Cell cycle kinases as therapeutic targets for cancer

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Abstract | Several families of protein kinases orchestrate the complex events that drive the cell cycle, and their activity is frequently deregulated in hyperproliferative cancer cells. Although several molecules that inhibit cell cycle kinases have been developed and clinically screened as potential anticancer agents, none of these has been approved for commercial use and an effective strategy to specifically control malignant cell proliferation has yet to be established. However, recent genetic and biochemical studies have provided information about the requirement for certain cell cycle kinases by specific tumours and specialized tissue types. Here, we discuss the potential and limitations of established cell cycle kinases as targets in anticancer drug discovery as well as novel strategies for the design of new agents.

Cyclins
A family of proteins that are involved in cell cycle progression. They are transiently expressed in response to growth signals in order to regulate the timely activation of cyclin-dependent kinases.

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Within the human genome, ~300 genes have been found to be mutated in cancer (Catalogue of Somatic Mutations in Cancer at the Wellcome Trust Sanger Institute; see Further information), and many more exhibit altered levels or patterns of expression. Such changes contribute to deregulation of cell cycle kinases, which is often associated with aberrant division and uncontrolled proliferation of cancer cells. Since the elucidation of the mechanisms of mammalian cell division in the 1970s and 1980s by the work of Lee Hartwell, Paul Nurse and Tim Hunt[1], gene products that regulate the key cell cycle machinery have been investigated as cancer drug targets. These include the cyclin-dependent kinases (CDKs), which have been established as master regulators of cell proliferation, and more recently identified potential targets, such as protein kinases that coordinate the cellular response to DNA damage and protein kinases that regulate mitosis (FIG. 1).

The search for synthetic inhibitors of protein kinases as anticancer drugs has been invigorated by the successful approval of a number of molecules that target tyrosine kinases, such as the BCR–ABL protein kinase inhibitor imatinib (Gleevec; Novartis) for the treatment of chronic myelogenous leukaemia. Moreover, a growing body of structural information about the cell cycle protein kinases, mainly CDKs in complex with their regulatory subunits and synthetic inhibitors, has facilitated the development of potential therapeutic compounds. However, the exploitation of cell cycle protein kinase inhibitors in clinical oncology has not yet achieved proof of concept, as no such molecule has been approved as an anticancer drug. Future approaches should therefore combine the lessons learned from the early work using small-molecule inhibitors with the recent increased understanding of the deregulation of cell cycle protein kinases in cancer.

Protein kinases in the mammalian cell cycle

During the mammalian cell cycle, progression through the first gap (G1) phase and initiation of the DNA synthesis (S) phase is cooperatively regulated by several cyclins and their associated CDKs, which integrate the flow of information from outside the cell, including growth factor signalling and the availability of nutrients. When cells in the quiescent (G0) phase enter the cycle, CDK4 and CDK6 form active complexes with D-type cyclins (D1, D2 and D3) and initiate phosphorylation of the retinoblastoma protein (RB1; also known as p105-RB) and possibly other members of the 'pocket' protein family[2,3] (BOX 1), which inactivates their function as transcriptional repressors. In late G1, active CDK2–cyclin E heterodimers reinforce RB1 phosphorylation on additional sites, in a feedforward action to irreversibly initiate the gene expression programme of the S phase. This stage, called the restriction point, is crucial in cancer as alterations in the key regulatory players in the G1 to S phase transition could allow cells to proliferate independently of mitogenic stimuli (BOX 2). Beyond the restriction point, RB1 is maintained in a hyperphosphorylated state by the sequential activities of cyclin A–CDK2, cyclin A–CDK1 and cyclin B–CDK1 complexes, thereby ensuring cell cycle progression. However, in the case of DNA damage, the

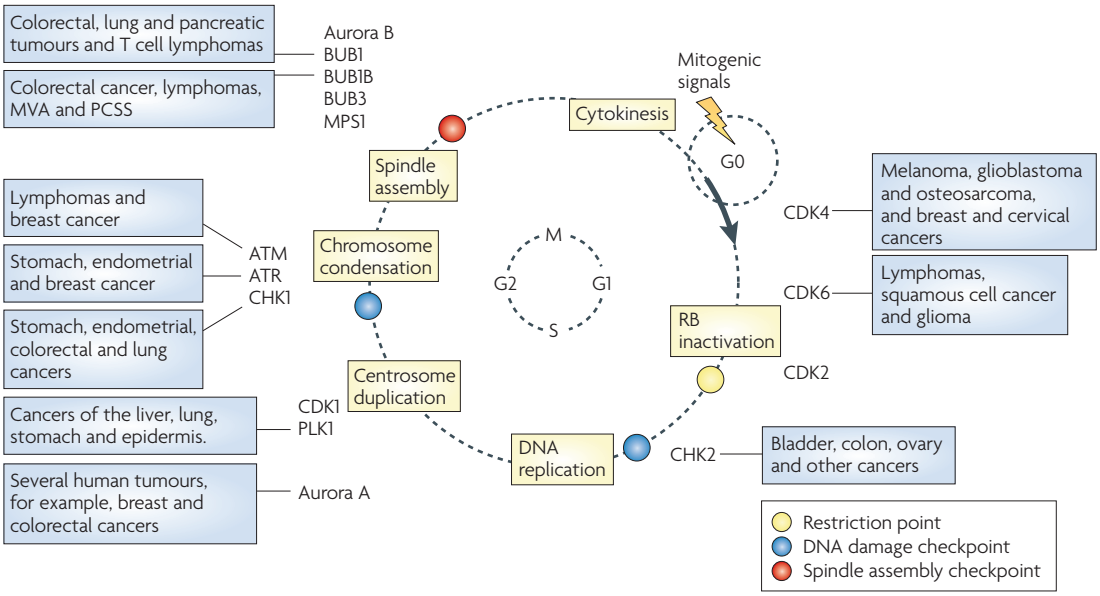


Figure 1 | **Cell cycle regulation by protein kinases of potential interest in cancer therapy.** Interphase cyclin-dependent kinases (CDKs) drive the cell out of quiescence (G0) and beyond the restriction point, resulting in the cell being irreversibly committed to the DNA synthesis (S) phase transition. The DNA-damage checkpoint kinases (CHKs) act as sensor proteins that can induce cell cycle arrest in the first gap (G1) and G1–S phases in response to DNA lesions. CDK1, CDK2, polo-like kinase 1 (PLK1) and aurora A are involved in the regulation of the centrosome cycle, whereas kinases that are involved in the spindle assembly checkpoint pathway ensure proper DNA segregation during mitosis (M) phase. Different kinases may act at several stages of the cell cycle and modulate the activities of other cell-cycle-related kinases. Cancers associated with genetic alteration of specific kinases are indicated in blue boxes. ATM, ataxia telangiectasia mutated (also known as serine protein kinase ATM); ATR, ataxia telangiectasia and RAD3-related protein (also known as serine–threonine protein kinase ATR); BUB1, budding uninhibited by benzimidazoles 1; BUB1B, BUB1 homologue beta (also known as BUBR1); MPS1, monopolar spindle 1; MVA, mosaic variegated aneuploidy; PCSS, premature chromatid separation syndrome; RB, retinoblastoma protein family members.

Cell cycle checkpoints
A series of surveillance pathways which ensure that cells pass accurate copies of their genome on to the next generation.

Centrosome
The main microtubule-organizing centre of the cell. It is formed by two centrioles, which are cylindrical structures made of bundles of microtubules.

Microtubule
A hollow tube made of polymers of α- and β-tubulin subunits.

Anaphase-promoting complex or cyclosome
A multi-subunit E3 ubiquitin ligase that targets key regulators of mitosis, such as cyclin B, aurora kinases and polo-like kinases, for destruction through direct polyubiquitylation.

Kinetochore
A multi-protein structure positioned at the central constriction of each mitotic chromosome (centromere) at which spindle microtubules attach. Unattached kinetochores are the signals for activation of the surveillance mechanism known as the spindle assembly checkpoint.

Spindle midzone
Organized bundles of antiparallel microtubules that form in late mitosis and are thought to be important for signalling the location of cleavage of the plasma membrane.

Anaphase
The process by which sister chromatids move to opposite spindle poles.

cell cycle is rapidly arrested by activation of the related kinases ataxia telangiectasia mutated (ATM; also known as serine protein kinase ATM), ataxia telangiectasia and RAD3-related protein (ATR; also known as serine–threonine protein kinase ATR) and their downstream effectors — checkpoint kinase 1 (CHK1) and checkpoint kinase 2 (CHK2). This gives the cell time to attempt the repair of the DNA lesion. The cell cycle checkpoints — such as the DNA damage checkpoint— are designed to preserve genome integrity and constitute a protective barrier against cancer. However, mutations in genes that are involved in the ATM–ATR cascade may impair the DNA damage response, favouring the development of cancer (BOX 3).

In parallel with DNA replication, the centrosome cycle begins. Centrosomes duplicate during late S phase to early G2 phase and separate to form the poles of the mitotic spindle at the beginning of mitosis. They then begin a process called maturation in which each centrosome nucleates its own aster of dynamic microtubules emanating from both poles of the mitotic spindle. These processes are controlled by both CDK1 and CDK2, which might have overlapping functions[4], as well as other serine–threonine protein kinases, including aurora A and polo-like kinase 1 (PLK1)[5,6]. In complex with cyclin A, CDK1 is abruptly activated at the transition from the second gap (G2) phase to the mitosis (M) phase to facilitate the commencement of mitosis through regulation of chromosome condensation and microtubule dynamics[7]. Following nuclear envelope breakdown, cyclin A is degraded and newly formed cyclin B–CDK1 complexes are required for progression through the M phase. Ultimately, for complete division into two daughter cells, CDK1 activity must be switched off; this occurs by proteolysis of cyclin B by the anaphase-promoting complex or cyclosome (APC–C). When mitosis begins, chromosomes become compacted ('condensed'). In the subsequent stage of metaphase, both sister chromatids of each duplicated chromosome pair are attached through their kinetochores to the microtubules of the mitotic spindle, and aligned to the spindle midzone for the following equal segregation at the two spindle poles during anaphase. A 'wait' anaphase signal is activated when even a single chromosome is not properly attached to the mitotic spindle. Arrest is mediated by the mitotic checkpoint, also known as the spindle assembly checkpoint (SAC), which is a circuit of signalling proteins including aurora B, mitotic arrest deficient protein 1 (MAD1), MAD2, monopolar spindle 1 (MPS1), budding uninhibited by benzimidazole 1 (BUB1), BUB3 and BUB1 homologue beta (BUB1B; also known as BUBR1). Their actions are required to rapidly halt mitotic progression by inactivation of the APC–C (BOX 4). Defects in the SAC can lead to premature separation of sister chromatids and could facilitate chromosomal instability (altered chromosome number) , which is a frequent characteristic of cancer

Box 1 | **The retinoblastoma protein family: cell cycle control and beyond**

Members of the retinoblastoma (RB) protein family, comprising RB1 (also known as p105-RB), retinoblastoma-like protein 1 (RBL1; also known as p107) and RBL2 (also known as p130), share sequence homology in a bipartite domain known as the pocket domain, which folds into a globular pocket-like structure owing to the presence of a flexible 'spacer' region. The pocket domain mediates interactions with members of the E2F family of transcription factors and with proteins containing an LXCXE motif, such as D-type cyclins (CYCDs), histone deacetylases and viral oncoproteins.

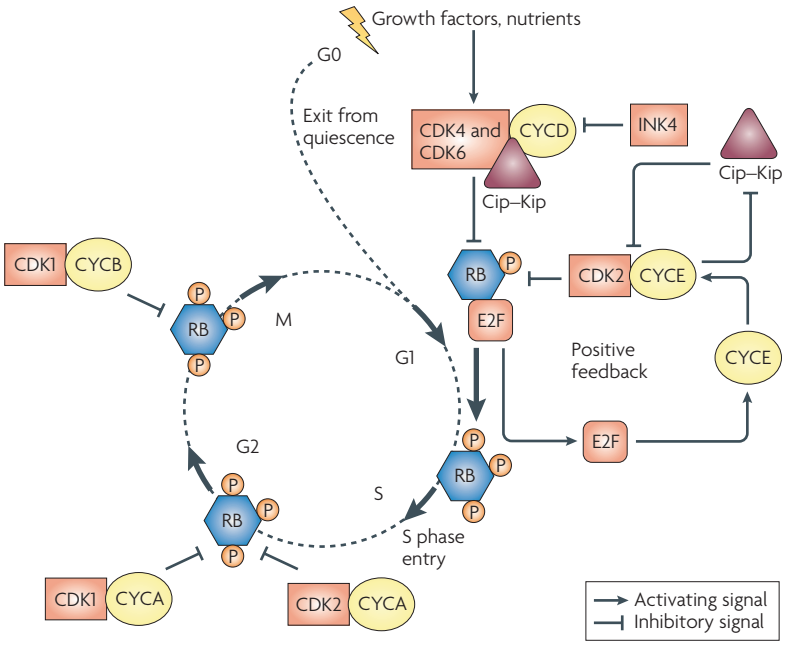
RB family members, or 'pocket proteins', play key parts in the control of cell proliferation. They negatively modulate the transition from the first gap (G1) phase to the DNA synthesis (S) phase (see figure), are growth-suppressive in a cell type-dependent manner, are implicated in various forms of differentiation and are crucial targets for inactivation by transforming oncoproteins of DNA tumour viruses[3,111].

The G1 to S phase transition is a complex process, involving the concerted actions of various cyclins and cyclin-dependent kinases (CDKs) in conjunction with the RB proteins (see figure). Mitogenic stimuli induce the release of the cyclin D-associated kinases, CDK4 and CDK6, from the inhibitory INK4 proteins (p16INK4A, p15INK4B, p18INK4C and p19INK4D) and initiate phosphorylation of RB1, RBL1 and RBL2. Cyclin D–CDK4 and cyclin D–CDK6 complexes also bind stoichiometrically to the potent CDK2 inhibitors of the Cip–Kip family (p27 (also known as CDKN1B and KIP1), p21 (also known as CDKN1A and CIP1) and p57 (also known as CDKN1C and KIP2)), sequestering them away from CDK2. Partially phosphorylated RB proteins release E2F transcription factors, enabling the expression of genes required for G1 to S phase transition and DNA synthesis. This includes the *cyclin E* gene, the protein product of which binds and allosterically regulates CDK2 activity in late G1, creating a positive feedback loop that antagonizes Cip–Kip inhibitors by signalling for their proteolysis, and reinforces RB inactivation, leading to an irreversible switch to S phase. Cyclin A- and cyclin B-dependent CDKs are activated at later phases of the cell cycle to maintain RB in a hyperphosphorylated form until the cell exits mitosis. RB family members[112] and Cip–Kip proteins[113] may also be involved in maintaining cells in a quiescent state (G0).

The functional overlap between the RB proteins does not seem to extend to complete redundancy. First, their expression levels differ in the various phases of the cell cycle: RBL2 expression is higher in the G0 phase, RBL1 expression peaks during the S phase, and RB1 expression is uniform throughout the cell cycle. Second, their expression is cell type-specific: RBL2 predominates in neurons and skeletal muscle, RBL1 expression is particularly high in breast and prostate epithelial cells, whereas RB1 is ubiquitously expressed in normal tissues[112]. Third, each of the pocket proteins interacts with specific subsets of E2F transcription factors, although overexpression of any RB family member causes cell cycle arrest in G1 in most cell types. Fourth, RB1, but not RBL1 or RBL2, can bind to the anaphase-promoting complex or cyclosome and stabilize p27, promoting cell cycle exit[114]. Finally, RBL1 and RBL2, but not RB1, associate stoichiometrically with either cyclin E–CDK2 or cyclin A–CDK2 complexes, thereby inhibiting the complexes[115,116]. RBL1 is thought to act as an E2F competitor by binding to CDK2–cyclin complexes through its amino-terminal region. By contrast, RBL2 could act as a direct CDK2 inhibitor[117,118]. Such activity was found to be mediated by the spacer region of RBL2, which has an amino-acid sequence that is unique among the other members of the RB family[119].

Retinoblastoma, a relatively rare cancer, has dramatically changed the way cancer is studied and understood, through important scientific advances such as the identification of *RB1* as the first tumour suppressor gene. Currently, loss of *RB1* is considered either a causal or an accelerating event in many cancer types[120,121]. Depending on the tumour type, loss of *RB1* function is associated with different responses to various therapeutic agents[122]. By contrast, much less is known regarding the tumour-suppressive functions of *RBL2* and *RBL1*, which are less frequently inactivated in human tumours compared with *RB1*. Several lines of evidence show that functional inactivation of *RBL2* or *RBL1* can provide a growth advantage during later stages of cancer[123].

A thorough grasp of the role of the *RB* gene family in cancer remains a challenge. Further studies to elucidate the relationships between the status of all members of the RB family of proteins and the response to different treatments in a cancer type-specific context will be crucial for accurate prognosis and optimal treatment.



M, mitosis phase.

Box 2 | **Cyclin-dependent kinase (CDK) deregulation in cancer**

The catalytic subunits of CDK–cyclin complexes are infrequently mutated in cancer. Notable exceptions to this include mutations of CDK4 and CDK6, which have been described in distinct subgroups of melanoma (CDK4) and other tumours (CDK4 and CDK6) (TABLE 1). More frequently observed in human malignancies are amplifications of the levels of the regulatory subunits of CDKs and cyclins, and mutations of endogenous CDK inhibitors. For example, cyclin D1 is overexpressed in several tumours, such as parathyroid adenoma, leukaemia, lymphomas and multiple myeloma, and colorectal, gastric, oesophageal, lung, kidney and breast cancer, as a result of gene amplification, rearrangement or translocation[124]. Cyclin D-dependent kinase activity might also be increased by other mechanisms, including inactivation of p16INK4A by gene deletion, point mutation or transcriptional silencing by methylation[124].

Aberrant activation of CDK1 has been observed in a number of primary tumours (for example, breast, colon, prostate, oral, lung and oesophageal carcinomas), most commonly owing to overexpression of cyclin B1, and in some cases correlates with poor prognosis[125]. CDK2 is deregulated in various malignancies, including lung carcinoma (cyclin A–CDK2), melanoma, osteosarcoma, ovarian carcinoma (cyclin E–CDK2), pancreatic neoplasia and sarcomas, most commonly owing to overexpression of cyclin E and/or cyclin A, or inactivation of Cip–Kip inhibitors[7]. The transcriptional kinase CDK9 is overexpressed in myeloma, prostate cancer and lung cancer[13], suggesting that this kinase could be a potential target for cancer therapy. CDK11 is an additional regulator of transcription, and recent evidence also indicates that it has a role in microtubule stabilization and in the control of sister chromatid cohesion[126,127]. The CDK11 gene (*CDC2L*) maps to a chromosome band region (1p36) which is frequently deleted in human cancers, and loss of one allele of *Cdc2l* facilitates skin carcinogenesis in mice[128].

cell lines[8]. Conversely, complete inhibition of the SAC causes death in human tumour cells[9,10], suggesting that pharmacological inactivation of mitotic checkpoint kinases could be a conceivable anticancer strategy.

Targeting cell cycle kinases in cancer therapy

CDKs. CDKs are often overactive in human cancer owing to various genetic and epigenetic events that affect their regulatory pathways (TABLE 1; BOX 2). The overall effect is loss of checkpoint integrity, resulting in uncontrolled proliferation[7]. Therefore, selective inhibition of CDKs may limit the progression of a tumour cell through the cell cycle and facilitate the induction of apoptotic pathways.

The therapeutic value of targeting members of the CDK family has been intensively studied, particularly for interphase CDKs (CDK2, CDK4 and CDK5); CDK1 is also emerging as a potential new target. Other CDKs that are not involved in the cell cycle have specific functions in particular processes or cell types; however, their potential usefulness as therapeutic targets for cancer treatment remains to be fully investigated. For example, CDK5 has been shown to play a part in controlling cell motility and metastatic potential in prostate cancer[11]. CDK7, a component of the CDK-activating complex, is necessary for assembly of the CDK1–cyclin B heterodimer[12]. It belongs to a second group of CDKs, also comprising CDK8, CDK9, CDK10 and CDK11, which are involved in the regulation of transcription[7]. In contrast to the cognate cyclins of cell cycle-related CDKs (cyclin types A, B, D and E), the expression levels of which fluctuate with cell cycle progression, the cyclin partners of transcription-related CDKs (cyclin types H, C and T) have steady expression levels through the various phases. For example, acting by phosphorylation of RNA polymerase II, the CDK9–cyclin T holoenzyme stabilizes the elongation of nascent mRNA transcripts and has a crucial role in several biological processes, such as cell growth, proliferation and differentiation[13]. Several ATP-competitive nonspecific CDK inhibitors are highly active against CDK9 and possibly other transcription-related CDKs, but these CDKs are seldom included in the panel of kinases used for screening drug specificity. Inactivation of these CDKs has a global impact on cellular transcription, affecting primarily the accumulation of mRNA transcripts that have a rapid turnover, such as those that encode cell cycle regulators, nuclear factor-κB- and p53-responsive gene transcripts, and anti-apoptotic factors. Consequently, chemicals that inhibit transcriptional CDKs could possess anticancer activity by augmenting apoptotic responses in cancer cells, particularly tumorous haematopoietic cells as their survival depends on the continuous expression of anti-apoptotic proteins[14]. However, such compounds may have toxic effects in non-tumorous cells owing to downregulation of crucial cellular factors, thereby limiting the therapeutic application of wide-spectrum CDK inhibitors as single agents. Early cancer treatment with combinations of DNA-damaging or other cytotoxic drugs could be advantageous with regard to therapeutic index and clinical efficacy.

Over the past two decades, there has been an intensive search for small molecules that target CDKs, but no CDK inhibitors have yet been approved for commercial use. First-generation compounds, such as the pan-CDK inhibitors flavopiridol, olomoucine and R-roscovitine generally did not meet the expectations following preclinical studies, exhibiting low activity and/or toxicity in the clinical trials (TABLE 2; for chemical structures of the compounds described in this section, see Supplementary information S1 (table); selected structures are shown in FIG. 2)[14]. This might reflect a failure to identify the key molecular targets or off-target proteins of the inhibitors and/or a failure to optimize the drug dosing schedule and pharmacokinetics. As pan-CDK inhibitors may act on many phases of the cell cycle, the drug must possess a suitable pharmacokinetic profile to allow for sufficient exposure of the damaged tissue over the entire duration of the cell cycle, using a reasonable schedule of administration. For example, whereas prolonged infusions of flavopiridol (FIG. 2) were largely inactive in many settings, a recent Phase I trial in patients with leukaemia implementing a 30-minute bolus dose followed by a 4-hour infusion produced sustained micromolar concentrations for several hours, allowing a 41% response rate in 22 assessable patients[15]. More trials of bolus–infusion flavopiridol in leukaemia and other cancers are anticipated. Another Phase I study established the safe concentration range of flavopiridol in combination with the cytotoxic agents paclitaxel and carboplatin, which led to partial responses or stable disease in patients with non-small-cell lung carcinoma (NSCLC)[16]. Given that resistance to radiotherapy and chemotherapy may arise during the treatment of NSCLC and other solid tumours, the potential of CDK inhibitors to improve

Pan-CDK inhibitor
A cyclin-dependent kinase (CDK) inhibitor with a broad specificity for CDKs.

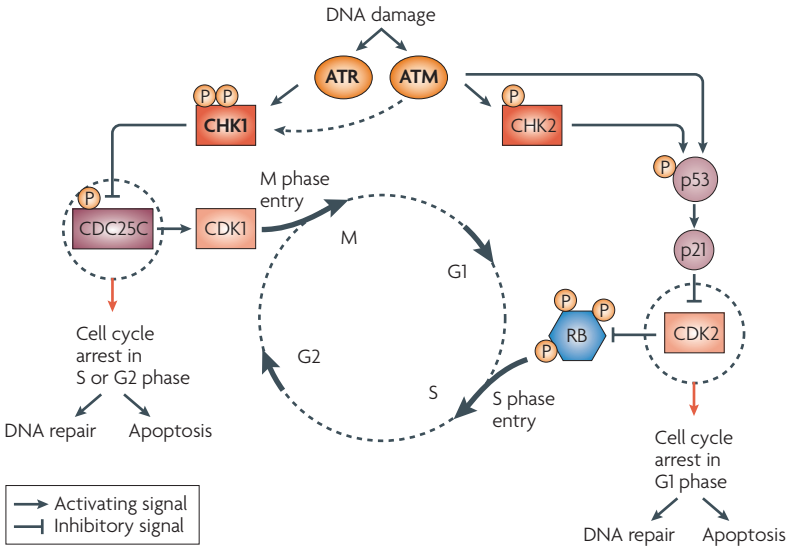
Box 3 | **The good and the bad of DNA damage checkpoints**

Induced or spontaneous DNA lesions are common events in the life of the cell. The ability of the cell to maintain homeostasis and protect itself from neoplastic transformation depends upon complex surveillance mechanisms and activation of repair pathways to preserve chromosomal integrity. The DNA damage checkpoint is a cardinal process. Genetic defects that perturb DNA repair mechanisms almost always cause severe diseases, including ataxia-telangiectasia and related syndromes, characterized by degeneration of the nervous and immune systems, sensitivity to ionizing radiation and DNA-damaging agents, and predisposition to cancer[129].

The serine–threonine protein kinases ataxia telangiectasia mutated (ATM; also known as serine protein kinase ATM) and ataxia telangiectasia and RAD3-related protein (ATR; also known as serine-threonine protein kinase ATR) are DNA damage sensor proteins that can induce cell cycle arrest, DNA damage repair or apoptosis, depending on the extent of the DNA lesions (see figure). Whereas ATM responds primarily to DNA double-strand breaks, which are generally caused by ionizing radiation and radiomimetic drugs, ATR also responds to damage caused by ultraviolet light and stalled replication forks[55].

The ATM–ATR cascade is activated within minutes of a DNA damage alarm. Both ATM and ATR can phosphorylate and activate the transcription factor p53, either directly or by means of prior activation of checkpoint kinase 2 (CHK2). Among the genes induced by p53 is the cyclin-dependent kinase 2 (CDK2) inhibitor *p21* (also known as *CDKN1A* and *CIP1*), the activity of which prevents damaged cells from entering the DNA synthesis (S) phase. Also, damaged cells that have already passed the transition from the first gap (G1) phase to S phase can be halted through the activation of another ATM–ATR effector, CHK1, which phosphorylates the dual-specificity phosphatase CDC25C, providing a signal that induces its sequestration in the cytoplasm. Because CDC25C is responsible for removing two inhibitory phosphates from CDK1, its inactivation prevents the cell from entry into the mitosis (M) phase. Cell cycle arrest in G1, S or G2 phase is maintained until DNA integrity is restored. If lesions are irreparable, programmed cell death is induced by the ATM–ATR signalling pathway[55]. The ATM–CHK2 pathway predominantly regulates the G1 checkpoint, whereas the ATR–CHK1 pathway predominantly regulates the S and G2 checkpoints, although there is crosstalk between these pathways.

In most human cancers, however, the function of the DNA damage checkpoint in G1 is impaired owing to mutations in *p53* or the gene encoding the retinoblastoma protein (*RB1*)[130]. Treatment of these tumour cells with DNA-damaging agents, such as ionizing radiation and DNA-targeting drugs, results in S or G2 checkpoint-mediated arrest. Nonetheless, some of these cells might use this remaining checkpoint to protect themselves from radiation or cytotoxic agents. These cancer-favouring circumstances may be tackled by the combination of DNA-damaging drugs or ionizing radiation with inhibitors of the S or G2 checkpoints, or 'S or G2 checkpoint abrogators'. Such a combination should force cancer cells carrying DNA lesions into mitosis, a condition which prompts mitotic catastrophe and associated cell death[131]. Abrogation of the DNA damage checkpoint in S or G2 is an attractive strategy for selectively targeting G1 checkpoint-defective cancer cells and is currently being explored in clinical trials.



current therapeutic regimens deserves further investigation. Indeed, a second-generation CDK inhibitor, the aminothiazole SNS-032 (FIG. 2; TABLE 2), was recently shown to sensitize radiotherapy-resistant NSCLC cells to ionizing radiation[17]. The inhibitory activity of this molecule on cell cycle-unrelated CDKs (CDK7 and CDK9) has been suggested as the mechanism underlying the modulation of DNA double-strand break repair by this compound[17]. SNS-032 is currently in Phase I clinical trials as an intravenous agent. Although bioavailability of SNS-032 is limited by a poor absorption owing to P glycoprotein-mediated efflux, a series of derivatives (for representative analogues, see BOX 5 and Supplementary information S2 (table)) have been recently shown to possess an improved permeability and lowered efflux *in vitro*[18,19].

New generation ATP-competitive CDK inhibitors are currently in clinical trials (TABLE 2 and Supplementary information S1 (table)). The synthetic flavone P276-00 (FIG. 2) belongs to the same chemical class as flavopiridol, but possesses a superior CDK selectivity, particularly for CDK4–cyclin D1, CDK1–cyclin B and CDK9–cyclin T1, and a twofold to threefold higher antiproliferative activity in human tumour cells[20,21]. This compound decreases cellular levels of cyclin D1, an effect which could depend on inhibition of CDK9 transcriptional activity[20]. P276-00 is currently in Phase I–II clinical trials for the treatment of multiple myeloma, mantle cell lymphoma and melanoma characterized by cyclin D overexpression.

The pyrazole-3-carboxamide AT7519 (FIG. 2) is another pan-CDK inhibitor that is currently in Phase I trials in patients with advanced solid tumours or refractory non-Hodgkin's lymphoma. Among cell cycle-related CDKs, AT7519 showed preference for CDK2; it is also highly active against CDK9 and CDK5, and relatively inactive against non-CDK proteins, with the exception of glycogen synthase kinase 3β. Preclinical testing of AT7519 showed that it had antiproliferative activity at submicromolar concentrations in numerous human tumour cell lines that was independent of their p53 and RB1 status, and reduced or no activity in fibroblast cells[22]. This compound also promoted tumour regression in colon cancer xenograft models[22].

The diaminopyrimidine R547 (FIG. 2)[23] is a potent inhibitor of CDK1, CDK2, CDK4, CDK7 and CDK9, exhibiting promising antitumour activity in preclinical models. Early Phase I studies have confirmed that R547 inhibits RB1 phosphorylation and has antitumour activity, and have established the adverse effects and tolerable dose of this compound[24]. Further trials in advanced solid tumours and haematological tumours are anticipated.

Another promising drug candidate is ZK 304709, a multitarget oral tumour growth inhibitor currently in Phase I trials. In enzymatic assays, this compound blocked various CDKs (CDK1, CDK2, CDK4, CDK7 and CDK9) as well as tyrosine kinases that are associated with angiogenesis (vascular endothelial growth factor receptor 1 (VEGFR1), VEGFR2, VEGFR3 and platelet-derived growth factor receptor), all at nanomolar concentrations[25]. In mouse models of pancreatic tumours,

Box 4 | **Aurora and mitotic checkpoint kinases: biological functions and alterations in cancer**

Aurora kinases (aurora A, aurora B and aurora C) are regarded as key regulators of the mitosis (M) phase of the eukaryotic cell cycle. Despite sharing a high degree of structural similarity, these proteins have distinct cellular localization and functions. Aurora A, the 'polar kinase', binds to its regulatory protein TPX2, concentrates on centrosomes at all stages of the cell cycle and is required for spindle assembly[5]. Aurora B, the 'equatorial kinase', is a component of the chromosome passenger complex, along with the inner centromere protein (INCENP), borealin and survivin. In early mitosis (prometaphase), this complex associates with the heterochromatin found around the centromeres but, once anaphase begins, it relocates to the microtubules that interdigitate at the spindle midzone[132]. In prophase, aurora B phosphorylates histone H3, which is necessary for chromosome bi-orientation. It also participates in the mitotic checkpoint by sensing tension between sister centromeres and regulates kinetochore function to impart correct chromosome alignment and segregation. Aurora B is also required for cell division (cytokinesis). Aurora C also accumulates at the centrosomes; its expression profile seems to be restricted to the testis, although it might also have an overlapping role with aurora B.

The expression levels of human aurora kinases are elevated in several primary tumours[125], including breast cancer and colon cancer. Moreover, *aurora A* maps to a locus (20q13) that is frequently amplified in a subset of these and other tumours (TABLE 1).

The mitotic checkpoint, or spindle assembly checkpoint (SAC), is normally activated at every cell cycle after entry into mitosis. It represents a 'wait anaphase' signal that is elicited by the presence of unattached kinetochores. This signalling pathway inhibits the proteolytic degradation of important regulators of mitosis, such as cyclin B (which is degraded by the anaphase-promoting complex or cyclosome (APC–C)), leading to mitotic arrest. The capture of microtubules at both kinetochores of sister chromatids results in SAC silencing and APC–C-mediated degradation of its components, after which anaphase is initiated and mitosis can be completed. Defects in the SAC may cause dysfunctional chromosome segregation and chromosome instability, which may favour tumour progression. Cancer-associated point mutations have been reported for several SAC regulators including the kinases budding uninhibited by benzimidazoles 1 (BUB1) and BUB1 homologue beta (BUB1B; also known as BUBR1). Inactivating mutations in BUB1B are responsible for cancer-predisposing disorders, including mosaic variegated aneuploidy and premature chromatid separation syndrome[8]. In addition, aurora B is frequently overexpressed in cancer. Mice carrying homozygous deletions of mitotic checkpoint proteins, such as mitotic arrest deficient protein 2 (MAD2), BUB3 and BUB1B, die at early stages of embryonic development as a result of massive chromosome loss[8]. Conversely, mice with reduced levels of any of these components display chromosome instability. At present, it is not clear whether chromosome misdistribution per se can drive oncogenesis, but evidence suggests that this event might facilitate tumour development[133].

ZK 304709 induced effects consistent with CDK inhibition (cell cycle arrest in G2 and apoptosis) and caused a reduction in tumour microvessel density, resulting in an 80% reduction in primary tumour growth[26].

The 2,6,9-trisubstituted purine family, which includes R-roscovitine (FIG. 2)[27] and the second-generation CDK inhibitors purvalanol A[28] and NU6140 (REF. 29) (BOX 5 and Supplementary information S2 (table)), has been the subject of intense exploration aimed at identifying novel synthetic analogues with superior activity. Recently developed R-roscovitine analogues include the pyrazolo triazine N-&-N1 (REF. 30) and the purine derivative CR8 (REF. 31) (BOX 5 and Supplementary information S2 (table)). Although both of these molecules show CDK selectivity (against CDK1, CDK2, CDK5, CDK7 and CDK9) and activity similar to that of the parent compound *in vitro*, CR8 was 25–50-fold more potent than R-roscovitine at inducing apoptosis in 25 cancer cell lines[31], and N-&-N1 was twice as potent at inhibiting tumour growth *in vivo*[32]. These findings warrant further investigation with regard to toxicity and antitumour activity.

Other broad-spectrum CDK inhibitors that are in advanced preclinical evaluation and have shown anti-tumour activity in animal models include the thiazole urea CDKi-277 (REF. 33), the indenopyrazole derivative RGB-286199 and the acyl-substituted triazole diamine JNJ-7706621 (BOX 5 and Supplementary information S2 (table)). JNJ-7706621 showed a unique inhibition profile of cell cycle regulatory proteins, as it potently inhibited both CDK1–CDK2 and aurora A–aurora B, and was less potent against CDK4 and CDK6 (REF. 34). The compound showed *in vivo* efficacy in a human melanoma tumour xenograft model in mice[34]. However, signs of resistance have been reported in cancer cells exposed to incrementally increasing concentrations of JNJ-7706621, as a result of overexpression of the ATP-binding cassette subfamily G member 2 drug transporter[35].

It has recently emerged that sensitivity to CDK inhibitors might depend on the tumour type. In mouse tumour models, cyclin D1-regulated CDK4 kinase activity is crucial for proliferation of breast cancer cells induced by the *Erbb2* (also known as *Neu* and *Her2*) and *Hras* oncogenes[36–39]. By contrast, ablation of *Cdk6* had little effect on the development of this mammary tumour[39]. These findings point to inhibition of cyclin D1-mediated CDK4 kinase activity as a promising strategy for patients with human epidermal growth factor receptor 2 (HER2)-positive breast cancer. Other studies indicate that CDK4 inhibition may be effective in diminishing *MYC*-mediated skin tumour growth[40,41], whereas CDK6 may be a suitable target in therapies against human lymphoid tumours[42]. Such observations support the concept that targeted inactivation of individual CDKs could protect against cancers in which tumour survival depends specifically on the expression and activation of these CDKs. For example, the orally active pyridopyrimidine PD-0332991 (FIG. 2; TABLE 2 and Supplementary information S1 (table)) is exquisitely selective for CDK4 and CDK6 (REF. 43), and hence could be useful to test the hypotheses arising from the genetic models in a clinical setting.

Erbb2
The rodent orthologue of the human epidermal growth factor receptor 2 (*HER2*) gene. At least one-quarter of human breast cancers overexpress *HER2*, an event which often leads to increased cyclin D1 expression levels.

Table 1 | **Genetic alterations of cell cycle kinases in human cancer**

Target	Main functions	Alteration	Biological effect	Cancer type	Refs
CDK4	A serine–threonine protein kinase involved in regulating progression through the G1 phase of the cell cycle by phosphorylation and inactivation of RB1	R24C	Inability to bind the INK4 inhibitor p16INK4A, causing deregulation of CDK4 kinase activity	Familial melanoma	134
		Gene amplification	CDK4 overexpression, leading to RB1 hyperphosphorylation and loss of antiproliferative control	Sporadic melanoma, glioblastoma and osteosarcoma, and cancers of the breast and uterine cervix	124,135
CDK6	A serine–threonine protein kinase involved in regulating progression through the G1 phase of the cell cycle by phosphorylation and inactivation of RB1	Chromosomal translocations	CDK6 overexpression, leading to RB1 hyperphosphorylation and loss of antiproliferative control	Splenic marginal zone lymphoma	124
		Gene amplification		Lymphoma, squamous cell carcinoma and glioma	124
ATM	A serine–threonine protein kinase of the PI3K-like family activated in response to genotoxic insults, primarily DNA DSBs; its activation triggers cellular repair mechanisms	Truncation or missense mutations	Loss of ATM function; homozygous carriers are affected by A-T syndrome	Lymphoid tumours, particularly thymic lymphomas	55,136
		Missense or nonsense mutations	Loss of ATM function; heterozygous carriers have increased sensitivity to ionizing radiation and a higher cancer incidence	Various tumours, particularly breast cancer	
ATR	A serine–threonine protein kinase of the PI3K-like family activated in response to genotoxic insults, including DNA DSBs, ultraviolet light damage and stalled replication forks; its activation triggers cellular repair mechanisms	Truncation or missense mutations	Loss of ATR function, causing decreased phosphorylation of p53 and other downstream effectors, and impairment of the DNA damage checkpoint	Stomach, breast and endometrial cancers	137,138
CHK1	A serine–threonine protein kinase that relays the DNA damage checkpoint signals from upstream ATM and ATR kinases; it is a labile protein, expressed only in S and G2 phases.	Frameshift mutations	Truncated or defective CHK1 protein; *CHK1* mutations may contribute to enhanced genomic instability in some tumours, but their overall effect on cancer is not yet clear	Colorectal, gastric, endometrial and small cell lung cancers	67,137, 138
CHK2	A serine–threonine protein kinase that relays the DNA damage checkpoint signals, mainly by ATM; it is a stable protein expressed throughout the cell cycle, but is inactive in the absence of DNA damage	Truncation or missense mutations	Unstable CHK2 protein, decreased or lost kinase activity, or defective substrate recognition; *CHK2* mutations have been associated with oncogenesis	Cancers of the breast, bladder, colon, ovary, lung, vulva and prostate; lymphomas and osteosarcomas; and Li–Fraumeni syndrome	67,139
Aurora A	A serine–threonine protein kinase that coordinates the centrosome cycle, mitotic entry and spindle assembly	Gene amplification	Aurora A overexpression, which leads to chromosome instability, characterized by chromosome amplification, aneuploidy and premature segregation of sister chromatids; these effects have been associated with tumorigenesis	Several human tumours, including breast, colorectal and bladder cancers	5,125
PLK1	A serine–threonine protein kinase involved in activation of CDK1–cyclin B at mitotic entry, centrosome maturation, spindle assembly and cytokinesis	Point mutations	Altered PLK1 stability	Cancers of the liver, lung, stomach and epidermis	86
BUB1	A serine–threonine protein kinase required for the spindle assembly checkpoint	Point mutations, deletions and/or frameshifts	Loss of BUB1 function, causing a weakened or unsustainable spindle assembly checkpoint	Colorectal, lung and pancreatic tumours, and T cell lymphomas	8
BUB1B	A serine–threonine protein kinase required for the spindle assembly checkpoint	Point mutations, deletions and/or frameshifts	Loss of BUB1B function, causing a weakened or unsustainable spindle assembly checkpoint	Colorectal cancers, lymphomas, mosaic variegated aneuploidy and premature chromatid separation syndrome	8,140

A-T, ataxia-telangiectasia; ATM, ataxia telangiectasia mutated (also known as serine protein kinase ATM); ATR, ataxia telangiectasia and RAD3-related protein (also known as serine–threonine protein kinase ATR); BUB1B, budding uninhibited by benzimidazoles 1 homologue beta (also known as BUBR1); CDK, cyclin-dependent kinase; CHK, checkpoint kinase; DSBs, double-strand breaks; G1, first gap; G2, second gap; PI3K, phosphoinositide 3-kinase; PLK1, polo-like kinase 1; RB1, retinoblastoma protein; S, synthesis.

Table 2 | **Selected inhibitors of cyclin-dependent kinases in clinical trials**

Inhibitor (company)	Main targets (other targets)	Preclinical study data	Clinical trials
AG-024322 (Pfizer)‡	CDK1, CDK2 and CDK4 (other CDKs)	• *In vitro*: K_i = 1–3 nM (CDK1, CDK2 and CDK4)[141]; displayed CDK selectivity over non-CDKs[141]; arrested multiple stages of the cell cycle and induced apoptosis in various human tumour cell lines (IC_{50} = 30–200 nM)[141] • Displayed dose-dependent antitumour activity in mice bearing human tumour xenografts[142] • Intravenous toxicity in cynomolgus monkey included bone marrow hypocellularity, lymphoid depletion and vascular irritation (at doses ≥ 6 mg per kg); the no-adverse-effect dose was 2 mg per kg[143]	• Phase I: advanced cancer; trials were discontinued (2007) owing to the inability of the compound to adequately differentiate from other treatment options
AT7519 (Astex)‡	CDK2, CDK4, CDK5 and CDK9 (CDK1, CDK4, CDK6 and GSK3β)	• *In vitro*: IC_{50} < 10 nM (CDK9–CYCT), IC_{50} = 13 nM (CDK5–p35), 47 nM (CCK2–CYCA), 100 nM (CCK4–CYCD1), 170 nM (CDK6–CYCD3), 210 nM (CDK1–CYCB), 2.4 μM (CDK7–CYCH–MAT1)[22]; inactivity against non-CDK kinases[22], except for GSK3β (IC_{50} = 89 nM); caused G0–G1 phase and G2–M phase cell cycle arrest followed by apoptosis in cancer cells at doses < 1 μM[22] • Showed potent antiproliferative activity in various human tumour cell lines, a lower activity in non-transformed fibroblasts and no activity in non-cycling cells[22]; promoted tumour growth inhibition or regression in human ovarian and colon carcinoma xenografts[22]	• Phase I: advanced or metastatic solid tumours, or refractory NHL
AZD5438 (AstraZeneca)*	NA	NA	• Phase I: advanced solid tumours • Trial discontinued (2009) owing to poor clinical tolerability and exposure data
Flavopiridol, also known as alvocidib (Sanofi–Aventis)‡	CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9 (GSK3β)	• *In vitro*: IC_{50} = 3 nM (CDK9–CYCT), 20–40 nM (CDK4–CYCD), 60 nM (CDK6–CYCD), 30–400 nM (CDK1–CYCB), 100 nM (CDK2–CYCA and CDK2–CYCE), 110–300 nM (CDK7–CYCH)[144]; inhibited GSK3β (IC_{50} = 450 nM); induced G1–S phase and G2–M phase arrest and apoptosis at 200–300 nM concentrations in many tumour cell types[144] • Significant clinical activity in refractory CLL[145]; clinical studies ongoing to determine efficacy of combination with anti-neoplastic agents[16]	• Phase I–II: various cancers, including leukaemia, multiple myeloma, lymphoma, sarcoma and solid tumours (as a single agent and in combination with DNA-damaging or other cytotoxic drugs)
Indisulam, also known as E7070 (Eisai)‡§	NA	• Induced G2 phase arrest in several human tumour cell lines[146] • Ongoing Phase II trials will determine its clinical efficacy in patients with solid tumours[147]	• Phase I–II: adult solid tumour, stage IV melanoma (as a single agent) • Phase I: gastrointestinal, pancreatic and lung cancer (in combination with irinotecan)
P1446A-05 (Nicholas Piramal)*	CDK4 (NA)	NA	• Phase I: advanced refractory malignancies including solid and haematological tumours
P276-00 (Nicholas Piramal)‡	CDK1, CDK4 and CDK9 (CDK2, CDK6 and CDK7)	• *In vitro*: IC_{50} = 20 nM (CDK9–CYCT1), 63 nM (CDK4–CYCD1), 79 nM (CDK1–CYCB), 224 nM (CDK2–CYCA), 396 nM (CDK6–CYCD3), 2.54 μM (CDK2–CYCE), 2.87 μM (CDK7–CYCH)[20]; inactive against several non-CDK kinases[20] • Induced G1–G2 arrest and caspase-dependent apoptosis in 16 tumour cell lines, including cisplatin-resistant cell lines, with a mean IC_{50} = 550 nM[21]; inhibited growth of human multiple myeloma, colon carcinoma and NSCLC xenografts[21,148]	• Phase I–II: multiple myeloma, mantle cell lymphoma, head and neck cancers, and cyclin D1-positive melanoma
PD-0332991 (Pfizer)*	CDK4 and CDK6 (NA)	• *In vitro*: IC_{50} = 11 nM (CDK4) and 16 nM (CDK6)[43] • Inhibited cellular proliferation with IC_{50} = 40–400 nM in RB1-positive cell lines and IC_{50} > 3 μM in RB1-negative cell lines[149]; induced G1 arrest in primary bone marrow myeloma cells and prevented tumour growth in disseminated human myeloma xenografts[150]; promoted tumour regression in mice with a human colon carcinoma xenograft[149] • Sensitized myeloma cells to killing by dexamethasone[150] and by bortezomib[151]	• Phase I: advanced cancer and mantle cell lymphoma • Phase I–II: multiple myeloma (in combination with bortezomib and dexamethasone), hormone receptor-positive advanced breast cancer (in combination with letrozole)

Moreover, preliminary data showed that pharmacological inhibition of CDK1 might have a therapeutic value against *MYC*-induced human tumours, but not against tumours overexpressing other oncogenes[44]. Among the more recently developed CDK inhibitors in preclinical evaluation, the quinolinyl thiazolinone RO-3306 (BOX 5; FIG. 2 and Supplementary information S2 (table)) has been reported to be a highly selective inhibitor of CDK1–cyclin B *in vitro*[45]. Treatment of proliferating cells with RO-3306 did not affect the G1 to S phase transition, but arrested cells at the point of transition from G2 to M phase, consistent with specific CDK1 inhibition. Sustained (72 hour) exposure to RO-3306 resulted in a larger apoptotic fraction in cancer cells compared with non-transformed cells. RO-3306 itself therefore deserves further investigation, and the thiazolinone class could be a source of lead compounds for the development of novel CDK1-specific inhibitors.

Studies in genetically engineered mouse models support the concept that CDKs that act during interphase (CDK2, CDK4 and CDK6) have compensatory roles in the control of the mammalian cell cycle[4,46–48], except for certain tissues in which they have specific functions. For example, *Cdk2* (the mouse orthologue of the human gene that encodes CDK2) is not essential for mouse survival[46,47], but is required for the development of germ cells[46]. CDK4- or CDK6-null mice are viable[48], although CDK4 is required for postnatal proliferation of pancreatic islet β-cells[49–51] and pituitary cells[52], and loss of CDK6 impairs erythroid cell count[48]. Interestingly, triple ablation of *Cdk*2, *Cdk*4 and *Cdk*6 is compatible with mouse embryo organogenesis until mid-gestation, when mutant embryos die owing to haematopoietic defects[4]. This implies that CDK1 is able to compensate for a lack of these interphase CDKs to fully suppress RB1 antiproliferative activity in most mammalian cell lineages.

Overall, genetic models could help to identify the potential toxic effects of drug candidates that selectively inhibit certain CDKs. For example, a small-molecule CDK2 inhibitor would be expected to cause male and female sterility; CDK4 inhibition could lead to specific endocrine phenotypes, such as insulin-dependent diabetes and female infertility; and CDK6 inhibition may provoke anaemia. Drugs that target CDK1 and/or CDK11, both of which are products of essential genes in mice[4,53], might be responsible for a more general cytotoxic effect. However, the vulnerability to CDK inactivation observed in mouse germ lines could differ in adult organisms, as these may be less susceptible to toxic effects arising from weakened or lost CDK activity. Indeed, in contrast to mouse embryos, double CDK2–CDK4 ablation does not lead to major proliferative defects in adult mice[54]. It is also important to consider that genetic ablation of a given CDK could have different phenotypical consequences to its pharmacological inhibition. The effect of most kinase inhibitors will not be as selective as genetic knockout experiments. Moreover, in the presence of an ATP-competitive CDK inhibitor, although the specific kinase function will be reduced by the inhibitor, the CDK will still be able to form complexes with its cognate cyclin and various physiological substrates or inhibitors, thereby sequestering them away from other CDK targets in the cell. This will give rise to a more complicated phenotype than seen with genetic knockouts. In this regard, generation of knock-in mice expressing mutated kinase targets, which retain their ability to bind partner proteins but lack their catalytic function, could aid the prediction of the pharmacodynamic effects of anticancer drug candidates and help distinguish between target and off-target effects.

DNA damage checkpoint kinases. In normal proliferating cells, the DNA damage checkpoint is in place to prevent erroneous DNA from being replicated before progression through mitosis (BOX 3). However, in cancer cells, the response to genome alterations is commonly impaired owing to accumulation of mutations in checkpoint regulators, particularly in ATM, CHK2 and p53 (TABLE 1).

ATM has an important role in maintaining genome integrity. In light of the fact that patients with ataxia-telangiectasia as well as heterozygous carriers of *ATM* mutations (estimated at 1–2% of the population) are hypersensitive to ionizing radiation and drugs that induce DNA double-strand breaks[55], it has been proposed that pharmacological inhibition of ATM might promote cellular radiosensitization and chemosensitization. Two small-molecule ATM inhibitors have been described: the morpholinyl-thianthrenyl-pyranone KU55933 (FIG. 2) and, more recently, the triazolamine CP466722 (BOX 5 and Supplementary information S2 (table)). These compounds target ATM by blocking its ATP-binding site and display higher specificity and potency than the previously used inhibitors, wortmannin and xanthines, which also target other members of the phosphoinositide 3-kinase (PI3K)–like kinase family (for example, ATR and DNA-dependent protein kinase). In cell model systems, KU-55933 prevented phosphorylation of ATM effectors, such as p53, in a concentration-dependent manner and sensitized cells expressing ATM to drugs that induce DNA double-strand breaks, namely the topoisomerase inhibitors[56]. Furthermore, both KU-55933 and CP466722 were shown to specifically and reversibly disrupt ATM-dependent cell cycle checkpoints in response to DNA damage induced by ionizing radiation[57]. Notably, transient exposure to either one of these compounds hypersensitized cultured cells to ionizing radiation. This suggests that long-lasting exposure might not be necessary to provide therapeutic benefit, which would lower the risk of potential adverse effects that may be associated with prolonged ATM inhibition. Although this concept remains to be fully investigated *in vivo*, the *in vitro* effects of these novel lead chemotypes are promising.

Unlike normal cells, which rely on a full arsenal of checkpoint responses, the majority of cancer cells lack the DNA-damage checkpoint in the G1 phase and hence depend on checkpoint-mediated arrest in the S phase or G2 phase for DNA repair and cell survival (BOX 3). ATR and CHK1 are key signalling protein kinases in this pathway and could be suitable targets to achieve selective killing of G1 checkpoint-deficient tumour cells. At present,

Table 2 (cont.) | **Selected inhibitors of cyclin-dependent kinases in clinical trials**

Inhibitor (company)	Main targets (other targets)	Preclinical study data	Clinical trials
R-roscovitine, also known as CYC202 and seliciclib (Cyclacel)*	CDK1, CDK2, CDK5, CDK7 and CDK9 (CK1, GSK3α–β, DYRK1A, ERK1, ERK2 and PDXK)	• *In vitro*: IC_{50} = 220 nM (CDK2–CYCA), 230 μM (CDK9–CYCT), 270 nM (CDK5–p25), 330 nM (CDK1–CYCB) and 800 nM (CDK7–CYCH)[32] • Induces S–G2 arrest and apoptosis[27]	• Phase I–II: NSCLC • Trials completed (2009); when administered at 800 mg twice daily for 7 days, it elicited limited tumour responses and dose-limiting toxicities (fatigue, skin rash and hypokalaemia)[152]
R547, also known as Ro-4584820 (Hoffmann–La Roche)‡	CDK1, CDK2, CDK4 and CDK7 (NA)	• *In vitro*: K_i = 1–3 nM (CDK1, CDK2 and CDK4); inactive (K_i > 5 mM) against > 120 non-CDK kinases[23]; induced G1–G2 arrest and apoptosis in tumour cell lines independently of RB1 or p53 status (IC_{50} < 0.60 μM)[23]; induced significant tumour growth reduction in human tumour xenografts and efficacious with daily oral dosing and weekly intravenous dosing[23] • Early clinical trials established tolerable dosage (155 mg per m^2 infusion on day 1 and day 8 during a 21-day cycle), related toxicities (nausea, emesis and hypotension) and confirmed antitumor activity[24]	• Phase I: advanced solid tumours; trial completed (2008)
SCH 727965 (Schering–Plough)‡	CDK1, CDK2, CDK5 and CDK9 (NA)	• Selectively inhibited CDK1, CDK2, CDK5 and CDK9 with IC_{50} < 5 nM[153]; induced apoptosis in tumour cell lines and growth inhibition or regression in xenograft models[153]	• Phase I: advanced solid tumours, NHL, multiple myeloma and CLL. • Phase II: advanced breast cancer, NSCLC, acute leukaemia and lymphoma; initial clinical results indicate good tolerability at 58 mg per m^2; dose-limiting toxicity was neutropaenia[153]
SNS-032, also known as BMS-387032 (Sunesis)‡	CDK2, CDK7 and CDK9 (CDK1 and CDK4)	• Inhibited CDK7–CYCH and CDK9–CYCT at low nM concentrations in biochemical assays[154]; also inhibited CDK2–CYCE (IC_{50} = 48 nM) 10- and 20-fold more potently than CDK1–CYCB (IC_{50} = 480 nM) and CDK4–CYCD (IC_{50} = 925 nM), respectively, *in vitro*[154]; high CDK selectivity over a panel of 12 unrelated kinases (IC_{50} > 25 μM)[154]; induced tumour growth reduction in a human ovarian carcinoma xenograft[154] • Sensitized radioresistant NSCLC cells to ionizing radiation[17] • Limited oral bioavailability (~31%) in rats[155], possibly owing to poor absorption rather than first-pass metabolism[155]	• Phase I: B-lymphoid malignancies and advanced solid tumours
Terameprocol, also known as EM-1421 (Erimos)‡	CDK1, survivin and VEGFRs (NA)	• Potent anticancer activity in tumour cell lines and animal models[156]; good safety and efficacy profile in patients with head and neck or squamous cell carcinoma (intratumoral administration) and in patients with cervical dysplasia (intravaginal administration)[156]	• Phase I: leukaemia, refractory solid tumours and lymphoma
ZK 304709, also known as MTGI and ZK-CDK (Schering AG)*	CDK1, CDK2, CDK4, CDK7 and CDK9 (VEGFR1 VEGFR2, VEGFR3 and PDGFRβ)	• *In vitro*: IC_{50} = 4 nM (CDK2–CYCE), 5 μM (CDK9–CYCT1), 50 nM (CDK1–CYCB), 61 nM (CDK4–CYCD1), 85 nM (CDK7–CYCH), 1 nM (VEGFR3), 10 nM (VEGFR1), 34 nM (VEGFR2) and 27 nM (PDGFRβ)[25]; blocked growth of human tumour cell lines at IC_{50} = 317 nM, by inducing a dose-dependent G1–S arrest followed by apoptosis[25] • Superior efficacy over standard chemotherapy in human tumour xenografts and orthotopic mouse models of human pancreatic cancer[25,26]	• Phase I: advanced solid tumours[157]

Data extracted from http://www.clinicaltrials.gov. *Oral. ‡Intravenous. §Indisulam is not a direct CDK inhibitor: it causes a depletion of cyclin E levels, which reduces CDK2 activity, and a depletion of cyclin H levels, which reduces CDK7 activity. CDK, cyclin-dependent kinase; CLL, chronic lymphocytic leukaemia; CYC, cyclin; DYRK1A, dual specificity tyrosine-phosphorylation-regulated kinase 1A; ERK, extracellular signal-regulated kinase; G1, first gap; G2, second gap; GSK3β, glycogen synthase kinase 3β; IC_{50}, compound concentration that caused 50% inhibition of kinase activity (*in vitro* kinase assays) or cellular proliferation (cell proliferation assays); K_i, inhibition constant; M, mitosis; NA, not available; NHL, non-Hodgkin's lymphoma; NSCLC, non-small-cell lung carcinoma; PDGFRβ, platelet-derived growth factor receptor-β; PDXK, pyridoxal kinase; RB1, retinoblastoma protein; S, synthesis; VEGFR, vascular endothelial growth factor receptor.

no specific ATR inhibitors have been identified. Existing drugs include caffeine, wortmannin and pentoxifylline, but these also target ATM and other PI3K-like kinases. However, there have been recent advances in the development of drugs that target CHK1, and several ATP-competitive inhibitors are currently in clinical evaluation (TABLE 3 and Supplementary information S1 (table)).

A natural metabolite from *Streptomyces* species, 7-hydroxystaurosporine (UCN-01), efficiently abrogates DNA damage checkpoints in cancer cells that lack p53 and have been treated with DNA-damaging drugs, resulting in mitotic catastrophe. These effects have been linked to its potent inhibitory activity against CHK1 and CDC25C-associated protein kinase 1 (CTAK1)[58]. However, UCN-01 also targets protein kinase C (PKC) isoforms and phosphoinositide-dependent protein kinase 1 (PDK1), as well as CDKs, which accounts for its complex pharmacodynamic profile. This compound is currently undergoing Phase I–II trials as a single agent and in combination with cytotoxic agents (TABLE 2).

Mitotic catastrophe
A form of apoptosis that occurs during mitosis and may result from deficient cell cycle checkpoints, particularly the DNA damage checkpoint and the spindle assembly checkpoint.

In 2005, the orally available aminopyrazine XL844 became the first CHK-selective inhibitor to enter clinical trials[59], but its structure has not yet been disclosed. In a human pancreatic carcinoma xenograft model, XL844 showed synergistic tumour growth inhibition with the DNA nucleoside analogue gemcitabine (Gemzar; Eli Lilly)[59]. A Phase I study of XL844 in combination with gemcitabine started in 2007 in advanced solid tumours and patients with lymphoma.

The structure and preclinical profile of AZD7762 (FIG. 2), a CHK1 and CHK2 inhibitor which has been in Phase I trials since 2006, have recently been disclosed[60]. AZD7762 is a thiophene urea carboxamide which has a kinase selectivity distinct from that of UCN-01 as it is more than 100-fold more selective for CHK1 than both PKC isozymes and CDKs, and does not affect PDK1 in cellular assays[60]. The compound abrogated DNA damage checkpoints in the S phase and G2 phase *in vivo*, and augmented cancer cell killing by a DNA anti-metabolite (that is, gemcitabine) and a topoisomerase inhibitor (that is, irinotecan) in rodent tumour models[60].

Whereas AZD7762 and XL844 are approximately equipotent against CHK1 and CHK2, the diazepinoindolone PF-477736 (FIG. 2) is almost 100-fold more selective for CHK1 than CHK2. Nonetheless, PF-477736 potentiated the effect of DNA-damaging therapies to a similar extent as these other two clinical candidates[61]. It is presently unclear whether different CHK selectivity profiles could lead to different clinical outcomes. Notably, depletion of CHK1 by RNA interference (RNAi) provoked premature entry into mitosis, despite incomplete DNA synthesis, in gemcitabine-treated cells[62]. By contrast, depletion of CHK2 had no effect on entry into mitosis[62]. Simultaneous knockdown of these two kinases was as efficacious as CDK1 depletion alone in enhancing sensitization to DNA-damaging agents, suggesting that CHK1 is the most relevant drug target[63].

Recently, two CHK2-selective ATP-competitive inhibitors have been described, the isothiazole carboximidamine VRX046617 (REF. 64) and the bis-guanylhydrazone NSC 109555 (REF. 65). In contrast to CHK1 inhibitors, which cause marked S phase and G2 phase checkpoint defects in response to ionizing radiation, the CHK2 inhibitor VRX046617 did not alter the cytotoxicity of the DNA-damaging drugs doxorubicin and cisplatin[64]. This is consistent with the phenotype of CHK2-knockout mice, which were more resistant to genotoxic agents owing to reduced transcriptional activation of p53-regulated genes[66]. The intriguing phenotype of CHK2-null mice fuelled the idea that chemical inhibition of CHK2 could be exploited to protect sensitive tissues, such as lymphoid tissue and epithelial tissue, from the side effects of radiotherapy or DNA-damaging drugs[67]. Indeed, initial experiments with CHK2 inhibitors suggested that they had potential radioprotective effects in thymocytes[64]. Considering that *CHK2* loss-of-function mutations may predispose to breast and colon cancer, as well as Li–Fraumeni syndrome (TABLE 1), a crucial issue here is the feasibility of targeting CHK2 in specific cancer therapy without increasing the incidence of other cancers.

Li–Fraumeni syndrome
A familial cancer syndrome that arises from a checkpoint kinase 2 (CHK2)-truncating mutation (1100delC). It is characterised by multiple tumours at a young age, particularly breast cancer and sarcoma.

The discovery of novel CHK inhibitors is a growing field of research. Further studies to elucidate the mechanisms by which defective checkpoint pathways arise in cancer could open new avenues for the development of tailor-made therapies, in which individual tumours are profiled for defects in the relevant pathways. Furthermore, identification of appropriate biomarkers for predicting the efficacy of targeted therapeutic agents could improve patient selection strategies. For example, cyclin B has recently been established as an efficacy-predictive biomarker for CDK1 inhibitors in various colon cancer cell lines, as its cellular levels positively correlated with the degree of sensitization to DNA-damaging agents[68]. These observations should be taken into consideration when planning clinical trials of CHK1 inhibitors.

Aurora and mitotic checkpoint kinases. Cells are more sensitive to apoptotic cell death during mitosis than at any other phase of the cell cycle. Indeed, many patients with cancer receive antimitotic agents, such as taxanes and vinca alkaloids, as first-line therapy. These drugs act as microtubule toxins by either stabilizing (taxanes) or depolymerizing (vinca alkaloids) microtubules, which disrupts their intrinsic dynamics and hence inhibits assembly of the bipolar spindle. When this happens, the mitotic checkpoint is activated, thereby inducing a prolonged mitosis which ends with cell death[69]. However, because microtubules have vital functions in both dividing and non-dividing cells, microtubule toxins cause a plethora of side effects, typically peripheral neuropathies. For this reason, during the past decade, much work has focused on the identification of new mitotic targets which could block spindle assembly but spare microtubules in resting cells. Among the several protein kinases required for spindle assembly, aurora kinases and PLKs have received particular attention, and numerous inhibitors are now in clinical development. In addition, SAC kinases are also emerging as potential anticancer drug targets.

Aurora kinases control chromosomal alignment and segregation during mitosis and are required to preserve genome stability[8]. Studies using RNAi and selective chemical inhibitors have shown that aurora A inhibition is characterized by a SAC-induced mitotic arrest and the formation of unipolar spindles, which can eventually lead to apoptosis. By contrast, inhibition of aurora B activity causes abrogation of the SAC, so that cells enter anaphase despite the presence of misaligned chromosomes. Cytokinesis also fails, leading to cells in the G1 phase that have four, rather than two, copies of each chromosome. These cells continue to grow, acquire enlarged polyploid nuclei, and eventually undergo apoptosis or senescence[70]. Ablation of aurora B activity thus produces a distinct phenotype from those caused by microtubule toxins, aurora A inhibitors and PLK inhibitors. Furthermore, as aurora B is required for SAC activation, aurora B inhibitors have a dominant effect on the cell phenotype when combined with aurora A inhibitors, or when dual aurora A and aurora B inhibitors are used.

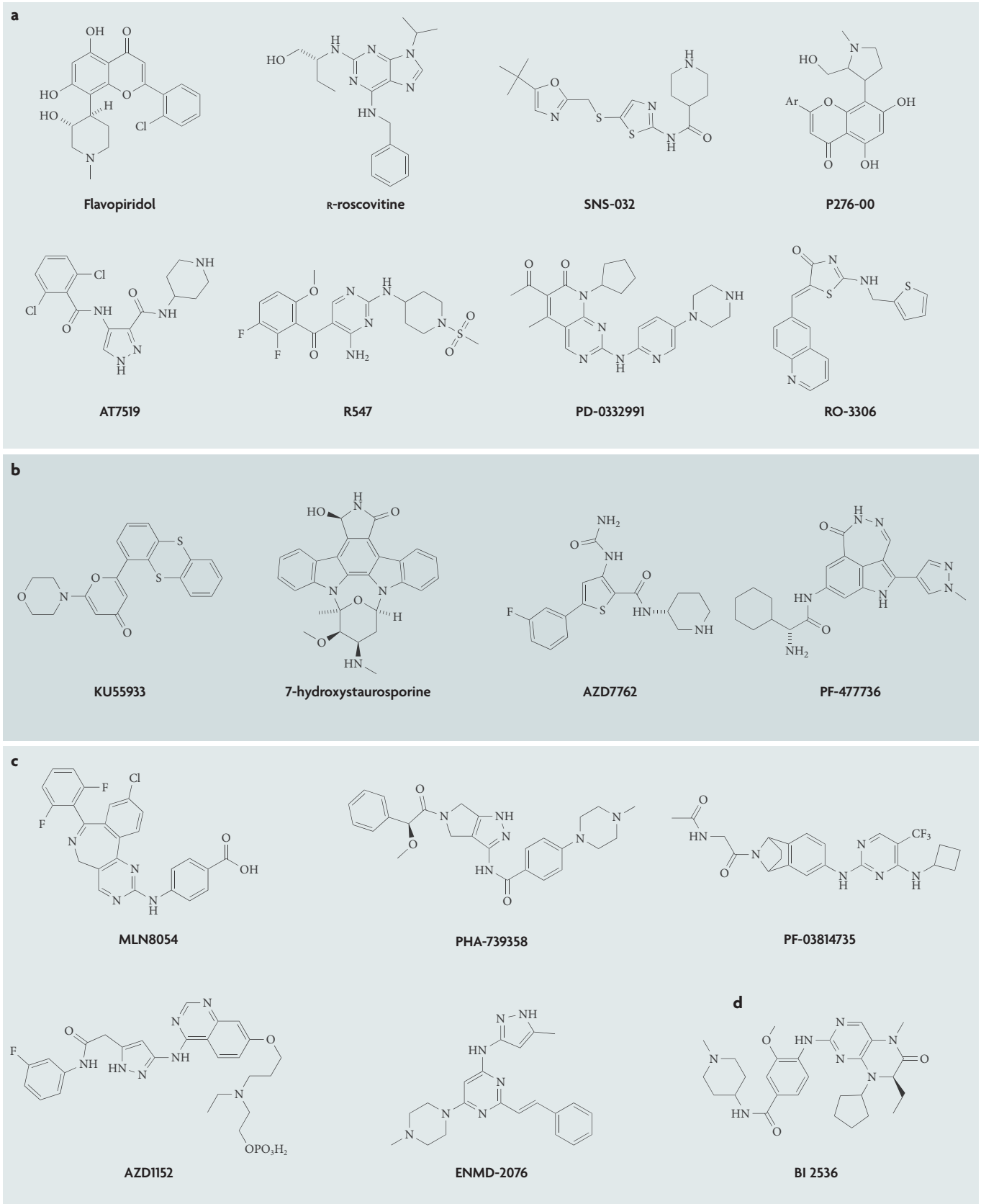


Figure 2 | **Selected chemical structures of kinase inhibitors. a** | Cyclin-dependent kinase inhibitors. **b** | DNA damage checkpoint kinase inhibitors. **c** | Aurora kinase inhibitors. **d** | A polo-like kinase inhibitor. For structures of other compounds discussed in this article, see Supplementary information S1 and S2 (tables).

Table 3 | **Selected inhibitors of checkpoint kinases CHK1 and CHK2 in clinical trials**

Inhibitor (company)	Main targets (other targets)	Preclinical study data	Clinical trials
AZD7762 (AstraZeneca)‡	CHK1 and CHK2 (various kinase families)	• Equal potency against CHK1 and CHK2 *in vitro* (IC_{50} = 5 nM; K_i = 3.6 nM), and 10- to > 100-fold CHK selectivity over 167 other kinases, particularly CDK1 (> 1,000-fold), PKC, CDKs, p38 and MAPK2 (> 100-fold)[60]; abrogated DNA damage-induced cell cycle checkpoints; potentiated the response to DNA-damaging agents in double-negative p53 cancer cell lines and rodent xenograft models in a dose-dependent manner[60]	• Phase I: advanced solid tumours (as a single agent and in combination with gemcitabine or irinotecan)
PF-477736 (Pfizer)‡	CHK1 (CHK2)	• *In vitro*: K_i = 0.49 nM (CHK1) and 47 nM (CHK2)[61]; reduced IR-induced G2 arrest in a clonogenic survival assay, consistent with abrogation of the G2 checkpoint; increased the number of cells entering mitosis following IR[61]; increased DNA damage in gemcitabine-treated tumour cells[61]	• Phase I: advanced solid tumours in combination with gemcitabine
7-hydroxy-staurosporine, also known as UCN-01‡§	CHK1 and MARK3 (PKC, PDK1, GSK3β, CDK1, CDK2 and CHK2)	• Inhibited CHK1 (IC_{50} = 11 nM) and MARK3 (IC_{50} = 27 nM)[158]; 100-fold less potent against CHK2 (IC_{50} = 1040 nM) than CHK1[158]; inhibited PKC isozymes (for example, Ca^{2+}-dependent PKC; IC_{50} = 30 nM), PDK1 (IC_{50} = 33 nM), GSK3β (IC_{50} = 70 nM), and CDK1 and CDK2 (IC_{50} = 300–600 nM) *in vitro* • Abrogated DNA damage-induced G2 arrest, leading to mitotic entry and subsequent cell death[131]	• Phase I: leukaemia and MDS (with perifosine); solid tumours (with irinotecan); lymphoma and solid tumours (with prednisone). • Phase II: RCC, melanoma and lymphoma (as a single agent); SCLC (with topotecan)
XL844, also known as EXEL-9844 (Exelixis)*	CHK1 and CHK2 (VEGFR2, VEGFR3, FLT3 and PDGFR)	• Inhibited CHK1 (K_i = 2.2 nM) and CHK2 (K_i = 0.07 nM)[59]; inhibited FLT3, VEGFR3, VEGFR2 and PDGFR with IC_{50} < 20 nM[59]; enhanced gemcitabine-induced cell killing in several cell lines and a human pancreas carcinoma xenograft model[59]	• Phase I: advanced solid tumours or lymphoma (as a single agent and in combination with gemcitabine)

Data extracted from http://www.clinicaltrials.gov. *Oral. ‡Intravenous. §This compound was developed by one or more academic groups; see references for details. CDK, cyclin-dependent kinase; CHK, checkpoint kinase; FLT3, fms-related tyrosine kinase 3; G2, second gap; GSK3β, glycogen synthase kinase 3β; IC_{50}, compound concentration that caused 50% inhibition of kinase activity (*in vitro* kinase assays) or cellular proliferation (cell proliferation assays); IR, ionizing radiation; K_i, inhibition constant; MAPK2, mitogen-activated protein kinase 2; MARK3, MAP–microtubule affinity-regulating kinase 3 (also known as CTAK1); MDS, myelodysplastic syndromes; PDGFR, platelet-derived growth factor receptor; PDK1, phosphoinositide-dependent protein kinase 1; PKC, protein kinase C; SCLC, small-cell lung carcinoma; RCC, renal cell carcinoma; VEGFR, vascular endothelial growth factor receptor.

The first aurora A-selective inhibitor to be described, MLN8054 (FIG. 2), has a benzazepine core fused to an aminopyrimidine ring (TABLE 4 and Supplementary information S1 (table)). *In vitro*, this compound inhibited aurora A and aurora B. When applied to cells, it produced a monopolar spindle phenotype consistent with aurora A depletion by RNAi[70]. However, at higher concentrations, it induced polyploidy, indicating that aurora B is also targeted *in vivo*. By contrast, the quinazoline derivative ZM447439 was shown to be 20-fold more active against aurora B than aurora A *in vitro*, and to produce a cellular phenotype consistent with aurora B inhibition[70]. Although less potent than ZM447439, the indolinone hesperadin is another aurora B-selective inhibitor that has been used extensively for probing aurora B function[70].

Aurora kinases have attracted much attention over the past few years, both in academia and the pharmaceutical industry, and a diverse array of ATP-competitive inhibitors are currently in clinical development (TABLE 4 and Supplementary information S1 (table)). These include pan-aurora kinase inhibitors, such as VX-680, PHA-739358 (FIG. 2), R763, CYC-116 and SNS-314; aurora A-specific agents, namely, MLN8054, MLN8237 and MP529; dual aurora A and aurora B inhibitors, namely, PF-03814735 (FIG. 2); dual aurora B and aurora C inhibitors, namely, AZD1152 and GSK1070916; multi-kinase inhibitors with potent activity against aurora kinases, namely, ENMD-2076 (selective for aurora A over aurora B), SU-6668 (moderately selective for aurora B over aurora A), AT-9283 (inhibits aurora A and aurora B) and PHA-739358 (a pan-aurora inhibitor).

Interestingly, in preclinical *in vivo* studies, aurora kinase inhibitors suppressed tumour growth regardless of their specificity profiles, highlighting the theraputic potential of aurora A and aurora B as candidate targets for anticancer drugs. For example, the aryl carboxylic acid MLN8237 was reported to produce less severe benzodiazepine-like central nervous system effects than its parent analogue, MLN8054, and had superior potency and equal oral availability. In preclinical models, MLN8237 showed *in vivo* activity against neuroblastoma, lymphoma, leukaemia and multiple myeloma through specific inhibition of aurora A[71–73]. Further investigation focusing on combinations with other agents is expected, and paediatric clinical trials of MLN8237 are in progress (TABLE 2).

AZD1152 (FIG. 2) is a dihydrogen phosphate prodrug which is rapidly converted in the plasma to a pyrazol-quinazoline analogue (AZD1152-hydroxyquinazoline pyrazol-anilide)[74]. This compound induces apoptosis in acute myeloid leukaemia cell lines and primary blasts[75,76], increases sensitivity to both a tubulin depolymerizing agent and a topoisomerase inhibitor[75], and enhances cancer cell response to ionizing radiation[77], providing rational bases for clinical trials in patients with leukaemia.

Table 4 | **Selected inhibitors of aurora kinases in clinical trials**

Inhibitor (company)	Main targets (other targets)	Preclinical study data	Clinical trials
AZD1152 (AstraZeneca)‡	Aurora B and aurora C (aurora A)	• *In vitro*: IC_{50} = 3.7 nM (aurora B), 4.6 nM (aurora C) and 687 nM (aurora A)[74] • Induced growth arrest, polyploidy and promoted apoptosis in various human tumour xenografts[75, 159] and AML cell lines[76] • Sensitized tumour cells to radiotherapy *in vitro* and *in vivo*[77]	• Phase I–II: AML and advanced solid tumours; dose-limiting toxicity was neutropaenia
AT-9283 (Astex)‡	Aurora A and aurora B (JAK2, JAK3, BCR–ABL, the BCR–ABL mutant T315I, TYK2 and RSK2)	• *In vitro*: IC_{50} = 3 nM (aurora A and aurora B); inhibited JAK2, JAK3 and the BCR–ABL mutant T315I with IC_{50} = 1.1–4.0 nM, and TYK2 and RSK2 with IC_{50} < 10 nM *in vitro*[160]; exhibited potent inhibitory effects in human leukaemia cell lines expressing wild-type BCR–ABL or its mutant forms[160]	• Phase I–II: adult solid tumours, NHL, AML, ALL, CML, MDS and myelofibrosis
CYC-116 (Cyclacel)*	Pan-aurora (VEGFR2)	• *In vitro*: IC_{50} = 19 nM (aurora B), 44 nM (aurora A), 65 nM (aurora C); inhibited VEGFR2 (IC_{50} = 69 nM)[161]; inhibited the spindle checkpoint and cytokinesis, resulting in polyploidy and induction of apoptosis[161]; antitumour activity in various human solid tumour and leukaemia xenograft models[161] • Orally bioavailable	• Phase I: advanced solid tumours
ENMD-2076 (Entremed)*	Aurora A (SRC, KIT, PTK2, VEGFR2 and aurora B)	• *In vitro* IC_{50} = 14 nM (aurora A) and 290 nM (aurora B) [78]; inhibits angiogenesis kinases with IC_{50} = 5 nM (PTK2), 40 nM (KIT), 80 nM (VEGFR2) and 100 nM (SRC)[78]; inhibited tumour growth and reduced microvessel density in various tumour xenografts[78]	• Phase I: advanced cancer and multiple myeloma
GSK1070916 (GlaxoSmith-Kline)	Aurora B and aurora C (aurora A)	• Inhibited aurora kinases with K_i = 0.38 nM (aurora B), 1.5 nM (aurora C), 490 nM (aurora A)[162]; induced tumour regression in mice bearing human colon carcinoma xenografts at a dose of 100 mg per kg (maximum tolerated dose) for each of 5 days with two cycles[162]; long dissociation half lives from aurora B–INCENP and aurora C–INCENP complexes[162]	• Phase I: advanced solid tumours
MLN8237 (Millennium)*	Aurora A (aurora B)	• Inhibited recombinant aurora A with IC_{50} = 1 nM; active against neuroblastoma xenografts *in vivo* (more than standard chemotherapy) and ALL xenografts[71] • Induced cytotoxicity and cell cycle arrest in G2–M phase in multiple myeloma cellular models (at 0.25 mM)[73] • Daily oral administration (20 mg per kg twice daily) to mice bearing lymphoma or leukaemia xenografts was well tolerated and significantly reduced tumour growth[72]	• Phase I–II: paediatric solid tumours or ALL • Phase II: adult NHL, leukaemia and MDS, and ovarian, fallopian tube and peritoneal carcinoma • Phase I: advanced solid tumours and haematological malignancies
MLN8054 (Millennium)*	Aurora A (aurora B)	• *In vitro*: IC_{50} = 4 nM (aurora A) and 172 nM (aurora B)[70]; in cells, showed selective aurora A inhibition at 1 μM, and unselective aurora A and aurora B inhibition at concentrations > 1 μM[70]; blocked cells in G2–M phase and induced spindle defects and apoptosis[70]; induced regression of human tumour xenografts[70]	• Phase I: advanced solid tumours and other malignancies; trials discontinued

The vinyl-pyrimidine ENMD-2076 (FIG. 2) is another orally active aurora kinase inhibitor, which shows selectivity for aurora A. It also targets angiogenesis kinases (that is, SRC, KIT, focal adhesion kinase and VEGFR2)[78]. ENMD-2076 inhibited tumour growth and reduced microvessel density in various tumour xenografts, providing a unique combination of antiproliferative and anti-angiogenic effects[78]. This compound is currently in Phase I trials in patients with advanced cancer and multiple myeloma.

With the exception of VX-680, the development of which was discontinued after reports of QT prolongation, preliminary data from clinical trials of aurora kinase inhibitors have generally indicated disease stabilization, the best response being achieved in solid tumours[79]. The main dose-limiting toxicity of most aurora kinase inhibitors was neutropaenia (loss of neutrophils), with consequent nausea and fatigue. Further investigation is necessary to assess the potential advantages of selectively targeting individual aurora kinases.

Several recently solved crystal structures of aurora A and aurora B in complex with either ADP or small-molecule inhibitors[80] have allowed for the synthesis of novel compounds with increased specificity for aurora A or aurora B (for example, see the aurora A-specific 2-furyl quinazoline TC-28 in BOX 5 and Supplementary information S2 (table)), which will be useful to decipher the therapeutic value of independently targeting these two kinases.

Recent *in vitro* studies with hypermutagenic cancer cell lines identified mutations in the ATP-binding site of aurora B which conferred resistance to several ATP-competitive inhibitors of different chemical classes, including ZM447439, MLN8054, VX-680 and hesperadin[81]. These observations indicate the risk of the emergence of drug-resistant aurora kinase mutants, as seen with BCR–ABL kinase inhibitors. It may therefore be valuable to generate and characterize different aurora kinase mutants in order to explore them as novel targets for structure-based drug design. In addition, the development of non-ATP-competitive aurora kinase antagonists, such as allosteric inhibitors (discussed below), could be of help in preventing resistance.

SAC kinases may be relevant targets in cancer therapy as part of strategies aimed at silencing the mitotic checkpoint. It is important to note that weakened SAC

Table 4 (cont.) | **Selected inhibitors of aurora kinases in clinical trials**

Inhibitor (company)	Main targets (other targets)	Preclinical study data	Clinical trials
PF-03814735 (Pfizer)*	Aurora A and aurora B (NA)	• Orally bioavailable ATP-competitive inhibitor of aurora A and aurora B; proof-of-mechanism data are yet to be disclosed	• Phase I: advanced solid tumours
PHA-739358 (Pfizer/Nerviano)‡	Pan-aurora (BCR–ABL, RET, TRKA and FGFR1)	• *In vitro*: IC_{50} = 13 nM (aurora A), 79 nM (aurora B), 61 nM (aurora C); inhibited FGFR1, BCR–ABL, RET and TRKA (IC_{50} = 25–47 nM)[163,164]; antitumour activity in different human tumour xenografts in nude mice and in spontaneous and transgenic animal tumour models[163]	• Phase II: CML that relapsed after imatinib or BCR–ABL-targeted therapy; MHRPC
R763, also known as AS703569 (Rigel)*	Pan-aurora (NA)	• Blocked cell cycle progression and induced apoptosis in several tumour cell lines and xenografts§ • Initial results from a Phase I trial established tolerated dose levels[165]	• Phase I: advanced solid tumours
SNS-314 (Sunesis)‡	Pan-aurora (VEGFR3, CSF1R, DDR2 and AXL)	• *In vitro*: IC_{50} = 3.4 nM (aurora C), 9 nM (aurora A) and 31 nM (aurora B); inhibited TRKA, TRKB, VEGFR3, CSF1R, DDR2 and AXL (IC_{50} < 100 nM)[166]; induced defects in spindle checkpoint and cytokinesis, leading to multiple rounds of endoreduplication and cell death; suppressed tumour growth in mice bearing a human colon carcinoma xenograft[166]	• Phase I: advanced solid tumours (completed in March 2009)
SU-6668 (Sugen)*	Aurora A and aurora B (VEGFR2, PDGFRβ, FGFR1 and TBK1)	• ATP-competitive inhibitor of multiple tyrosine kinases, with highest potency against PDGFR, aurora B and KIT (*in vitro* IC_{50} < 50 μM)[167]; *in vitro*: IC_{50} = 0.047 μM (aurora B), 0.85 μM (aurora A) and 1.4 μM (TBK1)[167] • Inhibited angiogenesis and tumour growth in a diverse panel of human tumour xenografts[168] • Prevented colon cancer liver metastasis in mice[169]	• Phase I: advanced solid tumours
VX-680, also known as MK-0457 (Vertex/Merck)‡	Pan-aurora (JAK and the BCR –ABL mutant T315I[170])	• *In vitro*: IC_{50} = 0.7 nM (aurora A), 18 nM (aurora B) and 4.6 nM (aurora C) *in vitro*[70]; blocked cell cycle progression and induced apoptosis in human leukaemia xenografts[70]	• Phase I–II: colorectal cancer, NSCLC, ALL, CML and solid tumours; trials discontinued owing to QT prolongation in 1 in 100 patients
VX-689, also known as MK-5108 (Vertex/Merck)*	Aurora kinases (NA)	NA	• Phase I: advanced or refractory solid tumours (as a single agent and in combination with docetaxel)

Data extracted from http://www.clinicaltrials.gov. *Oral. ‡Intravenous. §See http://www.rigel.com/rigel/oncology. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; CSF1R, macrophage colony-stimulating factor 1 receptor (also known as FMS); DDR2, discoidin domain-containing receptor 2; FGFR, fibroblast growth factor receptor; G2, second gap; IC_{50}, compound concentration that caused 50% inhibition of kinase activity (*in vitro* kinase assays) or cellular proliferation (cell proliferation assays); INCENP, inner centromere protein; JAK, janus kinase; M, mitosis; MDS, myelodysplastic syndromes; MHRPC, metastatic hormone-refractory prostate cancer; NA, not available; NHL, non-Hodgkin's lymphoma; NSCLC, non-small-cell lung carcinoma; PDGFRβ, platelet-derived growth factor receptor-β; PTK2, protein tyrosine kinase 2 (also known as FAK); RSK2, ribosomal S6 kinase 2; TBK1, tank-binding kinase 1; TYK2, tyrosine kinase 2; VEGFR, vascular endothelial growth factor receptor.

signalling, owing to mutations of checkpoint regulators or changes in their expression levels, might induce chromosomal instability and cell transformation (BOX 4). Tumour-associated mutations in SAC kinases have been shown to weaken the fidelity of checkpoint signalling[8]. However, further weakening or even silencing of the SAC pathway may be lethal for these cells[8,10]. Consequently, increasing the rate of chromosomal instability of tumour cells with drugs that target essential SAC kinases could be a successful strategy to selectively kill cancer cells. To date, among the SAC kinases, chemical inhibitors have been identified only for aurora B and MPS1. MPS1 inhibitors, namely cincreasin[82] and SP600125 (REF. 83), have been reported to block the mitotic checkpoint, causing chromosome mis-segregation. However, whether this can induce apoptosis in cancer cells remains to be elucidated. The discovery and characterization of new SAC inhibitors are eagerly awaited.

Clearly, the strategy of silencing the mitotic checkpoint also raises concerns regarding potential increases in chromosomal instability in proliferating non-cancerous cells when inhibition is incomplete, with consequent risks of associated tumorigenesis. Whether SAC inhibition might preferentially kill cancer cells over normal cells is currently unknown. However, this approach would not differ substantially from treatment with anticancer drugs such as taxanes or vinca alkaloids, which are largely non-selective for tumour cells or healthy cells but nevertheless have been successfully used for many years. Preclinical studies in animal models, such as the generation of strains with disabled SAC signalling, will be useful for evaluating the therapeutic validity of individual candidate targets in the context of a defined cancer course.

PLKs. In mammals, the PLK family comprises four structurally related but functionally distinct proteins: PLK1, PLK2 (also known as SNK), PLK3 (also known as FNK and PRK) and PLK4 (also known as SAK)[6]. PLK1 is the best-characterized family member, and several studies describe its essential and non-redundant functions for activation of the CDK1–cyclin B complex at the G2 to M phase transition, centrosome maturation, spindle formation, chromosome segregation and cytokinesis. Recently, a role for PLK1 in regulating aurora A localization and activity on centrosomes was also discovered[84].

Table 5 | **Selected inhibitors of polo-like kinases (PLKs) in clinical trials**

Inhibitor (company)	Main targets (other targets)	Preclinical study data	Clinical trials
BI 2536 (Boehringer Ingelheim)‡	PLK1 and PLK2 (PLK3, ERBB4, HGFR, PI3Kα and TIE2)	• *In vitro*: IC_{50} = 0.8 nM (PLK1), 3.5 nM (PLK2), 39.0 nM (PLK3); IC_{50} < 10nM for ERBB4, HGFR, PI3Kα and TIE2 (REF. 90) • Induced mitotic arrest and apoptosis (IC_{50} = 2-30 nM) in multiple tumour cell lines[89]; induced tumour regression in human colon carcinoma xenografts[89]	• Phase II: AML and solid tumours Phase I: NHL
GSK461364 (GlaxoSmith-Kline)‡	PLKs (aurora A and CDK2)	• *In vitro*: IC_{50} = 2.2 nM (PLK1), 9.1 nM (PLK3), 4.8 nM (aurora A) and 7.6 nM (CDK2)[171]; induced arrest in G2–M phase, mitotic spindle defects and apoptosis in tumour cells and, to a lesser degree, normal human fibroblasts[171]	• Phase I: advanced solid tumours and NHL
HMN-214 (Nippon Shinyaku)*	PLK1 (NA)	• Induced arrest in G2–M phase, showed antitumour activity in various human xenografts and was reported to interfere with the subcellular spatial location of PLK1 (REFS 172–174) • In a Phase I study in patients with advanced cancer, the maximum tolerated oral daily dose was 8 mg per m^2 per day[172-174]	• Phase I: advanced solid tumours
ON 01910 (Onconova)‡	NA	• Initially reported as a non-ATP-competitive PLK1 inhibitor but was subsequently found not to inhibit purified PLK1 up to 30 μM[89] • Cellular phenotype consistent with inhibition of microtubule dynamics[70]	• Phase I–II: MDS and ovarian cancer (as a single agent) • Phase I: lymphoid malignancies (as a single agent) • Phase I: hepatoma and other advanced solid tumours (in combination with irinotecan, oxaliplatin or gemcitabine)

Data extracted from http://www.clinicaltrials.gov. *Oral. ‡Intravenous. AML, acute myeloid leukaemia; G2, second gap; HGFR, hepatocyte growth factor receptor (also known as MET); IC_{50}, compound concentration that caused 50% inhibition of kinase activity (*in vitro* kinase assays) or cellular proliferation (cell proliferation assays); M, mitosis; MDS, myelodysplastic syndromes; NA, not available; NHL, non-Hodgkin's lymphoma; PI3Kα, phosphoinositide 3-kinase-α; TIE2, tunica interna endothelial cell kinase (also known as TEK).

PLK1 overexpression has been observed in a wide range of tumour types and is often associated with a poor prognosis[85]. Furthermore, *PLK1* mutations could play a part in tumorigenesis[86]. PLK1 is an attractive anticancer target because targeted interference in PLK1 function induces prolonged mitotic arrest and subsequent onset of apoptosis. Thus, PLK1 inhibition could potentially have the same effect as the microtubule toxins in causing mitotic arrest, without disrupting microtubule dynamics in non-cycling cells, thereby circumventing the adverse effects arising from tubulin interference in non-cancerous cells. Indeed, normal cells seemed to be less sensitive to PLK1 depletion than transformed cells[87,88].

Numerous compounds have been identified that potently block PLK1 activity in an ATP-competitive manner[70]. Among these, the dihydropteridinone BI 2536 (FIG. 2) (currently in Phase I–II trials), the thiophene benzimidazole GSK461364A (currently in Phase I trials) and the stilbene HMN-214 (currently in Phase I trials) are the most clinically advanced (TABLE 5 and Supplementary information S1 (table)). Another compound that is currently in clinical testing, ON01910, was initially described as a PLK1 inhibitor, but subsequent studies found this not to be so[89].

BI 2536 is a potent PLK1 inhibitor[90] with substantial antitumour activity in xenograft models[89]. Initial Phase I trials concluded that BI 2536 is well tolerated, with reversible neutropaenia representing the dose-limiting toxicity[91]. Interestingly, a chemical genetic study using this compound proved that aurora A autophosphorylation, necessary for its activation on the centrosome, is under the control of PLK1 (REF. 84). This finding suggests that the antimitotic efficacy of PLK1 inhibitors could be the result of a dual mechanism that affects both PLK and aurora A signalling pathways[84]. Similarly, a novel potent and selective PLK1 inhibitor, a thiazolidinone known as TAL (BOX 5 and Supplementary information S2 (table)), has been used to gain further insight into the regulation of PLK1 during mitotic exit and cytokinesis[92]. By contrast, another recently described PLK1 inhibitor, the benzothiazole *N*-oxide derivative cyclapolin 1, showed a cellular phenotype that was not fully consistent with selective PLK1 inhibition, as only a small fraction of treated cells were arrested in M phase[70].

A wealth of data on PLK1 inhibition is currently present in the literature, and the recent disclosure of the crystal structure of a PLK1 mutant in complex with a non-hydrolysable ATP analogue[93] could further encourage the discovery and optimization of small-molecule inhibitors, with the goal of achieving tumour cell antiproliferative activity at sub-micromolar concentrations. However, caution should be taken when postulating that the mode of action of a drug is PLK1 inhibition, as potential off-target effects may contribute to the observed phenotype. In addition, ATP-competitive antagonists of PLK1 might also inhibit other members of the PLK family, which seem to have a diverse range of functions in SAC response and centriole biogenesis[6], increasing the risk of side effects. Validation using PLK1-specific cellular assays is therefore necessary.

Novel strategies for cell cycle kinase inhibition

Mimicking RBL2 tumour suppressor functions. The growth suppression functions of retinoblastoma-like protein 2 (RBL2; also known as p130) have been established in certain tumour types[94,95] (BOX 1). Recently, a 39-amino-acid

peptide derived from the spacer region of RBL2, SPA310, was found to retain the CDK2 inhibitory activity and growth arrest properties of the full-length RBL2 spacer domain, both *in vitro* and *in vivo*[96]. SPA310 induced cell cycle arrest in the G0–G1 phase and suppressed tumour formation and progression in nude mice[96]. These observations point to SPA310 as a candidate template molecule for the design of novel CDK inhibitors that function as growth suppressors.

Cyclin-binding inhibitors. In their active complexes, the regulatory subunits of CDKs, the cyclins, contain an exposed, hydrophobic groove. This is conserved in cyclins A, B, D and E[97], located more than 40 Å from the catalytic site on CDKs and functions as an anchor point for substrate recruitment. This site is recognized by numerous cell cycle substrates (for example, RB1, RBL1 (also known as p107), RBL2, p53 and E2F transcription factors) and endogenous inhibitory proteins (for example, p27 (also known as CDKN1B and KIP1) and p21 (also known as CDKN1A and CIP1)), which contain an Arg/Lys–X–Leu ('RXL' or 'KXL') motif. Cell-permeable peptides containing this motif have been reported to induce apoptosis in tumour cells[98] and inhibit tumour growth *in vivo*[99], presumably through inhibition of E2F phosphorylation by CDK2–cyclin A in late S phase, which is necessary for the cell to avoid an apoptotic trigger. A similar mode of action has been proposed to explain the antitumour activity of SPA310 (REF. 100). Development of synthetic CDK inhibitors, based on small molecules such as SPA310 or other peptides containing the RXL or KXL motif, could offer the advantage of enhancing selectivity for CDKs over non-CDK protein kinases.

Protein–protein interactions have long been considered complicated drug targets owing to the inherent difficulty of designing small molecules that are able to disrupt interactions that occur over large, flat areas. However, novel approaches are beginning to emerge following identification of protein binding 'hot spots', such as key contact residues that mediate protein–protein interactions[101]. For example, a recent computational method was generated to replace key binding residues with non-peptidic fragments and applied to the design of pharmaceutically acceptable cyclin-binding inhibitors[102]. Another recent study describes peptide templates for blocking the formation of CDK–cyclin complexes[103].

Allosteric inhibition of mitotic kinases. Recent crystallographic studies of the AGC (cAMP-dependent, cGMP-dependent, protein kinase C) family of kinases revealed the presence of a conserved hydrophobic pocket that mediates interactions with substrates, which in some cases favour the catalysis of ATP by the kinases (allosteric activation)[104]. Analogous interactions through an equivalent hydrophobic pocket found in aurora kinases have been suggested to be responsible for the allosteric activation of aurora A and aurora B by their respective partner proteins, TPX2 (REF. 105) and inner centromere protein (INCENP)[106]. This hydrophobic pocket in aurora kinases might therefore provide an additional docking site for which novel allosteric small-molecule inhibitors could be designed.

Blocking the polo box domain of PLK1. The carboxy-terminal region of PLKs displays a characteristic fold of a six-stranded β-sheet and an α-helix, referred to as the 'polo box', and is responsible for substrate recognition and cellular localisation[6]. Peptides that target the polo box of PLK1 have been shown to decrease its kinase activity[107] and, recently, the first non-peptidic PLK1 polo box blocker, poloxin, has been described[108]. Poloxin (BOX 5 and Supplementary information S2 (table)) is a synthetic thymoquinone which was reported to induce mitotic arrest and apoptosis in HeLa cervical carcinoma cells. Because the polo box domain is unique to PLKs, its exploitation as an alternative docking site for new PLK inhibitors could overcome the hurdle posed by the conserved nature of the ATP-binding pocket of serine–threonine protein kinases for the development of monospecific inhibitors.

Conclusions

Since the elucidation of the mechanism of mammalian cell division almost four decades ago, it has been established that aberrant activity of cell cycle protein kinases is a hallmark of human cancer. Intensive research on small molecules that target cell cycle regulatory proteins has led to the identification of many candidate inhibitors that are able to arrest proliferation and induce apoptosis in neoplastic cells. On the basis of advanced technologies that allow structure-based drug design and chemical genetics, and powerful screening platforms to investigate drug activity and target selectivity[109,110], it is expected that novel compounds with increased potency, improved kinase specificity and favourable drug-like properties will soon be available for clinical evaluation. Furthermore, ongoing studies are providing new template molecules for inhibitor design, information about additional docking sites on kinase targets, as well as an improved list of potential kinase targets, with exciting implications for the generation of novel mechanism-based approaches for cancer drug development.

Box 5 | **Selected inhibitors of cell cycle kinases in preclinical screening**

Cyclin-dependent kinase inhibitors
CDKi-277 (REF. 33), RO-3306 (REF. 45), Purvalanol A (REFS 28,29,175), NU6140 (REF. 29), s-CR8 (REF. 31), N-&-N1 (also known as GP0210) (REFS 30,32), AZ703 (REFS 176,177), JNJ-7706621 (REFS 34,35,178), RGB-286199 (REFS 179,180), a 1,4-diaminocyclohexyl-substituted derivative of SNS-032 (REF. 18) and a nipecotic amide derivative of SNS-032 (REF. 19).

DNA damage checkpoint kinase inhibitors
KU-55933 (REFS 56,57,181), CP466722 (REF. 57), CBP501 (REF. 182), NSC 109555 (REF. 65) and VRX046617 (REF. 64).

Aurora kinase inhibitors
Hesperadin[70], TC-28 (REF. 183), VE-465 (REF. 184) and ZM447439 (REF. 70).

Polo-like kinase inhibitors
Cyclapolin 1 (REF. 70), DAP-81 (REF. 70), Poloxin (REF. 108) and TAL (REF. 92).

For chemical structures of the inhibitors and further information on the inhibitor targets and study data, see Supplementary information S2 (table).

In parallel, several promising strategies for therapeutic intervention are being investigated. Emerging insights into the specific requirement for particular interphase CDKs for the survival of specific tumour cells offer the opportunity to design clinical trials that match the target specificity of the drug candidate with the relevant cancer type. In this context, genomic technologies could be useful to identify the patients that are most likely to benefit from these on the basis of their profile of oncogenic mutations. Current research goals also include the establishment of rational bases for combining CDK inhibitors with classical chemotherapy drugs to enhance clinical efficacy, inhibiting kinases that signal DNA damage to enhance cancer cell sensitivity to genotoxic agents, and targeting SAC kinases to selectively kill tumour cells by increasing their intrinsic chromosomal instability. Further preclinical studies in animal models will be required to determine the validity of each of these strategies. Gains from these studies are expected to be huge, as they will provide a rational basis for designing more focused clinical trials and, consequently, for tailoring therapies to improve treatment for specific tumour types.

1. Boye, E. & Grallert, B. The 2001 Nobel Prize in Physiology or Medicine. *Tidsskr. Nor. Laegeforen.* **121**, 3500 (2001).
2. Weinberg, R. A. The retinoblastoma protein and cell cycle control. *Cell* **81**, 323–330 (1995).
3. Giacinti, C. & Giordano, A. RB and cell cycle progression. *Oncogene* **25**, 5220–5227 (2006).
4. Santamaria, D. *et al.* Cdk1 is sufficient to drive the mammalian cell cycle. *Nature* **448**, 811–815 (2007).
This study showed that genetic ablation of the mitotic kinase CDK1, but not the interphase kinases CDK2, CDK4 and CDK6, causes cell cycle arrest and is lethal to mouse embryos.
5. Barr, A. R. & Gergely, F. Aurora-A: the maker and breaker of spindle poles. *J. Cell Sci.* **120**, 2987–2996 (2007).
6. Archambault, V. & Glover, D. M. Polo-like kinases: conservation and divergence in their functions and regulation. *Nature Rev. Mol. Cell Biol.* **10**, 265–275 (2009).
7. Malumbres, M. & Barbacid, M. Cell cycle, CDKs and cancer: a changing paradigm. *Nature Rev. Cancer* **9**, 153–166 (2009).
8. Kops, G. J. P. L., Weaver, B. A. A. & Cleveland, D. W. On the road to cancer: aneuploidy and the mitotic checkpoint. *Nature Rev. Cancer.* **5**, 773 (2005).
An excellent Review illustrating the relationship between chromosomal instability and cancer, and the strategy of complete inhibition of the mitotic checkpoint to kill cancer cells.
9. Kops, G. J. P. L., Foltz, D. R. & Cleveland, D. W. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proc. Natl Acad. Sci. USA* **101**, 8699–8704 (2004).
10. Weaver, B. A. A., Silk, A. D. & Cleveland, D. W. Low rates of aneuploidy promote tumorigenesis while high rates of aneuploidy cause cell death and tumor suppression. *Cell. Oncol.* **30**, 453–453 (2008).
11. Strock, C. J. *et al.* Cyclin-dependent kinase 5 activity controls cell motility and metastatic potential of prostate cancer cells. *Cancer Res.* **66**, 7509–7515 (2006).
12. Larochelle, S. *et al.* Requirements for Cdk7 in the assembly of Cdk1/Cyclin B and activation of Cdk2 revealed by chemical genetics in human cells. *Mol. Cell* **25**, 839–850 (2007).
13. Romano, G. & Giordano, A. Role of the cyclin-dependent kinase 9-related pathway in mammalian gene expression and human diseases. *Cell Cycle* **7**, 3664–3668 (2008).
14. Shapiro, G. I. Cyclin-dependent kinase pathways as targets for cancer treatment. *J. Clin. Oncol.* **24**, 1770–1783 (2006).
15. Byrd, J. C. *et al.* Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. *Blood* **109**, 399–404 (2007).
16. George, S. *et al.* Phase I study of flavopiridol in combination with paclitaxel and carboplatin in patients with non-small-cell lung cancer. *Clin. Lung Cancer* **9**, 160–165 (2008).
17. Kodym, E. *et al.* The small-molecule CDK inhibitor, SNS-032, enhances cellular radiosensitivity in quiescent and hypoxic non-small cell lung cancer cells. *Lung Cancer* 1 Feb 2009 (doi:10.1016/j.lungcan.2008.12.026).
18. Choong, I. C. *et al.* A diaminocyclohexyl analog of SNS-032 with improved permeability and bioavailability properties. *Bioorg. Med. Chem. Lett.* **18**, 5763–5765 (2008).
19. Fan, J. *et al.* Modifications of the isonipecotic acid fragment of SNS-032: analogs with improved permeability and lower efflux ratio. *Bioorg. Med. Chem. Lett.* **18**, 6236–6239 (2008).
20. Joshi, K. S. *et al. In vitro* antitumor properties of a novel cyclin-dependent kinase inhibitor, P276-00. *Mol. Cancer Ther.* **6**, 918–925 (2007).
21. Joshi, K. S. *et al.* P276-00, a novel cyclin-dependent inhibitor induces G1–G2 arrest, shows antitumor activity on cisplatin-resistant cells and significant *in vivo* efficacy in tumor models. *Mol. Cancer Ther.* **6**, 926–934 (2007).
22. Squires, M. S. *et al.* Biological characterization of AT7519, a small-molecule inhibitor of cyclin-dependent kinases, in human tumor cell lines. *Mol. Cancer Ther.* **8**, 324–332 (2009).
23. DePinto, W. *et al. In vitro* and *in vivo* activity of R547: a potent and selective cyclin-dependent kinase inhibitor currently in phase I clinical trials. *Mol. Cancer Ther.* **5**, 2644–2658 (2006).
24. Diab, S. *et al.* A phase I study of R547, a novel, selective inhibitor of cell cycle and transcriptional cyclin dependent kinases (CDKs). *J. Clin. Oncol.* **25**, 3528 (2007).
25. Siemeister, G. *et al.* Molecular and pharmacodynamic characteristics of the novel multi-target tumor growth inhibitor ZK 304709. *Biomed. Pharmacother.* **60**, 269–272 (2006).
26. Scholz, A. *et al.* The oral multitarget tumour growth inhibitor, ZK 304709, inhibits growth of pancreatic neuroendocrine tumours in an orthotopic mouse model. *Gut* **58**, 261–270 (2009).
27. McClue, S. J. *et al. In vitro* and *in vivo* antitumor properties of the cyclin dependent kinase inhibitor CYC202 (R-roscovitine). *Int. J. Cancer* **102**, 463–468 (2002).
28. Villerbu, N., Gaben, A. M., Redeuilh, G. & Mester, J. Cellular effects of purvalanol A: a specific inhibitor of cyclin-dependent kinase activities. *Int. J. Cancer* **97**, 761–769 (2002).
29. Pennati, M. *et al.* Potentiation of paclitaxel-induced apoptosis by the novel cyclin-dependent kinase inhibitor NU6140: A possible role for survivin down-regulation. *Mol. Cancer Ther.* **4**, 1328–1337 (2005).
30. Bettayeb, K. *et al.* N-&-N, a new class of cell death-inducing kinase inhibitors derived from the purine roscovitine. *Mol. Cancer Ther.* **7**, 2713–2724 (2008).
31. Bettayeb, K. *et al.* CR8, a potent and selective, roscovitine-derived inhibitor of cyclin-dependent kinases. *Oncogene* **27**, 5797–5807 (2008).
32. Popowycz, F. *et al.* Pyrazolo[1,5-a]-1,3 5-triazine as a purine bioisostere: access to potent cyclin-dependent kinase inhibitor (R)-roscovitine analogue. *J. Med. Chem.* **52**, 655–663 (2009).
33. Payton, M. *et al.* Discovery and evaluation of dual CDK1 and CDK2 inhibitors. *Cancer Res.* **66**, 4299–4308 (2006).
34. Emanuel, S. *et al.* The *in vitro* and *in vivo* effects of jnj-7706621: a dual inhibitor of cyclin-dependent kinases and Aurora kinases. *Cancer Res.* **65**, 9038–9046 (2005).
35. Seamon, J. A. *et al.* Role of the ABCG2 drug transporter in the resistance and oral bioavailability of a potent cyclin-dependent kinase/Aurora kinase inhibitor. *Mol. Cancer Ther.* **5**, 2459–2467 (2006).
36. Yu, Q., Geng, Y. & Sicinski, P. Specific protection against breast cancers by cyclin D1 ablation. *Nature* **411**, 1017–1021 (2001).
37. Reddy, H. K. D. L. *et al.* Cyclin-dependent kinase 4 expression is essential for neu-induced breast tumorigenesis. *Cancer Res.* **65**, 10174–10178 (2005).
38. Landis, M. W., Pawlyk, B. S., Li, T., Sicinski, P. & Hinds, P. W. Cyclin D1-dependent kinase activity in murine development and mammary tumorigenesis. *Cancer Cell* **9**, 13–22 (2006).
39. Yu, Q. *et al.* Requirement for CDK4 kinase function in breast cancer. *Cancer Cell* **9**, 23–32 (2006).
References 36–39 showed that cyclin D1-dependent CDK4 activity is required for the development of *Erbb2*- and *Hras*-induced breast cancer in mice, suggesting possible therapeutic uses of CDK4 inhibitors in patients with HER2-positive breast cancer.
40. Rodriguez-Puebla, M. L. *et al.* Cdk4 deficiency inhibits skin tumor development but does not affect normal keratinocyte proliferation. *Am. J. Pathol.* **161**, 405–411 (2002).
41. Miliani de Marval, P. L. *et al.* Lack of cyclin-dependent kinase 4 inhibits c-myc tumorigenic activities in epithelial tissues. *Mol. Cell. Biol.* **24**, 7538–7547 (2004).
42. Hu, M. *et al.* A requirement for cyclin-dependent kinase 6 in thymocyte development and tumorigenesis. *Cancer Res.* **69**, 810–818 (2009).
This work showed that CDK6-deficient mice are resistant to AKT-induced lymphomagenesis, supporting possible therapeutic uses of CDK6 inhibitors against human lymphoid tumours.
43. Toogood, P. *et al.* Discovery of a potent and selective inhibitor of cyclin-dependent kinase 4/6. *J. Med. Chem.* **48**, 2388–2406 (2005).
44. Goga, A., Yang, D., Tward, A. D., Morgan, D. O. & Bishop, J. M. Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. *Nature Med.* **13**, 820–827 (2007).
This preliminary study showed that CDK1 inhibition might be a suitable therapeutic target in cells transformed by *Myc* but not other oncogenes.
45. Vassilev, L. T. *et al.* Selective small-molecule inhibitor reveals critical mitotic functions of human CDK1. *Proc. Natl Acad. Sci. USA* **103**, 10660–10665 (2006).
46. Ortega, S. *et al.* Cyclin-dependent kinase 2 is essential for meiosis but not for mitotic cell division in mice. *Nature Genet.* **35**, 25–31 (2003).
47. Berthet, C., Aleem, E., Coppola, V., Tessarollo, L. & Kaldis, P. Cdk2 knockout mice are viable. *Curr. Biol.* **13**, 1775–1785 (2003).
The work reported in references 46 and 47 showed that *Cdk2* is not an essential gene for the mitotic cell cycle in mice.
48. Malumbres, M. *et al.* Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. *Cell* **118**, 493–504 (2004).
49. Rane, S. G. *et al.* Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in β-islet cell hyperplasia. *Nature Genet.* **22**, 44–52 (1999).
50. Tsutsui, T. *et al.* Targeted disruption of CDK4 delays cell cycle entry with enhanced $p27^{Kip1}$ activity. *Mol. Cell. Biol.* **19**, 7011–7019 (1999).
51. Martin, J. *et al.* Genetic rescue of Cdk4 null mice restores pancreatic β-cell proliferation but not homeostatic cell number. *Oncogene* **22**, 5261–5269 (2003).
52. Jirawatnotai, S. *et al.* Cdk4 is indispensable for postnatal proliferation of the anterior pituitary. *J. Biol. Chem.* **279**, 51100–51106 (2004).

53. Li, T., Inoue, A., Lahti, J. M. & Kidd, V. J. Failure to proliferate and mitotic arrest of CDK11 (p110/p58)-null mutant mice at the blastocyst stage of embryonic cell development. *Mol. Cell. Biol.* **24**, 3188–3197 (2004).
54. Barrière, C. *et al.* Mice thrive without Cdk4 and Cdk2. *Mol. Oncol.* **1**, 72–83 (2007).
This article provided genetic evidence that both CDK2 and CDK4 are dispensable for homeostasis in adult mice.
55. Shiloh, Y. ATM and related protein kinases: safeguarding genome integrity. *Nature Rev. Cancer* **3**, 155–168 (2003).
56. Hickson, I. *et al.* Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. *Cancer Res.* **64**, 9152–9159 (2004).
57. Rainey, M. D., Charlton, M. E., Stanton, R. V. & Kastan, M. B. Transient inhibition of ATM kinase is sufficient to enhance cellular sensitivity to ionizing radiation. *Cancer Res.* **68**, 7466–7474 (2008).
58. Busby, E. C., Leistritz, D. F., Abraham, R. T., Karnitz, L. M. & Sarkaria, J. N. The radiosensitizing agent 7-hydroxystaurosporine (UCN-01) inhibits the DNA damage checkpoint kinase hChk1. *Cancer Res.* **60**, 2108–2112 (2000).
59. Matthews, D. J. *et al.* Pharmacological abrogation of S-phase checkpoint enhances the anti-tumor activity of gemcitabine *in vivo*. *Cell Cycle* **6**, 104–110 (2007).
60. Zabludoff, S. D. *et al.* AZD7762, a novel checkpoint kinase inhibitor, drives checkpoint abrogation and potentiates DNA-targeted therapies. *Mol. Cancer Ther.* **7**, 2955–2966 (2008).
61. Ashwell, S., Janetka, J. W. & Zabludoff, S. Keeping checkpoint kinases in line: new selective inhibitors in clinical trials. *Expert Opin. Investig. Drugs* **17**, 1331–1340 (2008).
62. Morgan, M. A. *et al.* Role of checkpoint kinase 1 in preventing premature mitosis in response to gemcitabine. *Cancer Res.* **65**, 6835–6842 (2005).
63. Xiao, Z., Xue, J., Sowin, T. J. & Zhang, H. Differential roles of checkpoint kinase 1, checkpoint kinase 2, and mitogen-activated protein kinase-2 in mediating DNA damage-induced cell cycle arrest: implications for cancer therapy. *Mol. Cancer Ther.* **5**, 1935–1943 (2006).
64. Carlessi, L. *et al.* Biochemical and cellular characterization of VRX0466617, a novel and selective inhibitor for the checkpoint kinase Chk2. *Mol. Cancer Ther.* **6**, 935–944 (2007).
65. Jobson, A. G. *et al.* Identification of a bis-guanylhydrazone [4, 4′-diacetyldiphenylurea-bis(guanylhydrazone); NSC 109555] as a novel chemotype for inhibition of Chk2 kinase. *Mol. Pharmacol.* **72**, 876–884 (2007).
66. Takai, H. *et al.* Chk2-deficient mice exhibit radioresistance and defective p53-mediated transcription. *EMBO J.* **21**, 5195–5205 (2002).
67. Bartek, J. & Lukas, J. Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell* **3**, 421–429 (2003).
68. Xiao, Z. *et al.* Cyclin B1 is an efficacy-predicting biomarker for Chk1 inhibitors. *Biomarkers* **13**, 579–596 (2008).
69. Jordan, M. A. & Wilson, L. Microtubules as a target for anticancer drugs. *Nature Rev. Cancer* **4**, 253–265 (2004).
70. Taylor, S. & Peters, J.-M. Polo and Aurora kinases: lessons derived from chemical biology. *Curr. Opin. Cell Biol.* **20**, 77–84 (2008).
This article reviews the use of small-molecule kinase inhibitors to elucidate the functions of specific mitotic kinases.
71. Smith, M. A. *et al.* Pediatric preclinical testing program (PPTP) stage 2 testing of the Aurora A kinase inhibitor MLN8237. 20th EORTC-NCI-AACR Symposium, 21–24 October 2008, Geneva, Switzerland.
72. Huck, J., Zhang, S., Hyer, M. L. & Manfredi, M. G. Anti-tumor activity of the aurora A inhibitor MLN8237 in diffuse large B-cell lymphoma preclinical models. 50th ASH annual meeting, 6–8 December, 2008, San Francisco, USA.
73. Gorgun, G. *et al.* A novel aurora-A kinase inhibitor MLN8237 induces cytotoxicity and cell cycle arrest in experimental multiple myeloma models. 50th ASH annual meeting, 6–8 December, 2008, San Francisco, USA.
74. Mortlock, A. A. *et al.* Discovery, synthesis, and *in vivo* activity of a new class of pyrazoloquinazolines as selective inhibitors of Aurora B kinase. *J. Med. Chem.* **50**, 2213–2224 (2007).
75. Yang, J. *et al.* AZD1152, a novel and selective aurora B kinase inhibitor, induces growth arrest, apoptosis, and sensitization for tubulin depolymerizing agent or topoisomerase II inhibitor in human acute leukemia cells *in vitro* and *in vivo*. *Blood* **110**, 2034–2040 (2007).
76. Walsby, E., Walsh, V., Pepper, C., Burnett, A. & Mills, K. Effects of the aurora kinase inhibitors AZD1152-HQPA and ZM447439 on growth arrest and polyploidy in acute myeloid leukemia cell lines and primary blasts. *Haematologica* **93**, 662–669 (2008).
77. Tao, Y. *et al.* Enhancement of radiation response in p53-deficient cancer cells by the aurora-B kinase inhibitor AZD1152. *Oncogene* **27**, 3244–3255 (2008).
78. Bray, M. R. ENMD-2076, an oral Aurora A and angiogenesis kinase inhibitor. AACR Annual Meeting, 12–16 April 2008, San Diego, USA.
79. Gautschi, O. *et al.* Aurora kinases as anticancer drug targets. *Clin. Cancer Res.* **14**, 1639–1648 (2008).
80. Matthews, N., Visintin, C., Hartzoulakis, B., Jarvis, A. & Selwood, D. L. Aurora A and B kinases as targets for cancer: will they be selective for tumors? *Expert Rev. Anticancer Ther.* **6**, 109–120 (2006).
81. Girdler, F. *et al.* Molecular basis of drug resistance in Aurora kinases. *Chem. Biol.* **15**, 552 (2008).
This work describes drug-resistant aurora B mutants. Such mutants could represent novel anticancer drug targets.
82. Dorer, R. K. *et al.* A small-molecule inhibitor of Mps1 blocks the spindle-checkpoint response to a lack of tension on mitotic chromosomes. *Curr. Biol.* **15**, 1070–1076 (2005).
83. Schmidt, M., Budirahardja, Y., Klompmaker, R. & Medema, R. H. Ablation of the spindle assembly checkpoint by a compound targeting Mps1. *EMBO Reports* **6**, 866–872 (2005).
84. Scutt, P. J. *et al.* Discovery and exploitation of inhibitor-resistant Aurora and Polo kinase mutants for the analysis of mitotic networks. *J. Biol. Chem.* **284**, 15880–15893 (2009).
This work showed that the generation and characterization of drug-resistant aurora kinase mutants and polo-like kinase mutants is a powerful approach to validate small-molecule inhibitor-induced phenotypes against target and off-target effects.
85. Takai, N., Hamanaka, R., Yoshimatsu, J. & Miyakawa, I. Polo-like kinases (Plks) and cancer. *Oncogene* **24**, 287–291 (2005).
86. Simizu, S. & Osada, H. Mutations in the Plk gene lead to instability of Plk protein in human tumour cell lines. *Nature Cell Biol.* **2**, 852–854 (2000).
87. Guan, R. *et al.* Small interfering RNA-mediated Polo-like kinase 1 depletion preferentially reduces the survival of p53-defective, oncogenic transformed cells and inhibits tumor growth in animals. *Cancer Res.* **65**, 2698–2704 (2005).
88. Liu, X., Lei, M. & Erikson, R. L. Normal cells, but not cancer cells, survive severe Plk1 depletion. *Mol. Cell. Biol.* **26**, 2093–2108 (2006).
89. Steegmaier, M. *et al.* BI 2536, a potent and selective inhibitor of Polo-like kinase 1, inhibits tumor growth *in vivo*. *Curr. Biol.* **17**, 316–322 (2007).
90. Lénárt, P. *et al.* The small-molecule inhibitor BI 2536 reveals novel insights into mitotic roles of Polo-like kinase 1. *Curr. Biol.* **17**, 304–315 (2007).
91. Mross, K. *et al.* Phase I dose escalation and pharmacokinetic study of BI 2536, a novel Polo-like kinase 1 inhibitor, in patients with advanced solid tumors. *J. Clin. Oncol.* **26**, 5511–5517 (2008).
92. Santamaria, A. *et al.* Use of the novel Plk1 inhibitor zk-thiazolidinone to elucidate functions of Plk1 in early and late stages of mitosis. *Mol. Biol. Cell* **18**, 4024–4036 (2007).
93. Kothe, M. *et al.* Structure of the catalytic domain of human Polo-like kinase 1. *Biochemistry (Mosc).* **46**, 5960–5971 (2007).
94. Claudio, P. P. *et al.* P130/pRb2 has growth suppressive properties similar to yet distinctive from those of retinoblastoma family members pRb and p107. *Cancer Res.* **54**, 5556–5560 (1994).
95. Claudio, P. P. *et al.* Functional analysis of pRb2/p130 interaction with cyclins. *Cancer Res.* **56**, 2003–2008 (1996).
96. Bagella, L. *et al.* A small molecule based on the pRb2/p130 spacer domain leads to inhibition of cdk2 activity, cell cycle arrest and tumor growth reduction *in vivo*. *Oncogene* **26**, 1829–1839 (2007).
This study provides the first description of an RBL2-derived small peptide with antitumour activity *in vivo*.
97. Brown, N. R. *et al.* The crystal structure of cyclin A. *Structure* **3**, 1235–1247 (1995).
98. Chen, Y.-N. P. *et al.* Selective killing of transformed cells by cyclin/cyclin-dependent kinase 2 antagonists. *Proc. Natl Acad. Sci. USA* **96**, 4325–4329 (1999).
99. Mendoza, N. *et al.* Selective cyclin-dependent kinase 2/cyclin A antagonists that differ from ATP site inhibitors block tumor growth. *Cancer Res.* **63**, 1020–1024 (2003).
100. Giordano, A., Bellacchio, E., Bagella, L. & Paggi, M. G. Interaction between the Cdk2/Cyclin A complex and a small molecule derived from the pRb2/p130 spacer domain: a theoretical model. *Cell Cycle* **6**, 2591–2593 (2007).
101. Wells, J. A. & McClendon, C. L. Reaching for high-hanging fruit in drug discovery at protein–protein interfaces. *Nature* **450**, 1001–1009 (2007).
102. Andrews, M. J. I. *et al.* Replace: a strategy for iterative design of cyclin-binding groove inhibitors. *Chembiochem.* **7**, 1909–1915 (2006).
103. Canela, N. *et al.* Identification of an hexapeptide that binds to a surface pocket in Cyclin A and inhibits the catalytic activity of the complex cyclin-dependent kinase 2–Cyclin A. *J. Biol. Chem.* **281**, 35942–35953 (2006).
104. Gold, M. G., Barford, D. & Komander, D. Lining the pockets of kinases and phosphatases. *Curr. Opin. Struct. Biol.* **16**, 693–701 (2006).
105. Anderson, K. *et al.* Binding of TPX2 to aurora A alters substrate and inhibitor interactions. *Biochemistry (Mosc).* **46**, 10287–10295 (2007).
106. Jeyaprakash, A. A. *et al.* Structure of a survivin-borealin-INCENP core complex reveals how chromosomal passengers travel together. *Cell* **131**, 271–285 (2007).
107. Garcia-Alvarez, B., de Carcer, G., Ibanez, S., Bragado-Nilsson, E. & Montoyade, G. Molecular and structural basis of polo-like kinase 1 substrate recognition: implications in centrosomal localization. *Proc. Natl Acad. Sci. USA* **104**, 3107–3112 (2007).
108. Reindl, W., Yuan, J., Krämer, A., Strebhardt, K. & Berg, T. Inhibition of Polo-like kinase 1 by blocking Polo-box domain-dependent protein–protein interactions. *Chem. Biol.* **15**, 459–466 (2008).
109. Whitehurst, A. W. *et al.* Synthetic lethal screen identification of chemosensitizer loci in cancer cells. *Nature* **446**, 815–819 (2007).
110. Karaman, M. W. *et al.* A quantitative analysis of kinase inhibitor selectivity. *Nature Biotech.* **26**, 127–132 (2008). .
111. Stiegler, P., Kasten, M. & Giordano, A. The RB family of cell cycle regulatory factors. *J. Cell. Biochem. Suppl.* **30–31**, 30–36 (1998).
112. Claudio, P. P., Tonini, T. & Giordano, A. The retino-blastoma family: twins or distant cousins? *Genome Biology* **3**, 1–9 (2002).
113. Pajalunga, D. *et al.* Critical requirement for cell cycle inhibitors in sustaining nonproliferative states. *J. Cell Biol.* **176**, 807–818 (2007).
114. Binne, U. K. *et al.* Retinoblastoma protein and anaphase-promoting complex physically interact and functionally cooperate during cell-cycle exit. *Nature Cell Biol.* **9**, 225–232 (2007).
115. Zhu, L., Harlow, E. & Dynlacht, B. D. P107 uses a p21cip1-related domain to bind cyclin/cdk2 and regulate interactions with E2F. *Genes Dev.* **9**, 1740–1752 (1995).
116. Woo, M. S., Sanchez, I. & Dynlacht, B. D. P130 and p107 use a conserved domain to inhibit cellular cyclin- dependent kinase activity. *Mol. Cell. Biol.* **17**, 3566–3579 (1997).
117. Claudio, P. P. *et al.* Mutations in the retinoblastoma-related gene rb2/p130 in lung tumors and suppression of tumor growth *in vivo* by retrovirus-mediated gene transfer. *Cancer Res.* **60**, 372–382 (2000).
118. Howard, C. M. *et al.* Inducible pRb2/p130 expression and growth-suppressive mechanisms: evidence of a pRb2/p130, $p27^{Kip1}$, and Cyclin E negative feedback regulatory loop. *Cancer Res.* **60**, 2737–2744 (2000).
119. De Luca, A. *et al.* A unique domain of pRb2/p130 acts as an inhibitor of Cdk2 kinase activity. *J. Biol. Chem.* **272**, 20971–20974 (1997).
120. Wikenheiser-Brokamp, K. Retinoblastoma family proteins: insights gained through genetic manipulation of mice. *Cell. Mol. Life Sci.* **63**, 767–780 (2006).
121. Dimaras, H. *et al.* Loss of RB1 induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. *Hum. Mol. Genet.* **17**, 1363–1372 (2008).
122. Knudsen, E. S. & Knudsen, K. E. Tailoring to RB: tumour suppressor status and therapeutic response. *Nature Rev. Cancer* **8**, 714–724 (2008).
123. Paggi, M. G. & Giordano, A. Who is the boss in the retinoblastoma family? The point of view of Rb2/p130, the little brother. *Cancer Res.* **61**, 4651–4654 (2001).

124. Ortega, S., Malumbres, M. & Barbacid, M. Cyclin D-dependent kinases, INK4 inhibitors and cancer. *Biochim. Biophys. Acta* **1602**, 73–87 (2002).
125. Perez de Castro, I., de Carcer, G. & Malumbres, M. A census of mitotic cancer genes: new insights into tumor cell biology and cancer therapy. *Carcinogenesis* **28**, 899–912 (2007).
126. Yokoyama, H. *et al.* Cdk11 is a RanGTP-dependent microtubule stabilization factor that regulates spindle assembly rate. *J. Cell Biol.* **180**, 867–875 (2008).
127. Hu, D., Valentine, M., Kidd, V. J. & Lahti, J. M. Cdk11p58 is required for the maintenance of sister chromatid cohesion. *J. Cell Sci.* **120**, 2424–2434 (2007).
128. Chandramouli, A. *et al.* Haploinsufficiency of the cdc2l gene contributes to skin cancer development in mice. *Carcinogenesis* **28**, 2028–2035 (2007).
129. Lavin, M. F. Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. *Nature Rev. Mol. Cell Biol.* **9**, 759–769 (2008).
130. Sherr, C. J. & McCormick, F. The RB and p53 pathways in cancer. *Cancer Cell* **2**, 103–112 (2002).
131. Vogel, C., Hager, C. & Bastians, H. Mechanisms of mitotic cell death induced by chemotherapy-mediated G2 checkpoint abrogation. *Cancer Res.* **67**, 339–345 (2007).
132. Ruchaud, S., Carmena, M. & Earnshaw, W. C. Chromosomal passengers: conducting cell division. *Nature Rev. Mol. Cell Biol.* **8**, 798–812 (2007).
133. Yuen, K. W. & Desai, A. The wages of CIN. *J. Cell Biol.* **180**, 661–663 (2008).
134. Zuo, L. *et al.* Germline mutations in the $p16^{INK4a}$ binding domain of CDK4 in familial melanoma. *Nature Genet.* **12**, 97–99 (1996).
135. Smalley, K. S. M. *et al.* Identification of a novel subgroup of melanomas with KIT/cyclin-dependent kinase-4 overexpression. *Cancer Res.* **68**, 5743–5752 (2008).
136. Bogdanova, N. *et al.* A nonsense mutation (E1978X) in the ATM gene is associated with breast cancer. *Breast Cancer Res. Treat.* 21 Sep 2008 (doi:10.1007/s10549-008-0189-9).
137. Menoyo, A. *et al.* Somatic mutations in the DNA damage-response genes ATR and CHK1 in sporadic stomach tumors with microsatellite instability. *Cancer Res.* **61**, 7727–7730 (2001).
138. Vassileva, V., Millar, A., Briollais, L., Chapman, W. & Bapat, B. Genes involved in DNA repair are mutational targets in endometrial cancers with microsatellite instability. *Cancer Res.* **62**, 4095–4099 (2002).
139. Wu, X., Dong, X., Liu, W. & Chen, J. Characterization of CHEK2 mutations in prostate cancer. *Hum. Mutat.* **27**, 742–747 (2006).
140. Matsuura, S. *et al.* Monoallelic BUB1B mutations and defective mitotic-spindle checkpoint in seven families with premature chromatid separation (PCS) syndrome. *Am. J. Med. Genet. A* **140**, 358–367 (2006).
141. Zhang, C. C. *et al.* AG-024322 is a potent and selective multi-targeted CDK inhibitor with broad spectrum anti-proliferative activity. *Proc Amer. Assoc. Cancer Res.* **46**, Abstr. 4415 (2005).
142. Zhang, C. C. *et al.* AG-024322 is a multi-targeted CDK inhibitor with potent antitumor activity *in vivo*. *Proc Amer. Assoc. Cancer Res.* **46**, Abstr. 4413 (2005).
143. Brown, A. *et al.* Toxicity and toxicokinetics of the cyclin-dependent kinase inhibitor AG-024322 in cynomolgus monkeys following intravenous infusion. *Cancer Chemother. Pharmacol.* **62**, 1091–1101 (2008).
144. Sedlacek, H. H. Mechanisms of action of flavopiridol. *Crit. Rev. Oncol. Hematol.* **38**, 139–170 (2001).
145. Christian, B. A., Grever, M. R., Byrd, J. C. & Lin, T. S. Flavopiridol in the treatment of chronic lymphocytic leukemia. *Curr. Opin. Oncol.* **19**, 573–578 (2007).
146. Owa, T. *et al.* Synthesis and biological evaluation of n-(7-indolyl)-3-pyridinesulfonamide derivatives as potent antitumor agents. *Bioorg. Med. Chem. Lett.* **12**, 2097–100 (2002).
147. Talbot, D. C. *et al.* A randomized phase II pharmacokinetic and pharmacodynamic study of indisulam as second-line therapy in patients with advanced non-small cell lung cancer. *Clin. Cancer Res.* **13**, 1816–22 (2007).
148. Raje, N. *et al.* Preclinical activity of P276–00, a novel small-molecule cyclin-dependent kinase inhibitor in the therapy of multiple myeloma. *Leukemia* (2009).
149. Fry, D. W. *et al.* Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. *Mol. Cancer Ther.* **3**, 1427–1438 (2004).
150. Baughn, L. B. *et al.* A novel orally active small molecule potently induces G1 arrest in primary myeloma cells and prevents tumor growth by specific inhibition of cyclin-dependent kinase 4/6. *Cancer Res.* **66**, 7661–7667 (2006).
151. Menu, E. *et al.* A novel therapeutic combination using PD 0332991 and bortezomib: study in the 5T33MM myeloma model. *Cancer Res.* **68**, 5519–5523 (2008).
152. Benson, C. *et al.* A phase I trial of the selective oral cyclin-dependent kinase inhibitor seliciclib (CYC202; R-roscovitine), administered twice daily for 7 days every 21 days. *Br. J. Cancer* **96**, 29–37 (2006).
153. Shapiro, G. I. *et al.* A phase I dose-escalation study of the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of the novel cyclin-dependent kinase inhibitor SCH 727965 administered every 3 weeks in subjects with advanced malignancies. *J. Clin. Oncol.* **26**, 3532 (2008).
154. Misra, R. N. *et al.* N-(cycloalkylamino)acyl-2-aminothiazole inhibitors of cyclin-dependent kinase 2. N-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl] thio]-2-thiazolyl]-4- piperidinecarboxamide (BMS-387032), a highly efficacious and selective antitumor agent. *J. Med. Chem.* **47**, 1719–1728 (2004).
155. Kamath, A. V., Chong, S., Chang, M. & Marathe, P. H. P-glycoprotein plays a role in the oral absorption of BMS-387032, a potent cyclin-dependent kinase 2 inhibitor, in rats. *Cancer Chemother. Pharmacol.* **55**, 110–116 (2005).
156. Smolewski, P. Terameprocol, a novel site-specific transcription inhibitor with anticancer activity. *IDrugs* **11**, 204–214 (2008).
157. Scott, E. *et al.* A phase I dose escalation study of the pharmacokinetics and tolerability of ZK 304709, an oral multi-targeted growth inhibitor (MTGI™), in patients with advanced solid tumours. *Cancer Chemother. Pharmacol.* 12 Mar 2009 (doi:0.1007/s00280-009-0968-y).
158. Seynaeve, C., Kazanietz, M., Blumberg, P., Sausville, E. & Worland, P. Differential inhibition of protein kinase C isozymes by UCN-01, a staurosporine analogue. *Mol. Pharmacol.* **45**, 1207–1214 (1994).
159. Wilkinson, R. W. *et al.* AZD1152, a selective inhibitor of aurora B kinase, inhibits human tumor xenograft growth by inducing apoptosis. *Clin. Cancer Res.* **13**, 3682–3688 (2007).
160. Howard, S. *et al.* Fragment-based discovery of the pyrazol-4-yl urea (AT9283), a multitargeted kinase inhibitor with potent aurora kinase activity. *J. Med. Chem.* **52**, 379–388 (2009).
161. Griffiths, G. *et al.* Anti-tumor activity of CYC116, a novel small molecule inhibitor of Aurora kinases and VEGFR2. AACR Annual Meeting, 12–16 April 2008, San Diego, USA.
162. Anderson, K. *et al.* Biochemical characterization of GSK1070916, a potent and selective inhibitor of aurora B and aurora C kinases with an extremely long residence time. *Biochem. J.* **420**, 259–265 (2009).
163. Carpinelli, P. *et al.* PHA-739358, a potent inhibitor of Aurora kinases with a selective target inhibition profile relevant to cancer. *Mol Cancer Ther* **6**, 3158–3168 (2007).
164. Fancelli, D. *et al.* 1,4,5,6-Tetrahydropyrrolo[3,4-c] pyrazoles: Identification of a potent Aurora kinase inhibitor with a favorable antitumor kinase inhibition profile. *J. Med. Chem.* **49**, 7247–7251 (2006).
165. Renshaw, J. S. *et al.* A phase I two arm trial of AS703569 (R763), an orally available aurora kinase inhibitor, in subjects with solid tumors: preliminary results. *J. Clin. Oncol. (Meeting Abstracts)* **25**, 14130 (2007).
166. Evanchik, M. J. *et al.* Sns-314, a potent inhibitor of Aurora kinases, has preclinical anti-activity and induces apoptosis. AACR Annual Meeting, 12–16 April 2008, San Diego, USA.
167. Godl, K. *et al.* Proteomic characterization of the angiogenesis inhibitor SU6668 reveals multiple impacts on cellular kinase signaling. *Cancer Res.* **65**, 6919–6926 (2005).
168. Laird, A. D. *et al.* SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Res.* **60**, 4152–4160 (2000).
169. Shaheen, R. M. *et al.* Tyrosine kinase inhibition of multiple angiogenic growth factor receptors improves survival in mice bearing colon cancer liver metastases by inhibition of endothelial cell survival mechanisms. *Cancer Res.* **61**, 1464–1468 (2001).
170. Carter, T. A. *et al.* Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc. Natl Acad. Sci. USA* **102**, 11011–11016 (2005).
171. Lansing, T. J. *et al. In vitro* biological activity of a novel small-molecule inhibitor of polo-like kinase 1. *Mol. Cancer Ther.* **6**, 450–459 (2007).
172. Garland, L. L., Taylor, C., Pilkington, D. L., Cohen, J. L. & Von Hoff, D. D. A Phase I pharmacokinetic study of HMN-214, a novel oral stilbene derivative with Polo-like kinase-1-interacting properties, in patients with advanced solid tumors. *Clin. Cancer Res.* **12**, 5182–5189 (2006).
173. Takagi, M. *et al. In vivo* antitumor activity of a novel sulfonamide, HMN-214, against human tumor xenografts in mice and the spectrum of cytotoxicity of its active metabolite, HMN-176. *Invest. New Drugs* **21**, 387–399 (2003).
174. Tanaka, H. *et al.* HMN-176, an active metabolite of the synthetic antitumor agent HMN-214, restores chemosensitivity to multidrug-resistant cells by targeting the transcription factor NF-Y. *Cancer Res.* **63**, 6942–6947 (2003).
175. Iizuka, D., Ogura, A., Kuwabara, M. & Inanami, O. Purvalanol A induces apoptosis and downregulation of antiapoptotic proteins through abrogation of phosphorylation of JAK2/STAT3 and RNA polymerase II. *Anticancer Drugs* **19**, 565–572 (2008).
176. Byth, K. F., Geh, C., Forder, C. L., Oakes, S. E. & Thomas, A. P. The cellular phenotype of AZ703, a novel selective imidazo[1,2-α]pyridine cyclin-dependent kinase inhibitor. *Mol. Cancer Ther.* **5**, 655–664 (2006).
177. Cai, D., Byth, K. F. & Shapiro, G. I. AZ703, an imidazo[1,2-α]pyridine inhibitor of cyclin-dependent kinases 1 and 2, induces E2F-1-dependent apoptosis enhanced by depletion of cyclin-dependent kinase 9. *Cancer Res.* **66**, 435–444 (2006).
178. Lin, R. *et al.* 1-acyl-1h-[1,2,4]triazole-3,5-diamine analogues as novel and potent anticancer cyclin-dependent kinase inhibitors: Synthesis and evaluation of biological activities. *J. Med. Chem.* **48**, 4208–4211 (2005).
179. Wang, S., Ayyub, E. & Lamphere, L. Evaluation of selective biomarkers of the broad spectrum CDK inhibitor: RGB-286199. *Proc. Amer. Assoc. Cancer Res.* **46**, Abstr. 4428 (2005).
180. Oalmann, C. J. *et al.* Indenopyrazoles: potent, broad-spectrum cyclin dependent kinase inhibitors. *232nd ACS National Meeting, 10–14 Sep 2006, San Francisco, USA.*
181. Ivanov, V., Zhou, H., Partridge, M. & Hei, T. Inhibition of ataxia telangiectasia mutated kinase activity enhances TRAIL-mediated apoptosis in human melanoma cells. *Cancer Res.* **69**, 3510 (2009).
182. Sha, S.-K. *et al.* Cell cycle phenotype-based optimization of G2-abrogating peptides yields CBP501 with a unique mechanism of action at the G2 checkpoint. *Mol. Cancer Ther.* **6**, 147–153 (2007).
183. Sardon, T., Cottin, T., Xu, J., Giannis, A. & Vernos, I. Development and biological evaluation of a novel aurora A kinase inhibitor. *Chembiochem.* **10**, 464–478 (2009).
184. Akahane, D., Tauchi, T., Okabe, S., Nunoda, K. & Ohyashiki, K. Activity of a novel Aurora kinase inhibitor against the T315I mutant form of BCR-ABL: *in vitro* and *in vivo* studies. *Cancer Sci.* **99**, 1251–1257 (2008).

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DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Erbb2 | *Hras*

OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
ataxia-telangiectasia

UniProtKB: http://www.uniprot.org
ATM | ATR | aurora A | aurora B | CDK1 | CDK2 | CDK4 | CDK5 | CDK6 | CDK7 | CDK8 | CDK9 | CDK10 | CDK11 | CHK1 | CHK2 | cyclin A | cyclin B | cyclin E | D1 | D2| D3| p53 | PLK1 | PLK2 | PLK3 | PLK4 | RB1 | RBL1 | RBL2

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The Human Health Foundation: http://www.hhfonlus.org/
The Sbarro Health Research Organization:
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