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Review article

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Targeting DNA damage response pathways in cancer

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Abstract

Cells have evolved a complex network of biochemical pathways, collectively known as the DNA damage response (DDR), to prevent detrimental mutations from being passed on to their progeny. The DDR coordinates DNA repair with cell-cycle checkpoint activation and other global cellular responses. Genes encoding DDR factors are frequently mutated in cancer, causing genomic instability, an intrinsic feature of many tumours that underlies their ability to grow, metastasize and respond to treatments that inflict DNA damage (such as radiotherapy). One instance where we have greater insight into how genetic DDR abrogation impacts on therapy responses is in tumours with mutated *BRCA1* or *BRCA2*. Due to compromised homologous recombination DNA repair, these tumours rely on alternative repair mechanisms and are susceptible to chemical inhibitors of poly(ADP-ribose) polymerase (PARP), which specifically kill homologous recombination-deficient cancer cells, and have become a paradigm for targeted cancer therapy. It is now clear that many other synthetic-lethal relationships exist between DDR genes. Crucially, some of these interactions could be exploited in the clinic to target tumours that become resistant to PARP inhibition. In this Review, we discuss state-of-the-art strategies for DDR inactivation using small-molecule inhibitors and highlight those compounds currently being evaluated in the clinic.

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Introduction

DNA is a relatively stable organic molecule, but our genomes are nonetheless subjected to constant assault from various sources of endogenous and exogenous damage. Cells have therefore evolved a complex system of biochemical pathways to deal with this threat, collectively termed the 'DNA damage response' (DDR)[1].

The physiological importance of the DDR in humans is highlighted by the fact that mutations in genes encoding enzymes involved in the DDR (DDR genes) are found in cancer cells and in the germ lines of individuals with a hereditary predisposition to cancer[2]. Indeed, genome instability caused by deficiencies in the DDR is a hallmark of cancer, because a greater mutational burden increases the chances of oncogene activation and loss of tumour suppressor genes, leading to tumorigenesis[3]. A more genetically diverse population of cancer cells within a tumour also increases the chances that resistant clones will arise after radiotherapy or chemotherapy, thus facilitating cancer recurrence[4].

However, the inherent genome instability of rapidly proliferating tumours also provides us with therapeutic opportunities to target DDR pathways and thus specifically kill cancer cells via additional replication stress, exogenous DNA damage and/or DDR inhibition. This probably explains in large part the early therapeutic success of radiotherapy and various DNA-damaging chemotherapeutics, such as alkylating agents and topoisomerase inhibitors[1]. However, such agents tend to be indiscriminate and damage healthy tissues, leading to substantial side effects.

Because specific genetic vulnerabilities have been identified in certain cancers, we are currently in a unique position to exploit the concept of synthetic lethality in the clinic, whereby loss of one cellular pathway results in high reliance on another pathway, which is not essential under normal settings (Tables 1, 2). This is perhaps best exemplified by the use of poly(ADP-ribose) polymerase (PARP) inhibitors to specifically target tumours deficient in the homologous recombination (HR) DNA repair factor BRCA1 or BRCA2 (BRCA1/2)[5,6] or tumours with otherwise compromised HR (Boxes 1, 2). It has subsequently become apparent that some tumours with wild type *BRCA1*/*BRCA2* genes can also be hypersensitive to PARP inhibition through mechanisms independent of HR inactivation[7]. In addition, targeting the DDR in cancer is not confined to PARP inhibition (Table 2), as other potential DDR targets have been identified in recent years; small-molecule inhibitors of several targets have been developed, and some are being tested in clinical trials (Table 1).

In this Review, we describe the major DNA damage checkpoint and repair pathways and discuss the strategies for DDR inactivation using small-molecule inhibitors that are currently being evaluated in the clinic. Furthermore, we highlight the mechanisms of DDR inhibitor drug resistance and how these might be overcome through understanding and targeting the molecular pathways underlying resistant tumours.

The DDR

DNA damage activates a signalling cascade mediated by checkpoint kinases

The DDR starts with lesion recognition and engagement of DNA repair pathways[8]. Depending on the type and complexity of genotoxic stress, cellular signalling cascades may be launched that alter the surrounding chromatin, activate cell-cycle checkpoints and modify gene expression via changes to transcription or translation. If lesions are not rapidly repaired, persistent DDR signalling can also alter cell fate by promoting differentiation, senescence or programmed cell death. The DDR thereby maintains genome stability to maximize the chances of the hereditary material being passed on to the next generation intact and unchanged.

ATM and ATR are the kinases responsible for orchestrating cellular responses to DNA double-strand breaks (DSBs) and replication stress (Fig. 1), which include DNA repair, checkpoint activation, apoptosis, senescence, and alterations in chromatin structure, transcription and pre-mRNA splicing[8]. To achieve this, they phosphorylate hundreds of substrates in response to DNA damage (see, for example, ref.[9]). The downstream cell-cycle checkpoint kinases CHK1 and CHK2 are major substrates for ATR and ATM, respectively, and are responsible for downregulating the activity of cyclin-dependent kinases (CDKs) to halt cell-cycle progression in response to genotoxic stress. This is achieved in G1 phase cells by ATM and CHK2 through phosphorylation and stabilization of p53, which results in transcription of *CDKN1A*, which encodes the CDK inhibitor p21 (ref.[10]) (Fig. 1). ATM is recruited at DSBs and promotes histone H2AX phosphorylation, which in turn recruits mediator of DNA damage checkpoint protein 1 (MDC1). In interphase cells, MDC1 recruitment and phosphorylation by ATM catalyse further phosphorylation and ubiquitylation events that result in recruitment of the DNA damage mediator proteins 53BP1 and BRCA1; however, neither 53BP1 nor BRCA1 are recruited during mitosis[11]. Instead, casein kinase 2 (CK2)-dependent MDC1 phosphorylation mediates the interaction of MDC1 and DNA topoisomerase 2-binding protein 1 (TOPBP1)[12], which is recruited to DSBs together with cellular inhibitor of PP2A (CIP2A)[13] (Fig. 1). Together, TOPBP1 and CIP2A form filamentous structures that can bridge two MDC1 foci, thus tethering both DSB ends together[12,13]. This ensures correct chromosome segregation until DSBs are repaired in the following G1 phase.

In S and G2 phases of the cell cycle, ATR and its downstream target CHK1 are primarily responsible for the checkpoint activation via phosphorylation and inactivation of the CDC25 family of phosphatases[14–16]. These remove the inhibitory phosphorylation from CDK1 and CDK2, deposited by the kinase WEE1 (refs.[17,18]) and the WEE1-like kinase PKMYT1 (refs.[19,20]) (Fig. 1). Moreover, ATR controls the S–G2 checkpoint that prevents entry into mitosis before replication is completed[21,22]. In contrast to ATM, which is activated by binding of the MRE11–RAD50–NBS1 (MRN) complex to DSBs[23,24], ATR is recruited by ATR-interacting protein (ATRIP), which binds to replication protein A (RPA)-coated single-stranded DNA (ssDNA) generated at stalled replication forks[25]. ATR is then activated by the replication stress response protein ETAA1 (refs.[26–28]) or TOPBP1 (ref.[29]), which bind to RPA at ssDNA or to the RAD9–RAD1–HUS1 (9-1-1) complex at ssDNA–double-stranded DNA junctions, respectively[8]. Therefore, ATR is activated in S phase in response to a broad range of genotoxic lesions, including stalled replication forks, leading or lagging strand ssDNA gaps or resected DSBs[21,22].

Multiple DNA repair pathways deal with different DNA lesions

The most common types of DNA damage are lesions where only one strand of the DNA double helix is affected, either by single-strand breaks (SSBs) in the phosphate backbone or by chemical modification of DNA bases[30]. Most such lesions are recognized and repaired by the base excision repair or nucleotide excision repair pathways or can be bypassed entirely during replication via translesion synthesis. During DNA replication, the wrong nucleotide can be incorporated, resulting in two mismatched bases; these are dealt with by the mismatch repair (MMR) pathway[31]. Alternatively, ribonucleotides can be incorporated into the genome instead of deoxyribonucleotides; these are excised

Table 1 | Selected clinical trials for DDR inhibitors

Target	Agent[a]	Combination	Phase	Cancer types	Biomarkers	Clinical trial ID[b] or reference
ATM	AZD0156	Monotherapy, olaparib, irinotecan or FOLFIRI	I	ASTs	–	NCT02588105
	AZD1390	Radiotherapy	I	Grade IV glioma	–	NCT05182905
			I	Brain	–	NCT03423628
			I	Soft tissue sarcoma	–	NCT05116254
			I	Lung	–	NCT04550104
	M4076	–	I	ASTs	–	NCT04882917
ATM and DNA-PKcs	XRD-0394	Radiotherapy (palliative)	I	ASTs, MSTs, RSTs	–	NCT05002140
ATR	ART0380	Monotherapy, gemcitabine or irinotecan	I/II	ASTs, MSTs	ATM deficiency	NCT04657068
	ATRN-119	–	I/II	ASTs	DDR gene mutations	NCT04905914
	BAY1895344	–	I	ASTs, lymphomas	–	NCT03188965 (ref.[196])
		Niraparib	I	ASTs, ovarian	–	NCT04267939
		Pembrolizumab	I	Solid tumours	DDR gene mutations	NCT04095273
	Berzosertib (VX-970, M6620, VE-822)	Veliparib or cisplatin	I	Solid tumours	–	NCT02723864
		Various chemotherapies	I	ASTs	–	NCT02157792
						Yap et al.[196]
		Gemcitabine±berzosertib	II	Ovarian	–	NCT02595892 (ref.[197])
		Topotecan	II	Lung	–	NCT02487095 (ref.[134])
		Avelumab	I/II	Solid tumours	DDR gene mutations	NCT04266912
	Ceralasertib (AZD6738)	Olaparib	II	Gynaecological	–	NCT04065269
		Durvalumab	I	Head and neck, lung	–	NCT02264678
	IMP9064	–	I	ASTs	–	NCT05269316
	M4344	Monotherapy or carboplatin	I	ASTs	*ARID1A*, *ATRX*, *DAXX* or *ATM* mutations	NCT02278250
	RP-3500	Monotherapy or talazoparib+gemcitabine	I/II	ASTs	–	NCT04497116
		Olaparib	I/II	CLL	*TP53*, *ATM*, *SF3B1*, *XPO1* or *POT1* mutations	NCT05405309
		Olaparib or niraparib	I/II	ASTs	–	NCT04972110
		RP-6306	I	ASTs	–	NCT04855656
CHK1	Prexasertib (LY2606368)	Irinotecan	I/II	DSRCT, rhabdomyosarcoma	–	NCT04095221
		–	II	Lung	–	NCT02735980
		–	II	Solid tumours	*MYC* or *CCNE1* amplification, *RB* loss or *FBXW7*, *BRCA1*/*BRCA2*, *PALB2*, *RAD51C*, *RAD51D*, *ATR*, *ATM*, *CHK2* or Fanconi anaemia gene mutations	NCT02873975
		–	II	Ovarian	*BRCA1*/*BRCA2* mutations	NCT03414047
		–	II	Breast, ovarian, mCRPC	*BRCA1*/*BRCA2* mutations	NCT02203513
	SRA737	Gemcitabine±cisplatin	I/II	ASTs	Predicted sensitivity to CHK1 inhibition[c]	NCT02797977
		–	I/II	ASTs, NHL	Predicted sensitivity to CHK1 inhibition[c]	NCT02797964
	LY2880070	Gemcitabine	I/II	ASTs, MSTs	–	NCT02632448
			II	Ewing sarcoma	–	NCT05275426

Table 1 (continued) | Selected clinical trials for DDR inhibitors

Target	Agent[a]	Combination	Phase	Cancer types	Biomarkers	Clinical trial ID[b] or reference
DNA-PKcs	AZD7648	Radiotherapy	I	Soft tissue sarcoma	–	NCT05116254
		Monotherapy or PLD	I	ASTs	–	NCT03907969
	M3814	–	I	ASTs, CLL	–	NCT02316197
		Lutetium Lu 177 dotatate	I	Neuroendocrine	–	NCT04750954
		Radiotherapy	I/II	Pancreatic	–	NCT04172532
		Radiotherapy + avelumab	I/II	ASTs, MSTs	–	NCT04068194
		Radiotherapy + capecitabine	I/II	Rectal	–	NCT03770689
		Radiotherapy + temozolomide	I	Glioblastoma	–	NCT04555577
		Radium-223 dichloride ± avelumab	I/II	Prostate	–	NCT04071236
DNA-PKcs and mTOR	CC-115	Enzalutamide	I	Prostate	–	NCT02833883
		–	I	ASTs	–	NCT01353625
	Samotolisib[d] (LY3023414)	Prexasertib	I	ASTs, MSTs	*PIK3CA* mutations	NCT02124148 (ref.[198])
		–	II	ASTs, MSTs, NHL	*TSC1*, *TSC2*, *PIK3CA* or *MTOR* mutations	NCT03155620
		–	II	AST, NHL, CNS tumours	*TSC1*, *TSC2*, *PI3K* or *MTOR* mutations	NCT03213678
		–	II	Metastatic breast cancers	–	NCT04032080
		–	II	Endometrial	–	NCT02549989
PKMYT1	RP-6306	FOLFIRI	I	ASTs	–	NCT05147350
		Gemcitabine	I	–	–	NCT05147272
		RP-3500	I	–	–	NCT04855656
WEE1	Adavosertib (AZD1775)	Gemcitabine, paclitaxel, carboplatin or PLD	II	Ovarian	-	NCT02272790 (ref.[199])
		Radiotherapy + gemcitabine	I	Pancreatic	–	NCT02037230 (ref.[200])
		–	II	ASTs, RSTs, lymphomas, plasma cell myeloma	*BRCA1*/*BRCA2* mutations	NCT04439227
		–	II	ASTs, MSTs	*SETD2* mutations	NCT03284385
	IMP7068	–	I	ASTs	–	NCT04768868

AST, advanced solid tumour; CLL, chronic lymphocytic leukaemia; DDR, DNA damage response; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSRCT, desmoplastic small round cell tumour; FOLFIRI, folinic acid, fluorouracil and irinotecan; mCRPC, metastatic castration-resistant prostate cancer; MST, metastatic solid tumour; RST, refractory solid tumour; NHL, non-Hodgkin lymphoma; PLD, pegylated liposomal doxorubicin. [a]Other names for agents are given in parentheses where applicable. [b]Clinical trials are accessible at https://clinicaltrials.gov/. [c]Predicted sensitivity to CHK1 inhibition includes DDR gene mutations, loss of tumour suppressor genes involved in G1 cell cycle progression (*RB* and *TP53*), gain of functions or amplification of oncogenic drivers (for example, *CCNE1*), amplification of *CHK1* or *ATR*, and human papilloma virus positivity. [d]Samotolisib also inhibits PI3K, whereas CC-115 does not.

by ribonuclease H2 (RNase H2)[32]. Occasionally, the two strands of the double helix can become crosslinked; depending on their chemical nature, such DNA interstrand crosslinks can be repaired by the Fanconi anaemia pathway, the DNA glycosylase NEIL3 or other incompletely characterized interstrand crosslink repair pathways[33]. Proteins can also become covalently trapped on DNA, requiring DNA–protein crosslink repair mediated by specialized proteases such as SPRTN[34].

SSBs, or single-strand nicks, are primarily recognized by the enzyme PARP1 or PARP2, which catalyse the formation of poly(ADP-ribose) (PAR) chains on themselves and adjacent target proteins[30]. PARP1 and its poly(ADP-ribosyl)ation (PARylation) activity at SSBs recruit the scaffold protein XRCC1, which brings DNA ligase 3 (LIG3) and accessory repair factors to re-ligate the break. PARylation is a highly dynamic and often transient process, because PAR chains can be degraded rapidly by poly(ADP-ribose) glycohydrolase (PARG). Rather than simply being a negative regulator of SSB repair, PARG activity restores PARPs and PARylated proteins to their de-(ADP-ribosylated) state to promote subsequent rounds of SSB repair. Thus, PARG inhibition, similarly to PARP inhibition, reduces the rate of SSB repair[35].

While SSBs are the most common DNA lesions, they are relatively easily repaired. By contrast, DNA DSBs, which pose a higher threat to genome integrity, are far more difficult to repair[8]. There are two major DSB repair pathways in human cells (Fig. 2). The first is the non-homologous end joining (NHEJ) pathway, which repairs the vast majority of two-ended DSBs. By contrast, if DNA replication forks collapse during S phase to form one-ended DSBs, NHEJ is toxic because it can

Table 2 | Selected clinical trials for PARP inhibitors

Agent[a]	Combination	Phase	Cancer type	Biomarkers	Clinical trial ID[b]
Monotherapy					
Fuzuloparib	–	III	Ovarian	–	NCT03863860
Olaparib	–	II	Breast	–	NCT04191135
		III	Lung	–	NCT04624204
Pamiparib (BGB-290)	–	III	Ovarian	–	NCT03519230
Rucaparib	–	III	Ovarian	Wild-type *BRCA1/BRCA2*	NCT04227522
Monotherapy in *BRCA1/BRCA2*-mutated cancers					
Fuzuloparib	–	III	Pancreatic	*BRCA1/BRCA2* mutation or *PALB2* mutation	NCT04300114
IMP4297	–	II	Ovarian	*BRCA1/BRCA2* mutation	NCT04089189
		III	Ovarian	*BRCA1/BRCA2* mutation	NCT04169997
Rucaparib	–	III	Ovarian	*BRCA1/BRCA2* mutation	NCT02855944 (ref.[201])
In prostate cancer					
AZD5305	Enzalutamide, abiraterone or darolutamide	I/II	Metastatic prostate cancer	–	NCT05367440
Fuzuloparib	Abiraterone and prednisone	II	mCRPC	–	NCT04691804
Olaparib	Abiraterone	III	Prostate	–	NCT03732820 NCT05171816
Rucaparib	Enzalutamide	III	Prostate	–	NCT04455750
Talazoparib	Enzalutamide	III	Prostate	–	NCT03395197
In DDR-deficient prostate cancer					
Fuzuloparib	Monotherapy or apatinib	II	mCRPC	HR gene mutations	NCT04869488
IMP4297	–	II	mCRPC	HR gene mutations	NCT04822961
Niraparib	Abiraterone and prednisone	III	Prostate	HR gene mutations	NCT04497844 NCT03748641
Rucaparib	–	III	Prostate	*BRCA1/BRCA2* mutation or *ATM* mutation	NCT02975934
Talazoparib	Enzalutamide	III	Prostate	DDR gene mutations	NCT04821622
In combination with chemotherapy					
AZD5305	Various chemotherapies	I/II	Solid tumours	–	NCT04644068
Olaparib	Paclitaxel	III	Gastric	–	NCT01924533 (ref.[202])
Veliparib	Carboplatin and paclitaxel	III	Lung	–	NCT02106546 (ref.[203])
			Lung	–	NCT02264990 (ref.[204])
			Breast	–	NCT02032277 (ref.[205])
			Breast	*BRCA1/BRCA2* mutation	NCT02163694 (ref.[206])
			Ovarian	HR deficiency or *BRCA1/BRCA2* mutation	NCT02470585 (ref.[207])
Fuzuloparib	Temozolomide	II	Glioblastoma	–	NCT04552977
Veliparib	Temozolomide	II/III	Glioblastoma	*MGMT* promoter hypermethylation	NCT02152982
In combination with immunotherapy					
Niraparib	Bevacizumab ± dostarlimab	III	Ovarian	–	NCT03602859
	Pembrolizumab	III	Lung	–	NCT04475939 (refs.[203,208])
Olaparib	Pembrolizumab	III	Lung	–	NCT03976362
					NCT03976323
					NCT04380636
Pamiparib (BGB-290)	Tislelizumab	I	Advanced solid tumours	HR deficiency or *BRCA1/BRCA2* mutation	NCT02660034 (ref.[209])
Pamiparib (BGB-290)	Tislelizumab or temozolomide	III	Advanced solid tumours	–	NCT04164199
Rucaparib	Nivolumab	III	Ovarian	–	NCT03522246

DDR, DNA damage response; HR, homologous recombination; mCRPC, metastatic castration-resistant prostate cancer; PARP, poly(ADP-ribose) polymerase. [a]Other names for agents are given in parentheses where applicable. [b]Clinical trials are accessible at https://clinicaltrials.gov/.

Box 1

Current clinical applications of PARP inhibitors

Since the discovery of the synthetic-lethal interaction between poly(ADP-ribose) polymerase (PARP) inhibition and BRCA1/2 deficiencies[5,6], multiple studies have demonstrated the clinical benefits of PARP inhibitors, in particular for patients with *BRCA1*/*BRCA2*-mutated tumours[4]. Consequently, six different PARP inhibitors have been approved for clinical use, including against specific subsets of *BRCA1*/*BRCA2*-mutated cancers: olaparib, rucaparib, niraparib, talazoparib, fuzuloparib and pamiparib. For example, the US Food and Drug Administration (FDA) has approved olaparib and talazoparib to treat advanced or metastatic HER2-negative breast cancers in patients carrying deleterious germ line *BRCA1*/*BRCA2* mutations[211,212]. In March 2022, olaparib was approved by the FDA for adjuvant treatment of patients with inherited *BRCA1*/*BRCA2* mutations and HER2-negative high-risk early breast cancer[213]. PARP inhibitors are also used in patients with germ line or somatic *BRCA1*/*BRCA2*-mutated ovarian cancer, as maintenance treatment (olaparib)[214] or treatment after chemotherapy (olaparib and rucaparib)[201]. Moreover, olaparib is used for maintenance treatment of patients with germ line *BRCA1*/*BRCA2*-mutated metastatic pancreatic cancers[215]. Rucaparib is used as second-line treatment for patients with germ line or somatic *BRCA1*/*BRCA2*-mutated metastatic castration-resistant prostate cancer[216].

The clinical applications of PARP inhibitors are not restricted to *BRCA1*/*BRCA2*-mutated tumours. Olaparib and niraparib are available to target advanced ovarian cancers associated with homologous recombination deficiency defined not only by *BRCA1*/*BRCA2* mutations but also by genomic instability evaluated with an approved companion diagnostic test[217,218] (Box 2). Moreover, olaparib has been approved for treatment of patients with metastatic castration-resistant prostate cancer whose tumours have progressed following anti-androgen therapy and who harbour germ line or somatic mutations in homologous recombination genes, including *BRCA1*, *BRCA2*, *PALB2*, *RAD51C*, *RAD51D* and *ATM*[219]. It is noteworthy that PARP inhibitors have also been approved for maintenance therapy in patients with recurrent or advanced ovarian cancers (olaparib, rucaparib and niraparib) and who were not selected on the basis of known *BRCA1*/*BRCA2* mutations or homologous recombination deficiencies[220–223].

These inhibitors have also been approved by the European Medicines Agency (EMA) to treat some of the tumour types mentioned above. Moreover, the China National Medical Products Administration (NMPA) has approved the use of PARP inhibitors to treat germ line *BRCA1*/*BRCA2*-mutated recurrent ovarian cancers (fuzuloparib)[224] or germ line *BRCA1*/*BRCA2*-mutated recurrent advanced ovarian cancers (pamiparib)[225].

However, the emergence of resistance to PARP inhibitors limits their clinical efficacy (see Box 2). Because PARP trapping mediates the antitumour activity of PARP inhibitors and acquired resistance[93,189], modulation of their trapping ability may affect their clinical efficacy. Therefore, novel PARP inhibitors with increased PARP trapping capacity, such as the recently developed veliparib derivative[226], are promising clinical candidates.

generate chromosomal rearrangements by re-ligating DNA ends on different chromosomes. Thus, NHEJ is actively suppressed at replication forks by components of the second major DSB repair pathway, HR. HR is also favoured in specific contexts, such as interstrand crosslink repair by the Fanconi anaemia pathway[36] or repair of programmed DSBs induced during meiosis[37], when NHEJ would be similarly disruptive.

NHEJ is often referred to as error-prone (for example, in the context of gene editing via CRISPR–Cas9[38]) because it does not use a homologous template for repair. In reality, NHEJ is remarkably efficient and accurate most of the time[8], which explains why cells have evolved to use it preferentially. The pathway is initiated when Ku, a basket-shaped heterodimeric protein complex consisting of Ku70 and Ku80 subunits[39], recognizes and binds broken DNA ends (Fig. 2). Ku primarily acts as a recruitment platform for downstream NHEJ components, while simultaneously protecting DNA ends from unwinding and degradation by cellular helicases and nucleases, respectively. Ku-bound DNA ends rapidly recruit the DNA-dependent protein kinase (DNA-PK) catalytic subunit (DNA-PKcs) to form the DNA-PK holoenzyme, which is essential for NHEJ in part by promoting DNA-end tethering and thus facilitating DNA ligation. Interestingly, the best-known substrate for DNA-PK kinase activity in NHEJ is DNA-PKcs itself. This autophosphorylation is nonetheless essential for the repair process because it promotes DNA-end synapsis[40] and DNA-PK dissociation from DNA ends[41,42]. DNA-end ligation is subsequently performed by LIG4 and its stable binding partner XRCC4 (refs.[43,44]), a step which also requires either XLF or PAXX, two partially redundant NHEJ core factors[45–49]. NHEJ accessory factors, including specialized nucleases (for example, Artemis) and polymerases (for example, DNA polymerase-μ and DNA polymerase-λ), also promote accurate DSB repair in specific circumstances but are not essential for all NHEJ repair[50].

In contrast to NHEJ, the HR pathway uses a homologous DNA molecule (usually the sister chromatid) as a template for repair. HR is initiated when nucleases digest the double-stranded DNA ends at DSB sites to produce ssDNA overhangs (Fig. 2), a process termed 'DNA-end resection'[51]. This results in removal of Ku from DNA ends and production of tracts of ssDNA, which become rapidly coated by RPA. DNA-end resection is tightly controlled during the cell cycle, such that it occurs only during S and G2, due to CDK-dependent phosphorylation of CtIP (also known as RBBP8), a factor known to stimulate end resection[52,53]. This also prevents diploid cells from using the homologous chromosome rather than the sister chromatid as a template for repair (which could otherwise lead to loss of heterozygosity). Exceptions may occur at repetitive regions of the genome, such as ribosomal or centromeric DNA, where resection and HR can occur even in G1 phase cells[54,55].

Two distinct but possibly redundant pathways promote end resection after initial ssDNA overhang production by CtIP and the MRN complex. The first pathway depends on the nuclease EXO1, while the second requires the nuclease DNA2 and the helicase BLM[56–58]. After resection, RPA is replaced on ssDNA with the recombinase RAD51, a step that requires BRCA1 and BRCA2 (ref.[36]). This is because BRCA1,

via its interaction with PALB2, promotes BRCA2 recruitment to DSB sites, where the latter loads RAD51 directly onto the ssDNA ends. RAD51 forms nucleoprotein filaments on ssDNA, which promotes strand invasion and displacement loop (D-loop) formation. This DNA structure allows DNA synthesis across the break site using the sister chromatid as the template so as to prevent alteration of genetic information. Finally, the invading strand can be displaced and HR completed via synthesis-dependent strand annealing. Alternatively, if second-end capture occurs, a double Holliday junction is formed, which is either dissolved by the Bloom syndrome complex (containing BLM and the topoisomerase TOP3A) or resolved by SLX4–MUS81 or GEN1, structure-specific nucleases[59]. The extent of DNA-end resection determines the use of alternative DNA repair pathways (Fig. 2), most notably single-strand annealing and alternative end joining (also known as DNA polymerase-θ (POLQ)-mediated end joining or microhomology-mediated end joining), which are reliant on RAD52 and POLQ, respectively. Single-strand annealing and alternative end joining act mainly during S phase and mitosis[60], respectively, as backup repair pathways when HR is inactivated (discussed later).

Box 2

Biomarkers and functional assays for patient selection

Patient stratification has a key role in the development and clinical application of targeted antitumour therapies, and novel biomarkers are being used to determine whether a patient will benefit from a given treatment. The presence of germ line or somatic mutations is a major criterion for patient selection, as illustrated by the use of poly(ADP-ribose) polymerase (PARP) inhibitors to target *BRCA1*-, *BRCA2*- or *ATM*-mutated tumours (Box 1, Tables 1, 2). Other companion diagnostic tests that measure complex ‘genomic scars’ are also being used to select eligible patients. For instance, one companion diagnostic test measures loss of heterozygosity, telomeric allelic imbalance and large-scale state transitions and calculates a genomic instability score[227].

As an alternative to genetic tests, functional assays measuring the formation of RAD51 foci, as a surrogate for homologous recombination activity, have been shown to predict PARP inhibitor response ex vivo. Indeed, in cells derived from patients with epithelial ovarian cancers that were challenged with the PARP inhibitor rucaparib ex vivo, the formation of RAD51 foci correlated with cell survival in congenic assays[228]. Recent studies demonstrated the possibility to perform formation of RAD51 foci assays in formalin-fixed, paraffin-embedded tumour samples, thereby removing the need to isolate live cancer cells and treat them ex vivo[229,230]. Functional homologous recombination assays in formalin-fixed, paraffin-embedded tumour samples from patients with germ line *BRCA1*/*BRCA2* mutations and treated with PARP inhibitors predicted clinical efficacy and drug resistance[230]. Altogether, the formation of RAD51 foci is a promising tool for the selection of patients who may benefit from PARP inhibitors.

Genetic and functional assays are likely to become routine for selection of patients for DNA damage response inhibitor therapy in the clinic. However, functional assays are available only for PARP inhibitors, and future efforts should focus on the development of functional assays predicting the efficacy of other DNA damage response-targeting therapies.

Chromatin modifications at DNA damage sites control DNA repair pathway choice

While cell cycle-dependent phosphorylation events (for example, CtIP phosphorylation by CDKs) enable initiation of DNA resection and HR during S and G2 phases of the cell cycle, NHEJ remains the predominant DSB repair pathway throughout interphase[61,62]. This is due, in part, to DSB repair regulation at the chromatin level by BARD1, the stable binding partner of BRCA1, and 53BP1, which compete to promote and antagonize end resection, respectively. BARD1 and 53BP1 act as readers of histone H2A Lys15 (H2AK15) ubiquitylation, a DNA damage-induced histone modification deposited by RNF168 (Fig. 1), and of histone H4 Lys20 (H4K20) methylation status. Histone H4 Lys20 dimethylation is a histone modification deposited on mature chromatin and is thus absent from the nascent daughter DNA strand during S phase[63,64]. By directly binding to nucleosomes containing H2AK15ub[65] and H4K20me2 (ref.[66]), 53BP1 recruits downstream effector proteins, including RIF1 and the shieldin complex, to counteract DNA-end resection by modulating 3D chromatin structure, protecting DSB ends and/or recruiting DNA polymerase-α to fill in ssDNA gaps generated during resection[67–70]. Thus, by inhibiting the production of ssDNA, 53BP1 and its binding partners ensure that RAD51 filaments of sufficient length cannot be assembled, thereby suppressing HR.

53BP1-mediated NHEJ is highly toxic during S phase, when collapsed replication forks lead to the production of one-ended DSBs[71]. When two such ends from different chromosomes are ligated, chromosomal translocations arise. Thus, DSBs that occur during replication must be repaired by HR rather than NHEJ. This is achieved by the BRCA1–BARD1 complex, which, like 53BP1, recognizes the RNF168-mediated histone H2A Lys15 ubiquitylation mark (Fig. 1), but binds unmethylated histone H4 Lys20 rather than dimethylated histone H4 Lys20 (refs.[72–75]). Importantly, nascent chromatin in the vicinity of a replication fork is enriched in unmethylated histone H4 Lys20, in contrast to mature chromatin, which becomes progressively methylated on H4K20 after replication. In this way, cells ensure that 53BP1 promotes NHEJ throughout interphase at DSBs that occur in mature chromatin, whereas BRCA1 is recruited to chromatin proximal to DSBs that occur during DNA replication and are repaired by HR. However, it is important to note that 53BP1 is still recruited to more distal chromatin sites at DSBs even in S phase. This contributes to maintaining HR accuracy by preventing extensive long-range resection that could otherwise activate single-strand annealing (Fig. 2), a RAD52-mediated repair process that produces large genomic deletions[76]. If DNA ends are not extensively resected, then alternative end joining (Fig. 2) can be used to repair DSBs[77]. However, the alternative end joining and single-strand annealing pathways are both highly mutagenic and are used as backups only when the more accurate repair pathways (NHEJ or HR) are compromised. This model is supported by recent evidence showing that POLQ-dependent DSB repair is restricted until the onset of mitosis, before cell division occurs[60]. This last-resort mutagenic DSB repair is preferable to unrepaired DSBs being carried into mitosis, where chromosome segregation could lead to micronuclei and catastrophic events such as breakage–fusion–bridge cycles and chromothripsis, both sources of chromosomal instability in cancer[11].

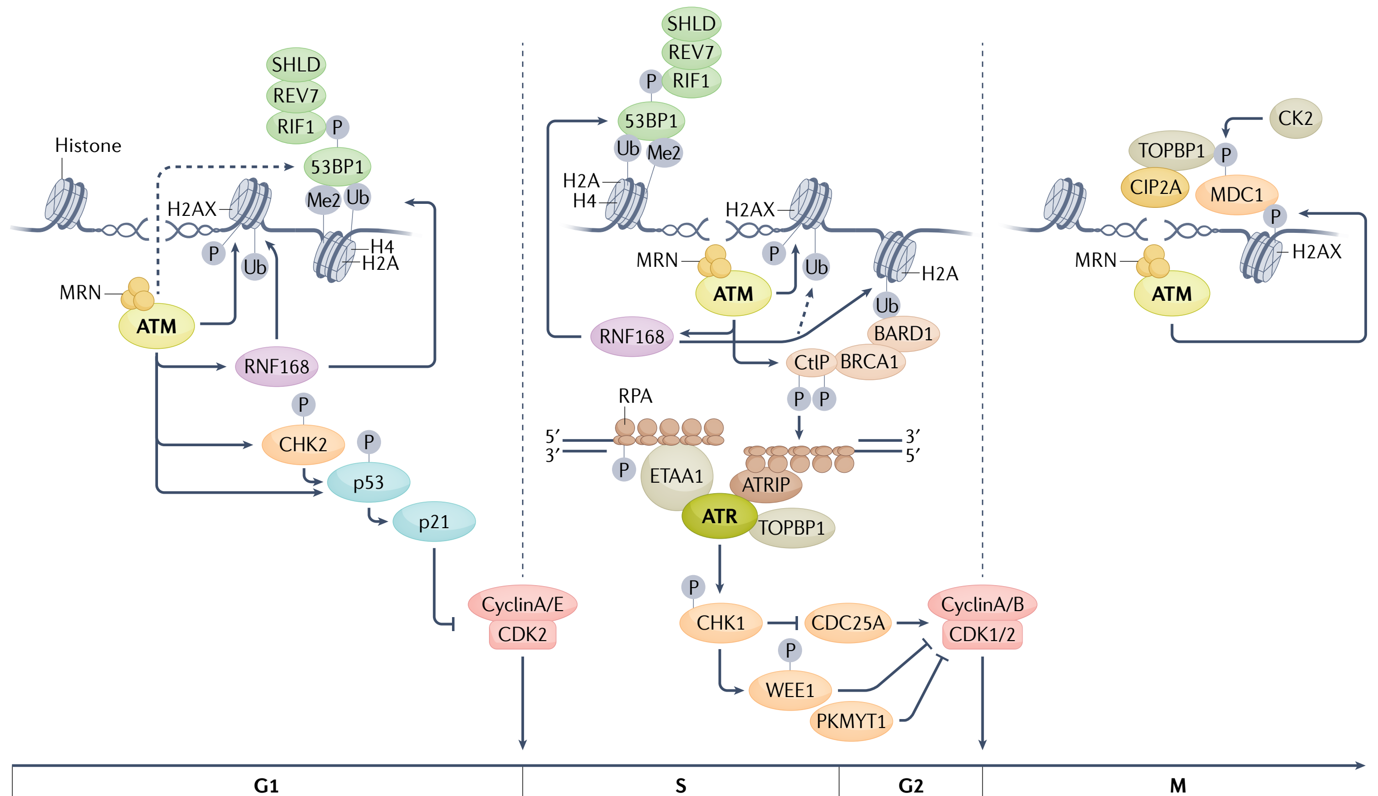

Fig. 1 | Cell cycle-dependent DDR activation at DSBs. The MRE11–RAD50–NBS1 (MRN) complex senses DNA double-strand breaks (DSBs) and recruits and activates ATM at break sites to orchestrate DNA damage response (DDR) signalling. Left: During G1, ATM promotes activation of RNF168, which ubiquitylates histone H2A. This modification, together with the histone H4 Lys20 dimethylation mark leads to the recruitment of 53BP1 and the ATM-mediated phosphorylation of 53BP1, which promotes its interaction with RIF1, as well as REV7 and other members of the shieldin complex (SHLD). ATM kinase activity also controls p53 stability, which triggers G1 arrest in response to DNA damage. Centre: During S phase, the BRCA1–BARD1 complex is recruited to DSBs and counteracts 53BP1 via recognition of unmethylated H4 Lys20 and H2A Lys15 ubiquitylation to promote DNA-end resection and RAD51 loading onto resected DNA ends. Replication protein A (RPA)-coated single-stranded DNA also recruits ATR, via interaction with ATR-interacting protein (ATRIP). ETAA1 binding to RPA-coated single-stranded DNA or topoisomerase 2-binding protein 1 (TOPBP1) binding to the RAD9–RAD1–HUS1 (9-1-1) complex (not shown) at double-stranded DNA–single-stranded DNA junctions activates ATR, which phosphorylates CHK1 to promote CDC25A degradation, thus preventing activation of cyclin-dependent kinase 1 (CDK1) and CDK2. The kinases WEE1 (a phosphorylation target of CHK1) and PKMYT1 mediate inhibitory phosphorylation of CDK1 and CDK2, which prevents cells progressing into mitosis. Right: In mitosis, ATM-dependent H2AX phosphorylation recruits mediator of DNA damage checkpoint protein 1 (MDC1), which binds TOPBP1 and cellular inhibitor of PP2A (CIP2A) via its casein kinase 2 (CK2)-mediated phosphorylation. TOPBP1 and CIP2A form filamentous structures which tether both DSB ends together (not shown). Me2, dimethylation; P, phosphorylation; Ub, ubiquitylation.

PARP inhibitors in targeted cancer therapy

Germ line heterozygous mutations in *BRCA1* or *BRCA2* predispose affected individuals to multiple types of cancer, including breast, ovarian, prostate and pancreatic cancer[78,79]. As discussed earlier herein, BRCA1 and BRCA2 promote accurate DNA repair by the HR pathway, which is essential for preserving genome integrity following exposure to DNA-damaging treatments such as ionizing radiation or DNA interstrand crosslinking agents. In the absence of exogenous challenges, BRCA1 and BRCA2 are crucial to maintaining genome stability during DNA replication[36]. Although not required for replication per se, BRCA1 and BRCA2 facilitate it by preventing nascent DNA degradation by nucleases and promoting HR repair of broken replication forks[80–83]. When BRCA1 or BRCA2 is abrogated, DNA lesions caused by failed replication accumulate. Their repair by error-prone backup pathways generates genomic alterations (insertions, deletions and chromosomal rearrangements[84]) that pose a threat to genome integrity and, in the long term, promote tumorigenesis[85,86].

As discussed earlier herein, PARP plays a key role in SSB repair. It was therefore originally postulated that PARP inhibition specifically kills BRCA1/2-deficient cells due to accumulation of SSBs, which are converted into DSBs when they are encountered by replication forks. Replication-associated DSBs are one-ended and must therefore be repaired by HR, as NHEJ can produce toxic genomic rearrangements. The exquisite hypersensitivity of BRCA1/2-deficient and, more generally, HR-deficient cells to PARP inhibition[5,6] was thought to be due to suppression of PARP-dependent SSB repair, leading to accumulation of DNA lesions (SSBs and DSBs) during replication. However, this model does not completely explain the observation that the degree to which BRCA1/2-deficient cells are sensitive to PARP inhibitors correlates with the extent to which they 'trap' PARP on DNA[87]. Indeed, loss or inhibition

of factors such as ALC1 (also known as CHD1L), RNase H2 or the ATPase p97 (also known as VCP), which increase PARP trapping, are synthetic lethal with BRCA1/2 loss and increase sensitivity to PARP inhibitors[88–93]. Furthermore, recent studies have demonstrated that PARP inhibition leads to accumulation of ssDNA gaps behind the replication fork, which originate from defects in processing Okazaki fragments[94–96]. Progression through mitosis and the subsequent S phase are required for conversion of these gaps into DSBs that require BRCA1/2 for repair[97,98].

Further work is required to establish the precise mechanism of PARP inhibitor-induced lethality in BRCA1/2-deficient cells. However,

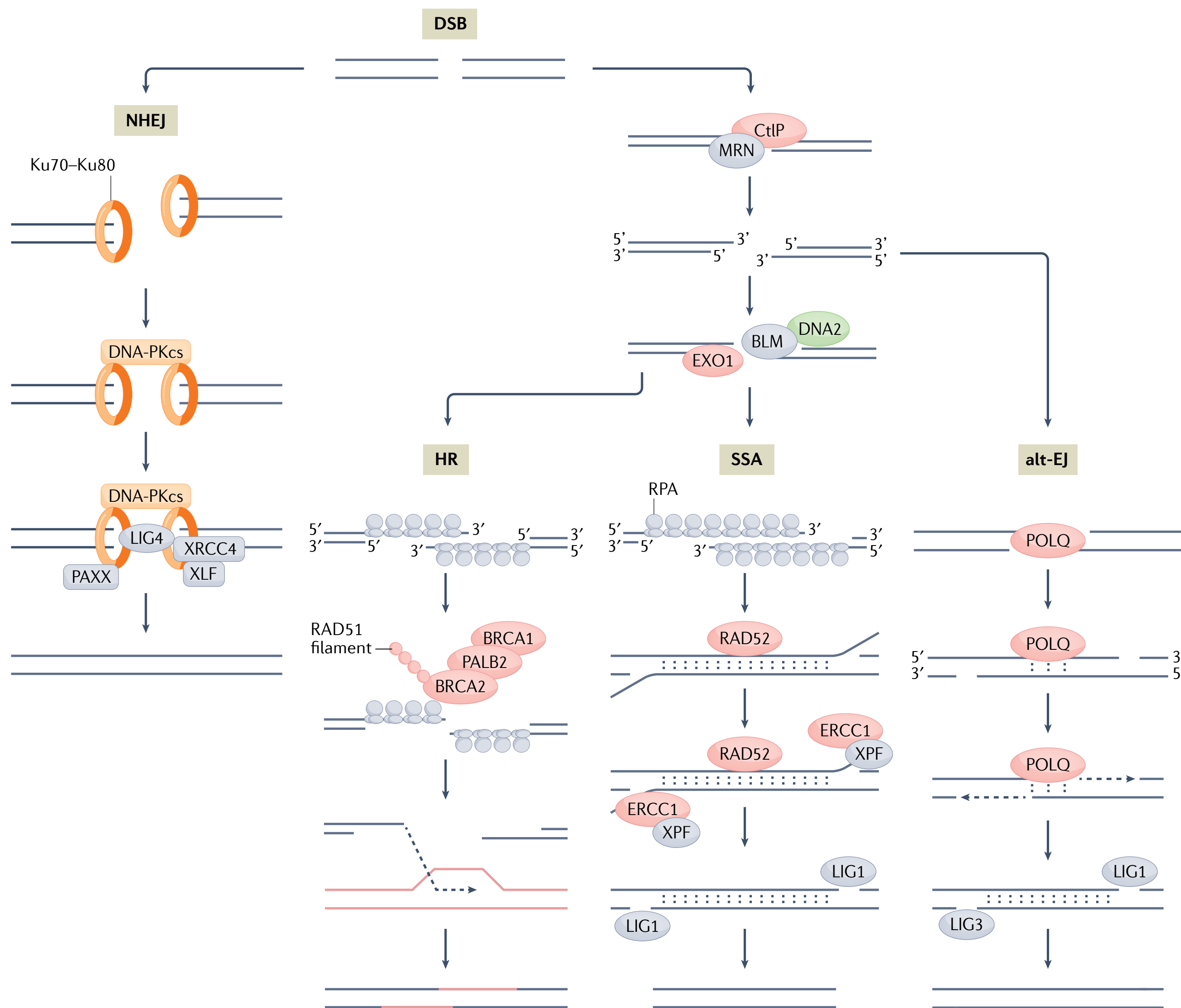

Fig. 2 | Major and backup pathways of DSB repair. DNA double-strand break (DSB) repair pathway choice depends on the cell-cycle stage and the extent of DNA-end resection. Most DSBs are repaired by non-homologous end joining (NHEJ), initiated by binding of Ku70–Ku80 heterodimer to DNA ends. The subsequent recruitment and autophosphorylation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) brings the DNA ends together and allows their ligation by XRCC4–DNA ligase 4 (LIG4), together with either XLF or PAXX. During S phase, MRE11 as part of the MRE11–RAD50–NBS1 (MRN) complex and CtIP initiate resection to promote homology-directed repair. Long-range resection requires BLM–DNA2 helicase–nuclease or EXO1 nuclease activities and leads to the formation of replication protein A (RPA)-coated single-stranded DNA overhangs. Homologous recombination (HR) requires the concerted action of BRCA1, PALB2 and BRCA2 to load RAD51 onto single-stranded DNA, displacing RPA. RAD51 nucleoprotein filaments promote invasion into the sister chromatid (pink), which serves as a template for DNA synthesis. Alternatively, extensive resection creates a substrate for single-strand annealing (SSA), where RAD52 promotes annealing of homologous sequences on each single-stranded DNA end. The subsequent processing of 3′ single-stranded flaps by ERCC1 and XPF allows LIG1-mediated DNA ligation. RAD52 is also involved in break-induced replication (not shown), which is distinct from SSA and may contribute to the genomic alterations in cells defective for HR[210]. By contrast, short-range resection is sufficient to activate alternative end joining (alt-EJ), where DNA polymerase-θ (POLQ) promotes the annealing of short homologous sequences, followed by DNA synthesis and LIG1- or LIG3-dependent re-ligation of DNA ends.

the discovery of the synthetic-lethal relationship between PARP inhibition and BRCA1/2 deficiency has established a paradigm for selective targeting of DDR factors in tumours with HR defects (Box 1). This provides an exciting field for future cancer research and clinical applications, despite the acquired drug resistance (Box 3), which still poses a major drawback to the use of PARP inhibitors in the clinic. Indeed, as we discuss next, novel synthetic-lethal interactions between DDR factors and pathways have been identified, some of which are already being tested in early clinical trials.

Targeting DDR kinases in the clinic

ATM inhibitors

ATM is the apical DDR kinase orchestrating DSB repair, and therefore multiple compounds have been developed for its selective inhibition (Table 1). Given the key role of ATM in DSB signalling and repair, ATM inhibition coupled with radiotherapy is an obvious and attractive therapeutic combination for tumour eradication, and is currently being explored in several clinical trials. Early on, it was revealed that specific ATM inhibitors can be used to increase the anticancer activity of DNA-damaging agents such as topoisomerase inhibitors[99] or PARP inhibitors[100]. In addition, ATM deficiency sensitizes cancer cells to topoisomerase 1 inhibitors[71] or PARP inhibitors[71,101]. This is of clinical relevance since *ATM* is frequently mutated or inactivated in sporadic cancers, including lung, breast, brain[102] or pancreatic[103] cancers. Mechanistically, PARP and topoisomerase 1 inhibitors lead to one-ended DSBs which require HR for their accurate repair. Loss of ATM signalling delays end resection and channels the repair of one-ended DSBs to NHEJ, which results in toxic chromosome fusions. Conversely, abrogation of XRCC4, LIG4 or XLF, which are critical for NHEJ, promotes resistance to PARP or topoisomerase 1 inhibitors in ATM-deficient cells, because one-ended DSBs can be repaired by HR[71]. However, ATM inhibition reverses PARP inhibitor resistance in BRCA1–53BP1-deficient or BRCA1–REV7-deficient mouse cells[104,105], an effect mediated by reduced end resection. Thus, the combination of ATM inhibitors with PARP or topoisomerase 1 inhibitors may be a promising therapeutic strategy, which is currently being investigated in the clinic (Table 1).

In addition, previous studies have indicated that ATM inhibition is synthetic lethal with loss of genes in the Fanconi anaemia pathway[106,107], an observation recently validated in multiple CRISPR–Cas9 screens[108,109]. Thus, tumours that are defective in one or more of the more than 20 Fanconi anaemia genes (for example, ~5% of ovarian cancers[110]) may be attractive candidates for therapy using ATM inhibitors.

Despite several ongoing clinical trials (Table 1), the clinical efficacy of ATM inhibitors is yet to be demonstrated. It is also noteworthy that germ line heterozygous *ATM* mutation predisposes to haematological malignancies and other cancer types[111,112]. Therefore, careful consideration should be given to ensure that therapeutic benefits of ATM inhibition outweigh the risks for patients with cancer, as prolonged exposure to ATM inhibitors might lead to de novo tumour formation in multiple tissues.

DNA-PKcs inhibitors

DNA-PKcs, the catalytic subunit of the holoenzyme DNA-PK, which also includes the proteins Ku70 and Ku80, is critical for DSB repair via NHEJ throughout the cell cycle. In line with this, cells lacking DNA-PKcs are sensitive to DSB-inducing agents[113–115], and treatment with DNA-PKcs inhibitors recapitulates this effect[116]. Similarly to ATM, DNA-PKcs inhibition in combination with radiotherapy is an attractive anticancer strategy[116,117]. Importantly, because ATM and DNA-PKcs display only partial redundancy in substrate phosphorylation in response to DSBs, their concomitant loss is synthetic lethal[118,119]. Therefore, DNA-PKcs inhibitors, especially those that are currently in clinical trials (Table 1), might also be effective against ATM-deficient tumours[120]. This concept would presumably not apply to inhibitors such as XRD-0394, a dual inhibitor

Box 3

Clinically relevant mechanisms of PARP inhibitor resistance

The major drawback to the use of poly(ADP-ribose) polymerase (PARP) inhibitors in the clinic is the emergence of resistant disease. Recent in vitro studies have identified several gene mutations that could underlie PARP inhibitor resistance. However, only a subset of these mutations was found in patients. Reactivation of the *BRCA1* gene or the *BRCA2* gene through reversion mutations that restore the open reading frames is one of the most common causes of PARP inhibitor resistance in the clinic[231]. Often, this is a partial open reading frame restoration, sufficient to sustain some of the key BRCA1/2 activities (for example, RAD51 loading at double-strand breaks). A frequent mechanism of BRCA1 inactivation in sporadic triple-negative breast tumours is *BRCA1* promoter hypermethylation, a predictor of PARP inhibitor sensitivity[232]. *BRCA1* silencing is reversed by demethylation, which enables residual transcription, sufficient to trigger resistance[229]. Resistant tumours that re-express *BRCA1* while retaining *BRCA1* promoter hypermethylation have been reported[233]. One such case was caused by a chromosomal rearrangement which placed the *BRCA1* gene under the control of a heterologous active promoter.

Another mechanism of PARP inhibitor resistance specific to *BRCA1*-mutated tumours is restoration of homologous recombination repair via abrogation of 53BP1 (ref.[234]) or of its interacting partners RIF1 (refs.[235–238]) and the shieldin complex (that is, REV7, SHLD1, SHLD2 and SHLD3)[239–242]. Abrogation of any of these factors in BRCA1-deficient cells reactivates end resection and enables loading of RAD51 at double-strand break sites to levels sufficient for partial homologous recombination restoration. This mechanism is clinically relevant because mutations in or downregulation of the genes encoding 53BP1 pathway components have been found in PARP inhibitor-resistant triple-negative human breast cancers[230,239].

Mechanisms of PARP inhibitor resistance independent of homologous recombination reactivation have also been reported in patients with cancer. For example, loss of expression of poly(ADP-ribose) glycohydrolase (PARG), the enzyme responsible for the degradation of poly(ADP-ribose) chains, stabilizes poly(ADP-ribosyl) ation and promotes PARP inhibitor resistance in BRCA1/BRCA2-deficient cells and tumours[243]. PARG suppression, as detected by immunohistochemistry, may be relevant to a wide spectrum of tumours. Similarly, loss of the helicase SLFN11, a factor with elusive roles in DNA replication and repair, promotes resistance to PARP inhibitors in BRCA1/2-deficient cells[143]. An *SLFN11* missense mutation was reported in a patient with PARP inhibitor-resistant *BRCA1*-deleted metastatic breast cancer[230].

Glossary

cGAS–STING axis
A pathway that detects cytosolic DNA and activates innate immune responses.

Chromothripsis
The process of chromosome shattering followed by incorrect repair.

Displacement loop
(D-loop). A DNA structure formed when a double helix is separated by invasion of a complementary single-stranded DNA end during homologous recombination.

Double Holliday junction
A four-way joint DNA molecule and homologous recombination intermediate that can form after second-end capture and ligation.

Micronuclei
Cytosolic structures containing a chromosome or chromosome fragment that are not incorporated in the nucleus during mitosis.

One-ended DSBs
Double-stranded DNA ends formed when a replication fork collapses, where there is no second DNA end available for ligation.

Second-end capture
The annealing step that pairs the second resected single-stranded DNA end from one side of a double-strand break with the joint molecule formed by invasion of a template DNA by the first resected DNA end during homologous recombination.

Shieldin complex
A protein complex consisting of SHLD1–SHLD2–SHLD3, which acts together with 53BP1–RIF1–REV7 to limit end resection at DNA double-strand breaks.

Two-ended DSBs
DNA double-strand breaks (DSBs) where both ends are available for ligation.

of both ATM and DNA-PKcs that has recently entered phase I clinical trials but for which no data have yet been made publicly available.

ATR inhibitors
The kinase ATR acts during S phase to ensure timely and accurate DNA replication by regulating origin firing and fork progression. Because cancer cells frequently accumulate stalled replication forks, they become reliant on ATR signalling to sustain DNA replication. Cells with defective control of the G1–S transition due to p53 deficiency or oncogene activation are also dependent on ATR signalling[121–123]. Thus, ATR is an attractive target for cancer therapy, with multiple specific and potent ATR inhibitors having been developed in recent years[123–125] (Table 1). ATR inhibitors increase replication fork stalling and promote chromosomal breakage, leading to cytotoxicity. Cancer cells with replication stress caused by overexpression of oncogenes such as the oncogene encoding cyclin E1 (ref.[123]) are particularly susceptible to ATR inhibitors. Similar results were observed in xenograft mouse models of *ALK*- and *MYCN*-amplified neuroblastoma, where ATR inhibition led to inhibition of tumour growth[126]. Cancer cells harbouring *ATM* mutations that interfere with DNA repair[127,128] are also susceptible to ATR inhibitors[129].

ATR inhibitors sensitize tumours to ionizing radiation, and their combination is effective against patient-derived xenograft (PDX) models of triple-negative breast cancer[130]. Interestingly, recent studies have demonstrated that ATR inhibitors potentiate radiation-induced interferon signalling[131,132], suggesting potential therapeutic combinations of ATR inhibitors with immunotherapy. This concept was validated in mouse models for castration-resistant prostate cancer, where ATR inhibitors combined with immunotherapy targeting the immune checkpoint molecule PDL1 showed synergistic antitumour activity[133]. Combinations of ATR inhibitors and immune checkpoint blockade are currently being tested in clinical trials (Table 1); for example, a combination of the ATR inhibitor VE-822 and the anti-PDL1 immunotherapy avelumab is being used to treat solid tumours with mutations in DDR genes.

Several studies have revealed synergies between ATR inhibitors and established anticancer drugs, which interfere with DNA replication or inflict DNA damage (for example, cisplatin, gemcitabine and temozolomide)[124,128,134–137]. Importantly, ATR inhibitors have also been reported to act synergistically with PARP inhibitors against tumours harbouring *BRCA1*/*BRCA2* mutations[138], Mechanistically, ATR inhibitors exacerbate some of the pathologies induced by PARP inhibitors in BRCA2-deficient cells, including accelerated mitotic entry, accumulation of chromatin bridges and broken and/or lagging chromosomes, leading to increased PARP inhibitor cytotoxicity[139]. The synergy between these two drugs has recently been extended to wild type *BRCA1*/*BRCA2* tumours[140,141].

One of the most promising clinical applications of ATR inhibitors is their potential to treat PARP inhibitor-resistant tumours. BRCA1-deficient cancer cells can overcome PARP inhibitor toxicity via BRCA1-independent loading of RAD51 at DSBs (Box 3). In vitro, ATR inhibitors block this BRCA1-independent function, leading to resensitization of tumour cells to PARP inhibition[142]. Furthermore, ATR inhibitors reversed PARP inhibitor resistance caused by inactivation of the helicase SLFN11 in both HR-proficient and HR-deficient cancer cells[143].

CHK1 inhibitors
As a downstream target of ATR, the kinase CHK1 is similarly activated by defects in DNA replication. CHK1 inhibitors selectively kill cancer cells with high levels of replication stress[140,144,145]. CHK1 inhibitors were shown to potentiate the cytotoxicity of DNA-damaging agents such as gemcitabine, cisplatin and camptothecin in p53-deficient cancer cells[146] and of irinotecan in p53-deficient mouse models of triple-negative breast cancer[147].

Similarly to ATR inhibitors, CHK1 inhibitors exacerbate the DNA damage caused by PARP inhibition[148]. Furthermore, treatment with the CHK1 inhibitor prexasertib activates type I interferon signalling, and its combination with anti-PDL1 immunotherapy results in synergistic antitumour responses in mouse models of small cell lung cancer[149]. Although some CHK1 inhibitors showed substantial toxicity in initial clinical trials, several novel candidate compounds with better safety profiles are currently being tested in the clinic (Table 1).

WEE1 and PKMYT1 inhibitors
The tyrosine kinase WEE1 is responsible for the inhibitory phosphorylation of CDK1, which prevents mitotic entry[17,18]. Therefore, treatment with WEE1 inhibitors results in aberrant entry into mitosis, which underlies the cytotoxicity of these compounds. CDK1 activation in S phase also leads to enhanced cleavage of stalled replication forks by the SLX4–MUS81 complex and toxic accumulation of DNA damage[150].

Importantly, inhibition of WEE1 is synthetic lethal with loss of histone H3 Lys36 trimethylation (H3K36me3), which is frequently found in cancers lacking the histone methyltransferase SETD2 or overexpressing the histone lysine demethylase KDM4A[151]. Given that *SETD2* is frequently mutated in cancer, the results obtained in xenograft models and cell lines[151] provided a rationale for the use of anti-H3K36me3 antibodies as biomarkers for the identification of *SETD2*-mutated tumours that are susceptible to WEE1 inhibitors, which is currently being tested clinically[152].

Recently, the WEE1-like kinase PKMYT1 was identified in a CRISPR–Cas9 screen for synthetic-lethal interactions with cyclin E overexpression[153]. Treatment with the PKMYT1 inhibitor RP-6306 triggers unscheduled CDK1 activation in cancer cells overexpressing cyclin E, and this inhibitor exhibits potent in vivo antitumour activity in xenograft or PDX models, alone or in combination with gemcitabine[153]. This inhibitor is currently being tested in phase I clinical trials in patients with solid tumours (Table 1).

Emerging therapies and new targets

In addition to PARP1 and the kinases discussed earlier herein, which have been validated as bona fide targets for cancer therapy (Tables 1, 2), several additional DDR factors have been identified as potential targets for specific tumours. These, together with inhibitory compounds currently in clinical development, are discussed in the following subsections.

POLQ inhibitors target HR-deficient tumours

POLQ has a central role in the alternative end joining pathway, which repairs resected DNA ends with internal microhomologies[154] (Fig. 2). POLQ contains a helicase-like ATPase domain required to displace RPA from resected ssDNA ends and to unravel homologous sequences[155]. Upon their alignment and annealing, the polymerase activity of POLQ fills in the gaps, which are subsequently sealed by LIG1 or LIG3 (refs.[156–158]). Reflecting its key role in DNA repair, POLQ inhibition has a radiosensitizing effect in a panel of different tumour cell lines in vitro[159].

The discovery of *POLQ* synthetic-lethal interactions with DNA repair genes, including *BRCA1*, *BRCA2* and *ATM*[84,160,161], has underscored POLQ as a promising target for elimination of tumours with compromised HR repair. Concerted efforts of several groups worldwide towards identification of POLQ inhibitors have led to the identification of two compounds, which showed efficacy in preclinical studies and for which clinical trials are currently being designed. The antibiotic novobiocin was identified as a specific and potent POLQ inhibitor in a small-molecule library screen[162]. Binding of novobiocin to the ATPase domain of POLQ triggers elimination of BRCA1/2-deficient cells and potentiates the toxic effects of PARP inhibitors. Importantly, novobiocin inhibits growth of *BRCA1*-deleted tumours in genetically engineered mouse models of triple-negative breast cancer, as well as in PDXs with acquired PARP inhibitor resistance through loss of 53BP1, indicating the unique clinical potential of this drug. Moreover, high *POLQ* mRNA levels could potentially be used as a predictive biomarker of novobiocin sensitivity in PARP inhibitor-resistant cancers.

A second POLQ inhibitor, ART558, shows nanomolar POLQ affinity in vitro and specifically inhibits growth of BRCA2-deficient cells, thus recapitulating the effect of *POLQ* genetic ablation[163]. Similarly to novobiocin, ART558 is toxic to *BRCA1*$^{-/-}$ cells, and organoids derived from a BRCA1-mutant breast cancer that have acquired resistance to PARP inhibitors. Although in vivo data for this inhibitor are not available, an additional POLQ inhibitor with increased bioavailability and lower clearance in animal models developed by the same company, ART812, suppresses the growth of PARP inhibitor-resistant *BRCA1*$^{-/-}$ tumours in rats.

USP1 inhibitors target BRCA1/2-deficient cancers

The deubiquitylating enzyme USP1 is a negative regulator of translesion synthesis via reversal of proliferating cell nuclear antigen (PCNA) ubiquitylation[164–167]. PCNA ubiquitylation is required for postreplicative DNA repair via translesion synthesis[168,169]. The persistence of monoubiquitylated PCNA upon USP1 inactivation destabilizes replication forks, which become reliant on BRCA1. Consequently, USP1 inactivation is synthetic lethal with BRCA1 loss in cancer cells. The recently developed USP1 inhibitor KSQ-4279 specifically targets BRCA1/2-deficient cancer cells. Importantly, its combination with PARP inhibitors led to a prolonged antitumour response in PDX models of triple-negative breast cancer, regardless of BRCA1/2 status[170]. KSQ-4279 is currently being tested in a phase I clinical trial in patients with advanced solid tumours[171].

RAD51 inhibitors

RAD51 is the central enzyme of the HR pathway of DSB repair, and as such is essential for cell survival. RAD51 assembles nucleoprotein filaments on ssDNA formed at resected DSBs, which catalyse strand invasion into a homologous DNA template to initiate HR repair. BRCA2 is the loader of RAD51 at DSB sites and, as expected, RAD51 abrogation is synthetic lethal with PARP inhibition[172–174]. Several compounds that modulate RAD51 activity and prevent RAD51 filament formation and/or inhibit HR repair in vitro and in vivo have been identified (reviewed in[175]). The RAD51 inhibitor CYT-0851 exhibits selective cytotoxic activity against activation-induced cytidine deaminase (AID)-expressing chronic lymphocytic leukaemia mouse models and human cancer cells[176,177] and is currently in a phase I/II clinical trial, alone or in combination with chemotherapies, for treatment of B cell haematological malignancies and advanced solid tumours[178].

Targeting the helicase WRN in cancers with microsatellite instability

Microsatellite instability (MSI) is a common hallmark of cancers with defective MMR (for example, *MLH1*, *MSH2*, *MSH3* or *MSH6* gene mutations) and is frequent in stomach (~28%), uterine (~22%) and colorectal (~15%) cancers[179–181]. The helicase WRN is a synthetic-lethal target in cells with MSI, particularly in hereditary non-polyposis colon cancer[182,183]. However, inactivation of MMR genes alone was not sufficient to explain the observed synthetic-lethal interaction. Instead, long-term MMR deficiency leads to the accumulation of large TA dinucleotide repeats[184], which form non-B-DNA secondary structures. In the absence of WRN, the SLX4–MUS81 endonuclease complex cleaves these structures, resulting in chromosomal fragmentation and apoptosis[184]. Therefore, inhibition of WRN helicase activity might be a potent and selective therapy for MSI-positive cancers.

Conclusion and perspectives

The DDR network is highly coordinated to ensure chromosome integrity during cellular processes involving DNA metabolic reactions (replication and repair). Given that these activities are essential for cell survival and proliferation, it became clear early on that targeting the DDR could be a feasible strategy for suppressing growth of cancer cells[1,185]. The obvious challenge to this approach is to target the DDR specifically in the tumour, leaving the normal tissues unaffected. Substantial progress has been made towards tackling this challenge with the identification of specific genetic alterations that promote tumour growth, while making the tumour vulnerable to DDR-targeting treatments with selective toxicity in this specific genetic background. The well-characterized paradigm of the synthetic-lethal interaction between *BRCA1*/*BRCA2* mutations in tumours and PARP inhibitors illustrates this concept. Applying the same principle, multiple studies have identified synthetic-lethal interactions between other DDR genes mutated in cancer, thereby supplying potential druggable targets

against tumours carrying those mutations. However, the approach has proven cumbersome. This is because three unique factors underlie the remarkable hypersensitivity of *BRCA1*/*BRCA2*-mutated cells and tumours to PARP inhibitors: (1) PARP inhibitors prevent processing of unligated Okazaki fragments, thereby generating SSBs[94]; (2) SSBs are the most common DNA lesions in cancers[186] and require PARP1 for repair; (3) unrepaired SSBs in BRCA1/2-mutated tumours treated with PARP inhibitors cause further DNA damage, which in turn triggers potent innate immune responses[187,188]. It is therefore possible that other synthetic-lethal interactions may not be able to replicate the success of PARP inhibitors against HR-deficient tumours.

Moreover, most tools used for the identification of novel druggable targets (for example, CRISPR–Cas9 screens) monitor synthetic-lethal interactions on the genetic level. Although this approach can lead to the identification of novel druggable targets (for example, RNase H in BRCA1-deficient cancer cells[88]), the deletion of a target protein may not have as strong anticancer effects as its chemical inhibition. For example, the cytotoxicity of PARP1 inhibitors with regard to HR-deficient cancers is thought to be mediated by PARP1 trapping rather than by loss of PARP activity[87,189]. In other cases, loss of an enzyme's catalytic activity is worse than not having the protein at all. For example, *Atm*-knockout mice are viable although tumour-prone, whereas mutation of the ATM catalytic site is embryonic lethal[190,191]. The same is true for DNA-PKcs kinase-dead mice, which are also embryonic lethal, although mice lacking DNA-PKcs are viable[192].

Furthermore, inactivation of DDR genes elicits genomic instability, leading to alterations in multiple other genes, making it difficult to ascertain the genetic origin of the tumour. The emergence of precision medicine emphasized the benefits of matching a specific genetic alteration found in patients with cancer with a targeted therapy for this alteration. The aim is to maximize therapeutic impact, while diminishing adverse effects (that is, toxicity and resistance). However, the advances made in precision medicine in recent years have also unravelled the complexity of the task. For example, although technologies that enable sequencing of patient samples are readily available, deconvoluting and interpreting the genomics and transcriptomics data remain major obstacles[193].

Another consequence of the genomic instability in DDR-mutated tumours is the rewiring of their genetic make-up to escape drug toxicity and restore tumour growth despite the targeted treatment. This phenomenon is known as acquired resistance to therapy, and is still an insurmountable obstacle in the clinic. The design of drug combinations, with optimized timing and dosing regimens, that reduce toxicity and limit or suppress resistance could tackle these problems[194]. In this respect, the use of DDR inhibitors together with immune checkpoint blockade is a promising therapeutic strategy. The DNA lesions inflicted by DDR inhibitors lead to the accumulation of cytosolic DNA fragments (for example, in the form of micronuclei), which activate the cGAS–STING axis and trigger antitumour immune responses. The interplay between the DDR and cGAS–STING pathways and clinical applications have been discussed in other recent reviews[187,195].

Multiple novel compounds targeting DDR pathways have been recently developed, as discussed in this Review, and those showing promising results in the preclinical setting are being evaluated in patients. However, it is important to recognize that such empirical approaches may not work in complex diseases such as cancer. It is imperative therefore to concentrate research efforts towards the discovery of novel targets for drug development, as well as towards the optimization of existing drugs through superior formulations or identification of potent drug combinations that can reduce side effects and maximize clinical efficacy.

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