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Targeting DNA double-strand break signalling and repair: recent advances in cancer therapy

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Abstract

Genomic instability, a hallmark of almost all human cancers, drives both carcinogenesis and resistance to therapeutic interventions. Pivotal to the ability of a cell to maintain genome integrity are mechanisms that signal and repair deoxyribonucleic acid (DNA) double-strand breaks (DSBs), one of the most deleterious lesions induced by ionising radiation and various DNA-damaging chemicals. On the other hand, many current therapeutic regimens that effectively kill cancer cells are based on the induction of excessive DSBs. However, these drugs often lack selectivity for tumour cells, which results in severe side effects for the patients, thus compromising their therapeutic potential. Therefore, the development of novel tumour-specific treatment strategies is required.

Unlike normal cells, however, cancer cells are often characterised by abnormalities in the DNA damage response including defects in cell cycle checkpoints and/or DNA repair, rendering them particularly sensitive to the induction of DSBs. Therefore, new anticancer agents designed to exploit these vulnerabilities are becoming promising drugs for enhancing the specificity and efficacy of future cancer therapies. Here, we summarise the latest preclinical and clinical developments in cancer therapy based on the current knowledge of DSB signalling and repair, with a special focus on the combination of small molecule inhibitors with synthetic lethality approaches.

Key words: *genomic instability; DNA damage response; DNA repair; cancer therapy; small molecule inhibitors; synthetic lethality*

Introduction

Cancer is the major cause of death in Switzerland among people aged 45–84 years [1]. The latest Swiss cancer statistics indicate that prostate cancer in men and breast cancer in women are the most common types, with 6,000 and 5,500 incidences per year, respectively. Notably, lung cancer is still the leading cause of cancer-related death in the Swiss population, accounting for approximately 3,000 deaths each year [2].

Almost all human cancers are characterised by genomic instability, which is considered to play a key role in the conversion of a normal cell into a premalignant cell [3]. Mechanisms contributing to genomic instability include aberrant repair of deoxyribonucleic acid (DNA) lesions as well as defective signalling to cell-cycle checkpoints and induction of apoptosis. Damaging agents, emanating from endogenous and environmental sources such as oxidative stress and ultraviolet (UV) radiation, constantly challenge the integrity of DNA. Remarkably, spontaneous DNA damage, mostly hydrolytic cytosine deamination and oxidative DNA base damage, occurs at a rate of up to 10^5 lesions per cell per day [4, 5].

In order to counteract these insults and preserve genome stability, cells activate a coordinated signal-transduction network, which is collectively known as the DNA damage response (DDR). Generally, this response consists of a series of events such as detection of the DNA damage by sensors, accumulation of repair factors by mediators and repair of the lesion by effectors [6]. Cells are equipped with a variety of distinct, but partially compensatory, DNA repair mechanisms, each addressing a specific type of lesion [5]. DNA double-strand breaks (DSBs) are considered to be the most hazardous lesions, since a single unrepaired DSB may trigger cell death whereas a misrepaired DSB potentially results in mutations such as chromosomal rearrangements, which can promote carcinogenesis. Therefore, activation of cell-cycle checkpoints and faithful repair in response to DSBs are a primary barrier to malignant transformation.

The fact that DSBs are highly cytotoxic is exploited in conventional cancer treatment with radiation therapy and certain chemotherapeutic drugs such as DNA crosslinkers and topoisomerase inhibitors. Although those agents induce DSBs in all cells, hyperproliferating cancer cells are much more susceptible to killing than normal cells. However, most of these well-established treatments cause a number of adverse effects, mainly by affecting the fast-dividing cells of the patient, such as haematopoietic stem cells, hair follicles and cells lining the stomach and intestines. Therefore, novel strategies to treat cancer are eagerly anticipated and the subject of extensive research.

In this review, we summarise how DSB repair and its genetic interactions have emerged as targets for improved cancer treatment strategies in the recent past. We also highlight the current knowledge of small molecule inhibitors

(SMIs) of DSB signalling and repair factors, which are promising candidates for clinical use. For a comprehensive overview of targeting DDR pathways for cancer therapy, we direct the reader to recently published reviews [7–9].

DNA damage response

The DDR is a multifaceted signalling network, which is elicited upon detection of DNA lesions in order to coordinate the cell cycle, DNA repair and possibly senescence or apoptosis (fig. 1). Three members of the phosphatidylinositol-3-kinase (PI3K) related kinases (PIKKs) – ATM (ataxia telangiectasia mutated), ATR (ATM- and Rad3-related) and DNA-PKcs (DNA-dependent protein kinase catalytic subunit) – become activated upon DNA damage to trigger the DDR [10]. Through a cascade of phosphorylation events, ATM and ATR activate multiple proteins, most notably p53 and the downstream checkpoint kinases CHK1 and CHK2, which in turn phosphorylate WEE1 kinase and CDC25 phosphatases. Consequently, through regulating the activity of cyclin-dependent kinases (CDKs), the progression from one cell cycle phase to another is delayed [11]. Depending on which CDK is inhibited; the cell cycle is arrested either at the G1/S or the G2/M transition. The resulting cell-cycle arrest allows time for repair, thereby preventing genome duplication or cell division in the presence of damaged DNA. Thus, cells with an abrogated DDR generally display an increased sensitivity towards DNA-damaging agents.

DNA repair pathways

Depending on the type of DNA damage, cells invoke specific DNA repair pathways in order to restore the genetic information (see fig. 1). Briefly, minor changes to DNA such as oxidised or alkylated bases, small base adducts and single-strand breaks (SSBs) are restored by the base excision repair (BER) pathway [4]. A key player in this process is poly-adenosine-diphosphate-ribose (PAR) polymerase (PARP). Upon detection of SSBs, PARP covalently transfers PAR chains to itself and to acceptor proteins in the vicinity of the lesion, thereby facilitating the repair of SSBs. More complex, DNA helix-distorting base lesions, such as those induced by UV light, are repaired by nucleotide excision repair (NER) [5]. Another kind of damage disturbing the helical structure of DNA is represented by base mismatches. Mismatch repair factors recognise and process misincorporated nucleotides as well as insertion or deletion loops that arise during recombination or from errors of DNA polymerases [12]. Covalent links between the two strands of the double helix represent a type of DNA damage referred to as interstrand crosslinks (ICLs). ICLs represent the most deleterious lesions produced by chemotherapeutic agents such as mitomycin C (MMC), cisplatin and cyclophosphamide. ICL repair is complex and involves the collaboration of several repair pathways, namely Fanconi anaemia, NER, translesion synthesis and homologous recombination (HR) [13].

DNA double-strand break repair

Thus far, four mechanistically distinct DSB repair mechanisms in mammalian cells have been described: nonhomologous end joining (NHEJ), alternative-NHEJ, single-strand annealing and HR [14]. NHEJ and HR represent the two major DSB repair pathways, with NHEJ operating throughout the cell cycle and HR being most active during S-phase (fig. 2) [15]. NHEJ starts with the binding of the Ku70/80 heterodimer to both ends of the break, followed by the recruitment of the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs). Subsequent phosphorylation events mediated by the DNA-PK holoenzyme lead to appropriate DNA end processing by the Artemis nuclease. DSB repair by NHEJ is completed by rejoining of the ends

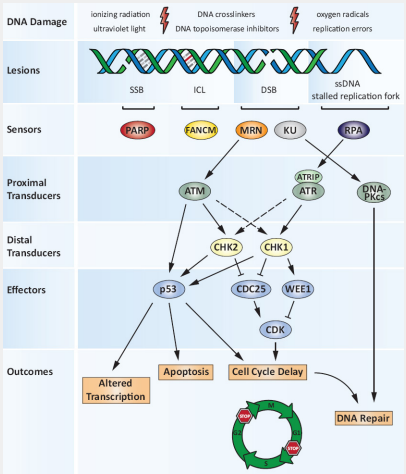

Figure 1

The DNA damage response.

Exogenous and endogenous DNA damaging agents generate various types of lesions including DNA single- and double-strand breaks (SSBs and DSBs). The multifunctional MRN complex detects DSBs, while FANCM is required for the DNA interstrand crosslink (ICL)-induced checkpoint response. PARP predominantly acts as a SSB sensor protein. RPA binds to regions of single-stranded DNA (ssDNA) that are exposed at stalled replication forks or after DSB resection. MRN and RPA mediate the recruitment of ATM and ATR-ATRIP, respectively, and the subsequent activation of the respective pathways, coordinating cell-cycle checkpoints, DNA repair and apoptotic responses to DNA damage. The Ku70/Ku80 heterodimer (KU) competes with MRN for binding to DSBs. KU recruits DNA-PKcs to form the catalytically active DNA-PK holoenzyme which is a major component of the canonical NHEJ machinery during DSB repair. MRN on the other hand initiates HR (see also fig. 2). Once activated, the DNA damage signalling cascade extends through multiple phosphorylation events primarily via the cell-cycle checkpoint kinases CHK1 and CHK2. Their signals converge on downstream effectors such as the tumour suppressor protein p53 or the CDC25 protein phosphatase and WEE1 tyrosine kinase. As a result, CDK activity is inhibited, delaying cell cycle progression from G1 to S (the G1/S checkpoint) or from G2 to M phase (the G2/M checkpoint). The DNA damage response (DDR) thus orchestrates a variety of cellular outcomes: the transcriptional programme of the damaged cell is altered and the cell cycle is transiently arrested, thereby facilitating repair of the DNA lesions. In situations where DNA damage is too severe and cannot be repaired, the DDR triggers apoptosis or senescence.

ADP = adenosine diphosphate; ATM = ataxia telangiectasia mutated protein; ATR = ATM- and Rad3-related; ATRIP = ATR-interacting protein; CDK = cyclin-dependeant kinase; DNA = deoxyribonucleic acid; DNA-PK = DNA-dependeant protein kinase; DNA-PKcs = DNA-PK catalytic subunit; FANCM = Fanconi anaemia complementation group M; HR = homologous recombination; ICL = interstrand crosslink; MRN = MRE11-RAD50-NBS1 complex; NEHJ = nonhomologous end joining; PARP = poly(ADP-ribose) polymerase; RPA = replication protein A

catalysed by a complex consisting of X-ray repair cross-complementing protein 4 (XRCC4), XRCC4-like factor (XLF) and DNA ligase IV. HR takes over if NHEJ is unsuccessful in ligating the broken DNA ends or when the DSB is first recognised by the MRE11-RAD50-NBS1 (MRN) complex rather than by Ku70/80 [16]. Together with CtBP-interacting protein (CtIP; CtBP = C-terminal binding protein), MRN resects DSBs to generate short 3'-single-stranded-DNA (ssDNA) tails that get immediately coated with replication protein A (RPA) [17]. The BRCA2-PALB2 complex promotes RAD51 nucleation onto ssDNA, thereby replacing RPA. The RAD51 nucleoprotein filament then invades the homologous, intact DNA template forming a displacement loop. The second end of the broken chromosome is captured and anneals to the complementary strand of the donor DNA molecule, resulting in the formation of two Holliday junctions (HJs). After DNA synthesis and ligation of both strands, the double HJ is either dissolved or is dismantled by the catalytic action of resolvases in order to complete repair [18]. Thus, repair by HR is error-free since it copies the missing genetic information from the undamaged sister chromatid, whereas NHEJ is error-prone since DNA ends without sequence homology are religated with the risk of causing mutations [19]. Given that a single unrepaired DSB has the potential to kill a cell, inhibition of repair by compounds that target factors involved in NHEJ or HR will increase the sensitivity of cancer cells to DSB-inducing anticancer agents.

Harnessing DNA damage signalling and repair for cancer therapy

The fact that cells with a compromised DDR are hypersensitive to DNA damage-inducing agents is currently under vigorous investigation for use in targeted cancer therapy. More precisely, during their pathogenesis, many cancer cells acquire defects in a certain DNA repair pathway and become dependent on a compensatory mechanism in order to survive. Hence, pharmacological inhibition of the "backup" pathway in combination with DNA damage will selectively kill cancer cells but spare their normal counterparts. Furthermore, highly proliferative cancer cells are inherently hypersensitive to DNA damage because S-phase, in which DNA replication takes place, is the most vulnerable period of the cell cycle.

Targeting DSB signalling pathways

As previously mentioned, cell-cycle checkpoint activation in response to DSBs gives a cell time for DNA repair before entry into S-phase or mitosis. Consequently, cell-cycle checkpoints reduce the efficacy of DNA-damaging agents used in cancer therapy. Therefore, selective abrogation of checkpoint signalling sensitises cancer cells to chemo- and radio-therapy, potentiating cancer treatment [20]. Importantly, more than 50% of human tumours are defective in p53 tumour suppressor function and cell-cycle checkpoint inhibitors have been demonstrated particularly to sensitise p53-deficient cancer cells to various anticancer agents in clinical use [21].

In the late 1960s, long before the discovery of cell-cycle checkpoints, the first attempts to sensitise cancer cells to standard cytotoxic therapy were made using ordinary compounds such as caffeine [22]. Later it was found that caffeine directly binds to and inhibits ATM and ATR *in vitro* and thus interferes with initiation of the DDR [23, 24]. However, since caffeine is a relatively nonselective agent, efforts have been made to develop more potent and selective inhibitors of the PIKK family members ATM, ATR and DNA-PKcs (table 1). In 2004, KuDOS Pharmaceuticals (now AstraZeneca) reported the identification of KU-55933, a specific SMI of ATM [25]. On the molecular level, KU-55933, like most kinase inhibitors, competes with the ATP-binding site of the enzyme, thereby inhibiting the catalytic activity of ATM [26]. Based on the promising preclinical results, KU-60019, a KU-55933 analogue with

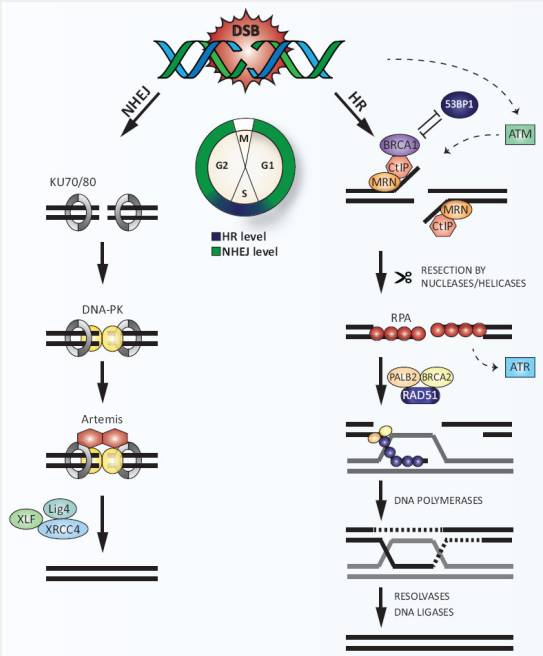

Figure 2

DNA double-strand break (DSB) repair.

DSBs are predominantly repaired by two distinct pathways: NHEJ or HR. NHEJ operates throughout the cell cycle, but mainly during the G1 and G2 phases, whereas HR peaks in S phase. Rapid association of the Ku70/80 heterodimer to DSBs promotes NHEJ by recruiting DNA-PKcs. DNA ends are processed by the nucleolytic activity of Artemis, followed by religation catalysed by a complex of XLF, Ligase IV (Lig4) and XRCC4. Alternatively, MRN, which is initially recruited to DSBs in competition with Ku70/80, initiates DSB resection together with CtIP thereby promoting HR. 53BP1 antagonises BRCA1 in DSB resection. Extensive DSB resection by other nucleases and formation of RPA-coated ssDNA stimulates the activation of ATR. Displacement of RPA by RAD51 is mediated by BRCA2 and PALB2, resulting in the formation of RAD51 nucleoprotein filaments. Subsequent strand invasion into the homologous DNA template and capturing of the second DNA end leads to the formation of a double Holliday junction, which is processed by resolvases. Finally, the DNA is sealed by ligases to accomplish error-free repair of the DSB.

53BP = p53 binding protein; ATM = ataxia telangiectasia mutated protein; CtBP = C-terminal binding protein; CtIP = CtBP-interacting protein; DNA = deoxyribonucleic acid; DNA-PK = DNA-dependent protein kinase; DNA-PKcs = DNA-PK catalytic subunit; HR = homologous recombination; MRN = MRE11-RAD50-NBS1 complex; NHEJ = nonhomologous end joining; PALB1/2 = partner and localiser of BRCA1/2; RPA = replication protein A; ssDNA = single-stranded DNA; XLF = XRCC4-like factor; XRCC4 = X-ray repair cross-complementing protein 4

improved pharmacokinetics and bioavailability, was synthesised and shown to radiosensitise glioma cells approximately 10 times more efficiently than KU-55933 [27]. Compounds selectively targeting ATR have long been awaited, particularly when inhibitors of CHK1, a direct downstream target of ATR, had proven to be clinically effective [28]. Finally, in 2011, three ATR inhibitors, NU-6027, VE-821 and ETP-46464 were described. NU-6027 is a pyrimidine analogue originally discovered as an adenosine triphosphate (ATP) competitive inhibitor of CDKs, but recently reported also to inhibit ATR at low micromolar concentrations and to confer cisplatin cytotoxicity independently of CDK inhibition [29, 30]. The ATR inhibitor VE-821 (Vertex Pharmaceuticals) was identified using a high-throughput screen against full-length recombinant ATR [31]. Preclinical testing of VE-821 using pancreatic cancer cells demonstrated its chemo- and radiosensitisation properties [32]. ETP-46464 was discovered by screening compounds with a previously reported activity against the related PI3Ks using a cell-based system assaying for ATR activity [33]. In the same study, NVP-BEZ235, a recognised dual PI3K/mTOR inhibitor (mTOR = mammalian target of rapamycin), was also reported to block efficiently ATM, ATR and DNA-PK activity. Furthermore, NVP-BEZ235 was found to act as a radio- and chemo-sensitiser in various cancer cell lines and is currently being tested as a single agent in various Phase I/II clinical trials [34, 35]. Importantly, most, if not all, of the aforementioned compounds are likely to inhibit additional protein kinases, especially when used at concentrations in the high micromolar range, thus potentially exhibiting "off-target" effects.

Downstream of ATM and ATR act the two transducer kinases CHK1 and CHK2, against which several inhibitors have emerged during recent years. One of the first SMIs, UCN-01, a derivative of staurosporine, was originally isolated from a *Streptomyces* strain as a protein kinase antagonist with cytotoxic effects [36]. UCN-01 was later shown to be a potent inhibitor of CHK1 and to block its kinase activity by interacting with the ATP-binding pocket [37, 38]. Six Phase II clinical trials of UCN-01, either as a single agent or in combination with other drugs, in patients with different types of advanced cancer have already been completed. Recently, three novel CHK1 inhibitors, GDC-0425 (Genentech Inc.), SCH900776 (now renamed MK-8776, Merck) and LY-2606368 (Eli Lilly), have entered Phase I clinical trials either as single agents or in combination with gemcitabine, a nucleoside analogue [39]. Another promising drug that interferes with checkpoint activation is the WEE1 tyrosine kinase inhibitor MK-1775 (Merck), which was discovered by screening a chemical library [40]. MK-1775 is already under investigation in a Phase II trial combined with carboplatin in order to assess the benefit for patients with p53-mutated epithelial ovarian cancer. Last but not least, efforts to target CDC25 phosphatases, which also represent key molecules in checkpoint regulation, led to the discovery of several CDC25 inhibitors, amongst which the most potent are quinonoid-based derivatives such as the bis-quinone compound IRC-08386 [41, 42].

In summary, several SMIs that interfere with checkpoint activation show great promise of advancing in clinical studies and eventually being used as chemo- or radio-sensitisers as well as monotherapeutic agents in cancer treatment. Nevertheless, since many of the SMIs have only very recently been discovered, their safety, tolerability and efficacy when used alone or in combination has to be further investigated.

Targeting DSB repair

Impairing the repair of DSBs using drugs that either inhibit the enzymatic activity or interfere with protein-protein interactions of repair factors provides another approach to sensitising cancer cells for chemo- and radio-therapy. A key player in DSB repair by NHEJ is DNA-PK (see fig. 1), which, like ATM and ATR, belongs to the PIKK family of protein kinases. In 2003, two DNA-PKcs-specific inhibitors, NU-7026 and NU-7441, were reported, both of which are practically inactive against ATM and ATR [43, 44]. Unfortunately, neither of them has progressed into clinical development. However, Celgene Corporation is currently recruiting patients with advanced tumours unresponsive to standard therapies in order to test the pharmacokinetics and preliminary efficacy of the dual DNA-PK/mTOR inhibitor, CC-115, in a Phase I trial. Moreover, a very recent preclinical study reported that KU-60648 (AstraZeneca), a dual inhibitor of DNA-PK and PI3K, acts as a chemosensitiser in cell-based assays and in mice xenografts [45]. More recent attempts to find novel DSB repair inhibitors led to the identification of mirin, the first inhibitor of the MRN complex that acts by blocking the nuclease activity of MRE11 [46]. Interestingly, mirin was shown to kill BRCA2-deficient cells, an effect that was even more pronounced when combined with a PARP inhibitor [47]. However, since mirin has to be applied at high micromolar concentrations to inhibit MRN, such treatment is prone to increase the risks of undesired "off-target" effects and the generation of more selective derivatives is eagerly anticipated.

During the course of DSB repair by HR, ssDNA is generated and immediately coated by RPA, which later on is replaced by the RAD51 recombinase (see fig. 2). Inhibiting the DNA-binding activity of RPA by the SMI MCI13E yielded encouraging preclinical results in combination with cisplatin [48]. Moreover, several means to prevent RAD51 action have been reported, including SMIs (B02, RI-1) as well as inhibitory peptides that interfere with the binding of BRCA2 to RAD51 [49–52]. Although peptides blocking protein-protein interactions represent an interesting concept for inhibiting DSB repair, their potential application in the clinics has yet to be established.

In summary, safety, tolerability, pharmacokinetics and efficacy of most of the aforementioned SMIs have still to be carefully validated before they may enter clinical trials to examine their benefit for cancer therapy.

Synthetic lethality approaches to target DSB repair-deficient cancers

Mutations in DSB repair genes render cancer cells dependent on alternative DNA repair pathways. Thus, comprom-

ised abilities to repair DSBs confer a weakness that can be therapeutically exploited on the basis of the concept of synthetic lethality, whereby inhibition of the "back-up" pathway induces greater toxicity in DSB repair-deficient cancer cells as compared with normal cells (fig. 3).

PARP inhibitors

The first "proof-of-principle" study verifying synthetic lethality as a suitable approach for targeted cancer therapy was published in 2005, after it had been demonstrated that HR-defective BRCA1- or BRCA2-deficient cell lines display dramatically increased sensitivity to inhibition of the SSB repair enzyme PARP [53, 54]. Subsequently, clinical development of potent small molecule PARP inhibitors (PARPis) rapidly advanced, and the first Phase II results in 2009 showed that monotherapy with the PARPi olaparib (AZD-2281; AstraZeneca) achieved encouraging response rates of 41% and 33% in patients with BRCA1- or BRCA2-mutated advanced breast and ovarian cancers, respectively [55, 56]. Furthermore, preclinical studies suggested the potential use of PARPi also in sporadic cancers that share phenotypical features with cancers arising from hereditary *BRCA* mutations, a phenomenon that is referred to as "BRCAness" [57]. Reasons for "BRCAness" can be the inactivation of BRCA1 or BRCA2 function caused by aberrant epigenetic or posttranslational modifications, or a wider range of mutations in other genes resulting in defective DSB signalling and HR. For example, it was reported that depletion of factors such as ATR, ATM, CHK1, CHK2, NBS1, CtIP and RAD51 in cultured cells synergistically increases PARPi cytotoxicity to an extent similar to mutations in *BRCA1/2*. This indicates that BRCA-deficient cells are, at least in part, sensitive to PARP inhibition because of a defect in HR [58, 59]. The current understanding suggests that inhibition of PARP leads to the accumulation of SSBs which are converted into DSBs upon encountering DNA replication forks during S-phase when HR is most active [60]. Consequently, in the absence of functional HR, such as in cancer cells lacking BRCA1 or BRCA2, PARP inhibition results in the accumulation of DSBs and, ultimately, in apoptosis or mitotic catastrophe. Importantly, normal cells survive the treatment owing to functional HR, providing the kind of selectivity that is considered the ultimate goal of cancer therapy. Nowadays, most PARPi in preclinical and clinical trials belong to the third generation of SMIs designed to compete with the substrate nicotine adenine dinucleotide (NAD^+) resulting in reversible inhibition of PARP. Recent reports indicate that in addition to catalytic inhibition, some PARPi induce cytotoxic PARP–DNA complexes, trapping PARP proteins on damaged DNA. Currently, PARPi are divided into two classes: catalytic inhibitors and dual inhibitors that not only block the enzymatic activity but also act as so-called PARP "poisons" [61, 62].

Today, 7 years after PARPi were first established for cancer therapy and despite some quite promising clinical studies, none of them has gained official approval for the treatment of cancer patients. In 2011, encouraging results from a

Table 1: Small molecule inhibitors of DNA damage response factors in preclinical or clinical development for cancer therapy.

Target	Inhibitor	Mono- or combination therapy / clinical study stage	Clinical trial identifier/ reference
ATM	KU-55933	IR, etoposide, doxorubicin, camptothecin, in preclinical testing	[25, 95]
	KU-60019	IR in preclinical testing using glioma cells	[27]
ATR	NU-6027	Hydroxyurea, cisplatin, temozolomide, rucaparib in preclinical testing	[29]
	VE-821	Cisplatin in breast and ovarian cell lines IR, gemcitabine in pancreatic cancer cells in preclinical testing	[31, 96] [32]
	ETP-46464	Single agent in p53-deficient cancer cells in preclinical testing	[33]
DNA-PKcs	NU-7441	IR, etoposide in preclinical testing of cancer cell lines and tumour xenografts	[44, 97]
	NU-7026	IR and combined with AG14361 (PARPi) in preclinical testing Anthracyclines, mitoxantrone, etoposide in preclinical testing using leukaemia cells	[43] [98]
DNA-PKcs/PI3K	KU-60648	Etoposide, doxorubicin in preclinical testing	[45]
DNA-PKcs/mTOR	CC-115	Single agent in Phase I safety and tolerability study (recruiting)	NCT01353625
PI3K/mTOR/PIKK	NVP-BEZ235	Single agent in several clinical trials IR, cisplatin in preclinical testing	www.clinicaltrials.gov [34, 35]
CHK1/(CHK2)	UCN-01	Single agent in Phase II for relapsed T-cell lymphoma (completed) Single agent in Phase II for metastatic melanoma (completed) Five-fluorouracil in Phase II for metastatic pancreatic cancer (completed) Topotecan in Phase II for various forms of ovarian cancer (completed) Topotecan in Phase II for small cell lung cancer (completed) Olaparib in pre-clinical testing for multiple mammary tumour types	NCT00082017 NCT00072189 NCT00045747 NCT00072267 NCT00098956 [99]
	GDC-0425	Single agent or with gemcitabine in Phase I dose-escalation study (recruiting)	NCT01359696
	MK-8776	Single agent or with gemcitabine in Phase I dose-escalation study (completed)	NCT00779584
	LY-2606368	Single agent in Phase I study in patients with advanced cancer (recruiting)	NCT01115790
WEE1	MK-1775	Carboplatin in Phase II for epithelial ovarian cancer	NCT01164995
CDC25	IRC-083864	Single agent in preclinical testing using pancreatic and prostate cancer cells	[42]
MRE11	mirin	Single agent or with olaparib (PARPi) in preclinical testing using BRCA2-deficient cells	[47]
RPA	MCI13E	Single agent or with cisplatin in preclinical testing	[48]
RAD51	B02	IR, mitomycin C, cisplatin in preclinical testing	[100]
	RI-1	Mitomyin C in preclinical testing	[50]

ADP = adenosine diphosphate; ATM = ataxia telangiectasia mutated protein; ATR = ATM- and Rad3-related; CHK = checkpoint kinase; DNA = deoxyribonucleic acid; DNA-PK = DNA-dependent protein kinase; DNA-PKcs = DNA-PK catalytic subunit; IR = ionising radiation; mTOR = mammalian target of rapamycin; PAR = poly(ADP-ribose); PARP = PAR polymerase; PARPi = PARP inhibitor; PI3K = phosphatidylinositol-3-kinase; PIKK = PI3K-related kinase; RPA = replication protein A

Phase II trial with iniparib (BSI-201, Sanofi-Aventis) in patients with triple negative breast cancer, which shares many features with BRCA-associated breast cancer, failed to translate into overall patient survival in a Phase III trial [63]. Later that year, AstraZeneca announced that olaparib would not progress into Phase III for hereditary *BRCA* mutation-associated breast cancer. This decision was possibly driven by economic concerns rather than by clinical issues [64]. Notwithstanding all setbacks, clinical development and research on the mechanism of action of PARPi is still ongoing (table 2). Despite controversies about its effectiveness as a PARPi, Sanofi's iniparib is under clinical investigation as a single agent and in combination with chemotherapeutic regimens in patients with recurrent solid tumours (NCT01455532), nonsmall-cell lung cancer (NCT01082549) and ovarian cancer (NCT01033292) [65, 66]. Likewise, AstraZeneca is continuing Phase II trials with olaparib to treat serous ovarian cancer, since it shares many features with BRCA1/2-mutated cancers. Indeed, activity of olaparib as a monotherapy was evident in women with pretreated high-grade serous ovarian cancer without germline *BRCA1/2* mutations [67]. This finding clearly demonstrates positive responses of a subpopulation of sporadic cancers to PARPi therapy and also underlines the importance of classifying patients according to biomarkers in order to predict the efficacy of PARPi. Such potential biomarkers also include deficiency of the phosphatase and tensin homologue (PTEN) tumour suppressor. Interestingly, due to its role in the regulation of RAD51 transcription, loss of PTEN is associated with defective HR [68, 69]. In general, detection of compromised HR provides a rationale to stratify patients for PARPi treatment. Several ways of identifying HR defects are under investigation, including gene expression profiling and gene copy number analysis of DNA repair factors [70, 71]. Further approaches assess the DSB repair capacity of tumours by measuring expression of the MRN complex, monitoring RAD51 foci formation and poly(ADP-ribosyl)ation as surrogate markers for DSB repair proficiency [72]. As for most cancer therapies, a major challenge of using PARPis is the acquired resistance of initially PARPi-sensitive cancer cells due, for example, to the loss of 53BP1 (a p53 binding protein) or to overexpression of multidrug-resistance efflux transporters [72, 73]. In addition, secondary *BRCA2* mutations have been identified, which restore the full-length protein thereby re-establishing BRCA2 functions and conferring PARPi resistance [74].

Thus, despite considerable efforts to develop PARPi for clinical use, conventional DNA-damaging chemo- and radio-therapy largely remains the mainstay of cancer treatment. However, several onging preclinical and clinical studies employ PARPi both as monotherapy and as chemo- or radio-sensitisers, because an improvement of current anti-cancer regimes is long-awaited.

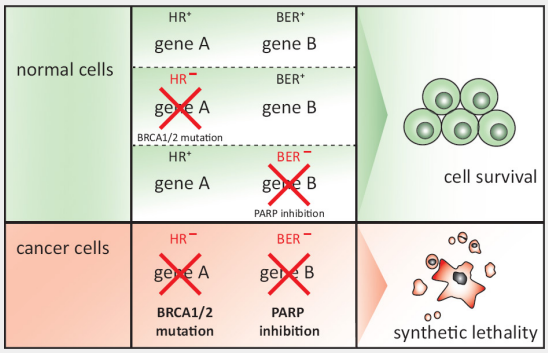

Figure 3

Synthetic lethality.

Synthetic lethality is defined as a combination of mutations or perturbations in two or more genes that leads to cell death, whereas inactivation of any one of the genes alone does not. Perturbation of genes can occur through genetic mutation or silencing, depletion by RNAi or inhibition by SMIs and is depicted by a red cross. If genes that are essential for a certain DNA repair pathway (e.g. gene A) are inactivated in normal cells, alternative pathways with functional genes (e.g. gene B) are utilised to respond to DNA damage. Conversely, cancer cells mutated or silenced for a component of a DDR pathway are compromised in their ability to process DSBs. These cells then rely on alternative DNA repair pathways to repair the breaks. Therefore, inhibition of the alternative pathway will cause cell death due to persisting DSBs. Please refer to main text for details about the example given for synthetic lethality between *BRCA1/2* mutation and PARP inhibition.

ADP = adenosine diphosphate; BER = base excision repair; DDR = DNA damage response; DNA = deoxyribonucleic acid; DSB = double-strand break; HR = homologous recombination; PARP = poly(ADP-ribose) polymerase; RNA = ribonucleic acid; RNAi = RNA interference; SMI = small molecule inhibitor

Synthetic lethal strategies emerging from preclinical research

As intensive basic research is leading towards a better understanding of cellular functions and their underlying genetic networks, more and more genetic interactions become apparent as potential targets for synthetic lethality in cancer therapy. Beyond BRCA1 and BRCA2, their joint interaction partner PALB2 is emerging as a breast cancer susceptibility gene, thus providing another opportunity for PARPi-based therapies [75].

PARP inhibition is not the only approach that takes advantage of synthetic lethal interactions between two DNA repair pathways, as inhibition of apurinic/apyrimidinic (AP) endonuclease 1, an essential component of BER, was recently shown to eliminate cancer cells with HR defects [76]. Moreover, synthetic lethality with components of the cell-cycle checkpoint machinery could be exploited in cancers harbouring activated oncogenes, since oncogene-induced replication stress activates the ATR-CHK1 signalling pathway. For example, exacerbated toxicity was reported upon inhibition of CHK1 in lymphoma cells with upregulated c-Myc expression [77]. This finding underscores the concept that cancers with elevated levels of replication stress rely on intact checkpoint signalling for cell survival. Replicative stress induces pan-nuclear distribution of phosphorylated histone variant H2AX (γ-H2AX), which is a useful biomarker for classification of tumour biopsies in order to stratify patients [78].

Finally, disruption of the FA repair pathway was shown to be synthetically lethal with abrogated checkpoint signalling. More precisely, inactivation of ATM or CHK1 resulted in reduced viability of FA-deficient cells, illustrating the concept that checkpoint signalling and FA are mutually compensatory pathways in the maintenance of genome integrity [79, 80]. These observations highlight the

usefulness of SMIs, as currently tested for CHK1, to treat tumours bearing a specific genetic background. Although many of the strategies that are based on the concept of synthetic lethality have so far only been investigated in preclinical settings, some hold great promise of entering clinical trials soon.

Haploinsufficiency of DDR factors

There is increasing evidence that haploinsufficiency of DDR components promotes genome instability and drives tumourigenesis. Dosage insufficiencies of DNA repair genes might, however, only be unmasked once a cell is challenged with an increased load of DNA damage such as oncogene-induced replicative stress [81, 82]. Synthetic lethal approaches might therefore be applicable not only in cancer cells with deficiencies, but also in those bearing haploinsufficiencies for DDR factors. Evidence from gene targeting studies in mice revealed that, for example, the loss of one allele of ATR or CtIP is sufficient to cause increased chromosomal aberrations, genomic instability and tumour susceptibility [83, 84]. This indicates that heterozygous carriers of DDR defects are more prone to develop tumours once the threshold of endogenous DNA damage is increased as, for example, in precancerous lesions [85]. However, scientists are just beginning to unravel how haploinsufficiency of DDR genes contributes to carcinogenesis and how these may be exploited for novel synthetic lethal approaches in cancer therapy.

Table 2: PARP inhibitors in preclinical or clinical development for cancer therapy*.

Inhibitor	Mono- or combination therapy	Preclinical and clinical study stage	Clinical trial identifier/ reference
Olaparib (AZD-2281, KU-59436) AstraZeneca	Single agent	Phase II trials showing with promising response rates in patients with BRCA1/ or BRCA2 mutated advanced breast and ovarian cancers	NCT01078662, NCT00494234 [55, 56]
	Single agent	Phase II trial demonstrating efficacy for advanced high-grade serous ovarian cancer without germline BRCA1/2 mutations, but not with TNBC	NCT00679783 [67]
	Cediranib	Phase I/II study for treatment of recurrent serous ovarian cancer and TNBC	NCT01116648
	Single agent and combinations with other drugs	Several ongoing Phase I/II trials for various cancers, dose-limiting adverse effects for combination of olaparib and topotecan	NCT00516438, NCT00819221, NCT01296763, NCT00912743, [101]
	Cisplatin, radiation	Phase I trial to test olaparib as a radio- and/or chemo-sensitiser in combination with high-dose radiotherapy with or without a daily cisplatin dose in locally advanced NSCLC	NCT01562210
Iniparib** (BSI-201) Sanofi-Aventis	Single agent and combinations with other drugs	Ongoing Phase I/II trials in solid tumours such as sarcomas as well as breast, uterine and ovarian cancers	NCT01455532, NCT01033292, NCT00687687
	Gemcitabine/ carboplatin	Promising results from a Phase II trial failed to translate into survival benefit for TNBC patients with unselected BRCA1/2 status in Phase III	NCT00938652, NCT01130259, [63]
	Radiotherapy	Ongoing Phase I trial of iniparib as radiosensitiser in nonoperable brain metastases	NCT01551680
	Temozolomide and radiotherapy	Ongoing Phase I/II trials for newly diagnosed malignant glioblastoma	NCT00687765
	Gemcitabine/ carboplatin	Ongoing Phase III trial in advanced squamous NSCLC	NCT01082549
Veliparib (ABT-888) Abbott Laboratories	Temozolomide, carboplatin/paclitaxel	International randomised Phase II trial of veliparib combined with chemotherapy in BRCA1/2-mutated, metastatic breast cancer	NCT01506609
	Single agent	Phase I trial for refractory BRCA 1/2-mutated solid cancers; platinum-refractory ovarian, fallopian tube, or primary peritoneal cancer or basal-like breast cancer Additional evaluation of BRCA1/2 expression and changes in PAR and γ-H2AX in peripheral blood mononuclear cells as diagnostic biomarkers	NCT00892736
	Single agent and combinations with chemo- and radio-therapy	Phase I and II trials to identify efficient combinatorial regimens in various solid and lymphoid tumours, promising results in combination with topotecan and cyclophosphamide	NCT01154426, NCT01282333, NCT01386385 and more, [102-104]
Rucaparib (AG-014699, PF-01367338, CO-338) Cancer Research UK Clovis Oncology Pfizer	Single agent	Phase I/II trials for BRCA1/2-mutated breast or ovarian cancer	NCT01482715
	Temozolomide	Initial Phase I trial as enhancer for chemotherapy in unselected solid tumours; severe myelosuppression of the combination in Phase II study for previously untreated metastatic melanoma	[105]
	Single agent and with carboplatin	Phase I/II testing in advanced solid tumors with and without BRCA mutations Several biomarkers for therapeutic response are being evaluated concurrently.	NCT01009190, NCT00664781
Niraparib (MK-4827) Merck Tesaro Inc.	Single agent	Phase I trial in advanced solid tumours showing that MK-4827 is well tolerated, blocks PARP and has promising antitumour activity in both BRCA-deficient and sporadic cancer	[106]
	Single agent and with temozolomide	Phase I dose-escalation study for solid tumours and haematological malignancies	NCT00749502, NCT01294735

* For a complete overview of PARPi currently used in clinical trials, we direct the reader to [61, 107].
** Since the primary mechanism of action for iniparib is likely not via inhibition of PARP activity, it is no longer considered to be a *bona fide* PARPi [65].
ADP = adenosine diphosphate; γ-H2AX = phosphorylated histone variant H2AX; NSCLC = nonsmall cell lung cancer; PARP = poly(ADP-ribose) polymerase; PARPi = PARP inhibitor; TNBC = triple negative breast cancer

Conclusions and future perspectives

To date, DSB-inducing agents have been the core components of conventional cancer therapy, confirming the rationale of inflicting excessive DNA damage in order to kill cancer cells. However, most chemotherapeutic regimens cause severe side effects that limit their therapeutic potential. As summarised in this review, SMIs and synthetic lethal approaches targeting the individual genetic profile of the tumours are under clinical development, with the aim to improve the patients' benefit by increasing the efficacy while lowering the toxicity of cancer treatments. A prerequisite for personalised therapy is the molecular characterisation of tumours with reliable biomarkers to assign patients the appropriate treatment. In order to stratify cancer patients according to their DNA repair status, tumour biopsies can be analysed with immunohistochemistry, fluorescence *in-situ* hybridisation (FISH), gene sequencing, expression profiling and other methods [86]. Relevant biomarker assays should ideally predict the functionality of DNA repair pathways, rather than just providing information about mutations or expression levels of proteins involved in the DDR. Certainly, such a detailed molecular profiling of cancer versus normal tissue from a given patient is critical to maximise the potential of personalised cancer drugs in terms of both therapeutic success and cost-effectiveness.

Recent *in-vitro* and *in-vivo* research has deepened our knowledge about synthetic genetic interactions and put forward alternative ways to treat cancer. Furthermore, by utilising ribonucleic acid (RNA) interference technologies, screens for synthetic lethal interactions of cancer-specific defects in DNA repair pathways have augmented the discovery of targets for cancer therapy. For example, studies using MMR-deficient cells lacking human muts homologue 2 (MSH2) revealed synthetic sickness with POLB, a DNA polymerase acting in BER [87]. Since MSH2 is mutated in 40% of patients with hereditary nonpolyposis colorectal cancer, targeted inhibition of POLB potentially opens new therapeutic applications. Moreover, gaining further insights into the structure and mechanism of action of DNA repair factors such as CtIP will aid the design of new and more efficient SMIs of the DDR. Recently discovered inhibitors of RPA and RAD51 are promising candidates, which are in preclinical testing in order to be approved for the use in clinical trials soon [48, 50].

Interestingly, the latest scientific progress in the field of microRNAs (miRNAs) has demonstrated an intensive interplay of these small regulatory RNAs with the DDR, including DSB repair. Recent studies revealed that DNA damage globally induces miRNA biogenesis and, *vice versa*, that numerous miRNAs modulate the expression of DDR factors [88–90]. Notably, BRCA1 expression was shown to be downregulated by miR-182, conferring hypersensitivity to PARPi [91]. Conversely, BRCA1 was demonstrated to suppress expression of miR-155, an oncogenic miRNA that is overexpressed in many cancers [92, 93]. These observations highlight the therapeutic potential of miRNA mimics or inhibitors in future approaches for cancer therapy [94].

In summary, as the concept of personalised medicine emerges, tumour-specific defects of DSB repair pathways represent a promising therapeutic target to be exploited for the selective elimination of cancer cells. Thus, there is an air of optimism for targeted cancer therapy through exploiting the DDR of tumour cells in the clinics.

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Figures (large format)

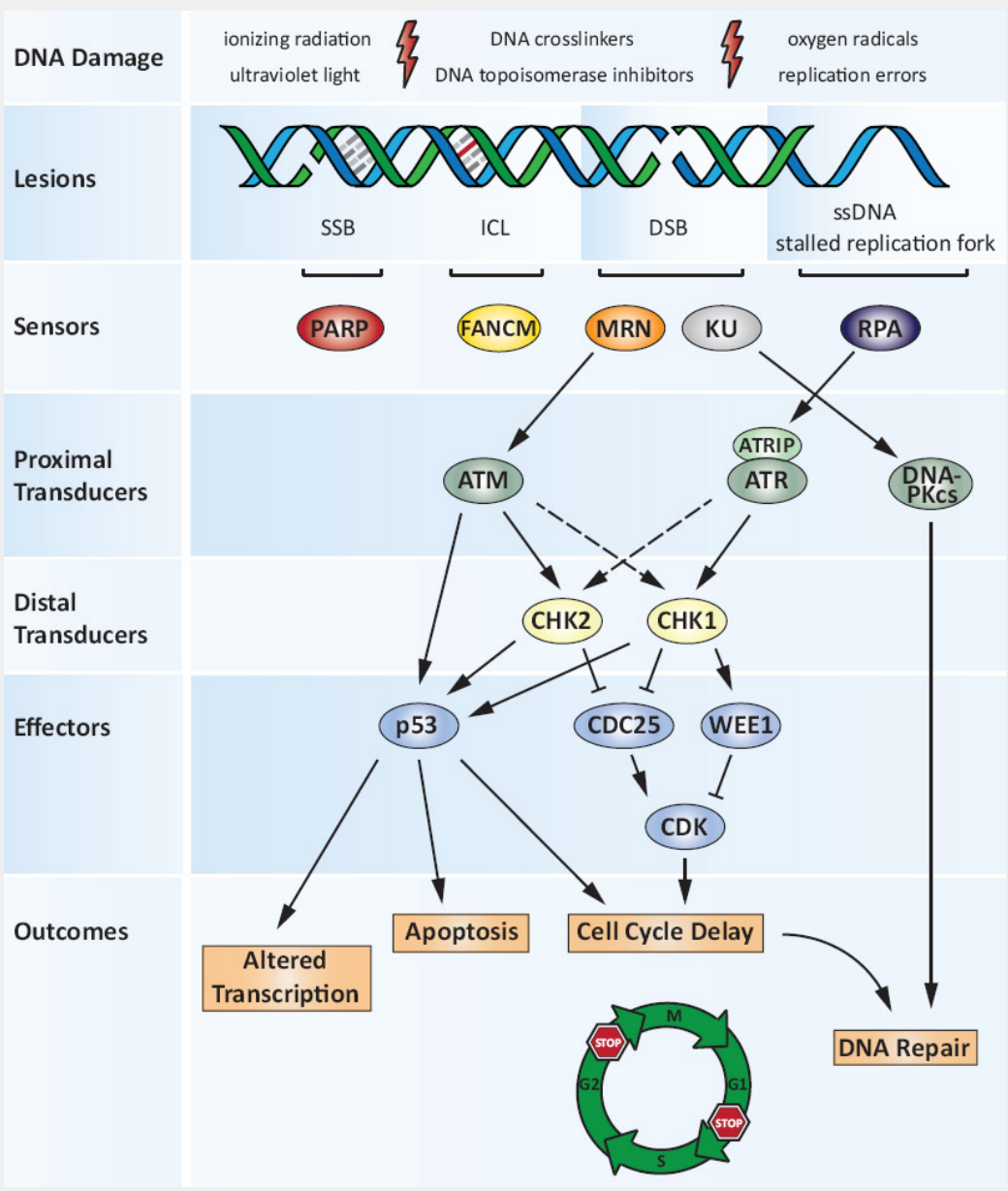

Figure 1

The DNA damage response.

Exogenous and endogenous DNA damaging agents generate various types of lesions including DNA single- and double-strand breaks (SSBs and DSBs). The multifunctional MRN complex detects DSBs, while FANCM is required for the DNA interstrand crosslink (ICL)-induced checkpoint response. PARP predominantly acts as a SSB sensor protein. RPA binds to regions of single-stranded DNA (ssDNA) that are exposed at stalled replication forks or after DSB resection. MRN and RPA mediate the recruitment of ATM and ATR-ATRIP, respectively, and the subsequent activation of the respective pathways, coordinating cell-cycle checkpoints, DNA repair and apoptotic responses to DNA damage. The Ku70/Ku80 heterodimer (KU) competes with MRN for binding to DSBs. KU recruits DNA-PKcs to form the catalytically active DNA-PK holoenzyme which is a major component of the canonical NHEJ machinery during DSB repair. MRN on the other hand initiates HR (see also fig. 2). Once activated, the DNA damage signalling cascade extends through multiple phosphorylation events primarily via the cell-cycle checkpoint kinases CHK1 and CHK2. Their signals converge on downstream effectors such as the tumour suppressor protein p53 or the CDC25 protein phosphatase and WEE1 tyrosine kinase. As a result, CDK activity is inhibited, delaying cell cycle progression from G1 to S (the G1/S checkpoint) or from G2 to M phase (the G2/M checkpoint). The DNA damage response (DDR) thus orchestrates a variety of cellular outcomes: the transcriptional programme of the damaged cell is altered and the cell cycle is transiently arrested, thereby facilitating repair of the DNA lesions. In situations where DNA damage is too severe and cannot be repaired, the DDR triggers apoptosis or senescence.

ADP = adenosine diphosphate; ATM = ataxia telangiectasia mutated protein; ATR = ATM- and Rad3-related; ATRIP = ATR-interacting protein; CDK = cyclin-dependant kinase; DNA = deoxyribonucleic acid; DNA-PK = DNA-dependant protein kinase; DNA-PKcs = DNA-PK catalytic subunit; FANCM = Fanconi anaemia complementation group M; HR = homologous recombination; ICL = interstrand crosslink; MRN = MRE11-RAD50-NBS1 complex; NEHJ = nonhomologous end joining; PARP = poly(ADP-ribose) polymerase; RPA = replication protein A

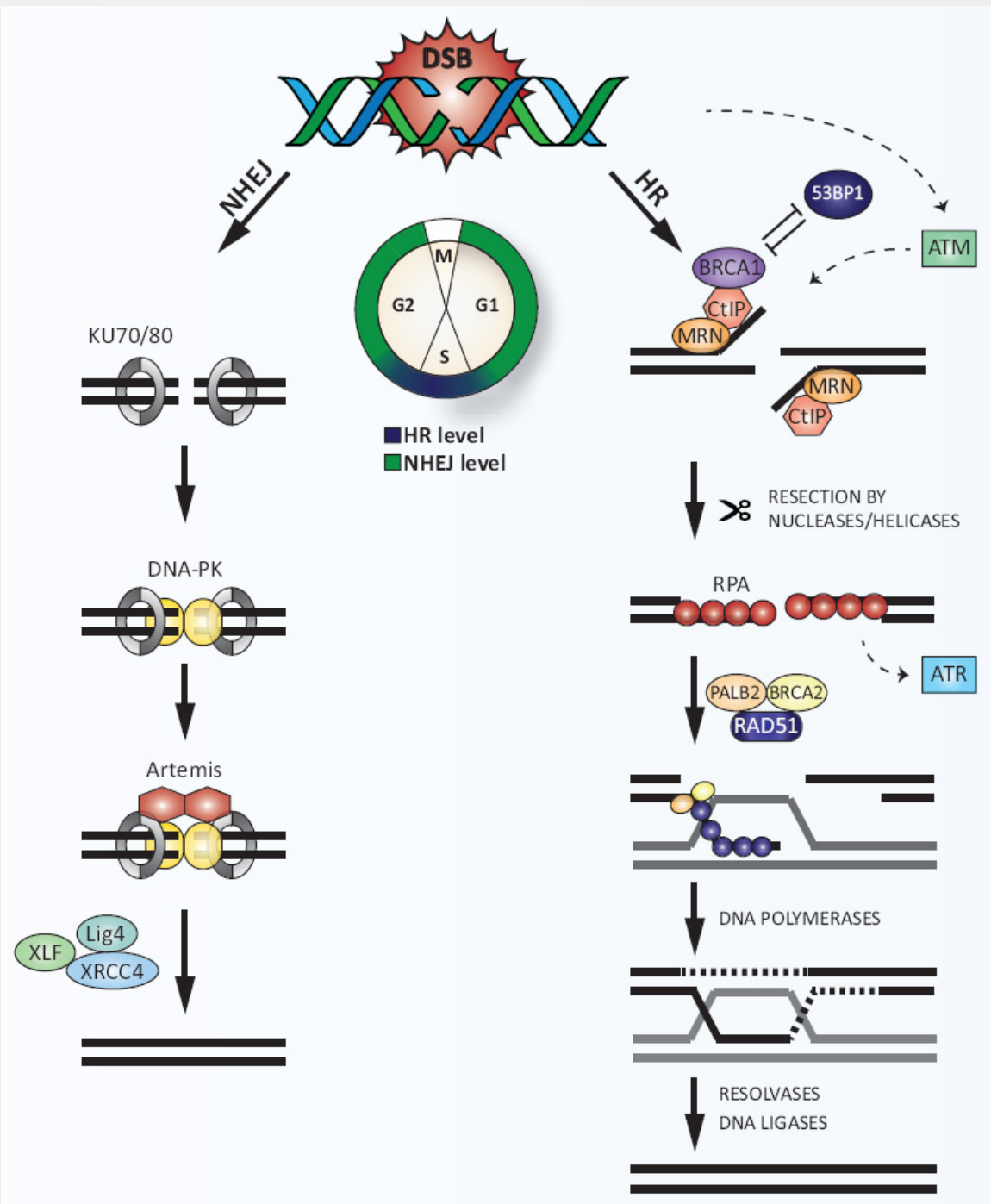

Figure 2

DNA double-strand break (DSB) repair.

DSBs are predominantly repaired by two distinct pathways: NHEJ or HR. NHEJ operates throughout the cell cycle, but mainly during the G1 and G2 phases, whereas HR peaks in S phase. Rapid association of the Ku70/80 heterodimer to DSBs promotes NHEJ by recruiting DNA-PKcs. DNA ends are processed by the nucleolytic activity of Artemis, followed by religation catalysed by a complex of XLF, Ligase IV (Lig4) and XRCC4. Alternatively, MRN, which is initially recruited to DSBs in competition with Ku70/80, initiates DSB resection together with CtIP thereby promoting HR. 53BP1 antagonises BRCA1 in DSB resection. Extensive DSB resection by other nucleases and formation of RPA-coated ssDNA stimulates the activation of ATR. Displacement of RPA by RAD51 is mediated by BRCA2 and PALB2, resulting in the formation of RAD51 nucleoprotein filaments. Subsequent strand invasion into the homologous DNA template and capturing of the second DNA end leads to the formation of a double Holliday junction, which is processed by resolvases. Finally, the DNA is sealed by ligases to accomplish error-free repair of the DSB.

53BP = p53 binding protein; ATM = ataxia telangiectasia mutated protein; CtBP = C-terminal binding protein; CtIP = CtBP-interacting protein; DNA = deoxyribonucleic acid; DNA-PK = DNA-dependant protein kinase; DNA-PKcs = DNA-PK catalytic subunit; HR = homologous recombination; MRN = MRE11-RAD50-NBS1 complex; NEHJ = nonhomologous end joining; PALB1/2 = partner and localiser of BRCA1/2; RPA = replication protein A; ssDNA = single-stranded DNA; XLF = XRCC4-like factor; XRCC4 = X-ray repair cross-complementing protein 4

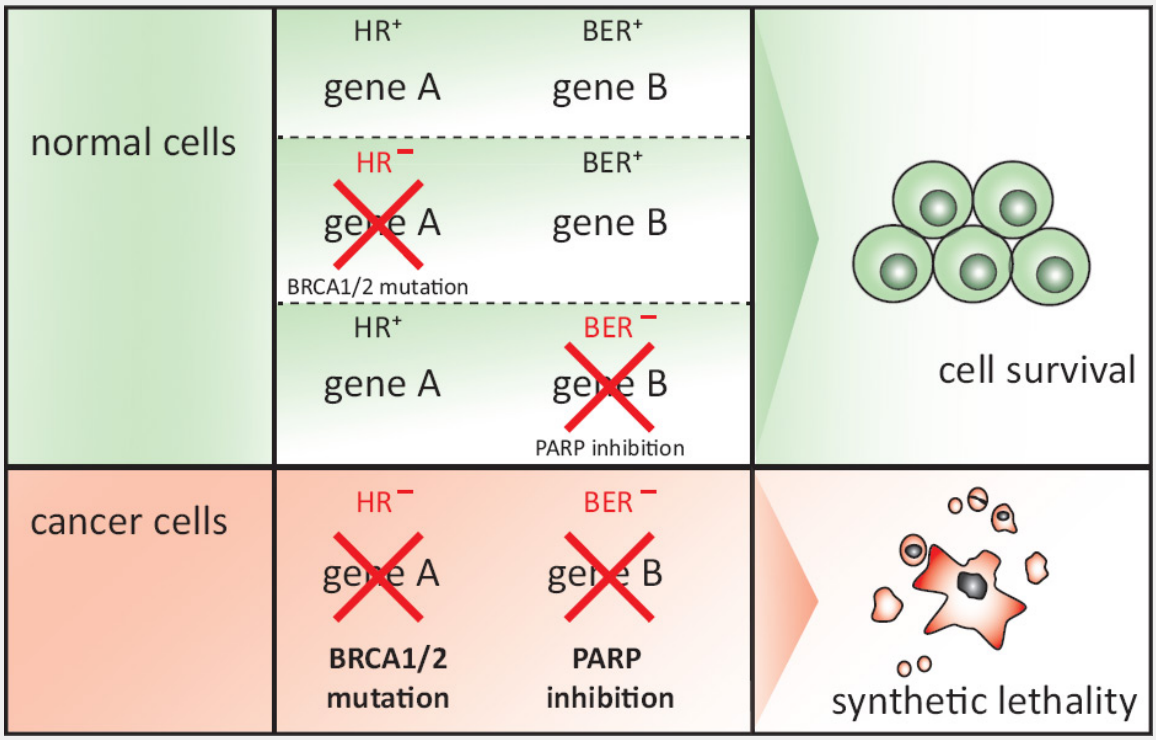

Figure 3

Synthetic lethality.

Synthetic lethality is defined as a combination of mutations or perturbations in two or more genes that leads to cell death, whereas inactivation of any one of the genes alone does not. Perturbation of genes can occur through genetic mutation or silencing, depletion by RNAi or inhibition by SMIs and is depicted by a red cross. If genes that are essential for a certain DNA repair pathway (e.g. gene A) are inactivated in normal cells, alternative pathways with functional genes (e.g. gene B) are utilised to respond to DNA damage. Conversely, cancer cells mutated or silenced for a component of a DDR pathway are compromised in their ability to process DSBs. These cells then rely on alternative DNA repair pathways to repair the breaks. Therefore, inhibition of the alternative pathway will cause cell death due to persisting DSBs. Please refer to main text for details about the example given for synthetic lethality between *BRCA1/2* mutation and PARP inhibition.

ADP = adenosine diphosphate; BER = base excision repair; DDR = DNA damage response; DNA = deoxyribonucleic acid; DSB = double-strand break; HR = homologous recombination; PARP = poly(ADP-ribose) polymerase; RNA = ribonucleic acid; RNAi = RNA interference; SMI = small molecule inhibitor