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DNA damage checkpoint kinases in cancer

Hannah L. Smith[1], Harriet Southgate[2], Deborah A. Tweddle[2] and Nicola J. Curtin[1]

[1]Newcastle Centre for Cancer Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK and [2]Wolfson Childhood Cancer Research Centre, Newcastle Centre for Cancer, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

Review

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Author for correspondence:
Nicola J. Curtin,
E-mail: nicola.curtin@ncl.ac.uk

Abstract

DNA damage response (DDR) pathway prevents high level endogenous and environmental DNA damage being replicated and passed on to the next generation of cells via an orchestrated and integrated network of cell cycle checkpoint signalling and DNA repair pathways. Depending on the type of damage, and where in the cell cycle it occurs different pathways are involved, with the ATM-CHK2-p53 pathway controlling the G1 checkpoint or ATR-CHK1-Wee1 pathway controlling the S and G2/M checkpoints. Loss of G1 checkpoint control is common in cancer through *TP53, ATM* mutations, Rb loss or cyclin E overexpression, providing a stronger rationale for targeting the S/G2 checkpoints. This review will focus on the ATM-CHK2-p53-p21 pathway and the ATR-CHK1-WEE1 pathway and ongoing efforts to target these pathways for patient benefit.

Introduction

The DNA damage response (DDR) is essential to maintain genomic integrity in the face of a continuous onslaught of DNA damage from endogenous and environmental sources. Activation of this response involves the close coordination of DNA repair pathways and signalling to cell cycle arrest to allow repair and prevent DNA damage being copied (G1 and S-phase checkpoint) or transmitted to the next generation (G2/M checkpoint). The kinases ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia mutated and rad3 related (ATR) are DNA damage sensors that are at the apex of a phosphorylation and dephosphorylation cascade signalling to both cell cycle arrest via inactivation of cyclin-dependent kinases (CDKs) (Fig. 1), and DNA repair. ATM and ATR have overlapping but non-redundant activities with substantial cross-talk between the two pathways (Ref. 1). This review will describe the role of these signalling cascades and the development of drugs targeting them for anti-cancer therapy.

The role of ATM/CHK2 pathway in cell cycle checkpoints

ATM is activated in response to DNA double-strand breaks (DSBs) (Ref. 2). In undamaged cells, ATM exists as a dimer. Upon recruitment by the MRE11/RAD50/NBS1 (MRN) complex to DSBs, ATM autophosphorylates at serine 367 (ser367), serine 1893 (ser1893), serine 1981 (ser1981) and serine 2996 (ser2996) resulting in monomerisation and activation (Refs 3, 4). Active ATM phosphorylates many target proteins regulating DNA repair, cell cycle arrest and apoptosis including CHK2, p53 and H2AX (Ref. 5). ATM plays a crucial role in the activation of the G1/S cell cycle checkpoint, primarily mediated through p53 activity. The most important transducer of ATM signalling is CHK2, a kinase that signals to DNA repair, cell cycle arrest and apoptosis. ATM phosphorylates CHK2 on threonine 68 (thr68) causing CHK2 dimerisation and autophosphorylation of the kinase domain, required for full activation (Ref. 6).

Active CHK2 phosphorylates the Cdc25A and Cdc25C phosphatases, which results in their inactivation/degradation. This promotes cell cycle arrest as active cdc25A/C remove inhibitory phosphorylation on CDKs that drive cell cycle progression. Cdc25A dephosphorylates CDK2, promoting progression into S phase. Cdc25C also dephosphorylates CDK1, which is usually held in the inactive state via phosphorylation by WEE1 and Myt1, promoting the transition into M phase (Refs 1, 7, 8). Although ATM can signal to G2 arrest via CHK2, the cell cycle defects observed in ATM-deficient cells are primarily G1/S checkpoint deficiency (Refs 9–11).

CHK2 also causes cell cycle arrest by phosphorylating the tumour suppressor p53 on ser15 and ser20 resulting in p53 stabilisation and activation (Ref. 12). p53 is a transcription factor which, when active, initiates the transcription of genes involved in DNA repair, cell cycle arrest, apoptosis and metabolism as well as its own negative regulators (Ref. 13) for example, mouse double minute 2 (MDM2), a ubiquitin ligase that targets p53 for degradation (Ref. 14). In response to DNA damage, p53 is phosphorylated by many kinases including ATR and CHK1 as well as ATM and CHK2, contributing to cross-talk between the two pathways. This phosphorylation blocks the interaction between p53 and MDM2 leading to p53 protein accumulation (Ref. 15). Active p53 promotes the transcription of *CDKN1A*, which encodes the cyclin-dependent kinase inhibitor $p21^{CIP1/WAF1}$ (Ref. 16). p21 mediates p53-dependent G1 cell cycle arrest (Refs 17, 18). p53 activation also leads to the transcription of pro-apoptotic genes including *Puma*, *Noxa*, *BAX* and *Apaf1,* resulting in apoptotic cell death if the damage is sustained (Ref. 19). The regulation of p53-mediated G1 checkpoint arrest and/or apoptosis by the

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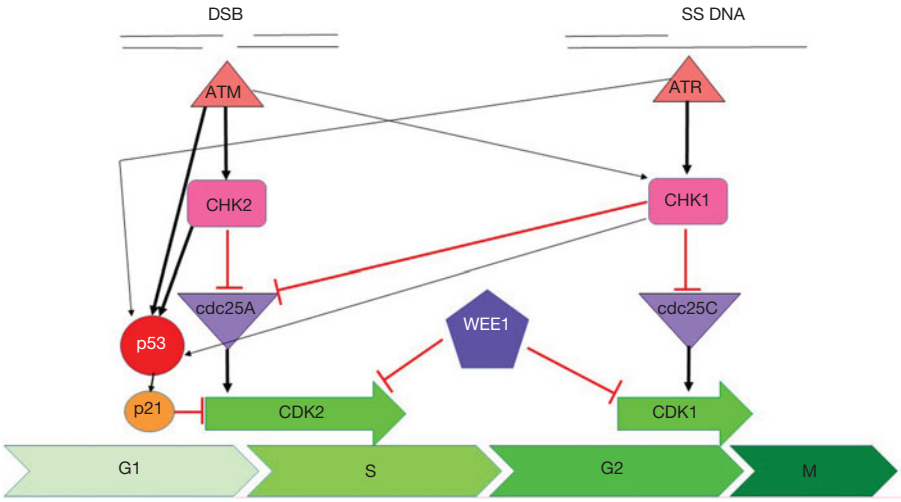

Fig. 1. Cell cycle checkpoint signalling. DNA double-strand breaks activate ATM, which phosphorylates and activates CHK2, which phosphorylates and inactivates cdc25A, preventing it from removing the inactivating phosphate on CDK2 thereby inhibiting S-phase entry and progression. Both ATM and CHK2 phosphorylate p53 resulting in transactivation of p21 to inhibit CDK2. SS-DNA (e.g. at stalled replication forks) activates ATR, which phosphorylates and activates CHK1, which phosphorylates and inactivates cdc25c, preventing it from removing the inactivating phosphate on CDK1 thereby inhibiting G2/M progression. There is substantial cross-talk between the two pathways with CHK1 also being a target of ATM and cdc25A a target of CHK1 and both ATR and CHK1 targeting p53. In addition, DNA damage activates WEE1 which phosphorylates and inactivates both CDK1 and CDK2. Black arrows indicate main activation pathways, grey ones are secondary pathways and red lines indicate inhibition.

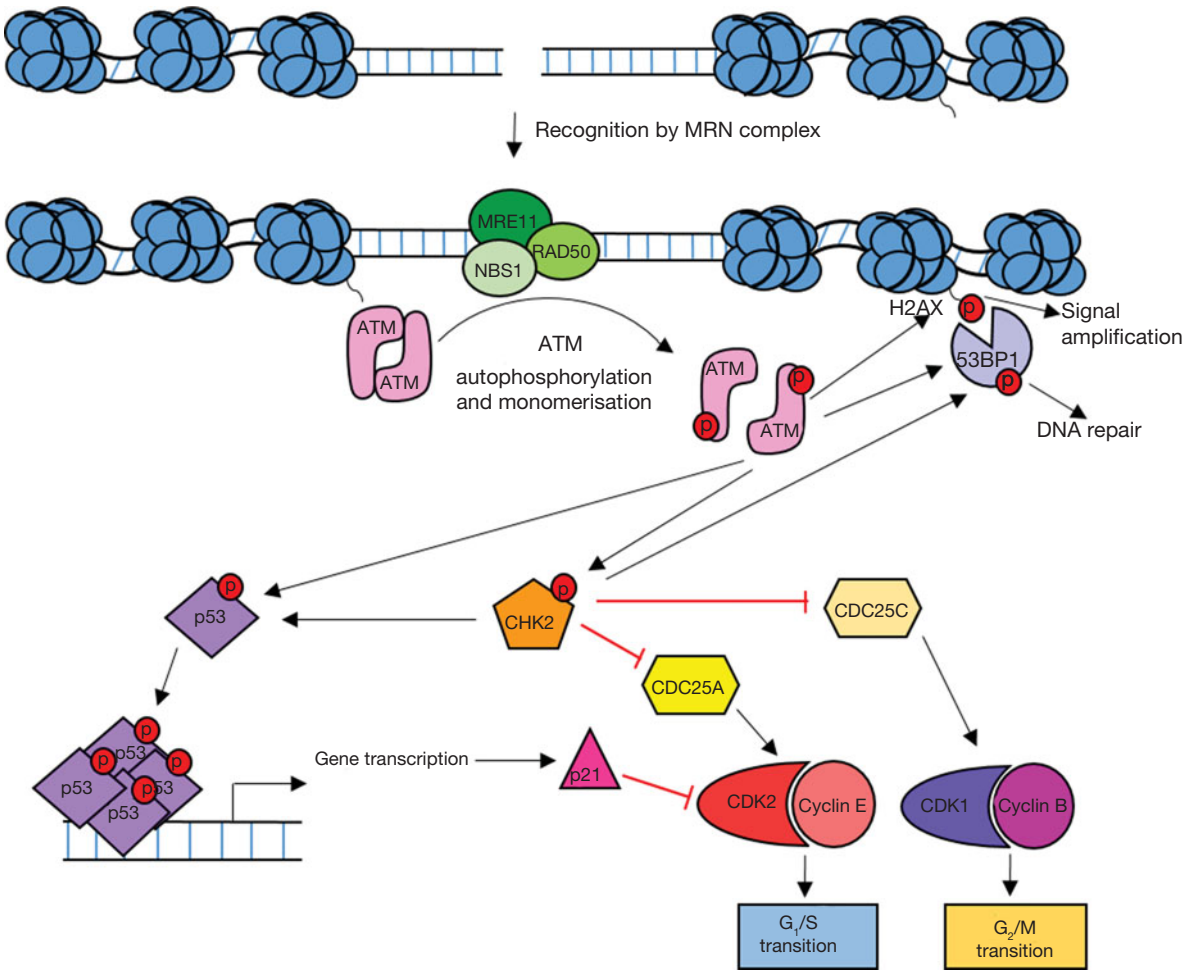

Fig. 2. Overview of ATM signalling in response to DNA damage. DNA double-strand breaks are recognised by the MRE11/RAD50/NBS1 (MRN) complex which recruits ATM leading to ATM activation. Active ATM phosphorylates the histone variant H2AX (γH2AX) leading to amplification and spreading of the damage signal. ATM-dependent phosphorylation of p53 and CHK2 leads to the activation of DNA repair processes and cell cycle arrest. Active p53 induces G_1 arrest through transcriptional activation of the CDKN1A gene which codes for the cyclin-dependent kinase (CDK) inhibitor p21. Active CHK2 also phosphorylates p53 as well as CDC25 phosphatases resulting in S and G_2 arrest. 53BP1 is recruited to yH2AX and phosphorylated by ATM and CHK2 leading to DNA repair.

DDR kinases is likely dependent on the context of the DNA damage, such as the type of DNA damage, cell cycle phase and molecular pathology of the cell type in question.

Figure 2 illustrates how the ATM/CHK2/p53 signalling pathway leads to cell cycle arrest and the maintenance of genome integrity. This pathway suppresses tumorigenesis and as a consequence, defects are often observed in cancer.

Role of ATM/CHK2 in DNA repair

Although not essential for the repair of the majority of DSBs, ATM activity is required for the repair of a subset of DSBs generally associated with heterochromatin (Ref. 20). In response to DNA DSBs and stalled replication forks the variant histone H2AX is phosphorylated at ser139 by DNA-PK, ATM and ATR resulting in the accumulation of γH2AX in the vicinity of the

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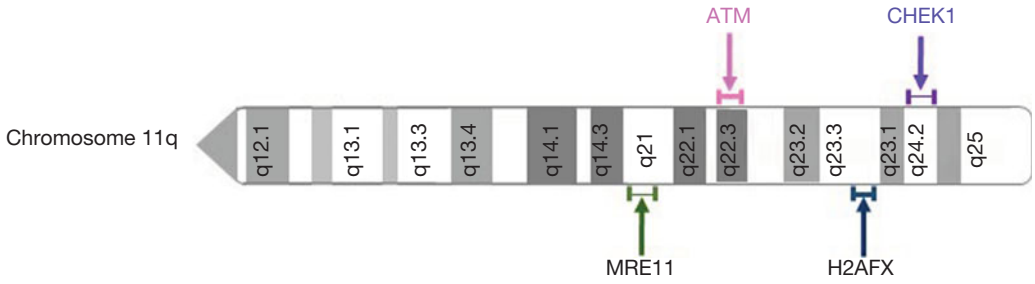

Fig. 3. Location of frequently deleted DNA damage response genes on chromosome 11q.

DNA lesion. In *ATM*-deficient cells, around 10–15% of DNA damage foci, identified by antibodies against γH2AX, are retained 72 hours after ionising radiation (IR) (Ref. 21). γH2AX recruits mediator of DNA damage checkpoint 1 (MDC1) (Ref. 22). Phosphorylation by ATM stabilises MDC1 on chromatin where it acts as a molecular scaffold recruiting chromatin modifiers to relax heterochromatin in the vicinity of the DSB (Ref. 23). More MRN complexes are recruited to the site of the DSB by the interaction between MDC1 and NBS1, which in turn recruits and activates further ATM kinases, resulting in amplification of the signal along chromatin (Refs 24, 25). ATM activation promotes DNA repair indirectly via both non-homologous end joining (NHEJ) and homologous recombination DNA repair (HRR), two prominent DSB repair pathways. ATM-dependent phosphorylation of 53BP1 recruited to damage markers on chromatin prevents DSB end resection, promoting NHEJ (Ref. 26). Conversely, ATM and CHK2 phosphorylate BRCA1 at the site of DNA damage (Ref. 27). BRCA1 plays a critical role in the initiation of HRR (described in the section 'Role in DNA repair') (Ref. 28). 53BP1 and BRCA1 show mutual antagonism and, although both proteins are present throughout the cell cycle, pathway dominance is largely governed by the cell cycle stage and cyclin-CDK activity (Refs 29, 30).

In addition to BRCA1, ATM-mediated regulation of nucleases is required for efficient DNA end resection and activation of ATR at DSBs (Refs 31, 32). ATR signalling is discussed in the section 'Role of ATR-CHK1-WEE1 in cell cycle checkpoints'. However, a direct role of ATM in DNA repair is unclear as ATM is dispensable for the repair of the majority of DNA DSBs, although it has an important role in initiating the chromatin remodelling cascade induced by phosphorylation of histone H2AX, and signalling to cell cycle checkpoint arrest (Ref. 22).

Pathway dysfunction in cancer

Aberrations in the *ATM* gene are commonly seen in cancer. Homozygous germline mutations in *ATM* result in ataxia telangiectasia (A-T), a well characterised recessive genetic disease which predisposes to the development of cancer (Ref. 33). Somatic mutations of *ATM* have been identified in many cancer types, most commonly lymphoid malignancies, suggesting that ATM loss contributes to tumorigenesis (Ref. 34). Loss of ATM expression has also been observed in many cancer types including colorectal cancer, breast cancer, non-small cell lung cancer, lung adenocarcinoma and pancreatic cancer (Refs 35–39). In addition to *ATM* mutation, *ATM* loss of heterozygosity (LOH) may arise through deletion of the long arm of chromosome 11 (11q). Interestingly, other DDR components are often co-deleted with *ATM*. Genes encoding MRE11, CHK1 and the histone variant H2AX are also located on chromosome 11q (Fig. 3), and are frequently deleted with ATM (Refs 40, 41). Allelic deletion of these genes may contribute to DNA damage repair deficiencies which could be targeted therapeutically. 11q deletion is commonly observed in breast cancer, chronic lymphocytic leukaemia (CLL), other lymphoid malignancies, and childhood neuroblastoma and is associated with poor survival (Refs 41–43).

CHK2 mutations, although less common than mutations in ATM, are also observed across cancer types including colon, kidney, breast and prostate cancer (Ref. 44). Homozygous germline *CHK2* mutation is rare and manifests in Li-Fraumeni syndrome, a cancer predisposition syndrome usually associated with mutations in the gene encoding p53, *TP53* (Ref. 45). Somatic mutations in *CHK2* have been observed across the entire amino acid sequence and lead to functionally null or unstable CHK2 protein.

The most commonly mutated gene across cancer types is *TP53*, which codes for the p53 protein. Around 80% of patients with Li-Fraumeni patients have germline mutations of *TP53* (Ref. 46). Somatic *TP53* mutations are observed in around 40% of all tumours (Refs 47, 48), the mutation rate varying between cancer types from nearly 100% in ovarian cancer to <10% for haematological malignancies (Ref. 47). Mutations in *TP53* result in a spectrum of p53 mutant proteins, from classical loss of transcriptional function to gain of function mutants which alter transcriptional networks and promote an oncogenic phenotype (Ref. 49).

As with *ATM*, *TP53* loss of heterozygosity through allelic deletion of chromosome 17p is frequently observed in cancer (Ref. 50). Loss of 17p is often accompanied by mutation of the other *TP53* allele, although 17p deletion alone has been shown to predict poor prognosis in some myeloid malignancies (Refs 51, 52).

Overall aberration in the ATM/CHK2/p53 axis frequently occurs in cancer. Targeting cancer-specific defects in this pathway could contribute to effective cancer treatments with reduced side effects.

Rationale for the development of inhibitors

Neither ATM nor CHK2 kinases are essential for life, indicating some redundancy with other DNA damage signalling and repair pathways.

In humans, *ATM* mutations lead to the autosomal recessive disease A-T. A-T patients are very radiosensitive and display increased adverse and sometimes fatal reactions to both radiotherapy and radio-mimetic chemotherapy (Ref. 33). In addition to the identification of *ATM* mutations in cancer (discussed in the section 'Pathway dysfunction in cancer'), heterozygous carriers also have an increased risk of developing cancer, particularly breast and lymphoid (Refs 34, 53). $ATM^{-/-}$ mice are viable and display many features of A-T including cerebellar dysfunction, infertility, radio-sensitivity and cancer predisposition (Refs 54, 55). Targeting ATM with small molecule inhibitors should sensitise cells to radio- and chemotherapy thus reducing the dose required and reducing off-target toxicities of these treatments. However,

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systemic ATM inhibition could also lead to increased toxicity from chemotherapy agents. For example, combining chemotherapy with other DDR inhibitors, such as MGMT and PARP inhibitors, led to dose reductions of both the chemotherapy and DDR inhibitors owing to increased toxicity (Refs 56, 57). Radiotherapy is more targeted towards the tumour and as techniques become more precise with improving technology. ATM inhibitors may be particularly useful in this context.

CHK2 kinase is also not essential. *CHK2* knockout (KO) mice show little to no phenotype but, in contrast to ATM KO, are resistant to ionising radiation (IR) and have defects in p53-mediated apoptosis pathways (Ref. 58). However, cancer-prone phenotypes associated with the absence of CHK2 become apparent when other DDR genes, such as *CHK1, MRE11* and *NBS1*, are impaired (Ref. 59). $CHK1^{+/-}$ $CHK2^{-/-}$ mice show high levels of spontaneous damage and decreased apoptotic responses, increasing cancer susceptibility showing some degree of co-operation and redundancy. Overall, the context in which CHK2 inhibitors will have therapeutic benefit remains unclear.

Preclinical development of ATM inhibitors

Many small-molecule ATP-competitive inhibitors of ATM have been developed and generally act as radiosensitisers *in vitro* (Ref. 60). The first described potent and specific ATM inhibitor, KU55933, was developed by KuDos pharmaceuticals (now part of AstraZeneca) (Ref. 61). It enhanced the cytotoxicity of IR and topoisomerase I and II poisons, but its poor aqueous solubility and *in vivo* bio-availability precluded advanced preclinical testing. KU60019, a structural derivative of KU55933 with improved potency and aqueous solubility, effectively radiosensitised glioblastoma *in vivo* when directly injected into the tumour (Ref. 62), but still had poor *in vivo* bioavailability (Ref. 1). KU60019 caused greater radiosensitisation in p53 deficient tumours. However, using KU59403, another derivative of KU55933, in matched p53 functional and dysfunctional cell lines showed that the radio- and chemosensitising effects of ATM inhibition was not p53 dependent (Ref. 63). While the pharmacodynamic properties of KU59403 were still not suitable for oral administration, systemic *in vivo* studies in mice were carried out by intraperitoneal injection. As well as sensitizing to IR, KU59403 also sensitised tumours to topoisomerase I and II inhibitors, irinotecan and etoposide respectively, *in vivo*.

AZ32 is a moderately potent ATM inhibitor discovered by chemical library screening at AstraZenena. The chemistry is different from that of the KuDos compounds and has been shown to be orally bioavailable as well as capable of crossing the blood-brain barrier in mice (Ref. 64). *In vivo* optimisation of AZ32 led to the development of AZD1390 sensitised brain tumours to radiotherapy in preclinical models justifying translation into a clinical trial (section 'Clinical trials with ATM and CHK2 inhibitors') (Ref. 65).

In addition to AZD1390, the compound AZD0156 was developed by AstraZeneca following optimisation of a different lead scaffold (Ref. 66). AZD0156 shows good pharmacodynamic properties and is synergistic with the topoisomerase I inhibitor irinotecan and the PARP inhibitor olaparib in tumour xenograft models. AZD0156 has also entered a phase 1 clinical trial.

CP-466722, another selective ATM inhibitor, was identified by Pfizer and showed similar radio-sensitising properties to KU55933 (Ref. 67). *In vivo* studies were not possible because of the compound having a short half-life in mice ($t_{1/2} < 1$ hour).

In 2017, Dohmen *et al.* identified GSK635416A as a novel radio-sensitiser in non-small cell lung cancer (NSCLC) cell lines from a screen of published GlaxoSmithKline protein kinase inhibitors, which was shown to act through inhibition of ATM (Ref. 68). When combined with the PARP inhibitor olaparib, GSK635416A showed an additive radio-sensitizing effect. No *in vivo* studies have been published for this compound to date.

Preclinical development of CHK2 inhibitors

In contrast to CHK1 inhibitors, few CHK2-specific inhibitors have been developed. In general, they show modest anti-proliferative effects when compared with ATM, ATR and CHK1 kinase inhibitors (Ref. 69).

A screen of the AstraZeneca compound library yielded AZD7762 as a potent CHK1 inhibitor with equal potency against CHK2 (Ref. 70). This dual inhibitor will be discussed with other CHK1 inhibitors in the section 'Preclinical development of CHK1 inhibitors'.

A 2-arylbenzamidazole compound (ABI) was the first CHK2-specific inhibitor to be proposed, showing high selectivity (IC50 = 15 nM) over CHK1 (IC50 > 10 μM) (Ref. 71). However, CHK2 inhibition in cells by ABI was greatly reduced compared with cell-free assays achieving 42% inhibition of CHK2 at 5 μM. When used as a tool, the compound showed dose-dependent *radioprotection* in human CD4+ and CD8+ T-cells, similar to the radioresistance of CHK2 null mice.

Attenuation of IR-induced apoptosis was seen in mouse thymocytes after treatment with three other structurally distinct CHK2 inhibitors, VRX0466617 (Ref. 72), PV1019 (Ref. 73) and CCT241533 (Ref. 74). Another CHK2 inhibitor, BML-277 (CHK2 inhibitor II), first disclosed by Arienti *et al.* (Ref. 71), was shown to be radioprotective in human glioma cell lines (Ref. 75). These data are consistent with the observation that CHK2 KO mice are radioresistant (section 'Rationale for the development of inhibitors' (Ref. 58)). Studies in HT-29 (human colon cancer) cells and HeLa (human cervical cancer) cells treated with CCT241533 failed to show any impact on the radiomimetic bleomycin cytotoxicity (Ref. 76). Interestingly, BML-277 antagonised oxaloplatin cytoxicity in colorectal cancer cell lines (Ref. 77). In contrast, survival analysis by colony formation assay in U251 human glioblastoma cell line showed potentiation of IR by PV1019. Whether these contrasting observations reflect the differing molecular pathology of the cell lines remains to be determined.

Although the role of CHK2 inhibition in response to IR is unclear, there is some evidence that the combination of CHK2i with topoisomerase I poisons and poly (ADP)-ribose polymerase (PARP) inhibitors might be effective. PV1019 was shown to potentiate the cytotoxic effects of topotecan and camptothecin in ovarian cancer cell lines (Ref. 73). Potentiation of the effects of the PARP inhibitors rucaparib and olaparib was seen with the addition of CCT241533 (Ref. 76).

Clinical trials with ATM and CHK2 inhibitors

Three ATM inhibitors are currently being investigated in phase 1 clinical trials (Table 1). To date, no specific CHK2 inhibitor has progressed to clinical trials but phase 1 studies of AZD7762, the dual CHK1/CHK2 inhibitor have been undertaken in combination with gemcitabine. The results published from the completed trial showed that AZD7762 leads to cardiac toxicity leading to 2 further trials being terminated and the clinical progression of the inhibitor being discontinued (Ref. 78).

Role of ATR-CHK1-WEE1 in cell cycle checkpoints

The ATR-CHK1-WEE1 pathway activates both intra-S and G2/M checkpoint control in response to replication stress (RS) and DNA damage. RS is the momentary slowing or stalling of replication fork progression that can be caused by replication outstripping the rate of dNTP production or lesions in the DNA. ATR is

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Table 1. ATM inhibitors currently in clinical trials

Drug name	Phase	Monotherapy/combination	Tumour type	NCT
M3541	I	In combination with palliative radiotherapy	Solid tumour	NCT03225105
AZD0156	I	In combination with olaparib, irinotecan, fluorouracil, folinic acid	Advanced solid tumours	NCT02588105
AZD1390	I	In combination with radiotherapy	Brain cancer	NCT03423628

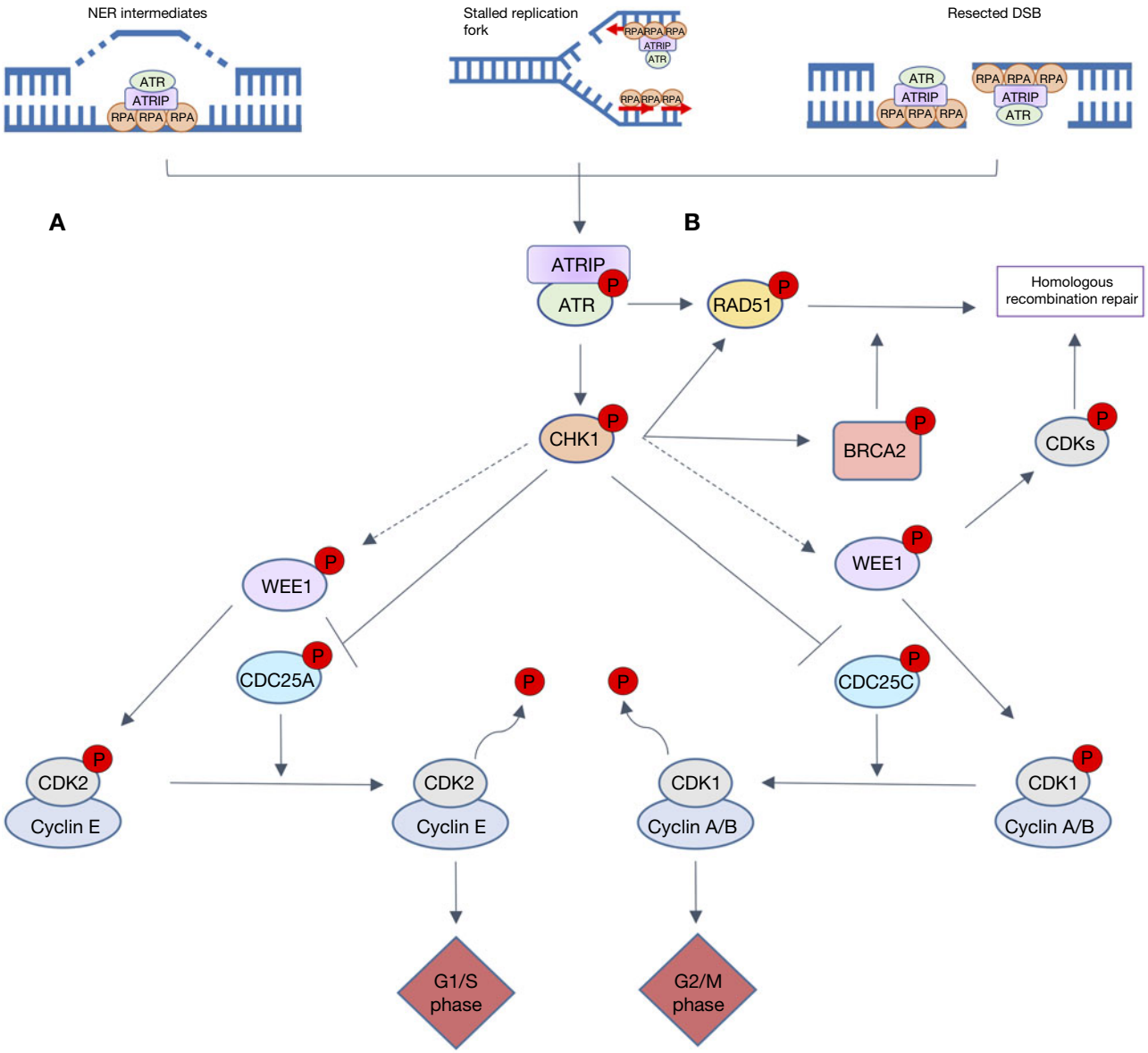

Fig. 4. Overview of ATR signalling in response to DNA damage. Resected double-strand breaks (DSBs), stalled replication forks and NER intermediates all lead to replication protein A (RPA) recruiting ATR via ATR-interacting protein (ATRIP). **A.** The ATR-CHK1 cascade is heavily involved in cell cycle checkpoint control**.** ATR activates CHK1 which causes the inactivating phosphorylation of both CDC25C and CDC25A, hence preventing the removal of inhibitory phosphorylation on CDK1 and 2, respectively. These lesions also activate WEE1, directly or via CHK1 (dashed grey arrows) to phosphorylate and inactivate CDK1 and 2. Progression through cell cycle G1/s and G2/M phases is reliant upon activation of CDK2/cyclin E and CDK1/cyclin A/B complexes, respectively. When WEE1 CHK1 or ATR are inhibited, CDK1 and CDK2 are activated so S-phase progression and mitotic entry occur with no delay to allow DNA repair. **B.** ATR, CHK1 and WEE1 also signal to key proteins involved in homologous recombination repair (HRR). ATR promotes repair protein RAD51 recruitment to DSBs and stalled replication forks, independent of BRCA. CHK1 phosphorylates key HRR proteins BRCA2 and RAD51. When activated WEE1 inhibitory phosphorylates CDKs, which play a key role in HRR end resection.

activated by a number of DNA damaging factors, including ultraviolet radiation, antimetabolite-induced dNTP depletion, topoisomerase poisons, alkylating agents or DNA crosslinking agents (Ref. 79). Many of these factors result in single-strand DNA (ss-DNA), which allows for the recruitment of ATR activating proteins TOPBP1 or ETAA1. Although ss-DNA arises primarily from RS it is also a result of resected DSBs and nucleotide excision repair (NER) intermediates. Replication protein A (RPA) coats the ss-DNA, protecting it from degradation and enabling recruitment of ATR via ATR-interacting protein (ATRIP). ATR is activated by either TOPBP1, which is recruited by interaction with MRN complex that resects DSB to give long ss-DNA overhangs and the 9-1-1 complex (a proliferating cell nuclear antigen (PCNA)-like clamp that binds SS-DS DNA

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junctions) or by ETAA1, which is recruited to ss-DNA by interaction with RPA (Ref. 80). Upon activation, ATR activates CHK1 by phosphorylation at serine 345 causing CHK1 to autophosphorylate at serine 296, to achieve full activation. Like CHK2, CHK1 causes the inactivating phosphorylation of CDC25A/C (Refs 81, 82), thereby preventing them from removing the inhibitory phosphorylation on CDK2 and 1, respectively (as described in Fig. 4). In yeast and xenopus, CHK1 phosphorylates and activates Wee1 kinase activity to phosphorylate, and hence inactivate, CDK 1 and 2 (Refs 83, 84). This is yet to be shown in mammalian cells. The net result of this kinase activation and phosphatase inhibition is inhibition of CDK2, and thus S-phase entry and progression, and inhibition of CDK1 preventing entry into mitosis.

Role in DNA repair

The ATR-CHK1-WEE1 cascade also plays a role in HRR, a high fidelity DSB repair pathway, restricted to late S and G2 phase as it uses the sister chromatid as a DNA template (Ref. 85). During HRR extensive DNA end-resection occurs, resulting in the ss-DNA overhang that leads to ATR activation. HRR is also responsible for the resolution of collapsed replication forks caused by RS – the prime activator of the ATR-CHK1-WEE1 pathway. It is therefore not surprising that these kinases are also associated with DNA repair by HRR (Ref. 86).

All three kinases in the cascade have demonstrated involvement in HRR. In *BRCA* defective cells, ATR can act independently of BRCA1 to recruit RAD51 to DSBs and stalled replication forks, inhibition of ATR disrupted RAD51 loading suggesting key involvement in HRR (Ref. 87). Similarly, CHK1 may promote HRR by phosphorylating key HRR components BRCA2 and RAD51 (Refs 81, 88). WEE1 is also involved in HRR owing to its inhibitory phosphorylation of CDKs upon activation. The resection of DNA ends, a necessary step in HRR is antagonised by CDK activity (Ref. 89), inhibition of CDK activity by WEE1 (or cdc25 inactivation downstream of CHK1) promotes HRR, therefore inhibition of either CHK1 or WEE1 will result in higher CDK activity and compromise HRR (Ref. 90).

Pathway dysfunction in cancer

The importance of the ATR-CHK1-WEE1 cascade is highlighted by the embryonic lethality of all three components (Refs 91–93). No humans are recorded as being born without these essential kinases but Seckel syndrome (SS) is a rare, autosomal recessive disorder owing to a hypomorphic mutation in *ATR,* resulting in delayed development but not cancer predisposition. SS mice are not cancer-prone, even when crossed with p53 defective mice (Ref. 94). There are contrasting data regarding the tumour predisposition of $ATR^{+/-}$ mice with one study reporting increased tumour incidence and others reporting no increase in tumour incidence (Refs 91, 95). $CHK1^{+/-}$ mice are not predisposed to tumourigenesis (Ref. 92), and no abnormalities are reported in adult $WEE1^{+/-}$ mice (Ref. 93).

The general consensus seems to be that complete loss of ATR, CHK1 or WEE1 signalling is incompatible with normal development but that compromising the pathway by hypomorphic mutation or heterozygous deletion does not predispose to tumour development. However, upregulation of the pathway in tumours may be indicators of poor prognosis. Two studies in breast cancer indicate that high pCHK1 levels correlated with local recurrence and worse cancer-specific survival (Refs 96, 97) and WEE1 overexpression have been observed in several tumour types: hepatocellular carcinoma (HCC), breast, glioblastoma, lung and colon (Ref. 98).

Rationale for targeting ATR-CHK1-WEE1

Cancer cells are considered to have higher levels of RS than normal cells. There are several causes for this: (i) increased expression of oncogenes or growth factor receptors that drive cells into S-phase, (ii) accelerated cell cycle progression owing to increased expression of CDKs or their cyclin partners or loss of their protein inhibitors, and (iii) loss of G1 checkpoint control (Refs 96–99). Additionally, RS results in genomic instability that is an enabling characteristic of cancer (Refs 100, 101), thereby creating a vicious circle. RS is the prime trigger for ATR-CHK1-WEE1 signalling and cancer cells are therefore highly dependent on this pathway (Refs 100, 102, 103). Thus, there is a potential to exploit the increased RS, coupled with the loss of G1 control, in cancer cells by targeting the ATR-CHK1-WEE1 pathway, without compromising normal cells with proficient G1 checkpoint control (Ref. 104).

In addition to their pivotal role in the S and G2/M cell cycle checkpoints, ATR-CHK1-WEE1 also promote HRR, as described above. Therefore, inhibiting these kinases has the potential to compromise HRR, thereby sensitising cells to DNA damaging anticancer agents.

Preclinical development of ATR inhibitors

An early study in 1998 found that overexpression of kinase-inactive ATR caused sensitivity to IR, cisplatin and methyl methanesulfonate (MMS) (Ref. 105). Caffeine was found to be a weak ATR inhibitor (1999) and, although it lacked specificity, it was still good enough to test the potential of ATR inhibition (Ref. 106). It was shown to inhibit ATR activity at a radio sensitising concentration (Ref. 107). Subsequently in 2002, Nghiem *et al.*, showed that expression of the kinase-dead ATR conferred sensitivity to multiple anti-cancer/DNA damaging agents (UV, hydroxyurea (HU), IR, cisplatin and aphidicolin). In terms of the potential of single-agent ATR inhibitors this study also showed that endogenous causes of replication stress (cyclin D, E, CDK2 overexpression or p53 inactivation by MDM2 or human papillomavirus (HPV) E6 expression) conferred sensitivity to kinase-dead ATR overexpression and/or caffeine (Ref. 108).

ATR inhibitor development was slow to take off from these early studies, possibly because of the difficulty in developing a cell-free assay. Nevertheless, in 2011, NU6027, originally developed as a CDK2 inhibitor, was found to be a more potent inhibitor of ATR that CDK2 in intact cells. NU6027 enhanced cisplatin and HU cytotoxicity in an ATR-dependent manner, and the major classes of DNA damaging anticancer drugs in MCF7 breast cancer cells, and attenuated G2 cell cycle arrest. Cells defective in HRR are exquisitely sensitive to PARP inhibitors and, in the first investigation of its kind, NU6027 inhibited HRR and increased PARP inhibitor cytotoxicity (Ref. 109).

In 2011 a novel ATRi screen identified ETP-46464 as an ATRi that had increased cytotoxicity in cells overexpressing cyclin E. It significantly sensitised cells to IR, abolishing the G2/M checkpoint in these cells, independent of p53 status (Ref. 110). In 2015, Teng *et al.*, went on to show that it also sensitised cells to cisplatin treatment (Ref. 111). However, ETP-46464 lacked specificity as it also inhibited mTOR, DNA-PKcs and P13Kα, and had poor *in vivo* pharmacological properties (Ref. 110).

AZ20 is an ATRi developed in 2013 from the P13K inhibitor, LY294002, with good potency and selectivity (Ref. 112). In acute myeloid leukaemia (AML) cell lines and patient samples, AZ20 acted synergistically with cytarabine, resulting in enhanced apoptosis and induced replication stress (Ref. 113). This drug also synergistically inhibited cell growth in combination with gemcitabine in pancreatic cancer cell lines (Ref. 114).

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AZD6738 was developed from AZ20 in 2018, with improved aqueous solubility and excellent pharmacokinetic qualities (Ref. 115). It sensitised non-small cell lung cancer (NSCLC) cells and xenografts to cisplatin and gemcitabine (Ref. 116), and a panel of human cancer cells to radiation (Ref. 117). AZD6738 suppressed tumour growth and increased apoptosis in ATM defective cells (Ref. 118).

VE-821 was one of the first potent ATRi with greatly improved selectivity for ATR over other PI3K-like kinases discovered in 2011 (ATM, DNAPKcs, mTOR). VE-821, as a single agent, increased apoptosis in cancer cells versus non-cancerous cells (Ref. 119). VE-821 can sensitise cells to IR (Refs 120–122), gemcitabine and camptothecin (Refs 119, 123). However, the strongest synergy observed thus far is with platinum-based therapies cisplatin and carboplatin (Refs 119, 124). Interestingly, when used in combination with the PARPi veliparib, VE-821 further sensitised *BRCA* defective cells beyond the sensitivity already observed owing to HRR status (Ref. 124). In 2015 Middleton *et al*., showed that defects in ATM, HRR (BRCA2, XRCC3) and BER (XRCC1) resulted in increased sensitivity to VE-821. Interestingly, defective Ku80 (involved in NHEJ) caused hypersensitivity to VE-821, but the loss of its binding subunit, DNA-PKcs, did not (Ref. 125).

M6620 (also known as VE-822/VX-970 developed in 2012 and, from the same chemical series as VE-821) was the first highly selective, potent ATR inhibitor to go into clinical trials and is currently in phase 2 trials. It potentiates a number of DNA damaging agents including carboplatin, cisplatin, gemcitabine, irinotecan and IR in a wide array of cancers (Refs 126–132). Nagel *et al*., found that M6620 combined with cisplatin showed a better response *in vivo* than cisplatin combined with etoposide, another chemotherapeutic, providing a solid rationale for combining cisplatin and M6620 in the clinic and limiting inevitable side effects with combining two chemotherapeutic agents (Ref. 132). As with other ATR inhibitors, it was found that ATM conferred sensitivity to ATR, both *in vitro* and *in vivo* (Refs 128, 133).

In 2017 Wengner *et al*., characterised a novel ATRi, BAY1895344, which inhibited cell proliferation in an array of human cancer cell lines as well as having a strong anti-tumour effect as monotherapy in xenograft models. Synergistic anticancer activity was reported when used in combination with Radium-223 in xenograft models (Ref. 134).

Preclinical development of CHK1 inhibitors

UCN-01 was a first-generation, potent CHK1 inhibitor originally developed in 1999 as a protein kinase C inhibitor (Ref. 135). *In vitro,* it abrogated G2 checkpoint control and sensitised p53 defective cancer cells to DNA damaging agents (cisplatin, camptothecin and IR). However, it has poor potency and specificity and struggled to bypass a radiation-induced G2/M checkpoint (Ref. 136).

In 2008 the biological effects of AZD7762, a potent dual CHK1 CHK2 inhibitor with equal potency against both kinases, was shown to result primarily from inhibition of CHK1 (Ref. 70). *In vitro* studies have demonstrated that AZD7762 potentiated the cytotoxic effects of the nucleoside analogue gemcitabine, topoisomerase inhibitors and cisplatin (Refs 137–141). These findings were reflected *in vivo* and AZD7762 showed good pharmacokinetics and tolerability in mice. However, the effect of dual CHK1/CHK2 inhibition showed no increased benefit compared with CHK1-specific targeting agents suggesting that most of the anti-tumour effects are through inhibition of CHK1.

PF-477736 is a potent ATP-competitive CHK1 inhibitor with >100-fold selectivity over CHK2 developed in 2008 (Ref. 142). It has potent single-agent activity in triple-negative breast and ovarian cancer cell lines (Ref. 143), as well as sensitising cells to chemotherapeutic drugs gemcitabine, carboplatin, doxorubicin, mitomycin C and toptotecan (Refs 142, 144–146). PF-477736 also sensitises HPV positive head and neck cancer cells to radiation (Ref. 147). PF-477736 caused synergistic cytotoxicity in combination with targeted therapies irutinib and bosutinib in mantle cell lymphoma (MCL) and chronic myeloid leukaemia (CML), respectively (Refs 148, 149). PF-477736 was more cytotoxic in p53 mutant and Myc-driven cancers (Refs 142, 150).

In 2017, a novel CHK1 inhibitor, MK-8776 (also known as SCH900776 and identified in 2011), abrogated IR-induced G2/M checkpoint activation, resulting in aberrant mitosis, and was a potent radiosensitiser in breast and cervical cell lines (Refs 151, 152). MK-8776 also radiosensitised non-small cell lung cancer and head and neck cancer cell lines, in p53 non-functional cells (Ref. 153). Montano *et al*., reported that MK-8776 sensitised cells to an array of DNA damaging agents: HU (20–70 fold), cytarabine (15–35 fold) and gemcitabine (5–10 fold), with no sensitisation reported with cisplatin or 5-fluorouracil (5-FU) (Ref. 154). However, a later study by Herudkova *et al*. found that MK-8776 significantly sensitised cells to cisplatin and another platinum-based therapy, LA-12 (Ref. 155).

SRA737 (previously CCT244747, which was discovered in 2012) is a novel, potent, orally active CHK1 inhibitor, with good selectivity (Refs 156, 157). It was developed at the ICR and is active as a single agent in *MYCN*-driven neuroblastoma and in combination with IR, gemcitabine and irinotecan (Refs 156, 157). SRA737 has synergistic antitumour activity with the PARP inhibitors niraparib and olaparib in mammary and ovarian cancer cells *in vitro* and *in vivo* (Ref. 158).

In 2015 LY2606368, a specific CHK1 inhibitor with strong single-agent activity *in vitro* and *in vivo* was discovered (Refs 159–162). It demonstrated synergy with PARP inhibitors olaparib and BMN673 in ovarian and gastric cancer, respectively (Refs 163, 164) and potentiated cisplatin even in a panel of platinum-resistant human cancers cell lines (Refs 161, 165).

Pre-clinical development of WEE1i

Despite the key role for WEE1 in S and G2 arrest, very few small-molecule inhibitors have been developed. In 2001, PD0166285 was the first potent WEE1 inhibitor. It radiosensitised ovarian, colon, lung and ovarian tumour cells in a p53-dependent manner (Ref. 166). It was a potent radiosensitiser in glioblastoma, but a major limitation to its development is its inability to penetrate the blood-brain-barrier (Refs 167–169). Furthermore, PD0166285 was non-selective, it also inhibited CHK1, Src non-receptor tyrosine kinase, epidermal growth factor receptor (EGFR), platelet-derived growth factor β (PDGF-β) and fibroblast growth factor receptor-1 (FGFR-1) (Ref. 170).

In 2009 AZD1775/MK-1775 was discovered as a specific, potent WEE1 inhibitor that showed excellent selectivity (Ref. 171). It was effective as a single agent (Ref. 172), as well as in synergistically increasing the cytotoxicity of various DNA damaging agents (IR, gemcitabine, carboplatin, cisplatin, 5-FU, pemetrexed, doxorubicin and camptothecin), *in vitro* and *in vivo* (Refs 171, 173–176). Earlier studies reported p53 status as a determinant for sensitivity to AZD1775 (Refs 171, 174). However, more recent studies have shown MK-1775 cytotoxicity to be independent of p53 (Refs 172, 177). Heijink *et al*. carried out a genome-wide unbiased screen and concluded that the activity of DNA replication proteins, beyond p53, is a key determinant of WEE1 inhibitor sensitivity (Ref. 177).

Synergy between inhibitors of ATR-CHK1-WEE1

Studies of the combination of inhibitors of the pathway with one another have thus far shown potential. ATR inhibition by VE-821 and CHK1 inhibition by AZD7762 caused synergistic cell death *in*

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vitro and *in vivo* (Ref. 178). VX-970 (VE-822) was well tolerated in combination with AZD7762 in mice and resulted in increased survival of tumour-bearing mice (Ref. 178). ATR inhibition by AZD6738 was also synergistic with WEE1 inhibition by AZD1775 in causing the accumulation of DNA damage, via forced mitotic entry, and growth inhibition. This combination also inactivated HRR- sensitising cells to cisplatin and PARP inhibition (Ref. 179). An anti-metastatic effect was observed *in vitro* when ATR inhibitors AZ06738 and ETP-46464 were combined with WEE1 inhibitor AZD1775 (Ref. 180).

CHK1 and WEE1 inhibitors are another well-tolerated, synergistic combination as demonstrated in several studies in a variety of different cancer models, a few examples are given here. CHK1 inhibitor MK-8776 sensitised AML cells to AZD1775 *ex-vivo* and the combination was effective against neuroblastoma xenografts (Refs 181, 182). A study by Hauge *et al.*, showed synergistic anti-tumour effects between the WEE1 inhibitor AZD1775 and CHK1 inhibitors AZD7762 and LY2603618. A combination of these inhibitors resulted in mitotic catastrophe and reduced cell survival because of the combined effects on S phase and DNA damage associated with unscheduled replication initiation (Ref. 183). Similarly, the combination of CHK1 inhibitor AZD7762 and WEE1 inhibitor AZD1775 caused increased cytotoxicity and apoptosis in metastatic melanoma cell lines (Ref. 184). Synergy was also reported with CHK1 inhibitor PF-477736 and WEE1 inhibitor AZD1775 in MCL cells (Ref. 185).

Pre-clinical synergy of ATR-CHK1-WEE1 inhibitors and PARP inhibitors

Inhibitors of the ATR-CHK1-WEE1 cascade have shown synergy with PARP inhibitors. Peasland *et al.*, were the first to show that the ATR inhibitor, NU6027, was synthetically lethal in combination with the PARP inhibitor, rucaparib in breast and ovarian cancer cells (Ref. 109). Subsequently, the ATR inhibitor VE-821 was found to sensitise *BRCA* mutant cells to veliparib (Ref. 125) and a synthetically lethal screen found VE-821 had profound synergy with PARP inhibition in both HRR competent and defective cells (Ref. 186). Kim *et al.*, showed that inhibiting PARP resulted in increased reliance on the ATR-CHK1 pathway for genomic stability and that the combination of olaparib with ATRi AZD6738 effectively reduced tumour burden in patient-derived xenografts of serous ovarian cancer (Ref. 187).

A series of PARP inhibitors (rucaparib, olaparib, veliparib and NU1025) synergised with various CHK1 inhibitors (UCN-01, AZD7762 and LY2603618) to increase DNA damage and apoptosis *in vitro* in breast cancer cells (Ref. 188). The PARPi olaparib in combination with CHK1 inhibitor MK-8776, supressed colony formation in *BRCA* mutant models to a greater degree than either inhibitor as a single agent (Ref. 187). The PARP inhibitor Talazoparib was also synergistic with LY2606368, both *in vitro* and *in vivo* in gastric cancer (Ref. 164). Similarly, LY2606368 also showed inhibited HRR function and synergised with olaparib to decrease cell survival in *BRCA* wild-type cells (Ref. 163).

Fewer studies have looked at PARP and WEE1 inhibition combined, perhaps owing to the availability of only one WEE1 inhibitor with the desired selectivity and specificity (AZD1775). AZD1775 has only been tested in combination with olaparib but these results have been promising and when used in combination, the inhibitors act synergistically to radiosensitise pancreatic, and *KRAS*-mutant NSCLC cells further than when either is used as a single agent (Refs 189, 190).

ATR inhibitors in clinical trials

M6620 (VE-822/VX-970) was the first ATR inhibitor to reach clinical trials. Thomas *et al.*, first reported ATR inhibition in combination with chemotherapy in patients, with the maximum dose of topotecan being well tolerated when used in combination with M6620 (Ref. 191). There are currently three active clinical trials using M6620, one of which is the first in a human study looking at the pharmacokinetics of M6620 in combination with gemcitabine, cisplatin, etoposide carboplatin and irinotecan (NCT02157792). A number of phase 1 and 2 studies are currently recruiting patients for the use of M6620 single agent and in combination with a number of DNA damaging agents including irradiation, cisplatin, carboplatin, gemcitabine, irinotecan and topotecan (see Table 2). Another selective, bioavailable ATR inhibitor currently in phase 1 and 2 trials is AZD6738. Of these trials, nine are investigating the use of AZD6738 with the PARP inhibitor olaparib, which has strong support from pre-clinical data (Ref. 187). The ATR inhibitor BAY1895344 is in a first in human phase I safety trial in patients with advanced solid tumours and lymphomas (NCT03188965). M4344/VX-803 is an orally bioavailable ATR inhibitor currently recruiting in phase 1 clinical trial where it will be used as a monotherapy and in combination with cisplatin, carboplatin or gemcitabine (NCT02278250). The ATR inhibitor BAY1895344 has recently shown to have anti-tumour activity and is well tolerated at active doses in cancers with defects in DDR, such as loss of ATM (Ref. 192) (NCT03188965).

CHK1 inhibitors in clinical trials

Although there is significant interest surrounding CHK1 inhibitors, clinical progression has often been hindered because of the lack of bio-availability and off-target effects (Ref. 157). UCN-01 was used in combination with carboplatin in a phase 1 study (NCT00036777) and progressed into phase 2 clinical trials in 2010 in patients with metastatic melanoma, but the trial was terminated prematurely because of discouraging results (NCT00072189). CHK1 inhibitors with greater specificity have now entered clinical evaluation. SRA737 is currently in phase 1/2 clinical trials, both as a monotherapy and in combination with gemcitabine +/− cisplatin (NCT02797964) (NCT02797977). MK-8776 is also in phase 1 and 2 trials as a monotherapy and in combination with gemcitabine, cytarabine and hydroxyurea (NCT00779584) (NCT01870596). Of the four clinical trials it is currently in, two have been completed, with one terminated and one withdrawn owing to a lack of patients (see Table 3). However, *in vitro* studies have shown MK-8776 to have a short half-life, as well as undergoing rapid demethylation *in vivo*, resulting in a less selective metabolite (MU379) (Ref. 193). Phase 1 clinical trials, in patients with advanced solid tumours, of PF-477736 in combination with gemcitabine were terminated early for business reasons, rather than safety concerns (NCT00437203). Prexasertib (LY2606368) is currently in phase 2 trials a monotherapy agent, specifically in patients with cancers that are p53 mutant, have DDR defects such as *BRCA* mutation, increased replication stress or CCNE1 amplification, as these are determinants of CHK1 inhibitor sensitivity (NCT02735980, NCT02203513, NCT03414047, NCT02873975). Prexasertib has also entered phase 1 and 2 clinical trials in combination with pemetrexed (NCT01296568, NCT00415636, NCT01139775, NCT00988858), gemcitabine (NCT01358968, NCT01341457, NCT01296568, NCT00839332) or cisplatin (NCT02555644, NCT01139775) but has currently only been in one clinical trial with a PARP inhibitor (NCT03057145), despite promising synergy being observed pre-clinically (Refs 163, 164).

WEE1 inhibitors in clinical trials

AZD17775 is the only WEE1 inhibitor to reach clinical development and is already in a number of phase 1 and 2 trials being used in combination with treatments such as carboplatin, gemcitabine, cisplatin, cytarabine and olaparib (Refs 194, 195, 196). Recently a dose-escalation trial of AZD1775 in

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Table 2. ATR inhibitors currently in clinical trials

Drug name	Phase	Monotherapy/combination	Tumour type	NCT
M6620/ VX-970	I	In combination with whole brain radiotherapy	NSCLC, SCLC or neuroendocrine tumours that have metastasised to the brain	NCT02589522
		In combination with irinotecan hydrochloride	Solid metastatic tumours that are unable to be operated on	NCT02595931
		Assessing pharmacokinetics of M6620 in combination with gemcitabine, cisplatin, etoposide, carboplatin or irinotecan	First-in-human study, in patients with advanced solid tumours	NCT02157792
		As monotherapy or in combination with carboplatin and paclitaxel. A rollover study from VX13-970-002	Advanced solid tumours	NCT03309150
		In combination with chemoradiotherapy treatment (cisplatin and capecitabine)	Oesophageal cancer	NCT03641547
		In combination with cisplatin and radiotherapy	Locally advanced head and neck squamous cell carcinoma	NCT02567422
	II	In combination with avelumab and carboplatin	PARPi-resistant, recurrent, platinum-sensitive ovarian, primary peritoneal or fallopian tube	NCT03704467
		In combination with cisplatin or gemcitabine hydrochloride	Metastatic urothelial cancer	NCT02567409
		In combination with carboplatin +/− docetaxel	Metastatic castration-resistant prostate cancer	NCT03517969
		In combination with gemcitabine	Recurrent ovarian, primary peritoneal or fallopian tube	NCT02595892
		In combination with topotecan	Relapsed SCLC or extrapulmonary small cell cancer	NCT03896503
		Monotherapy	Advanced solid tumour	NCT03718091
		In combination with irinotecan	Metastatic or unresectable *TP53* mutant gastric or gastroesophageal junction cancer	NCT03641313
AZD6738	I	In combination with acalabrutinib	Relapsed/refractory aggressive Non-Hodgkins Lymphoma	NCT03527147
		Monotherapy	Head and neck squamous cell carcinoma	NCT03022409
		Monotherapy and in combination with palliative radiotherapy	Solid tumours	NCT02223923
		Monotherapy	Relapsed/refractory CLL, PLL or B cell lymphomas	NCT01955668
		In combination with gemcitabine	Advanced solid tumours	NCT03669601
		In combination with paclitaxel	Metastatic cancer failed standard chemotherapy	NCT02630199
		Monotherapy	Chronic myelomonocytic leukaemia or myelodysplastic syndrome	NCT03770429
	I/II	In combination with carboplatin, olaparib or durvalumab	Head and neck squamous cell carcinoma, advanced solid malignancy, NSCLC, gastric and breast cancer	NCT02264678
		Monotherapy and in combination with acalabrutinib	Relapsed or refractory high-risk CLL	NCT03328273
	II	Monotherapy	Neoadjuvant chemotherapy-resistant residual triple negative breast cancer	NCT03740893
		Monotherapy and in combination with PARPi olaparib	Renal cell carcinoma, urothelial carcinoma, pancreatic cancers and solid tumours that have spread to nearby tissue	NCT03682289
		In combination with PARPi olaparib	Recurrent ovarian cancer	NCT03462342
		In combination with PARPi olaparib	SCLC	NCT03428607
		In combination with PARPi olaparib	IDH1 and IDH2 mutant tumours	NCT03878095
		In combination with PARPi olaparib	Second- or third-line triple-negative breast cancer	NCT03330847
		In combination with PARPi olaparib	Resistant prostate cancer	NCT03787680
		In combination with PARPi olaparib	Advanced solid tumour	NCT02576444
		In combination with PARPi olaparib	Platinum refractory extensive-stage SCLC	NCT02937818
		In combination with immunotherapy durvalumab	Metastatic NSCLC which has progressed on an anti-PD-1 therapy	NCT03334617

(*Continued*)

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Table 2. (*Continued.*)

Drug name	Phase	Monotherapy/combination	Tumour type	NCT
		In combination with immunotherapy durvalumab	NSCLC with PD-1 immune checkpoint inhibitor resistance	NCT03833440
		In combination with immunotherapy durvalumab	Solid tumour, gastric cancer with failed secondary chemotherapy, melanoma patients	NCT03780608
BAY1895344	I	Monotherapy (first-in-human)	Advanced solid tumours and lymphomas	NCT03188965
M4344/ VX-803	I	Monotherapy and in combination with cisplatin, carboplatin and gemcitabine	Advanced solid tumours	NCT02278250

Table 3. CHK1 inhibitors currently in clinical trials

Drug name	Phase	Monotherapy/combination	Tumour type	NCT
MK-8776/ SCH900776	I	In combination with and without cytarabine	Acute leukaemia	NCT00907517
		In combination with hydroxyurea	Advanced solid tumours	NCT01521299
	II	In combination with and without cytarabine	Acute myeloid leukaemia	NCT01870596
LY2606368/ prexasertib	I	In combination with desipramine, pemetrexed or gemcitabine	Drug interaction study	NCT01358968
		In combination with gemcitabine	Solid tumours	NCT01341457
		In combination with pemetrexed or gemcitabine	Advanced/metastatic solid tumours	NCT01296568
		In combination with pemetrexed	Advanced/metastatic solid tumours	NCT00415636
		Monotherapy	Japanese patients with advanced solid tumour	NCT02514603
		Monotherapy	Paediatric solid tumours	NCT02808650
		In combination with ralimetanib	Advanced solid tumour	NCT02860780
		In combination with olaparib	Advanced solid tumour	NCT03057145
		In combination with cisplatin, cetuximab or radiotherapy	Head and neck cancer	NCT02555644
		In combination with PD-L1 inhibitor	Advanced solid tumours	NCT03495323
		In combination with cytarabine	Chronic/acute myeloid leukaemia	NCT02649764
	I/II	In combination with and without gemcitabine	Pancreatic cancer	NCT00839332
		In combination with cisplatin or pemetrexed	NSCLC	NCT01139775
	II	Monotherapy	Extensive stage SCLC	NCT02735980
		Monotherapy	Platinum resistant ovarian cancer	NCT03414047
		Monotherapy	Solid tumour with HRR defects or CCNE1 amplification	NCT02873975
		In combination with or without pemetrexed	Advanced or metastatic NSCLC	NCT00988858
		Monotherapy	*BRCA* mutant breast or ovarian cancer Triple-negative breast cancer HGSOC Castrate-resistant prostate cancer	NCT02203513

combination with gemcitabine and radiation showed promising results with good tolerability in patients with locally advanced pancreatic cancer (Ref. 197). The majority of clinical trials are still recruiting, suggesting this kinase inhibitor has exciting potential once trials have been completed and data is collected and analysed (Table 4).

DNA checkpoint kinase and immune checkpoint inhibitor combinations

Immune checkpoint inhibitors block the immunosuppressive mechanisms employed by cancers to prevent an effective anti-tumour immune response and have been found to be efficacious in many types of cancer. There is increasing evidence that tumour mutational burden increases the immunogenicity of cancers through the production of mutation-associated neoantigens, including those associated with microsatellite instability from defective DNA mismatch repair (Ref. 198). Damaged cytosolic DNA may also directly activate the immune system by stimulating interferon via the STING pathway (Stimulation of Interferon Genes) leading to enhanced immune checkpoint inhibitor responses in pre-clinical models. In ATM-deficient mice and patients with ataxia telangiectasia enhanced interferon production through the STING pathway has been observed (Ref. 198). ATM inhibition has recently been found to increase type 1 interferon signalling in a STING independent manner (Ref. 199). Clinical

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Table 4. WEE1 inhibitors currently in clinical trials

Drug name	Phase	Monotherapy/combination	Tumour type	NCT
AZD1775/ MK-1775	I	Monotherapy	Advanced/metastatic cancers	NCT02610075
		Monotherapy	Ovarian cancer, SCLC, other solid tumours	NCT02482311
		In combination with carboplatin and paclitaxel	Asian patients with advanced solid tumours	NCT02341456
		In combination with olaparib	Refractory solid tumours	NCT02511795
		In combination with irinotecan	RAS or BRAF mutated second-line metastatic colorectal cancer	NCT02906059
		In combination with either gemcitabine, cisplatin or carboplatin	Advanced solid tumours	NCT00648648
		In combination with either cisplatin and radiotherapy	Head and neck cancer	NCT03028766
	II	Monotherapy	Relapsed SCLC	NCT02593019
		In combination with docetaxel	NSCLC	NCT02087176
		In combination with carboplatin-paclitaxel	SCLC	NCT02513563
		In combination with cisplatin	Triple-negative metastatic breast cancer	NCT03012477
		In combination with cytarabine	Advanced acute myeloid leukaemia or myelodysplastic syndrome	NCT02666950

trials evaluating DNA damage repair inhibitors with immune checkpoint inhibitors are ongoing, including PARP, ATR and CHK1 inhibitors (Table 2).

Concluding remarks and future directions

Targeting DDR checkpoint signalling has evolved to the clinic based on sound scientific hypotheses and preclinical data. Early less specific inhibitors may have clouded the case for development but now more specific inhibitors are under investigation both as monotherapy and in combination with conventional cytotoxic chemotherapy or novel agents. The preclinical data suggest that targeting the ATR-CHK1-WEE1 pathway is likely to be more fruitful than targeting ATM and CHK2 signalling. To date, the most active combinations for each class of kinase inhibitor include ATR inhibitors with cis/carboplatin and CHK1 inhibitors with gemcitabine. A developing field is a potentiation with immune checkpoint inhibitors via several mechanisms of action. Identification of predictive biomarkers, particularly for monotherapy, however, has been challenging, for example, whether the presence of *TP53* mutations confers sensitivity.

The potential for some of these agents to be associated with second malignancy must not be forgotten, which is particularly a concern for young patients treated with these agents. Since defects in ATM and CHK2 are associated with tumours, but defects in ATR, CHK1 and WEE1 are not, one might predict that as single agents, inhibitors of the former might be associated with second malignancies but not the latter. However, in combination with cytotoxics already associated with second malignancies, the incidence is likely to be increased unless lower doses of the primary cytotoxic can be used in combination to achieve the same efficacy. Nevertheless, we must remember that the malignancies for which these agents are most likely to be used, especially in children, are the ones that are most difficult to cure with current strategies in which cure has yet to be achieved for the majority at any cost rather than at least cost.

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