# Chapter 1
# Improving the Therapeutic Ratio of Radiotherapy by Targeting the DNA Damage Response

**Ross Carruthers and Anthony J. Chalmers**

**Abstract** In recent decades, technological advances in radiotherapy delivery have allowed dose escalation or reduction of toxicity for radiotherapy regimens used to treat several major tumour sites. However, tumour radioresistance remains a significant clinical problem. Although it is well established that the major biological effects of ionising radiation are mediated through DNA damage, our knowledge of the biological processes influencing tumour response to radiation is still relatively basic. It is known that tumour cells repair the vast majority of potentially lethal DNA damage inflicted by ionising radiation and that the cellular response to DNA damage is a major determinant of tumour radiosensitivity. Manipulation of tumour DNA damage repair mechanisms to modify the radiobiological response of malignant cells is therefore a very appealing idea with the potential to greatly amplify the therapeutic effects of radiation therapy.

**Keywords** DNA damage response • Radiotherapy • Poly(ADP-ribose) polymerase • Ataxia telangiectasia mutated • Cell cycle checkpoints • Radiosensitizers

## Introduction

In recent decades, technological advances in radiotherapy delivery have allowed dose escalation or reduction of toxicity for radiotherapy regimens used to treat several major tumour sites. However, tumour radioresistance remains a significant clinical problem. Although it is well established that the major biological effects of ionising radiation are mediated through DNA damage, our knowledge of the biological processes influencing tumour response to radiation is still relatively basic.

R. Carruthers • A.J. Chalmers (✉)
Translational Radiation Biology, Institute of Cancer Sciences, Wolfson Wohl Cancer Research Centre, University of Glasgow, Garscube Estate, Glasgow G61 1QH, UK
e-mail: Anthony.Chalmers@glasgow.ac.uk



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The components and mechanisms of DNA repair and cell cycle control pathways in normal mammalian cells have now been defined in some detail, and the potential to target and inhibit specific components of DNA damage response pathways is now a reality with the development in recent decades of small molecule inhibitors of some of the key components of these pathways. The current challenge facing radiation oncology is to integrate this knowledge in a manner which will allow specific manipulation of tumour radiobiological response in order to provide clinically useful, tumour-specific radiosensitisation. This chapter will summarise briefly the DNA damage response of cancer cells to ionising radiation and then describe various strategies to manipulate tumour radiobiology by inhibition of key DNA damage response proteins.

## The DNA Damage Response (DDR)

Upon encountering DNA damage of any variety, the normal response of mammalian cells, whether malignant or otherwise, is to attempt repair. Cells accomplish this via a complex network of protein signalling cascades and pathways. The term 'DNA damage response' (DDR) will be used to refer to this cellular repair network. There are multiple pathways involved in DDR, often with huge complexity and some redundancy in function. However in general the cellular response to DNA damage can be summarised by two processes: (1) activation of cell cycle checkpoints and (2) initiation and execution of DNA repair. These two processes are complementary; activation of cell cycle checkpoints provides time for the cell to repair damaged DNA before either replicating it or attempting it to undergo mitosis. If repair of DNA damage is successful, the cell will survive and retain reproductive integrity. If unsuccessful, the cell may die via apoptosis, mitotic catastrophe or an alternative cell death mechanism. This is summarised in Fig. 1.1. A brief overview of the cellular DDR to ionising radiation follows.

## Detection of Radiation-Induced DNA Damage and Initiation of DDR

Efficient DDR relies upon rapid detection of DNA damage and subsequent escalation of appropriate DDR pathways. The MRN complex consisting of MRE-11, NBS-1 and Rad50 proteins represents the major DNA DSB detector within mammalian cells. Ku70/Ku80 proteins, which are key effectors of the non-homologous

**Fig. 1.1** Responses of mammalian cells to DNA DSBs induced by gamma irradiation following induction of DNA DSBs by ionising radiation, a DNA damage response consisting of cell cycle checkpoint activation and DNA repair is generated. If successful, this will result in DNA repair and cell survival

end joining (NHEJ) pathway, also bind directly to DNA DSBs facilitating early repair of most DNA DSBs. Following detection of DNA damage, this signal must be amplified and coordinated in order to facilitate a cellular environment conducive to DNA repair. This is achieved by the actions of three apical DDR proteins: ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad 3 related (ATR) and DNA-dependent protein kinase (DNAPK) which are phosphatidylinositol 3-kinase-related kinases (PIKKs). The apical PIKKs phosphorylate a repertoire of DNA repair and checkpoint control proteins ensuring timely activation of cell cycle checkpoints and initiation of DNA repair mechanisms appropriate to DNA lesion stimuli and allow modification of heterochromatin and other more general intracellular environmental features in order to promote cellular survival. Apical PIKKs are appealing targets for radiosensitisation strategies and their functions and are described below.

## *Ataxia Telangiectasia Mutated (ATM)*

ATM is a highly prolific kinase which phosphorylates many substrates in response to DNA DSBs and has a dual role in both cell cycle control and repair of a subset of DNA DSBs. For a detailed review, see Shiloh et al. [1]. Mutations in ATM are responsible for the radiosensitivity syndrome 'ataxia telangiectasia', first described in 1975 [2]. Cells derived from patients with ataxia telangiectasia show deficient G1/S, S and G2/M checkpoints and deficient DNA DSB repair. ATM exists as an inactive dimer or multimer until DNA damage occurs, upon which autophosphorylation at serine 1981 occurs, allowing the dissociation of ATM dimers into active monomers. The exact mechanism of ATM activation is debated in current literature, and activation may occur via direct interaction with DNA DSBs, in response to conformational changes in heterochromatin structure or via the MRN complex [3, 4].

The proportion of DNA DSBs that cannot be repaired in ATM-mutant cells is estimated at around 10–20 % of the total DSB burden. ATM has a role in promoting DSB repair executed by NHEJ in G1 phase and by both NHEJ and HR in G2, and the proportion of ATM-dependent DSBs is similar in both phases of the cell cycle. Goodarzi et al. investigated the role of ATM in chromatin modification and demonstrated that ATM has a role in repair of heterochromatic DSBs [5]. This model proposes that in G1 phase, around 75 % of DNA DSBs occur in euchromatin regions and that ATM is not required for the repair of these lesions. However, in heterochromatic regions, nucleosome flexibility is constrained by factors such as KAP-1, which severely limits DSB repair. In this model, DSBs in heterochromatin are responsible for the slow phase of DSB repair, since the cell needs to execute additional steps to rejoin DSBs occurring in this relatively inaccessible chromatin context. ATM is able to phosphorylate KAP-1, thereby generating sufficient elasticity in DNA tertiary structure to allow repair. It has previously been suggested that ATM's primary role is to deal with complex DNA DSB lesions, since Artemis and ATM defects create epistatic DNA repair defects and Artemis has a vital role in end

resection for facilitation of NHEJ [6]. However, the proportion of ATM-dependent DNA DSBs appears not to increase following irradiation with high LET radiation types which cause more complex DSBs, which implies that ATM-dependent repair is not necessarily associated with complex DNA DSBs.

Nevertheless, ATM is also known to have roles in specialised DSB repair mechanisms that are not related to heterochromatin such as VDJ class switching and meiotic recombination. Alvarez-Quilon et al. demonstrated that ATM is necessary for the repair of DNA DSBs with blocked ends and that this requirement is independent of chromatin status [7]. The authors speculated that ATM could promote nucleolytic activity to eliminate blockage at DNA ends via the MRN complex, CtIP or Artemis or it could restrict excessive nucleolytic degradation of DNA ends by inhibiting these same nucleases or by phosphorylation of H2AX. These two models are not necessarily conflicting, since ATM may have roles in both complex DNA lesion repair and modification of chromatin.

## *Ataxia Telangiectasia and Rad 3 Related (ATR)*

ATR has a critical role in the DDR by protecting cells from replication stress. Replication stress can be defined as the slowing or stalling of replication forks during duplication of DNA and is characterised by the presence of single-stranded DNA (ssDNA) within the nucleus. Cancers in general are known to exhibit high levels of replication stress, which is thought to be induced primarily by oncogene activation, leading to upregulation and increased dependence upon the ATR-Chk1 pathway [8]. Furthermore, the DNA damage induced by ionising radiation (both SSBs and DSBs) is a significant source of replicative stress in the irradiated cell. The role of ATR in the DDR is reviewed in Marechal et al. [9]. ATR has an essential role in the survival of proliferating cells, and its deletion leads to embryonic lethality in mice and lethality in human cells [10]. ATM and ATR share many phosphorylation substrates; however, they have distinct roles in DDR and cannot be viewed as redundant in function. ATR is activated by RPA-coated ssDNA; hence, any situation leading to the formation of ssDNA will result in the activation of ATR. ATR phosphorylates Chk1 which leads to G2/M checkpoint activation, allowing time for damage repair. However, both ATR and Chk1 have additional important functions in maintaining the integrity of replication forks. Replication fork collapse is characterised by the dissociation of replisome contents and may result in generation of a DSB. This process is still poorly understood and may be the result of replisome dissociation/migration, nuclease digestion of a reversed fork or replication runoff [11]. ATR is activated by ssDNA generated at stalled replication forks and acts to stabilise the fork and initiate cell cycle checkpoint activation and inhibition of DNA replication origin firing on a global scale throughout the cell nucleus. ATR activation inhibits origin firing via the phosphorylation of the lysine methyltransferase MLL, which alters chromatin structure around replication origins [12]. In this manner, the stalled fork can then be restarted when the replication stress stimulus has been resolved.

### *DNA-Dependent Protein Kinase (DNA-PK)*

DNA-PK has a critical role in DDR via its function in NHEJ, as discussed below. It phosphorylates a smaller number of substrates in comparison to ATR and ATM. However, DNA-PK is able to phosphorylate some substrates of ATM in ATM-defective cells, allowing a degree of functional redundancy. In particular, DNA-PK is able to phosphorylate histone H2AX in the absence of ATM [13].

Activation of the apical DDR PIKKs results in cell cycle checkpoint initiation and attempted DNA repair. These processes will be considered separately as follows.

## Cell Cycle Checkpoint Control

Mammalian cells have three main cell cycle checkpoints that are activated following DNA damage: G1, intra-S and G2/M. These are shown in Fig. 1.2. The checkpoints regulate progression through the cell cycle, preventing a cell from progressing into the next phase of the cell cycle prior to satisfying the requirements of the previous phase.

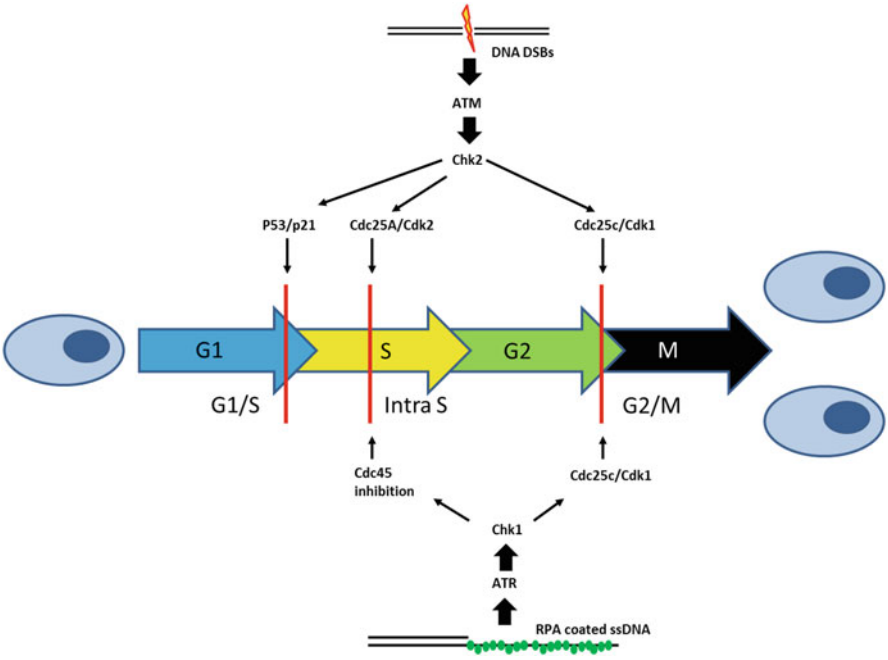


**Fig. 1.2** Cell cycle control in response to DNA damage. Simplified diagram of cell cycle control following activation of the upstream PIKKs ATR and ATM. ATM is activated by DNA DSBs and influences all three major checkpoints, whereas ATR is activated by RPA-coated ssDNA and has its major roles in the intra-S checkpoint and maintenance of the G2/M checkpoint

Progression through the cell cycle is controlled by cyclin-dependent kinases (CDKs) and cyclins, the names alluding to their cyclical accumulation and destruction through the cell cycle. These proteins form cyclin-CDK complexes whose activity ultimately regulates the machinery responsible for cycle progression. For a review of the cellular machinery controlling cell cycle checkpoints, see Lukas et al. [14].

The G1 checkpoint is usually very robust in eukaryotic cells; however, in malignant cells, the G1 checkpoint is frequently absent due to mutations affecting the p53 pathway. For example, glioblastoma and other cancer cells frequently fail to initiate a G1 checkpoint response to irradiation. Normal G1 checkpoint function requires functioning p53, which is phosphorylated in response to DNA damage by both ATM and Chk2 proteins. This leads to a reduction in the binding of MDM2 to p53, and subsequent p53 activation, resulting in its nuclear accumulation and stabilisation. Increased levels of p53 protein stimulate increased transcription of p21, which binds and inhibits CDK2-cyclin E activity, preventing the cell from entering S phase. The G1/S checkpoint is highly sensitive, but limited by the time required for p21 upregulation [15]. Alternative activation of the G1/S checkpoint is mediated via phosphorylation of Cdc25A, again by ATM and Chk2, which then targets Cdc25A for proteasomal degradation. Cdc25A removes inhibitory phosphate groups on CDK2, allowing progression into S phase [16].

The intra-S checkpoint is activated in response to replication stress or other difficulties encountered by the cell during S phase. It operates to slow DNA replication rather than stop it entirely and is p53 independent. The components of the S phase checkpoint suppress origin firing and slow replication fork progression to reduce the rate of DNA replication. Abnormalities in S phase checkpoints result in the radioresistant DNA synthesis (RDS) phenotype, i.e. cells are unable to stop or delay the synthesis of DNA following induction of DNA damage by radiation.

Cancer cells frequently demonstrate an increased dependency upon G2/M checkpoint activation to allow repair of DNA damage prior to entering mitosis, since the G1/S phase checkpoint is often dysfunctional in malignant cells due to deficiencies in the p53 pathway. Progression through the G2/M checkpoint with unrepaired DNA damage can result in cell death, and therefore it is essential that control of the G2/M checkpoint is maintained. Activation of the G2/M checkpoint occurs via ATM and ATR which phosphorylate Chk1 and Chk2, leading to phosphorylation of Cdc25 phosphatases. The G2/M checkpoint has a defined threshold of sensitivity, with activation and maintenance of G2/M arrest appearing to require 10–15 DSBs [17]. The G2/M cell cycle checkpoint arrests heavily damaged cells in G2 to provide time for repair of DSBs, and it is proposed that this may be important for slow phase repair in G2 via homologous recombination. However, the G2/M checkpoint is inherently insensitive and allows cells to enter mitosis carrying a measurable number of unrepaired DSB [18].

## DNA Repair Processes

Exposure to a 2Gy dose of radiation will produce on average around 2000 SSBs and 80 DSBs. DNA DSBs are much more difficult for cells to repair and have long been considered the lesions responsible for lethality following irradiation.

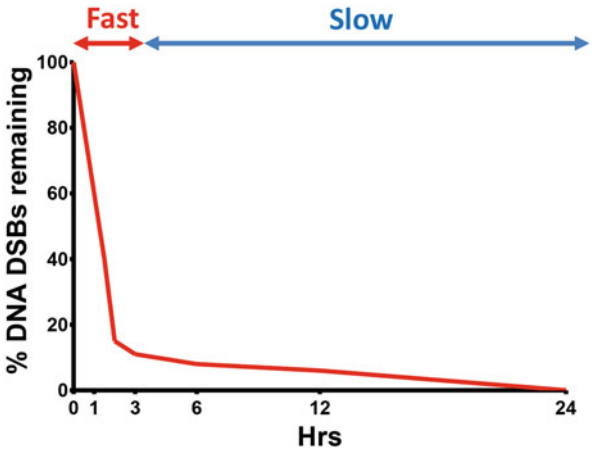


**Fig. 1.3** Illustrative schematic of kinetics of DNA DSB repair following irradiation in mammalian cells. The majority of DSBs are repaired a short time after irradiation in the 'fast' phase of DNA DSB repair via NHEJ. However, a subset of DNA DSBs requires much more time for repair, due to complexity and/or chromatin context, and is represented by a 'slow' phase tail on the above illustration. Slow phase repair is achieved via NHEJ in G1 phase and HR repair in G2 phase. Adapted from [19]

Figure 1.3 illustrates DNA DSB repair kinetics in mammalian cells following gamma radiation adapted from Goodarzi et al. [19]. There is an initial fast phase of repair lasting 1–3 h which represents DNA DSBs that can be efficiently repaired by the cell. In addition to the fast phase of repair, there is a longer 'tail' which is termed the slow phase of DNA DSB repair and can extend past 24 h. Both slow phase and fast phase repair occur simultaneously. If left unrepaired, even a single DNA DSB can result in loss of genetic information and cell death [20] so it is unsurprising that mammalian cells have developed complex and highly efficient systems for their repair. DNA DSBs are repaired predominantly by two pathways, homologous recombination (HR) and non-homologous end joining (NHEJ), although back up pathways such as microhomology-mediated end joining (MMEJ) also exist. For a review of DNA DSB repair, see Shibata and Jeggo [21].

## *Non-homologous End Joining (NHEJ)*

The bulk of DNA DSB repair in mammalian cells is undertaken by NHEJ, exclusively so in G1 cell cycle phase where cells have a diploid DNA content. NHEJ is involved in both fast phase repair and slow phase repair in G1 cells and in the fast phase of repair in G2 cells [6]. NHEJ involves the processing of broken DNA termini to form compatible ends which can then be ligated back together. NHEJ is a relatively simple, rapid and efficient method of DNA DSB repair but is error prone and associated with loss of genetic information. The mechanisms of NHEJ can be

simplified into three steps: (for a comprehensive review, see Weterings et al. [22]) (1) capture of both ends of the broken DNA molecule, (2) bridging of the two broken DNA ends and (3) religation of the broken DNA molecule. NHEJ is thought to make the first attempt at rejoining the majority of DNA DSBs, even in G2 phase where HR is competent, due partly to the cellular abundance of Ku70 and Ku80 and their high affinity for DNA termini [23, 24]. NHEJ and its major protein components are summarised in simplified form in Fig. 1.4.

An alternative mechanism of NHEJ is thought to occur via microhomology-mediated end joining (MMEJ) [25, 26]. For a detailed review, see McVey et al. [27]. MMEJ has a requirement for limited MRN-dependent end resection and relies upon homologous matching of 5–25 base pairs on both strands in order to correctly align the DNA DSB ends. Any overhanging or mismatched bases are removed and missing bases inserted. The process is particularly error prone, since it does not identify sequences lost around the DSB. MMEJ appears to act as a reserve DSB repair pathway but can also repair DSBs generated at collapse of replication forks. The process is dependent upon ATM, PARP-1, MRE-11, CtIP and DNA ligase IV but operates independently of Ku or DNA-PKcs [27]. The extent to which MMEJ contributes to DSB repair in normal cells is unknown, but it has been shown to assume importance in cancer cells bearing defects in other DSB repair pathways [28].

### *Homologous Recombination (HR)*

The homologous recombination (HR) pathway represents a more complex and sophisticated mechanism of DNA DSB repair. Although NHEJ repairs the majority of DNA DSBs, HR contributes to the repair of DSBs in specific circumstances, such as the one-ended DSB created by the collapse of DNA replication forks and a subset of DNA DSBs in G2 that are repaired with slow kinetics [23, 29, 30]. HR is conventionally considered to be limited to S and G2 phases of the cell cycle, since it relies upon homologous DNA sequences (in the form of the duplicated DNA strand of a sister chromatid) to effect repair. Because of this, however, it is highly accurate. For a more detailed review of the process, see Filippo et al. [31], Li et al. [32] and Krejci et al. [33]. In brief, HR is initiated by resection of the 5′ DNA end of the DSB in order to create 3′ SS DNA which can then invade a partner chromosome. End processing creates 3′ ends following resection of nucleotides from the 5′ break ends. Extension of resection is tightly regulated by the repositioning of 53BP1 via a BRCA 1-dependent process (9 Jeggo 2014 review). Resected 3′ ends are then quickly bound by replication protein A (RPA), which protects ssDNA and removes DNA secondary structure in order to facilitate formation of a ‘presynaptic filament’ consisting of Rad51-coated ssDNA [34, 35]. Rad51 is a recombinase, i.e. an enzyme which facilitates genetic recombination and forms a helical filament on ssDNA which holds it in an extended conformation to aid the search for homology. BRCA 2 has an essential role in the loading of Rad51 onto ssDNA.

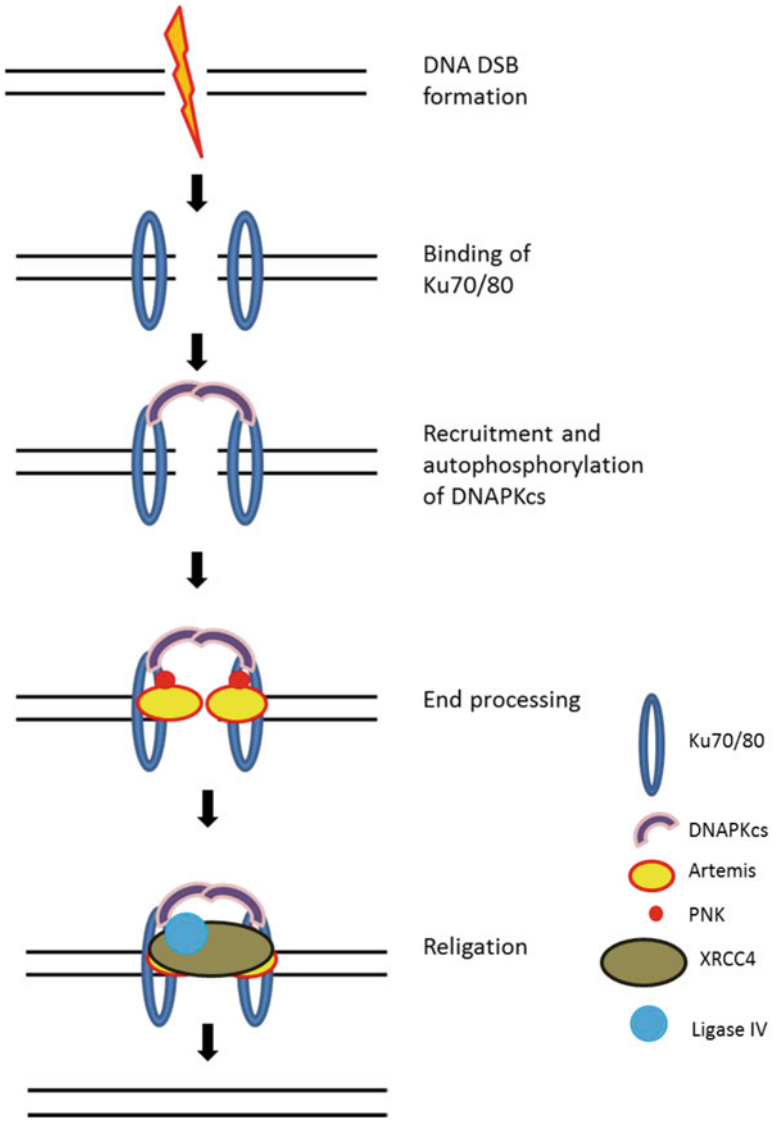


**Fig. 1.4** Schematic diagram of non-homologous end joining (NHEJ) repair. NHEJ is initiated by the binding of Ku70/Ku80, followed by the recruitment of DNA-PKcs and its subsequent autophosphorylation. End processing is achieved via Artemis, and additional factors before the broken DNA ends are ligated

Once assembled, the presynaptic filament captures a duplex DNA molecule and begins its search for the homologous sequence. Rad51 facilitates the physical connection between the invading DNA strand and the DNA duplex structure leading to the formation of heteroduplex DNA ('D loop') with a Holliday junction (HJ), as described in Fig. 1.5. Synthesis of DNA and repair of the DSB lesion then occurs

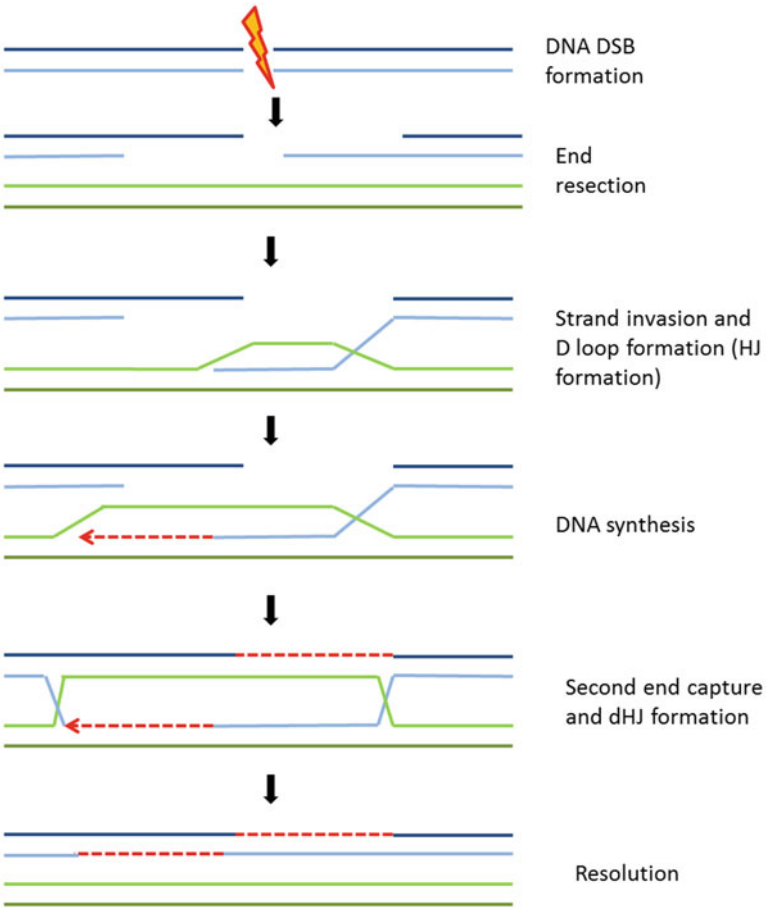


**Fig. 1.5** Schematic diagrams of homologous recombination (HR) repair. HR repair is initiated by end resection and coating of ssDNA by RPA and subsequently Rad51. The search for a homologous sequence on the sister chromatid is initiated by strand invasion and subsequent Holliday junction formation. Synthesis of new complementary DNA sequence and Holliday junction resolution results in successful DNA DSB repair

using the undamaged DNA strand of the heteroduplex DNA molecule as a template. Following successful repair, resolution of the heteroduplex DNA molecule occurs, generating crossover or non-crossover products.

## Poly (ADP-Ribose) Polymerases (PARPs)

Whilst the main features of the DDR to DNA DSB have been explored, it should not be forgotten that responses to single-strand DNA breaks also influence the eventual outcome of radiation-induced DNA damage. Gamma or X-radiation

induces around 25-fold more SSBs than DSBs, but these are usually repaired promptly. If SSBs are not resolved efficiently, however, they can have significant effects on cell survival via the generation of DSBs. The PARP family of proteins is known to facilitate base excision repair (BER) which is one of the main cellular single-strand break repair pathways.

PARPs form a large protein family with diverse cellular functions including DNA repair, mitotic segregation, telomere homeostasis and cell death. PARPs are characterised by their catalytic function, which is poly(ADP-ribosylation). There are 18 reported family members; however, not all have definite poly(ADP-ribose) catalytic function, and only PARPs 1–3 have well-characterised roles in DNA repair. For an in-depth review of PARP function, see D'Amours and Burkle [36, 37]. PARP-1 is the most abundant and best understood family member, so the term 'PARP' will be used to refer to the actions of PARP-1 for the rest of this chapter.

Activated PARP modifies its substrates via covalent, sequential addition of ADP-ribose molecules that form branching poly(ADP-ribose) (PAR) polymers on its targets. The substrate from which PAR is formed is nicotinamide adenine dinucleotide (NAD+). Poly(ADP-ribosylation) is a commonly occurring post-translational modification in the cell. It creates negative charge on target proteins altering their three-dimensional structure and regulating interactions with other proteins and with DNA [38].

PARP is an efficient sensor of DNA damage and its rapid binding to damaged DNA results in its activation (Fig. 1.6). PARP can bind to a variety of DNA damage structures including SSBs and DSBs [39–42] and plays a major role in PAR synthesis following DNA damage: approximately 90% of PAR production is attributable to PARP-1 in this context [43]. DNA-bound PARP undergoes automodification via the addition of long, negatively charged PAR polymers [36]. This autoPARylation promotes dissociation of PARP from the DNA molecule, allowing access of other DNA repair components to the damaged DNA [44–46] and facilitating their recruitment to the damaged sites. The list of substrates of PARP is extensive, and their DDR function can be modified both by PARylation and by direct interaction with PARP.

Although the precise role of PARP in DNA repair is still being elucidated, an important contribution to the repair of SSB lesions is well documented. Rather than being essential for SSB repair, however, PARP appears to increase the efficiency and kinetics of this process [47–49]. Activation of PARP promotes recruitment of the scaffold protein XRCC1 to damages sites [50]; PARP modifies and interacts directly with XRCC1 during this process. Lesions then undergo end processing before being repaired by either short patch or long patch mechanisms. PARP is known to interact with and modulate many SSB repair proteins, including DNA Lig III, DNA Pol Beta and others, whilst playing a clear role in base excision repair (BER) does not appear to be an absolute requirement for the function of this pathway [49]. The radiosensitising effects of PARP inhibition will be discussed below.

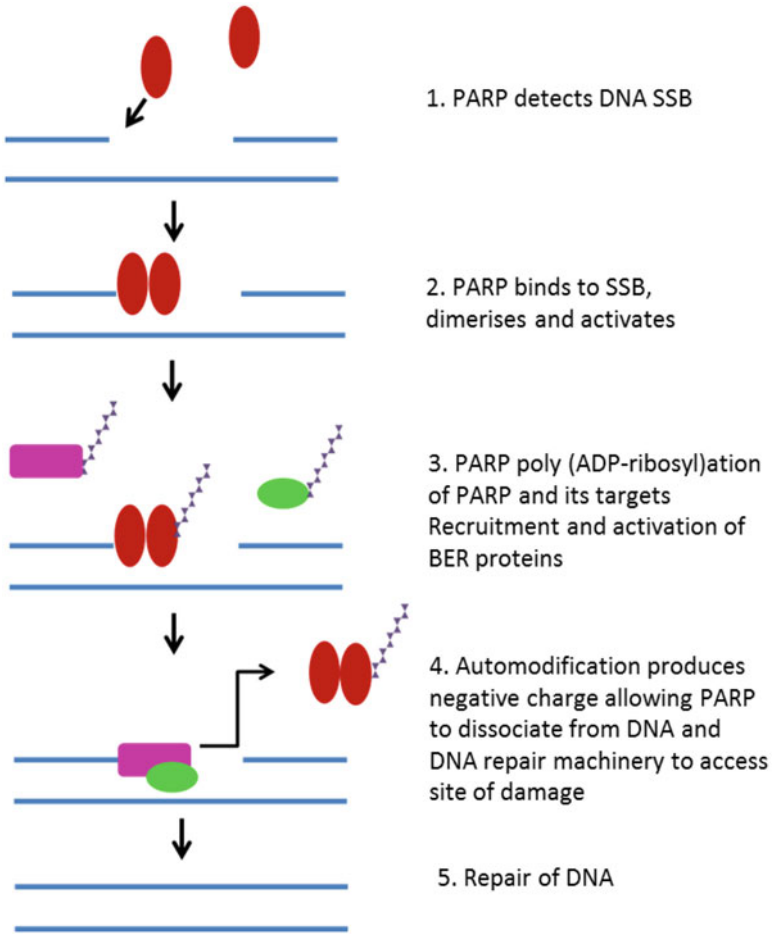


**Fig. 1.6** The role of PARP in SSB repair. PARP detects SSBs and facilitates efficient repair via interactions with a variety of base excision repair (BER) factors. Automodification of PARP facilitates its dissociation from the damaged site

## DNA Damage Response as a Therapeutic Target

From the discussion above, it can be predicted that targeting of the tumour cell DDR will lead to radiosensitisation via two distinct mechanisms. Inhibition of cellular checkpoint activation will promote transit of malignant cells into mitosis before DNA damage can be completed, thus increasing the probability of cell death, whilst inhibition of DNA repair will increase the incidence and persistence of unrepaired DNA breaks, thus enhancing the lethal effects of irradiation. Some of the key DDR effectors (e.g. ATM) are involved in both of these processes.

Exploitation of DDR as a therapeutic target often raises understandable concerns regarding toxicity to normal tissues. The concept of ‘tumour specificity’ is vitally important in cancer therapy and particularly so when considering strategies that increase the biological effects of ionising radiation. If DDR inhibition were to sensitise normal tissues to the same degree as tumour cells, then no therapeutic gain would be made, since any increased tumour effect would be accompanied by an unacceptable increase in normal tissue toxicity.

Supporting the prospect of tumour-specific radiosensitisation, important differences between the DDR of tumours and normal tissues have been well documented. At the most fundamental level, the DDR presents a barrier to carcinogenesis during the early stages of tumour development [51]. Cellular populations in the process of carcinogenesis face selective pressures that promote survival of cells bearing mutations associated with altered DDR that increase their ability to tolerate oncogenic proliferative stress. At the population level, dysfunctional DDR can be advantageous, endowing a minority of tumour cells the capacity to generate and tolerate genomic instability and heterogeneity, leading to adaptability and a survival advantage in the hostile tumour microenvironment. Consistent with this, there is evidence to suggest that tumours may be profoundly deficient in some aspects of DDR, rendering them overly dependent on other DDR pathways to carry out necessary DNA repair. Examples of this behaviour are seen in the widespread loss of G1/S checkpoint integrity in solid tumours due to p53 mutation and resulting dependence upon G2/M checkpoint integrity. A further example is seen in the context of ‘synthetic lethality’ in HR-deficient tumours, which are sensitive to therapies such as PARP inhibitors that create DNA lesions requiring HR for repair. Given that genomic instability is now considered a ‘hallmark’ of cancer, it is likely that DDR abnormalities are common in cancer cells [52]. Indeed the main reason why radiotherapy is a successful cancer treatment is because tumour cells are less able than the surrounding normal tissues to deal with the DNA damage caused by ionising radiation. The intact DDR of normal tissues ensures that a therapeutic ratio exists between tumour and normal tissue, allowing radiation to eradicate tumour cells whilst normal tissues are able to survive or tolerate the resulting DNA damage. Therefore, pharmacological inhibition of DDR exploits an inherent vulnerability of many cancer cells and represents a valid and promising therapeutic strategy.

Recently, a variety of small molecule inhibitors have become commercially available that possess the ability to specifically and potently inhibit individual DDR proteins. Although many of these are not yet sufficiently advanced to be anything more than laboratory tools, others such as the PARP inhibitor class have been licensed as single agents and are entering phase I and II clinical trials in combination with radiotherapy. A discussion on the current landscape of DDR inhibition in the context of radiation therapy now follows.

## *PARP Inhibition*

PARP inhibitors represent the most developed class of DDR modifiers, largely due to early successful trials as monotherapy in the 'synthetic lethality' setting [53]. There are now several PARP inhibitors entering clinical trials as radiosensitisers including AZD2281 (olaparib), AG014699 (rucaparib) and ABT888 (veliparib). Extensive preclinical investigation into their role as radiosensitising agents has been carried out and is summarised below.

In vitro work has demonstrated that PARP inhibitors (PARPi) provide modest radiosensitisation. Sensitiser enhancement ratios (SER), which are a measure of the fold increase in radiation dose necessary to produce a given level of survival observed in the absence of the sensitising drug, have been reported in the range of 1.1–1.7, depending on the PARP inhibitor and cell line tested.

Brock et al. [54] demonstrated this effect in fibroblast and murine sarcoma cell lines, with SERs (at 10 % survival) of 1.4–1.6 using the PARP inhibitor INO-1001. Interestingly they also showed an enhanced sensitisation effect when INO-1001 was combined with fractionated radiotherapy, suggesting that PARPi was able to block interfraction repair of sublethal damage. This effect was also reported in a study of glioblastoma cell lines [55].

Other authors have confirmed the radiosensitising effects of PARPi in vitro in a variety of different tumour cell lines; these are summarised in Table 1.1 and include head and neck squamous cancer; prostate cancer; glioblastoma; pancreatic, colon and cervix cancer; and lung carcinoma cell lines.

PARP inhibitors have been shown to decrease clonogenic survival and increase apoptosis and mitotic catastrophe in irradiated cells in vitro. The pro-apoptotic effects of PARPi vary between studies and are likely to be cell line dependent. Noel et al. demonstrated lack of radiosensitisation of asynchronously dividing human cell lines treated with PARPi, whilst HeLa cells synchronised in S phase were significantly sensitised to radiation by the addition of PARPi, suggesting that sensitisation was dependent upon DNA replication [61]. This was confirmed by Dungey et al. [55] who showed that radiosensitisation was enhanced by synchronisation in S phase and abrogated by aphidicolin (which creates an early S phase block). PARPi delayed repair of DNA damage and was associated with a replication-dependent increase in DNA DSBs as measured by gamma H2AX and Rad51 foci. Again radiosensitisation was increased with a fractionated schedule, indicating impaired repair of sublethal damage in PARPi-treated cultures. The authors proposed a mechanism whereby PARPi reduced the rate of SSB repair which, in replicating cells, increased the burden of DSBs due to generation of collapsed replication forks during S phase (see Fig. 1.7). They also proposed that the DNA lesions produced by collapsed replication forks in the presence of PARPi might be more complex and hence more difficult to repair. Persistent binding of chemically inhibited PARP to DNA (via steric hindrance) would prevent efficient recruitment of DNA repair proteins to the lesion, providing a potential explanation for this theory [62]. The observation that DNA replication is required in order for PARP inhibition to radiosensitise cells indicates that direct effects on DSB repair are unlikely.

**Table 1.1** Summary of in vitro studies of radiosensitising effects of PARP inhibitors

| Author | Parp inhibitor and radiation dose | Cell line | Assays | Outcome |
|---|---|---|---|---|
| Brock et al. [54] | INO-1001 10 μM, IR 0–8 Gy | CHO rodent fibroblast, c37 human fibroblast, SaNH murine sarcoma cell lines | Clonogenic survival and apoptosis | Decreased clonogenic survival in PARPi plus IR, effect enhanced by fractionation<br>No increase in apoptosis |
| Albert et al. [56] | ABT888 (veliparib) 5 μM, IR 0–6 Gy | H460 lung carcinoma cell lines | Clonogenic survival, apoptosis, endothelial damage assay | Decreased clonogenic survival in PARPi plus IR vs. IR alone<br>Increased apoptosis<br>Inhibition of endothelial tubule formation |
| Dungey et al. [55] | AZD2881 (olaparib) 1 μM , IR 0–5 Gy | T98G and U87MG glioblastoma cell lines | Clonogenic survival, gamma H2AX foci | Decreased clonogenic survival in PARPi plus IR vs. IR alone, decreased DNA repair, DNA replication-dependent effect of PARPi, fractionation-sensitive effect |
| Loser et al. [57] | AZD2881 (olaparib) 500 nmol/l plus IR 0–8 Gy | Human and murine primary cells defective in Artemis, ATM, DNA ligase IV | Clonogenic survival, alkaline comet assay, gamma H2AX foci | PARPi radiosensitisation enhanced in ATM, Artemis and DNA ligase IV-deficient cells.<br>Clonogenic survival decreased in rapidly dividing and DNA repair-deficient cells |
| Calabrese et al. [58] | AG14361 0.4 μM plus IR 8 Gy | LoVo and SW620 human colonic carcinoma cell lines | Clonogenic survival | PARPi plus IR decreased survival by inhibiting recovery from potentially lethal damage |
| Russo et al. [59] | E7016 3–5 μM plus IR 0–8 Gy | U251 glioblastoma, MiaPaCa pancreatic, DU145 prostatic carcinoma cell lines | Clonogenic survival, gamma H2AX foci, mitotic catastrophe, apoptosis | PARPi plus IR increased clonogenic cell kill and mitotic catastrophe, however no increase in apoptosis |
| Liu et al. [60] | ABT 888 (veliparib) 2.5 μM plus IR 5 Gy | H1299 lung cancer cells, DU145 and 22RV1 prostate carcinoma cell lines | Clonogenic survival, repair foci assay | PARPi plus IR reduced clonogenic survival, with effect seen in acute hypoxic cells and oxic cells |

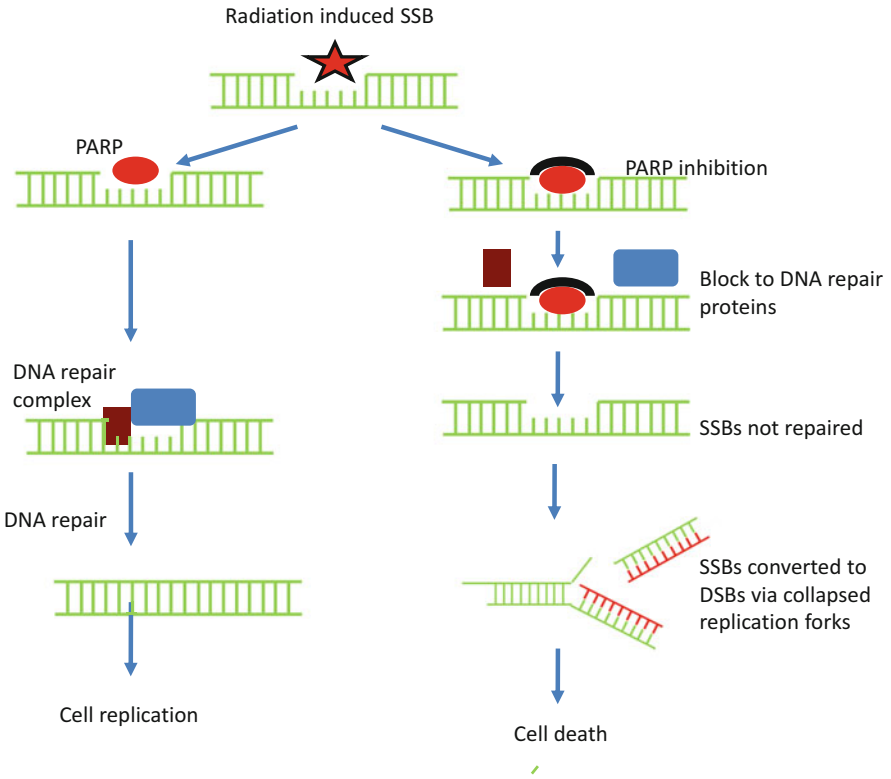


**Fig. 1.7** Mechanism of radiosensitisation by PARP-1 inhibition. PARP inhibition does not affect binding of PARP-1 to DNA SSBs but prevents their efficient repair by inhibiting recruitment of key BER effectors and by blocking access of repair elements to damaged sites. This results in delayed SSB repair and increases the likelihood of replication fork collapse by which mechanism SSBs are converted into cytotoxic DSBs during S phase

Loser et al. investigated the radiosensitising effects of PARPi on cells that were deficient in various DDR pathways, an effect which has been termed 'synthetic sickness'. Pre-existing DDR pathway abnormalities were found to enhance the radiosensitising effects of PARPi when compared with effects in DDR competent cell lines. Whilst the underlying mechanism varied according to the specific DDR pathway abnormality, the addition of PARPi appeared to render DDR-deficient cells more vulnerable to radiation-induced DNA lesions that would otherwise have been repaired by alternative pathways [57].

Important work by Liu and colleagues [60] examined the effects of acute hypoxia on radiosensitisation by PARPi. Firstly, the clinical PARPi ABT-888 was shown to inhibit intracellular PARP activity in prostate and non-small cell lung carcinoma cell lines under conditions of hypoxia. Secondly, tumour cells under conditions of acute hypoxia were radiosensitised to the same degree as oxic cells. The authors concluded that ABT-888 remained an effective radiosensitiser under conditions of

acute hypoxia, which is an important consideration in translating PARPi into clinical practice because most tumours are hypoxic to some degree [63, 64]. Chronic hypoxia induces downregulation of HR, which may allow targeting of chronically hypoxic cancer cells with a PARPi synthetic lethal strategy. Chan et al. have shown that PARPi-treated tumour xenografts with hypoxic subregions exhibited increased gamma H2AX signalling and reduced survival in an ex vivo clonogenic assay. However, the specific radiosensitising effects of PARPi in the context of chronic hypoxia were not investigated [65]. Nevertheless, the ability of PARPi to selectively target chronically hypoxic cancer cells is of significant clinical interest.

The radiosensitising effects of PARPi have been replicated by several authors in in vivo models. The results of these studies are summarised in Table 1.2. As an example, a recent paper by Tuli et al. demonstrated tumour growth inhibition and prolonged survival in an in vivo orthotopic model of pancreatic carcinoma [69].

Reviewing these data, there is an indication that the radiosensitising effects of PARPi are enhanced in in vivo models, with several studies showing radiosensitising effects that exceed those predicted by in vitro data. This is unlikely to be explained by radiotherapy fractionation effects alone, since several of the studies used large single fraction radiotherapy doses similar to those used in vitro. The enhanced effects observed in vivo may be at least partly explained by effects of PARPi on the tumour vasculature, which may in turn be attributed to the structural similarities of many PARPi to nicotinamide, which is a potent vasodilator. Vasodilatory effects of PARPi on tumour blood vessels might alleviate tumour hypoxia whilst simultaneously increasing drug delivery and enhancing radiosensitisation [58, 70]. As yet, the clinical relevance and therapeutic potential of these effects remain unproven.

The normal tissue toxicity implications of a PARPi radiosensitisation strategy have not been extensively investigated, partly because few animal models yield clinically meaningful radiation toxicity data. However, several mechanistic arguments predict at least a degree of tumour specificity, as described below. Likely toxicities will of course depend upon the tumour site irradiated. As single agents, PARP inhibitors have been shown to have highly favourable toxicity profiles [53], so toxicities outwith the irradiated field would be unexpected, unless concomitant chemotherapy was also incorporated into the treatment regimen.

Since PARP inhibition requires DNA replication to produce a radiosensitising effect, rapidly dividing tissues are likely to be radiosensitised by PARP inhibition. Hence, squamous cell carcinomas, glioblastoma and other highly mitotically active tumours may be most sensitised by PARPi. This also has implications for normal tissue toxicity, however, since tissues with high cellular turnover such as the skin, bone marrow and mucosal surfaces of the oesophagus, oropharynx and bowel might also be radiosensitised by PARPi, although only if these tissues were irradiated of course. Tissues such as the brain, spinal cord, heart and muscle, which are comprised mainly of infrequently dividing cells, are predicted not to be radiosensitised by PARPi, although it should be remembered that these tissues are heterogeneous and contain additional cell types such as vascular endothelial cells, which may have higher mitotic indices.

**Table 1.2** Summary of in vivo studies of radiosensitising effects of PARP inhibitors

| Author | PARP inhibitor and radiation dose | Cell line | Assay | Outcome |
|---|---|---|---|---|
| Khan et al. [66] | GPI-15427 10, 30, 100, 300 mg/kg po, IR 2 Gy for 2 days | JHU012 and JHU012 head and neck cancer xenografts | Tumour growth delay apoptosis | PARPi plus IR inhibited tumour regrowth vs. IR Increased apoptosis |
| Clarke et al. [67] | ABT 888 7.5 mg/kg po bd, Temozolomide 33 mg/kg/day, IR 20 Gy over 11 days | Glioblastoma intracranial xenografts (MGMT hypermethylated) | Animal survival, body weight | PARPi-TMZ-IR prolonged survival vs. IR alone, minimal weight loss |
| Donawho et al. [68] | ABT 888 25 mg/kg/day via osmotic pumps, IR 20 Gy over 10 days | HCT116 xenograft human colorectal carcinoma | Animal survival | PARPi plus IR increased mean survival time vs. IR alone |
| Albert et al. [56] | ABT 888 25 mg/kg ip for 5 days, IR 10 Gy over 5 days | H460 xenograft, human lung carcinoma | Tumour growth delay, Ki67 staining, apoptosis, blood vessel density | PARPi plus IR delayed tumour regrowth vs. IR alone Decreased tumour vasculature Decreased proliferation Increased apoptosis |
| Calabrese et al. [58] | AG143615 or 15 mg/kg/day ip, IR 10 Gy over 5 days | SW620 human colon carcinoma | Tumour growth delay | PARPi plus IR delayed tumour regrowth vs. IR alone |
| Russo et al. [59] | E7016 30 mg/kg po, IR 4 Gy single fraction | U251 glioblastoma xenograft | Tumour growth delay | PARPi-TMZ-IR delayed tumour regrowth vs. IR alone |
| Tuli et al. [69] | ABT 888 25 mg/kg, IR 5 Gy single fraction | Pancreatic carcinoma | Tumour growth delay and survival | PARPi plus IR delayed tumour regrowth and prolonged survival |

Since vascular endothelial cells are present in every organ and tumour treated, they are worthy of specific consideration. The cell doubling time of endothelial cells in culture has been estimated from labelling studies to be in the region of 93 to 2300 days, which would classify the endothelium as an intermediate to late-responding tissue [71]. However, there is evidence to suggest that irradiation provides a proliferative stimulus that decreases cell doubling time and hence might increase the radiosensitising effects of PARPi on endothelial cell radiosensitivity [72]. To date, there is no direct evidence to support or refute such an effect either in animals or in patients.

It is also unknown whether the progenitor stem cells of slowly dividing tissues might be sensitised by PARPi strategies; this issue clearly has implications for late normal tissue toxicities. Intermediate tissues such as type I and II pneumocytes and the bladder epithelium would be expected to experience less radiosensitisation with PARPi than malignant tumours.

Considering potential mechanisms of tumour specificity, PARPi have been observed to accumulate in malignant tissue, an effect that might be related to increased levels of DNA damage (which would therefore bind more PARP) in malignant tissue. In theory, this phenomenon would increase the tumour-sensitising effects of PARPi [58] whilst limiting normal tissue toxicity [73] and might also have implications for scheduling of PARPi, particularly if cytotoxic chemotherapy agents form part of the therapeutic schedule and there is a risk of increased haematological toxicity in combinations with continuous PARPi dosing.

Taking the tumour selectivity argument a step further, there are theoretical grounds on which to predict that PARP inhibition could protect certain late-responding normal tissues from the adverse effects of radiation. In a variety of normal tissue models, damage-induced activation of PARP has been shown to deplete cells of NAD+, preventing them from activating energy-dependent apoptotic pathways and thereby promoting necrotic cell death and a consequent inflammatory cascade that exacerbates and disseminates tissue damage. If PARP activity is inhibited prior to the toxic insult, NAD+ levels are preserved, and cells are more likely to die via apoptosis, thus reducing overall levels of tissue damage. A broad and expanding literature describes the protective application of PARPi in animal models of myocardial reperfusion injury and acute lung injury that lend some support to this theory [74, 75]. Furthermore, it has been reported that PARP inhibition is protective in mouse models of irinotecan-induced gastrointestinal toxicity [76].

## *ATM Inhibition*

The development of radiosensitisation strategies based on ATM inhibition is at a much earlier stage of development. Much of the in vitro work in this area has explored the use of ATM inhibition as a laboratory tool rather than preclinical investigation as a therapeutic radiosensitiser.

In recent studies, Golding et al. [77] evaluated ATM inhibition as a radiosensitiser for GBM. They demonstrated highly potent radiosensitisation of commercially available GBM cell lines using the ATM inhibitor KU-60019 and concluded that ATM inhibition had clinical potential as a highly effective radiosensitiser and inhibitor of DDR in this disease. In a subsequent paper, the team explored the combination of ATM inhibition with radiation and temozolomide on commercially available GBM cell lines [78]. $SER_{37}$ values for radiation were calculated to be 1.8–2.1 depending on the dose of KU-60019 used, whilst the addition of temozolomide did not enhance the radiosensitising effects of ATM inhibition (nor did temozolomide radiosensitise in the absence of ATM inhibitor). In coculture models of glioma cells and human astrocytes, the combination of

temozolomide and ATM inhibition reduced glioma cell growth by around 70%, but astrocytes did not exhibit in vitro radiosensitisation after exposure to KU-60019. Biddlestone-Thorpe et al. explored similar combinations in an orthotopic in vivo GBM model [79]. In vivo administration of KU-60019 required the use of intracranial osmotic pumps and convection-enhanced delivery, since the drug did not reach therapeutic concentrations in plasma following oral or intraperitoneal administration. In this context, KU-60019 delayed tumour growth and significantly prolonged survival when added to radiation treatment. The investigators also reported that p53 status had an important effect on the radiosensitising effects of ATM inhibition. U87 cells, which express wild-type p53, were infected with a mouse retrovirus expressing the p53-281G allele, generating p53-mutant U87 cells that were shown to be more susceptible to the radiosensitising effects of ATM inhibition in vitro than the parental cell populations. Similarly, mice bearing U87-281G xenografts experienced prolonged overall survival when treated with the combination of ATM inhibition and radiation in comparison to mice bearing U87 parental xenografts. Whilst the authors concluded that ATM inhibition may be of potential benefit in combination with radiotherapy for p53-mutant GBM, it should be recognised that aberrations in the p53 signalling pathway are observed in about 90% of GBM even though p53 mutations are seen in only 30–40% of cases [80].

These three papers represent the most in-depth preclinical studies of ATM inhibition to date. Other studies have demonstrated the potentiating effects of ATM inhibition on cisplatin-mediated radiosensitisation of non-small cell lung cancer cells and radiosensitisation of head and neck squamous carcinoma cell lines by ATM downregulation via RNA interference [81, 82]. Rainey et al. demonstrated that transient ATM inhibition for a period of 4 h was able to potently radiosensitise HeLa cells in vitro [83], whilst Choi et al. demonstrated distinct effects of ATM inhibition versus ATM loss, manifested by reduced sister chromatid exchange (a marker of homologous recombination) in ATM inhibited irradiated cells which was not apparent in irradiated ATM null cells [84].

Current dogma might suggest that inhibition of ATM in combination with radiotherapy would lead to overwhelming normal tissue toxicity, since ATM is one of the central DDR kinases. However, there is evidence to suggest that radiosensitivity following ATM inhibition may be tissue specific. A study by Schneider et al. demonstrated that astrocytes downregulate ATM expression but retain DNA repair competency via NHEJ [85]. In support of this, Gosink et al. demonstrated that astrocyte radiosensitivity was unaffected by ATM deficiency [86]. A further recent study by Moding et al. using a murine sarcoma model demonstrated that deletion of the ATM gene had much less of a radiosensitising effect on normal cardiac endothelia than on rapidly proliferating tumour endothelial cells [87]. These data suggest that ATM inhibition as a radiosensitising strategy may be clinically achievable; however, further study of the normal tissue effects of ATM inhibition is clearly required.

The low bioavailability of compounds used to inhibit ATM to date has been a barrier to both preclinical in vivo studies and clinical trials in combination with radiation. Recently however, a highly potent inhibitor of ATM that exhibits blood-brain barrier penetration has been described by Valerie et al. AZ32 in combination with a fractionated radiotherapy schedule significantly increased median survival in an orthotopic human glioma murine model [88].

## *ATR Inhibition*

The effects of ATR inhibition on radiosensitivity have been the subject of several preclinical studies. Wang et al. investigated the effects of kinase-dead ATR expression on cellular radiosensitivity and demonstrated that loss of ATR kinase function radiosensitised cells through deficient S and G2 cell checkpoints and reduced HR [89]. Gilad et al. demonstrated a requirement for malignant cells to engage the ATR-Chk1 pathway in order to maintain genome stability following oncogenic expression of Ras, implying indirectly that suppression of ATR signalling may sensitise cancer cells to DNA-damaging agents such as radiation [90].

Until a recent study by Reaper et al. of the compound VE821, specific and potent inhibitors of ATR had not been available. VE-821 was shown to potentiate the lethal effects of cisplatin and ionising radiation, effects that were enhanced in cells with a deficiency in the ATM-p53 axis. The authors speculated that ATR inhibition generated DSBs via collapse of replication forks which would normally induce an ATM-dependent S phase checkpoint response. Cells deficient in ATM or p53 were unable to activate this response and exhibited increased sensitivity to ATR inhibition [91].

Prevo et al. investigated the radiosensitising effects of ATR inhibition in pancreatic carcinoma models using VE821, which was shown to ablate induction of Chk1 phosphorylation by radiation or gemcitabine. It also increased the sensitivity of established and primary pancreatic cancer cells to the combination of radiation and gemcitabine under both normoxic and hypoxic conditions and effectively inhibited radiation-induced G2/M arrest. ATR inhibition also appeared to increase DNA DSBs following treatment with radiation as assessed by persistent gamma H2AX and 53BP1 foci. In contrast, Rad51 foci formation was reduced 24 h after treatment with IR and VE821, suggesting inhibition of HR [92].

Fokas et al. subsequently used a more potent analogue, VE822, to study the effects of ATR inhibition on pancreatic cancer cell radiosensitivity in vivo. VE822 was found to inhibit Chk1 phosphorylation and sensitise pancreatic cancer cells to radiation, both alone and in combination with gemcitabine. In contrast, it had no effect on tube formation by human dermal microvascular endothelial cells after radiotherapy and did not affect clonogenic survival of fibroblasts, indicating favourable tumour specificity. As before, radiation-induced DSB repair foci (gamma H2AX and 53BP1) were increased by the combination of ATR and radiotherapy, whilst Rad51 foci were decreased, strengthening the concept that ATR inhibition is associated with an HR defect. In vivo, the combination of IR and ATR inhibition produced a significant increase in tumour growth delay in subcutaneous pancreatic tumour xenografts. This study also attempted to quantify the toxic effects of the IR plus ATR inhibitor combination on critical normal tissues by assessing apoptosis of jejunal cells and villus tip loss in mice treated with the combination. Neither of these parameters when compared with controls indicated additional toxicity with the addition of ATR inhibition [93].

In further studies on radiotherapy-resistant hypoxic tumour cells, Pires et al. demonstrated that inhibition of ATR with VE821 sensitised a wide variety of commercially available cancer cell lines to radiation with no evidence of a relationship with p53 mutation in these experiments. Severe hypoxia is known to cause replicative

stress and consequent activation of ATM and ATR signalling; in this study, VE821 was demonstrated to abrogate hypoxia-mediated ATR signalling and to increase radiation-induced cell killing in physiologically relevant hypoxic conditions [94].

Finally, Sankunny et al. demonstrated that siRNA knockdown of ATR could radiosensitise oral squamous cell carcinoma with distal chromosome arm 11q loss (a marker of relative radioresistance and poor prognosis) [95], whilst Vavrova et al. have also demonstrated radiosensitisation of p53-deficient promyelocytic leukaemia cells by ATR inhibition [96].

### *Chk1 Inhibition*

The radiosensitising effects of Chk1 have been investigated by several authors in various tumour models. Since Chk1 has important effects on G2/M checkpoint control and in the promotion of Rad51-mediated DNA DSB homologous recombination repair, Chk1 inhibitors are predicted to have potent radiosensitising effects. Many studies have addressed this question in p53-mutant models since these cells are expected to display increased dependency on G2/M checkpoint arrest. Koniaras et al. demonstrated that the G2/M checkpoint was independent of p53 and then showed that expression of a dominant negative Chk1 construct resulted in increased radiosensitivity [97]. Sorensen et al. further defined the role of Chk1 as an essential kinase for the maintenance of genomic integrity [98]. They demonstrated Chk1 inhibition with two different compounds (UCN01 and CEP3891) and noted an increase in phosphorylation of ATR targets, increased initiation of DNA replication and generation of DNA DSBs. Chen et al. investigated the role of Chk1 inhibition as a potential sensitiser to DNA-damaging agents [99] by comparing radiation responses of p53-mutated cancer cell lines following Chk1 inhibition to those of p53 wild-type cell lines and normal human fibroblasts. Chk1 inhibition was found to potentiate the effects of radiation in p53-mutant cells only.

Radiosensitising effects of additional Chk1 inhibitor compounds have subsequently been published in preclinical models of breast cancer and pancreatic cancer [100, 101].

### *Inhibition of NHEJ*

Inhibition of NHEJ can be achieved using inhibitors of DNAPK, the apical kinase that plays a central role in this pathway. Since NHEJ is the predominant mechanism of DSB repair in normal mammalian cells, its inhibition might be predicted to cause non-specific radiosensitisation and severe normal tissue toxicity, an argument often used to suggest that NHEJ is not a promising therapeutic target. Nevertheless, it should be remembered firstly that malignant cells do not possess normal DDR and

secondly that back up repair pathways such as MMEJ exist in normal cells. Inhibition of NHEJ thus remains an area of active interest as a radiosensitisation strategy.

DNAPK-deficient cell lines have been shown to be highly radiosensitive [102], but whilst several inhibitors of DNAPK are available, none as yet have been used in preclinical in vivo studies in combination with radiation. In cellular models, Veuger et al. demonstrated effective radiosensitisation in vitro using NU7026, which was shown to be a potent and specific DNAPK inhibitor in this study [102].

### *Rad51 Inhibition*

Rad51 is a key element of the HR DSB repair pathway, and inhibition of this protein would be predicted to have significant effects on the repair of DSBs following irradiation. Investigating this hypothesis, Short et al. found that levels of Rad51 in human glioma cell lines were inversely related to their radiosensitivity and that knockdown of Rad51 led to increased sensitivity to both radiation and temozolomide (an alkylating cytotoxic agent). They and others have proposed that Rad51 inhibition represents a promising radiosensitisation strategy [103] but development of pharmacological inhibitors of Rad51 has lagged behind work on other DNA repair targets. Huang et al. recently described the development of a small molecule inhibitor of Rad51 which increased the chemosensitivity of in vitro cancer cells; however, the effects on radiosensitivity were not explored [104].

### *Combination DDR Inhibition*

The ability to inhibit different targets within the DDR allows the prospect of inhibiting combinations of DDR proteins in order to manipulate radiation sensitivity. To date, only a few studies have adopted this approach. Vance et al. investigated radiosensitisation of pancreatic cancer cells exposed to combinations of Chk1 and PARP inhibitors [105]. This study demonstrated radiosensitisation of both p53 wild type and p53 mutants in isogenic cell lines by the combination treatment; however, radiosensitisation was greater in the p53-mutated cell lines. Single-agent sensitiser enhancement ratios for PARP and Chk1 were modest (1.5); however, the combination of agents produced sensitiser enhancement ratios of greater than 2. The combination of Chk1 and PARP inhibition caused G2/M dysfunction, inhibition of HR and persistent DDR in tumour cells but did not appear to radiosensitise normal intestinal epithelial cells in vitro. The authors speculated that the HR deficiency induced by Chk1 inhibition may sensitise to PARP inhibition via generation of a ‘BRCAness’ phenotype.

Hoglund et al. demonstrated that the combination of PARP inhibition and Chk2 functional loss elicits a synthetic lethal response in Myc-overexpressing lymphoma cells [106], whilst Booth et al. observed that combining PARP inhibition and Chk1

inhibition produced cytotoxic effects in mammary cells even in the absence of any exogenous DNA-damaging agent [107]. Furthermore, the actions of PARP and Chk1 inhibition were enhanced by ATM knockdown. Similarly Peasland et al. documented a synthetic lethal effect of combining the ATR inhibitor NU-6027 and PARP inhibition [108]. None of these studies evaluated the impact of adding ionising radiation.

Clearly the combination of different DDR inhibitors has the potential to enhance the effects of radiation, and given the redundancy encountered within DDR pathways, this may represent a particularly effective way of inducing potent radiosensitisation of resistant cancers. Nevertheless, the effects of combination DDR inhibition on normal tissue toxicity will require careful consideration.

## DDR Kinase Inhibition and Cancer Stem Cell Theory

Cancer stem cell theory has gained prominence in a variety of solid tumour sites in the last decade. This theory states that only a subpopulation of tumour cells (cancer stem cells) possesses the ability to initiate tumour growth and that this subpopulation exhibits some of the features of normal tissue stem cells. Cancer stem cells have been shown by several authors to be resistant to conventional cancer treatments and in particular to be radiation resistant [109, 110]. These observations implicate the cancer stem cell population in tumour recurrence following treatment; hence, efforts to develop therapies that specifically target the cancer stem cell populations of solid tumours are urgently required.

Bao et al. demonstrated the radioresistance of glioblastoma cancer stem cells (GBM CSCs) and subsequently showed GBM CSCs to exhibit upregulated DNA damage responses [110]. Subsequent studies have demonstrated that GBM CSCs exhibit enhanced activation of the G2/M checkpoint and more efficient DNA DSB repair in G2 phase of the cell cycle following irradiation [109] compared to other GBM cell populations which did not exhibit the CSC phenotype. ATM inhibition was shown to be a potent radiosensitiser of GBM CSCs and was effective in abrogating both enhanced G2/M checkpoint activation and G2 DNA DSB repair advantage following radiation in the GBM CSC population. Ahmed et al. recently demonstrated that selective inhibition of parallel DNA damage response pathways optimised radiosensitisation of GBM CSCs. Individually, inhibition of ATR, PARP, Chk1 and ATM all radiosensitised GBM CSCs; however, only ATM inhibition or dual inhibition of ATR and PARP delivered increases in GBM CSC radiosensitivity that were significantly greater than those observed in tumour bulk (non-CSC) populations. These data demonstrate that multiple, parallel pathways contribute to GBM CSC radioresistance and that combined inhibition of cell cycle checkpoint and DNA repair targets provides the most effective means of overcoming radioresistance of GBM CSCs [111]. They also support

the concept that upregulated DDR is integral to the radioresistance seen in GBM CSCs and that DDR inhibition is a promising radiosensitising strategy for this problematic cellular subpopulation.

## Combining Radiotherapy and DDR Kinase Inhibition in the Clinic

Combining DDR kinase inhibition with radiotherapy in the clinic poses several challenges. Many DDR kinase inhibitors used for in vitro studies are potent and specific inhibitors of their targets but lack the bioavailability, tumour penetration or blood-brain barrier penetration necessary for them to be clinically useful compounds. In recent years, a number of clinically useful DDR kinase inhibitors have been developed, and these agents are starting to be combined with radiotherapy in early-phase clinical trials.

As discussed above, there is compelling evidence to suggest that a DDR inhibitor radiosensitiser strategy has the potential to provide tumour-specific radiosensitisation but that concomitant administration of cytotoxic systemic agents can complicate delivery of this strategy by increasing the risk of systemic toxicities. Many curative radiotherapy regimens now incorporate systemic chemotherapy agents, which have been demonstrated to provide small benefits in terms of tumour control, but which increase toxicity towards the ceiling of tolerance. DDR inhibition has been demonstrated to increase the haematological toxicity of chemotherapy drugs: early combination trials of PARP inhibitors with systemic cytotoxic agents reported severe haematological toxicity that limited the usefulness of the combination approach. However, improved scheduling of these agents with systemic treatments may provide a solution to this problem. Another solution would be to pioneer early-phase clinical trials in palliative (non-curative) radiotherapy treatments which do not include concurrent chemotherapy.

## Clinical Trials of DDR Kinase Inhibition

Clinical trials of several DDR kinase inhibitor agents combined with radiation are either in progress or in advanced stages of development. Most are investigating the combination of PARP inhibitors with radiation, since these compounds are the most advanced in their clinical development. There are now several phase I clinical trials of PARP inhibitors in combination with radiotherapy in various tumour sites including breast cancer, non-small cell lung cancer, oesophageal cancer, brain metastases and glioblastoma. Many of the studies have adopted palliative (non-curative) radiotherapy regimens for locally advanced cancer in order that toxicity of combined PARP inhibitor and radiation therapy can be fully explored without compromising chances of cure. An example of one of these trials is the ‘PARADIGM’ study which is currently recruiting patients in the United Kingdom. This study will investigate

the combination of olaparib with hypofractionated radiotherapy (40 Gy in 15 fractions) in glioblastoma patients who are unsuitable for concurrent chemoradiation. An initial phase I trial will identify a maximum tolerated dose of olaparib with radiotherapy before progressing to a randomised phase II trial which will investigate whether olaparib in combination with radiotherapy increases survival in this population, with a view to justifying a subsequent phase III trial.

The results of a phase I trial of the PARP inhibitor ABT888 (veliparib) in combination with palliative whole brain radiotherapy for brain metastases have been published recently [112]. This trial showed that the combination of whole brain radiotherapy and PARP inhibition was well tolerated; indeed a maximum tolerated dose of veliparib was not reached because predefined dose-limiting toxicities were not observed at any dose level. The toxicity of the combined regimen at the recommended phase 2 dose of veliparib was felt to be similar to that of whole brain radiation alone. Comparison to historical controls suggested an improvement in survival in patients receiving veliparib and radiotherapy; however, this was a phase 1 trial in a highly selected patient population, preventing any robust conclusions regarding efficacy. Nevertheless, the study provides promising evidence that PARP inhibition can be delivered in combination with whole brain radiotherapy with relatively modest toxicity.

The ‘PATRIOT’ study is the only ‘non-PARP’ DDR inhibitor/radiotherapy combination study currently under way. This phase I study is evaluating the ATR inhibitor AZD6738 both as monotherapy and in combination with radiation in solid tumours exhibiting abnormalities in the p53 pathway. The trial design incorporates three stages that enable investigation of optimal dose, optimal scheduling and overall safety of the combination of AZD6738 with palliative radiotherapy (20 or 30Gy). Recruitment to this trial has commenced, and results are eagerly awaited. Other inhibitors of the DDR are yet to be combined with radiation in a clinical setting, and to the authors’ knowledge, no clinical trials of inhibitors of ATM or Chk1 in combination with radiotherapy are yet underway.

## Biomarkers

Clinical application of DDR inhibitor radiosensitisation strategies will require the development of companion biomarkers that allow rational patient selection whilst ensuring optimal tumour radiosensitisation and minimal normal tissue toxicity. Next-generation sequencing technologies have enabled comprehensive sequencing of tumour genomes, facilitating detailed analysis of mutations, copy number variants and deletions in individual tumours. This information has the potential to enable selection of patients that will benefit from DDR inhibition and to identify the DDR inhibitor that will deliver optimal radiosensitisation. For example, a tumour deficient in the HR pathway may benefit from PARP inhibition, or cancers with high levels of replication stress may be optimally radiosensitised by ATR inhibition. Tumours lacking p53-mediated G1/S checkpoint may usefully be radiosensitised by Chk1 or ATM inhibition. In this way, radiosensitiser strategies could in the future be

tailored to a patient's tumour allowing manipulation of the therapeutic ratio of radiation treatment to its maximum extent. Whilst the building blocks of this personalisation strategy are in place in the form of extensive, detailed understanding of the DDR pathways involved, clinically meaningful and deliverable molecular biomarkers have yet to be identified and will need to be validated in randomised clinical trials before they can be adopted in routine clinical practice.

## Conclusion

Tumour radioresistance has been a fundamental problem facing radiation oncologists since ionising radiation was first used to treat cancer over one hundred years ago. Despite the knowledge that radiotherapy is essentially a DNA-damaging agent and that repair of radiation-induced DNA damage is a major determinant of tumour radioresistance, manipulation of the radiobiological response of tumours has not been a feasible prospect until the last few years. Recent advances in molecular biology have described the vast interconnected pathways responsible for maintaining the integrity of mammalian DNA, and it is clear that during the process of carcinogenesis fundamental alterations to the normal DNA damage response are necessary in order to generate the hallmark feature of genomic instability in cancer cells. Given the presence of altered DDR in many tumour cells, the targeting of specific DDR pathways by small molecule inhibitors provides the exciting prospect of tumour-specific radiosensitisation.

Recent research has centred upon inhibition of central DDR kinases such as ATM, ATR, DNAPKcs and Chk1. These agents deliver potent radiosensitisation in vitro, and there is some evidence to indicate tumour specificity in their actions. The effects of PARP inhibition on tumour radiation response have also been investigated by a number of authors, and this approach has been shown to be a promising way of radiosensitising normoxic and hypoxic tumour cells both in vitro and in vivo, in a potentially tumour-specific manner. Clinical development of DDR inhibitors is progressing, with PARP inhibitors entering phase I and II trials in combination with radiotherapy in a variety of tumour sites. Entry of other DDR inhibition strategies into clinical trials has been somewhat slower; however, ATR inhibitors are soon to enter phase I trials in combination with radiation.

The manipulation of DDR in radioresistant tumours will greatly enhance the biological effects of radiotherapy, allowing the treatment of cancers which have in the past proven difficult or impossible to cure using radiation. One of the challenges of developing DDR radiosensitiser strategies will be to identify which elements of DDR in a particular tumour can be safely targeted by inhibitors to produce tumour-specific radiosensitisation. Only a fuller understanding of the DDR mechanisms that determine radioresistance in tumours will achieve this aim, coupled with the development of clinically useful biomarkers. DDR inhibition has significant potential to enhance the beneficial biological effects of radiation on tumours and to open a new frontier in the treatment of malignant disease.

## References


1. Shiloh Y, Ziv Y (2013) The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nat Rev Mol Cell Biol 14(4):197–210
2. Taylor AM, Harnden DG, Arlett CF, Harcourt SA, Lehmann AR, Stevens S et al (1975) Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. Nature 258(5534):427–429
3. Bakkenist CJ, Kastan MB (2003) DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 421(6922):499–506
4. You Z, Bailis JM, Johnson SA, Dilworth SM, Hunter T (2007) Rapid activation of ATM on DNA flanking double-strand breaks. Nat Cell Biol 9(11):1311–1318
5. Goodarzi AA, Noon AT, Deckbar D, Ziv Y, Shiloh Y, Lobrich M et al (2008) ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. Mol Cell 31(2):167–177
6. Riballo E, Kuhne M, Rief N, Doherty A, Smith GC, Recio MJ et al (2004) A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. Mol Cell 16(5):715–724
7. Alvarez-Quilon A, Serrano-Benitez A, Lieberman JA, Quintero C, Sanchez-Gutierrez D, Escudero LM et al (2014) ATM specifically mediates repair of double-strand breaks with blocked DNA ends. Nat Commun 5:3347
8. Halazonetis TD, Gorgoulis VG, Bartek J (2008) An oncogene-induced DNA damage model for cancer development. Science 319(5868):1352–1355
9. Marechal A, Zou L. DNA damage sensing by the ATM and ATR kinases. Cold Spring Harb perspect Biol 2013;5(9). doi:10.1101/cshperspect.a012716
10. Brown EJ, Baltimore D (2000) ATR disruption leads to chromosomal fragmentation and early embryonic lethality. Genes Dev 14(4):397–402
11. Zeman MK, Cimprich KA (2014) Causes and consequences of replication stress. Nat Cell Biol 16(1):2–9
12. Liu H, Takeda S, Kumar R, Westergard TD, Brown EJ, Pandita TK et al (2010) Phosphorylation of MLL by ATR is required for execution of mammalian S-phase checkpoint. Nature 467(7313):343–346
13. Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Lobrich M, Jeggo PA (2004) ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. Cancer Res 64(7):2390–2396
14. Lukas J, Lukas C, Bartek J (2004) Mammalian cell cycle checkpoints: signalling pathways and their organization in space and time. DNA Repair 3(8-9):997–1007
15. Deckbar D, Stiff T, Koch B, Reis C, Lobrich M, Jeggo PA (2010) The limitations of the G1-S checkpoint. Cancer Res 70(11):4412–4421
16. Mailand N, Falck J, Lukas C, Syljuasen RG, Welcker M, Bartek J et al (2000) Rapid destruction of human Cdc25A in response to DNA damage. Science 288(5470):1425–1429
17. Deckbar D, Birraux J, Krempler A, Tchouandong L, Beucher A, Walker S et al (2007) Chromosome breakage after G2 checkpoint release. J Cell Biol 176(6):749–755
18. Deckbar D, Jeggo PA, Lobrich M (2011) Understanding the limitations of radiation-induced cell cycle checkpoints. Crit Rev Biochem Mol Biol 46(4):271–283
19. Goodarzi AA, Jeggo PA (2012) Irradiation induced foci (IRIF) as a biomarker for radiosensitivity. Mutat Res 736(1–2):39–47
20. Frankenberg D, Frankenberg-Schwager M, Blocher D, Harbich R (1981) Evidence for DNA double-strand breaks as the critical lesions in yeast cells irradiated with sparsely or densely ionizing radiation under oxic or anoxic conditions. Radiat Res 88(3):524–532
21. Shibata A, Jeggo PA (2014) DNA double-strand break repair in a cellular context. Clin Oncol 26(5):243–249
22. Weterings E, Chen DJ (2008) The endless tale of non-homologous end-joining. Cell Res 18(1):114–124

23. Beucher A, Birraux J, Tchouandong L, Barton O, Shibata A, Conrad S et al (2009) ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. EMBO J 28(21):3413–3427
24. Shibata A, Conrad S, Birraux J, Geuting V, Barton O, Ismail A et al (2011) Factors determining DNA double-strand break repair pathway choice in G2 phase. EMBO J 30(6):1079–1092
25. Roth DB, Wilson JH (1986) Nonhomologous recombination in mammalian cells: role for short sequence homologies in the joining reaction. Mol Cell Biol 6(12):4295–4304
26. Wang H, Perrault AR, Takeda Y, Qin W, Wang H, Iliakis G (2003) Biochemical evidence for Ku-independent backup pathways of NHEJ. Nucleic Acids Res 31(18):5377–5388
27. McVey M, Lee SE (2008) MMEJ repair of double-strand breaks (director's cut): deleted sequences and alternative endings. Trends Genet 24(11):529–538
28. Bentley J, Diggle CP, Harnden P, Knowles MA, Kiltie AE (2004) DNA double strand break repair in human bladder cancer is error prone and involves microhomology-associated end-joining. Nucleic Acids Res 32(17):5249–5259
29. Jeggo PA, Geuting V, Lobrich M (2011) The role of homologous recombination in radiation-induced double-strand break repair. Radiother Oncol 101(1):7–12, Epub 2011/07/09
30. Helleday T, Lo J, van Gent DC, Engelward BP (2007) DNA double-strand break repair: from mechanistic understanding to cancer treatment. DNA Repair 6(7):923–935
31. San Filippo J, Sung P, Klein H (2008) Mechanism of eukaryotic homologous recombination. Annu Rev Biochem 77:229–257
32. Li X, Heyer WD (2008) Homologous recombination in DNA repair and DNA damage tolerance. Cell Res 18(1):99–113
33. Krejci L, Altmannova V, Spirek M, Zhao X (2012) Homologous recombination and its regulation. Nucleic Acids Res 40(13):5795–5818
34. Wold MS (1997) Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. Annu Rev Biochem 66:61–92
35. Eggler AL, Inman RB, Cox MM (2002) The Rad51-dependent pairing of long DNA substrates is stabilized by replication protein A. J Biol Chem 277(42):39280–39288
36. D'Amours D, Desnoyers S, D'Silva I, Poirier GG (1999) Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J 342(Pt 2):249–268
37. Burkle A, Virag L (2013) Poly(ADP-ribose): PARadigms and PARadoxes. Mol Aspects Med 34:1046–1065
38. Krishnakumar R, Kraus WL (2010) The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. Mol Cell 39(1):8–24
39. Khodyreva SN, Prasad R, Ilina ES, Sukhanova MV, Kutuzov MM, Liu Y et al (2010) Apurinic/apyrimidinic (AP) site recognition by the 5'-dRP/AP lyase in poly(ADP-ribose) polymerase-1 (PARP-1). Proc Natl Acad Sci U S A 107(51):22090–22095
40. Chasovskikh S, Dimtchev A, Smulson M, Dritschilo A (2005) DNA transitions induced by binding of PARP-1 to cruciform structures in supercoiled plasmids. Cytometry A 68(1):21–27
41. Lonskaya I, Potaman VN, Shlyakhtenko LS, Oussatcheva EA, Lyubchenko YL, Soldatenkov VA (2005) Regulation of poly(ADP-ribose) polymerase-1 by DNA structure-specific binding. J Biol Chem 280(17):17076–17083
42. Potaman VN, Shlyakhtenko LS, Oussatcheva EA, Lyubchenko YL, Soldatenkov VA (2005) Specific binding of poly(ADP-ribose) polymerase-1 to cruciform hairpins. J Mol Biol 348(3):609–615
43. Langelier MF, Ruhl DD, Planck JL, Kraus WL, Pascal JM (2010) The Zn3 domain of human poly(ADP-ribose) polymerase-1 (PARP-1) functions in both DNA-dependent poly(ADP-ribose) synthesis activity and chromatin compaction. J Biol Chem 285(24):18877–18887
44. Zahradka P, Ebisuzaki K (1982) A shuttle mechanism for DNA-protein interactions. The regulation of poly(ADP-ribose) polymerase. Eur J Biochem 127(3):579–585
45. Ferro AM, Olivera BM (1982) Poly(ADP-ribosylation) in vitro. Reaction parameters and enzyme mechanism. J Biol Chem 257(13):7808–7813

46. Lindahl T, Satoh MS, Poirier GG, Klungland A (1995) Post-translational modification of poly(ADP-ribose) polymerase induced by DNA strand breaks. Trends Biochem Sci 20 (10):405–411
47. Fisher AE, Hochegger H, Takeda S, Caldecott KW (2007) Poly(ADP-ribose) polymerase 1 accelerates single-strand break repair in concert with poly(ADP-ribose) glycohydrolase. Mol Cell Biol 27(15):5597–5605
48. Satoh MS, Lindahl T (1992) Role of poly(ADP-ribose) formation in DNA repair. Nature 356(6367):356–358
49. Strom CE, Johansson F, Uhlen M, Szigyarto CA, Erixon K, Helleday T (2011) Poly (ADP-ribose) polymerase (PARP) is not involved in base excision repair but PARP inhibition traps a single-strand intermediate. Nucleic Acids Res 39(8):3166–3175
50. El-Khamisy SF, Masutani M, Suzuki H, Caldecott KW (2003) A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. Nucleic Acids Res 31(19):5526–5533
51. Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K et al (2005) DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 434(7035):864–870
52. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5): 646–674
53. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M et al (2009) Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 361(2):123–134
54. Brock WA, Milas L, Bergh S, Lo R, Szabo C, Mason KA (2004) Radiosensitization of human and rodent cell lines by INO-1001, a novel inhibitor of poly(ADP-ribose) polymerase. Cancer Lett 205(2):155–160
55. Dungey FA, Caldecott KW, Chalmers AJ (2009) Enhanced radiosensitization of human glioma cells by combining inhibition of poly(ADP-ribose) polymerase with inhibition of heat shock protein 90. Mol Cancer Ther 8(8):2243–2254
56. Albert JM, Cao C, Kim KW, Willey CD, Geng L, Xiao D, et al. Inhibition of poly(ADP-ribose) polymerase enhances cell death and improves tumor growth delay in irradiated lung cancer models. Clin Cancer Res. 2007;13(10):3033–42.
57. Loser DA, Shibata A, Shibata AK, Woodbine LJ, Jeggo PA, Chalmers AJ (2010) Sensitization to radiation and alkylating agents by inhibitors of poly(ADP-ribose) polymerase is enhanced in cells deficient in DNA double-strand break repair. Mol Cancer Ther 9(6):1775–1787
58. Calabrese CR, Almassy R, Barton S, Batey MA, Calvert AH, Canan-Koch S et al (2004) Anticancer chemosensitization and radiosensitization by the novel poly(ADP-ribose) polymerase-1 inhibitor AG14361. J Natl Cancer Inst 96(1):56–67
59. Russo, A. L.; Kwon, H. C.; Burgan, W. E.; Carter, D.; Beam, K.; Weizheng, X.; Zhang, J.; Slusher, B. S.; Chakravarti, A.; Tofilon, P. J.; Camphausen, K., In vitro and in vivo radiosensitization of glioblastoma cells by the poly (ADPribose) polymerase inhibitor E7016. Clin. Cancer. Res. 2009, 15(2), 607–12.
60. Liu SK, Coackley C, Krause M, Jalali F, Chan N, Bristow RG (2008) A novel poly(ADP-ribose) polymerase inhibitor, ABT-888, radiosensitizes malignant human cell lines under hypoxia. Radiother Oncol 88(2):258–268
61. Noel G, Godon C, Fernet M, Giocanti N, Megnin-Chanet F, Favaudon V (2006) Radiosensitization by the poly(ADP-ribose) polymerase inhibitor 4-amino-1,8-naphthalimide is specific of the S phase of the cell cycle and involves arrest of DNA synthesis. Mol Cancer Ther 5(3):564–574
62. Langelier MF, Planck JL, Roy S, Pascal JM (2012) Structural basis for DNA damage-dependent poly(ADP-ribosyl)ation by human PARP-1. Science 336(6082):728–732
63. Meng AX, Jalali F, Cuddihy A, Chan N, Bindra RS, Glazer PM et al (2005) Hypoxia down-regulates DNA double strand break repair gene expression in prostate cancer cells. Radiother Oncol 76(2):168–176

64. Bindra RS, Schaffer PJ, Meng A, Woo J, Maseide K, Roth ME et al (2004) Down-regulation of Rad51 and decreased homologous recombination in hypoxic cancer cells. Mol Cell Biol 24(19):8504–8518
65. Chan N, Pires IM, Bencokova Z, Coackley C, Luoto KR, Bhogal N et al (2010) Contextual synthetic lethality of cancer cell kill based on the tumor microenvironment. Cancer Res 70(20):8045–8054
66. Khan K, Araki K, Wang D, Li G, Li X, Zhang J, Xu W, Hoover RK, Lauter S, O'Malley B Jr, Lapidus RG, Li D. Head and neck cancer radiosensitization by the novel poly(ADP-ribose) polymerase inhibitor GPI-15427. Head Neck. 2010 Mar;32(3):381–91.
67. Clarke MJ, Mulligan EA, Grogan PT, Mladek AC, Carlson BL, Schroeder MA, Curtin NJ, Lou Z, Decker PA, Wu W, Plummer ER, Sarkaria JN. Effective sensitization of temozolomide by ABT-888 is lost with development of temozolomide resistance in glioblastoma xenograft lines. Mol Cancer Ther. 2009 Feb;8(2):407–14.
68. Donawho CK, Luo Y, Luo Y, Penning TD, Bauch JL, Bouska JJ, Bontcheva-Diaz VD, Cox BF, DeWeese TL, Dillehay LE, Ferguson DC, Ghoreishi-Haack NS, Grimm DR, Guan R, Han EK, Holley-Shanks RR, Hristov B, Idler KB, Jarvis K, Johnson EF, Kleinberg LR, Klinghofer V, Lasko LM, Liu X, Marsh KC, McGonigal TP, Meulbroek JA, Olson AM, Palma JP, Rodriguez LE, Shi Y, Stavropoulos JA, Tsurutani AC, Zhu GD, Rosenberg SH, Giranda VL, Frost DJ. ABT-888, an orally active poly(ADP-ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical tumor models. Clin Cancer Res. 2007 May 1;13(9):2728–37.
69. Tuli R, Surmak AJ, Reyes J, Armour M, Hacker-Prietz A, Wong J et al (2014) Radiosensitization of pancreatic cancer cells in vitro and in vivo through poly (ADP-ribose) polymerase inhibition with ABT-888. Transl Oncol. doi:10.1016/j.tranon.2014.04.003
70. Ali M, Kamjoo M, Thomas HD, Kyle S, Pavlovska I, Babur M et al (2011) The clinically active PARP inhibitor AG014699 ameliorates cardiotoxicity but does not enhance the efficacy of doxorubicin, despite improving tumor perfusion and radiation response in mice. Mol Cancer Ther 10(12):2320–2329
71. Hobson B, Denekamp J (1984) Endothelial proliferation in tumours and normal tissues: continuous labelling studies. Br J Cancer 49(4):405–413
72. Haveman J, Rodermond H, van Bree C, Wondergem J, Franken NA (2007) Residual late radiation damage in mouse stromal tissue assessed by the tumor bed effect. J Radiat Res 48(2):107–112
73. Galia A, Calogero AE, Condorelli R, Fraggetta F, La Corte A, Ridolfo F et al (2012) PARP-1 protein expression in glioblastoma multiforme. Eur J Histochem 56(1), e9
74. Roesner JP, Mersmann J, Bergt S, Bohnenberg K, Barthuber C, Szabo C et al (2010) Therapeutic injection of PARP inhibitor INO-1001 preserves cardiac function in porcine myocardial ischemia and reperfusion without reducing infarct size. Shock 33(5):507–512
75. Hamahata A, Enkhbaatar P, Lange M, Yamaki T, Sakurai H, Shimoda K et al (2012) Administration of poly(ADP-ribose) polymerase inhibitor into bronchial artery attenuates pulmonary pathophysiology after smoke inhalation and burn in an ovine model. Burns 38(8):1210–1215
76. Tentori L, Leonetti C, Scarsella M, Muzi A, Mazzon E, Vergati M et al (2006) Inhibition of poly(ADP-ribose) polymerase prevents irinotecan-induced intestinal damage and enhances irinotecan/temozolomide efficacy against colon carcinoma. FASEB J 20(10):1709–1711
77. Golding SE, Rosenberg E, Valerie N, Hussaini I, Frigerio M, Cockcroft XF et al (2009) Improved ATM kinase inhibitor KU-60019 radiosensitizes glioma cells, compromises insulin, AKT and ERK prosurvival signaling, and inhibits migration and invasion. Mol Cancer Ther 8(10):2894–2902
78. Golding SE, Rosenberg E, Adams BR, Wignarajah S, Beckta JM, O'Connor MJ et al (2012) Dynamic inhibition of ATM kinase provides a strategy for glioblastoma multiforme radiosensitization and growth control. Cell Cycle 11(6):1167–1173
79. Biddlestone-Thorpe L, Sajjad M, Rosenberg E, Beckta JM, Valerie NC, Tokarz M et al (2013) ATM kinase inhibition preferentially sensitizes p53-mutant glioma to ionizing radiation. Clin Cancer Res 19(12):3189–3200

80. Cancer Genome Atlas Research N (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455(7216):1061–1068
81. Toulany M, Mihatsch J, Holler M, Chaachouay H, Rodemann HP (2014) Cisplatin-mediated radiosensitization of non-small cell lung cancer cells is stimulated by ATM inhibition. Radiother Oncol 111(2):228–236
82. Zou J, Qiao X, Ye H, Yang Y, Zheng X, Zhao H et al (2008) Antisense inhibition of ATM gene enhances the radiosensitivity of head and neck squamous cell carcinoma in mice. J Exp Clin Cancer Res 27:56
83. Rainey MD, Charlton ME, Stanton RV, Kastan MB (2008) Transient inhibition of ATM kinase is sufficient to enhance cellular sensitivity to ionizing radiation. Cancer Res 68(18):7466–7474
84. Choi S, Gamper AM, White JS, Bakkenist CJ (2010) Inhibition of ATM kinase activity does not phenocopy ATM protein disruption: implications for the clinical utility of ATM kinase inhibitors. Cell Cycle 9(20):4052–4057
85. Schneider L, Fumagalli M (2012) d'Adda di Fagagna F. Terminally differentiated astrocytes lack DNA damage response signaling and are radioresistant but retain DNA repair proficiency. Cell Death Differ 19(4):582–591
86. Gosink EC, Chong MJ, McKinnon PJ (1999) Ataxia telangiectasia mutated deficiency affects astrocyte growth but not radiosensitivity. Cancer Res 59(20):5294–5298
87. Moding EJ, Lee CL, Castle KD, Oh P, Mao L, Zha S et al (2014) Atm deletion with dual recombinase technology preferentially radiosensitizes tumor endothelium. J Clin Invest 124(8):3325–3338
88. Blood-brain barrier penetrating ATM inhibitor (AZ32) radiosensitises intracranial gliomas in mice. Steve T. Durant, Jeremy Karlin, Kurt Pike, Nicola Colclough, N Mukhopadhyay, S F. Ahmad, J M. Bekta, M Tokarz, Catherine Bardelle, Gareth Hughes, Bhavika Patel, Andrew Thomason, Elaine Cadogan, Ian Barrett, Alan Lau, Martin Pass, Kristoffer Valerie DOI:10.1158/1538-7445.AM2016-3041 Published 15 July 2016
89. Wang H, Wang H, Powell SN, Iliakis G, Wang Y (2004) ATR affecting cell radiosensitivity is dependent on homologous recombination repair but independent of nonhomologous end joining. Cancer Res 64(19):7139–7143
90. Gilad O, Nabet BY, Ragland RL, Schoppy DW, Smith KD, Durham AC et al (2010) Combining ATR suppression with oncogenic Ras synergistically increases genomic instability, causing synthetic lethality or tumorigenesis in a dosage-dependent manner. Cancer Res 70(23):9693–9702
91. Reaper PM, Griffiths MR, Long JM, Charrier JD, Maccormick S, Charlton PA et al (2011) Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR. Nat Chem Biol 7(7):428–430
92. Prevo R, Fokas E, Reaper PM, Charlton PA, Pollard JR, McKenna WG et al (2012) The novel ATR inhibitor VE-821 increases sensitivity of pancreatic cancer cells to radiation and chemotherapy. Cancer Biol Ther 13(11):1072–1081
93. Fokas E, Prevo R, Pollard JR, Reaper PM, Charlton PA, Cornelissen B et al (2012) Targeting ATR in vivo using the novel inhibitor VE-822 results in selective sensitization of pancreatic tumors to radiation. Cell Death Dis 3, e441
94. Pires IM, Olcina MM, Anbalagan S, Pollard JR, Reaper PM, Charlton PA et al (2012) Targeting radiation-resistant hypoxic tumour cells through ATR inhibition. Br J Cancer 107(2):291–299
95. Sankunny M, Parikh RA, Lewis DW, Gooding WE, Saunders WS, Gollin SM (2014) Targeted inhibition of ATR or CHEK1 reverses radioresistance in oral squamous cell carcinoma cells with distal chromosome arm 11q loss. Genes Chromosomes Cancer 53(2):129–143
96. Vavrova J, Zarybnicka L, Lukasova E, Rezacova M, Novotna E, Sinkorova Z et al (2013) Inhibition of ATR kinase with the selective inhibitor VE-821 results in radiosensitization of

cells of promyelocytic leukaemia (HL-60). Radiat Environ Biophys 52(4):471–479, Epub 2013/08/13
97. Koniaras K, Cuddihy AR, Christopoulos H, Hogg A, O'Connell MJ (2001) Inhibition of Chk1-dependent G2 DNA damage checkpoint radiosensitizes p53 mutant human cells. Oncogene 20(51):7453–7463
98. Sorensen CS, Hansen LT, Dziegielewski J, Syljuasen RG, Lundin C, Bartek J et al (2005) The cell-cycle checkpoint kinase Chk1 is required for mammalian homologous recombination repair. Nat Cell Biol 7(2):195–201
99. Chen Z, Xiao Z, Gu WZ, Xue J, Bui MH, Kovar P et al (2006) Selective Chk1 inhibitors differentially sensitize p53-deficient cancer cells to cancer therapeutics. Int J Cancer 119(12):2784–2794
100. Engelke CG, Parsels LA, Qian Y, Zhang Q, Karnak D, Robertson JR et al (2013) Sensitization of pancreatic cancer to chemoradiation by the Chk1 inhibitor MK8776. Clin Cancer Res 19(16):4412–4421
101. Ma Z, Yao G, Zhou B, Fan Y, Gao S, Feng X (2012) The Chk1 inhibitor AZD7762 sensitises p53 mutant breast cancer cells to radiation in vitro and in vivo. Mol Med Rep 6(4):897–903
102. Veuger SJ, Curtin NJ, Richardson CJ, Smith GC, Durkacz BW (2003) Radiosensitization and DNA repair inhibition by the combined use of novel inhibitors of DNA-dependent protein kinase and poly(ADP-ribose) polymerase-1. Cancer Res 63(18):6008–6015
103. Short SC, Giampieri S, Worku M, Alcaide-German M, Sioftanos G, Bourne S et al (2011) Rad51 inhibition is an effective means of targeting DNA repair in glioma models and CD133+ tumor-derived cells. Neuro Oncol 13(5):487–499
104. Huang F, Mazin AV (2014) A small molecule inhibitor of human RAD51 potentiates breast cancer cell killing by therapeutic agents in mouse xenografts. PLoS One 9(6), e100993
105. Vance S, Liu E, Zhao L, Parsels JD, Parsels LA, Brown JL et al (2011) Selective radiosensitization of p53 mutant pancreatic cancer cells by combined inhibition of Chk1 and PARP1. Cell Cycle 10(24):4321–4329
106. Hoglund A, Stromvall K, Li Y, Forshell LP, Nilsson JA (2011) Chk2 deficiency in Myc overexpressing lymphoma cells elicits a synergistic lethal response in combination with PARP inhibition. Cell Cycle 10(20):3598–3607
107. Booth L, Cruickshanks N, Ridder T, Dai Y, Grant S, Dent P (2013) PARP and CHK inhibitors interact to cause DNA damage and cell death in mammary carcinoma cells. Cancer Biol Ther 14(5):458–465
108. Peasland A, Wang LZ, Rowling E, Kyle S, Chen T, Hopkins A et al (2011) Identification and evaluation of a potent novel ATR inhibitor, NU6027, in breast and ovarian cancer cell lines. Br J Cancer 105(3):372–381
109. Carruthers R, Ahmed SU, Strathdee K, Gomez-Roman N, Amoah-Buahin E, Watts C et al (2015) Abrogation of radioresistance in glioblastoma stem-like cells by inhibition of ATM kinase. Mol Oncol 9(1):192–203
110. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB et al (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444(7120):756–760
111. Ahmed SU, Carruthers R, Gilmour L, Yildirim S, Watts C, Chalmers AJ (2015) Selective Inhibition of Parallel DNA Damage Response Pathways Optimizes Radiosensitization of Glioblastoma Stem-like Cells. Cancer Res 75(20):4416–4428
112. Mehta MP, Wang D, Wang F, Kleinberg L, Brade A, Robins HI et al (2015) Veliparib in combination with whole brain radiation therapy in patients with brain metastases: results of a phase 1 study. J Neurooncol 122(2):409–417