TARGETING DNA REPAIR IN CANCER: BEYOND PARP INHIBITORS

Jessica S. Brown[1], Brent O'Carrigan[1], Stephen P. Jackson[2,3] and Timothy A. Yap[1,4]

[1]Royal Marsden NHS Foundation Trust, London SM2 5PT, UK

[2]The Wellcome Trust/Cancer Research UK Gurdon Institute and Department of Biochemistry, University of Cambridge, Cambridge CB2 1QN, UK.

[3]The Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK.

[4]The Institute of Cancer Research, London SM2 5NG, UK

Running title: Targeting DNA repair in cancer.

Key words: DNA repair, DNA damage response, DDR, PARP, DNAPK, ATM, ATR, CHK1, WEE1.

***Corresponding author:**

Dr Timothy A Yap

Clinician Scientist and Consultant Medical Oncologist

Drug Development Unit

Royal Marsden NHS Foundation Trust and The Institute of Cancer Research

Downs Road, London SM2 5PT, United Kingdom.

Tel: 44-20-8661-3539

Fax: 44-20-8642-7979

E-mail: timothy.yap@icr.ac.uk

Conflict of interest:

TAY has received research support from AstraZeneca and Vertex, travel support from Vertex and Merck, and has served on Advisory Boards of Clovis Oncology, Pfizer and Bristol-Myers Squibb. JSB, BO and SPJ have no disclosures to declare.

ABSTRACT

Germline aberrations in critical DNA repair and DNA-damage response (DDR) genes cause cancer predisposition, while various tumors harbor somatic mutations causing defective DDR/DNA repair. The concept of synthetic lethality can be exploited in such malignancies, as exemplified by approval of poly(ADP-ribose) polymerase inhibitors for treating *BRCA1/2* mutated ovarian cancers. Herein, we detail how cellular DDR processes engage various proteins that sense DNA damage, initiate signaling pathways to promote cell cycle checkpoint activation, trigger apoptosis and coordinate DNA repair. We focus on novel therapeutic strategies targeting promising DDR targets and discuss challenges of patient selection and the development of rational drug combinations.

SIGNIFICANCE: Various inhibitors of DDR components are in preclinical and clinical development. A thorough understanding of DDR pathway complexities must now be combined with strategies and lessons learnt from the successful registration of PARP inhibitors in order to fully exploit the potential of DDR inhibitors and to ensure their long-term clinical success.

INTRODUCTION

Failure to accurately repair damaged DNA in cells manifests in various clinical phenotypes, including neurodegeneration, infertility, immunodeficiencies and cancer susceptibility (1,2). Furthermore, the production of DNA damage in cells following exposure to carcinogens increases cancer risk (3). Germline mutations in genes encoding key players in the DNA damage response (DDR), including *BRCA1, BRCA2, BLM, FANCA, TP53, RAD51C* and *MSH2*, result in cancer susceptibility syndromes (1), in part because failure to adequately protect the genome against endogenous and exogenous sources of DNA damage results in the accumulation of oncogenic mutations. Genomic instability is therefore a recognised hallmark of cancer (3).

Cancer cells often harbor a reduced repertoire of DNA repair and DNA-damage signalling capabilities compared to normal cells, and in some cases cancers also upregulate mutagenic repair pathways that drive oncogenesis (4). Consequently, cancer cells are often more reliant on a subset of repair pathways and are therefore more susceptible to DDR inhibition than are normal cells that maintain full DNA repair/DDR capacity. Faulty cell cycle checkpoint activation and suboptimal DNA repair capability in cancer cells also results in replication stress and subsequent accumulation of DNA damage in tumors. In addition, cancer cells often have dysfunctional redox homeostasis and therefore rely heavily on mechanisms that repair oxidative DNA damage, as well as on enzymes that counteract the incorporation of oxidised DNA precursors into genomic DNA (5,6). Both replication and oxidative stress, as well as other processes such as telomere attrition, provide

a background of ongoing DNA damage in cancer cells that can provide potential therapeutic windows for compounds that exacerbate these processes. Such compounds may achieve this by stressing replication further, impairing the ability of cancer cells to handle high levels of replicative or oxidative pressures, or potentially inhibiting DNA repair and associated processes (5–7). Such issues have led to intense interest in the therapeutic development of specific inhibitors of a range of components of the DDR network, several of which are now in clinical testing (8).

A well-recognised sensor of DNA damage is the protein poly(ADP-ribose) polymerase (PARP), which is best known for its role in DNA base excision repair (BER) and repair of DNA single-strand breaks (SSBs; **Table 1 + Figure 1**) (9)**,** although it also has a less well-defined role in DNA double-strand break (DSB) repair by alternative non-homologous end-joining (alt-NHEJ; **Table 1 + Figure 1)** (10). The clinical development of PARP inhibitors in patients with germline *BRCA1/2* mutations stemmed from the robust pre-clinical data that demonstrated exquisite sensitivity of *BRCA1/2* mutant cells and tumors to PARP inhibition (11,12). It was correctly hypothesised that in these patients, the cancer cells (in which both alleles of either *BRCA1* or *BRCA2* have been mutated or deleted) would depend on PARP activity for survival, whereas normal cells (that maintain a fully functional copy of *BRCA1* or *BRCA2*) would not. While these findings were initially thought to be due to a reliance of *BRCA1/2* mutant cells on SSB-repair for survival, it has since become well-recognised that PARP trapping and the subsequent generation of replication-dependent DSBs also contributes significantly to the synthetic

lethal relationship between PARP and BRCA. In addition, the PARP family of enzymes plays key roles in multiple cellular processes beyond DNA repair, including cellular differentiation, gene transcription, inflammation, mitosis, cell death and metabolism, which may contribute to the antitumor activity of PARP inhibitors (13,14). Synthetic lethality between repair pathways has provided a paradigm for many current clinical strategies targeting DNA repair/DDR. Detailed reviews of the underlying mechanisms-of-action and clinical applications of PARP inhibitors have previously been published and are beyond the scope of this article (15–19).

In this review, we summarise the current status with PARP inhibitors and then look beyond these, focusing on other protein components of the cellular DDR, which includes proteins that sense DNA damage, initiate signaling pathways that promote cell cycle checkpoint activation and coordinate the repair of damaged DNA with various other cellular processes. We highlight how such DDR enzymes represent rational targets for the discovery of novel therapeutics **(Figure 1)** and detail the development and future potential of such compounds. We also discuss the numerous challenges of discovering predictive biomarkers of response for optimal patient selection and the development of promising DDR combinations, including molecularly-targeted agents, compounds inhibiting epigenetic targets and immune checkpoint inhibitors.

DRUG TARGETING OF DNA DAMAGE SENSOR PROTEINS

DDR sensor proteins detect the region of damaged DNA and direct ensuing cellular responses that include activation of one or more repair pathways. For DSBs, Ku (comprising the Ku70/Ku80 protein heterodimer) and MRN (MRE11-RAD50-NBS1) are the predominant sensor protein complexes. Ku binds DNA DSBs within seconds of them being generated, and serves as a platform for the subsequent recruitment of classical non-homologous end-joining (NHEJ) proteins. The MRN complex plays key roles in triggering activation of the DNA-damage signalling kinase ATM, the initiation of DNA end-resection, and promotion of repair by HR. Chromatin context, transcriptional status, cell cycle stage and extent of end-resection are all factors that contribute to the selection of DNA repair, either by HR or NHEJ, and the mechanisms determining the choice of DNA repair pathway is an intense area of active research (20,21). Other DNA-damage sensors include components of the Fanconi anemia core complex (FANCA, B, C, E, F, G, L and M), mismatch repair proteins (MSH2, MSH3, MSH6, MLH1 and PMS2) and nucleotide excision repair proteins (XPC, DDB2 and CSA), which are sensors of DNA inter-strand crosslinks, base-base mismatches or insertion-deletion loops and UV-induced photo-lesions (in particular cyclobutane pyrimidine dimers and pyrimidine 6-4 pyrimidone photoproducts), respectively **(Table 1)**.

There are 17 PARP family members, of which PARP-1 has the predominant role in DNA repair, with PARP-2 and to a lesser extent PARP-3 functioning in fewer, but overlapping DNA repair processes (22). Through binding to single-

stranded DNA breaks (SSBs), DNA nicks or DSBs, PARP catalytic function is activated to generate extensive poly(ADP-ribose) chains (PAR chains) on itself and proteins in the vicinity of DNA damage. These PAR chains and PARP itself then promote the recruitment of critical SSB repair proteins, such as XRCC1 to SSBs and modify chromatin structure to facilitate DNA repair (9). PARP auto-PARylation is also required for the dissociation of PARP from DNA-damage sites (23,24). Enzymatic inhibition by PARP inhibitors therefore results in both the suppression of SSB repair and BER, which molecularly converges with SSB repair in its downstream stages. Inhibition also results in the trapping of PARP to SSBs, causing the stalling and subsequent collapse of DNA replication forks, resulting in replication-dependent DNA DSBs (23,24). Such DSBs would normally be repaired by HR; however in HR deficient cells, such as *BRCA1/2* mutant tumors, less effective and lower fidelity methods of repair are utilised, which result in an unsustainable levels of damage, chromosomal fusions/translocations and ultimately cell death (20,25).

Following strong preclinical findings predicting a therapeutic rationale, early clinical trials assessing the PARP inhibitor olaparib (Lynparza; AstraZeneca) demonstrated multiple durable antitumor responses in patients with advanced germline *BRCA1/2* mutated ovarian, breast or castration resistant prostate cancer (CRPC) (26,27). This patient benefit was confirmed in later-phase clinical trials (28–31) eventually leading to clinical registration. Olaparib is now approved by the European Medicines Agency (EMA) as maintenance therapy for responding patients with *BRCA1/2* mutant ovarian cancer following platinum-based chemotherapy. It was also granted accelerated approval by

the US Food and Drug Administration (FDA) for use in patients with advanced *BRCA1/2* mutant ovarian cancers, while confirmatory trials are being completed. Most recently, olaparib was given breakthrough therapy designation for treatment of *BRCA1/2* or *ATM* gene mutated metastatic CRPC in patients who have received a prior taxane-based chemotherapy and at least one newer hormonal agent. Another potent PARP inhibitor, rucaparib (Clovis), has also recently been granted breakthrough therapy status by the FDA following the results of the Phase II ARIEL2 trial (32) for use as monotherapy in patients with *BRCA1/2* mutant (germline or somatic) advanced ovarian cancer after at least two prior lines of platinum-containing therapies (33). There are additional potent and selective PARP inhibitors in late phase monotherapy and combination clinical trial development, including niraparib (MK4827; Tesaro), talazoparib (BMN673; Medivation) and veliparib (ABT-888; Abbvie) **(Table 2)**.

Although PARP inhibition is undeniably an effective treatment for *BRCA1/2* mutated cancers, with response rates in the region of 50% for platinum-sensitive ovarian cancers (33), the overwhelming majority of patients will ultimately develop tumor resistance. Genetic reversion events that restore *BRCA1/2* gene function have been identified in PARP inhibitor resistant cell lines, platinum-resistant patient-derived cell lines and tumors from patients that have developed clinical resistance to PARP inhibitors (34). In addition, in *Brca1-null* mouse embryonic stem cells, loss of DDR factors such as 53BP1 at least partially rescues HR by removing a barrier to DNA end-resection (35,36), although this has yet to be rigorously identified as a resistance

mechanism in the clinic. A number of critical challenges therefore remain to optimise the clinical efficacy and widen the utility of PARP inhibitors. Identifying mechanisms of PARP inhibitor resistance remains a critical challenge; others include the development of promising PARP inhibitor combination regimens and the analytical validation of clinically meaningful predictive biomarker assays to identify HR-deficient tumors caused by *BRCA1/2* mutations or by other mechanisms (33).

TARGETING OF DNA DAMAGE SIGNALLING PROTEINS

By triggering various protein post-translational modifications and promoting the assembly of protein complexes, DDR signalling proteins amplify and diversify the damage signal within a cell and coordinate the most appropriate cellular responses, including transcriptional changes, cell cycle checkpoint activation, alternative splicing, engagement of DNA repair processes, or in the context of overwhelming damage, activation of cell senescence or apoptotic pathways (1). DNA DSB signalling events are largely coordinated by the apical phosphatidylinositol 3-kinase-related kinases (PIKKs) DNA-PKcs (DNA-dependent serine/threonine protein kinase catalytic subunit), ATM (ataxia telangiectasia mutated), and ATR (ataxia telangiectasia and Rad3-related protein;

Figure 2).

DNA-PK

DNA-PKcs activity is essential for effective repair by classical NHEJ, which is the predominant DNA repair pathway of DSBs in human cells, occurring through all phases of the cell cycle **(**

Figure 2**A)**. DNA-PK is composed of Ku plus a ~460 kDa catalytic subunit (DNA-PKcs), the activity of which is dependent on Ku-mediated DNA DSB binding (37). Ku binds to DNA DSBs and serves as a platform for the recruitment of other core NHEJ proteins including DNA-PKcs, XRCC4, LIG4, XLF and PAXX amongst others (38,39). Upon DNA binding, autophosphorylation of DNA-PKcs induces a conformational change that destabilises the NHEJ core complex, causing inward sliding of Ku on the DNA and enabling access of end-processing and ligation enzymes to DNA ends to facilitate repair (37). Autophosphorylation also stimulates the dissociation of DNA-PKcs from DNA and Ku, and inactivates the kinase activity of DNA-PK (38). The best described substrate for DNA-PK is itself, with autophosphorylation occurring at multiple sites (37).

As well as being important for the repair of exogenous DNA DSBs, classical NHEJ also plays crucial roles in the repair of endogenous DSBs arising during physiological process, such as V(D)J and class-switch recombination (40). Mice with a disrupted *Prkdc* gene have severe combined immunodeficiency, as well as being radiosensitive (40). In addition, DNA-PK has an established role in innate immunity and pro-inflammatory signalling, which are important issues to consider with respect to long-term treatment with DNA-PK inhibitors, as well as the potential for combination approaches with immune-modulating agents (37). Interestingly, DNA-PK has also long been known to have links to transcription (41), and studies are currently ongoing to investigate the mechanisms by which transcriptional regulation by DNA-PK affects DNA repair (42).

DNA-PK mutants lacking kinase activity, or treatment of cells with small molecule inhibitors of DNA-PK kinase activity cause the latter to be stabilised on DNA ends, impeding NHEJ and also likely interfering with other repair processes, including HR by obstructing DNA end-resection (38). As it plays key roles in repair by NHEJ, DNA-PK inhibition profoundly hypersensitises cells and tumor xenografts to replication-independent DSB-inducing agents, such as radiotherapy and topoisomerase 2 inhibitors (43). In contrast, DNA-PK inhibition alone has very little effect on cancer cell or tumor viability (43), perhaps because most endogenous DSBs arise in the context of DNA replication, where the preferred repair pathway is HR. These compounds are therefore predicted to be associated with modest antitumor activity as monotherapy, while, there is potential for antitumor synergy in combination with DNA damaging agents, albeit with a potentially narrow therapeutic index because of associated effects on normal cells in the patient.

The clinical development of DNA-PK inhibitors with high potency and selectivity *in vitro* has been complicated by inadequate pharmacokinetic (PK) properties. However, a number of novel DNA-PK inhibitors have recently entered clinical development (**Table 2**). For instance, MSC2490484A (Merck KGaA; NCT02316197) is being evaluated in phase I trials as monotherapy and in combination with radiotherapy, while a phase I trial of VX-984 (Vertex pharmaceuticals) in combination with liposomal doxorubicin has recently started recruitment (NCT02644278). A dual inhibitor of DNA-PK and TOR kinase (a downstream effector of the PI3K-AKT pathway signaling and

another member of the PIKK family), is CC-115 (Celgene), which was developed through lead optimisation of existing mTOR inhibitors (44). In pre-clinical studies, CC-115 inhibits proliferation and induces caspase-dependant cell death in chronic lymphoid leukaemia (CLL) cells, and also leads to death of CLL cells resistant to the PI3Kδ inhibitor idelalisib (45). The relative importance of DNA-PK versus TOR inhibition in this setting has however not been fully elucidated. In a recently reported phase I trial involving patients with advanced solid and hematological malignancies, CC-115 was well tolerated, with preliminary antitumor activity observed (46). There has been some suggestion that CC-115 may have greater activity in patients with CLL harboring biallelic *ATM* loss, although the mechanism for this has yet to be established (45).

ATM

Similar to DNA-PK, ATM promotes DNA DSB repair in cells and responds to DSBs generated throughout the cell cycle. Inherited mutations in the *ATM* gene result in the autosomal recessive condition Ataxia Telangiectasia, a syndrome characterised by progressive cerebellar ataxia, oculocutaneous telangiectasia, radiosensitivity, predisposition to lymphoid malignancies and immunodeficiency, with defects in both cellular and humoral immunity (47). A number of different factors have now been identified that promote ATM activation; however, following DNA DSBs, ATM is predominantly activated through interactions with NBS1 of the MRN complex (**Figure 2B;** (48,49). ATM is the principal kinase responsible for the phosphorylation of histone H2AX on serine 139 (known as γH2AX) (50), although some functional

redundancy exists with ATR and DNA-PK. MDC1 (mediator of DNA damage checkpoint protein-1) binds directly to γH2AX (51) and potentiates the DNA damage signal, leading to the spreading of γH2AX to over a megabase from its initial lesion (52). This amplification is thought to help sustain the DDR signal to enable sufficient recruitment and retention of DNA damage mediator proteins such as 53BP1 at sites of DNA damage, which can be visualised as foci in DNA damaged cells.

Phospho-proteomic studies have identified hundreds of ATM substrates (53), although the physiological relevance of many of these proteins is currently unknown. A well-recognised substrate of ATM is CHK2, the activity of which is predominantly, but not exclusively important for G1-S phase checkpoint activation (54). ATM is also important for the stabilisation of p53 through the phosphorylation and subsequent inhibition of proteosomal degradation by MDM2 (54).

ATM inhibition has been demonstrated to hypersensitize cells to ionizing radiation and the DNA DSB-inducing agents etoposide, camptothecin and doxorubicin (55). A phase I trial of the ATM inhibitor AZD0156 (AstraZeneca) is currently underway as monotherapy and in combination with olaparib and other cytotoxic or molecularly targeted agents (NCT02588105; **Table 2**). While there are likely to be other ATM inhibitors in development, to our knowledge, none have reached clinical studies.

ATR

Although also important for DSB repair, the context for ATR activation is different to ATM and DNA-PK. ATR is activated by RPA (replication protein A) bound ssDNA, which can arise as a result of stalled replication forks and also occurs following DNA end-resection during the early stages of homologous recombination (**Figure 2C)** (56). ATR is recruited to RPA-ssDNA by its obligate binding partner ATRIP (ATR-interacting protein), and is activated by TOPBP1 (topoisomerase binding partner 1) in complex with the Rad17-Rfc2-5 clamp loader, the 9-1-1 complex (Rad9-Rad1-Hus1), Claspin and RHINO (57). CHK1 is the best described substrate of ATR and once activated by ATR, CHK1 serves to inhibit cyclin-dependent kinase (CDK) activity through the phosphorylation of CDC25A. As such, CHK1 is a critical regulator of the G2-M and intra-S cell cycle checkpoints (**Figure 2C**) (58). Interestingly, recent preclinical studies have demonstrated that both ATR and CHK1 have distinct roles in the regulation of the intra-S checkpoint. ATR appears particularly important for the suppression of replication catastrophe in early S-phase cells through the promotion of ribonucleotide reductase accumulation and by limiting origin firing (59). In contrast, other S-phase cells are capable of recovering from replication insults through a CHK1-mediated back-up mechanism (59). A synthetic lethal relationship has now been established between ATR and CHK1 inhibition, with combination blockade leading to replication fork arrest, DNA SSB accumulation, replication collapse and synergistic cell death in cancer cells *in vitro* and *in vivo* (60).

In addition, a third checkpoint kinase has now been identified (MK2; MAPKAP-K2), which functions independently of CHK1, downstream of ATM

and ATR to maintain G2/M and intra-S phase arrest (61–63). To our knowledge, there are currently no MK2 inhibitors in clinical development, although pre-clinical work has demonstrated interesting synergy between MK2 and CHK1 inhibitors, particularly in *KRAS* mutant tumors (64).

VX-970 (Vertex pharmaceuticals) is a first-in-class ATR inhibitor, with preclinical data demonstrating chemosensitization of lung cancer cells predominantly to chemotherapeutics that result in replication fork collapse, such as cisplatin and gemcitabine *in vitro*, and increased antitumor activity in combination with cisplatin *in vivo* (65,66). Preliminary phase I trial data have shown that VX-970 is well tolerated as monotherapy with no dose limiting toxicities or grade 3-4 adverse events demonstrated up to weekly intravenous (IV) doses of 480mg/m^2 (67). A durable RECIST (response evaluation criteria in solid tumors) complete response was observed in a patient with metastatic ATM-loss colorectal cancer, who remained on single agent VX-970 for more than 20 months. When VX-970 was combined with carboplatin, although the maximum tolerated dose was not established, because there was no more than 1 dose-limiting toxicity at each dose level, the recommended phase II dose (RP2D) was VX-970 90 mg/m^2 + carboplatin area under the curve (AUC) 5 based on carboplatin dose delays observed at higher dose levels. Crucially, paired tumor biopsy studies undertaken at this RP2D showed significant inhibition of ATR-targeted phosphorylation of Ser-345 on CHK1, confirming target modulation. At the RP2D, a patient with platinum-refractory, PARP inhibitor resistant, germline *BRCA1* and *TP53* mutant advanced high-grade serous ovarian cancer achieved a RECIST partial response and gynecologic

cancer intergroup (GCIG) CA125 tumor marker response lasting 6 months. As expected from the predicted mechanism-based toxicity profile of an ATR inhibitor and platinum chemotherapy, myelosuppression (neutropenia and thrombocytopenia) was the most commonly observed treatment-related toxicity (68). Combination trials with VX-970 and a number of other chemotherapeutics are ongoing, including cisplatin and gemcitabine, with promising antitumor responses observed in chemotherapy-resistant patients with advanced solid cancers (69,70) (**Table 2**). AZD6738 (AstraZeneca) is an oral ATR inhibitor currently being assessed in phase I clinical trials as monotherapy or in combination regimens with olaparib, carboplatin, radiotherapy or the immune-checkpoint inhibitor durvalumab (MEDI4736; AstraZeneca). The optimal scheduling and sequencing of these agents with their respective partners, in order to balance the trade-off between antitumor activity and bone marrow toxicity, is not yet clear and full results of these trials are awaited with interest.

CHK1

Pre-clinically, CHK1 inhibitors have demonstrated most synergy with drugs that generate replication dependent DNA damage such as anti-metabolites, and therefore clinical development has focused on their use in combination with such drugs (71). MK8776 (Merck & Co) is a potent and selective CHK1 inhibitor that is well tolerated as a monotherapy, as well as in combination with gemcitabine (**Table 2**) (72). Results from a recently published phase I trial of MK8776 as monotherapy or in combination with gemcitabine, has shown preliminary evidence of clinical efficacy, with 2/30 patients (7%) having

a partial response and 13/30 (43%) demonstrating stable disease (72). As expected, toxicity was more frequent in combination, and included fatigue, nausea, anorexia, thrombocytopenia, neutropenia and transient, dose-related electrocardiogram (ECG) abnormalities, specifically QTc prolongation. The recommended phase II dose of MK8776 is 200mg, with gemcitabine administered at 1000 mg/m^2 on days 1 and 8 of a 21-day cycle. LY2603618 (Eli Lilly) is a selective CHK1 inhibitor being evaluated at both 170mg and 230mg in combination with gemcitabine (**Table 2**) (73). Preliminary results reported RECIST partial responses in 4/17 patients, with mainly hematological toxicities observed, and three patients discontinuing treatment because of adverse events. A phase I trial of CHK1 inhibitor CCT245737 (Sareum Holdings plc) as monotherapy, and in combination with cisplatin and gemcitabine has recently started accrual (NCT02797977, NCT02797964) (**Table 2**).

CHK2

There is some uncertainty as to whether the inhibition of CHK2 will be beneficial in the clinical setting, and at present there are no selective CHK2 inhibitors in clinical development (71). Genetic deletion of the mouse *Chek2* gene alleviates p53-dependent cell death following irradiation and although the mechanisms for this protection have not been fully defined, there is a hypothetical risk that inhibition of CHK2 may be radio-protective in a clinical setting (74). Further studies are required to determine the appropriate clinical context in which CHK2 inhibition may lead to antitumor activity (75). Several of the early cell cycle checkpoint inhibitors, such as LY2606368 (Prexasertib;

Eli Lilly), are dual inhibitors of CHK1 and CHK2, and many of these have now discontinued clinical development due to lack of efficacy. LY2606368 has undergone evaluation in a phase I trial, defining a RP2D of 105mg/m^2 every 14 days, with a predominant toxicity of myelosuppression (**Table 2**) (76). Evidence of single agent activity was observed, with 2/45 patients achieving a RECIST partial response (one with anal cancer and one with head and neck squamous cell carcinoma), while 15/45 (33.3%) patients obtained clinical benefit with radiological stable disease. The trial is expanding, preferentially in patients with squamous histology tumors, and several combination strategies are currently ongoing.

WEE1

Working in parallel with CHK1, the WEE1 protein kinase also plays a critical role in the activation of the G2-M checkpoint through the regulation of cyclin dependent kinases (77,78). Unlike CHK1, however, WEE1 is not directly regulated by DNA damage, but is required for physiological cell cycle progression. CDK1 Tyr15 phosphorylation by WEE1 inhibits CDK1 activity resulting in inactivation of the CDK1/CCNB1 complex and G2-M checkpoint activation (79). The predominant mechanism-of-action of WEE1 inhibitors was initially believed to be failure of the G2-M checkpoint due to inappropriate CDK1/CCNB1 activation, resulting in mitotic catastrophe (79). More recently, however, it has become clear that WEE1 inhibition also generates replication-dependent DNA damage in cells, due to aberrant DNA replication through CDK2 inhibition (80,81). The first-in-class WEE1 kinase inhibitor AZD1775 (MK1775; AstraZeneca) has been shown to potentiate the cytotoxic effects of

a range of DNA damaging agents and demonstrate single agent activity In preclinical models (77). AZD1775 has been evaluated in a single agent phase I clinical trial (82), where a maximum tolerated dose of 225mg twice daily for 2.5 days per week for two weeks in three-weekly cycles was established (**Table 2**). Dose limiting toxicities reported were reversible supraventricular tachycardia and myelosuppression, with common toxicities including myelosuppression and diarrhea. The study noted evidence of single agent activity with RECIST partial responses in two germline *BRCA1* mutant patients (papillary serous ovarian and squamous cell carcinoma of the head and neck). Proof-of-mechanism target modulation was demonstrated in paired tumor biopsies demonstrating reduced CDK1 Tyr15 phospho levels and increased γH2AX levels after treatment.

Recently published data from a phase I trial of AZD1775 in combination with gemcitabine, cisplatin or carboplatin demonstrated that chemotherapy combinations with AZD1775 were safely tolerated, with superior response rates in *TP53*-mutated (21%) compared to *TP53* wild-type patients (12%) (83). Interestingly, there is also evidence that WEE1 inhibition may reverse platinum resistance, as the combination of AZD1775 and carboplatin has shown antitumor activity in patients with *TP53* mutant, platinum resistant/refractory ovarian cancer, with a published RECIST partial response rate of 38% (n=8) and complete response rate of 5% (n=1) (**Table 2**) (84). Preliminary data from a phase II trial of AZD1775 in combination with carboplatin and paclitaxel versus chemotherapy alone in patients with platinum-sensitive *TP53*-mutant ovarian cancer demonstrated a superior

progression free survival (PFS) benefit [hazard ratio (HR) 0.55, CI 0.32-0.95, p=0.030], with common toxicities including nausea, diarrhea, alopecia and fatigue (**Table 2**) (85). There is now a need to better define the patient populations predicted to respond to AZD1775 monotherapy and novel combination regimens, and numerous biomarker-driven clinical trials with AZD1775 are currently ongoing to address such issues (**Table 2**).

TARGETING DDR EFFECTOR PROTEINS AND REPAIR PATHWAYS

DDR events converge on one or more repair pathways that are dedicated to specific types of DNA damage **(Figure 1 and Table 1)**. Some established antitumor agents result in a single type of DNA lesion (e.g. topoisomerase inhibitors), while others generate a heterogenous mixture of DNA damage types, engaging multiple repair pathways simultaneously (e.g. radiotherapy). While NHEJ and HR remain the predominant DSB repair pathways, the importance of alternative homology-directed repair mechanisms is also now recognised (20). These are mutagenic pathways that are able to 'back-up' standard repair processes, which have either been genetically or chemically compromised. While there are multiple attractive DNA repair and DDR effector protein targets, there are still only a limited number of drugs currently in clinical development that target such proteins.

APE1 (AP endonuclease-1) recognises abasic (AP) sites generated following the removal of damaged bases by DNA glycosylases, and its endonuclease activity is essential for BER (86). In addition, APE1 harbors exonuclease activity important for the removal of 3' obstructive lesions in DNA, including

chain-terminating nucleoside analogs (87). TRC102 (Methoxyamine; TRACON pharmaceuticals) reacts with abasic sites to cause an AP-adduct that is resistant to APE1 action (88), exacerbating the cytotoxicity of alkylating agents and anti-metabolites in cells. Hematological toxicities were dose limiting for TRC102 in combination with pemetrexed or temozolomide (89,90), and other early phase combination trials are ongoing (**Table 2**).

Recent studies have demonstrated that HR deficient cells rely on error-prone microhomology-mediated end-joining (MMEJ; also known as alt-NHEJ) for survival (25,91). The polymerase activity of POLQ (DNA polymerase theta) is required for gap-filling during MMEJ, and POLQ also prevents hyper-recombination by limiting RAD51 accumulation at resected DNA ends (25,91). POLQ is therefore an attractive drug target, particularly in the context of HR deficient tumors. The development of small molecule inhibitors that target protein-protein interactions of the RAD51 recombinase family are also ongoing (92). Targeting protein-protein interactions is challenging and has had limited success, although compounds have been identified that disrupt the self-association of RAD51 and successfully inhibit the interaction between RAD51 and BRCA1 (92). These compounds have the potential to inhibit RAD51-dependent HR in cells, and the demonstration of such effects in functional cellular assays is awaited.

FINE-TUNING THE DDR

Each step of the DDR is tightly regulated by reversible post-translational modifications (PTMs) including: phosphorylation, ADP-ribosylation,

methylation, acetylation, ubiquitylation, sumoylation and neddylation (93–96). While DDR-specific kinase and ADP-ribosylation inhibitors are already in clinical development/use, given the essential role of ubiquitylation and de-ubiquitylation in the DDR, modulating DNA repair through the use of specific inhibitors of ubiquitylation, deubiquitylation or the ubiquitin-proteasome system is an active area of research (96,97). HR is particularly sensitive to proteasome inhibition (98) and proteasome inhibitors such as bortezomib (Velcade; Takeda Pharmaceutical Company Ltd) have been shown to block global ubiquitylation in cells, and disrupt protein turnover of several DDR proteins, such as MDC1, BRCA1 and RPA (99–101). Inhibiting a subset of ubiquitin ligases, namely the cullin-ring-ligases (CRLs), through inhibition of neddylation (the covalent attachment of the ubiquitin-like protein, NEDD8 to target proteins) in cells, also affects the DDR (93,102). Pevonedistat (MLN4924; Takeda Pharmaceutical Company Ltd) inhibits the NEDD8 E1, blocking NEDD8 conjugation and CRL activity in cells (103). A phase I study of pevonedistat showed that an intermittent dosing schedule was generally well tolerated, with hepatotoxicity being dose-limiting (104). In pre-clinical studies, pevonedistat exhibited particular synergy with DNA cross-linking agents (105,106) and phase I combination studies are currently ongoing.

PTMs are reversible, with such turnover being important for various cellular processes. It’s perhaps unsurprising therefore that deubiquitylating enzymes (DUBs) also play key roles in promoting DNA repair in cells. Indeed, various DUBs are attractive drug targets (96), with several DUB inhibitors currently in pre-clinical development. Analogously, poly(ADP-ribose) glycohydrolase

(PARG) catalyses the hydrolysis of poly(ADP-ribose) and therefore reverses the effects of PARP. Inhibition of PARG, in a similar fashion to PARP inhibition, leads to DNA damage that depends on HR for repair (107), and efforts are ongoing to generate specific PARG inhibitors for clinical use (108).

Several other classes of compounds have demonstrated inhibitory effects on the DDR, which may potentially be exploited in a clinical setting. Chromatin compaction significantly affects DNA repair (21) and the chromatin modifying inhibitors vorinostat (Zolonza^{TM}; Merck) and romidepsin (Istodax; Celgene) are both approved for the treatment of cutaneous T cell lymphoma (109). As well as relieving chromatin compaction, these histone deactylase (HDAC) inhibitors also transcriptionally down-regulate a number of DSB repair proteins, thereby hypersensitising cells to DSB-inducing agents and providing strong rationale for combination treatment with DNA-damaging compounds (110,111). There are numerous other compounds in pre-clinical and clinical development, which have inhibitory effects on DNA repair through targeting epigenetic modifier enzymes, such as EZH2 (H3K27 methyltransferase) (112), histone deacetylases (HDACs) (111) and G9A (histone lysine N-methyl-transferase) (113).

PATIENT SELECTION

PARP inhibitors are selectively toxic to tumor cells with biallelic mutations/loss of *BRCA1* or *BRCA2* (*BRCA1/2*) (11,12), and olaparib is the first oncology drug to be licensed with a companion genetic diagnostic (BRCAnalysis CDx^{TM}). Such a level of “synthetic lethality” has yet to be reproduced with any

other DDR inhibitor. The effectiveness of PARP inhibitors is not restricted to patients with germline or somatic *BRCA1/2* mutations however, and significant efforts are underway to determine tumors that are essentially HR deficient through other mechanisms (15). Genomic approaches to achieve this include studies undertaken to identify mutations in single HR and/or other genes that predict for PARP inhibitor sensitivity (114,115). In addition, DNA repair dysfunction has the potential to lead to global DNA aberrations; the presence of a genome-wide mutational signature (or genomic scar) that occurs in the context of chronic HR deficiency, may thus also be a useful biomarker that is predictive of PARP inhibitor sensitivity (116). Scoring systems that measure genomic defects reflective of HR deficiency are also being utilised, including those that quantify loss of heterozygosity (LOH), telomeric allelic imbalance and large scale state transitions (defined as a chromosomal break between adjacent segments of DNA of at least 10 Mb) within tumors (117).

The hypermethylation of genes and other epigenetic effects mean that focusing entirely on genomics will ultimately fail to identify all patients who are likely to benefit from a molecularly-targeted cancer therapy. To address this issue, functional assays of HR deficiency have been pursued and have included the detection of RAD51 foci at DNA-damage sites in breast cancer biopsies following neo-adjuvant chemotherapy, where a failure to generate RAD51 foci in cells was strongly predictive of a pathological complete response ($p=0.011$) (118). To date, however, there is no robust strategy to clinically determine which patients will benefit from PARP inhibitors outside of

the *BRCA1/2* mutant population and it may be that a combination of functional and genomic approaches is required.

Beyond PARP inhibitors, preclinical data have demonstrated synergy between ATR inhibition and impaired ATM signalling, particularly in the context of exogenous DNA damage (119). The mechanism for this synergy has yet to be defined; however, clinical studies exploring ATR inhibition in the context of ATM loss are ongoing (68). Certainly, a number of studies suggest that high levels of replication stress and consequently, increased endogenous DNA damage in tumors may be required for hypersensitivity to ATR inhibitor monotherapy. Overexpression of oncogenes such as *CCNE1*, *CCND2* and *MYC,* adversely affects DNA replication by disrupting origin firing and replication progression, resulting in oncogene-induced replication stress (7). In keeping with this, *CCNE1* amplification exaggerates the hypersensitivity of *TP53* deficient cells to ATR inhibition (120), and both ATR and CHK1 inhibitors are particularly toxic for *Myc*-driven lymphomas in mice (121). In addition, oncogenic stress as a result of activating *KRAS* mutations has been shown to hypersensitise cells to ATR inhibition (122), and selecting tumors with oncogene-induced replication stress has the potential to provide a much-needed therapeutic window for ATR inhibitor/chemotherapy combination strategies.

The physical ends of linear chromosomes, telomere ends, are naturally occurring DNA DSBs in cells that are protected by the Shelterin protein complex in order to prevent DDR activation (123). Maintaining telomere length

is essential for the genomic stability of replicating cells and involves the concerted actions of several key DDR players, including ATM, ATR, DNA-PK and Ku (124,125). Telomere maintenance is achieved through telomerase activation in 85% of cancers and telomerase recruitment to telomeres is dependent on ATM and ATR activity in human cells (125). ATR activity has also been shown to be important for alternative lengthening of telomeres (ALT) (126), a mechanism which relies on recombination events to maintain telomere length. In keeping with this, preclinical data have been published demonstrating hypersensitivity of ALT tumor cells to ATR inhibition (126). Given the role of the DDR PIKKs in telomere maintenance, DNA-PK, ATM and ATR inhibitors, all have the potential to negatively affect telomere length in cells. While this may potentially contribute to their antitumor effects, it may also result in detrimental genomic instability in replicating non-cancer cell populations.

It is currently too preliminary to establish if *TP53* deficiency, ATM loss/mutation, ALT reliance or *CCNE1*, *CCND2* or *MYC* oncogene activation will predict for sensitivity to ATR inhibitors in the clinic, but it is likely that this will be a far from exhaustive list of putative genomic predictive biomarkers, and having a functional marker of replication stress in tumors will be helpful. Surrogate markers of replication stress might include phosphorylation of ATR substrates (e.g. CHK1pS345 and RPApS33) or levels of single-stranded DNA (59). How these markers might change over time and with treatment is of course currently unknown however, and the usefulness of measuring these biomarkers in archival tumor specimens has yet to be tested.

COMBINATION STRATEGIES

Combining DDR inhibitors with DNA damaging agents has been the natural first step in the clinical development of combination strategies for DDR inhibitors **(Table 2)**. A thorough understanding of the DNA lesions induced by different chemotherapies, and inhibition of the respective pathways required for repair will ultimately maximise the odds of synergistic antitumor efficacy. Nevertheless, toxicities will in many cases likely limit drug doses used in such combinations. Indeed, combining olaparib with carboplatin and paclitaxel chemotherapies in the clinic has been challenging because of myelosuppression, and reductions in the full single-agent doses of all drugs had to be undertaken to enable the combination to be administered safely (127,128). While olaparib showed promising data in a phase II trial of patients with advanced gastric cancer harboring ATM loss when combined with the paclitaxel chemotherapy (129), there was no statistically significant survival benefit in the phase III GOLD trial (according to a May 18th, 2016 AstraZeneca press release). Optimizing drug scheduling may enable potential differences in repair kinetics of normal versus cancer cells to be exploited and may therefore, increase damage in tumors while sparing normal tissue. Careful consideration of the sequence of combination drug administration is required to optimize synergistic effects. Equally, selecting patients with tumors of specific genotypes or phenotypes may produce a therapeutic window for such combinations.

Combining DDR inhibitors with small molecule inhibitors of other cellular signalling pathways also shows promise, and PARP inhibition has been tested in combination with a number of agents. For example, an EGFR inhibitor combination has been explored following clinical data from the EURTAC trial showing that low BRCA1 mRNA levels were associated with longer PFS to erlotinib (Tarceva; Genentech) (130). A phase I trial of olaparib with gefitinib (Iressa; AstraZeneca) demonstrated safety and tolerability, as well as promising signals of antitumor activity (131); a phase 2 trial is now accruing (132). Recent pre-clinical data also suggest that inhibition of the receptor tyrosine kinase c-MET hypersensitises cells to PARP inhibition and the clinical evaluation of this finding is likely to follow (133). Preclinical evidence of phosphatidylinositide 3-kinase (PI3K) inhibition impairing BRCA1/2 expression and sensitising tumor cells to PARP inhibition in both *BRCA1/2*-mutant and *BRCA1/2*-wild type breast cancers (134,135) has led to phase I combination trials of olaparib with the PI3K inhibitor BKM120 (Buparlisib; Novartis) (136) and the AKT inhibitor AZD5363 (AstraZeneca) (137), respectively. Preliminary data suggest that these combinations are tolerable and effective, with final results awaited with interest.

Preclinical evidence suggests that hypoxia results in impaired HR through the down-regulation of HR-related genes (138–140). This provided the rationale for a phase 1/2 trial of the pan-vascular endothelial growth factor (VEGF)1-3 inhibitor cediranib (AZD2171; AstraZeneca) with olaparib in patients with platinum-sensitive ovarian cancer (141). A PFS benefit of 8.7 months (HR 0·42 [95% CI 0·23–0·76; p=0·005]) was demonstrated with the combination

versus olaparib alone in the overall patient population, with predominant toxicities of fatigue, diarrhea and hypertension in the combination arm observed. Interestingly, however, no PFS difference was observed between the two treatment arms in patients with *BRCA1/2* mutated ovarian cancer.

Apart from molecularly targeted agents, we are also beginning to appreciate the considerable crosstalk between DNA repair and endocrine signalling (142). Steroid hormone signaling has been shown to promote NHEJ through transcriptional regulation of NHEJ components such as *PRKDC*, and has been demonstrated to have both positive and negative effects on HR depending on tumor model and context (142). In prostate cancer models, PARP1 has been demonstrated to support androgen transcriptional function, and is required for transcriptional activation of the oncogenic fusion TMPRSS2-ERG protein found in >50% of prostate cancers. Duel blockade of PARP activity and androgen receptor signaling delays tumor growth compared to either as monotherapy in mouse xenograft prostate cancer models (143). This has led to trials combining PARP inhibitors with hormonal manipulation, such as olaparib with the CYP17 inhibitor abiraterone (Zytiga; Janssen Biotech) (144).

There are also now multiple combination studies involving immune checkpoint inhibitors with DDR inhibitors, such as PARP and ATR inhibitors (**Table 2**). There is pre-clinical evidence to suggest that immune checkpoint inhibition synergizes with PARP inhibitor treatment in BRCA1 deficient tumors and clinical trials investigating this hypothesis are ongoing (145). In addition, the

success of anti-PD-1/PD-L1 therapeutics in MMR deficient tumors (146) raises the intriguing question as to whether increasing mutational load with DDR inhibitors might increase the immunogenicity of cancers and subsequent responses to immunotherapy. Further studies are required to substantiate this hypothesis, and while high levels of microsatellite instability might prove to be a useful biomarker of response to immune checkpoint inhibitors, alternative mechanisms that might be driving sensitivity should not be discounted (147). Equally, we must be mindful that an intact DDR plays an important role in innate immunity (148). DDR signalling is important for the activation of inflammatory cytokines and induces the expression of immune-receptor ligands on damaged cells. As such, inhibitors of DDR signalling may in fact attenuate the immune response following DNA damage and therefore immunotherapy-DDR inhibitor combination studies need to be carefully considered.

FUTURE PERSPECTIVES AND CONCLUSIONS

With multiple DDR inhibitors now in preclinical and clinical pipelines, careful consideration of their mechanisms-of-action is required in order to maximise their potential. DDR-deficient tumors should not be grouped indiscriminately into a class of tumors that may respond to any DDR inhibitor. Through closer collaborations between scientists and clinicians, we must insist on a rational rather than empirical approach to the clinical development of DDR inhibitors. A number of factors will be critical to ensure clinical success, including the development of analytically validated pharmacodynamic assays and predictive biomarkers of response and resistance. As we have observed with

PARP inhibitors, managing the toxicities of DDR inhibitor/DNA-damaging agent combinations is likely to be challenging, and so clinicians should not shy away from aiming for a single agent synthetic lethal approach that has already led to some success in the clinic. Much attention has focused on genetic alterations to key DDR drivers, but the relative contribution of somatic epigenetic loss of such DDR players has not been extensively explored. For example, the silencing of the *BRCA1* gene through promoter hypermethylation has been demonstrated in breast and ovarian cancers (149), which highlights the importance of also considering functional biomarker assays, rather than relying on genomics in isolation.

Modern clinical trial designs will need to incorporate translational studies, which may be used to guide patient selection, drug scheduling and treatment response (150). Early phase trials should aim to consolidate preclinical understandings of drug mechanisms-of-action. Notably, understanding how many successful drugs function, including the PARP inhibitors, has changed over time, meaning that compounds showing preclinical promise should not be discounted on the basis of hypotheses that later appear to be incorrect. It is likely that combination regimens, either with drugs given together or sequentially to overcome resistance, will be required for the optimal application of these DDR inhibitors in the clinic. The use of longitudinal genomic profiling of circulating free DNA to support adaptive drug administration will also be important.

The long-term effects of inhibiting the DDR in patients are still not known and needs further study. One recognised risk of DNA damage to normal tissue is the emergence of secondary cancers, particularly hematological malignancies, following chemotherapy treatment and the potential mutagenic effects of inhibiting DNA repair. We will need to increase our clinical experience of DDR inhibitors before the long-term effects of these compounds are realised. Nevertheless, as PARP inhibitors move into the neo-adjuvant and adjuvant settings, the malignant potential of these drugs must be monitored.

Precision medicine has heralded the advent of sophisticated modern technologies, which have permitted genomic profiling of both normal and tumor tissue at greater speeds and at lower costs than before. This has enabled the “real-time” identification of germline and somatic DNA repair gene aberrations, which has critical implications both for identifying families at risk of cancer predisposition, and also for predicting therapeutic responses to DDR inhibitors. Now that olaparib has been approved for clinical use and others will hopefully soon follow, we must not forget the lessons learned from the successful development of PARP inhibitors, nor ignore the multitude of opportunities that still exist within the DDR network, which now need to be exploited to impact positively on cancer medicine.

GRANT SUPPORT

JB, BO and TAY acknowledge support from the Experimental Cancer Medicine Centre (to The Institute of Cancer Research) and the National Institute for Health Research Biomedical Research Centre (jointly to the Royal

Marsden NHS Foundation Trust and The Institute of Cancer Research). Research in the Jackson laboratory is funded by Cancer Research UK (CRUK) program grant number C6/A11224. Core funding is provided by CRUK (C6946/A14492) and the Wellcome Trust (WT092096). S.P.J. receives his salary from the University of Cambridge, UK, supplemented by CRUK

References:

1. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461:1071–8.
2. Yap TA, Sandhu SK, Carden CP, de Bono JS. Poly(ADP-Ribose) polymerase (PARP) inhibitors: Exploiting a synthetic lethal strategy in the clinic. CA Cancer J Clin. 2011;61:31–49.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
4. Jeggo PA, Löbrich M. How cancer cells hijack DNA double-strand break repair pathways to gain genomic instability. Biochem J. 2015;471:1–11.
5. Huber KVM, Salah E, Radic B, Gridling M, Elkins JM, Stukalov A, et al. Stereospecific targeting of MTH1 by (S)-crizotinib as an anticancer strategy. Nature. 2014;508:222–7.
6. Gad H, Koolmeister T, Jemth A-S, Eshtad S, Jacques SA, Ström CE, et al. MTH1 inhibition eradicates cancer by preventing sanitation of the dNTP pool. Nature. 2014;508:215–21.
7. Gaillard H, García-Muse T, Aguilera A. Replication stress and cancer. Nat Rev Cancer. Nature Publishing Group; 2015;15:276–89.
8. Jackson SP, Helleday T. Drugging DNA repair. Science (80-).

2016;352:1178–9.

9. Caldecott KW. DNA single-strand break repair. Exp Cell Res. 2014;329:2–8.

10. Frit P, Barboule N, Yuan Y, Gomez D, Calsou P. Alternative end-joining pathway(s): bricolage at DNA breaks. DNA Repair. 2014;17:81–97.

11. Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434:917–21.

12. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434:913–7.

13. Weaver AN, Yang ES. Beyond DNA Repair: Additional Functions of PARP-1 in Cancer. Front Oncol. 2013;3:1–11.

14. Bock FJ, Chang P. New Directions in PARP Biology. FEBS J. 2016;1–15.

15. Lord CJ, Ashworth A. BRCAness revisited. Nat Rev Cancer. 2016;16:110–20.

16. Kaye S. Progress in the treatment of ovarian cancer . Lessons from homologous recombination deficiency – the first 10 years. Ann Oncol. 2016;27:i1–3.

17. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer. Cancer Discov. 2015;5:1137–54.

18. Scott CL, Swisher EM, Kaufmann SH. Poly (ADP-Ribose) Polymerase Inhibitors: Recent Advances and Future Development. J Clin Oncol.

2015;33:1397–406.

19. Sonnenblick A, de Azambuja E, Azim HA, Piccart M. An update on PARP inhibitors-moving into the adjuvant setting. Nat Rev Clin Oncol. 2015;27–41.
20. Ceccaldi R, Rondinelli B, D'Andrea AD. Repair Pathway Choices and Consequences at the Double-Strand Break. Trends Cell Biol. 2015;26:52–64.
21. Lemaître C, Soutoglou E. Double strand break (DSB) repair in heterochromatin and heterochromatin proteins in DSB repair. DNA Repair. 2014;19:163–8.
22. De Vos M, Schreiber V, Dantzer F. The diverse roles and clinical relevance of PARPs in DNA damage repair: Current state of the art. Biochem Pharmacol. 2012;84:137–46.
23. Helleday T. The underlying mechanism for the PARP and BRCA synthetic lethality: Clearing up the misunderstandings. Mol Oncol. 2011;5:387–93.
24. Murai J, Huang SYN, Das BB, Renaud A, Zhang Y, Doroshow JH, et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res. 2012;72:5588–99.
25. Mateos-Gomez PA, Gong F, Nair N, Miller KM, Lazzerini-Denchi E, Sfeir A. Mammalian polymerase θ promotes alternative NHEJ and suppresses recombination. Nature. 2015;518:254–7.
26. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009;361:123–34.

27. Fong PC, Yap TA, Boss DS, Carden CP, Mergui-Roelvink M, Gourley C, et al. Poly(ADP)-ribose polymerase inhibition: Frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol. 2010;28:2512–9.

28. Kaufman B, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmana J, et al. Olaparib Monotherapy in Patients With Advanced Cancer and a Germline BRCA1/2 Mutation. J Clin Oncol. 2014;33:244–50.

29. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol. 2014;15:852–61.

30. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: A proof-of-concept trial. Lancet. 2010;376:235–44.

31. Audeh MW, Carmichael J, Penson RT, Friedlander M, Powell B, Bell-McGuinn KM, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: A proof-of-concept trial. Lancet. 2010;376:245–51.

32. McNeish I, Oza A, Coleman R, Scott C, Konecny G, Tinker A, et al. Results of ARIEL2: A Phase 2 trial to prospectively identify ovarian cancer patients likely to respond to rucaparib using tumor genetic analysis. J Clin Oncol 33, 2015 (suppl; abstr 5508).

33. Brown JS, Kaye SB, Yap TA. PARP inhibitors: the race is on. Br J Cancer. 2016;114:713–5.

34. Bouwman P, Jonkers J. Molecular pathways: How can BRCA-mutated tumors become resistant to PARP inhibitors? Clin Cancer Res. 2014;20:540–7.

35. Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat Struct Mol Biol. 2010;17.

36. Bunting SF, Callén E, Wong N, Chen H-T, Polato F, Gunn A, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell. 2010;141:243–54.

37. Jette N, Lees-Miller SP. The DNA-dependent protein kinase: A multifunctional protein kinase with roles in DNA double strand break repair and mitosis. Prog Biophys Mol Biol. 2015;117:194–205.

38. Davis A, Chen D. DNA double strand break repair via non-homologous end-joining. Transl Cancer Res. 2013;2:130–43.

39. Ochi T, Blackford AN, Coates J, Jhujh S, Mehmood S, Tamura N, et al. DNA repair. PAXX, a paralog of XRCC4 and XLF, interacts with Ku to promote DNA double-strand break repair. Science. 2015;347:185–8.

40. Blunt T, Finnie NJ, Taccioli GE, Smith GC, Demengeot J, Gottlieb TM, et al. Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. Cell. 1995;80:813–23.

41. Jackson SP, MacDonald JJ, Lees-Miller S, Tjian R. GC box binding

induces phosphorylation of Sp1 by a DNA-dependent protein kinase. Cell. 1990;63:155–65.

42. Goodwin JF, Knudsen KE. Beyond DNA repair: DNA-PK function in cancer. Cancer Discov. 2014;4:1126–39.

43. Zhao Y, Thomas HD, Batey MA, Cowell IG, Richardson CJ, Griffin RJ, et al. Preclinical evaluation of a potent novel DNA-dependent protein kinase inhibitor NU7441. Cancer Res. 2006;66:5354–62.

44. Mortensen DS, Perrin-Ninkovic SM, Shevlin G, Elsner J, Zhao J, Whitefield B, et al. Optimization of a Series of Triazole Containing Mammalian Target of Rapamycin (mTOR) Kinase Inhibitors and the Discovery of CC-115. J Med Chem. 2015;58:5599–608.

45. Thijssen R, Ter Burg J, Garrick B, van Bochove GGW, Brown JR, Fernandes SM, et al. Dual TORK/DNA-PK inhibition blocks critical signaling pathways in chronic lymphocytic leukemia. Blood. 2016;128:574–83.

46. Munster PN, Mahipal A, Nemunaitis JJ, Mita MM, Paz-Ares LG, Massard C, et al. Phase I trial of a dual TOR kinase and DNA-PK inhibitor (CC-115) in advanced solid and hematologic cancers. J Clin Oncol 34, 2016 (suppl; abstr 2505).

47. Lavin MF, Shiloh Y. The Genetic Defect in Ataxia-Telangiectasia. Annu Rev Immunol. 1997;15:177–202.

48. Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature. 2005;434:605–11.

49. Lee J-H, Paull TT. ATM activation by DNA double-strand breaks

through the Mre11-Rad50-Nbs1 complex. Science. 2005;308:551–4.

50. Rogakou E, Pilch D, Orr A, Ivanova V, Bonner W. DNA Double-stranded Breaks Induce Histone H2AX Phosphorylation on Serine 139. J Biol Chem. 1998;5858–68.

51. Stucki M, Clapperton J a, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell. 2005;123:1213–26.

52. Rogakou EP, Boon C, Redon C, Bonner WM. Megabase chromatin domains involved in DNA double-strand breaks in vivo. J Cell Biol. 1999;146:905–16.

53. Matsuoka S, Ballif B a, Smogorzewska A, McDonald ER, Hurov KE, Luo J, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science. 2007;316:1160–6.

54. Shiloh Y, Ziv Y. The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nat Rev Mol Cell Biol. 2013;14:197–210.

55. Hickson I, Zhao Y, Richardson CJ, Green SJ, Martin NMB, Orr AI, et al. Identification and Characterization of a Novel and Specific Inhibitor of the Ataxia-Telangiectasia Mutated Kinase ATM Identification and Characterization of a Novel and Specific Inhibitor of the Ataxia-Telangiectasia Mutated Kinase ATM. Cancer Res. 2004;64:9152–9.

56. Costanzo V, Shechter D, Lupardus PJ, Cimprich KA, Gottesman M, Gautier J. An ATR- and Cdc7-dependent DNA damage checkpoint that inhibits initiation of DNA replication. Mol Cell. 2003;11:203–13.

57. Maréchal A, Zou L. DNA damage sensing by the ATM and ATR kinases. Cold Spring Harb Perspect Biol. 2013;5:1–18.

58. Stracker TH, Usui T, Petrini JHJ. Taking the time to make important decisions: the checkpoint effector kinases Chk1 and Chk2 and the DNA damage response. DNA Repair. 2009;8:1047–54.

59. Buisson R, Boisvert JL, Benes CH, Zou L. Distinct but Concerted Roles of ATR, DNA-PK, and Chk1 in Countering Replication Stress during S Phase. Mol Cell. 2015;59:1011–24.

60. Sanjiv K, Hagenkort A, Calderón-Montaño JM, Koolmeister T, Reaper PM, Mortusewicz O, et al. Cancer-Specific Synthetic Lethality between ATR and CHK1 Kinase Activities. Cell Rep. 2015;298–309.

61. Manke IA, Nguyen A, Lim D, Stewart MQ, Elia AEH, Yaffe MB. MAPKAP kinase-2 is a cell cycle checkpoint kinase that regulates the G2/M transition and S phase progression in response to UV irradiation. Mol Cell. 2005;17:37–48.

62. Reinhardt HC, Aslanian AS, Lees JA, Yaffe MB. p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage. Cancer Cell. 2007;11:175–89.

63. Blasius M, Wagner S, Choudhary C, Bartek J, Jackson SP. A quantitative 14-3-3 interaction screen connects the nuclear exosome targeting complex to the DNA damage response. Genes Dev. 2014;28:1977–82.

64. Dietlein F, Kalb B, Jokic M, Noll EM, Strong A, Tharun L, et al. A Synergistic Interaction between Chk1- and MK2 Inhibitors in KRAS-

Mutant Cancer. Cell. 2015;162:146–59.

65. Fokas E, Prevo R, Hammond EM, Brunner TB, McKenna WG, Muschel RJ. Targeting ATR in DNA damage response and cancer therapeutics. Cancer Treat Rev. Elsevier Ltd; 2014;40:109–17.

66. Hall AB, Newsome D, Wang Y, Boucher DM, Eustace B, Gu Y, et al. Potentiation of tumor responses to DNA damaging therapy by the selective ATR inhibitor VX-970. Oncotarget. 2014;5:5674–85.

67. Yap TA, Luken MJ de M, O'Carrigan B, Roda D, Papadatos-Pastos D, Lorente D, et al. Abstract PR14: Phase I trial of first-in-class ataxia telangiectasia-mutated and Rad3-related (ATR) inhibitor VX-970 as monotherapy (mono) or in combination with carboplatin (CP) in advanced cancer patients (pts) with preliminary evidence of target modulation and antitumor activity. Mol Cancer Ther. 2016 Jan 7;14(12 Supplement 2):PR14.

68. O'Carrigan B, Jose de Miguel Luken M, Papadatos-Pastos D, Brown J, Tunariu N, Perez Lopez R, et al. Phase I trial of a first-in-class ATR inhibitor VX-970 as monotherapy (mono) or in combination (combo) with carboplatin (CP) incorporating pharmacodynamics (PD) studies. J Clin Oncol 34, 2016 (suppl; abstr 2504).

69. Plummer ER, Dean EJ, Evans TRJ, Greystoke A, Herbschleb K, Ranson M, et al. Phase I trial of first-in-class ATR inhibitor VX-970 in combination with gemcitabine (Gem) in advanced solid tumors (NCT02157792). J Clin Oncol 34, 2016 (suppl; abstr 2513).

70. Shapiro G, Wesolowski R, Middleton M, Devoe C, Constantinidou A, Papadatos-Pastos D, et al. Abstract CT012: Phase 1 trial of first-in-

class ATR inhibitor VX-970 in combination with cisplatin (Cis) in patients (pts) with advanced solid tumors (NCT02157792). Cancer Res. 2016 Jul 22;76(14 Supplement):CT012.

71. Garrett MD, Collins I. Anticancer therapy with checkpoint inhibitors: what, where and when? Trends Pharmacol Sci. 2011;32:308–16.

72. Daud AI, Ashworth MT, Strosberg J, Goldman JW, Mendelson D, Springett G, et al. Phase I dose-escalation trial of checkpoint kinase 1 inhibitor MK-8776 as monotherapy and in combination with gemcitabine in patients with advanced solid tumors. J Clin Oncol. 2015;33:1060–6.

73. Doi T, Yoshino T, Shitara K, Matsubara N, Fuse N, Naito Y, et al. Phase I study of LY2603618, a CHK1 inhibitor, in combination with gemcitabine in Japanese patients with solid tumors. Anticancer Drugs. 2015;26:1043–53.

74. Antoni L, Sodha N, Collins I, Garrett MD. CHK2 kinase: cancer susceptibility and cancer therapy – two sides of the same coin? Nat Rev Cancer. 2007;7:925–36.

75. Matthews TP, Jones AM, Collins I. Structure-based design, discovery and development of checkpoint kinase inhibitors as potential anticancer therapies. Expert Opin Drug Discov. 2013;8:621–40.

76. Hong D, Infante J, Janku F, Jones S, Nguyen LM, Burris H, et al. Phase I Study of LY2606368, a Checkpoint Kinase 1 Inhibitor, in Patients With Advanced Cancer. J Clin Oncol. 2016;34:1764–71.

77. Do K, Doroshow JH, Kummar S. Wee1 kinase as a target for cancer therapy. Cell Cycle. 2013;12:3159–64.

78. Mueller S, Haas-kogan DA. Wee 1 Kinase as a target for cancer

therapy. J Clin Oncol. 2015;33:3485–6.

79. Aarts M, Sharpe R, Garcia-Murillas I, Gevensleben H, Hurd MS, Shumway SD, et al. Forced mitotic entry of S-phase cells as a therapeutic strategy induced by inhibition of WEE1. Cancer Discov. 2012;2:524–39.

80. Beck H, Nahse V, Larsen MSY, Groth P, Clancy T, Lees M, et al. Regulators of cyclin-dependent kinases are crucial for maintaining genome integrity in S phase. J Cell Biol. 2010;188:629–38.

81. Guertin AD, Li J, Liu Y, Hurd MS, Schuller AG, Long B, et al. Preclinical evaluation of the WEE1 inhibitor MK-1775 as single-agent anticancer therapy. Mol Cancer Ther. 2013;12:1442–52.

82. Do K, Wilsker D, Ji J, Zlott J, Freshwater T, Kinders RJ, et al. Phase I Study of Single-Agent AZD1775 (MK-1775), a Wee1 Kinase Inhibitor, in Patients With Refractory Solid Tumors. J Clin Oncol. 2015;33:JCO.2014.60.4009-.

83. Leijen S, van Geel RMJM, Pavlick AC, Tibes R, Rosen L, Razak ARA, et al. Phase I Study Evaluating WEE1 Inhibitor AZD1775 As Monotherapy and in Combination With Gemcitabine, Cisplatin, or Carboplatin in Patients With Advanced Solid Tumors. J Clin Oncol. 2016; DOI: 10.1200/JCO.2016.67.5991.

84. Leijen S, van Geel RMJM, Sonke GS, de Jong D, Rosenberg EH, Marchetti S, et al. Phase II Study of WEE1 Inhibitor AZD1775 Plus Carboplatin in Patients With TP53-Mutated Ovarian Cancer Refractory or Resistant to First-Line Therapy Within 3 Months. J Clin Oncol. 2016; DOI: 10.1200/JCO.2016.67.5942.

85. Oza AM, Weberpals JI, Provencher DM, Grischke E-M, Hall M, Uyar D, et al. An international, biomarker-directed, randomized, phase II trial of AZD1775 plus paclitaxel and carboplatin (P/C) for the treatment of women with platinum-sensitive, TP53-mutant ovarian cancer. J Clin Oncol 33, 2015 (suppl; abstr 5506).

86. Dianov GL, Hübscher U. Mammalian base excision repair: the forgotten archangel. Nucleic Acids Res. 2013;41:3483–90.

87. Chou K, Cheng Y. An exonucleolytic activity of human apurinic / apyrimidinic endonuclease on 3’ mispaired DNA. Nature. 2002;415:655–9.

88. Liuzzi M, Talpaert-Borlé M. A new approach to the study of the base-excision repair pathway using methoxyamine. J Biol Chem. 1985;260:5252–8.

89. Gordon MS, Rosen LS, Mendelson D, Ramanathan RK, Goldman J, Liu L, et al. A phase 1 study of TRC102, an inhibitor of base excision repair, and pemetrexed in patients with advanced solid tumors. Invest New Drugs. 2013;31:714–23.

90. Meehan RS, Chen AP, O’Sullivan Coyne GH, Collins JM, Kummar S, Anderson L, et al. A phase 1 trial of TRC102 (methoxyamine HCl) with temozolomide (TMZ) in patients with solid tumors and lymphomas. J Clin Oncol 34, 2016 (suppl; abstr 2556).

91. Ceccaldi R, Liu JC, Amunugama R, Hajdu I, Primack B, Petalcorin MIR, et al. Homologous-recombination-deficient tumours are dependent on Polθ-mediated repair. Nature. 2015;518:258–62.

92. Scott DE, Coyne AG, Venkitaraman A, Blundell TL, Abell C, Hyvonen M.

Small-molecule inhibitors that target protein-protein interactions in the RAD51 family of recombinases. ChemMedChem. 2015;10:296–303.

93. Brown JS, Jackson SP. Ubiquitylation, neddylation and the DNA damage response. Open Biol [Internet]. 2015 Apr 1;5(4). Available from: http://dx.doi.org/10.1098/rsob.150018.

94. Jackson SP, Durocher D. Regulation of DNA damage responses by ubiquitin and SUMO. Mol Cell. 2013;49:795–807.

95. Ulrich HD. Ubiquitin and SUMO in DNA repair at a glance. J Cell Sci. 2012;125:249–54.

96. Jacq X, Kemp M, Martin NMB, Jackson SP. Deubiquitylating enzymes and DNA damage response pathways. Cell Biochem Biophys. 2013;67:25–43.

97. Pal A, Young MA, Donato NJ. Emerging Potential of Therapeutic Targeting of Ubiquitin-Specific Proteases in the Treatment of Cancer. Cancer Res. 2014;74:4955–66.

98. Murakawa Y, Sonoda E, Barber LJ, Zeng W, Yokomori K, Kimura H, et al. Inhibitors of the proteasome suppress homologous DNA recombination in mammalian cells. Cancer Res. 2007;67:8536–43.

99. Galanty Y, Belotserkovskaya R, Coates J, Jackson SP. RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. Genes Dev. 2012;26:1179–95.

100. Wu W, Sato K, Koike A, Nishikawa H, Koizumi H, Venkitaraman AR, et al. HERC2 is an E3 ligase that targets BRCA1 for degradation. Cancer Res 2010;70:6384–92.

101. Shi W, Ma Z, Willers H, Akhtar K, Scott SP, Zhang J, et al. Disassembly

of MDC1 foci is controlled by ubiquitin-proteasome-dependent degradation. J Biol Chem. 2008;283:31608–16.

102. Brown JS, Lukashchuk N, Sczaniecka-Clift M, Britton S, le Sage C, Calsou P, et al. Neddylation Promotes Ubiquitylation and Release of Ku from DNA-Damage Sites. Cell Rep. 2015;704–14.

103. Soucy TA, Smith PG, Milhollen M a, Berger AJ, Gavin JM, Adhikari S, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature. 2009458:732–6.

104. Sarantopoulos J, Shapiro GI, Cohen RB, Clark JW, Kauh JS, Weiss GJ, et al. Phase I Study of the Investigational NEDD8-activating Enzyme Inhibitor Pevonedistat (TAK-924/MLN4924) in Patients with Advanced Solid Tumors. Clin Cancer Res. 2015;1–12.

105. Garcia K, Blank JL, Bouck DC, Liu XJ, Sappal DS, Hather G, et al. Nedd8-Activating Enzyme Inhibitor MLN4924 Provides Synergy with Mitomycin C through Interactions with ATR, BRCA1/BRCA2 and Chromatin Dynamics Pathways. Mol Cancer Ther. 2014;13:1625–35.

106. Kee Y, Huang M, Chang S, Moreau L a, Park E, Smith PG, et al. Inhibition of the Nedd8 system sensitizes cells to DNA interstrand cross-linking agents. Mol Cancer Res. 2012;10:369–77.

107. Fathers C, Drayton RM, Solovieva S, Bryant HE. Inhibition of poly(ADP-ribose) glycohydrolase (PARG) specifically kills BRCA2-deficient tumor cells. Cell Cycle. 2012;11:990–7.

108. James D, Jordan A, Hamilton N, McGonagle A, Smith K, Stowell A, et al. Abstract 2745: Pharmacological characterisation of cell active inhibitors of Poly(ADP-ribose) glycohydrolase (PARG). Cancer Res.

2014;74:2745–2745.

109. West AC, Johnstone RW. New and emerging HDAC inhibitors for cancer treatment. J Clin Invest. 2014;124:30–9.

110. Bose P, Dai Y, Grant S. Histone deacetylase inhibitor (HDACI) mechanisms of action: Emerging insights. Pharmacol Ther. 2014;143:323–36.

111. Miller KM, Tjeertes J V, Coates J, Legube G, Polo SE, Britton S, et al. Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol. 2010;17:1144-51.

112. Campbell S, Ismail IH, Young LC, Poirier GG, Hendzel MJ. Polycomb repressive complex 2 contributes to DNA double-strand break repair. Cell Cycle. 2013;12:2675–83.

113. Agarwal P, Jackson SP. G9a inhibition potentiates the anti-tumour activity of DNA double-strand break inducing agents by impairing DNA repair independent of p53 status. Cancer Lett. 2016;380:467–75.

114. Konstantinopoulos PA, Spentzos D, Karlan BY, Taniguchi T, Fountzilas E, Francoeur N, et al. Gene expression profile of BRCAness that correlates with responsiveness to chemotherapy and with outcome in patients with epithelial ovarian cancer. J Clin Oncol. 2010;28:3555–61.

115. Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. N Engl J Med. 2015;373:1697–708.

116. Helleday T, Eshtad S, Nik-Zainal S. Mechanisms underlying mutational signatures in human cancers. Nat Rev Genet. 2014;15:585–98.

117. Timms KM, Abkevich V, Hughes E, Neff C, Reid J, Morris B, et al. Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. Breast Cancer Res. 2014;16:1–9.
118. Graeser M, McCarthy A, Lord CJ, Savage K, Hills M, Salter J, et al. A marker of homologous recombination predicts pathologic complete response to neoadjuvant chemotherapy in primary breast cancer. Clin Cancer Res. 2010;16:6159–68.
119. Reaper PM, Griffiths MR, Long JM, Charrier J-D, MacCormick S, Charlton PA, et al. Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR. Nat Chem Biol. 2011;7:428–30.
120. Toledo LI, Murga M, Zur R, Soria R, Rodriguez A, Martinez S, et al. A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. Nat Struct Mol Biol. 2011;18:721–7.
121. Murga M, Campaner S, Lopez-Contreras AJ, Toledo LI, Soria R, Montaña MF, et al. Exploiting oncogene-induced replicative stress for the selective killing of Myc-driven tumors. Nat Struct Mol Biol. 2011;18:1331–5.
122. Grabocka E, Commisso C, Bar-Sagi D. Molecular pathways: Targeting the dependence of mutant RAS cancers on the DNA damage response. Clin Cancer Res. 2015;21:1243–7.
123. Schmutz I, de Lange T. Shelterin. Curr Biol. 2016;26:R397–9.
124. Grundy GJ, Moulding H a, Caldecott KW, Rulten SL. One ring to bring them all-The role of Ku in mammalian non-homologous end joining.

DNA Repair. 2014 [cited 2014 Apr 1];12–5.

125. Tong AS, Stern JL, Sfeir A, Kartawinata M, de Lange T, Zhu XD, et al. ATM and ATR Signaling Regulate the Recruitment of Human Telomerase to Telomeres. Cell Rep. 2015;13:1633–46.

126. Flynn R, Cox K, Jeitany M, Wakimoto H, Bryll A, Ganem N, et al. Alternative Lengthening of Telomeres Renders Cancer Cells Hypersensitive to ATR Inhibitors. Science. 2015;347:273–7.

127. Oza AM, Cibula D, Benzaquen AO, Poole C, Mathijssen RHJ, Sonke GS, et al. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial. Lancet Oncol. 2015;16:87–97.

128. Dent RA, Lindeman GJ, Clemons M, Wildiers H, Chan A, McCarthy NJ, et al. Safety and efficacy of the oral PARP inhibitor olaparib (AZD2281) in combination with paclitaxel for the first- or second-line treatment of patients with metastatic triple-negative breast cancer: Results from the safety cohort of a phase I/II multicenter. J Clin Oncol 28:15s, 2010 (suppl; abstr 1018).

129. Bang Y-J, Im S-A, Lee K-WKH, Cho JY, Song E-K, Lee K-WKH, et al. Randomized, Double-Blind Phase II Trial With Prospective Classification by ATM Protein Level to Evaluate the Efficacy and Tolerability of Olaparib Plus Paclitaxel in Patients With Recurrent or Metastatic Gastric Cancer. J Clin Oncol. 2015;33:3858–65.

130. Karachaliou N, Costa C, Gimenez-Capitan A, Molina-Vila MA, Bertran-Alamillo J, Mayo C, et al. BRCA1, LMO4, and CtIP mRNA expression in erlotinib-treated non-small-cell lung cancer patients with EGFR

mutations. J Thorac Oncol. 2013;8:295–300.

131. Campelo RG, Felip E, Massuti B, Majem M, Carcereny Costa E, Palmero R, et al. Phase IB study to evaluate efficacy and tolerability of olaparib (AZD2281) plus gefitinib in patients (P) with epidermal growth factor receptor (EGFR) mutation positive advanced non-small cell lung cancer (NSCLC) (NCT=1513174/GECP-GOAL). J Clin Oncol 32:5s, 2014 (suppl; abstr 8079).
132. Massuti B, Garcia Campelo R, Rodriguez Abreu D, Remon J, Majem M, Galvez E, et al. Open, phase II randomized trial of gefitinib alone versus olaparib (AZD2281) plus gefitinib in advanced non-small cell lung cancer (NSCLC) patients (P) with epidermal growth factor receptor (EGFR) mutations: Spanish Lung Cancer Group trial (NCT=1513174/GECP-GOAL). J Clin Oncol 32:5s, 2014 (suppl; abstr TPS8127).
133. Du Y, Yamaguchi H, Wei Y, Hsu JL, Wang H-L, Hsu Y-H, et al. Blocking c-Met–mediated PARP1 phosphorylation enhances anti-tumor effects of PARP inhibitors. Nat Med. 2016;22.
134. Juvekar A, Burga LN, Hu H, Lunsford EP, Ibrahim YH, Balmañà J, et al. Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer. Cancer Discov. 2012;2:1048–63.
135. Ibrahim YH, García-García C, Serra V, He L, Torres-Lockhart K, Prat A, et al. PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov. 2012;2:1036–47.

136. Matulonis U, Wulf GM, Birrer MJ, Westin SN, Quy P, Bell-McGuinn KM, et al. Phase I study of oral BKM120 and oral olaparib for high-grade serous ovarian cancer (HGSC) or triple-negative breast cancer (TNBC). J Clin Oncol 32:5s, 2014 (suppl; abstr 2510).

137. Michalarea V, Roda D, Drew Y, Carreira S, O'Carrigan BS, Shaw H, et al. Abstract CT010: Phase I trial combining the PARP inhibitor olaparib (Ola) and AKT inhibitor AZD5363 (AZD) in germline (g)BRCA and non-BRCA mutant (m) advanced cancer patients (pts) incorporating noninvasive monitoring of cancer mutations. Cancer Res. 2016 Jul 22;76(14 Supplement):CT010.

138. Bindra RS, Gibson SL, Meng A, Westermark U, Jasin M, Pierce AJ, et al. Hypoxia-induced down-regulation of BRCA1 expression by E2Fs. Cancer Res. 2005;65:11597–604.

139. Bindra RS, Schaffer PJ, Meng A, Woo J, Måseide K, Roth ME, et al. Down-Regulation of Rad51 and Decreased Homologous Recombination in Hypoxic Cancer Cells. Mol Cell Biol. 2004;24:8504–18.

140. Lim JJ, Yang K, Taylor-Harding B, Wiedemeyer WR, Buckanovich RJ. VEGFR3 Inhibition Chemosensitizes Ovarian Cancer Stemlike Cells through Down-Regulation of BRCA1 and BRCA2. Neoplasia. Neoplasia Press, Inc.; 2014;16:343–353.e2.

141. Liu JF, Barry WT, Birrer M, Lee J-M, Buckanovich RJ, Fleming GF, et al. Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. Lancet Oncol. 2014;15:1207–14.

142. Schiewer MJ, Knudsen KE. Linking DNA Damage and Hormone

Signaling Pathways in Cancer. Trends Endocrinol Metab. 2016;27:216–25.

143. Schiewer MJ, Goodwin JF, Han S, Chad Brenner J, Augello MA, Dean JL, et al. Dual roles of PARP-1 promote cancer growth and progression. Cancer Discov. 2012;2:1134–49.

144. Clarke NW, Shepard R, Spencer S, Jones RH. Olaparib combined with abiraterone in patients with metastatic prostate cancer: Safety run-in from a phase II study. J Clin Oncol 33, 2015 (suppl; abstr e16026).

145. Higuchi T, Flies DB, Marjon NA, Mantia-Smaldone G, Ronner L, Gimotty PA, et al. CTLA-4 Blockade Synergizes Therapeutically with PARP Inhibition in BRCA1-Deficient Ovarian Cancer. Cancer Immunol Res. 2015;3:1257–68.

146. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372:2509–20.

147. Kelderman S, Schumacher TN, Kvistborg P. Mismatch Repair-Deficient Cancers Are Targets for Anti-PD-1 Therapy. Cancer Cell. 2015;28:11–3.

148. Chatzinikolaou G, Karakasilioti I, Garinis GA. DNA damage and innate immunity: Links and trade-offs. Trends Immunol. 2014;35:429–35.

149. Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, et al. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst. 2000;92:564–9.

150. Yap T a, Sandhu SK, Workman P, de Bono JS. Envisioning the future of early anticancer drug development. Nat Rev Cancer. 2010;10:514–23.

151. Ciccia A, Elledge SJ. The DNA Damage Response: Making It Safe to

Play with Knives. Mol Cell. 2010;40:179–204.

152. Deriano L, Roth DB. Modernizing the Nonhomologous End-Joining Repertoire: Alternative and Classical NHEJ Share the Stage. Annu Rev Genet. 2013;47:451–73.

153. Deans AJ, West SC. DNA interstrand crosslink repair and cancer. Nat Rev Cancer. 2011;11:467–80.

154. Fu D, Calvo JA, Samson LD. Balancing repair and tolerance of DNA damage caused by alkylating agents. Nat Rev Cancer. 2012;12.

155. Sale JE. Competition, collaboration and coordination--determining how cells bypass DNA damage. J Cell Sci. 2012;125:1633–43.

156. Fousteri M, Mullenders LHF. Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. Cell Res. 2008;18:73–84.

157. Jiricny J. The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol. 2006;7:335–46.

158. Karp JE, Thomas BM, Greer JM, Sorge C, Gore SD, Pratz KW, et al. Phase I and pharmacologic trial of cytosine arabinoside with the selective checkpoint 1 inhibitor Sch 900776 in refractory acute leukemias. Clin Cancer Res. 2012;18:6723–31.

159. Calvo E, Chen VJ, Marshall M, Ohnmacht U, Hynes SM, Kumm E, et al. Preclinical analyses and phase I evaluation of LY2603618 administered in combination with pemetrexed and cisplatin in patients with advanced cancer. Invest New Drugs. 2014;32:955–68.

160. Bauer TM, Fields Jones S, Greenlees C, Cook C, Jewsbury PJ, Mugundu G, et al. A Phase Ib, Open-Label, Multi-Center Study to

Assess the Safety, Tolerability, Pharmacokinetics, and Anti-tumor Activity of AZD1775 Monotherapy in Patients with Advanced Solid Tumors: Expansion Cohorts. J Clin Oncol 34, 2016 (suppl; abstr TPS2608).

161. Hamilton EP, Wang JSZ, Falchook G, Fields Jones S, Cook C, Mugundu G, et al. A phase Ib study of AZD1775 and olaparib combination in patients with refractory solid tumors. J Clin Oncol 34, 2016 (suppl; abstr 5562).

162. Chera BS, Gupta GP, Weiss J, Grilley-Olson JE, Moore DT, Zevallos J, et al. Phase ib trial of dose-escalating AZD1775 in combination with concurrent radiation and cisplatin for intermediate and high risk head and neck squamous cell carcinoma. J Clin Oncol 34, 2016 (suppl; abstr TPS6106).

Double strand break repair pathways	
Classical (c)-NHEJ	• Predominant DNA DSB repair pathway in human cells, functioning throughout the cell cycle. • Involves the relatively rapid ligation of broken DNA ends, mediated by the core NHEJ complex including, DNA-PK, XRCC4, LIG4, XLF and PAXX amongst others. • DNA end-processing and DNA polymerase action may be required before ligation can occur, making NHEJ inherently error-prone. • NHEJ maintains genome stability however, by rapidly repairing DSBs in circumstances where recombinogenic events would likely result in gross chromosomal rearrangements; in non-cycling or G1 cells for example (38,39).
Homology-directed repair	
Homologous recombination (HR)	• Relatively slow and restricted to late-S phase/G2 as it generally relies on a homologous sister chromatid DNA strand for repair. • Extensive DNA end-resection by helicases and exonucleases such as DNA2, BLM, WRN and EXO1 results in a 3′ –ssDNA overhang, committing the break to repair by HR. • RPA coats and stabilizes the ssDNA, leading to ATR activation and subsequent signaling events. • BRCA2, with the help of BRCA1 and PALB2, load RAD51 onto the RPA-coated ssDNA leading to strand invasion, with a number of factors negatively regulating this process to prevent hyper-recombination such as POLQ, PARI, RECQL5, FANCJ and BLM (151).
Alternative (Alt)- NHEJ or microhomology mediated end-joining (MMEJ)	• Ligation pathway for DSBs when c-NHEJ is genetically compromised (152). • Occurs following limited DNA end resection. • Contributes to the excessive genomic deletions and chromosomal translocations seen in tumors and may also provide a back-up repair pathway in HR deficient cells (10,20).
Single-strand annealing (SSA)	• Mutagenic, RAD51 independent repair pathway, involving annealing of short or longer complimentary DNA sequences on resected DNA with subsequent deletion of the intervening DNA sequence. The detailed mechanism has yet to be defined in mammalian cells (20).
Other repair pathways	
Inter-strand crosslink (ICL) repair	• ICLs cause DNA replication fork stalling and collapse, resulting in DNA DSBs. • ICLs are recognised by the FANCONI core complex, which engages HR, TLS and NER pathways to repair the DNA lesion (153).
Single-strand break (SSB) repair	• SSBs usually arise following the removal of a damaged nucleotide (154). • PARP1 (poly-ADP-ribose polymerase 1) is the DNA damage sensor protein for DNA strand breaks. PARP1 localises to sites of DNA damage, generating extensive PAR (poly ADP-ribose) chains. • Ribosylated PARP1 promotes recruitment of SSB-repair proteins to DNA damage sites (9).
Base excision repair (BER)	• DNA glycosylases recognize and remove damaged bases leading to basic sites that are processed by APE1 (AP-endonuclease 1). • Results in SSB generation, repaired using SSB repair pathways (86).
Tran-lesion synthesis (TLS)	• DNA damage tolerance pathway that helps prevent replication fork stalling (155). • Engages low-fidelity DNA Y-family polymerases (e.g. REV1, POLH, POLI, POLK) that accommodate the damaged lesion, replicating past it, at the expense of increased mutagenesis.
Nucleotide excision repair (NER)	• Removes helix-distorting lesions from DNA, in particular the UV-induced photo lesions. • Involves removal of a short oligonucleotide including the damaged lesion using structure specific endonucleases and subsequent restoration of the DNA sequence by DNA polymerases (156).

Mismatch repair (MMR)	• MSH2, MSH3 and MSH6 recognize base-base mismatches and IDLs, where they recruit MLH1 and PMS2 to damaged sites. The concerted actions of the mismatch repair proteins, engage EXO1 to remove the mismatch and then POLD and LIG1 to fill the gap and seal the nick respectively (157).

Table 1: Predominant DNA repair pathways.

Target	Agent	Phase	Intervention	Cancer(s) enrolled	Status / results	Trial identifier	Ref.
DNA-PK	MSC2490484A	I	MSC2490484A	Solid tumors, CLL	Recruiting	NCT02316197	-
	MSC2490484A	I	MSC2490484A ± RT	Solid tumors	Recruiting	NCT02516813	-
	VX-984	I	VX-984 ± PLD	Solid tumors	Recruiting	NCT02644278	-
	CC-115[1]	I	CC-115	GBM, HNSCC, Prostate, ES, CLL	37% (3/8) PR in relapsed ATM^{mut} CLL	NCT01353625	(45,46)
ATM	AZD0156	I	AZD0156 ± olaparib	Solid tumors	Recruiting	NCT02588105	-
ATR	VX-970 ± chemotherapy	I	VX-970 ± carboplatin	Solid tumors	Recruiting	EudraCT: 2013-005100-34	(67,68)
		I	VX-970 ± gemcitabine, etoposide, cisplatin or carboplatin	Solid tumors	Recruiting	NCT02157792	(69,70)
		II	Gemcitabine ± VX-970 (randomised)	Ovarian, primary peritoneal or Fallopian tube	Recruiting	NCT02595892	-
		I	VX-970 + irinotecan	Solid tumors	Recruiting	NCT02595931	-
		II	Carboplatin + gemcitabine ± VX-970 (randomised)	Advanced gynecologic cancers	Not yet recruiting	NCT02627443	-
		I / II	VX-970 + topotecan	Advanced NSCLC, SCLC, Gynae or neuroendocrine	Recruiting	NCT02487095	-
		II	Cisplatin + gemcitabine ± VX-970 (randomised)	Advanced urothelial	Recruiting	NCT02567409	-
	VX-970 ± RT	I	Cisplatin + RT ± VX-970	Locally advanced HNSCC	Recruiting	NCT02567422	-
		I	Whole brain RT + VX-970	NSCLC brain metastases	Recruiting	NCT02589522	-

	VX-970 + targeted therapy	I	VX-970 + veliparib + cisplatin	Solid tumors	Recruiting	NCT02723864	-
	AZD6738	I	AZD6738	Relapsed CLL, PLL, B cell lymphomas	Complete, full results awaited	NCT01955668	-
	AD6738 ± chemotherapy	I	AZD6738 ± carboplatin, olaparib or MEDI4736	Solid tumors, HNSCC, ATM^{loss} NSCLC, gastric or GOJ carcinoma	Recruiting	NCT02264678	-
		I	AZD6738 + paclitaxel	Solid tumors	Recruiting	NCT02630199	-
	AZD6738 + RT	I	AZD6738 + palliative RT	Solid tumors	Recruiting	NCT02223923	-
CHK1	MK8776 (SCH 900776)	II	Cytarabine ± MK8776 (randomised)	Relapsed AML	Complete, full results awaited	NCT01870596	-
		I	Gemcitabine + MK8776	Relapsed lymphoma	Complete, full results awaited	NCT00779584	(72)
		I	Cytarabine + MK8776	Relapsed AML	33% (8/24) CR	NCT00907517	(158)
	LY2603618	I / II	Cisplatin / pemetrexed ± LY2603618	NSCLC	14% (2/14) PR	NCT01139775	(159)
		I / II	Gemcitabine ± LY2603618	Pancreatic carcinoma	Complete, full results awaited	NCT00839332	-
		II	Pemetrexed + LY2603618	NSCLC	Complete, full results awaited	NCT00988858	-
		I	LY2603618 + pemetrexed or gemcitabine	Solid tumors	Complete, full results awaited	NCT01296568	-
		I	LY2603618 + gemcitabine	Solid tumors	Complete, full results awaited	NCT01341457	(73)
		I	LY2603618 + pemetrexed	Solid tumors	Complete, full results awaited	NCT00415636	-
	CCT245737	I	CCT245737	Solid tumors	Recruiting	NCT02797964	-
		I	CCT245737 + cisplatin and/or gemcitabine	Solid tumors	Recruiting	NCT02797977	-

	GDC-0575 ± chemotherapy	I	GDC-0575 ± gemcitabine	Solid tumors, relapsed lymphoma	Complete, full results awaited	NCT01564251	-
CHK1/2	LY2606368	II	LY2606368	Refractory SCLC	Recruiting	NCT02735980	-
		II	LY2606368	Ovarian, breast, prostate	Recruiting	NCT02203513	-
		II	LY2606368	Solid tumors	Recruiting	NCT02873975	-
		I	LY2606368	Solid tumors	4% (2/45) PR – anal SCC, HNSCC	NCT01115790	(76)
		I	LY2606368	Solid tumors	Recruiting	NCT02778126	-
		I	LY2606368	Solid tumors	Recruiting	NCT02514603	-
		I	LY2606368	Pediatric solid tumors	Recruiting	NCT02808650	-
	LY2606368 + chemotherapy	I	LY2606368 + fludarabine + cytarabine	Relapsed AML, high risk MDS	Recruiting	NCT02649764	-
		I	LY2606368 + cisplatin, cetuximab, pemetrexed, fluorouracil and/or leucovorin	Solid tumors	Recruiting	NCT02124148	-
	LY2606368 + targeted therapy	I	LY2606368 + ralimetinib (MAPK inhibitor)	Solid tumors	Recruiting	NCT02860780	-
	LY2606368 + RT	I	LY2606368 + RT + cisplatin or cetuximab	Locally advanced HNSCC	Recruiting	NCT02555644	-
WEE1	AZD1775	II	AZD1775	SCLC	Recruiting	NCT02593019	-
		I	AZD1775	Solid tumors	8% (2/25) PR: $BRCA^{mut}$	NCT01748825	(77,82)

					ovarian, BRCA^{mut} HNSCC		
		I	AZD1775	Solid tumors	Recruiting	NCT02482311	(160)
		I	AZD1775	Solid tumors	Recruiting	NCT02610075	-
	AZD1775 + chemotherapy	II	Carboplatin / paclitaxel ± AZD1775	Ovarian, TP53^{mut}	Complete, full results awaited	NCT01357161	(85)
		II	Carboplatin + AZD1775	Ovarian, TP53^{mut} or platinum resistant	ORR 9/21 (43%), with prolonged CR in 1/21 (5%). Median PFS: 5.3 months. Median OS: 12.6 months; two patients with ongoing response for more than 31 and 42 months at data cutoff.	NCT01164995	(84)
		II	Gemcitabine ± AZD1775	Ovarian, Primary Peritoneal, or Fallopian	Recruiting	NCT02101775	-
		II	Carboplatin / pemetrexed ± AZD1775	NSCLC, 1st line	Complete, full results awaited	NCT02087241	-
		II	Docetaxel + AZD1775	NSCLC, 2nd line	Closed	NCT02087176	-
		II	Carboplatin / Paclitaxel + AZD1775	NSCLC	Recruiting	NCT02513563	-
		II	Paclitaxel weekly + AZD1775	Gastric carcinoma	Recruiting	NCT02448329	-
		II	Cisplatin ± AZD1775	HNSCC	Complete, full results awaited	NCT02196168	-
		I / II	Gemcitabine / nab-	Pancreatic	Recruiting	NCT02194829	-

			paclitaxel ± AZD1775				
		I / II	Irinotecan + AZD1775	Pediatric solid tumors	Recruiting	NCT02095132	-
		I	Cisplatin / docetaxel + AZD1775	Locally advanced HNSCC	Recruiting	NCT02508246	-
		I	Gemcitabine, cisplatin or carboplatin + AZD1775	Solid tumors	PR in 17/176 (10%). RR higher in TP53-mutated vs wild-type patients: 21% (n = 19) vs 12% (n=33)	NCT00648648	(83)
	AZD1775 + targeted therapy	I	AZD1775 + olaparib (PARP inhibitor)	Solid tumors	Complete, full results awaited	NCT02511795	(161)
			AZD1775 + Belinostat (HDAC inhibitor)	AML, other myeloid malignancies	Recruiting	NCT02381548	-
	AZD1775 + RT	I / II	Radiation + gemcitabine + AZD1775	Pancreatic	Recruiting	NCT02037230	-
		I	Radiation + cisplatin + AZD1775	HNSCC	Recruiting	NCT02585973	(162)
		I	RT + cisplatin + AZD1775	Locally advanced cervical cancer	Recruiting	NCT01958658	-
		I	Radiation + temozolomide + AZD1775	GBM	Recruiting	NCT01849146	-
		I	Radiation + AZD1775	Pediatric DIPG	Recruiting	NCT01922076	-
	AZD1775 + immune	I	MEDI4736 + AD1775	Solid tumors	Recruiting	NCT02617277	-

	checkpoint inhibitor						
BER	TRC102	I	TRC102	Solid tumors, lymphoma	Complete, full results awaited	NCT01851369	(90)
	TRC102 + chemotherapy	II	TRC102 + temozolomide	GBM	Recruiting	NCT02395692	-
		I / II	TRC102 + cisplatin and/or pemetrexed	Solid tumors	Recruiting	NCT02535312	-
		I	TRC102 + pemetrexed	Solid tumors	4% (1/28) PR: HNSCC	NCT00692159	(89)
		I	TRC102 + fludarabine	Haematologic malignancies	Complete, full results awaited	NCT01658319	-
	TRC102 + RT	I	TRC + RT + cisplatin ± pemetrexed	NSCLC	Recruiting	NCT02535325	-

Table 2: Monotherapy and combination trials involving DDR inhibitors that have completed or are still active (www.clinicaltrials.gov). CLL, chronic lymphocytic leukemia; CR, complete response; CRUK, Cancer Research UK; DIPG, diffuse intrinsic pontine gliomas; ES, Ewing's sarcoma; GBM, glioblastoma multiforme; GOJ, gastro-oesphageal carcinoma; HDAC, Histone deacetylase; HGSOC, high grade serous ovarian carcinoma; HNSCC, head and neck squamous cell carcinoma; mAb, monoclonal antibody; NCI, National Cancer Institute; NSCLC, non-small cell lung carcinoma; ORR, objective response rate; PLD, pegylated liposomal doxorubicin; PLL, prolymphocytic leukaemia; PR, partial response; RT, radiotherapy; SCLC, small cell lung carcinoma. [1] Dual DNA-PK and mTOR inhibitor.

FIGURE LEGENDS

Figure 1

Table showing predominant sensors, signaling and effector proteins for major DNA repair pathway. Main targets of drug development are in red (see text for details).

Repair pathway	NHEJ	HR	alt-NHEJ/ MMEJ	SSA	ICL repair	SSB repair	BER	TLS	NER	MMR
Source of DNA damage	IR, radiomimetics, topo II inhibitors	X-linking agents, replication inhibitors, anti-metabolites, topo I inhibitors			X-linking agents	IR, ROS, radiomimetics topo I inhibitors H2O2, alkylating agents	Alkylating agents	UV, alkylating agents	Alkylating agents, X-linkers	DNA pol proofreading errors
Damage sensors	Ku70/Ku80	MRN	PARP	MRN	FA core complex (FANCA, B, C,E, F, G, L and M)	PARP	DNA glycosylases, APE1	PCNA	XPC DDB2 CSA	MSH2, MSH3, MSH6, MLH1, PMS2
Signalling/ mediator proteins	DNAPK	ATM, ATR, MK2, CtIP, BRCA1/BARD1, BRCA2, PALB2, RPA		CtIP	FANCD1 [BRCA2] D2, I J [BRIP1] N [PALB2] O [RAD51C] P [SLX4]			RAD6 RAD18	XPA, XPF RPA	
Effector proteins	XRCC4 XLF LIG4 APLF Artemis PAXX WRN	RAD51 MUS81/EME1 SLX1/SLX4 RTEL1 BLM TOPOIII POLQ PARI RECQL5 FANCJ, BLM	XRCC1 LIG3, LIG1 CtIP POLQ	RAD52 others?	Shared with HR, TLS and NER.	XRCC1 PNKP POLBeta FEN1, TDP1 Aprataxin, LIG1, LIG3A	As SSB repair	REV1, POLH POLI, POLK.	XPG ERCC1 POLE POLD1 LIG1, LIG3	EXO1 POLD LIG1

Figure 1

Figure 2

DNA DSB repair signaling pathways through the apical DDR kinases.
A. DNA-PK: Ku binds to DNA DSBs and recruits DNA-PKcs. Upon DNA binding, autophosphorylation of DNA-PKcs induces a conformational change that destabilises the NHEJ core complex, causing sliding of Ku inwards on the DNA and enabling access of end-processing and ligation enzymes to DNA ends and facilitation of repair.
B. ATM: Following DSBs ATM is predominantly activated through interactions with NBS1 of the MRN complex. ATM is the principle kinase responsible for phosphorylation of histone H2AX on serine 139 (known as γH2AX). MDC1 (mediator of DNA damage checkpoint protein1) directly binds γH2AX and potentiates DNA damage signaling leading to spreading of γH2AX to over a megabase from its initial lesion. This in turn promotes recruitment and retention of DNA damage mediator proteins such as 53BP1. CHK2 is a well-studied ATM substrate.
C. ATR: ATR is activated by RPA (replication protein A) bound to ssDNA. The ATR-CHEK1 signaling cascade activates the G2-M checkpoint, promotes replication fork stabilisation and slows DNA replication by suppressing origin firing.

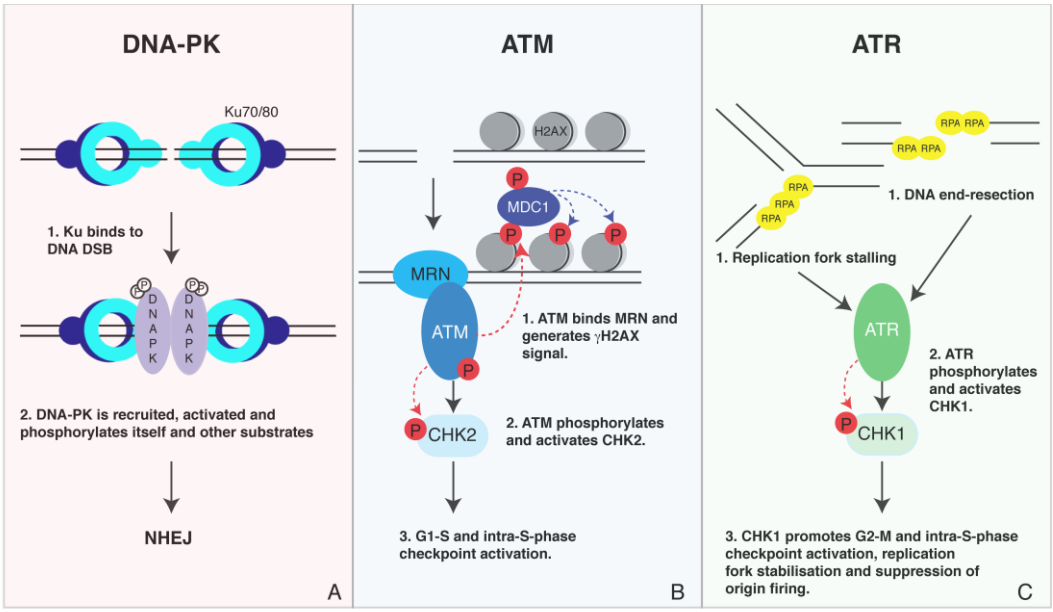

Figure 2