



HAL
open science

DNA repair inhibitors to enhance radiotherapy: progresses and limitations

S Ferreira, M. Dutreix

► **To cite this version:**

S Ferreira, M. Dutreix. DNA repair inhibitors to enhance radiotherapy: progresses and limitations. Cancer/Radiothérapie, 2019, 10.1016/j.canrad.2019.08.008 . hal-02408159

HAL Id: hal-02408159

<https://hal.science/hal-02408159v1>

Submitted on 21 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

DNA repair inhibitors to enhance radiotherapy: progresses and limitations

★

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

Sofia FERREIRA ^{a,b,c,d,e}, Marie DUTREIX ^{a*,b,c,d,e}

^a Institut Curie, UMR « Etic », bâtiment 110, centre universitaire, 91405 Orsay cedex, France

^b Université PSL, 91405 Orsay, France

^c CNRS, UMR 3347, 91405, Orsay, France

^d Inserm, UMR 3347, 91405, Orsay, France.

^e Université Paris-Sud université Paris-Saclay, 91405 Orsay, France

*Corresponding author; e-mail: marie.dutreix@curie.fr

* This article was presented at the 30th annual meeting of Société française de radiothérapie oncologique, Friday 11th October, 2019 (session: 21th Century radiobiology).

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.

Acknowledgements

This work was supported by the Siric-Curie, the institut Curie, the Centre national de la recherche scientifique (CNRS) and the IRS “NanoTheRad” of University Paris-Sud (Paris-Saclay) and CNRS. SF was supported by a Marie Sklodowska-Curie European fellowship ITN-RADIATE No. 642623 and by Espoirs de la Recherche 2018 program from the Fondation pour la recherche médicale (FRM).

References

1. Lindahl T. Instability and decay of the primary structure of DNA. *Nature* 1993;362(6422):709-15.
2. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100(1):57-70.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144(5):646-74.
4. Oksenyich V, Kumar V, Liu X, Guo C, Schwer B, Zha S, et al. Functional redundancy between the XLF and DNA-PKcs DNA repair factors in V(D)J recombination and nonhomologous DNA end joining. *Proc Natl Acad Sci U S A* 2013;110(6):2234-9.
5. Callen E, Jankovic M, Wong N, Zha S, Chen HT, Difilippantonio S, et al. Essential role for DNA-PKcs in DNA double-strand break repair and apoptosis in ATM-deficient lymphocytes. *Mol Cell* 2009;34(3):285-97.
6. Zha S, Jiang W, Fujiwara Y, Patel H, Goff PH, Brush JW, et al. Ataxia telangiectasia-mutated protein and DNA-dependent protein kinase have complementary V(D)J recombination functions. *Proc Natl Acad Sci U S A* 2011;108(5):2028-33.

7. Gapud EJ, Dorsett Y, Yin B, Callen E, Bredemeyer A, Mahowald GK, et al. Ataxia telangiectasia mutated (Atm) and DNA-PKcs kinases have overlapping activities during chromosomal signal joint formation. *Proc Natl Acad Sci U S A* 2011;108(5):2022-7.
8. Herring CJ, West CM, Wilks DP, Davidson SE, Hunter RD, Berry P, et al. Levels of the DNA repair enzyme human apurinic/apyrimidinic endonuclease (APE1, APEX, Ref-1) are associated with the intrinsic radiosensitivity of cervical cancers. *Br J Cancer* 1998;78(9):1128-33.
9. Tobin LA, Robert C, Nagaria P, Chumsri S, Twaddell W, Ioffe OB, et al. Targeting abnormal DNA repair in therapy-resistant breast cancers. *Mol Cancer Res* 2012;10(1):96-107.
10. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH. Integrating genetic approaches into the discovery of anticancer drugs. *Science* 1997;278(5340):1064-8.
11. Begg AC, Stewart FA, Vens C. Strategies to improve radiotherapy with targeted drugs. *Nat Rev Cancer* 2011;11(4):239-53.
12. Delaney G, Jacob S, Featherstone C, Barton M. The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. *Cancer* 2005;104(6):1129-37.
13. Pastwa E, Neumann RD, Mezhevaya K, Winters TA. Repair of radiation-induced DNA double-strand breaks is dependent upon radiation quality and the structural complexity of double-strand breaks. *Radiat Res* 2003;159(2):251-61.
14. Povirk LF. Biochemical mechanisms of chromosomal translocations resulting from DNA double-strand breaks. *DNA Repair* 2006;5(9-10):1199-212.
15. Bentzen SM, Heeren G, Cottier B, Slotman B, Glimelius B, Lievens Y, et al. Towards evidence-based guidelines for radiotherapy infrastructure and staffing needs in Europe: the ESTRO QUARTS project. *Radiother Oncol* 2005;75(3):355-65.
16. Taylor AM, Harnden DG, Arlett CF, Harcourt SA, Lehmann AR, Stevens S, et al. Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. *Nature* 1975;258(5534):427-9.
17. Chistiakov DA, Voronova NV, Chistiakov PA. Genetic variations in DNA repair genes, radiosensitivity to cancer and susceptibility to acute tissue reactions in radiotherapy-treated cancer patients. *Acta Oncol* 2008;47(5):809-24.
18. Jeggo P, Lavin MF. Cellular radiosensitivity: how much better do we understand it? *Int J Radiat Biol* 2009;85(12):1061-81.

19. Emami B, Lyman J, Brown A, Coia L, Goitein M, Munzenrider JE, et al. Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys* 1991;21(1):109-22.
20. Bentzen SM, Constine LS, Deasy JO, Eisbruch A, Jackson A, Marks LB, et al. Quantitative Analyses of Normal Tissue Effects in the Clinic (QUANTEC): an introduction to the scientific issues. *Int J Radiat Oncol Biol Phys* 2010;76(3 Suppl):S3-9.
21. Ward JF. Mechanisms of DNA repair and their potential modification for radiotherapy. *Int J Radiat Oncol Biol Phys* 1986;12(7):1027-32.
22. Paull TT, Gellert M. Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. *Genes Dev* 1999;13(10):1276-88.
23. de Jager M, Dronkert ML, Modesti M, Beerens CE, Kanaar R, van Gent DC. DNA-binding and strand-annealing activities of human Mre11: implications for its roles in DNA double-strand break repair pathways. *Nucleic Acids Res* 2001;29(6):1317-25.
24. Walker JR, Corpina RA, Goldberg J. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 2001;412(6847):607-14.
25. Lempiainen H, Halazonetis TD. Emerging common themes in regulation of PIKKs and PI3Ks. *EMBO J* 2009;28(20):3067-73.
26. Lovejoy CA, Cortez D. Common mechanisms of PIKK regulation. *DNA Repair* 2009;8(9):1004-8.
27. Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y. Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J* 2003;22(20):5612-21.
28. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 2008;27(41):5497-510.
29. Durant ST, Zheng L, Wang Y, Chen K, Zhang L, Zhang T, et al. The brain-penetrant clinical ATM inhibitor AZD1390 radiosensitizes and improves survival of preclinical brain tumor models. *Sci Adv* 2018;4(6):eaat1719.
30. Quanz M, Berthault N, Roulin C, Roy M, Herbette A, Agrario C, et al. Small-molecule drugs mimicking DNA damage: a new strategy for sensitizing tumors to radiotherapy. *Clin Cancer Res* 2009;15(4):1308-16.

31. Quanz M, Chassoux D, Berthault N, Agrario C, Sun JS, Dutreix M. Hyperactivation of DNA-PK by double-strand break mimicking molecules disorganizes DNA damage response. *PLoS One* 2009;4(7):e6298.
32. Croset A, Cordelieres FP, Berthault N, Buhler C, Sun JS, Quanz M, et al. Inhibition of DNA damage repair by artificial activation of PARP with siDNA. *Nucleic Acids Res* 2013;41(15):7344-55.
33. Biau J, Devun F, Jdey W, Kotula E, Quanz M, Chautard E, et al. A preclinical study combining the DNA repair inhibitor Dbait with radiotherapy for the treatment of melanoma. *Neoplasia* 2014;16(10):835-44.
34. Jdey W, Kozlak M, Alekseev S, Thierry S, Lascaux P, Girard PM, et al. AsiDNA treatment induces cumulative antitumor efficacy with a low probability of acquired resistance. *Neoplasia* 2019;21(9):863-71.
35. Jdey W, Thierry S, Russo C, Devun F, Al Abo M, Nogueiez-Hellin P, et al. Drug-driven synthetic lethality: bypassing tumor cell genetics with a combination of AsiDNA and PARP inhibitors. *Clin Cancer Res* 2017;23(4):1001-11.
36. Le Tourneau C, Dreno B, Kirova Y, Grob JJ, Jouary T, Dutriaux C, et al. First-in-human phase I study of the DNA-repair inhibitor DT01 in combination with radiotherapy in patients with skin metastases from melanoma. *Br J Cancer* 2016;114(11):1199-205.
37. Thierry S, Jdey W, Alculumbre S, Soumelis V, Nogueiez-Hellin P, Dutreix M. The DNA repair inhibitor Dbait is specific for malignant hematologic cells in blood. *Mol Cancer Ther* 2017;16(12):2817-27.
38. Schlegel A, Buhler C, Devun F, Agrario C, Urien S, Lokiec F, et al. Pharmacokinetics and toxicity in rats and monkeys of coDbait: a therapeutic double-stranded DNA oligonucleotide conjugated to cholesterol. *Mol Ther Nucleic Acids* 2012;1:e33.
39. Burma S, Chen BP, Chen DJ. Role of non-homologous end joining (NHEJ) in maintaining genomic integrity. *DNA Repair* 2006;5(9-10):1042-8.
40. Mahaney BL, Meek K, Lees-Miller SP. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. *Biochem J*. 2009;417(3):639-50.
41. Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *J Biol Chem* 2001;276(45):42462-7.
42. Lavin MF. ATM and the Mre11 complex combine to recognize and signal DNA double-strand breaks. *Oncogene* 2007;26(56):7749-58.

43. Williams RS, Williams JS, Tainer JA. Mre11-Rad50-Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. *Biochem Cell Biol* 2007;85(4):509-20.
44. Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. *Cell* 1993;72(1):131-42.
45. DeFazio LG, Stansel RM, Griffith JD, Chu G. Synapsis of DNA ends by DNA-dependent protein kinase. *EMBO J* 2002;21(12):3192-200.
46. Hammel M, Yu Y, Mahaney BL, Cai B, Ye R, Phipps BM, et al. Ku and DNA-dependent protein kinase dynamic conformations and assembly regulate DNA binding and the initial non-homologous end joining complex. *J Biol Chem* 2010;285(2):1414-23.
47. Reddy YV, Ding Q, Lees-Miller SP, Meek K, Ramsden DA. Non-homologous end joining requires that the DNA-PK complex undergo an autophosphorylation-dependent rearrangement at DNA ends. *J Biol Chem* 2004;279(38):39408-13.
48. Neal JA, Meek K. Choosing the right path: does DNA-PK help make the decision? *Mutat Res* 2011;711(1-2):73-86.
49. Neal JA, Dang V, Douglas P, Wold MS, Lees-Miller SP, Meek K. Inhibition of homologous recombination by DNA-dependent protein kinase requires kinase activity, is titratable, and is modulated by autophosphorylation. *Mol Cell Biol* 2011;31(8):1719-33.
50. Abe T, Ishiai M, Hosono Y, Yoshimura A, Tada S, Adachi N, et al. KU70/80, DNA-PKcs, and Artemis are essential for the rapid induction of apoptosis after massive DSB formation. *Cell Signal* 2008;20(11):1978-85.
51. Goodarzi AA, Yu Y, Riballo E, Douglas P, Walker SA, Ye R, et al. DNA-PK autophosphorylation facilitates Artemis endonuclease activity. *EMBO J* 2006;25(16):3880-9.
52. Grawunder U, Zimmer D, Fugmann S, Schwarz K, Lieber MR. DNA ligase IV is essential for V(D)J recombination and DNA double-strand break repair in human precursor lymphocytes. *Mol Cell* 1998;2(4):477-84.
53. Grawunder U, Zimmer D, Kulesza P, Lieber MR. Requirement for an interaction of XRCC4 with DNA ligase IV for wild-type V(D)J recombination and DNA double-strand break repair in vivo. *J Biol Chem* 1998;273(38):24708-14.
54. Critchlow SE, Bowater RP, Jackson SP. Mammalian DNA double-strand break repair protein XRCC4 interacts with DNA ligase IV. *Curr Biol* 1997;7(8):588-98.

55. Guirouilh-Barbat J, Rass E, Plo I, Bertrand P, Lopez BS. Defects in XRCC4 and KU80 differentially affect the joining of distal nonhomologous ends. *Proc Natl Acad Sci U S A* 2007;104(52):20902-7.
56. Shrivastav M, De Haro LP, Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. *Cell Res* 2008;18(1):134-47.
57. Mau-Sorensen M, van Bussel M, Kuipers M, Nielsen DL, Verheul HM, Aftimos P, et al. 1845PSafety, clinical activity and pharmacological biomarker evaluation of the DNA-dependent protein kinase (DNA-PK) inhibitor M3814: Results from two phase I trials. *Ann Oncol* 2018;29(suppl_8).
58. Munster PN, Mahipal A, Nemunaitis JJ, Mita MM, Paz-Ares LG, Massard C, et al. Phase I trial of a dual TOR kinase and DNA-PK inhibitor (CC-115) in advanced solid and hematologic cancers. *J Clin Oncol* 2016;34(15_suppl):2505.
59. Weterings E, Gallegos AC, Dominick LN, Cooke LS, Bartels TN, Vagner J, et al. A novel small molecule inhibitor of the DNA repair protein Ku70/80. *DNA Repair* 2016;43:98-106.
60. Srivastava M, Nambiar M, Sharma S, Karki SS, Goldsmith G, Hegde M, et al. An inhibitor of nonhomologous end-joining abrogates double-strand break repair and impedes cancer progression. *Cell* 2012;151(7):1474-87.
61. Liccardi G, Hartley JA, Hochhauser D. EGFR nuclear translocation modulates DNA repair following cisplatin and ionizing radiation treatment. *Cancer Res* 2011;71(3):1103-14.
62. Chakravarti A, Chakladar A, Delaney MA, Latham DE, Loeffler JS. The epidermal growth factor receptor pathway mediates resistance to sequential administration of radiation and chemotherapy in primary human glioblastoma cells in a RAS-dependent manner. *Cancer Res* 2002;62(15):4307-15.
63. Lammering G, Hewit TH, Hawkins WT, Contessa JN, Reardon DB, Lin PS, et al. Epidermal growth factor receptor as a genetic therapy target for carcinoma cell radiosensitization. *J Natl Cancer Inst* 2001;93(12):921-9.
64. Ang KK, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. *Cancer Res* 2002;62(24):7350-6.

65. Huhn SL, Mohapatra G, Bollen A, Lamborn K, Prados MD, Feuerstein BG. Chromosomal abnormalities in glioblastoma multiforme by comparative genomic hybridization: correlation with radiation treatment outcome. *Clin Cancer Res* 1999;5(6):1435-43.
66. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013;369(11):1023-34.
67. Stintzing S, Modest DP, Rossius L, Lerch MM, von Weikersthal LF, Decker T, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab for metastatic colorectal cancer (FIRE-3): a post-hoc analysis of tumour dynamics in the final RAS wild-type subgroup of this randomised open-label phase 3 trial. *Lancet Oncol* 2016;17(10):1426-34.
68. Dattatreya S, Goswami C. Cetuximab plus radiotherapy in patients with unresectable locally advanced squamous cell carcinoma of head and neck region--a open labelled single arm phase II study. *Indian J Cancer* 2011;48(2):154-7.
69. Pilepich MV, Krall JM, al-Sarraf M, John MJ, Doggett RL, Sause WT, et al. Androgen deprivation with radiation therapy compared with radiation therapy alone for locally advanced prostatic carcinoma: a randomized comparative trial of the Radiation Therapy Oncology Group. *Urology* 1995;45(4):616-23.
70. Pilepich MV, Caplan R, Byhardt RW, Lawton CA, Gallagher MJ, Mesic JB, et al. Phase III trial of androgen suppression using goserelin in unfavorable-prognosis carcinoma of the prostate treated with definitive radiotherapy: report of Radiation Therapy Oncology Group Protocol 85-31. *J Clin Oncol* 1997;15(3):1013-21.
71. Bolla M, Gonzalez D, Warde P, Dubois JB, Mirimanoff RO, Storme G, et al. Improved survival in patients with locally advanced prostate cancer treated with radiotherapy and goserelin. *N Engl J Med* 1997;337(5):295-300.
72. Goodwin JF, Schiewer MJ, Dean JL, Schrecengost RS, de Leeuw R, Han S, et al. A hormone-DNA repair circuit governs the response to genotoxic insult. *Cancer Discov* 2013;3(11):1254-71.
73. Polkinghorn WR, Parker JS, Lee MX, Kass EM, Spratt DE, Iaquina PJ, et al. Androgen receptor signaling regulates DNA repair in prostate cancers. *Cancer Discov.* 2013;3(11):1245-53.
74. Tarish FL, Schultz N, Tanoglidi A, Hamberg H, Letocha H, Karaszi K, et al. Castration radiosensitizes prostate cancer tissue by impairing DNA double-strand break repair. *Sci Transl Med* 2015;7(312):312re11.

75. Spina CS. Androgen deprivation therapy and radiation therapy for prostate cancer: the mechanism underlying therapeutic synergy. *Transl Cancer Res* 2018:S695-S703.
76. Warde P, Mason M, Ding K, Kirkbride P, Brundage M, Cowan R, et al. Combined androgen deprivation therapy and radiation therapy for locally advanced prostate cancer: a randomised, phase 3 trial. *Lancet* 2011;378(9809):2104-11.
77. Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, et al. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO J* 1998;17(18):5497-508.
78. Sonoda E, Sasaki MS, Morrison C, Yamaguchi-Iwai Y, Takata M, Takeda S. Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells. *Mol Cell Biol* 1999;19(7):5166-9.
79. Huertas P, Jackson SP. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. *J Biol Chem* 2009;284(14):9558-65.
80. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 2003;300(5625):1542-8.
81. Deng SK, Chen H, Symington LS. Replication protein A prevents promiscuous annealing between short sequence homologies: Implications for genome integrity. *Bioessays* 2015;37(3):305-13.
82. Baumann P, West SC. Role of the human RAD51 protein in homologous recombination and double-stranded-break repair. *Trends Biochem Sci* 1998;23(7):247-51.
83. Johnson RD, Liu N, Jasin M. Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination. *Nature* 1999;401(6751):397-9.
84. French CA, Masson JY, Griffin CS, O'Regan P, West SC, Thacker J. Role of mammalian RAD51L2 (RAD51C) in recombination and genetic stability. *J Biol Chem* 2002;277(22):19322-30.
85. Maloisel L, Fabre F, Gangloff S. DNA polymerase δ is preferentially recruited during homologous recombination to promote heteroduplex DNA extension. *Mol Cell Biol* 2008;28(4):1373-82.
86. Wu L, Hickson ID. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 2003;426(6968):870-4.

87. Kharbanda S, Ren R, Pandey P, Shafman TD, Feller SM, Weichselbaum RR, et al. Activation of the c-Abl tyrosine kinase in the stress response to DNA-damaging agents. *Nature* 1995;376(6543):785-8.
88. Yuan ZM, Huang Y, Ishiko T, Nakada S, Utsugisawa T, Kharbanda S, et al. Regulation of Rad51 function by c-Abl in response to DNA damage. *J Biol Chem* 1998;273(7):3799-802.
89. Pollack IF, Jakacki RI, Blaney SM, Hancock ML, Kieran MW, Phillips P, et al. Phase I trial of imatinib in children with newly diagnosed brainstem and recurrent malignant gliomas: a Pediatric Brain Tumor Consortium report. *Neuro Oncol* 2007;9(2):145-60.
90. Sanford-Crane H, Pejovic T, Xiao X. Drugging homologous recombination: back to the future. *Future Med Chem* 2018;10(11):1279-81.
91. Simsek D, Jasin M. Alternative end-joining is suppressed by the canonical NHEJ component Xrcc4-ligase IV during chromosomal translocation formation. *Nat Struct Mol Biol* 2010;17(4):410-6.
92. Roth DB, Porter TN, Wilson JH. Mechanisms of nonhomologous recombination in mammalian cells. *Mol Cell Biol* 1985;5(10):2599-607.
93. Wang M, Wu W, Wu W, Rosidi B, Zhang L, Wang H, et al. PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. *Nucleic Acids Res* 2006;34(21):6170-82.
94. Yan CT, Boboila C, Souza EK, Franco S, Hickernell TR, Murphy M, et al. IgH class switching and translocations use a robust non-classical end-joining pathway. *Nature* 2007;449(7161):478-82.
95. Decottignies A. Microhomology-mediated end joining in fission yeast is repressed by pku70 and relies on genes involved in homologous recombination. *Genetics* 2007;176(3):1403-15.
96. Bennardo N, Cheng A, Huang N, Stark JM. Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. *PLoS Genet* 2008;4(6):e1000110.
97. Letsolo BT, Rowson J, Baird DM. Fusion of short telomeres in human cells is characterized by extensive deletion and microhomology, and can result in complex rearrangements. *Nucleic Acids Res* 2010;38(6):1841-52.
98. Truong LN, Li Y, Shi LZ, Hwang PY, He J, Wang H, et al. Microhomology-mediated end joining and homologous recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. *Proc Natl Acad Sci U S A* 2013;110(19):7720-5.

99. Audebert M, Salles B, Calsou P. Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. *J Biol Chem* 2004;279(53):55117-26.
100. Mansour WY, Borgmann K, Petersen C, Dikomey E, Dahm-Daphi J. The absence of Ku but not defects in classical non-homologous end-joining is required to trigger PARP1-dependent end-joining. *DNA Repair* 2013;12(12):1134-42.
101. Xie A, Kwok A, Scully R. Role of mammalian Mre11 in classical and alternative nonhomologous end joining. *Nat Struct Mol Biol* 2009;16(8):814-8.
102. Deng Y, Guo X, Ferguson DO, Chang S. Multiple roles for MRE11 at uncapped telomeres. *Nature* 2009;460(7257):914-8.
103. Lee-Theilen M, Matthews AJ, Kelly D, Zheng S, Chaudhuri J. CtIP promotes microhomology-mediated alternative end joining during class-switch recombination. *Nat Struct Mol Biol* 2011;18(1):75-9.
104. Zhang Y, Jasin M. An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway. *Nat Struct Mol Biol* 2011;18(1):80-4.
105. Daley JM, Wilson TE. Rejoining of DNA double-strand breaks as a function of overhang length. *Mol Cell Biol* 2005;25(3):896-906.
106. Villarreal DD, Lee K, Deem A, Shim EY, Malkova A, Lee SE. Microhomology directs diverse DNA break repair pathways and chromosomal translocations. *PLoS Genet* 2012;8(11):e1003026.
107. Mateos-Gomez PA, Gong F, Nair N, Miller KM, Lazzarini-Denchi E, Sfeir A. Mammalian polymerase theta promotes alternative NHEJ and suppresses recombination. *Nature* 2015;518(7538):254-7.
108. Kent T, Chandramouly G, McDevitt SM, Ozdemir AY, Pomerantz RT. Mechanism of microhomology-mediated end-joining promoted by human DNA polymerase theta. *Nat Struct Mol Biol* 2015;22(3):230-7.
109. Simsek D, Brunet E, Wong SY, Katyal S, Gao Y, McKinnon PJ, et al. DNA ligase III promotes alternative nonhomologous end-joining during chromosomal translocation formation. *PLoS Genet* 2011;7(6):e1002080.

110. Ira G, Pelliccioli A, Balijja A, Wang X, Fiorani S, Carotenuto W, et al. DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature* 2004;431(7011):1011-7.
111. Huertas P, Cortes-Ledesma F, Sartori AA, Aguilera A, Jackson SP. CDK targets Sae2 to control DNA-end resection and homologous recombination. *Nature* 2008;455(7213):689-92.
112. Wu W, Wang M, Wu W, Singh SK, Mussfeldt T, Iliakis G. Repair of radiation induced DNA double strand breaks by backup NHEJ is enhanced in G2. *DNA Repair* 2008;7(2):329-38.
113. Sfeir A, de Lange T. Removal of shelterin reveals the telomere end-protection problem. *Science*. 2012;336(6081):593-7.
114. Deng SK, Gibb B, de Almeida MJ, Greene EC, Symington LS. RPA antagonizes microhomology-mediated repair of DNA double-strand breaks. *Nat Struct Mol Biol* 2014;21(4):405-12.
115. Ceccaldi R, Liu JC, Amunugama R, Hajdu I, Primack B, Petalcorin MI, et al. Homologous-recombination-deficient tumours are dependent on Poltheta-mediated repair. *Nature* 2015;518(7538):258-62.
116. Iliakis G, Murmann T, Soni A. Alternative end-joining repair pathways are the ultimate backup for abrogated classical non-homologous end-joining and homologous recombination repair: Implications for the formation of chromosome translocations. *Mutat Res Genet Toxicol Environ Mutagen* 2015;793:166-75.
117. Reiss KA, Herman JM, Armstrong D, Zahurak M, Fyles A, Brade A, et al. A final report of a phase I study of veliparib (ABT-888) in combination with low-dose fractionated whole abdominal radiation therapy (LDFWAR) in patients with advanced solid malignancies and peritoneal carcinomatosis with a dose escalation in ovarian and fallopian tube cancers. *Gynecol Oncol* 2017;144(3):486-90.
118. Chabot P, Hsia TC, Ryu JS, Gorbunova V, Belda-Iniesta C, Ball D, et al. Veliparib in combination with whole-brain radiation therapy for patients with brain metastases from non-small cell lung cancer: results of a randomized, global, placebo-controlled study. *J Neurooncol* 2017;131(1):105-15.

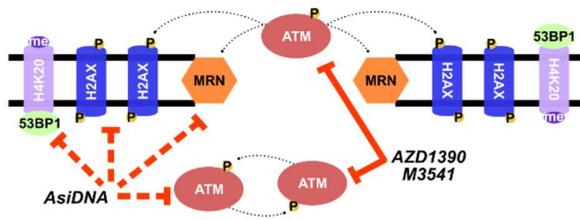
Figure legends

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.

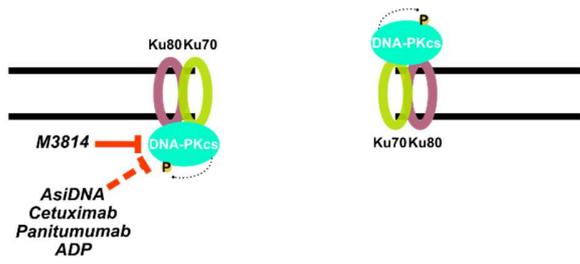
Figure 2. Mechanism of homologous recombination in humans. (1) sensing of DNA double-strand breaks by MRN complex, and signalling by ATM kinase; (2) DNA end resection by CtIP and RPA binding to 5' single-strand which is later replaced by Rad51 monomers; (3) further recruitment of effectors like BRCA1, 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.

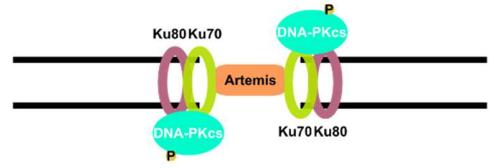
1 Sensing and signal transduction of DNA damage



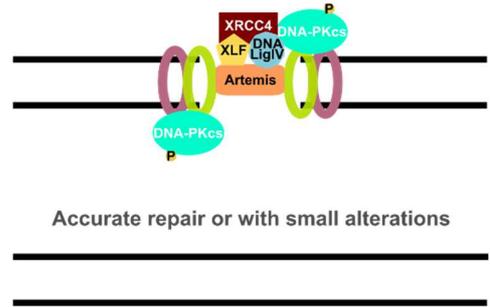
2 DNA end binding and protection



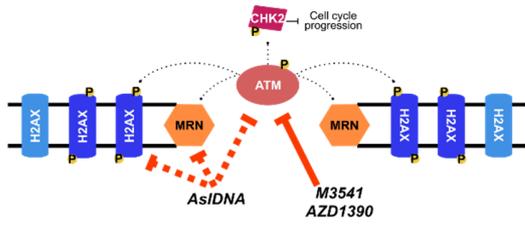
3 Physically proximal DNA ends bridging



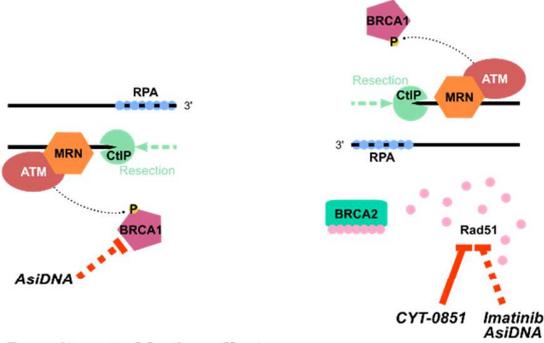
4 DNA ends processing and ligation



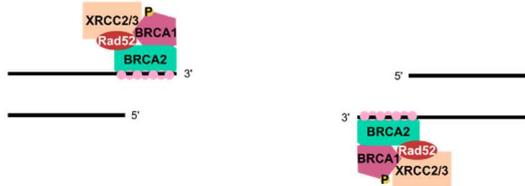
1 Sensing and signal transduction of DNA damage



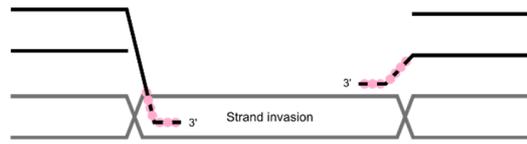
2 DNA resection and single-strand protection



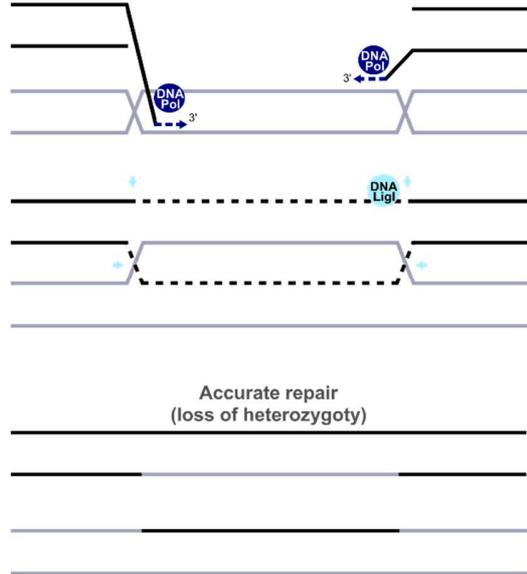
3 Recruitment of further effectors



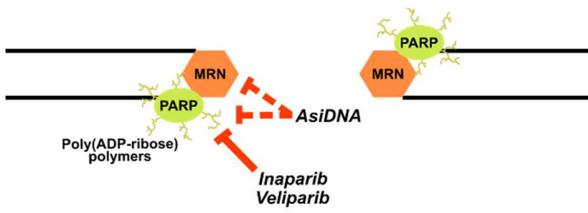
4 Homology search and D-loop formation



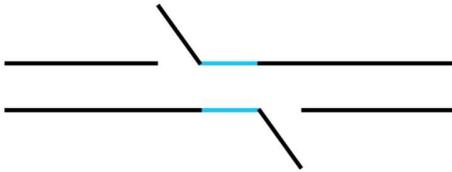
5 DNA synthesis, formation and ligation of Holliday junctions



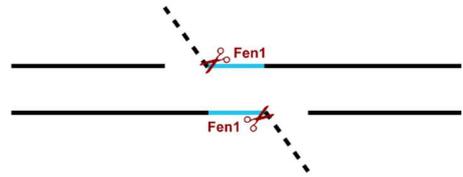
1 Sensing and signal transduction of DNA damage



2 Annealing of micro-homologies



3 Resection of heterologous 3' flaps



4 Fill-in DNA synthesis and ligation

