulators of damage signalling, which act on very early stages, seem to have more effective implications on DNA repair outcome, rather than inhibition of a single enzyme acting downstream in the repair pathways cascade. Next years will confirm if we have succeeded to improve radiotherapy efficacy by targeting DNA repair.

The development and use of DNA repair inhibitors is still callow, as many are still in early phases of clinical tests. However, some inhibitors have raised some issues concerning toxicity and effectiveness.

Deeper understanding of the underlying mechanistic differences of DNA repair in tumour and normal cells could help to identify the most effective with the least toxicity therapeutic targets. The ongoing and future preclinical and clinical studies will provide researchers with a wealth of information within the next years. A careful evaluation will help determined whether we have succeeded to improve radiotherapy efficacy and tumour selectivity using DNA repair.

Author contribution

SF and MD certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

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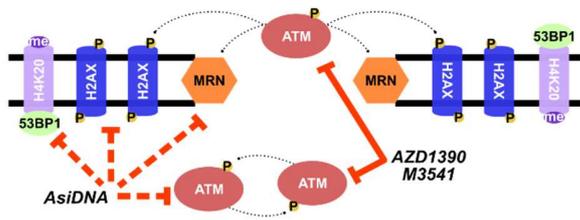
Figure legends

Figure 1. Mechanism of non-homologous end joining in humans. (1) Sensing and signalling of DNA double-strand breaks is made by MRN complex and ATM, respectively. ATM will phosphorylate the H2AX histones flanking the DNA break; (2) Ku70 and Ku80 will assemble to the DNA double-strand break end and will activate DNA-PKcs activating them; (3) DNA-PK complex will bring the two ends together and recruit Artemis and (4) finally recruiting XRCC4-XLF and DNA ligase IV (DNA Lig IV), which will ligate both ends.. Red arrows indicate the inhibitors being tested in the clinic in combination with radiotherapy; continuous line represents the direct inhibitors of the represented enzyme, while the discontinued line represents the indirect inhibitors that dysregulate their signalling or regulation. (P) phosphorylated ; (me) methylated form.

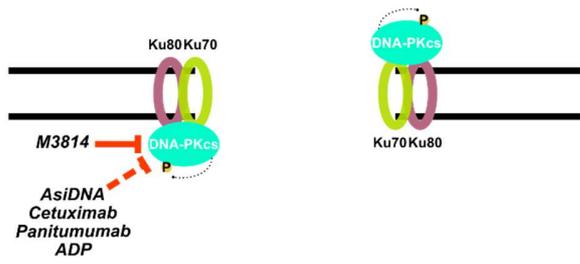
Figure 2. Mechanism of homologous recombination in humans. (1) sensing of DNA double-strand breaks by MRN complex, and signalling by ATM kinase; (2) DNA end resection by CtIP and RPA binding to 5' single-strand which is later replaced by Rad51 monomers; (3) further recruitment of effectors like BRCA1, BRCA2, Rad52 and XCC2/3 and Rad51 complex; (4) Rad51-mediated homology search and D-loop strand formation, resynthesis of the DNA by DNA polymerase δ , formation and ligation of the Holliday junctions by DNA ligase I and finally resolution with or without crossover. Red arrows indicate the inhibitors being tested in the clinic in combination with radiotherapy; continuous line represents the direct inhibitors of the represented enzyme, while the discontinued line represents the indirect inhibitors that dysregulate their signalling or regulation. (P) phosphorylated.

Figure 3. Mechanism of microhomology end joining in humans. (1) sensing of DNA double-strand breaks by MRN complex, and signalling by PARP which will produce poly(ADP-ribose) polymers; (2) microhomologies will anneal and (3) heterologous 3' flaps will be resected by Fen1; (4) fill-in synthesis by DNA polymerase θ and DNA ligation by DNA ligase III scaffolded by XRCC1. Red arrows indicate the inhibitors being tested in the clinic in combination with radiotherapy; continuous line represents the direct inhibitors of the represented enzyme, while the discontinued line represents the indirect inhibitors that dysregulate their signalling or regulation.

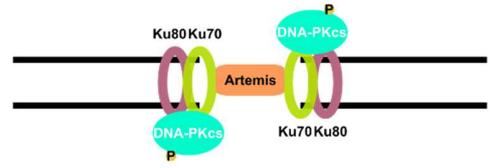
1 Sensing and signal transduction of DNA damage



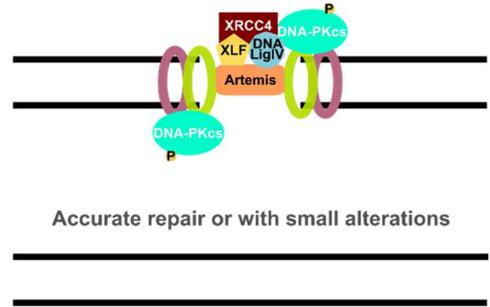
2 DNA end binding and protection



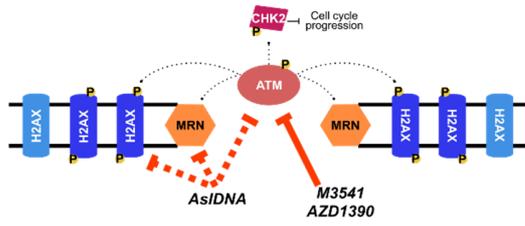
3 Physically proximal DNA ends bridging



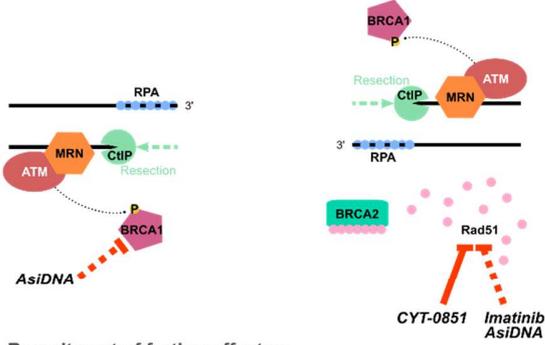
4 DNA ends processing and ligation



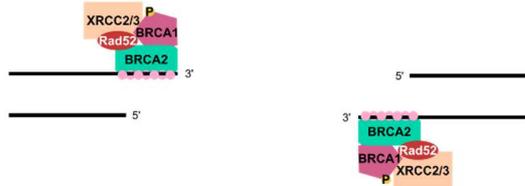
1 Sensing and signal transduction of DNA damage



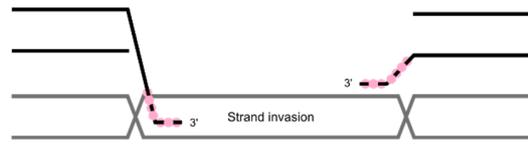
2 DNA resection and single-strand protection



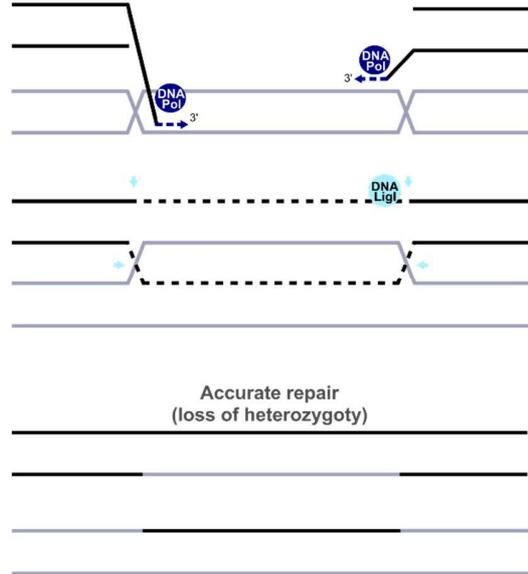
3 Recruitment of further effectors



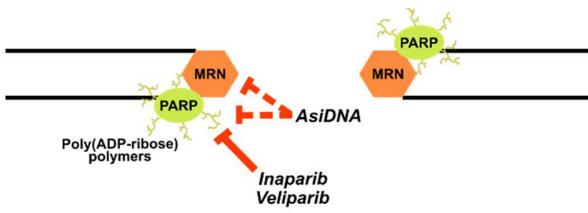
4 Homology search and D-loop formation



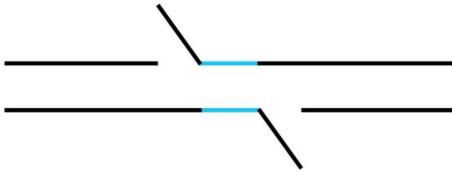
5 DNA synthesis, formation and ligation of Holliday junctions



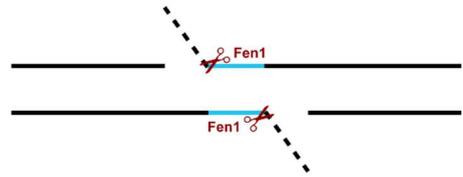
1 Sensing and signal transduction of DNA damage



2 Annealing of micro-homologies



3 Resection of heterologous 3' flaps



4 Fill-in DNA synthesis and ligation

