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Death by releasing the breaks: CHK1 inhibitors as cancer therapeutics

Cynthia X. Ma¹, James W. Janetka², Helen Piwnica-Worms^{1,3,4,5}

¹Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110

²Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110

³Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110

⁴BRIGHT Institute, Washington University School of Medicine, St. Louis, MO 63110

⁵Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110

Abstract

Defects in p53 function, which occur frequently in human cancers due to mutations in *TP53* or disruptions in the p53 regulatory pathway, render cells dependent on CHK1 (Checkpoint Kinase 1) to activate cell cycle checkpoints. In the presence of DNA damage or replication stress, inhibition of CHK1 leads to “mitotic catastrophe” and cell death in p53-deficient tumors while sparing p53-proficient cells. CHK1 inhibitors sensitize tumors to a variety of DNA damaging agents or antimetabolites in preclinical models and are being evaluated in early phase clinical trials. In this review, we summarize recent advances and controversies in the development and application of CHK1 inhibitors as cancer therapeutics.

Keywords

checkpoints; DNA damage; p53

Corresponding author: Piwnica-Worms, H. (hpiwnica@wustl.edu).

Disclosures

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Synthetic lethal strategy for killing p53-deficient cancers: the concept and its evolution

In 1982, Lau and Pardee reported that caffeine treatment forced BHK (Baby Hamster Kidney) cells arrested in G2 (Gap 2) of the cell cycle with nitrogen mustard (HN2) to enter into a premature, lethal mitosis. The authors concluded that caffeine potentiated the lethality of HN2 by forcing cells with damaged DNA to undergo mitosis before properly repairing the DNA lesions [1]. Subsequent studies have demonstrated that caffeine could also drive S (Synthesis)-phase arrested cells into a lethal mitosis by inducing premature chromosome condensation [2]. It was later recognized that the ability of caffeine to induce bypass of the DNA damage and replication checkpoints was selective for p53-deficient cells [3–5]. This finding created great excitement because it suggested the possibility of employing a synthetic lethal strategy (see Glossary) to specifically target p53-deficient tumors in cancer patients (Figure 1). Unfortunately, caffeine cannot be administered at high enough doses to induce checkpoint bypass in the therapeutic setting.

UCN-01 (7-hydroxystaurosporine), a nonselective protein kinase inhibitor, was subsequently shown to potently induce S- and G2- checkpoint bypass in cells experiencing replication or genotoxic stress and this property is selective for p53-deficient cells or for cells with wild-type p53 but defective p53-signaling [6–9]. Key molecular targets of UCN-01 and caffeine were later identified as CHK1 and ATM (ataxia-telangiectasia mutated)/ATR (ATM and RAD3-related), respectively [10–13]. Although UCN-01 is a nonselective protein kinase inhibitor, lowering CHK1 levels with CHK1-specific small interfering RNAs induces bypass of both the S- and G2-checkpoints in p53-deficient cells thereby phenocopying the checkpoint effects of UCN-01 treatment [14]. These results argue that the ability of UCN-01 to induce checkpoint bypass results from CHK1 inhibition.

The CHK1 pathway

ATM, ATR and CHK1 are key components of cell cycle checkpoints (Figure 2 and Box 1) that become engaged when cells experience replication stress (ATR/CHK1) or when DNA double strand breaks are generated (ATM/ATR/CHK1) [15]. CHK1 is phosphorylated by ATR under these conditions and signals to the cell cycle machinery to arrest cells in the S- and G2-phases of the cell division cycle. The cell division cycle (CDC)25A protein phosphatase is a key downstream effector of CHK1 [14, 16]. CHK1 negatively regulates CDC25A by promoting its ubiquitin-mediated proteolysis and by preventing it from interacting with and activating the cyclin dependent protein kinases (CDKs) at inappropriate times during the cell division cycle [14, 16–18]. Thus, by eliminating CDC25A, cells can temporarily arrest progression through the cell division cycle while they repair DNA damage. Although not required to initiate the S- and G2-checkpoints, p53 reinforces these checkpoints through the transcriptional activation of downstream targets including p21 and 14-3-3s [19, 20]. Thus, not only do p53-deficient cells lack a G1 checkpoint, they are also impaired in their ability to sustain S- and G2-checkpoints. This makes p53-deficient cells particularly vulnerable to agents such as caffeine and UCN-01 that abrogate the S- and G2-checkpoints (Figure 1).

Deregulation of CHK1 in human cancers

Hereditary *CHK1* mutations have not been identified in cancer predisposition syndromes [21]. This is in contrast to several other checkpoint-pathway components such as *TP53*, *CHK2*, *BRCA1* and *ATM*, whose mutation gives rise to familial cancer syndromes. However, low levels of CHK1 expression and deletions of the distal end of chromosome 11q, including 11q24 containing the *CHK1* locus, frequently accompany amplification of the Cyclin D1 locus at 11q13 and contribute to tamoxifen resistance in estrogen receptor positive (ER+) breast cancers [22]. Mice disrupted for *Chk1* die during early development [23, 24] and conditional deletion of *Chk1* in proliferating mouse mammary epithelial cells (MECs) induces apoptosis and developmental defects. Importantly, haploinsufficiency is observed in mice heterozygous for *Chk1* [25–28]. For example, MECs heterozygous for *Chk1* inappropriately enter S-phase, accumulate DNA damage during replication and prematurely enter mitosis [28]. Thus, low levels of CHK1 could contribute to the evolution of human cancers by promoting genome instability.

By contrast, high levels of CHK1 mRNA and/or protein have also been reported in certain human tumors, including colorectal cancers and triple negative breast cancers (negative for ER, progesterone receptor and HER2 overexpression) [29–31]. Both transcriptional and post-transcriptional mechanisms could contribute to CHK1 overproduction in these cancers. For example, as an E2F1 target gene, transcription of CHK1 can be upregulated in human cancers where E2F1 is overproduced [29–31]. In addition, CHK1 protein levels are regulated by ubiquitin-mediated proteolysis by two distinct E3 ligase complexes, which include DDB1 (DNA-damage binding protein)/CUL4 (cullin 4) and FBX6 (F box protein 6)/CUL complexes [32, 33]. An inverse correlation between levels of CHK1 and FBX6 has been observed in several cultured cancer cell lines and breast tumor tissues, suggesting that the failure to appropriately degrade CHK1 could account for the high levels of CHK1 in some cancers [33]. The inability to downregulate CHK1 may provide a selective advantage to cancer cells by conferring resistance to anticancer therapy [34, 35].

Clinical translation of CHK1 inhibitors

UCN-01, a nonselective CHK1 inhibitor, has been tested in phase I trials in patients with cancer either as a single agent or in combination with a variety of chemotherapy agents including irinotecan [36, 37], topotecan [38, 39], cisplatin [40, 41], carboplatin [42], 5-fluorouracil [43] or cytarabine [44]. Results indicate UCN-01 has disappointing pharmacokinetic profiles and has produced limited antitumor outcomes. In contrast to predictions from the preclinical pharmacokinetic data, UCN-01 had a small volume of distribution, low systemic clearance and a prolonged half-life of elimination owing to its high binding affinity to α 1-acid glycoprotein (AAG) in human plasma [36, 37, 41, 45]. Assessment of the pharmacodynamic (PD) effects of UCN-01 on tumor cells was performed only for a limited number of patients. Perez et al. (2006) collected tumor biopsies pre- and post-therapy from three patients in a phase I study of cisplatin in combination with UCN-01 [41]. Biopsies were analyzed by immunohistochemistry (IHC) for geminin, an S- and G2-phase marker [46]. Geminin levels increased after cisplatin treatment consistent with an S- and G2- cell cycle arrest and decreased after UCN-01 treatment consistent with checkpoint

bypass [41]. In the study testing the combination of cytarabine and UCN-01 in patients with acute myeloid leukemia, decreased CHK1 phosphorylation, inhibition of the AKT pathway (possibly owing to inhibition of PDK1 (3-phosphoinositide-dependent protein kinase 1) and activation of JNK (Janus kinase) were observed during the course of therapy [44]. In addition to confirming CHK1 target inhibition, these results also demonstrated good tumor bioavailability and PD effect of UCN-01.

Results of a phase I study combining irinotecan with UCN-01 in patients with resistant solid tumor malignancies was recently reported [36]. Serial biopsies from a small cohort of patients were evaluated for p53 status and response to therapy. A significant decrease in phosphorylated ribosomal protein S6 (pS6) staining in tumor specimens post-treatment was observed. γ H2AX (Histone 2AX) staining revealed significantly more DNA double strand breaks in tumor samples following the combination treatment compared to baseline. Interestingly, two patients with triple negative breast cancers responded to therapy and both of their tumors had defective p53. One patient with ER+ breast cancer progressed after one cycle of therapy and her tumor was wild type for *TP53*. These results are consistent with the preclinical findings that UCN-01 works synergistically with DNA damaging agents specifically in *TP53* mutated tumors.

As a result of its extended half-life of several weeks, the dose of UCN-01 is generally reduced after the first cycle and the administration is delayed relative to the chemotherapeutic agent [36]. Levels of phospho-ribosomal protein S6 (pS6) were measured in peripheral blood mononuclear cells (PBMCs) collected at various time points post UCN-01 administration to determine the bioavailability and PD effect of UCN-01. As expected, we observed a reduced level of pS6 in PBMCs collected 24 hrs post UCN-01 therapy, due to the inhibitory effect of UCN-01 on PDK1. However, at 7 days post UCN-01 therapy, the level of pS6 in PBMCs had returned to baseline. These data indicate that UCN-01 is bioavailable and displays a measurable PD effect for at least 24 hrs after the first dose but not 7 days later when the second dose of irinotecan was given. Thus, the delayed scheduling of UCN-01 relative to the DNA damaging agent has likely been suboptimal for tumor chemosensitization in the trials conducted to date.

Newer generation selective CHK1 inhibitors

Unfavorable pharmacokinetics and untoward toxicities have hindered further clinical development of UCN-01. In addition to CHK1, UCN-01 is also a potent inhibitor of several additional protein kinases including PKC (Protein kinase C), PDK1, CDK1 and CDK2. In the past several years, many next generation ATP-competitive inhibitors (Table 1) with improved selectivity for CHK1, such as AZD7762, PF477736, and SCH900776, have been developed. These newer inhibitors are showing promise in preclinical models and are currently being evaluated in phase I clinical trials [47].

AZD7762

AZD7762 is a potent and selective urea-based CHK1/CHK2 inhibitor discovered at AstraZeneca (Waltham, MA, USA) by medicinal chemistry optimization of a high-throughput screening hit [48]. AZD7762 inhibits CHK1 kinase activity with a half maximal

inhibitory concentration (IC₅₀) of 5 nM (K_i or inhibitor binding affinity of 3.6 nM). This inhibitor is equally potent against CHK2 but has good enzyme selectivity (>10-fold) against more than 100 kinases tested, including CDK1 (>1000-fold), other CDKs, PKC isoforms, p38, and MAPKAP (Mitogen-activated protein kinase-activated protein) kinase 2 (>100-fold). By contrast, AZD7762 has <10-fold selectivity against some members of its parent CAMK (Ca²⁺/calmodulin-dependent kinase) family and SRC-like kinases [YES, FYN, LYN, HCK (hematopoietic cell kinase), LCK (lymphocyte-specific cell kinase)] although not SRC.

AZD7762 enhances the antitumor activity and abrogates S and/or G2 phase checkpoints mediated by both antimetabolites and DNA damaging agents, including gemcitabine, irinotecan (SN-38), topotecan, and ionizing radiation (IR) in preclinical models of various p53-deficient tumor types [48–50]. In a recent report, AZD7762 sensitized the pancreatic cell line MiaPaCa-2 to the combination of IR and gemcitabine *in vivo* [50]. Knockdown of p53 in the colorectal cancer cell line HCT116 resulted in a marked potentiation of clonogenic loss following treatment with gemcitabine and AZD7762 [48], consistent with the overall hypothesis that checkpoint inhibitors specifically enhance the cytotoxicity of DNA-damaging agents in p53-deficient tumors.

Three phase I dose escalation studies are being conducted to assess the safety, tolerability and pharmacokinetics of AZD7762 when administered as a single intravenous (IV) agent and in combination with a weekly standard dose of gemcitabine (Study D1040C00002 and Study D1040C00008) or with irinotecan (Study D1040C00004) in patients with advanced solid tumor malignancies [47]. The results of these trials have not yet been reported.

PF477736

PF477736 is a selective and potent diazepinoindolone CHK1 inhibitor identified at Agouron (Pfizer) (LaJolla, CA, USA) that inhibits CHK1 with a K_i of 0.49 nM. PF477736 also significantly inhibits CHK2 (K_i = 47 nM) although the selectivity ratio for CHK1 versus CHK2 is 100-fold [51]. Evaluation of the inhibitor across a panel of over 100 receptor tyrosine and serine/threonine protein kinases revealed seven kinases that were inhibited by PF477736 with <100-fold selectivity. These included VEGFR2 (Vascular endothelial growth factor receptor 2), Aurora-A, FGFR3 (Fibroblast growth factor receptor 3), FLT3 (FMS-like tyrosine kinase 3), FMS (CSF1R), RET and YES.

PF477736 abrogates the induction of cell cycle checkpoints and potentiates the activity of several DNA damaging agents including gemcitabine, irinotecan, carboplatin, doxorubicin, and mitomycin C, across a broad spectrum of p53-deficient human cancer (colon, breast, prostate, and leukemia) preclinical models [51]. In addition, PF477736 significantly enhances the antitumor effects of docetaxel, an antimicrotubule agent, in colon cancer (COLO205) and breast cancer (MDA-MB-231) xenograft models [52]. Cotreatment of PF477736 with docetaxel abrogated cell cycle arrest at M-phase and potentiated tumor cell apoptosis, suggesting the ability of PF477736 to override the spindle assembly checkpoint.

Preliminary data from the phase I trial of gemcitabine in combination with PF477736 was reported at the 2010 ASCO (American Society of Clinical Oncology) meeting [53].

PF477736 was administered as a 3 hour (hrs) or 24 hrs infusion on days one and eight of cycle 0, and on days two and nine following gemcitabine administered on days one and eight of subsequent 21-day cycles. Dose limiting toxicities (DLT) included a grade 4 increase in lipase and grade 4 neutropenia at 65 mg of PF477736 (3 hrs infusion); a grade 4 neutropenia with grade 3 thrombocytopenia and the sudden death of one patient treated at the higher dose level (340 mg) of PF477736 (24 hrs infusion). A maximum tolerated dose (MTD) was not identified for PF477736 administered over 3 hrs. The MTD for PF477736 infused over 24 hrs was identified as 270 mg in combination with gemcitabine 750 mg/m². Across all cohorts combined, common drug-related adverse events were pyrexia, fatigue, neutropenia, nausea, vomiting, and diarrhea. Partial responses were observed in patients with squamous cell carcinomas of the skin (n=2), non-small cell lung cancer (n=1) and mesothelioma (n=1). An additional nine patients exhibited stable disease (median 101 days). On the basis of this study, PF477736 infused over 24 hrs was selected for further development. However, as of September 2010, Pfizer has discontinued further development of PF477736.

SCH900776

SCH900776 is a potent ATP-competitive inhibitor of CHK1 (IC₅₀=3 nM) to move into the clinic. SCH900776, a Schering-Plough (Merck, Rahway, NJ, USA) compound, shows the best reported selectivity for CHK1 to date and is effective at abrogating both the S- and G2 - checkpoints caused by IR or various DNA alkylating agents [54]. In contrast to AZD7762 and PF477736, SCH900776 does not substantially inhibit CHK2 (IC₅₀=1.5 μM). However, it does inhibit CDK2 (IC₅₀=160 nM). The latter off-target activity might reduce the overall effectiveness of SCH900776, depending on dosing and scheduling, because the inhibition of CDK2 could induce cell cycle arrest and prevent checkpoint bypass in response to CHK1 inhibition.

A phase I dose-escalation study of SCH900776 in combination with gemcitabine in subjects with advanced solid tumors was reported at the 2010 ASCO meeting [55]; escalation was achieved for gemcitabine (800 mg/m²) followed by SCH900776 (112 mg/m²) at the maximally administered doses defined by the protocol on days one and eight every 21 days. DLTs included supraventricular tachycardia with atrial fibrillation or atrial fibrillation and grade 4 thrombocytopenia. Clinical activity was noted in five subjects: one patient with melanoma had a partial response; one patient with spindle cell sarcoma and one patient with cholangiocarcinoma had prolonged stable disease, and two patients with pancreatic cancer previously treated with gemcitabine had stable disease. The exposure threshold for preclinical activity (>0.5 μM C_{max}-peak drug concentration) and PD evidence of target engagement were achieved in the first cohort of SCH900776 dosed at 10 mg/m². Part B of the phase I study, with further dose escalation of SCH900776 in combination with gemcitabine (1000 mg/m²), is ongoing to define the recommended phase II treatment dosing [47].

CHK1 inhibitors in combination with IR as a therapeutic strategy

CHK1 inhibitors and CHK1 knockdown enhance the cytotoxicity of IR in various cancer cell lines *in vitro* and xenograft models *in vivo* [9, 49, 50, 56, 57]. Morgan et al (2010)

monitored the ability of the selective CHK1 inhibitor AZD7762 to further enhance the gemcitabine-mediated radiosensitization in pancreatic cell lines and xenografts [50]. Despite the positive results reported in these studies, CHK1 inhibitors have not been tested as radiosensitizers in cancer patients. As discussed above the clinical trials conducted to date have combined CHK1 inhibitors with drugs that induce replication stress (to activate the ATR pathway) rather than DNA double strand breaks (to activate the ATM pathway). This is likely due to the fact that double strand breaks have to be processed to single strand DNA bound to RPA (Replication Factor A) in order to activate the ATR/CHK1 pathway. This is in contrast to drugs that induce replication stress and immediately and robustly activate the ATR/CHK1 pathway (Figure 2).

Outstanding issues in the development of CHK1 inhibitors

CHK1 or CHK2?

The CHK1 inhibitors described above all potently inhibit CHK1 but have varying degrees of activity against CHK2. Although AZD7762 is equipotent against CHK2, PF477736 is 100-fold more selective for CHK1, and SCH900776 is 500-fold more selective for CHK1 than CHK2. Clinically effective doses of either AZD7762 or PF477736 are likely to potently inhibit both CHK1 and CHK2. By contrast, drug levels of SCH900776 required for PD activity targeting CHK1 are not expected to reach levels that would inhibit CHK2 to any significant degree. Thus, it is fair to ask which of the observed preclinical and clinical responses observed with AZD7762 and PF477736 result from CHK1 inhibition as opposed to CHK2 inhibition (Box 2). Knockdown of *CHK1* in the presence of endogenous CHK2 is sufficient to abrogate S- and G2-checkpoints in cells with DNA damage [14]. By contrast, *CHK2* knockdown does not induce checkpoint bypass nor does *CHK2* knockdown synergize with *CHK1* knockdown to potentiate checkpoint bypass [56, 58, 59]. Knockdown of *CHK2* does not sensitize cells to either radiation or gemcitabine and inhibition of CHK2 by VRX0466617, a potent and selective CHK2 inhibitor, does not synergize with either doxorubicin or cisplatin [60]. In pancreatic cancer cells, knockdown of *CHK1* but not *CHK2* increases sensitivity to 5-Fluoro-2'-deoxyuridine (FdUrd) and gemcitabine [59] as well as IR [50]. These data strongly argue that CHK1 inhibition and not CHK2 inhibition is responsible for the antitumor effects observed with these inhibitors. It will be interesting to perform parallel comparisons of the newer generation CHK1 inhibitors in preclinical models in order to elucidate the importance of CHK1 selectivity to clinical outcome.

Development of PD biomarkers and predictors of tumor response to CHK1 inhibitors

To ascertain target inhibition, assessment of PD biomarkers on serially collected tumor and surrogate tissues is an important task during the early phase clinical development of CHK1 inhibitors. In this regard, IHC analysis of phosphorylated histone H3 (a marker of mitotic entry), Geminin (a marker of cells in S- and G2- phases), γ H2AX (a marker for double strand DNA breaks), cleaved caspase 3 or TUNEL stain (Terminal deoxynucleotidyl transferase dUTP nick end labeling; markers for apoptosis) as well as phosphorylated CDK1 and CHK1 are well established assays. In response to replication stress or DNA damage, ATR phosphorylates CHK1 on serine (S) 317 and 345 [61]. Phosphorylation of CHK1 on these carboxy-terminal residues activates CHK1. Paradoxically, CHK1 phosphorylation

increases at these sites in cells treated with either nonselective (UCN-01, Gö6976) or selective (AZD7762) CHK1 inhibitors [32, 50, 62, 63]. This results from perturbations in the positive feedback loop that exists between CHK1 and PP2A (Protein phosphatase 2A) [64] and from ATR activation in cells exposed to CHK1 inhibitors during more prolonged incubation periods [63]. In contrast to the other CHK1 inhibitors, PF477736 treatment causes a decrease in CHK1 S345 phosphorylation [65]. The reason for this difference is not clear but may suggest that PF477736 affects the CHK1/PP2A regulatory circuit. Assessment of CHK1 activation (by DNA damaging agent or antimetabolite) and CHK1 inhibition (by CHK1 inhibitor) is probably best accomplished by monitoring phosphorylation of S296, the CHK1 autophosphorylation site [50].

A major challenge in validating target specificity and mechanism of action during clinical trials is the difficulty in obtaining serial biopsies from patients with lesions that are not easily accessible. The development of noninvasive imaging modalities to monitor tumor response to novel targeted therapies, including CHK1 inhibitors, is clearly needed. McArthur et al. (2006) used the Positron Emission Tomography (PET)-tracer, 18F-fluorine-L-thymidine (FLT), to noninvasively monitor the effects of gemcitabine followed by PF477736 in nude mice harboring prostate cancer (PC-3) xenografts [66]. An increase in FLT uptake into the tumor (FLT-flare), indicative of S-phase arrest, was measured 24 hrs after gemcitabine-treatment. Subsequent treatment with the CHK1 inhibitor abrogated the FLT-flare, suggestive of checkpoint bypass. The functional imaging results were correlated with several *ex-vivo* biomarkers. The results indicate that functional imaging with FLT-PET is a promising strategy for monitoring responses to therapeutic agents that target cell cycle checkpoints and its inclusion in clinical trials assessing CHK1 inhibitors should be considered.

Long-term effects of CHK1 inhibition in normal cells and tissues

A key function of CHK1 is to monitor the integrity of the replication fork during every cell division cycle even in the absence of exogenously introduced DNA damage [14, 67]. In cells exposed to compounds that stall replication forks, CHK1 activity is necessary to prevent late origin firing and irreversible replication fork collapse [67]. CHK1 phosphorylates several substrates to regulate cell cycle progression [CDC25s, NEK11 (NIMA-related kinase 11), B56delta subunit of PP2A, Retinoblastoma (Rb)]; checkpoint signaling [Claspin, MDM4/MDMX]; DNA repair [RAD51, FANCD2 (Fanconi anemia group D2), FANCE (Fanconi anemia group E)]; and chromatin dynamics [Histone H3, TLK1 (Tousled-like 1)] [68–71]. Genomic integrity depends on the ability of CHK1 to impact these cellular processes. As such, long-term or repeated exposure of “normal” cells and tissues to CHK1 inhibition needs to be considered and it remains to be seen what the long-term effects will be in cancer patients treated with CHK1 inhibitors.

Although the newer generation CHK1 inhibitors are indeed more selective than UCN-01 for CHK1, none are exclusively selective for the CHK1 protein kinase. Furthermore, similar to other ATP-competitive kinase inhibitors, CHK1 inhibitors mimic the structure of the protein kinase cofactor ATP, so it is possible that they might also inhibit other ATP-utilizing enzymes and receptors besides protein kinases. It is well established that protein kinase

inhibitors can cause serious toxicities such as cardiotoxicity [72–74]. Although these effects might be partly explained by off-target kinase inhibitory properties [73], it is also plausible that secondary pharmacology resulting from the ability of CHK1 inhibitors to affect other nonkinase ATP-dependent proteins, could contribute to unwanted side effects. Innovative design of drugs that target more selective allosteric sites on CHK1 with inhibitors not competitive with ATP is a new and promising strategy for further increasing selectivity towards CHK1, reducing secondary pharmacology and improving the safety of next generation CHK1 inhibitors [75, 76].

Mechanisms of tumor cell death induced by CHK1 inhibitors

It has been proposed that the simultaneous loss of the G1-, S- and G2- checkpoints in tumor cells with DNA damage forces them into a premature lethal mitosis. However, checkpoint abrogation has never been formally established as the cause of tumor cell death. In fact, the loss of two checkpoints does not significantly affect cell viability [77]. Importantly, CHK1 interacts with and phosphorylates RAD51 to regulate homologous recombination (HR) repair [78]. Thus, CHK1 inhibition impairs HR repair and this may also contribute to the effectiveness of combining agents that induce DNA double strand breaks with CHK1 inhibitors [50]. PARP (poly(ADP-ribose) polymerase) inhibitors have shown great promise in treating tumors deficient in HR repair, such as BRCA1 (Breast cancer type 1 susceptibility protein)-deficient tumors [79–81]. One might consider future clinical trials that test the synthetic lethal relationship between CHK1 loss (CHK1 inhibitor) and base excision repair loss (PARP inhibitor). CHK1 inhibition synergizes with ATM loss or cisplatin in cells with Fanconi anemia DNA repair defects [82] and cells deficient in BRCA2, XRCC3 (X-ray repair cross complementing protein 3) or DNA-PK are sensitized to the combination of gemcitabine and AZD7762 [83]. These studies lend credence to the concept of using CHK1 inhibitors to treat DNA repair deficient tumors. There is also data indicating that CHK1 inhibition can sensitize tumor cells to antimicrotubule agents such as taxol [84] and therapies combining antimetabolites with CHK1 inhibitors warrant preclinical testing.

Correlation between p53 status and tumor response

There are conflicting reports regarding whether p53 status correlates with tumor response in studies that have combined DNA damage with CHK1 inhibition. Some studies have demonstrated that p53-proficient tumors are killed by this combination [58, 85–87], whereas a large number of studies report preferential killing of p53-deficient cells (reviewed in [7]). Many of these discrepancies can be explained if one takes into account dosing schedules and the integrity of the p53 pathway in the cell lines being treated. Loss of viability is observed when cells knocked down for *CHK1* are exposed to antimetabolites to activate the S-phase checkpoint and then released from the S-phase block. This response is independent of p53 status [58]. Thus, p53-proficient tumors could potentially be targeted by concurrent administration of an antimetabolite and a CHK1 inhibitor. The differential response of p53 proficient- versus p53 deficient-tumors to combination therapy is most readily observed when cells treated with a DNA damaging agent are given time to arrest at the G2 checkpoint and then exposed to CHK1 inhibitors. Under these conditions only p53-deficient cells are observed to enter into a lethal mitosis [9]. These findings are important to consider when designing clinical trials involving CHK1 inhibitors. Another important point is that the p53

pathway can be inactivated by genetic or epigenetic events that occur upstream or downstream of p53. Thus, the integrity of the p53 pathway needs to be assessed in order for CHK1 inhibitors to be optimally administered to patients and for correlations to be drawn between p53 functional status and tumor response.

Concluding remarks

A new generation of more selective CHK1 inhibitors are currently undergoing clinical evaluation in combination with agents that either induce DNA damage (i.e. Topoisomerase I inhibitors) or interfere with DNA replication (antimetabolites) with the goal of enhancing the cytotoxicity of these agents. Although originally developed to selectively target p53-deficient tumors, CHK1 inhibitors may be equally effective in killing tumors with an intact p53 pathway. Activating the S-phase checkpoint with antimetabolites while simultaneously inhibiting CHK1 has shown good efficacy in preclinical models. CHK1 inhibitors are also showing promise in tumors with DNA repair deficiencies. Thus, CHK1 inhibitors may be useful in treating a wide variety of tumors that differ in their tissue and cell type of origin. Determining the molecular profile of individual tumors in order to select appropriate patient populations and performing correlative studies to validate target specificity will be the key to the successful development and application of these exciting, new generation potent and selective CHK1 inhibitors.

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Glossary Box

Synthetic lethal strategy

Cancer researchers borrowed the term “synthetic lethality” from classical genetics to describe situations where a cancer mutation (in this case, p53 loss) and a drug (in this case, DNA damaging agent combined with a CHK1 inhibitor) together cause the tumor cell’s death. (“Synthetic” is used in the sense of “synthesis,” or coming together). Neither the mutation nor the drug alone is capable of killing the tumor but rather it is the combination that kills the tumor cells [93]

Immunohistochemistry

A procedure used to detect the expression of an antigen (e.g. protein) in cells of a tissue section using specific antibodies.

IC₅₀ (Half maximal inhibitory concentration)

The concentration of a compound required to reach 50% of the maximum inhibitory effect in an assay of biological function. A lower number signifies a more effective inhibitor (or antagonist) of the activity being assayed.

K_i (inhibitor binding constant)

Describes the affinity or dissociation constant of an enzyme-inhibitor complex. A lower value of K_i indicates a greater binding affinity and more potent inhibitor of enzyme function.

m²

in this article, refers to the unit of body surface area that is calculated based on height and weight.

C_{max}

A pharmacokinetic term describing the peak blood plasma concentration of a drug.

Clearance

A pharmacokinetic term representing the volume of plasma cleared of the drug per unit time.

Half-life

A pharmacokinetic term indicating the time required for half of the original drug concentration to be cleared from the plasma.

Pharmacodynamic (PD)

A pharmacology term generally referring to the physiological consequence(s) of a drug administered to a living organism. A pharmacodynamic result is defined as a biological or phenotypic effect(s) (either desired or undesired) as a consequence of drug-receptor and/or other drug-target interactions.

Pharmacokinetics (PK)

A pharmacology term generally referring to the fate of a drug when administered to a living organism. Pharmacokinetic properties include absorption, distribution, metabolism, and excretion (ADME) of the drug.

Fanconi Anemia (FA)

An inherited genomic instability disorder, caused by mutations in genes regulating replication-dependent removal of interstrand DNA crosslinks. FA patients are hypersensitive to DNA damaging agents that create DNA interstrand crosslinks such as mitomycin C. The FA pathway coordinates several distinct repair pathways, including nucleotide excision repair (NER), translesion synthesis (TLS), and homologous recombination (HR), in order to remove interstrand crosslinks [94].

Base excision repair

A type of DNA repair whereby an altered base is removed by a DNA glycosylase enzyme, followed by excision of the resulting sugar phosphate. The small gap left in the DNA helix is filled in by the sequential action of DNA polymerase and DNA ligase.

Double-strand break repair

DNA double-strand breaks are repaired by two types of repair mechanisms. One type takes advantage of proteins that promote homologous recombination (HR) to obtain instructions from the sister or homologous chromosome for proper repair of breaks. The other type called non-homologous end joining (NHEJ) permits ligation of ends even if there is no sequence similarity between them.

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Box 1.**Cell cycle checkpoints and relevance to cancer therapy**

- Cell cycle checkpoints are signal transduction pathways that become activated when replication forks stall in S-phase (DNA replication checkpoint), when DNA is damaged in G1-, S-, or G2-phase (DNA damage checkpoint) or when microtubules fail to attach to kinetochores in mitosis (spindle assembly checkpoint) [89].
- p53 is essential for activating the G1 checkpoint and reinforces the S- and G2-checkpoints [90, 91].
- CHK1 regulates the S- and G2-checkpoints but not the G1-checkpoint [14, 16, 84].
- Cancer cells deficient for p53 function rely on CHK1 to activate the S- and G2-checkpoints in response to stalled replication forks or DNA damage [14, 16, 84].
- Treating p53-deficient tumors with agents that induce replication- or genotoxic-stress followed by a CHK1 inhibitor causes tumor cells to undergo a specialized form of cell death known as mitotic catastrophe and in some cases apoptosis [92].

Box 2.**Outstanding questions**

- Is inhibition of CHK1, CHK2 or both responsible for observed antitumor effects of CHK1 inhibitors?
- What is the best way to validate target specificity and mechanism of action of CHK1 inhibitor in clinical trials?
- What are the best antimetabolites or DNA damaging agents to combine with selective CHK1 inhibitors and what are the optimal scheduling parameters?
- What are the long-term effects of CHK1 inhibition in cancer patients?
- Does p53 status determine response of tumor cells to therapies that combine CHK1 inhibitor with DNA damaging agents or antimetabolites?

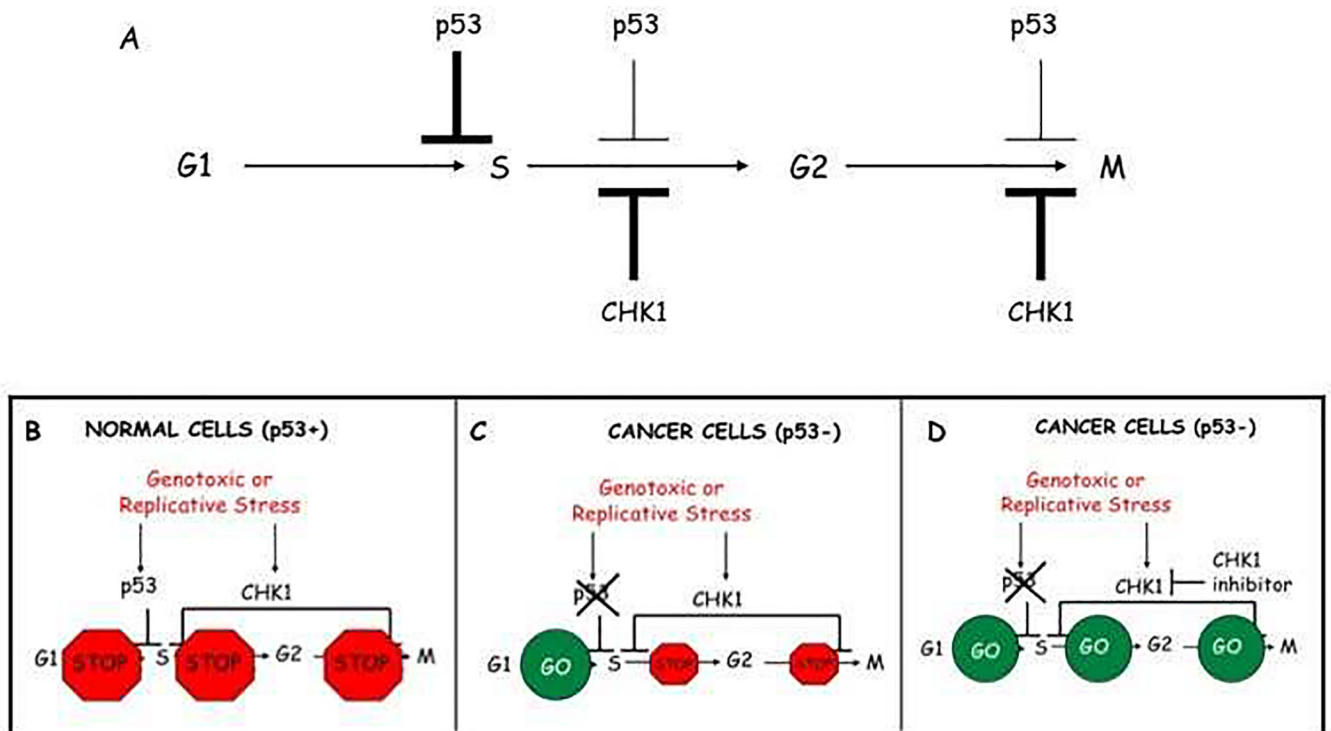


Figure 1. Cell cycle checkpoints

The eukaryotic cell cycle consists of four phases called G1 (Gap 1), S (Synthesis), G2 (Gap 2) and M (Mitosis). Genotoxic and replicative stress activate checkpoints in order to delay cells from transitioning from one cell cycle phase to the next. p53 is required for cells to stop at the G1/S-border (G1 checkpoint) whereas CHK1 is required to prevent new replication origins from firing in S-phase (S-phase checkpoint) and to prevent cells from exiting G2 and entering into M-phase (G2 checkpoint). Although cells are able to activate the S- and G2- checkpoints in the absence of p53, they are unable to sustain these checkpoints for as long as normal cells (A, B). Most cancer cells lack a functional p53 pathway and therefore are unable to arrest in G1 when their DNA is damaged, but they are able to activate the S- and G2-checkpoints through the CHK1 pathway. This gives the tumor cells time to repair any DNA damage and this promotes their survival (C). When p53 deficient cancer cells are subjected to genotoxic or replicative stress in combination with a CHK1 inhibitor they lose all three checkpoints, and progress through the cell cycle without repairing their DNA damage. This results in preferential killing of p53-deficient tumor cells (D).

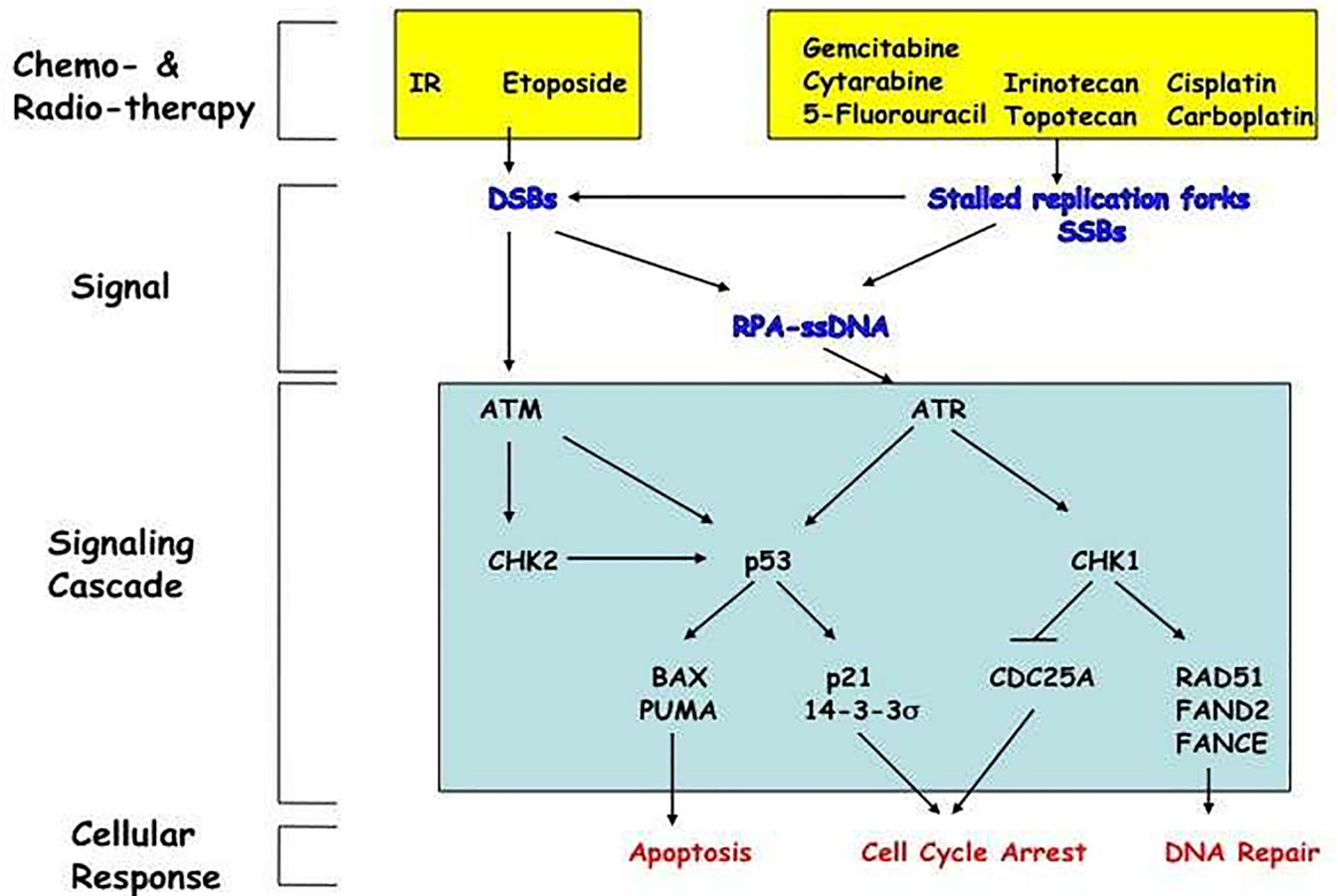


Figure 2. DNA damage response pathway

Exposure of cells to IR or etoposide induces double strand breaks (DSBs) in DNA whereas exposure of cells to various chemotherapeutic agents (irinotecan, topotecan, cisplatin, carboplatin) or antimetabolites (gemcitabine, 5-fluorouracil, cytarabine) results in replication fork stalling and the generation of single strand breaks (SSBs). This, in turn, activates checkpoints that mobilize DNA repair pathways and either signals to the cell cycle machinery to prevent progression or induces apoptosis. ssDNA becomes coated with replication protein A (RPA), which recruits ATR as well as additional proteins thereby leading to full ATR activation. ATR phosphorylates many intracellular substrates including p53 and CHK1. ATR phosphorylates CHK1 on serines 317 and 345 resulting in CHK1 autophosphorylation on serine 296. Activated CHK1, in turn, phosphorylates the Cdc25A protein phosphatase to promote its ubiquitin-mediated proteolysis. Loss of Cdc25A results in cell cycle arrest in the S- and G₂-phases of the cell division cycle. CHK1 also phosphorylates RAD51, FANCD2 and FANCD3 to activate DNA repair pathways. DSBs activate ATM, which in turn phosphorylates both CHK2 and p53. p53 is also phosphorylated by CHK2. This leads to p53 accumulation and activation of its downstream target genes. Transcriptional activation of genes encoding BAX and PUMA lead to apoptosis whereas transcriptional activation of genes encoding p21 and 14-3-3s lead to G₁ cell cycle arrest and also function to enforce the S- and G₂-cell cycle arrests regulated by CHK1. Crosstalk exists between these pathways as stalled replication forks can lead to DSBs leading to ATM

activation and the repair of DSBs can produce RPA-coated ssDNA that activates that ATR pathway [88].

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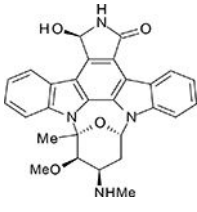
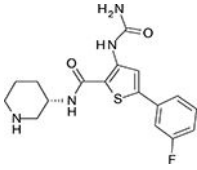
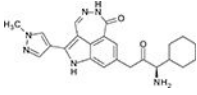
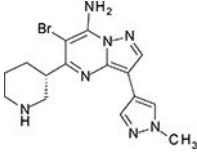
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Table 1.

Structure and kinase selectivity of clinically advanced CHK1 inhibitors.

Inhibitor	Structure	CHK1 IC ₅₀ (nM)	CHK2 IC ₅₀ (nM)	CDK1 IC ₅₀ (nM)	CDK2 IC ₅₀ (nM)	PKC IC ₅₀ (nM)
UCN-01		11	1040	31	30	7
AZD7762		5	<10	>5000	>500	>500
PF477736		0.49	47	9900	-	-
SCH900776		3	1500	-	160	-