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New Insights into Checkpoint Kinase 1 (Chk1) in the DNA Damage Response (DDR) Signaling Network: Rationale for Employing Chk1 Inhibitors in Cancer Therapeutics

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Abstract

The DNA damage response (DDR) represents a complex network of multiple signaling pathways involving cell cycle checkpoints, DNA repair, transcriptional programs, and apoptosis, through which cells maintain genomic integrity following various endogenous (metabolic) or environmental stresses. In cancer treatment, the DDR occurs in response to various genotoxic insults by diverse cytotoxic agents and radiation, representing an important mechanism limiting chemo- and radio-therapeutic efficacy. This has prompted the development of agents targeting DDR signaling pathways, particularly checkpoint kinase 1 (Chk1), which contributes to all currently defined cell cycle checkpoints, including G1/S, intra-S phase, G2/M, and the mitotic spindle checkpoint. While numerous agents have been developed with the primary goal of enhancing the activity of DNA-damaging agents or radiation, the therapeutic outcome of this strategy remains to be determined. Recently, new insights into DDR signaling pathways support the notion that Chk1 represents a core component central to the entire DDR, including, in addition to checkpoint regulation, direct involvement in DNA repair and apoptotic events. Together, these new insights into the role of Chk1 in the DDR machinery could provide an opportunity for novel approaches to the development of Chk1 inhibitor strategies.

Background

The DNA damage response (DDR) represents a signaling network involving multiple pathways including checkpoints, DNA repair, transcriptional regulation, and apoptosis(1). Various endogenous/metabolic (e.g., reactive oxygen species/ROS, stalled replication forks) or environmental insults (e.g., UV, ionizing radiation, genotoxic agents(2)) cause DNA damage (e.g., single strand breaks/SSBs, double strand breaks/DSBs, chemical adducts, mismatches) (3). When damage occurs, distinct, albeit overlapping and cooperating checkpoint pathways are activated, which block S-phase entry (the G1/S-phase checkpoint), delay S-phase progression (the intra-S or S-phase checkpoint), or prevent mitotic entry (the G2/M-phase checkpoint)(4). These events direct phase-specific repair mechanisms (e.g., base excision repair/BER, nucleotide excision repair/NER, mismatch repair/MMR, or DSB [double-strand break] repair including homologous recombination/HR and non-homologous end joining/NHEJ) through repair-specific gene transcription. For example, DSBs are repaired predominantly via NHEJ in G1-phase, but via HR in S- and G2-phases(3). If repair fails, checkpoints trigger p53-dependent or -independent apoptosis. Thus, checkpoints represent central orchestrators of the DDR network ranging from damage sensing to repair or apoptosis.

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Significantly, checkpoints are characteristically defective in transformed cells(5). This review summarizes recent insights into checkpoint signaling pathways, focusing on checkpoint kinase 1 (Chk1), and opportunities to exploit alternative strategies for Chk1 inhibitor development.

Checkpoint signaling cascades

Checkpoint signaling pathways are classified as sensors, mediators, transducers, and effectors (2). Following DNA damage, “**sensor**” multiprotein complexes (e.g., Mre11-Rad50-Nbs1/MRN complex for ATM; Rad17 and Rad9-Rad1-Hus1/9-1-1 complex for ATR) recognize damage, and recruit “**proximal transducers**” (ATM and ATR) to lesions where they are initially activated. ATM and ATR transduce signals to “**distal transducer**” checkpoint kinases (i.e., Chk1 and Chk2). Generally, ATM activates Chk2, while ATR primarily activates Chk1, although considerable cross-talk between ATM and ATR occurs. MAPKAP kinase 2 (MK2), a downstream target of the stress-response p38 MAPK pathway, may represent third “distal transducer” (“Chk3”?).

ATM/ATR activation and ATM/ATR-mediated phosphorylation of “sensors” recruit and phosphorylate “**mediators**” (e.g., 53BP1, BRAC1, MDC1, SMC1, FANCD2, Claspin, TopBP1, Timeless, Tipin, and H2A.X etc.). Once activated, these “mediators” remain at the site of damage, while Chk1/Chk2 are released to activate soluble targets. “Mediator” activation facilitates ATM/ATR-induced Chk1/Chk2 activation.

Activated “distal transducers” phosphorylate and promote degradation or sequestration of “**effector**” Cdc25s (e.g., Cdc25A, B, and C), specialized phosphatases that activate cyclin-dependent kinases (e.g., Cdk1/cdc2 and Cdk2) through inhibitory site (Tyr15 and Thr14) dephosphorylation. Chk1/Chk2 and ATM/ATR also phosphorylate the “**effector**” p53, increasing its stability. Cdc25 inactivation and p53 accumulation halt cell cycle progression at specific phases.

Chk1 activation upstream signals

Whereas Chk2 activation is largely restricted to DSBs (e.g., by IR) via ATM, Chk1 is activated by a diverse stimuli (e.g., UV, replication stresses, DNA-damaging agents) via both ATR and ATM. Generally, Chk1 activation is initiated by single strand DNA (ssDNA) breaks.

Stalled replication forks(6)—The genome is particularly vulnerable during DNA replication. In S phase, endogenous/exogenous insults hinder replication fork progression, resulting in stalled forks that are unstable and breakage-prone. When a fork encounters a lesion, DNA polymerase stalls while helicase unwinds DNA, generating a large stretch of ssDNA. ssDNA lesions are then coated by replication protein A (RPA), recruiting ATR-ATRIP (ATR-interacting protein) complexes via recognition and association of RPA-ssDNA by ATRIP. ATR/ATRIP activation requires Rad17/9-1-1 complex loading, which is also essential for ATR-mediated Chk1 activation.

Double strand breaks(7)—Following DSBs, MRN complexes interact with DSB lesions to recruit/activate ATM, leading to Chk2 activation. Meanwhile, MRN and ATM also mediate DSB resection, resulting in ssDNA formation as a DNA repair intermediate structure, which promotes slower activation of Chk1 via the RPA-ATR/ATRIP process.

Single strand breaks(1)—As above, RPA bound to ssDNA presenting at SSBs or gaps recruits Rad17/9-1-1 and ATR/ATRIP complexes, triggering Chk1 phosphorylation.

Current models for Chk1 activation mechanisms

Recruitment/activation of ATM/ATR and “sensor” proteins recruits Chk1/Chk2 at damage sites where the latter are activated. Chk1 and Chk2 are structurally unrelated kinases and activated through different processes. ATM predominantly phosphorylates Chk2 at Thr68, promoting homodimerization and activation via intramolecular trans-autophosphorylation at Thr383/387. In contrast, Chk1 activation does not require dimerization or trans-autophosphorylation. ATR (predominantly) or ATM (to a lesser extent) phosphorylates Chk1 at Ser317/345, directly leading to activation. Chk1 activation by ATR also requires 9-1-1 complex loading by the Rad17-RFC complex as well as several essential “mediators”. For example, **Claspin** directly binds to Chk1 and increases the stability of both. Claspin phosphorylation promotes **BRCA1** recruitment and phosphorylation, followed by recruitment of Chk1 to ATR. **TopBP1** directly activates ATR/ATRIP and promotes ATR-mediated Chk1 phosphorylation(8). **Timeless** (Tim/Tim1)(9) and **Tipin**(10) form stable complexes associated with chromatin via binding of Tipin to RPA, an event critical for chromatin association of Claspin and S317/345 phosphorylation of Chk1.

Currently, there are two models of Chk1 activation, a) Phosphorylations at the C-terminal residues (e.g., S317/S345) block intramolecular interactions, uncovering the N-terminal kinase domain(11); and b) S317/S345 phosphorylation results in release of Chk1 (inactive) from chromatin to accumulate at the centrosome where it prevents Cdk1 activation and mitotic entry (12). Notably, whereas S345 is essential for kinase activation and function, S317 plays only a contributory role(13;14). Moreover, different phosphorylation sites also play disparate roles in “essential” cell survival (S345) or “non-essential” checkpoint activation (S317) functions.

Chk1 in DNA damage checkpoints

DNA damage checkpoints are generally mediated by two pathways(15;16): the ATM/ATR-Chk1/Chk2-Cdc25s pathway (for fast, reversible responses), and the p53-dependent pathway (for slower, irreversible responses). Whereas Chk1 is the key “distal transducer” in the former, Chk1/Chk2, along with ATM/ATR, phosphorylate either p53 or its ligase Mdm2, promoting p53 stabilization. Moreover, these “transducers” also phosphorylate multiple other “effectors” involved in checkpoints (e.g., FancD2, SMC1, Rad9, Rad17, and Plk3), as well as other DDR mechanisms e.g., transcriptional regulation (e.g., E2F1, BRAC1, p53), DNA repair (e.g., Nbs1, Artemis, H2A.X, BLM1, BRAC1, p53), apoptosis (e.g., p53, Mdm2, E2F1, Che1, and Pml1), and chromatin remodeling (e.g., Tlk1/2). A model summarizing the diverse roles of Chk1 in the DDR is depicted in Figure 1.

S-phase checkpoint—At least two pathways are involved in the S-phase checkpoint, i.e., the ATM/ATR-Chk1/Chk2-Cdc25A-Cdk2 pathway and the Nbs1-dependent pathway that includes the ATM/Nbs1/Smc1 and the ATM/Nbs1/FANCD2 pathways(17). Chk1 activation via ATR plays a dominant role in response to replication stresses (the replication checkpoint). Chk1 is also required for amplification of DSB-initiated Cdc25A signaling mediated by ATM/Chk2. Moreover, Chk1 directly phosphorylates essential S-phase kinases (Cdc7 and Tlk1). Cdc7 phosphorylation/activation is required for initiation of DNA replication via the Mcm2-7 complex, which with Cdk2, mediates efficient loading of Cdc45 to replication origins(18). Tlk1 phosphorylation by Chk1 leads to inhibition of Tlk1 activity which is required for chromatin assembly. Furthermore, Chk1 activation impairs elongation during DNA replication and is required for inhibition of mRNA elongation of p53 target genes (e.g., p21) when DNA replication is blocked(19).

Chk1 has recently been implicated in translesion DNA synthesis (TLS) mediated by ubiquitinated PCNA(20). Whereas ATR/Chk1 is critical for stabilizing stressed replication forks, TLS allows replication forks to progress through certain DNA lesions. Both are

important for continuous replication of damaged DNA and avoidance of fork collapse. Chk1 is required for efficient PCNA ubiquitination mediated by the E2-E3 complex of Rad6 and Rad18.

G2/M-phase checkpoint(21)—Cdk1/cdc2 governs mitotic entry and exit. Cdk1/cdc2 activation involves Tyr15/Thr14 dephosphorylation, regulated by Wee1- and Myt1-mediated phosphorylation and Cdc25C-mediated dephosphorylation. Cdc25A may also be involved in Cdk1 dephosphorylation in the G2/M-phase checkpoint. Chk1 is a major kinase phosphorylating Cdc25A (Ser76/124) and Cdc25C (Ser216), leading to a) Cdc25A: ubiquitination (via ubiquitin ligases APC/C and Skp1/Cullin/F-box proteins/SCF) and proteasomal degradation; or b) Cdc25C: nuclear exclusion/cytoplasmic sequestration via binding to 14-3-3 proteins (Rad24 and Rad25). Chk1 also phosphorylates and stabilizes Wee1. Long-term Cdk1/cyclin B silencing for a sustained G2/M-phase checkpoint requires transcriptional induction of endogenous Cdk1 inhibitors (e.g., p21, Gadd45, and 14-3-3 σ) via p53-dependent or -independent (e.g., via BRAC1) mechanisms that also involve Chk1.

G1/S-phase checkpoint(22)—Cdk2-cyclin E/A is inactivated via Cdc25A-mediated dephosphorylation (Tyr15/Thr14; fast and reversible) or p53-induced p21 (slow/sustained), causing G1 arrest and preventing S-phase entry following DNA damage. Whereas these events are primarily mediated by Chk2, basal Chk1 activity is required for constitutive Cdc25A turnover in unperturbed cells.

Other Chk1 functions

Mitotic spindle checkpoint—The spindle checkpoint delays anaphase onset in cells with mitotic spindle defects. Following spindle toxin (e.g., taxol) exposure, Chk1 associates with kinetochores and is phosphorylated at non-canonical sites, thereby, phosphorylating Aurora-B and enhances its catalytic activity. This event in turn mediates phosphorylation and kinetochore localization of BubR1(23). Abrogation of Chk1 induces multiple mitotic defects and mislocalized Aurora B(24). In addition, Chk1 also negatively regulates another important mitotic substrate, Plk1(25).

DNA damage/repair—Chk1 is involved in DNA repair by targeting repair kinases (e.g., DNA-PK), which, together with Ku70-K80 (designed the DNA-PK complex), are important for DSB repair(26). Moreover, Chk1-dependent phosphorylation of Rad51 is required for DNA damage-induced homologous repair/HRR(27). Lastly, Chk1-mediated FANCE phosphorylation is critical for the FA (Fanconi Anemia)/BRCA-mediated DNA repair pathway (28;29). Conversely, abrogation of Chk1 by either inhibitors or siRNA causes ssDNA formation and DNA strand breaks(30).

Apoptosis—p53 is a central downstream checkpoint signaling protein responsible for apoptotic responses. However, ATR/Chk1 signaling is essential for suppression of a caspase-3-dependent apoptotic response following replication stress(31). Moreover, Chk1, but not Chk2, also blocks a caspase-2-dependent apoptotic response independently of p53, Bcl-2, and caspase-3(32). Interestingly, caspase-mediated Chk1 cleavage (Asp299/Asp351) promotes its activation(33), raising the possibility of unexplored, direct links between Chk1 and apoptotic signaling.

Transcription—Chk1 phosphorylates histone H3 (Thr11), responsible for DNA damage-induced transcriptional repression of cell cycle-regulatory genes (e.g., cyclin B1 and Cdk1) through loss of histone acetylation(34).

Clinical-translational advances

Chk1 vs Chk2 as anti-cancer targets

Proximal (i.e., ATM/ATR) and distal (i.e., Chk1/Chk2) “transducers” comprise the core of DDR signaling networks. Theoretically, inhibition of each could improve chemo- or radio-therapeutic efficacy. Currently, no ATR-specific inhibitor has been developed. ATM is a rational candidate, but ATM inhibitors (e.g., KU-55933 and KU-60019(35); Kudos) are at early preclinical stages of development. Therefore, whether targeting ATM, ATR, or both will be effective strategies remains to be determined. Despite similarities in substrate phosphorylation, Chk1 and Chk2 functions in cell survival and checkpoint regulation differ strikingly. Chk2 function is time- and cell type-dependent, and generally limited to DSB-induced checkpoints (by IR). Chk1 is involved in checkpoints induced by diverse stimuli (e.g., UV and numerous DNA-damaging agents), as well as DNA replication stresses (even in unperturbed cells). Thus, Chk1 is an extremely attractive target for multiple reasons e.g., a) Chk1 is associated with all checkpoints, e.g., G2/M, G1/S, S, and most recently, the mitotic spindle checkpoint; b) Chk1 is essential for maintenance of genomic integrity, whereas Chk2 is conditional; c) Chk2 function is to some extent replaceable by Chk1 (or other kinases), but the reverse is not true; d) Chk1 plays a central role in DNA replication checkpoints e.g., by exposure to agents (e.g., nucleoside analogs) that target replication; and e) Chk1 is involved in other critical functions (e.g., DNA repair and apoptotic inhibition). Therefore, Chk1 has been viewed as the “workhorse”, whereas Chk2 is the “amplifier” kinase(36). Consequently, Chk1 currently represents one of the most important targets for anti-cancer therapeutics directed at the DDR network.

Novel checkpoint abrogators

The clinical utility of UCN-01, the first Chk1 inhibitor evaluated in humans, is limited by its prolonged plasma half-life due to extensive plasma binding to α 1-acidic glycoprotein, and off-target actions (e.g., inhibition of multiple other kinases) resulting in toxicity (e.g., hyperglycemia)(37). These have prompted extensive efforts to develop a new generation of more specific and less toxic inhibitors targeting checkpoint kinases. However, as in the case of UCN-01, the major goal in developing these new agents continues to involve disrupting DNA damage checkpoint responses to genotoxic agents or radiation. Whether strategies combining newer checkpoint abrogators and cytotoxic agents will result in improved therapeutic activity or selectivity is currently the subject of intense interest. Nevertheless, numerous clinical trials involving checkpoint abrogators are ongoing based on this rationale. In such studies, phosphorylation of Chk1 (e.g., S345 or S296 [autophosphorylation]), histone H3 (e.g., Ser10), Cdc25C (e.g., Ser216), and histone H2A.X (Ser139, designated γ H2A.X) currently as serve as potential biomarker for Chk1 inhibition(38).

A brief summary of newer checkpoint abrogators, including those at early stages of clinical development (see Table 1), or at the preclinical development stage, follows below.

AZD7762 (AstraZeneca)—A potent, selective Chk1 inhibitor binds to the ATP-binding site of Chk1 and *in vitro* inhibits Chk1-mediated phosphorylation of Cdc25C peptide (IC₅₀, 5nM) (39). AZD7762 is equally potent against Chk2 *in vitro*. AZD7762 abrogates the S-phase checkpoint (via the Cdc25A/Cdk2 pathway) by gemcitabine or the G2/M-phase checkpoint (via the Cdc25C/Cdk1 pathway) by irinotecan (SN38), resulting in enhanced activity in solid tumors cell lines (particularly p53-mutant cells) and murine xenografts.

LY2603618 (Lilly)—This inhibitor binds to and blocks Chk1 activity, thereby potentiating the efficacy of various chemotherapeutic agents, possibly by interfering with DNA repair. Preclinical data involving LY2603618 has not been published.

CBP501 (CanBas)—A peptide corresponding to aa211-221 of Cdc25C inhibits Chk1 (IC₅₀, 3.4μM) and Chk2 (IC₅₀, 6.5μM) *in vitro*(40). CBP501 diminishes Cdc25C Ser216 phosphorylation, accompanied by Cdk1/cdc2 Tyr15 dephosphorylation and increased histone H3 Ser10 phosphorylation, leading to G2/M checkpoint abrogation and enhanced cytotoxicity of bleomycin or cisplatin (CDDP) *in vitro* and in murine xenografts.

PF-00477736 (Pfizer)—A selective, potent ATP-competitive Chk1 inhibitor, derived from PF-00394691, inhibits Chk1 (K_i, 0.49nM) and Chk2 (K_i, 47nM) *in vitro*. PF-00477736 abrogates both G2/M-phase (e.g., by camptothecin) and S-phase checkpoints (e.g., by gemcitabine)(41). The latter enhances gemcitabine cytotoxicity in p53-defective tumor cells and in murine xenografts. PF-00477736 also significantly enhances docetaxel efficacy *in vitro* and *in vivo*, in association with decreased Cdc25C cytoplasmic phosphorylation (Ser216) and histone H3 phosphorylation (Ser10)(42).

SCH-900776 (Schering-Plough)—This compound specifically binds to and inhibits Chk1, abrogating the S-phase or G2/M-phase checkpoints, thereby sensitizing tumor cell to IR and alkylating agents. These preclinical data have not yet been published.

XL844 (Exelixis)—A potent ATP-competitive inhibitor of Chk1 (K_i, 2.2nM) and Chk2 (K_i, 0.07nM)(43). XL844 blocks Cdc25A degradation, abrogates the S-phase checkpoints, increases DNA damage in response to gemcitabine, and potentiates gemcitabine activity *in vitro* and in xenografts.

CEP-3891 (Cephalon)—This specific Chk1 inhibitor, currently at the preclinical development stage, potently inhibits Chk1 (IC₅₀, 4nM) as well as other kinases, including TrkA (IC₅₀, 9nM), MLK1 (IC₅₀, 42nM), and VEGFR2 (IC₅₀, 164nM) *in vitro*. CEP-3891 abrogates S-phase and G2/M-phase checkpoints induced by IR(44). The former event is likely related to delayed IR-induced Cdc25A phosphorylation (Ser123, a residue critical for protein stability). CEP-3891 also accelerates IR-induced mitotic nuclear fragmentation stemming from defective chromosome segregation, accompanied by enhanced lethality(45).

CHIR-124 (Chiron)—This potent, selective Chk1 inhibitor, which occupies the ATP-binding site, inhibits Chk1 (IC₅₀, 0.3nM) 2,000-fold more potently than Chk2 (IC₅₀, 0.7μM). *In vitro*, CHIR-124 also potently targets other kinases such as PDGFR (IC₅₀, 6.6nM) and FLT3 (IC₅₀, 5.8nM). CHIR-124 interacts synergistically with topoisomerase I poisons (e.g., camptothecin) in p53-mutant tumor cells and in an orthotopic breast cancer xenograft(46). CHIR-124 also abrogates SN38-induced S-phase (by restoring Cdc25A) and G2/M-phase (via Cdc25C hyperphosphorylation) checkpoints, triggering apoptosis. In addition, CHIR-124 also sensitizes p53^{-/-} HCT116 cells to IR. CHIR-124 is currently in the preclinical development stage.

PD-321852 (Pfizer)—This compound catalytically inhibits Chk1, leading to Cdc25A stabilization and premature mitotic entry in response to gemcitabine. Inhibition of Chk1-mediated Rad51 responses to gemcitabine-induced replication stress also contributes to chemosensitization by PD-321852(47). PD321852 is currently in preclinical development.

MK-1775 (Merck)—This Wee1 inhibitor (IC₅₀, 5.2nM) potentiates the activity of DNA-damaging agents (e.g., gemcitabine, cisplatin, carboplatin) *in vitro* and *in vivo*, particularly in p53-negative cancers(48;49).

PD0166285 (Pfizer)—This potent, preclinical inhibitor of Wee1 (IC₅₀, 24nM) and Myt1 (IC₅₀, 72nM) inhibits Cdk1/cdc2 phosphorylation at inhibitory sites (i.e., Tyr15/Thr14),

independently of p53 status. PD0166285 abrogates IR-induced G2/M-phase checkpoints and enhances p53-dependent cell killing(50). In addition, PD0166285 also stabilizes microtubules and downregulates cyclin D(51).

17-AAG (Tanespimycin or KOS-953, Kosan)—Chk1, but not Chk2, is one of many client proteins of the molecular chaperone Hsp90. Exposure to the Hsp90 inhibitor 17-AAG downregulates Chk1(52), leading to Cdc25A stabilization and sensitization to gemcitabine, etoposide, and SN38 particularly in p53^{-/-} cells(53). In addition to multiple trials involving 17-AAG that focus on other client proteins, one ongoing clinical trial is based on Chk1 downregulation(54).

Alternative strategies: An example emphasizing the link between Chk1 inhibitors and the Ras/MEK/ERK survival pathway

A requirement for ERK1/2 (extracellular-regulated kinase 1/2) activation in progression across the G2-M boundary and through mitosis(55), as well as functional roles for MEK1/2 (MAP kinase kinase 1/2)/ERK1/2 signaling in DNA damage checkpoint(56) and repair responses (57) to genotoxic stresses, have been documented. We reported that UCN-01 markedly activated MEK1/2/ERK1/2 in malignant hematopoietic cells, while blockade of this event by MEK1/2 inhibitors strikingly induced apoptosis(58). Subsequently, it was shown that targeting Ras (e.g., by farnesyltransferase inhibitors/FTIs or HMG Co-A reductase inhibitors/statins) blocks UCN-01-induced ERK1/2 activation and dramatically increases lethality *in vitro* and *in vivo*(59;60). Analogous phenomena have also been reported in breast and prostate cancers, and with newer, clinically relevant Chk1 inhibitors (Dai and Grant, unpublished observations). Notably, whereas the activity of Chk1 inhibitor/DNA-damaging agent regimens is largely p53-dependent, Chk1/Ras/MEK1/2 inhibitor strategies act independently of p53 status. These findings suggest that combining Chk1 (or potentially ATM/ATR) inhibitors with agents that disrupt compensatory activation of the Ras/MEK/ERK signaling cascade, rather than DNA-damaging agents, may represent a novel treatment paradigm.

Future challenges for the Chk1 inhibitor field include a) exploiting rapidly emerging insights into DDR signaling networks, particularly those reflecting differences between normal and transformed cells; b) identifying intracellular signaling responses to DDR-targeting agents (e.g., Chk1 inhibitors), with the goal of inhibiting these responses to potentiate therapeutic activity; c) extending this strategy to include, in addition to DNA-damaging agents, newer survival signaling pathway antagonists; d) developing agents that interrupt more upstream targets within DDR signaling cascades (e.g., ATM, ATR, and “mediators”), which may circumvent intra-network compensatory responses to inhibition of single “distal transducer” like Chk1. Although much work clearly lies ahead, the future of this field appears promising.

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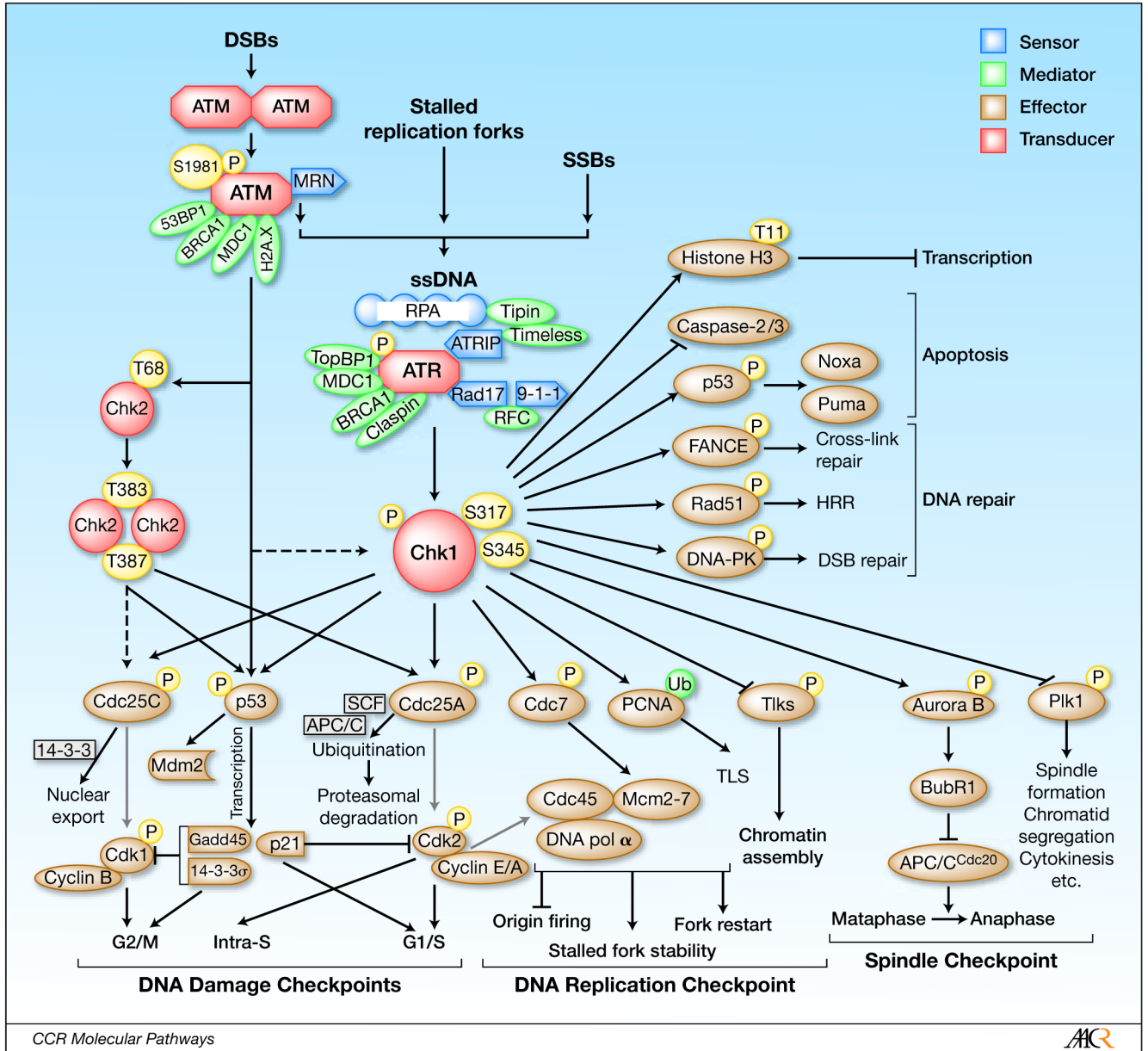


Figure 1. Chk1 in DDR signaling network

DNA damage (e.g., double strand breaks/DSBs, single-strand breaks/SSBs, and stalled replication forks) generates single-strand DNA (ssDNA) that initiates ATR-mediated Chk1 activation. In this context, the ATR/ATRIP complex is recruited to ssDNA lesions via binding of ATRIP with RPA that recognizes and coats ssDNA. In conjunction with recruited/activated “sensors” and “mediators”, ATR phosphorylates Chk1 at two canonical sites (Ser345 and S317), directly leading to its activation without the homodimerization and intramolecular trans-autophosphorylation that is required for Chk2 activation. Activated Chk1 then phosphorylates diverse downstream “effectors”, which in turn are involved in cell cycle checkpoints (i.e., intra-S-, G2/M-, and G1/S-phase checkpoints), the DNA replication checkpoint, the mitotic spindle checkpoint, as well as DNA repair, apoptosis, and transcription. Consequently, Chk1 is a kinase central for the DDR signaling network, thereby representing a particularly attractive target in anti-cancer therapeutics.

Table 1Checkpoint abrogators in clinical trials (<http://www.cancer.gov>; <http://www.clinicaltrial.gov>)

Compound	Target	Agents in combination	Cancer types	Status (number of trials)
UCN-01	Chk1, other kinases	monotherapy	refractory systemic anaplastic large cell and mature T-cell lymphoma	Phase II, active
		irinotecan	resistant solid tumors or locally recurrent or metastatic triple-negative breast cancer	Phase I, active
		perifosine	relapsed or refractory acute leukemia, CML, high risk MDS	Phase I, active
		monotherapy	advanced or metastatic renal cell carcinoma	Phase II, closed
		monotherapy	metastatic melanoma	Phase II, closed
		monotherapy	refractory solid tumors or lymphoma	Phase I, closed
		monotherapy	advanced solid tumors and chronic lymphoproliferative disorder	Phase I, completed
		fludarabine	relapsed or refractory CLL or small lymphocytic lymphoma	Phase I and II, closed
		fludarabine	recurrent or refractory low-grade or indolent lymphoid malignancies	Phase I, completed
		fluorouracil	gemcitabine-refractory metastatic pancreatic cancer	Phase II, completed
		fluorouracil	advanced or refractory solid tumors	Phase I, completed
		fluorouracil + leucovorin (triple)	metastatic or unresectable solid tumors	Phase I, completed
		topotecan	recurrent, persistent, or progressive advanced ovarian cancers	Phase I and II, completed
		topotecan	relapsed or progressed small cell lung cancer platinum-based chemotherapy	Phase II, closed
		cytarabine	refractory or relapsed AML or MDS	Phase I, closed
		cisplatin	advanced solid tumors	Phase I(2), completed
		carboplatin	advanced solid tumors	Phase I, completed
gemcitabine	unresectable or metastatic adenocarcinoma of the pancreas	Phase I, completed		
prednisone	refractory solid tumors or lymphomas	Phase I, closed		
AZD7762	Chk1, Chk2	gemcitabine	advanced solid malignancies	Phase I (2), active
		irinotecan	advanced solid malignancies	Phase I, active
LY2603618	Chk2	gemcitabine	pancreatic cancer	Phase I, active
		pemetrexed	metastatic non-small cell lung cancer	Phase I, active
CBT501	Chk1, Chk2	cisplatin	solid tumors	Phase I, active (1)/closed (1)
		cisplatin	malignant pleural mesothelioma	Phase II, active
		pemetrexed + cisplatin (triple)	non-small cell lung cancer	Phase II, active
PF-00477736	Chk1>Chk2	gemcitabine	advanced solid tumors	Phase I, active
SCH 900776	Chk1	gemcitabine	solid tumors or lymphoma	Phase I, active
		cytarabine	acute leukemia	Phase I, active

Compound	Target	Agents in combination	Cancer types	Status (number of trials)
XL844	Chk1, Chk2	gemcitabine	advanced malignancies(solid tumor and lymphoma)	Phase I, closed
MK-1775	Wee1	monotherapy gemcitabine cisplatin carboplatin	advanced solid tumors	Phase I, active
7-AAD	Hsp90, Chk1	irinotecan	solid tumors	Phase I, active