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ATR/CHK1 inhibitors and cancer therapy

Zhaojun Qiu[a], **Nancy L. Oleinick**[a,b], and **Junran Zhang**[a,b,*]

[a]Department of Radiation Oncology, School of Medicine, Case Western Reserve University, Cleveland, USA

[b]Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, USA

Abstract

The cell cycle checkpoint proteins ataxia-telangiectasia-mutated-and-Rad3-related kinase (ATR) and its major downstream effector checkpoint kinase 1 (CHK1) prevent the entry of cells with damaged or incompletely replicated DNA into mitosis when the cells are challenged by DNA damaging agents, such as radiation therapy (RT) or chemotherapeutic drugs, that are the major modalities to treat cancer. This regulation is particularly evident in cells with a defective G1 checkpoint, a common feature of cancer cells, due to p53 mutations. In addition, ATR and/or CHK1 suppress replication stress (RS) by inhibiting excess origin firing, particularly in cells with activated oncogenes. Those functions of ATR/CHK1 make them ideal therapeutic targets. ATR/CHK1 inhibitors have been developed and are currently used either as single agents or paired with radiotherapy or a variety of genotoxic chemotherapies in preclinical and clinical studies. Here, we review the status of the development of ATR and CHK1 inhibitors. We also discuss the potential mechanisms by which ATR and CHK1 inhibition induces cell killing in the presence or absence of exogenous DNA damaging agents, such as RT and chemotherapeutic agents. Lastly, we discuss synthetic lethality interactions between the inhibition of ATR/CHK1 and defects in other DNA damage response (DDR) pathways/genes.

Keywords

ATR; CHK1; DNA damage response; Cell cycle checkpoints; DNA replication stress; Synthetic lethality

Human genomic DNA is threatened by a variety of exogenous and endogenous agents, such as ultraviolet radiation (UV), ionizing radiation (IR) and chemotherapeutic drugs [1], reactive oxygen species, lipid peroxidation products and DNA replication stress (RS; defined as any situation leading to instability of replication forks) [2,3]. Defending the integrity of the genome is the DNA damage response (DDR), an extensive network of pathways which detects and signals DNA damage for subsequent processing. The two major components of DDR are (a) the activation of cell cycle checkpoints, which is thought to allow cells time to repair their damaged DNA before moving to the next stage of the cell cycle and (b)

*Corresponding author at: Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, USA. Junran.zhang@case.edu (J. Zhang).

Conflicts of interest

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facilitation of direct DNA repair. When DNA double-strand breaks (DSBs), the most dangerous type of DNA damage, are generated, the MRE11/NBS1/RAD5 complex activates the ataxia telangiectasia mutated (ATM) /checkpoint kinase 2 (CHK2) pathway to promote S-phase cell cycle arrest and the p53-associated G1/S-phase checkpoint [4]. When single-strand DNA (ssDNA) is created at sites of DNA damage or stressed replication forks, RPA-coated ssDNA activates ATR and its binding partner ATRIP (Fig. 1) [3,5,6]. The serine/threonine kinase CHK1 is the key downstream regulator of the ATR response and is phosphorylated by ATR on Ser-317 and Ser-345 [7]. Activated CHK1 triggers the intra-S- and G2/M-phase checkpoints [8–11]. Inhibitory phosphorylation by CHK1 of the phosphatase CDC25A and its subsequent proteasomal degradation leads to a decrease in CDK2 activity in S-phase [12]. CHK1-mediated phosphorylation of CDC25B at Ser-323 and CDC25C at Ser-216 also promotes their degradation, which abolishes the activation of CDK1/cyclin B kinases, thereby causing G2/M arrest [13,14]. The tyrosine kinase Wee1 is a dual-specificity kinase that can phosphorylate CDK1 at Tyr15 and inhibit CDK1 kinase activity [15]. In yeast and Xenopus egg extracts [16,17], CHK1 could activate Wee1 to halt the G2/M transition and prevent cells with damaged DNA from entering mitosis. However, it is not clear if a similar regulation exists in mammalian cells. There is crosstalk between the ATM/CHK2 and ATR/CHK1 pathways, and they share many substrates [18]. However, they cannot compensate for the loss of each other [19,20]. Most cancer cells have dysregulated G1 checkpoints, making them dependent on S and G2 checkpoints, which are activated by ATR/CHK1 signaling.

In addition to their regulation of cell cycle checkpoints, ATR/CHK1 have multiple additional roles in protecting cells from DNA damage, including the stabilization of stalled replication forks, the control of replication origin firing at specialized start sites, and the promotion of homologous recombination (HR). During replication stress, ATR/CHK1 stabilizes stalled replication forks to prevent their collapse and DSB formation and helps to restart stalled replication forks [18,21,22]. In addition, ATR/CHK1 suppresses replication initiation, particularly in cells expressing activated oncogenes [23] (Fig. 2, see discussion below). These properties of the ATR/CHK1 pathway have made it an attractive target for therapeutic intervention. ATR/CHK1 signaling regulates HR, a major pathway required for repair of DSBs/collapsed replication forks. The role of ATR/CHK1 in promoting HR, particular gene-conversion-mediated HR, has been well established [9,24–30]. It is believed that ATR/CHK1 promotes HR *via* regulation of HR factors, such as facilitation of phosphorylation of RAD51 [26], FANCE [29] and FANCD2 [30]. In addition, CHK1 recruits the HR proteins BRCA2 and RAD51 [9,26] to DNA damage foci. In most of the studies demonstrating a role for ATR/CHK1 in HR repair, DR-GFP, an HR reporter designed to detect gene conversion non-crossover recombination was used [24–28,31]. Interestingly, we demonstrated that ATR depletion increased HR, as detected by sister chromatid exchange [27], which is mediated by recombination during replication in vertebrates [32] and is a reflection of crossover recombination. Thus, ATR/CHK1 may have a different impact on the various subtypes of HR.

Inhibitors of both ATR and CHK1 have been developed and are being tested in clinical trials. However, the development and clinical application of CHK1 inhibitors are more advanced than that of ATR inhibitors for several reasons. First, it was thought that inhibition of ATR

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might affect normal cells extensively *in vivo*, because ATR has a broader biological function than CHK1 [33,34]. Second, it is difficult to purify active ATR protein for the development of ATR inhibitors. ATR is a large protein with 2644 amino acids that makes compound screening in kinase assays challenging. Thus, an enzymatic analysis is more difficult to perform on the upstream ATR than on the downstream CHK1 [35]. Third, the crystal structure of ATR has not been reported, and hence there is no clue as to possible allosteric binding regions for drug design [36]. Thus, results from CHK1 inhibitor-related studies are far richer than from ATR inhibitors. Nevertheless, there has been some recent progress in the development of small molecule ATR inhibitors. In this review, we first discuss the chemo- and radio-sensitization activities of ATR/CHK1 inhibitors, with a comparison of mechanisms by which ATR/CHK1 inhibition acts as a chemosensitizer and radiosensitizer. All the inhibitors discussed in this review are listed in Table 1. In addition, we also discuss recent new discoveries that ATR and CHK1 inhibitors can be used as single agents. Last, we summarize the additional opportunities for new use of ATR/CHK1 inhibitors based on synthetic lethal interactions. The aims of this review are to emphasize the promise of ATR and CHK1 inhibitors for cancer therapy and that the need to exploit biomarkers to guide the use of these agents.

ATR/CHK1 inhibitors and chemo- and radio-sensitization

The initial idea is that ATR/CHK1 inhibitors would enhance killing of tumor cells by cytotoxic drugs or by radiotherapy through blocking cell cycle checkpoints, especially in p53-deficient cells [11,37,38]. Thus, the early studies focused on the activities of ATR/CHK1 inhibitors in chemo- and radio-sensitization. It has been reported that the inhibition or knockdown of ATR and/or CHK1 renders cells sensitive to a wide variety of DNA-damaging agents, including IR, antimetabolites (hydroxyurea, gemcitabine, cytarabine, 5-fluorouracil, gemcitabine), DNA cross-linking agents (cisplatin), topoisomerase I and II poisons (topotecan, SN-38, etoposide, doxorubicin) and alkylating agents (methyl-methane sulfonate). A recent review has summarized this broad panel of sensitized agents [39]. We will discuss selected combinations in this review.

CHK1 inhibitors

UCN-01 (7-hydroxystaurosporine) is the first CHK1 inhibitor with broad-spectrum efficacy against the protein kinase C family [40]. However, further application of UCN-01 has been hindered by its lack of specificity and by its long half-life, due to its binding to alpha acidic-glycoprotein that leads to hyperglycemia [41–44]. XL844 is a potent ATP-competitive inhibitor of CHK1, CHK2, VEGFR-2 and VEGFR-3. XL844 in combination with gemcitabine blocks CDC25A degradation, abrogates the S-phase checkpoints and increases DNA damage. Thus, XL844 potentiates gemcitabine activity *in vitro* and in xenografts [45]. However, clinical evaluation of XL844 was prematurely terminated (NCT00475917 and NCT00234481) for unknown reasons. CBP501 inhibits kinases that phosphorylate Ser-216 of CDC25C, including MAPKAP-K2, C-TAK1, and CHK1 [46]. Although a phase I study demonstrated that CBP501 is well tolerated in patients with advanced solid tumors, both as monotherapy and in combination with cisplatin [47], in a randomized phase II trial the addition of CBP501 failed to improve the efficacy of pemetrexed/cisplatin treatment in

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patients with advanced malignant pleural mesothelioma [48]. Because of the nonspecific targeting of UCN-01, XL844 and CBP501, their clinical applications have been limited.

Subsequently, more potent and specific CHK1 inhibitors have been developed. Most are ATP-competitive but with varying potencies and specificities. As a potent CHK1/2 dual inhibitor, AZD7762 is an ATP-competitive drug which suppresses CHK1-mediated phosphorylation of CDC25C with an IC50 of 5 nmol/L [49]. It has been suggested that AZD7762 treatment impairs CHK1 autophosphorylation (S296 CHK1), stabilizes CDC25A and potentiates the ATR/ATM-mediated CHK1 phosphorylation (S345 CHK1) [50]. Thus, it has been proposed that increased pS296 CHK1 and/or pS345 CHK1 can serve as a predictive biomarker of CHK1 inhibitor sensitivity [51,52]. However, a recent study demonstrated that p-CHK1 may not be a readout for ATR/CHK1 inhibition in some cancer cells and that the enhanced CDC25A expression is strongly associated with ATR/CHK1 inhibition [53]. Interestingly, AZD7762 sensitizes pancreatic cancer cells and tumors to gemcitabine, in association with induction of pS345 CHK1, with a strict schedule in which gemcitabine is administered concurrently with or before AZD7762 [52]. Although AZD7762 is a dual inhibitor that potently inhibits both CHK1 and CHK2 [49,54], its function mainly depends on the suppression of CHK1, because neither siRNA-mediated CHK2 knockdown [55,56] nor a CHK2-specific inhibitor [57] led to the sensitization to DNA damaging agents by AZD7762. AZD7762 in combination with chemotherapeutic drugs decreases tumor growth, especially cancers with p53 mutations or deletion [37,49,57–59]. Also, AZD7762 increased IR-induced apoptosis in cancer cells with compromised p53 [50,57,60,61]. However, effects of CHK1 inhibitors independent of p53 were also observed, *e.g.*, UCN-01 or SB-218078 on cell survival [62,63]. Similarly, short-term cell viability, as well as the long-term ability to form colonies under CHK1 depletion, was similar in isogenic cell lines regardless of the status of p53, although abrogation of the DNA-damage-induced cell cycle arrest by CHK1 inactivation was p53 dependent [64]. These results indicate that p53 is not the only determinant. Indeed, CHK1 inhibition by AZD7762 preferentially kills HR-deficient tumor cells, because HR-deficient cells rely more heavily on ATR/CHK1 for survival [65]. Interestingly, AZD7762 sensitizes pancreatic cells to IR *via* inhibition of the G2/M checkpoint and HR, regardless of p53 status [50]. In support of this result, we recently demonstrated that AZD7762 was more effective in targeting radioresistant breast cancer cells that are proficient in HR independent of p53 status, although CHK1 inhibition abrogated the IR-induced G2/M-phase checkpoint [66]. Therefore, p53 may not be the sole determinant of the efficacy of ATR/CHK1 inhibitors, and CHK1 signaling driving cell cycle checkpoints may not be the only mechanism contributing to cell killing. Unfortunately, the clinical trial of AZD7762 was terminated due to cardiac toxicity [67] and multiple adverse effects [68].

LY2603618 is the first selective and potent CHK1 inhibitor in which replacement of the piperidine moiety with a morpholine guarantees the potent inhibition of CHK1 but reduces the risk of cardiac toxicity [69]. In preclinical studies, LY2603618 increased the *in vitro* potency of gemcitabine in p53-mutant HT29 cells but not in p53-wild type HCT116 cells, and it abrogated the G2/M checkpoints triggered by treatment with gemcitabine in xenograft models [69,70]. A Phase I clinical trial demonstrated that LY2603618 administered approximately 1 day after pemetrexed showed acceptable safety and pharmacokinetic

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profiles [71]. Two out of 14 non-small-cell lung cancer (NSCLC) patients achieved a confirmed partial response after treatment with a combination of LY2603618, pemetrexed and cisplatin [70]. LY2603618 in combination with gemcitabine showed acceptable safety and tolerability in Japanese patients with solid tumors [72]. One out of 50 patients with NSCLC achieved a partial response in a Phase I study of LY2603618 in combination with gemcitabine [73]. Unfortunately, p53 status appeared to be not important for LY2603618 efficacy when combined with pemetrexed in a Phase II evaluation of patients with advanced or metastatic NSCLC [74]. Thus, the clinical studies of LY2603818 did not produce promising results.

MK-8776 (SCH 900776) is a selective CHK1 inhibitor that induces DSBs and cell death when combined with the DNA antimetabolites hydroxyurea, gemcitabine or pemetrexed in xenograft models [75–79]. In acute myelogenous leukemia cells, MK-8776 abolished the cytarabine-induced S-phase checkpoint and increased Ara-C (cytarabine; cytosine arabinoside)-induced apoptosis [80]. Moreover, MK-8776 radiosensitized p53-defective NSCLC and HNSCC tumors by abrogation of G2/M arrest and by inhibition of DSB repair [81]. A phase I trial suggested that MK-8776 is well tolerated both as a single agent and in combination with gemcitabine in patients with refractory acute leukemias [82]. Two out of 30 patients with advanced solid tumors showed a partial response when treated with MK-8776 in combination with gemcitabine [83]. A phase II clinical trial using Ara-C with and without MK-8776 in patients with refractory acute leukemia (NCT01870596) has been completed. However, no results have yet been reported.

PF-00477736 is a potent, selective ATP-competitive CHK1 inhibitor that abrogated cell cycle arrest induced by gemcitabine and carboplatin in multiple p53-defective cancer cell lines *in vitro* [84]. In xenografts, PF-00477736 augmented the antitumor activity of gemcitabine and docetaxel by inducing checkpoint abrogation and mitotic catastrophe [84,85]. PF-00477736 also potentiated the anti-proliferative effect of gemcitabine against both parental and cancer stem cell-like NSCLC populations [86]. The combination of PF-00477736 and gemcitabine enhanced ^{177}Lu-anti-EGFR-directed radioimmunotherapy against pancreatic ductal adenocarcinoma both *in vitro* and *in vivo* [87]. In addition, PF-0047736 sensitized HPV-positive HNSCC cell lines to IR [88]. Unfortunately, a Phase I trial of PF-00477736 (NCT00437203) in combination with gemcitabine in patients with advanced solid tumors was terminated due to business reasons.

LY2606368 (Prexasertib) is a CHK1/CHK2 dual inhibitor that preferentially binds to and inhibits CHK1 *in vitro* [89]. Treatment of cancer cells with LY2606368 resulted in DNA damage, premature entry into mitosis and cell death [89]. Similar results were obtained in xenograft tumor models where tumor growth was significantly inhibited after LY2606368 treatment [89]. Several clinical trials of LY2606368 have been initiated, but only one Phase I clinical trial to determine biosafety and dose for LY2606368 as monotherapy has been completed [90], and no data have yet been published.

New CHK1 inhibitors are still in preclinical development. 2e and CCT244747 are potent and selective CHK1 inhibitors that abrogated G2 and S checkpoint arrest caused by doxorubicin and camptothecin and increased cytotoxicity of several anticancer drugs both *in vivo* and *in*

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vitro [91–93]. CCT244747 radiosensitized p53-deficient head-and-neck squamous cell carcinoma (HNSCC) cells to paclitaxel-based chemoradiotherapy [94]. CHIR-124 is a selective, quinolone-based CHK1 inhibitor that is structurally unrelated to other known CHK1 inhibitors [95]. CHIR-124 interacted synergistically with topoisomerase poisons in p53-mutant solid tumor cell lines and in an orthotopic breast cancer xenograft model [95]. Treatment with CHIR-124 potentiated the efficacy of IR, histone deacetylase inhibitors and gemcitabine in inhibiting cancer proliferation [96,97]. A comparison study of two structurally related CHK1 inhibitors revealed that GNE-783 was more effective at sensitizing cells to antimetabolite-based DNA-damaging agents, whereas GNE-900 preferentially enhanced the activity of gemcitabine [98]. Treatment with two CHK1/Wee1 dual inhibitors, PD-321852 or PD-407824, led to sensitization of pancreatic cancer cells to gemcitabine [99,100]. SAR-020106 is a potent and highly selective isoquinoline CHK1 inhibitor [101,102] that augmented the efficacies of irinotecan and gemcitabine in human colon carcinoma xenograft models [101] and sensitized p53-deficient cancer cells to IR [103,104]. An indolocarbazole inhibitor of CHK1, SB-218078, sensitized p53 mutant cancer lines to IR, topotecan or quinacrine by abolishing the G2 checkpoint [105–107]. Two newly developed CHK1 inhibitors, S1181 and the CHK1/CHK2 dual inhibitor V158411, potentiated p53-defective cancer cells to different chemotherapeutic drugs [108,109].

In summary, despite the promising preclinical studies of CHK1 inhibitors in chemo- and radio-sensitization, results from clinical trials have been less impressive. In addition, independence of p53 has been observed in both preclinical studies and in clinical trials. Thus, other new factors/biomarkers affecting the efficacy of CHK1 inhibitors need to be identified. The most important break-through to be made in cancer treatment with these inhibitors may be to establish inhibitor-specific patient stratification criteria, since a lower dose of a CHK1 inhibitor is expected to be effective if the appropriate patients were selected. In addition, a new generation of CHK1 inhibitors with reduced toxicity needs to be developed in the future.

ATR inhibitors

The natural product Schisandrin B is the first ATR-specific inhibitor that reduced the phosphorylation of CHK1 by inhibiting ATR protein kinase activity following UV treatment of lung cancer cells [110,111]. Nevertheless, application of this drug is limited, due to the high dose that is required (30 μM for cellular assays) [110]. More recently, NU6027, NVP-BEZ235, torin 2, and ETP-46464 were reported to be more potent ATR inhibitors that sensitized tumor cells to a variety of genotoxic chemotherapies, but they also inhibited other kinases, such as CDK2, PIK3, mTOR, and ATM [112–115]. Compound 45 is a novel ATR inhibitor that showed potent activity against ATM-defective HCT116 cells and spared normal HFL1 cells [116]. It also synergistically enhanced the killing effect of IR and cisplatin in multiple cell lines [116].

VE-821 is a potent and selective ATR inhibitor that was discovered in a high-throughput screen [117]. It inhibited the phosphorylation of CHK1 at Ser345 and sensitized cancer cells to IR [117–121] or chemotherapeutic drugs, including gemcitabine [117,122], cisplatin [53,123], topotecan [53], indotecan [124] and camptothecin [124]. Thus, decreased pCHK1

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Ser345 can be used as a biomarker for ATR inhibition [125]. The chemosensitization effect of VE-821 was further confirmed in p53-defective, ATM-defective or inhibited cells [120,122]. The novel inhibitor VE-822 (VX970) that is developed by Vertex is a close analog of VE-821 but has optimized solubility and pharmacokinetic properties. This inhibitor selectively inhibits ATR kinase activity. Targeting ATR *in vivo* with VE-822 resulted in selective sensitization of pancreatic tumors to IR [126] and augmented the *in vivo* anti-tumor response to the topoisomerase I inhibitor irinotecan without additional toxicity to normal cells [124]. VE-822 also potentiates the effect of cisplatin in NSCLC cells *in vitro* and in human lung tumor xenografts [127]. In a study of esophageal cancer, VE-822 improved response of cells to cisplatin, carboplatin or radiation and increased tumor growth delay from radiotherapy *in vivo* [128]. In this study, the radiation experiments were also carried out under hypoxic conditions with the same conclusion. Given its minimal toxicity, VE-822 is the first ATR-specific inhibitor entering clinical trials. Nine phase I/II trials of VE-822 in combination with radiotherapy or chemotherapy are currently ongoing.

AZ20 is another selective and potent inhibitor of ATR that inhibited the proliferation of LoVo colorectal adenocarcinoma cells *in vitro* as a single agent [129]. AZD6738, an analog of AZ20, is the second ATR inhibitor in clinical trials. The solubility, bioavailability and pharmacokinetic properties of AZD6738 are better than those of AZ20. These advantages of AZD6738 make it suitable for oral dosing [130]. AZD6738 potentiated cisplatin effects against ATM-deficient NSCLC *in vivo* [131]. A recent study suggested radiosensitization by the ATR inhibitor AZD6738 through generation of acentric micronuclei. The induction of micronuclei was significantly more prominent for AZD6738 in comparison to CHK1 inhibition at isotoxic doses [132]. Furthermore, AZD6738 sensitized p53- or ATM-defective primary chronic lymphocytic leukemia (CLL) cells to chemotherapy and ibrutinib both in *in vitro* and *in vivo* assays [133]. However, the selectivity of ATR inactivation might not be restricted to p53-defective cells. ATR silencing sensitized HeLa (p53-defective) and U2OS (p53-wild-type) cells to topoisomerase I poisons [134]. Radiosensitization by AZD6738 to clinically relevant doses of fractionated radiation was demonstrated *in vitro* using a 3D tumor spheroid model. The radiosensitization by AZD6738 is independent of both p53 and BRCA2 status [132]. Thus, p53 status may not be the sole determinant of the outcome of ATR inhibitors. Three phase I clinical trials of AZD6738 in combination with radiotherapy or chemotherapy are currently recruiting patients (Table 1). Compared to studies related of CHK1 inhibitors, investigations of ATR inhibition are still in an early stage. It is hoped that results from the current clinical trials will validate those from preclinical studies. We look forward to the development of new improved ATR inhibitors in the future.

The mechanisms by which ATR/CHK1 inhibitors sensitize cells to chemotherapy and radiotherapy

ATR/CHK1 signaling regulates cell cycle checkpoints, replication fork stability, replication origin firing and HR. It remains unknown whether it is the disruption of one or all of these pathways by ATR/CHK1 inhibition that results in the potentiation of the response to radiotherapy or chemotherapeutic drugs. The mechanisms of chemosensitization and radiosensitization by the various ATR/CHK1 inhibitors may overlap but with differences, given the multiple mechanisms by which the majority of cancer chemotherapeutic agents

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work; for example, both antimetabolites and cytotoxic drugs inhibit DNA synthesis in S-phase either by inhibiting production of the necessary deoxyribonucleotide precursors or by directly damaging DNA, but IR causes DNA damage, particularly DSBs, throughout all stages of the cell cycle. ATR/CHK1 signaling is primarily activated by RPA-coated ssDNA in S-phase cells during replication stress, a situation typically caused by most chemotherapeutic drugs, whereas DSBs induced by IR predominately activate ATM. The most dramatic sensitization has been observed when CHK1 inhibitors are combined with hydroxyurea or gemcitabine, two antimetabolites that inhibit ribonucleotide reductase, thereby starving cells of deoxyribonucleotides and leading to stalling of replication fork progression. For instance, a 100-fold decrease in the IC_{50} for hydroxyurea has been reported upon addition of the CHK1 inhibitor MK-8776 [76,135]. The sensitization to gemcitabine *in vitro* was about 10-fold with other CHK1 inhibitors [45,49,84,101,135]. CHK1 inhibitors do not cause cell cycle delay when combined with hydroxyurea or gemcitabine, because cell cycle arrest induced by antimetabolites does not require checkpoint activation, as cells cannot complete DNA replication without dNTPs. Thus, the effects of ATR/CHK1 inhibitors on S-phase checkpoints may not be important to their ability to sensitize cells to antimetabolites. It is generally believed that the antimetabolites alone result in the accumulation of stalled replication forks. In the absence of CHK1, the stalled forks are not protected by DNA2 or SMARCAL1 and would become susceptible to MUA81 cleavage and increased cytotoxicity by causing fork collapse/DSBs [135]. The detailed mechanism has been discussed in a recent review [135].

Of note, CHK1 inhibitors do not appear to sensitize cells to all antimetabolites. For instance, no sensitization activity of MK-8776 was observed when combined with 5-FU [76], a drug that inhibits thymidylate synthase, thus depleting cells of thymidine and stalling replication fork progression. Therefore, stabilization of replication forks by CHK1 may not be the sole mechanism explaining the sensitization by CHK1 inhibition when combined with antimetabolites. The work from Engelke et al. has demonstrated that HR is involved in radiosensitization by CHK1 inhibition, because HR-proficient cells, MiaPaCa-2, BxPC-3, and AsPC-1, were sensitized to gemcitabine by MK8776, whereas HR-deficient Capan-1 cells were only minimally sensitized [77]. Another study suggested that CHK1 inhibition sensitizes cells to gemcitabine and/or hydroxyurea by abolishing G2/M arrest and inhibition of HR [50], although disruption of replication fork stability is thought to be critical for sensitization to antimetabolites (discussed above). In addition, it has been demonstrated that interference with cell cycle progression may be important for the CHK1 inhibitor UCN-01-induced cell killing when combined with cytotoxic drugs, the topoisomerase I inhibitor camptothecin/SN38 [136,137] or the crosslinking agent cisplatin [138]. ATR/CHK1 inhibitors potentiate the response to cisplatin by decreasing the drug-induced G2/M arrest and leading to polyploidy and subsequent mitotic catastrophe [47,48,53,70,116,123,127,131]. Therefore, ATR/CHK1 inhibition sensitizes the response to many chemotherapeutic drugs that act through different mechanisms; the sensitization mechanisms may overlap, depending on the mode of action of the chemotherapeutic drugs.

In contrast to the extensive sensitization of chemotherapeutic drugs by ATR/CHK1 inhibitors, more modest radiosensitization is observed; however, variations in experimental conditions, such as cell lines, types of drugs, testing models, experimental assays, endpoints

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and p53 status, may contribute to differences. A recent study evaluated the effect of UCN-01 and MK-8776 on cell viability after exposure to IR using a clonogenic survival assay. The sensitizer enhancement ratios (SERs), which is defined as the ratio of radiation doses resulting in 10% survival (LD_{90} doses) under non-sensitized vs. sensitized conditions, for UCN-01 and MK-8776, were found to be 1.13 and 1.22 in EMT6 cells, and 1.07 and 1.39 in HeLa cells, respectively. Although AZD7762 was a very effective radiosensitizer in both *in vitro* and *in vivo* assays, MK8776 produced less radiosensitization [50,60,77]; radiosensitization by AZD7762 may be in part attributable to its activity against CHK2 or some other targets. Others have found that inhibition of CHK2 radiosensitizes cancer cells [139]. Clonogenic assays showed radiosensitization by AZD6738 in both p53 wild-type and p53 mutant cells with SERs from 1.43 to 2.27 at the 37% survival condition [132]. In pancreatic cancer cells, a low dose of AZD7762 reduced cell survival with an SER of 1.5 at the LD_{90} [50].

It is generally accepted that the mechanisms of ATR/CHK1 inhibition-mediated radiosensitization are related to abrogation of the G2 checkpoint, particularly in p53-deficient cells [50,57,60, 61,81,88,96,103–105]. In the accepted model, p53 triggers cell-cycle arrest in response to genotoxic stress: normal cells arrest in G1 (*via* p53), which is thought to allow time for DNA repair, whereas, p53-deficient tumor cells rely on CHK1 to arrest cell-cycle progression in the S- and G2-phases. Cells deficient in p53 undergo mitotic catastrophe and apoptosis when the S and G2 checkpoints are abrogated by inhibition of ATR or CHK1 [37,40,44,81,133]. Although HR may also be important for radiosensitization by ATR/CHK1 inhibition [28], it was also found that radiosensitization by the CHK1 inhibitor AZD7762 involves abrogation of the G2 checkpoint and inhibition of HR in a p53-independent manner [50], and the ATR inhibitor AZD6738 worked similarly in a manner independent of p53 and the HR protein BRCA2 [132]. Interestingly, HR may not be involved in radiosensitization when a CHK1 inhibitor is applied post-IR, as evidenced by a recent study demonstrating that CHK1 inhibition by MK-8776 had no influence on the radiation-induced DSB levels or repair kinetics if the drug was applied immediately after irradiation [140], a finding that differs from a previous study in which MK-8776 was applied prior to IR [81]. Thus, some mechanisms of radiosensitization and chemo-sensitization by ATR/CHK1 inhibition are shared, but they can also be different. These mechanisms are far more sophisticated than we discussed. A combination of ATR/CHK1 inhibitors with a variety of chemotherapeutic drugs has been discussed in a recent review [39].

Given the fact that the combination of radiation with chemotherapy is the most effective therapy for some types of cancer, such as unresectable pancreatic cancer, the role of CHK1 inhibitors on chemoradiation also has been studied. The work of Engelke et al. suggested that CHK1 inhibition by AZD7762 could sensitize pancreatic cancer cells and tumors to combinations of gemcitabine and radiation, with inhibition of HR repair as an important underlying mechanism of this sensitization [50]. A similar result was obtained with a second inhibitor MK8776 [78]. These results supported clinical investigation of CHK1 inhibitors with gemcitabine-radiation in locally advanced pancreatic cancer patients [77]. The same study also demonstrated that MK8776 combined with gemcitabine or gemcitabine-radiation produced a significantly greater increase in pCHK1 (S345) in tumors relative to small intestine. Thus, pCHK1 (S345) may be a useful biomarker of the inhibition of CHK1 and

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sensitization activity as well as for CHK1 inhibition and DNA damage. However, correlation of pCHK1 (S345) status with clinical response needs to be validated.

In summary, ATR/CHK1 inhibition can sensitize cells to a broad array of DNA damaging agents, although the mechanisms of sensitization may differ with different treatment modalities. It is possible that biomarkers to predict radiosensitization and chemosensitization may differ although they overlap. Thus, it will be important to identify biomarkers which predict the superiority of one modality over others. The future challenge is to identify the patient populations that are sensitive to particular treatment regimens.

Applications of ATR/CHK1 inhibitors as single agents

Recent studies suggest that inactivation of ATR or CHK1 could target cancer cells as single agents. Results exploring this strategy are starting to emerge.

Targeting cancer cells with ATR and CHK1 inhibitors, used as single agents

Despite their well-established role in cell cycle regulation following DNA damage, the essential function of CHK1/ATR in this response is poorly understood. The initial thoughts that ATR and CHK1 were tumor suppressors are countered by several recent studies in different models demonstrating that ATR/CHK1 signaling may promote tumor progression. An extra allele of CHK1 limits oncogene (RAS/E1A)-induced RS and DSB formation, and therefore promotes transformation [141]. In addition, suppression of ATR function in oncogenic KRAS-expressing cells synergistically increases genomic instability and DNA damage-induced cell death [142]. In support of the idea that ATR/CHK1 is indispensable for tumor growth, amplification of ATR or CHK1 is frequently observed in genomically unstable cancers, including squamous cell lung carcinoma, ovarian cancer and head-and-neck cancer [65]. In addition, expression of ATR or CHK1 is increased in triple-negative breast cancer (TNBC) [143], neuroblastoma [144], T-cell acute lymphoblastic leukemia [145], acute myeloid leukemia [146] and hepatocellular carcinoma [147]. The oncogenes MYC and E2F1 upregulate CHK1 mRNA and protein expression both *in vitro* and *in vivo* [148,149]. Thus, compared to normal cells, cancer cells that carry oncogene-induced RS rely more heavily on ATR/CHK1 for survival. The reliance on ATR/CHK1 signaling for growth of tumor cells provides a therapeutic window for ATR/CHK1 inhibitors as single agents. Thus, the value of CHK1 inhibitors, and perhaps ATR inhibitors, as single agents has been recognized.

In a comprehensive loss-of-function screen of the protein kinome for neuroblastoma, CHK1 was identified as a candidate therapeutic target [144]. Oncogene expression is a major cause of RS [150,151]. In support of the hypothesis that inhibition of the ATR-CHK1 pathway is antineoplastic when targeted to cancers with high levels of RS [51,141,152–154], the CHK1 inhibitor UCN-01 decreased the clonogenicity of AML samples with high levels of RS and complex karyotypes [146]. The CHK1 inhibitor CCT244747 showed marked antitumor activity as a monotherapeutic agent in a MYCN-driven neuroblastoma [92]. As single agents, V158411, PF-477736 and AZD7762 potently inhibited the proliferation of TNBC [51]. In MYC-overexpressing B-cell lymphoma cells, CHK1 inhibition by siRNA or the inhibitor Chekin led to increased cell death both *in vitro* and *in vivo via* caspase-dependent

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apoptosis pathways [148]. CHK1 inhibitors, as single agents, specifically target a subtype of cancer cells expressing Cyclin E and MYC [152]. We also reported that a CHK1 inhibitor has antitumor activity in radioresistant breast cancer cells *via* enhancing RS, perhaps caused by the oncogenes CDC25A/Myc/-Src/H-ras/E2F1 [66].

Treatment with the CHK1 inhibitor PF-00477736 increased DNA damage and DDR signaling and led to caspase-dependent apoptosis and cell death in Eμ-myc lymphoma cells [153]. The two CHK1-specific inhibitors AR323 and AR678 were shown to be selectively cytotoxic to melanoma cell lines with endogenous RS [154]. In addition, a hypomorphic ATR pathway was synthetic lethal in the combination with oncogenic RAS expression in cultured cells [142] or in oncogenic RAS-driven murine tumors *in vivo* [155]. In the murine ATR-Seckel syndrome model, a decrease in ATR expression completely prevented the development of Myc-induced lymphomas or pancreatic tumors, which had high levels of RS [152]. A cell-based screen identified ATR inhibitors with synthetic lethal properties for cancer cells overexpressing cyclin E [114]. Pharmacologic inhibition of ATR led to chromosome breakage under conditions that stall replication forks. ATR inhibition is particularly toxic for p53-deficient cells, this toxicity being exacerbated by overexpression of cyclin E, a condition causing RS. In this study, the compound NVP-BEZ235, a dual PI3K/mTOR inhibitor, was found to be very potent against ATM, ATR and DNA-PKcs [114]. In addition, lack of p53 exacerbated RS induced by the ATR deficiency and augmented the detrimental effect [156]. Combined deletion of p53 and ATR in the mouse hindered tissue regeneration, induced high levels of DNA damage and led to synthetic lethality [157]. Furthermore, ATR inhibition by VE-821 in U2OS cells was shown to rewire cellular signaling networks induced by RS and activate DSB repair driven by ATM [158]. A study of LY2606368 in patients with solid tumors with RS or HR deficiency (NCT02873975) is ongoing. This study is testing if the CHK1 inhibitor is a possible treatment for advanced solid tumors that harbor genetic alterations in the HR pathway or with genetic alterations that indicate RS. Eight clinical trials using CHK1 inhibitors as single agents have reported results [47,83,159–163], while three clinical trials (NCT01564251, NCT01115790 and NCT02203513) are still recruiting patients. However, all of these trials focus on finding the maximum tolerated dose or the tolerance of the tested drugs. None of the clinical trials of ATR inhibitors as single agents to treat cancers has been completed at the time of preparation of this manuscript.

ATR/CHK1 inhibitors target cancer cells, as single agents, by regulating RS

DNA replication is a tightly regulated process. In late M- and early G1-phases of the cell cycle when CDK activity is low, replication is initiated by the association of origin recognition complex proteins with DNA replication origins, followed by the loading of CDT1 and CDC6 proteins [164]. Then, the six minichromosome maintenance (MCM) proteins that are the core components of the replicative DNA helicase are recruited to the origins to complete the pre-replication complexes. Activation of DBF4-dependent kinase and CDKs subsequently trigger the formation of functional bidirectional replication forks by recruiting replication initiation factor CDC45, GINS complex, RPA and DNA polymerases [164]. There is a regulation mechanism to ensure that all of the licensed replication origins do not fire simultaneously. Although how oncogenes cause RS is not fully understood,

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uncoordinated replication initiation, reduced licensing and re-usage of origins could be involved [165–167] (Fig. 2). For instance, overexpression of the oncogene cyclin E causes increased replication initiation [167] and also reduces the number of licensed origins by impairing prereplication complex assembly [165,166]. In that model of oncogene-induced replication initiation/firing, activation of oncogenes results in increased origin firing due to increased CDK activity, which in turn depletes the dNTP pool, as too many ongoing replication forks consume the limited dNTP pool. Replication stalling produces massively elongated ssDNA regions that are protected by RPA coating. With extended RS time and a limited supply of RPA, uncoated ssDNA causes DSB formation [168,169] (Fig. 2). CHK1/ATR suppresses oncogene-induced RS [51,141,152–154,170,171] by controlling replication initiation (Fig. 2). In the presence of replication stalling, activated CHK1/ATR phosphorylates CDC25A and promotes its degradation. ATR/CHK1-mediated loss of CDC25A activity suspends CDKs, such as CDK2, in an inactive phosphorylated state, blocking initiation of DNA replication origins (Fig. 2). The resulting inhibition of CDK2 activity prevents accumulation of the initiation factor CDC45 at unfired origins. Without CHK1 to regulate CDC25A, CDK2 activity is increased, leading to uncoordinated and excessive DNA replication initiation [172] and consequently the slowing and stalling of replication forks, due to an insufficient supply of replication factors [173–175]. Although ATR/CHK1 suppress replication stress *via* inhibition of CDK-induced replication initiation, it is not clear if ATR/CHK1 also suppresses CDK-mediated regulation on the licensed replication origins. In addition, the promotion of HR by ATR/CHK1 may contribute to its suppression of RS. Thus, the inhibition of ATR/CHK1 enhances RS *via* multiple mechanisms.

In support of the hypothesis that the conditions causing RS likely result in dependence on ATR/CHK1, hypoxia, a common biological source of RS frequently found in the majority of solid tumors [176], activates ATR/CHK1 signaling [177]. Severe levels of hypoxia (<0.1% O_2) induce DDR and RS that activates both ATR and ATM signaling for survival [178–180]. Thus, it is not surprising to find that inducible knockdown of ATR and siRNA knockdown of CHK1 lead to increased DNA damage and decreased survival of hypoxic cells [177]. Cazares-Korner et al. suggested that the CHK1/Aurora A dual inhibitor CH-1 specifically decreases the viability of cancer cell lines under hypoxic conditions [181]. A unique aspect of this study is the development of a bioreductive prodrug of CH-01, which functions specifically in hypoxic conditions. A significant loss of viability of cancer cells exposed to hypoxia was observed when CH-01 is present [181]. The mechanism by which ATR/CHK1 inhibition targets hypoxic cells may be related to a defect in HR, since HR-defective cells are more reliant on ATR/CHK1 signaling for survival [65], and decreased HR in hypoxic cancer cells was observed to result from down-regulation of Rad51 and other proteins [182–185]. Thus, targeting hypoxic cells through targeting DDR is a promising strategy for cancer therapy [180]. Specifically, inhibition of ATR/CHK1 may be a promising therapy for tumors with high levels of hypoxia-induced RS, which needs to be further explored. Interestingly, a very recent study from Foskolou et al. suggested that ribonucleotide reductase (RNR), the only enzyme capable of *de novo* synthesis of dNTPs, requires subunit switching in hypoxia to maintain DNA replication. RNR consists of two homodimeric subunits, RRM1/RRM2 and RRM1/RRM2B. RRM1/RRM2B is favored over RRM1/RRM2 in order to preserve

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ongoing replication and avoid the accumulation of DNA damage [186]. Thus, this study raises the possibility of targeting tumors with hypoxia by targeting RRM2B.

Synthetic lethality between inhibition of ATR/CHK1 signaling and inhibition of other DDR pathways/genes

Synthetic lethality has attracted much attention from cancer biologists recently, as it provides a new angle for cancer therapy. It is a genetic concept describing any situation in which a combination of deficiencies in the expression of two genes leads to cell death, whereas a deficiency in only one of these genes does not. The potential synthetic lethality interactions with ATR/CHK1 are listed in Fig. 3. The synergistic cytotoxic effect of combined inhibition of WEE1 and CHK1 has been observed in preclinical research both *in vitro* and *in vivo* [187–193]. These two proteins have complementary but distinct roles. Both WEE1 and CHK1 suppress the activity of CDK and subsequent origin firing, but CHK1 has additional targets, not shared with WEE1, to promote fork progression and stability [194], providing a rationale for combination. Double knockdown of WEE1 and CHK1 by siRNA did not increase γ-H2AX levels more than those of single knockdowns; however, most of the cells lacking WEE1 and CHK1 underwent apoptosis [195]. Inhibition of WEE1 by siRNA or MK-1775, the only WEE1 inhibitor in clinical trial, potentiates the anti-cancer efficacy of the CHK1 inhibitor AR458323 and increases the level of apoptosis in prostate and lung cancer cell lines [187]. In a high-throughput synthetic lethality screen, WEE1 was also identified as a target that sensitized human ovarian cancer OVCAR-5 cells to the CHK1 inhibitor PF-0077736 [188]. The proposed mechanism for the synergistic effect is that inhibition of these two kinases interrupts DNA replication, induces DNA damage, allows the entry of unrepaired DNA into mitosis and results in apoptosis [188]. A similar synergistic effect was found between the CHK1 inhibitor MK-8776 and MK-1775 both *in vitro* and *in vivo* [189–191]. The CHK1/2 inhibitor AZD7762 synergized with MK-1775 in malignant melanoma cell lines both *in vitro* (2D and 3D cultures) and in xenograft models [196]. Consistent with the results with CHK1 inhibitors, ATR inhibition has a synthetic lethality with WEE1 inhibition in U2OS cells [197]. The ATR inhibitor VE-821, as a single agent, had no effect on the cell cycle of HeLa cells, but premature mitosis was induced when combined with sublethal concentrations of the WEE1 inhibitor MK-1775 [192]. In addition, two siRNA screens to identify synthetic lethal genes toward ATR inhibition suggested that knockdown of CHK1 is synthetic lethal to ATR inhibition [197,198]. Most interestingly, a combination of inhibitors of CHK1 (AZD77762) and ATR (VE-821), which are thought to be in the same pathway, led to DNA damage accumulation and subsequent cell death [199]. It was found that either the ATR or the CHK1 inhibitor alone leads to mitotic catastrophe, while the combination results in extensive DNA damage that likely leads to replication catastrophe [200]. It is proposed that cancer cells with disrupted CHK1 rely more heavily on ATR for survival, because the DNA damage caused by CHK1 inhibition activates ATR [199]. Of course, other mechanisms may also be involved; for instance, there is an ATR-dependent, CHK1-independent, intra-S-phase checkpoint that suppresses origin firing [200]. Interestingly, inhibition of CHK1 had no effect on B-cell transformation, but specific inhibition of ATR dramatically decreased the transformation efficiency of EBV [201]. Furthermore, ATR and CHK1 may not act linearly in the kinase cascade pathway and have

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distinct functions [199,202]. All the findings above warrant further investigation in clinical trials.

ATR or CHK1 inhibitor also undergoes synthetic lethality with a defect in other genes that are involved in DDR. The ATR inhibitor AZD6738 was reported to have single-agent anti-tumor activity in ATM-deficient xenograft models [130], and induced synthetic lethality in p53- or ATM-defective CLL cells both *in vitro* and *in vivo* [133]. The authors proposed that in p53- or ATM-defective CLL cells, inhibition of ATR signaling by AZD6738 can cause an accumulation of unrepaired DNA damage, which would be carried through into mitosis because of defective cell cycle checkpoints, resulting in cell death by mitotic catastrophe. In addition, a synthetic lethality screen revealed that the sensitivity to an ATR inhibitor treatment was enhanced in mantle cell lymphoma with ATM Loss-of-Function. The mechanism of this synthetic lethality is that cancers with *ATM* mutations have impaired DSB repair pathways and depend on compensatory repair mechanisms for survival [197]. Therefore, impairing ATR activity may selectively sensitize cancer cells to killing.

The CHK1 inhibitor UCN-01 suppressed the growth of pancreatic cancer cell lines with deficient BRCA2 [203]. CHK1 inhibition by siRNA knockdown or UCN-01 was more effective in cell lines deficient in Fanconi's Anemia (FA) DNA repair pathway [204]. In addition, the replication factor C (RFC)-related protein RAD17 was identified to be synthetic lethal to multiple CHK1 inhibitors [193]. Similar results were also found with the ATR inhibitor VE-821 [122]. ATR inhibition by NU6027 or VE-821 was shown to be synthetic lethal with XRCC1 loss in multiple ovarian cancer lines [205]. Loss of the structure-specific endonuclease ERCC1-XPF (ERCC4), which is important for nucleotide excision repair, was synthetic lethal with the ATR inhibitor VE-821 or the CHK1 inhibitor AZD7762 [198]. The synthetic lethality between inhibition of ATR and loss of MRE11A was also confirmed [129]. In a synthetic lethality screen directed against 288 DNA-repair genes using DLD1 colorectal cancer cells, POLD1 was identified to have synthetic lethality with ATR and CHK1 inhibitors [206]. However, those synthetic lethalities described above have not yet been confirmed in clinical trials.

Inhibition of ATR/CHK1 also sensitizes cells to PARP inhibition. Both ATR- and CHK1-deficient cells were sensitive to the PARP inhibitor KU0058684 in an RNAi screen [207]. Multiple types of breast cancers were effectively killed by a combination of PARP inhibitors and CHK1 inhibitors both *in vitro* and *in vivo* [61,208]. Similar results were also found in prostate [209] and p53-mutant pancreatic cancer cells [38]. Another study demonstrated that the CHK1/2 dual inhibitor AZD7762 synergized with the MEK1/2 inhibitor AZD6244 by inducing massive apoptosis of myeloma cells [210,211]. The ATR inhibitor NU6027 was synthetic lethal with the PARP inhibitor PF-01367338 in breast and ovarian cancer cells [113]. It was found that NU6027 impaired G2/M arrest and HR, which could explain the increasing sensitivity to DNA-damaging agents and PARP inhibitors, providing proof-of-concept data for clinical development of ATR inhibitors [113]. The ATR inhibitor VE-821 and the CHK1 inhibitor MK-8776 further sensitized HR-deficient ovarian cancer cells to the PARP inhibitor veliparib (ABT-888) [53]. The synergy between an ATR inhibitor and a PARP inhibitor was observed in more than 100 cell lines with p53 mutations [212]. Although synthetic lethality between ATR/CHK1 inhibition and a defect in various other

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factors involved in DDR might have a variety of explanations; most mechanisms are related to the fact that a defect in DDR factors, such as WEE1, PARP, HR proteins or replication proteins, results in increased RS which activates ATR/CHK1 signaling; thus in the presence of DDR defects that can cause RS, cells rely more heavily on ATR/CHK1 signaling for survival.

Last, ATR/CHK1 inhibition also has a synthetic lethal interaction with inhibitors targeting the mitogen-activated protein kinase (MAPK) signaling pathway. CHK1 inhibition was synergistically lethal with HMG-CoA reductase inhibitors [213], MEK and farnesyl-transferase inhibitors [214] and SRC family inhibitors [215,216]. A combination of the nonspecific CHK1 inhibitor UCN-01 and a MAPK inhibitor led to mitochondrial dysfunction and apoptosis in human leukemia cells [217]. A similar result was reported in human multiple myeloma cells [218]. It is believed that the MAPK pathway is activated following exposure of leukemic cells to UCN-01. Interruption of this process by MEK inhibition triggers perturbations in cell cycle regulatory pathways and several signaling cascades that culminate in mitochondrial injury, caspase activation and subsequent apoptosis. Thus, a combination of UCN-01 with MEK inhibitors may represent a novel anti-leukemic strategy.

Altogether, inhibition of ATR/CHK1 signaling had greater antitumor efficacy when combined with other inhibitors targeting DDR pathways or signaling pathways. Thus, identification of new synthetic lethal combinations will significantly improve the outcome of ATR/CHK1-associated cancer therapy.

Conclusions and future prospects

Extensive preclinical data support the clinical application of ATR/CHK1 inhibitors for targeted cancer therapy with or without combination with radiotherapy and chemotherapy. However, compelling results from clinical trials are lacking for most combinations. The clinical efficacy of ATR/CHK1 inhibitors as single agents remains unknown. One of the keys to improving the efficacy of ATR/CHK1 inhibitors is to identify the appropriate patient population to target and to develop more highly specific ATR/CHK1 inhibitors. New synthetic lethality interactions between ATR/CHK1 inhibitors and other inhibitors targeting DDR pathways need to be identified. The rapid progress of high throughput synthetic lethality screening and next generation sequencing should dramatically improve the ability to verify unknown targets. However, only a small portion of potential synthetic lethality situations has been confirmed. In addition, development of new generations of pharmacological inhibitors with improved bioavailability and substrate specificity are also critical for clinical implementation. Lastly, successful application of ATR/CHK1 inhibitors in clinic trials will also rely on optimized schedules to increase their efficacy and on the identification of biomarkers that are suitable for these agents. The results from ongoing and future clinical trials will help to elucidate the role of ATR/CHK1 inhibitors in cancer therapeutics.

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Abbreviations

RT	radiation therapy
ATR	ataxia-telangiectasia-mutated-and-Rad3-related kinase
ATM	ataxia-telangiectasia mutated
CHK1	checkpoint kinase 1
CHK2	checkpoint kinase 2
IR	ionizing radiation
DDR	DNA damage response
RS	replication stress
DSBs	DNA double-strand breaks
ssDNA	single-strand DNA

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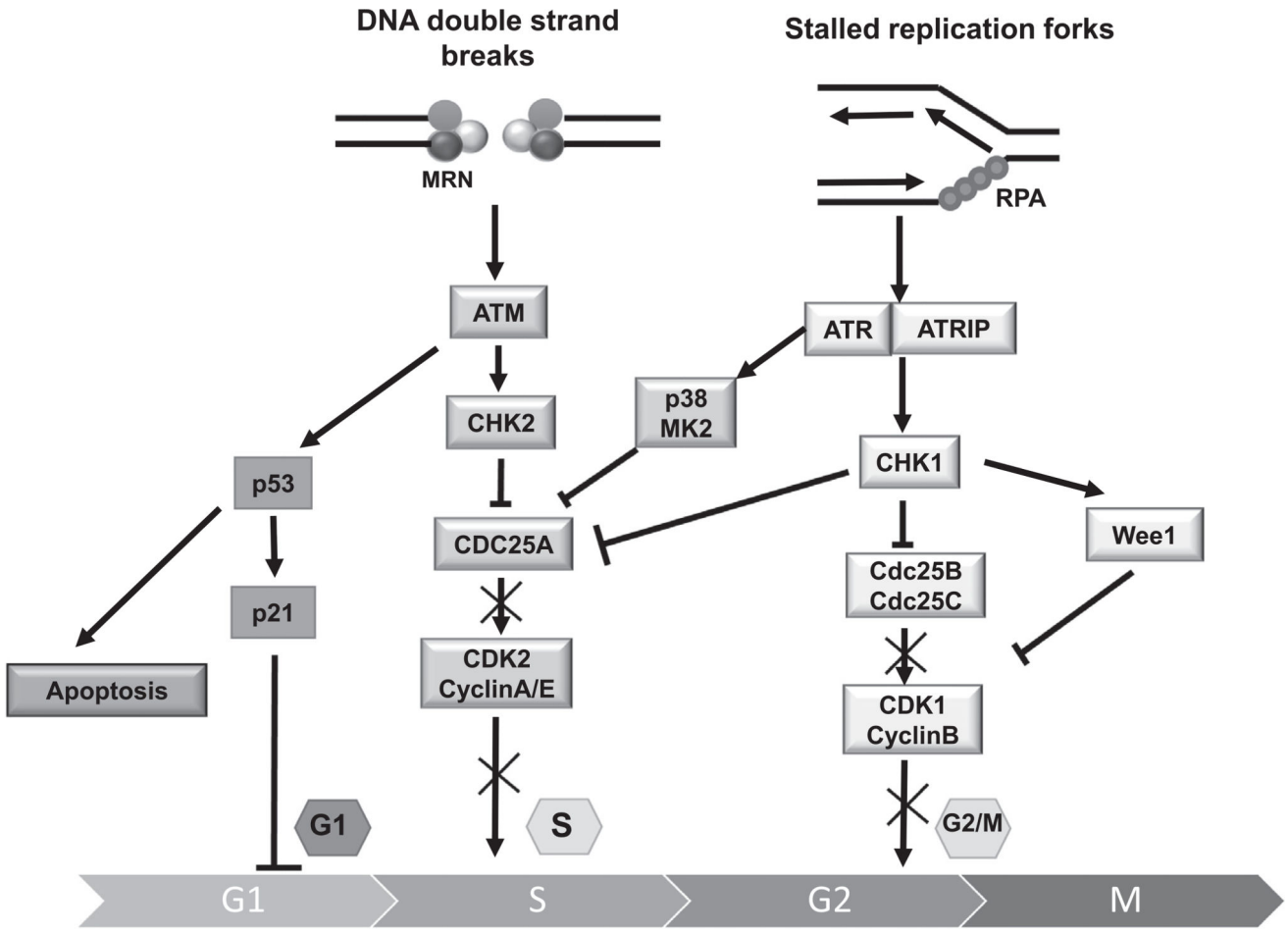

Fig. 1.

Overview of the DNA damage-induced checkpoint pathways. The ATM/CHK2 and ATR/CHK1 pathways are activated by DNA double-strand breaks or by DNA single-strand breaks and replication stress, respectively. Cell cycle checkpoints are induced primarily through p53, CHK2, CHK1 and p38/MK2, which are phosphorylated by ATM and ATR. Activated p53 leads to G1-phase arrest and induces apoptosis. The phosphorylation of CDC25 by CHK2 and CHK1 abolishes the activation of CDKs, thus stopping cell cycle progression either in S-phase or at the boundary of G2/M. Cancer cells deficient in p53 due to mutation or deletion lack the G1 checkpoint and are more dependent on the intra-S and G2/M checkpoints.

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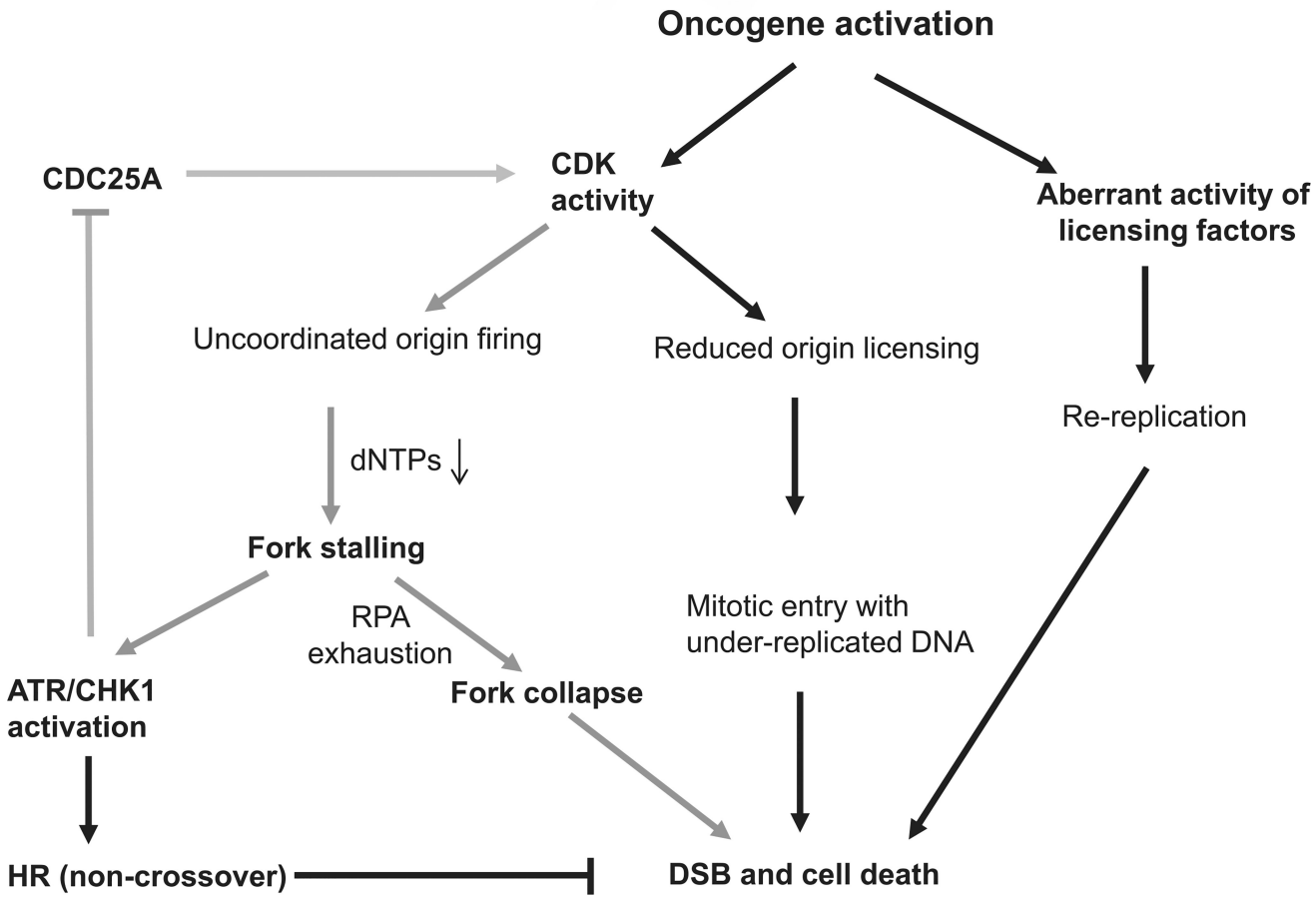

Fig. 2.

ATR/CHK1 suppresses oncogene-induced RS. Oncogene activation causes replication stress *via* uncoordinated origin firing, a reduction in licensed replication origins and inappropriate re-licensing of newly replicated DNA. Increased CDK activity leads to not only dysregulated replication initiation but also a deceased number of licensed replication origins, leading to under-replicated DNA. ATR/CHK1 signaling inhibits oncogene-induced replication stress *via* CDKs. Although it has been demonstrated that ATR/CHK1 suppresses replication stress *via* inhibition of CDK-induced replication initiation, it is not clear if ATR/CHK1 suppress CDK-mediated regulation of the number of licensed replication origins. CDK signaling suppressed by ATR/CHK1 is presented by gray arrows.

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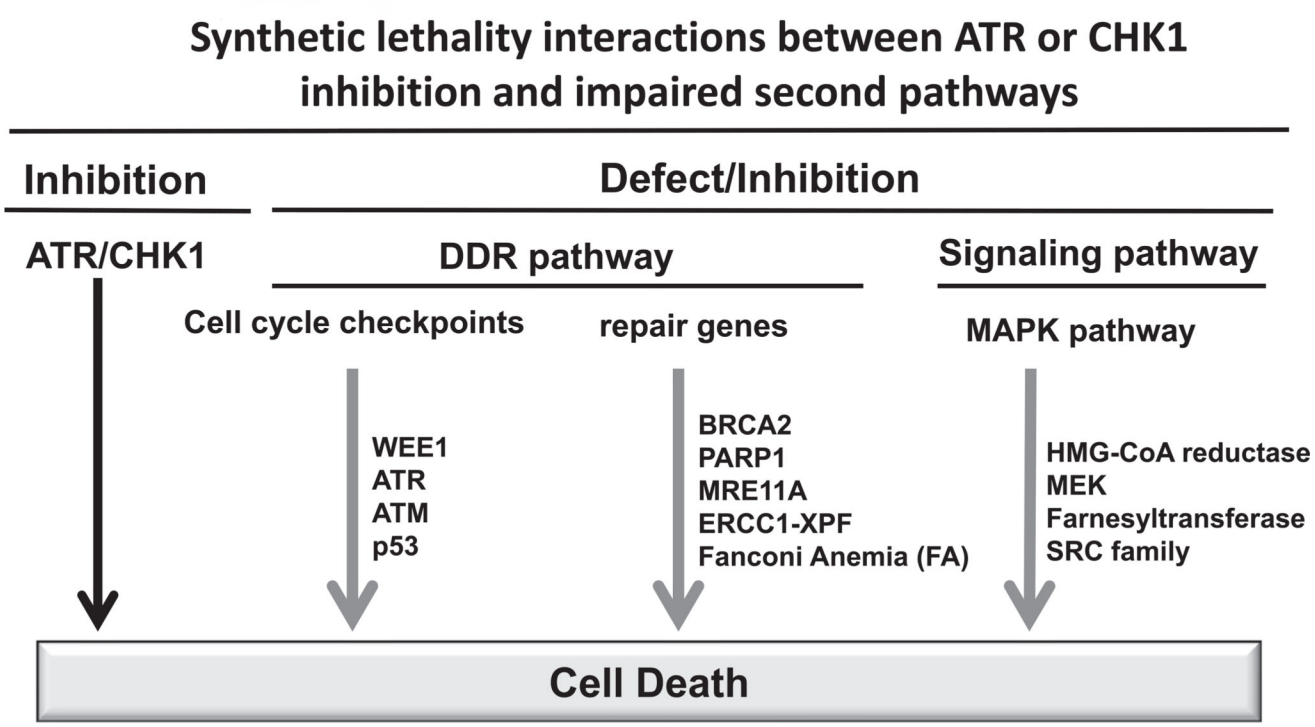

Fig. 3.

The potential synthetic lethality interactions with ATR/CHK1 inhibitions. See text for description. The defect in the second pathway is presented by gray arrows.

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Table 1

ATR/CHK1 inhibitors discussed in this review.

Agents	Targets	Preclinical research	Treatment	Clinical trials	Detail	References
UCN-01	CHK1	cellular/xenograft	Cisplatin	NCT00045513	Phase I/II combined with fludarabine phosphate	[40–44,62,136– 138,146,203,217]
	CHK2		Doxorubicin	NCT00006464	Phase I combined with cisplatin	
	CDK1		HDAC inhibitor	NCT00004059	Phase I combined with fluorouracil	
	CDK2			NCT00030888	Phase II single	
	MK2			NCT00072267	Phase II combined with topotecan hydrochloride	
	PKC			NCT00019838	Phase I combined with fludarabine phosphate	
				NCT00003289	Phase I single	
				NCT00042861	Phase I combined with multiple chemo drugs	
				NCT00047242	Phase I combined with irinotecan hydrochloride	
				NCT00045500	Phase I combined with prednisone	
				NCT00045747	Phase II combined with fluorouracil	
				NCT00045175	Phase I combined with topotecan hydrochloride	
				NCT00001444	Phase I single	
				NCT00072189	Phase II single	
				NCT00012194	Phase I combined with cisplatin	
				NCT00301938	Phase I combined with perifosine	
				NCT00098956	Phase II combined with topotecan hydrochloride	
				NCT00031681	Phase I combined with irinotecan hydrochloride	
				NCT00039403	Phase I combined with gemcitabine	
				NCT00004263	Phase I combined with cytarabine	
				NCT00082017	Phase II single	
				NCT00036777	Phase I combined with carboplatin	
XL844	CHK1	cellular/xenograft	Gemcitabine	NCT00475917	Phase I single/combined with gemcitabine	[45]
	CHK2			NCT00234481	Phase I single	
	VEGFR-2					
	VEGFR-3					
CBP501	CHK1	cellular/xenograft	CDDP	NCT00700336	Phase I/II combined with pemetrexed and cisplatin	[46–48]
	CHK2		Bleomycin	NCT00551512	Phase I combined with cisplatin	

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Agents	Targets	Preclinical research	Treatment	Clinical trials	Detail	References
	MAPKAP-K2		Cisplatin	NCT00942825	Phase II combined with cisplatin and pemetrexed	
	c-Tak1		Pemetrexed			
	PLK1					
AZD7762	CHK1	Cellular/xenograft	Nocodazole	NCT00937664	Phase I single/combined with gemcitabine	[49–59,65– 68,139,196,210,211]
	CHK2		Camptothecin	NCT00413686	Phase I single/combined with gemcitabine	
			Gemcitabine	NCT00473616	Phase I single/combined with irinotecan	
			Irinotecan			
			Gemcitabine			
			Radiation			
			Cisplatin			
			Temozolomide			
			HDAC inhibitor			
LY2603618	CHK1	Cellular/xenograft	Gemcitabine	NCT00415636	Phase I combined with pemetrexed	[69–74]
			Pemetrexed	NCT00839332	Phase I/II combined with gemcitabine	
			Cisplatin	NCT00988858	Phase II combined with pemetrexed	
				NCT01296568	Phase I single	
				NCT01139775	Phase I/II combined with gemcitabine or pemetrexed	
				NCT01341457	Phase I combined with gemcitabine	
				NCT01358968	Phase I single	
MK-8776	CHK1	Cellular/xenograft	Antimetabolites	NCT01870596	Phase II combined with cytarabine	[75–83,135,140,189,196]
			Gemcitabine	NCT00779584	Phase I/II combined with gemcitabine	
			Radiation	NCT00907517	Phase I combined with cytarabine	
			Pemetrexed	NCT01521299	Phase I combined with hydroxyurea	
			Cytarabine			
PF-00477736	CHK1	Cellular/xenograft	Gemcitabine	NCT00437203	Phase I combined with gemcitabine	[84–88,153,188]
			Carboplatin			
			Docetaxel			
			Radioimmunotherapy			
			Radiation			
LY2606368	CHK1	Cellular/xenograft	BMN673	NCT01115790	Phase I single	[89,90]
	CHK2			NCT02873975	Phase II single	

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Agents	Targets	Preclinical research	Treatment	Clinical trials	Detail	References
				NCT02203513	Phase II single	
				NCT02808650	Phase I single	
				NCT02514603	Phase I single	
				NCT02860780	Phase I combined with ralimetinib	
				NCT02555644	Phase I combined with radiation and multiple chemo drugs	
				NCT02735980	Phase II single	
				NCT02124148	Phase I combined with multiple chemo drugs	
				NCT02778126	Phase I single	
				NCT02649764	Phase I combined with fludarabine and cytarabine	
2e	CHK1	Cellular/xenograft	Doxorubicin			[91]
			Camptothecin			
CCT244747	CHK1	Cellular	Radiation			[92–94]
			Gemcitabine			
CHIR-124	CHK1	Cellular/xenograft	Radiation			[95–97]
			Top1 inhibitor			
			HDAC inhibitor			
GNE-783	CHK1	Cellular	Temozolomide			[98]
GNE-900	CHK1	Cellular/xenograft	Gemcitabine			[98]
			CPT-11			
			Temozolomide			
PD-321852	CHK1	Cellular	Gemcitabine			[99]
	Wee1					
PD-407824	CHK1	Cellular	Gemcitabine			[100]
	Wee1					
SAR-020106	CHK1	Cellular/xenograft	Radiation			[101–104]
			Radiochemo			
SB-218078	CHK1	Cellular	Doxorubicin			[62,105–107]
			HSP inhibitor			
S1181	CHK1	Cellular	Gemcitabine			[108]
V158411	CHK1	Cellular/xenograft	Irinotecan			[109]
	CHK2					

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Agents	Targets	Preclinical research	Treatment	Clinical trials	Detail	References
CH-1	CHK1 Aurora A	Cellular	Hypoxia			[181]
AR323	CHK1	Cellular				[154]
AR678	CHK1	Cellular				[154]
AR458323	CHK1	Cellular	MK-1775			[187]
Schisandrin B	ATR	Cellular	UV			[110,111]
NU6027	CDK2	Cellular	PARP inhibitor			[112,113,205]
	CDK1		Hydroxyurea			
	DNA-PK		Cisplatin			
	ATR					
NVP-BEZ235	PI3K	Cellular				[114]
	MTOR					
	ATR					
	ATM					
	DNA-PKcs					
Torin 2	MTOR	Cellular				[115]
	ATR					
	ATM					
	DNA-PKcs					
ETP-46464	MTOR	Cellular				[114]
	ATR					
	ATM					
	DNA-PKcs					
	PI3K					
Compound 45	ATR	Cellular	Cisplatin			[116]
VE-821	ATR	Cellular/xenograft	Radiation			[53,117– 125,158,192,199,205]
			Cisplatin			
			Topotecan			
			Veliparib			
VE-822 (VX-970)	ATR	Cellular/xenograft	Radiation	NCT02157792	Phase I combined with multiple chemo drugs	[124,126–128]
			Cisplatin	NCT02627443	Phase II combined with multiple chemo drugs	

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Agents	Targets	Preclinical research	Treatment	Clinical trials	Detail	References
				NCT02567409	Phase II combined with multiple chemo drugs	
				NCT02487095	Phase I/II combined with Topotecan	
				NCT02595892	Phase II combined with Gemcitabine	
				NCT02589522	Phase I combined with radiotherapy	
				NCT02567422	Phase I combined with Cisplatin	
				NCT02595931	Phase I combined with Irinotecan	
				NCT02723864	Phase I combined with Veliparib and Cisplatin	
AZ20	ATR	Cellular				[129]
AZD6738	ATR	Cellular/xenograft	Radiation	NCT02264678	Phase I/Ib combined with multiple chemo drugs	[130–133]
			Cisplatin	NCT02223923	Phase I single/combined with radiotherapy	
			Top1 inhibitor	NCT01955668	Phase I single	
				NCT02630199	Phase I combined with Paclitaxel	

Clinical trial data are from https://clinicaltrials.gov/.