Journal of
Cancer Science & Therapy

Salles et al., J Cancer Sci Ther 2011, S8
DOI: 10.4172/1948-5956.S8-001

Commentary Open Access

# DNA-PK, a Pharmacological Target in Cancer Chemotherapy and Radiotherapy?

**Bernard Salles[1]*, Patrick Calsou[2] and Gladys Mirey[1]**

[1]INRA, Université de Toulouse III, UMR1331, Toxalim (Research Centre in Food Toxicology), F-31027 Toulouse, France
[2]CNRS; IPBS (Institut de Pharmacologie et de Biologie Structurale); 205 route de Narbonne, F-31077 Toulouse, France

## Abstract

In the search for ways of sensitizing tumor cells to chemotherapy or radiotherapy, the inhibition of DNA repair has recently been proposed as a target of clinical interest. Ionizing radiation, as well as several antitumor drugs, induce the formation of DNA double-strand breaks (DSBs), that are highly damaging to the DNA, leading to cell death and genomic instability. DSBs are mainly repaired by the Non-Homologous End-Joining (NHEJ) process, in which DNA dependent protein kinase (DNA-PK) is the key complex. Consequently, specific DNA-PK inhibitors have been selected and evaluated for sensitizing cells to chemotherapy or radiotherapy. The choice of DNA-PK as a pharmacological target of interest in cancer treatment is discussed.

**Keywords:** DNA repair inhibition; DNA Double-strand break (DSB); DNA-dependent Protein Kinase (DNA-PK); Non-homologous end-joining (NHEJ)

## Introduction

Radiotherapy induces a variety of DNA damage including oxidized base damage, abasic sites, single-strand breaks (SSBs) and double-strand breaks (DSBs). This DNA damage, if unrepaired, triggers cell death through mitotic catastrophe and apoptosis. Amongst these lesions, DSBs are considered to be major actors in cell death [1]. Similarly to ionizing radiation, most untargeted antitumor drugs cause DNA damage that induces death signals in cancer cells as well as in normal cells. DNA lesions trigger a cell response through an interconnected network called the DNA damage response (DDR) that tends to maintain cell viability and genomic stability [2,3]. The DDR relies on a complex network of proteins that initiate and coordinate DNA repair activity by halting the cell cycle through the activation of checkpoints that block cells at the G1-S transition, the intra-S phase or the G2/M boundary [4]. When DNA repair fails, the DDR plays a key role in the induction of apoptosis. A defective DDR, for instance in the control of cell cycle blockage or in DNA repair processes, is commonly reported in many cancers and some cancer-prone human syndromes arise from defects in specific DDR and DNA repair genes [2].

Within the DDR, DNA damage repair determines the cell response, as illustrated with ionizing radiation [5,6]. Thus, an excess of DNA lesions or a specific localization of lesions in the genome may overcome the cell repair capacity and trigger cell death. In the case of DNA damage following radiotherapy, it has been largely documented that cell survival correlates with the number of DSBs in the genome [1]. Consequently, any increase or decrease in the repair capacity and/or signaling will lead to cell resistance or sensitivity, respectively. Based on such considerations, pharmaceutical companies have undertaken the development of new compounds aimed at modulating DDR processes and/or DNA repair, particularly DSB repair, after chemotherapy and radiotherapy, with the ultimate goal of sensitizing tumor cells to the treatment [7,8]. For example, inhibitors of the checkpoint kinases Chk1 and Chk2 have recently been shown to sensitize tumor cells to DNA damaging agents [9-12].

In translational research dealing with potential DNA repair proteins as pharmacological targets, the DNA dependent proteine kinase (DNA-PK), an heterotrimer comprising the regulatory subunit Ku70/Ku80 bound to the catalytic subunit DNA-PKcs, is of interest since its represents a major actor in DSBs repair. Cells deficient in Ku70/Ku80 or DNA-PKcs are sensitive to DSBs induced by IR or chemotherapeutic agents [13,14] supporting the idea that DNA-PK may represent a good target in cancer chemotherapy. We discuss here the rationale of this approach in the field of cancer treatment.

## Double strand break induction by radiotherapy/ chemotherapy and the biological consequences

DNA double strand breaks (DSBs) are considered the most severe DNA lesions: unrepaired DSBs can induce apoptosis or mitotic cell death or when repaired incorrectly, they can lead to carcinogenesis through mutagenic genome rearrangements [15]. DSBs are produced exogenously by ionizing radiation (IR) or chemicals, but also endogenously during DNA replication fork collapse or physiological processes such as V(D)J recombination and meiotic exchange [1].

Mainly through water molecule radiolysis, IR induces a plethora of DNA damage whose complexity increases with the value of the linear transfer energy (LET) [16,17]. DNA damage includes double-strand breaks (DSBs), single-strand breaks (SSBs), damaged bases and abasic sites located at a distance from each other when induced by low-LET irradiation. By contrast, high-LET provokes the formation of complex DNA damage within one or two DNA helical turns [18], although this value is under discussion since it has been reported recently that it could cover regions extending over several kilobases of the DNA molecule [19]. The biological consequences of complex DNA damage range from point mutations and loss of genetic material to cell death,

***Corresponding author:** Bernard Salles, DVM, PhD, INRA, Université de Toulouse III, UMR1331, Toxalim (Research Centre in Food Toxicology), F-31027 Toulouse, France, E-mail: bernard.salles@toulouse.inra.fr

**Received** October 17, 2011; **Accepted** December 01, 2011; **Published** December 03, 2011

**Citation:** Salles B, Calsou P, Mirey G (2011) DNA-PK, a Pharmacological Target in Cancer Chemotherapy and Radiotherapy? J Cancer Sci Ther S8:001. doi:10.4172/1948-5956.S8-001

due to repair impairment or repair-intermediate persistency. Clustered lesions induce intra- or interchromosomal insertions, and inversions, often in association with large deletions,- that appear to promote genome instability that may lead to carcinogenesis [19-21].

Antitumor chemotherapy has evolved from non-specific cytotoxic agents to targeted therapies, which are directed at unique molecular signatures of cancer cells to produce greater efficacy with less toxicity. However, untargeted drugs are still widely used and most of these compounds induce DNA damage directly or indirectly. Thus, DSBs arise from cell treatments with various anticancer agents such as (i) topoisomerase II inhibitors like anthracyclines and epipodophyllotoxins that trap the DNA–enzyme intermediate complex in a so-called cleavable complex; cellular processing of the cleavable complex converts the protein–DNA cleavable complex into DSBs [22]; (ii) topo I inhibitors like camptothecin that block the protein–DNA complex leading to single strand breaks converted into DSBs when the replication machinery encounters the lesion [23]; (iii) cross-linking bifunctional agents like cisplatin or chlorambucil that produce DSBs either during the repair processing of cross-links [24,25] or following replication fork collapse [26]; (iv) radiomimetic agents like enediynes that induce a low ratio of SSBs to DSBs (about 5:1 for neocarzinostatin, 2:1 for C-1027) unlike IR, which induces up to 100 SSBs for every DSB [27-31]. Overall, the induction of DNA breaks correlates with toxicity to the cells and cells usually respond to enediyne-induced damage as they do to IR-induced damage [32].

## DSD repair pathways

Whatever the origins of DSBs, such DNA damage can be repaired by two distinct and complementary mechanisms: the Homologous Recombination (HR) or the Non-Homologous End Joining (NHEJ) processes [33,34]. The activation of one or more of the three related phosphatidylinositol 3-kinase–like kinases (PI3KK) in response to DNA damage is required for the completion of the HR or NHEJ processes. While NHEJ allows fast but possibly error-prone repair during the entire cell cycle, the slower but high fidelity HR pathway is restricted to the S and G2 phases of the cell cycle. The PI3KK involved in DSB repair are ATM (Ataxia Telangiectasia Mutated) and ATR (ATM and Rad3-related) that are associated with HR and typically activated by DNA breaks or after replication fork collapse. The third one, the DNA protein kinase catalytic subunit, is involved in NHEJ that operates throughout the cell cycle in response to DSBs. Despite competition between HR and NHEJ during the S-G2 phases, it has recently been reported that DNA-PK was able to modulate HR activity through its phosphorylation status (more than 30 phosphosites have been determined in DNA-PKcs) [35]. These kinases belong to the family of transducer proteins that relay and amplify the damage signal to receptor proteins. A common substrate of the three PI3KKs mentioned above is the histone variant, H2AX, which is phosphorylated on serine 139 and subsequently called γ-H2AX. γ-H2AX has been widely used as a sensitive and early marker of DSBs in various areas including cancer research [36-38]. In line with its high sensitivity of detection (one DSB corresponds to one γ-H2AX focus determined by immunofluorescence) [39], it has been assumed that there is a direct relationship between γ-H2AX labeling and the existence of DSBs. However, since activation of PI3KK could occur in the absence of DSBs under certain circumstances [40-43], γ-H2AX labeling does not account solely for DSB occurrence. For instance, DNA-PK is activated in hypoxic cells independently of DNA breaks by a new mechanism relying on chromatin modifications [44].

## The Non-Homologous End-Joining Pathway

In mammalian cells, NHEJ is the predominant repair pathway for DSB repair which, throughout the cell cycle, ligates the two DNA ends together with minimal end processing [45,46]. NHEJ consists of at least two genetically and biochemically distinct sub-pathways (Figure 1): (i) a main canonical end-joining pathway (C-NHEJ) and (ii) an alternative NHEJ (A-NHEJ) or backup NHEJ (B-NHEJ) (hereafter referred to as A-NHEJ) [47-49].

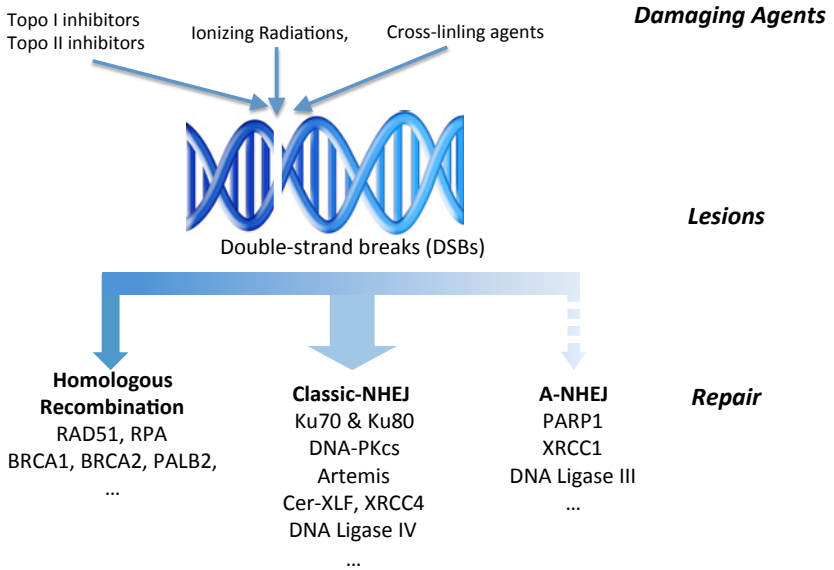


**Figure 1: DNA double-strand breaks damage and repair mechanisms.** In normal mammalian cells, the classic NHEJ (C-NHEJ) pathway is the major repair pathway, as Homologous Recombination needs the sister chromatid and preferentially takes place in the S/G2 phases. At the DSBs, C-NHEJ proceeds through the recruitment of Ku70/80 and DNA-PK catalytic subunit (cs), XLF, XRCC4 and DNA Ligase IV. Although A-NHEJ is a minor DSB repair pathway, it may take over in specific situations (e.g. when Ku is absent) therefore leading to error-prone repair of DNA-damage.

Since C-NHEJ is essential both for cell survival after IR treatment and V(D)J recombination, which generates the antibody and T cell receptor diversity required for lymphocyte maturation, cells from RS-SCID (radiosensitive-severe combined immunodeficiency) patients have helped to genetically define the NHEJ components [50]. C-NHEJ is a multi-step process involving several essential factors [51,52] (Figure 2).

The prerequisite event for all the subsequent steps is the binding of the Ku70/Ku80 heterodimer to DNA ends [53]. In the most recent model, drawn from live cell imaging following nuclear laser micro-irradiation experiments, the other core components of the reaction are then independently recruited to Ku-bound DSBs [54]. These include the DNA-PKcs subunit, Cernunnos-XLF (Cer-XLF) and the XRCC4/DNA Ligase IV (LIG4) complex, which is preassembled by a tight association between the two partners [55]. Multiple interactions then take place among these factors resulting in a stable assembly of the NHEJ machinery. As a result, the NHEJ complex associates more tightly with damaged sites and becomes resistant to biochemical extraction from the damaged chromatin, at least during the time of the repair [56-58]. The DNA-PK holoenzyme (Ku/DNA-PKcs) recognizes, protects and bridges the DNA-ends in addition to having a serine/threonine protein kinase activity [59]. DNAPK conformational change mediated by autophosphorylation is necessary for activation of end-processing enzymes, such as the Artemis nuclease [60]. Ligation requires the concerted action of LIG4, XRCC4 and Cer-XLF, the latter promoting readenylation of LIG4 [61]. The ligation complex also has a role upstream of the ligation reaction, since it stimulates processing of DNA ends [62,63]. At a later stage in the NHEJ process, this molecular machinery must be disassembled and released from the re-ligated DNA by a still unknown mechanism.

A-NHEJ is not a robust or a particularly important DSB repair pathway because it has been detected in the absence of C-NHEJ. A-NHEJ mechanistically results in deletions that are often accompanied by microhomology at the repair junction (for reviews, see [49,64,65]). A-NHEJ may also operate at telomeres in telomerase-deficient mouse cells [66] or following a defect in Ku or DNA-PKcs [67,68]. This pathway relies on factors different from those involved in the C-NHEJ route, such as poly (ADPribose) polymerase-1 (PARP-1), X-ray cross complementing factor 1 (XRCC1), DNA ligase III (LIG3), polynucleotide kinase, or Flap endonuclease 1 [48,69-73]. Our group and others have characterized some features of A-NHEJ using biochemical assays with cell extracts. It has been shown in vitro that Ku competes with PARP1 DNA end-binding, that PARP1 can carry out a synapsis activity thanks to short homology at the DNA ends - generally a few nucleotides- and that PARP1 activity is required for a subsequent XRCC1/LIG3 joining step favored by regions of microhomology [69,71,73,74]. More recently, the Mre11:Rad50:Nbs1 (MRN) complex has been implicated in A-NEHJ [75-81], but it is clear that additional factors await identification. A-NHEJ activity appears to be reduced in the plateau phase of growth, while no effect of the growth phase has been reported for C-NHEJ [82]. Established features of the A-NHEJ pathway are the following: (i) the kinetics of DSB repair appear slower than in C-NHEJ [69] and are enhanced in G2 [83]; (ii) it is repressed by Ku under normal conditions [69,84-89]; (iii) it relies preferentially on resection of the DNA ends and end annealing driven by microhomology > 4bp for intrachromosomal substrates [88-91], V(D)J junctions [92] or CSR joins [86,93], although this feature has been questioned in some reports [94].

This alternative pathway may be particularly relevant to genomic instability associated with tumor development. For example, frequent translocations lead to a high level of lymphomagenesis and other cancers in C-NHEJ deficient animal models [50,95]. In addition, chromosomal translocations, like those at the origin of leukemia, are mediated by a rejoining pathway described as Ku- and XRCC4/LIG4-independent [84,93,96,97].

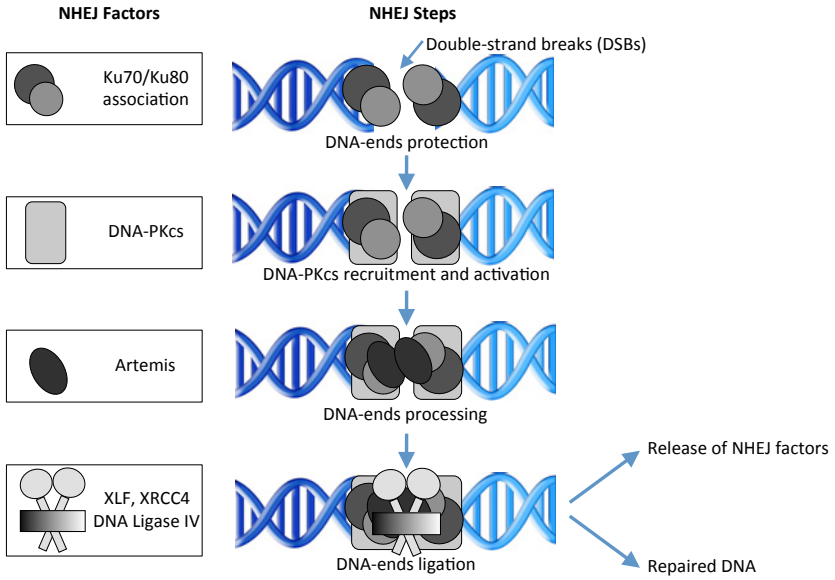


**Figure 2: NHEJ repair of DNA double-strand breaks (DSBs).** DSBs can be produced by endogenous or exogenous damaging agents or during physiological processes as V(D)J recombination. Classic-NHEJ proceeds through the recruitment of Ku70/80 heterodimer at the ends of the DSBs, followed by the DNAPK catalytic subunit (cs) recruitment and activation. The DNA damage processing involves Artemis, through its DNA-PK interaction, or the Mre11 protein. Finally, a DNA polymerase (as Pol μ, λ) may synthesize new DNA ends before the ligation step, involving XLF, XRCC4 and DNA Ligase IV. The NHEJ factors and the repaired DNA are then released.

## Structure-Function of The DNA-PK Repair Complex

The DNA-PK holoenzyme (Ku/DNA-PKcs) recognizes, protects and bridges the DNA ends. The Ku70/Ku80 heterodimer (Ku), present in the cell as a pre-assembled heterodimer, recognizes and binds the DNA ends of the DSB [53,98]. The recruitment of catalytic subunit DNA-PKcs occurs via the Ku80 C-terminal domain (Ku80-CTD). A truncated form of Ku70/Ku80 has been crystallized and shows a ringshaped form [98]. In addition, NMR studies of Ku80-CTD show a helical structure for the fragment comprising residues 592–709, although the extreme C-terminal portion of Ku80-CTD (residues 720–732) is disordered [99,100]. A structural model of the functions of the C-terminal domains in the context of the full-length Ku70/Ku80 protein has also been reported [101].

When bound to DNA-ends, Ku recruits the DNA-PKcs, which by itself has a weak protein kinase activity, strongly stimulated through the Ku interaction [102,103]. The phosphorylation occurs on an S/T-Q motif, although some serine/threonine in other sequences/targets could be phosphorylated [59,104]. The phosphorylation of Artemis may help to activate its endonuclease activity [60,105]. However, although DNA-PK also phosphorylates Ku, XRCC4 and Cer-XLF in the cell, mutational studies concluded that these phosphorylations are not functionally important, at least for NHEJ [59]. Thus, like ATM, DNA-PK may phosphorylate unknown substrates in vivo involved in processes other than DNA repair [106,107]. More likely, DNA-PKcs is the relevant target of its own enzymatic activity. Indeed, DNA-PKcs is autophosphorylated after ionizing radiation treatment [108,109]. Sixteen in vitro autophosphorylation sites in DNA-PKcs were identified and classified as two major clusters: the ABCDE cluster [110] and the PQR cluster [111,112]. A further autophosphorylation site was identified at Thr 3950, within the kinase domain, involved in the regulation of the kinase activity of DNA-PKcs [113]. It has been suggested that the structural plasticity of DNA-PK is highly affected by autophosphorylation at those two clusters [110,111]. Moreover, it was recently reported that there are more than 30 autophosphorylation sites within DNA-PKcs; a model was proposed in which phosphorylation induced conformational changes regulate the interaction of DNA-PKcs with its partners Ku and DNA [59,114]. This confirmed previous results showing that autophosphorylation of DNA-PKcs was a key event in the dissociation of DNA-PK from DNA [108,109,113-116]. On the other hand, biochemical studies on the mechanism of DNA-PK autophosphorylation indicate that it occurs in trans, both in vitro and in vivo [109].

Knowledge of the 3-D structure of DNA-PKcs contributes to a better understanding of its role in the NHEJ mechanism, illustrated for instance by the autophosphorylation reaction. However, structural studies of DNA-PKcs are challenging, due to its large size and poor recombinant protein production hence requiring complex purification from natural sources. Electron microscopy (EM) studies of the catalytic subunit DNAPKcs, a 469 kDa single-polypeptide chain, have produced a structure at 20Å resolution, defining the general architecture of DNA-PKcs into three main regions, namely a head, a palm and a connecting arm [117,118]. More recently, a 13Å resolution cryo-electron microscopy (cryo-EM) structure of DNA-PKcs revealed α-helices throughout the molecule and a model was proposed which localized the kinase domain in the head region [119,120]. Studies have shown that up to eightn repeats of the HEAT domain can fit into the cryoEM density model [120]. The HEAT domain (Huntington, Elongation factor 3, α regulatory subunit of PR65/A, TOR1) consists of repeats of 37 to 47 residues forming a rod-like helical structure.

In addition, DNA-PKcs has recently been crystallized with Ku80-CTD at 6.6Å, highlighting the overall topology and the formation of synaptic dimers [121,122]. Negative staining electron microscopy, single particle or X-ray analysis indicates that DNA-PKcs autophosphorylation induced significant conformational changes that were postulated to function as a DNA release mechanism [114,123,124].

Finally, the phosphorylated form of DNA-PKcs is a substrate for serine/threonine phosphatases that play a role in the DDR. The catalytic subunits of PP2A (PP2Ac), PP4 (PP4c), and PP6 (PP6c) belong to a subgroup referred to as the PP2A-like protein phosphatases (reviewed in [125]). Inhibition of PP2A-like protein phosphatases increases the phosphorylation status of DNA-PKcs and reduces its protein kinase activity [126]. In parallel, PP2A-like phosphatases (PP4 and PP2A) are involved in γ-H2AX dephosphorylation and have been shown to play a role in the DDR [127-130]. More recently, PP6 was reported to be recruited by DNA-PKcs to DSBs, a step involved in the regulation of dephosphorylation of γ-H2AX, the dissolution of IR-induced foci and the release from the G2/M checkpoint [131]. Thus, DNA-PKcs is involved in the recruitment of multiple protein phosphatases to DSB sites and might interact through the series of HEAT repeats [131].

## Inhibition of DNA-PK

Regardless of what the physiological substrates of DNA-PK are, the ability of small molecule inhibitors of DNA-PKcs to radiosensitize cells suggests that DNA-PK may be a good therapeutic target as a radiation sensitizer (reviewed in [132, 133]). In addition, DNA-PK has been implicated in the repair of chlorambucil-induced crosslinks, because increased DNA-PK activity in CLL cells correlates with clinical resistance to chlorambucil [134-137]. Furthermore, non-homologous end joining and DNA-PK activity are increased or upregulated in radioresistant compared with radiosensitive CLL cells.

Thus, the inhibition of NHEJ through DNA-PK may rely on different strategies (Figure 3): regulation of Ku or DNA-PKcs expression, inhibition of Ku/DNA-PKcs interaction, modulation of DNA-PKcs kinase activity, regulation of DNA-PKcs autophosphorylation, modulation of phosphatases activity. However, in the search for drugs usefull in therapy, almost all the research activity is being devoted to the specific inhibition of the kinase activity.

### Protein expression

RNA interference is being investigated as a therapeutic mechanism in the treatment of cancer, despite intrinsic problems like concentration, targeting to cancer cells and the metabolic stability of the miRNAs [138]. However, a decreased expression following RNAi treatment may not be sufficient to induce a strong phenotype, as reported for LIG3 and LIG4 functions [139]. A radiosensitizing effect has recently been reported, in vitro and in vivo, for miR-101 that targets DNA-PKcs and ATM via its binding to the 3'- UTR of DNA-PKcs or ATM mRNA [140]. In the case of Ku, in addition to the difficulty inherent in its very high level of expression and with pleiotropic localization and activities [141-148], targeting its expression does not appear to be of interest because it negatively controls the activity of the A-NHEJ mutagenic pathway [81].

### Ku/DNA-PKcs interaction

Since the C-terminal portion of Ku80-CTD that recruits DNA-PKcs is structurally disordered [99,100], the approach of drug design

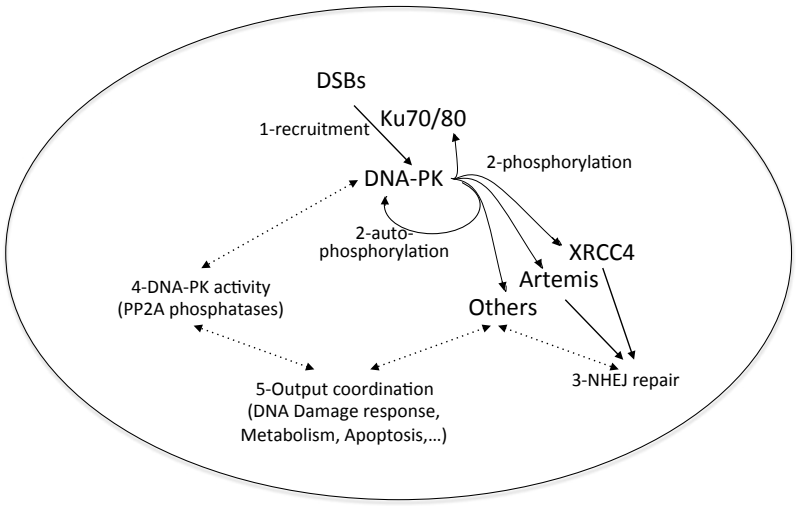


**Figure 3: DNA double-strand break repair and DNA-PK as pharmaceutical target.** Different steps may be used as targets in the NHEJ pathway. Recruitment of Ku70/Ku80 is essential for DNA-PK interaction (1) and activation; autophosphorylation of DNA-PK and phosphorylations of substrates (2) are involved in the repair process. Regulation of the DNA-PK activity is key and could be achieved via nphosphatases (4) or by inhibiting the previous steps. The output signals may coordinate different cell processes (5) such as the DNA damage response and some metabolic adaptations. (see text for details).

modelization is impractical. An indirect way has been developed by using short DNA molecules (Dbait) that mimic DSB in order to down regulate the kinase activity by competing with DNA-PKc [149]. Dbait molecules sensitize xenografted tumors to radiotherapy, not by inhibiting the kinase activity of DNA-PK, but by acting through the induction of "false" DNA damage signaling [149-151].

### DNA-PKcs kinase activity

Preliminary investigations of the inhibition of DNA-PK were undertaken by using wortmannin and LY294002, two nonselective PIKK inhibitors [152]. These drugs were shown to sensitize tumor cells to radiotherapy and chemotherapeutic agents and were used as a basis to develop more specific compounds. A flavone derivative, IC87361, led to tumor radiosensitization in both in vitro cell models and tumor xenograft in vivo models [133]. A more specific DNA-PK inhibitor, NU7026, has been reported to radiosensitize tumor cells [153]; similarly, NU7026 increased chlorambucil-sensitivity in CLL, correlated with DNA-PK inhibition and sensitization to chlorambucil [154]. Subsequently, a highly potent and selective DNA-PK inhibitor (NU7441) has been identified and showed an IC50 of 13nM [155]. NU7441 induced sensitization of CLL when treated with fludarabine and chlorambucil [156] or mitoxanthorone [157]. In addition, DNA-PKcs inhibitors synergize with irinotecan to improve the killing of colon cancer cell lines in vitro [158]. A number of other agents are currently in preclinical trials [159].

### DNA-PKcs autophosphorylation

A radiosensitizing effect of a single chain variable antibody fragment (scFv) against DNA-PKcs has been reported in vitro [160]. Very recently, epitopes in the autophosphorylation cluster domain were expressed as antigens to screen a phage antibody library. The selected antibody increased sensitivity to IR, decreased DSB repair capability along with decreased kinase activity and autophosphorylation on S2056 induced by radiation [161]. Another way to inhibit DNA-PKcs was developed by using a subtractive combinatorial selection to identify peptide ligands able to bind DNA-PKcs. A peptide was selected that specifically bound and non-competitively inactivated DNA-PKcs [162]. This peptide sensitizes BRCA-deficient tumor cells to genotoxic therapy.

### Phosphatase activity

Due to the role of DNA-PK in the PP2A-like phosphatase recruitment at the break site involved in the dephosphorylation step of DNA-PKcs itself and γ-H2AX turnoverthese proteins might be considered as pharmacological targets. However, various drawbacks could be raised such as the multiplicity of phosphatases, their lack of specificity and, as in the case of kinases, the difficulty in obtaining highly specific inhibitors.

## Discussion

DNA-PKcs is required for C-NHEJ, V(D)J recombination and telomere length maintenance but it has recently been shown to contribute to other pathways: (i) it is involved in the G2 checkpoint in response to IR [163]; (ii) it mediates metabolic gene activation in response to insulin [164]; (iii) it may also function outside DNA repair through phosphorylation of other substrates [165,166]). Also, and unexpectedly, the activation of cellular DDR pathways (ATM and DNA-PK) does not always require DNA damage but can be triggered by the stable association of single repair factors with chromatin [40]. Thus, hypoxia, by modifying higher-order chromatin structure and chromatin-remodeling complexes [167], triggers a DNA-PK-dependent DDR pathway. A key regulator of the cellular response to oxygen deprivation is the transcription factor, hypoxia-inducible factor 1 (HIF-1), whose function results in the induction of a plethora of target genes that collectively confer cellular adaptation to hypoxia [168]. Indeed, DNA-PK protects HIF-1α from degradation, indicating that DNA-PK controls the amplitude of HIF-1α accumulation under hypoxia [44]. These novel findings expand the cellular importance of DNA-PK [169] but paradoxically, compromise the therapeutic interest of its inhibition that may therefore induce side effects in uncharacterized metabolic networks.

In some cases, DNA-PK either shows variation in expression or is mutated in tumor cells. Despite a high level of expression of Ku and DNA-PKcs, an up-regulation of DNA-PKcs was reported in some tumors or IR-resistant cell lines, suggesting a role in tumor growth and survival [170-173]. Moreover, overexpression or increased activity of DNA-PKcs in various cancers is closely associated with metastases, poor prognosis and radioresistance [156,171,174,175]. Indeed, up-regulation of DNA-PK activity was shown to impair apoptosis in B-cell chronic lymphocytic leukemia [176]. Finally, in colorectal mismatch repair-deficient tumor cells (MSI), mutations in genes involved in DDR and DNA repair, including DNA-PKcs , have been reported [177]. Taken together, all these alterations in DNA-PK expression or activity suggest that the consequences of its inhibition should be useful against tumors. However, in tumor tissues, the expression of DNA-PK shows intratumor heterogeneity, suggesting difficulty in predicting the radio- or chemo-sensitivity of the tumor as well as when a DNA-PK inhibitor may be beneficial [174].

Strategies that block DNA repair will increase damage in the treated cells and result in increased cell death. Such approaches enhance sensitivity to treatment, although they do not provide selectivity against cancer cells as they increase the radiosensitivity or chemosensitivity of normal cells as well. Therefore the use of a DNA-PK inhibitor, in combination with genotoxic treatment, would allow the dose of irradiation or drug to be lowered without any gain in selectivity. It has recently been reported that monotherapy with DNA repair inhibitors could be successful with PARP inhibitors that can selectively kill BRCA1- and BRCA2-defective tumors [178,179], with promising results in phase II/III clinical studies [180,181]. The BRCA1 and BRCA2 genes encode large proteins that coordinate the homologous recombination DSB repair pathway [182]. Since BRCA1/2- deficient cells cannot repair DSBs by homologous recombination, PARP inhibitors will lead to the accumulation of DNA damage, genomic instability and cell death. Interestingly, these effects may rely on DNA-PK-dependent NHEJ activity [183]. This is the first example of a successful monotherapy, where the strategy is reminiscent of the synthetic lethality process [184-186]. Synthetic lethality is obtained when the simultaneous loss of two non-essential mutations results in cell death, which does not occur if either gene product is present and functional. Treatment of solid tumors partially deficient for DNA repair pathways opens a therapeutic window of opportunity. In contrast, for patients without inherited defects in DNA repair pathways, the combination of DNA repair inhibitors with genotoxic chemotherapy remains logical [187]. However, many tumor cells have specific genetic lesions, which could then be exploited by targeting synthetic lethal partner genes [188].

In the case of NHEJ inhibition, XRCC4/XLF/LigIV may be a better pharmacological target than DNA-PK itself, since the inhibition of the ligation step will not allow the ANHEJ pathway to proceed due to the remaining Ku binding to DNA. However, despite our knowledge of the structure of the ligation complex, inhibition of protein/protein interactions is a difficult task as is the specific inhibition of LigIV activity [55,58]. Indeed, most drugs bind at the biological sites of action and this implies, for a compound to be biologically active on LigIV, it must be similar to its endogenous ligand [189], that is in this case, the DNA molecule.

In conclusion, in cancer chemotherapy, the target is usually thought to be the tumor cells. Because of the lack of selectivity against the tumor cells, a recent alternative research field now favors the use of drugs directed against the tumor microenvironment. Results combining these two approaches are beginning to appear, for instance, in radiotherapy. Nonetheless, in the search for DNA-PK inhibitors more needs to be learnt about the structure of the repair complex, currently only understood at a low level of resolution that cannot help in drug design and docking approaches. Moreover, we need new insights on postranslational modifications –other than phosphorylations- of partners or substrates, on their eventual roles in metabolic pathways relevant to cell survival and adaptability, and on the coordinated manner in which they repair DSBs. Indeed, this may help in understanding the alternative pathways the tumor cells may find and avoid unpredictable outputs. Particularly, in the case of DSB repair, the A-NHEJ pathway should be bypassed; different strategies may help to reach this goal, such as synthetic lethality approaches or targeting downstream C-NHEJ effectors. Altogether, all these different possibilities indicate that integrated programs will be the key in the future.

## Acknowledgments

Work from the team “Radiobiology and DNA repair” at CNRS IPBS has been supported by grants from Ligue nationale contre le cancer (équipe labelisée), Electricité de France (EDF), Institut National du Cancer (INCA) and Cancéropole Grand-Sud Ouest. Bernard Salles and Gladys Mirey are Professor and Associate Professor at Faculty of Pharmacy and Patrick Calsou is a scientist from INSERM.

## References

1. Jeggo PA, Löbrich M (2007) DNA double-strand breaks: their cellular and clinical impact? Oncogene 26: 7717-7719.
2. Jackson SP, Bartek J (2009) The DNA-damage response in human biology and disease. Nature 461: 1071-1078.
3. Kastan MB (2008) DNA damage responses: mechanisms and roles in human disease: 2007 G.H.A. Clowes Memorial Award Lecture. Mol Cancer Res 6: 517-524.
4. Lobrich M, Jeggo PA (2007) The impact of a negligent G2/M checkpoint on genomic instability and cancer induction. Nat Rev Cancer 77: 861-869.
5. Jeggo P, Lavin MF (2009) Cellular radiosensitivity: how much better do we understand it? Int J Radiat Biol 85: 1061-1081.
6. O'Driscoll M, Jeggo PA (2006) The role of double-strand break repair - insights from human genetics. Nat Rev Genet 67: 45-54.
7. Begg AC, Stewart FA, Vens C (2011) Strategies to improve radiotherapy with targeted drugs. Nat Rev Cancer 11: 239-253.
8. Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA (2008) DNA repair pathways as targets for cancer therapy. Nat Rev Cancer 8: 193-204.
9. Guzi TJ, Paruch K, Dwyer MP, Labroli M, Shanahan F, et al. (2011) Targeting the replication checkpoint using SCH 900776, a potent and functionally selective CHK1 inhibitor identified via high content screening. Mol Cancer Ther 10: 591-602.
10. McNeely S, Conti C, Sheikh T, Patel H, Zabludoff S, et al. (2010) Chk1 inhibition after replicative stress activates a double strand break response mediated by ATM and DNA-dependent protein kinase. Cell Cycle 9: 995-1004.
11. Morgan MA, Parsels LA, Zhao L, Parsels JD, Davis MA, et al. (2010) Mechanism of radiosensitization by the Chk1/2 inhibitor AZD7762 involves abrogation of the G2 checkpoint and inhibition of homologous recombinational DNA repair. Cancer Res 70: 4972-4981.
12. Walton MI, Eve PD, Hayes A, Valenti M, De Haven Brandon A, et al. (2010) The preclinical pharmacology and therapeutic activity of the novel CHK1 inhibitor SAR-020106. Mol Cancer Ther 9: 89-100.
13. Ouyang H, Nussenzweig A, Kurimasa A, Soares VC, Li X, et al. (1997) Ku70 is required for DNA repair but not for T cell antigen receptor gene recombination *In vivo*. J Exp Med 186: 921-929.
14. Lees-Miller SP, Godbout R, Chan DW, Weinfeld M, Day RS 3rd, et al. (1995)

Absence of p350 subunit of DNA activated protein kinase from a radiosensitive human cell line. Science 267: 1183-1185.

15. Burma S, Chen BP, Chen DJ (1995) Role of non-homologous end joining (NHEJ) in maintaining genomic integrity. DNA Repair (Amst) 5: 1042-1048.
16. Goodhead DT (1994) Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. Int J Radiat Biol 65: 7-17.
17. Ward JF (1994) DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Prog Nucleic Acid Res Mol Biol 35: 95-125.
18. Blaisdell JO, Harrison L, Wallace SS (2001) Base excision repair processing of radiation induced clustered DNA lesions. Radiat Prot Dosimetry 97: 25-31.
19. Asaithamby A, Hu B, Chen DJ (2011) Unrepaired clustered DNA lesions induce chromosome breakage in human cells. Proc Natl Acad Sci U S A 108: 8293-8298.
20. Sedelnikova OA, Redon CE, Dickey JS, Nakamura AJ, Georgakilas AG, et al. (2010) Role of oxidatively induced DNA lesions in human pathogenesis. Mutat Res 704: 152-159.
21. Singleton BK, Griffin CS, Thacker J (2002) Clustered DNA damage leads to complex genetic changes in irradiated human cells. Cancer Res 62: 6263-6269.
22. Pommier Y, Leo E, Zhang H, Marchand C (2010) DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem Biol 17: 421-433.
23. Strumberg D, Pilon AA, Smith M, Hickey R, Malkas L, et al. (2000) Conversion of topoisomerase I cleavage complexes on the leading strand of ribosomal DNA into 5'-phosphorylated DNA double-strand breaks by replication runoff. Mol Cell Biol 20: 3977-3987.
24. Akkari YM, Bateman RL, Reifsteck CA, Olson SB, Grompe M (2000) DNA replication is required To elicit cellular responses to psoralen-induced DNA interstrand cross-links. Mol Cell Biol 20: 8283-8289.
25. De Silva IU, McHugh PJ, Clingen PH, Hartley JA (2000) Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. Mol Cell Biol 20: 7980-7990.
26. Räschle M, Knipscheer P, Enoiu M, Angelov T, Sun J, et al. (2008) Mechanism of replication-coupled DNA interstrand crosslink repair. Cell 134: 969-980.
27. Kennedy DR, Gawron LS, Ju J, Liu W, Shen B, et al. (2007) Single chemical modifications of the C-1027 enediyne core, a radiomimetic antitumor drug, affect both drug potency and the role of ataxia-telangiectasia mutated in cellular responses to DNA double-strand breaks. Cancer Res 67: 773-781.
28. Povirk LF (1996) DNA damage and mutagenesis by radiomimetic DNA-cleaving agents: bleomycin, neocarzinostatin and other enediynes. Mutat Res 355: 71-89.
29. Sutherland BM, Bennett PV, Sidorkina O, Laval J (2000) Clustered damages and total lesions induced in DNA by ionizing radiation: oxidized bases and strand breaks. Biochemistry 39: 8026-8031.
30. Van Lanen SG, Shen B (2008) Biosynthesis of enediyne antitumor antibiotics. Curr Top Med Chem 8: 448-459.
31. Xu YJ, Zhen YS, Goldberg IH (1994) C1027 chromophore, a potent new enediyne antitumor antibiotic, induces sequence-specific double-strand DNA cleavage. Biochemistry 33: 5947-5954.
32. Elmroth K, Nygren J, Mårtensson S, Ismail IH, Hammarsten O (2003) Cleavage of cellular DNA by calicheamicin gamma1. DNA Repair (Amst) 2: 363-374.
33. Mao Z, Bozzella M, Seluanov A, Gorbunova V (2008) DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. Cell Cycle 7: 2902-2906.
34. Shrivastav M, De Haro LP, Nickoloff JA (2008) Regulation of DNA double-strand break repair pathway choice. Cell Res 18: 134-147.
35. Neal JA, Dang V, Douglas P, Wold MS, Lees-Miller SP, et al. (2011) Inhibition of homologous recombination by DNA-dependent protein kinase requires kinase activity, is titratable, and is modulated by autophosphorylation. Mol Cell Biol 31: 1719-1733.
36. Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, et al. (2008) GammaH2AX and cancer. Nat Rev Cancer 8: 957-967.
37. Lord CJ, Ashworth A (2009) Bringing DNA repair in tumors into focus. Clin Cancer Res 15: 3241-3243.
38. Bañuelos CA, Banáth JP, Kim JY, Aquino-Parsons C, Olive PL (2009) GammaH2AX expression in tumors exposed to cisplatin and fractionated irradiation. Clin Cancer Res 15: 3344-3353.
39. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 273: 5858-5868.
40. Soutoglou E, Misteli T (2008) Activation of the cellular DNA damage response in the absence of DNA lesions. Science 320: 1507-1510.
41. Misteli T, Soutoglou E (2009) The emerging role of nuclear architecture in DNA repair and genome maintenance. Nat Rev Mol Cell Biol 10: 243-254.
42. Pospelova TV, Demidenko ZN, Bukreeva EI, Pospelov VA, Gudkov AV, et al. (2009) Pseudo-DNA damage response in senescent cells. Cell Cycle 8: 4112-4118.
43. Toledo LI, Murga M, Gutierrez-Martinez P, Soria R, Fernandez-Capetillo O (2008) ATR signaling can drive cells into senescence in the absence of DNA breaks. Genes Dev 22: 297-302.
44. Bouquet F, Ousset M, Biard D, Fallone F, Dauvillier S (2011) A DNA-dependent stress response involving DNA-PK occurs in hypoxic cells and contributes to cellular adaptation to hypoxia. J Cell Sci 124: 1943-1951.
45. Wyman C, Kanaar R (2006) DNA double-strand break repair: all's well that ends well. Annu Rev Genet 40: 363-383.
46. Pardo B, Gómez-González B, Aguilera A (2009) DNA repair in mammalian cells: DNA double-strand break repair: how to fix a broken relationship. Cell Mol Life Sci 66: 1039-1056.
47. Ma JL, Kim EM, Haber JE, Lee SE (2003) Yeast Mre11 and Rad1 proteins define a Kuindependent mechanism to repair double-strand breaks lacking overlapping end sequences. Mol Cell Biol 23: 8820-8828.
48. Wang H, Perrault AR, Takeda Y, Qin W, Wang H, et al. (2003) Biochemical evidence for Ku-independent backup pathways of NHEJ. Nucleic Acids Res 31: 5377-5388.
49. Nussenzweig A, Nussenzweig MC (2003) A backup DNA repair pathway moves to the forefront. Cell 131: 223-225.
50. Soulas-Sprauel P, Rivera-Munoz P, Malivert L, Le Guyader G, Abramowski V, et al. (2007) V(D)J and immunoglobulin class switch recombinations: a paradigm to study the regulation of DNA end-joining. Oncogene 26: 7780-7791.
51. Weterings E, Chen DJ (2008) The endless tale of non-homologous end-joining. Cell Res 18: 114-124.
52. Lieber MR (2010) The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem 79: 181-211.
53. Downs JA, Jackson SP (2004) A means to a DNA end: the many roles of Ku. Nat Rev Mol Cell Biol 5: 367-378.
54. Yano K, Morotomi-Yano K, Wang SY, Uematsu N, Lee KJ, et al. (2008) Ku recruits XLF to DNA double-strand breaks. EMBO Rep 9: 91-96.
55. Wu PY, Frit P, Meesala S, Dauvillier S, Modesti M, et al. (2009) Structural and functional interaction between the human DNA repair proteins DNA ligase IV and XRCC4. Mol Cell Biol 29: 3163-3172.
56. Drouet J, Delteil C, Lefrançois J, Concannon P, Salles B, et al. (2005) DNA-dependent protein kinase and XRCC4-DNA ligase IV mobilization in the cell in response to DNA double strand breaks. J Biol Chem 280: 7060-7069.
57. Drouet J, Frit P, Delteil C, de Villartay JP, Salles B, et al. (2006) Interplay between Ku, Artemis, and the DNA-dependent protein kinase catalytic subunit at DNA ends. J Biol Chem 281: 27784-27793.
58. Wu PY, Frit P, Malivert L, Revy P, Biard D, et al. (2007) Interplay between Cernunnos-XLF and nonhomologous end-joining proteins at DNA ends in the cell. J Biol Chem 282: 31937-31943.
59. Meek K, Dang V, Lees-Miller SP (2008) DNA-PK: the means to justify the ends? Adv Immunol 99: 33-58.

60. Goodarzi AA, Yu Y, Riballo E, Douglas P, Walker SA, et al. (2006) DNA-PK autophosphorylation facilitates Artemis endonuclease activity. EMBO J 25: 3880-3889.

61. Riballo E, Woodbine L, Stiff T, Walker SA, Goodarzi AA, et al. (2009) XLF-Cernunnos promotes DNA ligase IV-XRCC4 re-adenylation following ligation. Nucleic Acids Res 37: 482-492.

62. Budman J, Kim SA, Chu G (2007) Processing of DNA for nonhomologous end-joining is controlled by kinase activity and XRCC4/ligase IV. J Biol Chem 282: 11950-11959.

63. Akopiants K, Zhou RZ, Mohapatra S, Valerie K, Lees-Miller SP, et al. (2009) Requirement for XLF/Cernunnos in alignment-based gap filling by DNA polymerases lambda and mu for nonhomologous end joining in human whole-cell extracts. Nucleic Acids Res 37: 4055-4062.

64. Haber JE (2008) Alternative endings. Proc Natl Acad Sci USA 105: 405-406.

65. McVey M, Lee SE (2008) MMEJ repair of double-strand breaks (director's cut): deleted sequences and alternative endings. Trends Genet 24: 529-538.

66. Maser RS, Wong KK, Sahin E, Xia H, Naylor M, et al. (2007) DNA-dependent protein kinase catalytic subunit is not required for dysfunctional telomere fusion and checkpoint response in the telomerase-deficient mouse. Mol Cell Biol 27: 2253-2265.

67. Bombarde O, Boby C, Gomez D, Frit P, Giraud-Panis MJ, et al. (2010) TRF2/RAP1 and DNA-PK mediate a double protection against joining at telomeric ends. EMBO J 29: 1573-1584.

68. Riha K, Heacock ML, Shippen DE (2006) The role of the nonhomologous end-joining DNA double-strand break repair pathway in telomere biology. Annu Rev Genet 40: 237-277.

69. Wang M, Wu W, Wu W, Rosidi B, Zhang L, et al. (2006) PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. Nucleic Acids Res 34: 6170-6182.

70. Liang L, Deng L, Chen Y, Li GC, Shao C, et al. (2005) Modulation of DNA end joining by nuclear proteins. J Biol Chem 280: 31442-31449.

71. Audebert M, Salles B, Calsou P (2004) Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. J Biol Chem 279: 55117-55126.

72. Audebert M, Salles B, Weinfeld M, Calsou P (2006) Involvement of Polynucleotide Kinase in a Poly(ADP-ribose) Polymerase-1-dependent DNA Double-strand Breaks Rejoining Pathway. J Mol Biol 356: 257-265.

73. Wang H, Rosidi B, Perrault R, Wang M, Zhang L, et al. (2005) DNA ligase III as a candidate component of backup pathways of nonhomologous end joining. Cancer Res 65: 4020-4030.

74. Audebert M, Salles B, Calsou P (2008) Effect of double-strand break DNA sequence on the PARP-1 NHEJ pathway. Biochem Biophys Res Commun 369: 982-988.

75. Deng Y, Guo X, Ferguson DO, Chang S (2009) Multiple roles for MRE11 at uncapped telomeres. Nature 460: 914-918.

76. Deriano L, Stracker TH, Baker A, Petrini JH, Roth DB (2009) Roles for NBS1 in alternative nonhomologous end-joining of V(D)J recombination intermediates. Mol Cell 34: 13-25.

77. Dinkelmann M, Spehalski E, Stoneham T, Buis J, Wu Y, et al. (2009) Multiple functions of MRN in endjoining pathways during isotype class switching. Nat Struct Mol Biol 16: 808-813.

78. Rass E, Grabarz A, Plo I, Gautier J, Bertrand P, et al. (2009) Role of Mre11 in chromosomal nonhomologous end joining in mammalian cells. Nat Struct Mol Biol 16: 819-824.

79. Xie A, Kwok A, Scully R (2009) Role of mammalian Mre11 in classical and alternative nonhomologous end joining. Nat Struct Mol Biol 16: 814-818.

80. Zhuang J, Jiang G, Willers H, Xia F (2009) Exonuclease function of human Mre11 promotes deletional nonhomologous end joining. J Biol Chem 284: 30565-30573.

81. Cheng Q, Barboule N, Frit P, Gomez D, Bombarde O, et al. (2011) Ku counteracts mobilization of PARP1 and MRN in chromatin damaged with DNA double-strand breaks. Nucleic Acids Res [Epub ahead of print].

82. Singh SK, Wu W, Zhang L, Klammer H, Wang M, et al. (2011) Widespread dependence of backup NHEJ on growth state: ramifications for the use of DNA-PK inhibitors. Int J Radiat Oncol Biol Phys 79: 540-548.

83. Wu W, Wang M, Wu W, Singh SK, Mussfeldt T, et al. (2008) Repair of radiation induced DNA double strand breaks by backup NHEJ is enhanced in G2. DNA Repair (Amst) 7: 329-338.

84. Weinstock DM, Brunet E, Jasin M (2007) Formation of NHEJ-derived reciprocal chromosomal translocations does not require Ku70. Nat Cell Biol 9: 978-981.

85. Fattah F, Lee EH, Weisensel N, Wang Y, Lichter N, et al. (2010) Ku regulates the non-homologous end joining pathway choice of DNA double-strand break repair in human somatic cells. PLoS Genet 6: e1000855.

86. Boboila C, Yan C, Wesemann DR, Jankovic M, Wang JH, et al. (2010) Alternative end-joining catalyzes class switch recombination in the absence of both Ku70 and DNA ligase 4. J Exp Med 207: 417-427.

87. Mansour WY, Schumacher S, Rosskopf R, Rhein T, Schmidt-Petersen F, et al. (2008) Hierarchy of nonhomologous endjoining, single-strand annealing and gene conversion at site-directed DNA double-strand breaks. Nucleic Acids Res 36: 4088-4098.

88. 88. Guirouilh-Barbat J, Rass E, Plo I, Bertrand P, Lopez BS (2007) Defects in XRCC4 and KU80 differentially affect the joining of distal nonhomologous ends. Proc Natl Acad Sci USA 104: 20902-20907.

89. Schulte-Uentrop L, El-Awady RA, Schliecker L, Willers H, Dahm-Daphi J (2008) Distinct roles of XRCC4 and Ku80 in non-homologous end-joining of endonuclease- and ionizing radiation-induced DNA double-strand breaks. Nucleic Acids Res 36: 2561-2569.

90. Kuhfittig-Kulle S, Feldmann E, Odersky A, Kuliczkowska A, Goedecke W, et al. (2007) The mutagenic potential of non-homologous end joining in the absence of the NHEJ core factors Ku70/80, DNA-PKcs and XRCC4-LigIV. Mutagenesis 22: 217-233.

91. Guirouilh-Barbat J, Huck S, Bertrand P, Pirzio L, Desmaze C, et al. (2004) Impact of the KU80 pathway on NHEJ-induced genome rearrangements in mammalian cells. Mol Cell 14: 611-623.

92. Corneo B, Wendland RL, Deriano L, Cui X, Klein IA, et al. (2007) Rag mutations reveal robust alternative end joining. Nature 449: 483-486.

93. Yan CT, Boboila C, Souza EK, Franco S, Hickernell TR, et al. (2007) IgH class switching and translocations use a robust non-classical end-joining pathway. Nature 449: 478-482.

94. Mansour WY, Rhein T, Dahm-Daphi J (2010) The alternative end-joining pathway for repair of DNA double-strand breaks requires PARP1 but is not dependent upon microhomologies. Nucleic Acids Res 38: 6065-6077.

95. Rooney S, Chaudhuri J, Alt FW (2004) The role of the non-homologous end-joining pathway in lymphocyte development. Immunol Rev 200: 115-131.

96. Boboila C, Jankovic M, Yan CT, Wang JH, Wesemann DR, et al. (2010) Alternative end-joining catalyzes robust IgH locus deletions and translocations in the combined absence of ligase 4 and Ku70. Proc Natl Acad Sci USA 107: 3034-3039.

97. Simsek D, Jasin M (2010) Alternative end-joining is suppressed by the canonical NHEJ component Xrcc4-ligase IV during chromosomal translocation formation. Nat Struct Mol Biol 17: 410-416.

98. Walker JR, Corpina RA, Goldberg J (2001) Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. Nature 412: 607-614.

99. Zhang Z, Hu W, Cano L, Lee TD, Chen DJ, et al. (2004) Solution structure of the C-terminal domain of Ku80 suggests important sites for protein-protein interactions. Structure 12: 495-502.

100. Harris R, Esposito D, Sankar A, Maman JD, Hinks JA, et al. (2004) The 3D solution structure of the C-terminal region of Ku86 (Ku86CTR). J Mol Biol 335: 573-582.

101. Rivera-Calzada A, Spagnolo L, Pearl LH, Llorca O (2007) Structural model of full-length human Ku70-Ku80 heterodimer and its recognition of DNA and DNA-PKcs. EMBO Rep 8: 56-62.

102. DeFazio LG, Stansel RM, Griffith JD, Chu G (2002) Synapsis of DNA ends by DNA-dependent protein kinase. EMBO J 21: 3192-3200.

103. Hammarsten O, Chu G (1998) DNA-dependent protein kinase: DNA binding and activation in the absence of Ku. Proc Natl Acad Sci USA 95: 525-530.

104. Lees-Miller SP, Sakaguchi K, Ullrich SJ, Appella E, Anderson CW (1992) Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. Mol Cell Biol 12: 5041-5049.

105. Niewolik D, Pannicke U, Lu H, Ma Y, Wang LC, et al. (2006) DNA-PKcs dependence of Artemis endonucleolytic activity, differences between hairpins and 5' or 3' overhangs. J Biol Chem 281: 33900-33909.

106. Shiloh Y (2006) The ATM-mediated DNA-damage response: taking shape. Trends Biochem Sci 31: 402-410.

107. Lavin MF, Kozlov S (2007) ATM activation and DNA damage response. Cell Cycle 6: 931-942.

108. Chan DW, Lees-Miller SP (1996) The DNA-dependent protein kinase is inactivated by autophosphorylation of the catalytic subunit. J Biol Chem 271: 8936-8941.

109. Meek K, Douglas P, Cui X, Ding Q, Lees-Miller SP (2007) trans Autophosphorylation at DNA-dependent protein kinase's two major autophosphorylation site clusters facilitates end processing but not end joining. Mol Cell Biol 27: 3881-3890.

110. Douglas P, Sapkota GP, Morrice N, Yu Y, Goodarzi AA, et al. (2002) Identification of in vitro and in vivo phosphorylation sites in the catalytic subunit of the DNA-dependent protein kinase. Biochem J 368: 243-251.

111. Cui X, Yu Y, Gupta S, Cho YM, Lees-Miller SP, et al. (2005) Autophosphorylation of DNA-dependent protein kinase regulates DNA end processing and may also alter double-strand break repair pathway choice. Mol Cell Biol 25: 10842-10852.

112. Chen BP, Chan DW, Kobayashi J, Burma S, Asaithamby A, et al. (2005) Cell cycle dependence of DNA-dependent protein kinase phosphorylation in response to DNA double strand breaks. J Biol Chem 280: 14709-14715.

113. Douglas P, Cui X, Block WD, Yu Y, Gupta S, et al. (2007) The DNA-dependent protein kinase catalytic subunit is phosphorylated in vivo on threonine 3950, a highly conserved amino acid in the protein kinase domain. Mol Cell Biol 27: 1581-1591.

114. Hammel M, Yu Y, Mahaney BL, Cai B, Ye R, et al. (2010) Ku and DNA-dependent protein kinase dynamic conformations and assembly regulate DNA binding and the initial non-homologous end joining complex. J Biol Chem 285: 1414-1423.

115. Merkle D, Douglas P, Moorhead GB, Leonenko Z, Yu Y, et al. (2002) The DNA-dependent protein kinase interacts with DNA to form a protein-DNA complex that is disrupted by phosphorylation. Biochemistry 41: 12706-12714.

116. Uematsu N, Weterings E, Yano K, Morotomi-Yano K, Jakob B, et al. (2007) Autophosphorylation of DNA-PKCS regulates its dynamics at DNA double-strand breaks. J Cell Biol 177: 219-229.

117. Chiu CY, Cary RB, Chen DJ, Peterson SR, Stewart PL (1998) Cryo-EM imaging of the catalytic subunit of the DNA-dependent protein kinase. J Mol Biol 284: 1075-1081.

118. Leuther KK, Hammarsten O, Kornberg RD, Chu G (1999) Structure of DNA-dependent protein kinase: implications for its regulation by DNA. Embo J 18: 1114-1123.

119. Rivera-Calzada A, Maman JD, Spagnolo L, Pearl LH, Llorca O (2005) Three-dimensional structure and regulation of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). Structure 13: 243-255.

120. Williams DR, Lee KJ, Shi J, Chen DJ, Stewart PL (2008) Cryo-EM structure of the DNA-dependent protein kinase catalytic subunit at subnanometer resolution reveals alpha helices and insight into DNA binding. Structure 16: 468-477.

121. Sibanda BL, Chirgadze DY, Blundell TL (2010) Crystal structure of DNA-PKcs reveals a large open-ring cradle comprised of HEAT repeats. Nature 463: 118-121.

122. Ochi T, Sibanda BL, Wu Q, Chirgadze DY, Bolanos-Garcia VM, et al. (2010) Structural biology of DNA repair: spatial organization of the multicomponent complexes of nonhomologous end joining. J Nucleic Acids pii: 621695.

123. Dobbs TA, Tainer JA, Lees-Miller SP (2010) A structural model for regulation of NHEJ by DNA-PKcs autophosphorylation. DNA Repair (Amst) 9: 1307-1314.

124. Morris EP, Rivera-Calzada A, da Fonseca PC, Llorca O, Pearl LH, et al. (2011) Evidence for a remodelling of DNAPK upon autophosphorylation from electron microscopy studies. Nucleic Acids Res 39: 5757-5767.

125. Moorhead GB, Trinkle-Mulcahy L, Ulke-Lemée A (2007) Emerging roles of nuclear protein phosphatases. Nat Rev Mol Cell Biol 8: 234-244.

126. Douglas P, Moorhead GB, Ye R, Lees-Miller SP (2001) Protein phosphatases regulate DNA-dependent protein kinase activity. J Biol Chem 276: 18992-18998.

127. Nakada S, Chen GI, Gingras AC, Durocher D (2008) PP4 is a gamma H2AX phosphatase required for recovery from the DNA damage checkpoint. EMBO Rep 9:1019-1026.

128. Wang B, Zhao A, Sun L, Zhong X, Zhong J, et al. (2008) Protein phosphatase PP4 is overexpressed in human breast and lung tumors. Cell Res 18: 974-977.

129. Chowdhury D, Xu X, Zhong X, Ahmed F, Zhong J, et al. (2008) A PP4-phosphatase complex dephosphorylates gamma-H2AX generated during DNA replication. Mol Cell 31: 33-46.

130. Keogh MC, Kim JA, Downey M, Fillingham J, Chowdhury D, et al. (2006) A phosphatase complex that dephosphorylates gamma-H2AX regulates DNA damage checkpoint recovery. Nature 439: 497-501.

131. Douglas P, Zhong J, Ye R, Moorhead GB, Xu X, et al. (2010) Protein phosphatase 6 interacts with the DNA-dependent protein kinase catalytic subunit and dephosphorylates gamma-H2AX. Mol Cell Biol 30: 1368-1381.

132. O'Connor MJ, Martin NM, Smith GC (2007) Targeted cancer therapies based on the inhibition of DNA strand break repair. Oncogene 26: 7816-7824.

133. Shinohara ET, Geng L, Tan J, Chen H, Shir Y, et al. (2005) DNA-dependent protein kinase is a molecular target for the development of noncytotoxic radiation-sensitizing drugs. Cancer Res 65: 4987-4992.

134. Muller C, Dusseau C, Calsou P, Salles B (1998) Human normal peripheral blood B-lymphocytes are deficient in DNA-dependent protein kinase activity due to the expression of a variant form of the Ku86 protein. Oncogene 16: 1553-1560.

135. Muller C, Christodoulopoulos G, Salles B, Panasci L (1998) DNA-Dependent protein kinase activity correlates with clinical and in vitro sensitivity of chronic lymphocytic leukemia lymphocytes to nitrogen mustards. Blood 92: 2213-2219.

136. Muller C, Calsou P, Salles B (2000) The activity of the DNA-dependent protein kinase (DNAPK) complex is determinant in the cellular response to nitrogen mustards. Biochimie 82: 25-28.

137. Christodoulopoulos G, Muller C, Salles B, Kazmi R, Panasci L (1998) Potentiation of chlorambucil cytotoxicity in B-cell chronic lymphocytic leukemia by inhibition of DNA-dependent protein kinase activity using wortmannin. Cancer Res 58: 1789-1792.

138. Weidhaas JB, Babar I, Nallur SM, Trang P, Roush S, et al. (2007) MicroRNAs as potential agents to alter resistance to cytotoxic anticancer therapy. Cancer Res 67: 11111-11116.

139. Windhofer F, Wu W, Iliakis G (2007) Low levels of DNA ligases III and IV sufficient for effective NHEJ. J Cell Physiol 213: 475-483.

140. Yan D, Ng WL, Zhang X, Wang P, Zhang Z, et al. (2010) Targeting DNA-PKcs and ATM with miR-101 sensitizes tumors to radiation. PLoS One 5: e11397.

141. Takeda AA, de Barros AC, Chang CW, Kobe B, Fontes MR (2011) Structural Basis of Importin-alpha- Mediated Nuclear Transport for Ku70 and Ku80. J Mol Biol 412: 226-234.

142. Ohno M, Kunimoto M, Nishizuka M, Osada S, Imagawa M (2009) Ku proteins function as corepressors to regulate farnesoid X receptor-mediated gene expression. Biochem Biophys Res Commun 390: 738-742.

143. Kim E, Li K, Lieu C, Tong S, Kawai S, et al. (2008) Expression of apolipoprotein C-IV is regulated by Ku antigen/peroxisome proliferator-activated receptor gamma complex and correlates with liver steatosis. J Hepatol 49: 787-798.

144. Bednarek R, Boncela J, Smolarczyk K, Cierniewska-Cieslak A, Wyroba E, et al. (2008) Ku80 as a novel receptor for thymosin beta4 that mediates its intracellular activity different from G-actin sequestering. J Biol Chem 283: 1534-1544.

145. Shi L, Qiu D, Zhao G, Corthesy B, Lees-Miller S, et al. (2007) Dynamic binding of Ku80, Ku70 and NF90 to the IL-2 promoter in vivo in activated T-cells. Nucleic Acids Res 35: 2302-2310.

146. Munakata Y, Saito-Ito T, Kumura-Ishii K, Huang J, Kodera T, et al. (2005) Ku80 autoantigen as a cellular coreceptor for human parvovirus B19 infection. Blood 106: 3449-3456.

147. Muller C, Paupert J, Monferran S, Salles B (2005) The double life of the Ku protein: facing the DNA breaks and the extracellular environment. Cell Cycle 4: 438-441.

148. Monferran S, Paupert J, Dauvillier S, Salles B, Muller C (2004) The membrane form of the DNA repair protein Ku interacts at the cell surface with metalloproteinase 9. EMBO J 23: 3758-3768.

149. Quanz M, Berthault N, Roulin C, Roy M, Herbette A (2009) Small-molecule drugs mimicking DNA damage: a new strategy for sensitizing tumors to radiotherapy. Clin Cancer Res 15: 1308-1316.

150. Quanz M, Chassoux D, Berthault N, Agrario C, Sun JS, et al. (2009) Hyperactivation of DNA-PK by double-strand break mimicking molecules disorganizes DNA damage response. PLoS One 4: e6298.

151. Berthault N, Maury B, Agrario C, Herbette A, Sun JS, et al. (2011) Comparison of distribution and activity of nanoparticles with short interfering DNA (Dbait) in various living systems. Cancer Gene Ther 18: 695-706.

152. Rosenzweig KE, Youmell MB, Palayoor ST, Price BD (1997) Radiosensitization of human tumor cells by the phosphatidylinositol3-kinase inhibitors wortmannin and LY294002 correlates with inhibition of DNA-dependent protein kinase and prolonged G2-M delay. Clin Cancer Res 3: 1149-1156.

153. 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: 6008-6015.

154. Amrein L, Loignon M, Goulet AC, Dunn M, Jean-Claude B, et al. (2007) Chlorambucil cytotoxicity in malignant B lymphocytes is synergistically increased by 2-(morpholin-4-yl)-benzo[h]chomen-4-one (NU7026)-mediated inhibition of DNA double-strand break repair via inhibition of DNA-dependent protein kinase. J Pharmacol Exp Ther 321: 848-855.

155. Zhao Y, Thomas HD, Batey MA, Cowell IG, Richardson CJ, et al. (2006) Preclinical evaluation of a potent novel DNA-dependent protein kinase inhibitor NU7441. Cancer Res 66: 5354-5362.

156. Willmore E, Elliott SL, Mainou-Fowler T, Summerfield GP, Jackson GH, et al. (2008) DNA-dependent protein kinase is a therapeutic target and an indicator of poor prognosis in B-cell chronic lymphocytic leukemia. Clin Cancer Res 14: 3984-3992.

157. Elliott SL, Crawford C, Mulligan E, Summerfield G, Newton P, et al. (2011) Mitoxantrone in combination with an inhibitor of DNA-dependent protein kinase: a potential therapy for high risk B-cell chronic lymphocytic leukaemia. Br J Haematol 152: 61-71.

158. Davidson D, Coulombe Y, Martinez-Marignac VL, Amrein L, Grenier J, et al. (2011) Irinotecan and DNA-PKcs inhibitors synergize in killing of colon cancer cells. Invest New Drugs [Epub ahead of print].

159. Martin SA, Lord CJ, Ashworth A (2008) DNA repair deficiency as a therapeutic target in cancer. Curr Opin Genet Dev 18: 80-86.

160. Li S, Takeda Y, Wragg S, Barrett J, Phillips A, et al. (2003) Modification of the ionizing radiation response in living cells by an scFv against the DNA-dependent protein kinase. Nucleic Acids Res 31: 5848-5857.

161. Du L, Zhou LJ, Pan XJ, Wang YX, Xu QZ, et al. (2010) Radiosensitization and growth inhibition of cancer cells mediated by an scFv antibody gene against DNA-PKcs in vitro and in vivo. Radiat Oncol 5: 70.

162. Moeller BJ, Sidman RL, Pasqualini R, Arap W (2011) Discovery of DNA repair inhibitors by combinatorial library profiling. Cancer Res 71: 1816-1824.

163. Shang ZF, Huang B, Xu QZ, Zhang SM, Fan R, et al. (2010) Inactivation of DNA-dependent protein kinase leads to spindle disruption and mitotic catastrophe with attenuated checkpoint protein 2 Phosphorylation in response to DNA damage. Cancer Res 70: 3657-3666.

164. Wong RH, Chang I, Hudak CS, Hyun S, Kwan HY, et al. (2009) A role of DNA-PK for the metabolic gene regulation in response to insulin. Cell 136: 1056-1072.

165. Britton S, Froment C, Frit P, Monsarrat B, Salles B, et al. (2009) Cell nonhomologous end joining capacity controls SAF-A phosphorylation by DNA-PK in response to DNA double-strand breaks inducers. Cell Cycle 8: 3717-3722.

166. Shi M, Vivian CJ, Lee KJ, Ge C, Morotomi-Yano K, et al. (2009) DNA-PKcs-PIDDosome: a nuclear caspase-2-activating complex with role in G2/M checkpoint maintenance. Cell 136: 508-520.

167. Johnson AB, Denko N, Barton MC (2008) Hypoxia induces a novel signature of chromatin modifications and global repression of transcription. Mutat Res 640: 174-179.

168. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3: 721-732.

169. Kong X, Shen Y, Jiang N, Fei X, Mi J (2011) Emerging roles of DNA-PK besides DNA repair. Cell Signal 23: 1273-1280.

170. Söderlund Leifler K, Queseth S, Fornander T, Askmalm MS (2010) Low expression of Ku70/80, but high expression of DNA-PKcs, predict good response to radiotherapy in early breast cancer. Int J Oncol 37: 1547-1554.

171. Beskow C, Skikuniene J, Holgersson A, Nilsson B, Lewensohn R, et al. (2009) Radioresistant cervical cancer shows upregulation of the NHEJ proteins DNA-PKcs, Ku70 and Ku86. Br J Cancer 101: 816-821.

172. Hosoi Y, Watanabe T, Nakagawa K, Matsumoto Y, Enomoto A, et al. (2004) Up-regulation of DNA-dependent protein kinase activity and Sp1 in colorectal cancer. Int J Oncol 25: 461-468.

173. Ader I, Muller C, Bonnet J, Favre G, Cohen-Jonathan E, et al. (2002) The radioprotective effect of the 24 kDa FGF-2 isoform in HeLa cells is related to an increased expression and activity of the DNA dependent protein kinase (DNA-PK) catalytic subunit. Oncogene 21: 6471-6479.

174. Tonotsuka N, Hosoi Y, Miyazaki S, Miyata G, Sugawara K, et al. (2006) Heterogeneous expression of DNA-dependent protein kinase in esophageal cancer and normal epithelium. Int J Mol Med 18: 441-447.

175. Shintani S, Mihara M, Li C, Nakahara Y, Hino S, et al. (2003) Up-regulation of DNA-dependent protein kinase correlates with radiation resistance in oral squamous cell carcinoma. Cancer Sci 94: 894-900.

176. Deriano L, Guipaud O, Merle-Béral H, Binet JL, Ricoul M, et al. (2005) Human chronic lymphocytic leukemia B cells can escape DNA damage-induced apoptosis through the nonhomologous end-joining DNA repair pathway. Blood 105: 4776-4783.

177. Miquel C, Jacob S, Grandjouan S, Aimé A, Viguier J, et al. (2007) Frequent alteration of DNA damage signalling and repair pathways in human colorectal cancers with microsatellite instability. Oncogene 26: 5919-5926.

178. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, et al. (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434: 913-917.

179. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, et al. (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434: 917-921.

180. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, et al. (2009) Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 361: 123-134.

181. Fong PC, Yap TA, Boss DS, Carden CP, Mergui-Roelvink M, et al. (2010) Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol 28: 2512-2519.

182. Carden CP, Yap TA, Kaye SB (2010) PARP inhibition: targeting the Achilles' heel of DNA repair to treat germline and sporadic ovarian cancers. Curr Opin Oncol 22: 473-480.

183. Patel AG, Sarkaria JN, Kaufmann SH (2011) Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. Proc Natl Acad Sci USA 108: 3406-3411.

184. Helleday T (2010) Homologous recombination in cancer development, treatment and development of drug resistance. Carcinogenesis 31: 955-960.

185. Chan N, Pires IM, Bencokova Z, Coackley C, Luoto KR, et al. (2010) Contextual synthetic lethality of cancer cell kill based on the tumor microenvironment. Cancer Res 70: 8045-8054.

186. Helleday T (2011) The underlying mechanism for the PARP and BRCA synthetic lethality: clearing up the misunderstandings. Mol Oncol 5: 387-393.

187. Bolderson E, Richard DJ, Zhou BB, Khanna KK (2009) Recent advances in cancer therapy targeting proteins involved in DNA double-strand break repair. Clin Cancer Res 15: 6314-6320.

188. Yap TA, Sandhu SK, Carden CP, de Bono JS (2011) Poly(ADP-ribose) polymerase (PARP) inhibitors: Exploiting a synthetic lethal strategy in the clinic. CA Cancer J Clin 61: 31-49.

189. Fauman EB, Rai BK, Huang ES (2011) Structure-based druggability assessment--identifying suitable targets for small molecule therapeutics. Curr Opin Chem Biol 15: 463-468.

This article was originally published in a special issue, **DNA Damage and Repair in Cancer Therapy** handled by Editor(s). Dr. Samy L Habib, The University of Texas, USA