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Inhibitors of Cell Cycle Kinases: Recent Advances and Future Prospects as Cancer Therapeutics

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ABSTRACT: The cell cycle is a tightly regulated series of events that governs cell replication and division. Deregulation of cell cycle kinases, e.g., cyclin-dependent kinases (CDKs), can initiate a hyper-proliferative cell phenotype and cause genomic instability, thus facilitating malignant transformation. Pharmacological agents targeting CDKs have been developed as potential anti-cancer agents for over 20 years, evolving from early pan-CDK inhibitors to second-generation inhibitors with much greater specificity and selectivity. Despite these advances in drug design and highly successful preclinical investigations, CDK inhibitors have yet to achieve their expected efficacy in clinical trials. In addition, inhibitors of other cell cycle kinases are currently progressing through clinical trials. Recent biochemical and genetic studies might be used to improve the effectiveness of cell cycle kinase inhibitors as anti-cancer agents through better drug design, therapeutic combinations, and patient selection.

KEY WORDS: cell cycle, cyclin dependent kinase, aurora kinase, polo-like kinase, drug discovery

ABBREVIATIONS

AML: acute myeloid leukemia; **AMPK:** adenosine monophosphate-activated protein kinase; **ATP:** adenosine triphosphate; **CDC7:** cell division cycle 7-related protein kinase; **CDK:** cyclin dependent kinase; **FLT:** Fms-like tyrosine kinase; **GSK3:** glycogen synthase kinase 3; **HER2:** human epidermal growth factor receptor 2; **HIPK:** homeodomain-interacting protein kinase; **JAK2:** Janus kinase 2; **KO:** knockout; **MAPK:** mitogen activated protein kinase; **MCL:** mantle cell lymphoma; **NFkB:** nuclear factor kappa-light-chain-enhancer of activated B cells; **NSCLC:** non-small cell lung cancer; **PDGFR:** platelet derived growth factor receptor; **PLK:** Polo-like kinase; **RB:** retinoblastoma protein; **SAC:** spindle assembly checkpoint; **VEGFR:** vascular endothelial growth factor receptor

I. INTRODUCTION

Essential cellular processes such as proliferation, differentiation, DNA repair, and apoptosis signal to the cell cycle machinery through intricate networks of protein kinases. Cell cycle kinases ensure timely and accurate cell replication by orchestrating DNA synthesis and cell division while protecting the cell from DNA damage.[1–2] Deregulation of these kinases can cause cells to proliferate uncontrollably and leads to genetic instability, tumorigenesis, and malignancy, and can potentially be targeted to inhibit cancer progression.[2–5] In this review, we discuss the potential and limitations of current cyclin-dependent kinase (CDK) inhibitors. We also summarize progress in evaluating other cell cycle kinases as potential therapeutic agents.

II. FUNCTIONS OF CELL CYCLE KINASES AND THEIR DEREGULATION IN CANCER

The CDKs are a family of serine/threonine kinases, five of which (CDK1, CDK2, CDK3, CDK4, and CDK6) have been directly implicated in driving the cell cycle (Fig. 1).[1] These CDKs act sequentially to govern the transitions of the cell cycle, i.e., from G_o (quiescence), through G_1, and into DNA synthesis (S phase), followed by G_2 and mitosis (M phase). Upon mitogenic stimulation through signaling routes such as the Ras/mitogen-activated protein kinase (MAPK) pathway, D-type cyclins (D1, D2, and D3) are transcriptionally induced.[6–7] These cyclins are the cognate binding partners for CDK4 and CDK6, and following heterodimerization, these active cyclin-CDK complexes

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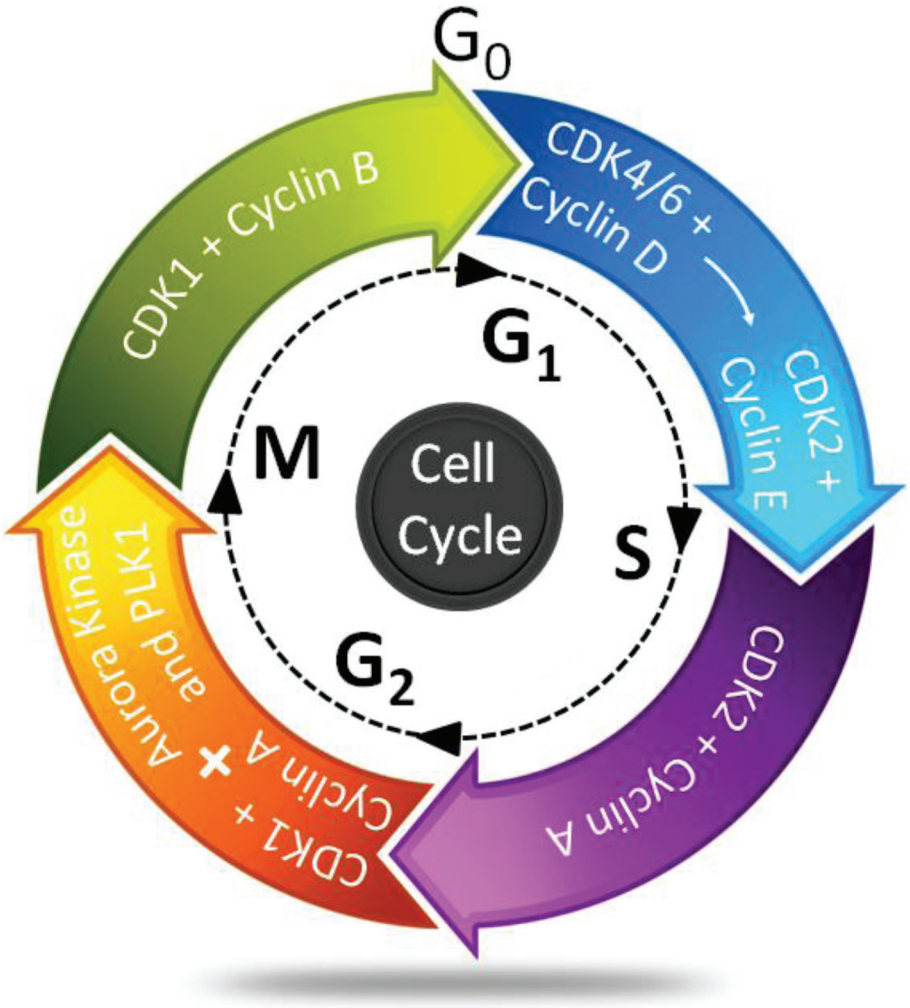

FIGURE 1. Cell cycle kinases known to be deregulated in cancer and their association with cell cycle progression. A schematic representation of the various stages of the cell cycle, i.e., from quiescence G_0, through G_1 and the DNA synthesis phase (S), progressing through G_2, and finally into mitosis (M), resulting in cell replication. The various CDK/Cyclin complexes and other cell cycle kinases that drive each particular stage of the cell cycle are also illustrated.

phosphorylate pocket proteins such as retinoblastoma protein (RB).[8] In its unphosphorylated form, RB is a repressor of transcription that binds and inactivates transcription factors such as E2F family members.[8–9] When RB is phosphorylated by cyclin D-CDK4/6, transcription factors are released, thus facilitating the transcription of cyclin E, the binding partner for CDK2. Cyclin E-CDK2 heterodimers reinforce the phosphorylated state of RB, irreversibly initiating a positive feedback loop that leads to the expression of genes necessary to promote cell cycle progression from G_1 to S phase.[1,10] Beyond this point progression through the cycle is independent of mitogenic stimuli, and RB is maintained in a hyperphosphorylated state by the sequential activities of cyclin A-CDK2, cyclin A-CDK1, and cyclin B-CDK1 complexes.[1] CDK3 is expressed at low levels in mammals, and although phosphorylation of RB by cyclin C-CDK3 is necessary for exit from G_0, its function remains largely undetermined.[1,11]

Aberrant expression of components that govern G_1-S phase transition has the potential to permit cells

to proliferate independently of mitogenic stimuli, and thus their role in malignant transformation has been intensively investigated. CDKs are deregulated in numerous cancers, including those of the breast, lung, pancreas, prostate, liver, and skin.[2–4] This deregulation is most commonly attributed to the increased expression of CDKs and cyclins, and mutation or deactivation of endogenous CDK inhibitors. CDK4 is most commonly amplified and overexpressed in gliomas,[12] sarcomas, and breast and cervical carcinoma,[13–15] and CDK6 is amplified in gliomas and lymphoid tumors.[16–17] However, over-activity of both CDK4 and CDK6 can be attributed to increased cyclin D1 expression in parathyroid adenoma, leukemia, lymphomas and multiple myeloma, and colorectal, gastric, esophageal, lung, kidney, and breast cancer.[18] Cyclin D-CDK4/6 activity can also be enhanced by inactivation of the endogenous CDK4/6 inhibitor p16, which can be caused by gene deletion (leukemia and bladder cancer), point mutation (melanomas and pancreatic cancer), or epigenetic mechanisms such as DNA methylation (gliomas, lung cancer, and head and neck tumors).[19–20] There have also been reports of mutation in CDK4 that render it unable to bind p16 and chromosomal translocations, leading to overexpression of CDK6 in distinct tumor subgroups.[21–22] However, these genomic alterations are relatively infrequent. CDK2 is deregulated in lung carcinoma, melanoma, osteosarcoma, ovarian carcinoma, pancreatic neoplasia, and sarcomas, mostly due to the overexpression of its binding partners cyclin E and cyclin A, or inactivation of endogenous inhibitors.[2]

CDK1 co-operates with other kinases, including Aurora and Polo-like kinases (PLK), to facilitate the transition from G_2 to M-phase, serving to regulate chromosome condensation and microtubule dynamics in preparation for cell division.[23–24] Aberrant overexpression of these kinases causes chromosomal instability and has been implicated in the oncogenic transformation of a variety of tissues.[25–27] Increased activation of CDK1 has been observed in primary breast, colon, prostate, and lung cancers, often due to the overexpression of cyclin B1.[28–31] Aurora kinase A (the aurora family member most consistently associated with cancer) is amplified and/or overexpressed in several malignancies, including breast, colon, pancreas, ovarian, bladder, liver, and gastric cancer,[32–36] and PLK1 overexpression is described in breast, ovary, colon, stomach, pancreas, lung, head and neck, skin, esophagus, and brain cancer, and correlates with poor outcome.[37–45] Since inhibition of these mitotic components activates the "spindle assembly checkpoint" and causes apoptosis through an undefined mechanism, pharmacological agents targeting these mitotic kinases are being investigated as potential therapeutics.[46]

III. CYCLIN-DEPENDENT KINASE INHIBITORS

The integral role played by CDKs in the progression of the cell cycle coupled with the high incidence of CDK deregulation in cancer provides a strong rationale for the development of CDK inhibitors as novel therapeutic agents. Over a period spanning more than two decades, many molecules that inhibit CDKs have been identified, the most well studied of which is the pan-CDK inhibitor Flavopiridol. Following successful pre-clinical development in which Flavopiridol inhibited the proliferation of colorectal (colo 205), lymphoma (HL60), head and neck (HN12), and prostate (LnCap/DU145) cancer cells in culture and xenograft models, it progressed to phase I and II clinical trials.[47] Unfortunately, Flavopiridol failed to achieve clinical efficacy in these early studies; however, much was learnt about the potential limitations of CDK inhibitors in terms of target specificity, development of resistance, activation of compensatory mechanisms, and poor pharmacokinetics. As well as giving rise to a new generation of more specific CDK inhibitors, the lessons learned from the development of Flavopiridol have reignited interest in the compound as more advantageous therapeutic strategies are devised.

A. Target Specificity

First-generation ATP-competitive CDK inhibitors, such as Flavopiridol, olomoucine, and roscovitine, were relatively nonspecific, inhibiting numerous CDKs with varying selectivity. In the case of Flavopiridol, early clinical trials were carried out in patients

with multiple myeloma, melanoma, and endometrial adenocarcinoma.[48] Only modest responses were observed, and treatment was associated with severe side effects such as diarrhea and myelosuppression. It was hypothesized that the associated toxicity was due to the agent's significant affinity for CDKs 7, 8, 9, 10, and 11, a distinct subfamily of CDKs whose function is primarily to promote transcription. Inactivation of these transcriptional CDKs has a global impact, primarily reducing the accumulation of RNA transcripts that have a rapid turnover, such as those that encode cell cycle regulators, NFκB- and p53-responsive transcripts, and anti-apoptotic factors, thus inducing cell death.[4,49] This detrimental effect on normal cells prevented the administration of a therapeutic dose, thus limiting the benefit that could be achieved. Interestingly, subsequent clinical trials have been completed using Flavopiridol in hematological malignancies with great success, as the survival of malignant hematopoietic cells is dependent on the continuous expression of anti-apoptotic proteins.[50–51] These studies highlighted how crucial it was to define which specific CDKs were required to drive the proliferation of cancer cells in order to eliminate undesired toxicities in normal cells. Although the close sequence and structural conservation between different members of the CDK family, particularly in the kinase domain, makes it challenging to develop compounds with specificity for a single CDK, second-generation compounds with improved selectivity for CDKs known to play a prominent role in tumorigenesis were subsequently developed, and many are currently undergoing clinical testing (Table 1). These include the selective CDK4/6 inhibitor PD0332991, which causes cultured cells to arrest in G_1 phase and inhibits the proliferation of xenografts of RB-positive breast, ovarian, lung, colon, and prostate cancer cell lines, and glioblastoma, leukemia, myeloma, and mantle cell lymphoma (MCL) cell lines.[52–57] It is currently undergoing phase II clinical testing as a single agent for advanced or metastatic liposarcoma, RB-positive glioblastoma, liver cancer, refractory solid tumors, and non-small cell lung cancer (NSCLC), and as a combination therapy for multiple myeloma (with bortezomib and dexamethasone) and hormone receptor positive advanced breast cancer (in combination with letrozole) (see Table 1). P276-00 is another promising inhibitor that is highly specific to CDK4. It has been used *in vitro* to demonstrate a significant anti-proliferative effect in numerous human cancer cell lines, including MCF-7 breast cancer cells and H-460 lung cancer cells.[58] Several phase I/II clinical studies with P276-00 are underway (Table 1) including its assessment as a combination therapy with gemcitabine in advanced pancreatic cancer patients, with radiation in subjects with advanced head and neck cancer, and with gemcitabine or carboplatin in patients with metastatic triple negative breast cancer. It is also being tested as a single agent in patients with refractory multiple myeloma, cyclin D-positive malignant melanoma, and relapsed and/or refractory mantle cell lymphoma in phase II studies (Table 1).

B. Pharmacokinetics

As with other therapies that act at specific phases of the cell cycle, the pharmacokinetics of CDK inhibitors provides a challenge. The heterogeneity of cell populations *in vivo* ensures that not all cells will be actively progressing through the cell cycle at any one time, and those that are "in cycle" will be distributed throughout the cell cycle. CDKs are typically active during only a limited window of the cell cycle, and therefore, unless an exogenous CDK inhibitor is present at inhibitory concentrations for a time exceeding the entire duration of a cell cycle, many cells may not pass through the vulnerable portion of the cell cycle. Although some compounds with cell cycle phase-specific actions are in routine clinical use (e.g., the antiestrogen tamoxifen, which acts during a specific window within G_1 phase, and gemcitabine, which blocks DNA synthesis),[59–60] the high concentration of CDK inhibitor required to induce a therapeutically effective dose over such a long period of time increases the likelihood of nonspecific inhibition of other kinases, potentially contributing to dose-limiting toxicity and in turn reducing the therapeutic benefit achieved. This limitation is likely to be reduced by the improved selectivity of second-generation compounds; however, it is evident that the dosing regimen with which a CDK inhibitor is administered is critical to

TABLE 1. Second-Generation CDK Inhibitors Undergoing Clinical Investigation (http://clinicaltrials.gov)

Compound	Primary CDK Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
AG024322	CDK1-B	n/a	I	NCT00147485	Advanced Cancer	Terminated
	CDK2-E (30-200 nM)					
	CDK4-D					
AT7519M	CDK1-B (210 nM)	GSK3β (89 nM)	I	NCT00390117	Advanced or Metastatic Solid Tumors or Refractory Non-Hodgkin's Lymphoma	Ongoing
	CDK2-A (47 nM)	CDK7-H-MAT1 (2.8 μM)	I/II	NCT01183949	Previously Treated Multiple Myeloma	Recruiting
	CDK4-D1 (100 nM)	Aurora A (>10 μM)				
	CDK5-p35 (13 nM)	c-abl (>10 μM)				
	CDK6-D3 (170 nM)	cSrc (>10 μM)				
	CDK9-T (<10 nM)	Chk 1 (>10 μM)				
		PKBbeta (>10 μM)				
		EGFR (>10 μM)				
		FGFR3 (>10 μM)				
		IR (>10 μM)				
		Jnk2 (>10 μM)				
		MAPK 1 (>10 μM)				
		MEK1 (>10 μM)				
		met (>10 μM)				
		P38 (>10 μM)				
		p70S6K (>10 μM)				
		PDGFR (>10 μM)				
		PDK1 (>10 μM)				
		VEGFR 1 (>10 μM)				
AZD5438	CDK1-B (16 nM)	n/a	I	NCT00088790	Advanced Solid Malignancies	Completed
	CDK2-A (45 nM)					
	CDK2-E (6 nM)					
	CDK9-T (20 nM)					
BAY1000394	CDK1-B (7 nM)	n/a	I	NCT01335256	Advanced Malignancies	Ongoing
	CDK2-E (9 nM)		I	NCT01188252	Advanced Malignancies	Recruiting

TABLE 1. (Continued)

Compound	Primary CDK Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
	CDK4-D1 (11 nM)					
	CDK9-T1 (<10 nM)					
P1446A-05	CDK1-B (n/a)	n/a	I	NCT00772876	Advanced Refractory Malignancies	Ongoing
	CDK4-D1 (n/a)		I	NCT00840190	Advanced Refractory Malignancies	Ongoing
	CDK9-T (n/a)					
P276-00	CDK1-B (79 nM)	CDK2-E (2.54 μM)	I/II	NCT00898287	With Gemcitabine in Patients with Advanced Pancreatic Cancer	Ongoing
	CDK4-D1 (63 nM)	CDK2-A (224 nM)	I/II	NCT00899054	With Radiation in Subjects with Advanced Head and Neck Cancer	Ongoing
	CDK9-T1 (20 nM)	CDK6-D3 (396 nM)	I/II	NCT00882063	Refractory Multiple Myeloma	Ongoing
		CDK7-H (2.87 μM)	II	NCT01333137	Metastatic Triple Negative Breast Cancer +/-Gemcitabine or Carboplatin	Recruiting
		GSK3β (2.771 μM)	I	NCT00407498	Advanced Refractory Neoplasms	Completed
			II	NCT00835419	Malignant Melanoma Positive for Cyclin D1 Expression	Ongoing
			II	NCT00843050	Relapsed and/or Refractory Mantle Cell Lymphoma	Ongoing
			I	NCT00547404	Myeloma	Withdrawn
			II	NCT00824343	Recurrent and/or Locally Advanced Head and Neck Cancer	Ongoing
			I	NCT00408018	Advanced Refractory Neoplasms	Terminated
PD0332991	CDK4-D1 (11 nM)	CDK2-E2 (>10 μM)	II	NCT01209598	Advanced or Metastatic Liposarcoma	Recruiting
	CDK4-D3 (9 nM)	CDK2-A (>10 μM)	I/II	NCT00555906	With Velcade and Dexamethasone in Patients with Pre-Treated Multiple Myeloma	Recruiting
	CDK6-D2 (15 nM)	CDK1-B (>10 μM)	I	NCT00141297	Advanced Cancer	Ongoing
		CDK5/p25 (>10 μM)	II	NCT01227434	Recurrent Rb Positive Glioblastoma	Recruiting
		EGFR (>10 μM)	II	NCT01356628	Liver Cancer	Recruiting
		FGFR (>10 μM)	I	NCT01111188	With Bortezomib in Patients with Relapsed Mantle Cell Lymphoma	Recruiting
		PDGFR (>10 μM)	II	NCT01037790	Refractory Solid Tumors	Recruiting
		IR (>10 μM)	I	NCT01320592	Advanced Breast Cancer	Recruiting

TABLE 1. (Continued)

Compound	Primary CDK Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
		VEGFR (>10 μM)	I/II	NCT00721409	With or Without Letrozole for Hormone-Receptor Positive Advanced Breast Cancer	Recruiting
		c-Src (>10 μM)	II	NCT01291017	Advanced Non-small Cell Lung Cancer NSCLC	Recruiting
		Erk2 (>10 μM)	I	NCT00420056	Pre-Treated Mantle Cell Lymphoma	Ongoing
		PKB (>10 μM)				
		PKC (>10 μM)				
PHA793887	CDK1-B (60 nM)	GSK3β (79 nM)	I	NCT00996255	Advanced/Metastatic Solid Tumors	Terminated
	CDK2-A (8 nM)					
	CDK4-D (62 nM)					
	CDK9-T (138 nM)					
PHA848125	CDK1-B (400 nM)	TRK-A (50nM)	II	NCT01011439	Thymic Carcinoma	Recruiting
	CDK2-A (32 nM)		II	NCT01301391	Malignant Thymoma Previously Treated with Multiple Lines of Chemotherapy	Recruiting
	CDK4-D (160 nM)		I	NCT01300468	Advanced/Metastatic Solid Tumors	Completed
R547	CDK1-B (0.2 nM)	n/a	I	NCT00400296	Advanced Solid Tumors	Completed
	CDK2-A (0.1 nM)					
	CDK2-E (0.4 nM)					
	CDK4-D (n/a)					
	CDK7-H (171 nM)					
RGB286638	CDK1-B (<5 nM)	GSK3β	I	NCT01168882	Relapsed or Refractory Hematological Malignancies	Withdrawn
	CDK2-A (<5 nM)	cSrc (<100 nM)				
	CDK4-D (44 nM)	MEK				
	CDK6-D (55 nM)	JNK				
	CDK9-T (<5 nM)					
SCH727965	CDK1-B (3 nM)	n/a	I	NCT00871910	Advanced Cancer	Completed
	CDK2-E (1 nM)		II	NCT00798213	Acute Myelogenous Leukemia and Acute Lymphoblastic Leukemia	Completed

TABLE 1. (Continued)

Compound	Primary CDK Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
	CDK5-p35 (1 nM)		II	NCT00871546	Mantle Cell Lymphoma or B-Cell Chronic Lymphocytic Leukemia	Ongoing
	CDK9-T (4 nM)		II	NCT00732810	Advanced Breast and Lung Cancers	Completed
			I	NCT00871663	Advanced Cancer	Recruiting
			I	NCT01434316	With or Without Carboplatin in Patients with Advanced Solid Tumors	Not yet open
			I/II	NCT01026324	Stage III or Stage IV Malignant Melanoma	Not yet open
			II	NCT01096342	Relapsed or Refractory Multiple Myeloma	Recruiting
			II	NCT00937937	Stage IV Melanoma	Ongoing
SNS032	CDK2-A (62 nM)	n/a	I	NCT00446342	Advanced B-Lymphoid Malignancies	Unknown
	CDK7-H (38 nM)		I	NCT00292864	Advanced Solid Tumors	Unknown
	CDK9-T (4 nM)					
TG02	CDK1-B (9 nM)	Lck (11 nM)	I	NCT01204164	Advanced Hematological Malignancies	Recruiting
	CDK2-A (5 nM)	TYK2 (14 nM)				
	CDK3-E (8 nM)	Fyn (15 nM)				
	CDK5-p35 (4 nM)	JAK2 (19 nM)				
	CDK7-H (37 nM)	FLT3 (19 nM)				
	CDK9-T (3 nM)	Fms (27 nM)				
		TYRO3 (36 nM)				
		ERK5 (43 nM)				
		p38δ (56 nM)				
		JAK1 (59 nM)				
ZK304709	CDK1-B (50 nM)	VEGFR1 (10 nM)	I	see Ref 82	Advanced Solid Tumors	Completed
	CDK2-E (4 nM)	VEGFR2 (34 nM)				
	CDK4-D1 (61 nM)	VEGFR3 (1 nM)				
	CDK7-H (85 nM)	PDGFRβ (27 nM)				
	CDK9-T1 (5 nM)					

achieve the greatest therapeutic benefit. For example, clinical trials with Flavopiridol administered as prolonged infusions were largely ineffective in many settings; however, recent clinical trials administering the drug as 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 leukemia patients.[61]

C. Resistance and Compensatory Mechanisms

Despite the promise second generation CDK inhibitors have shown as anti-cancer agents in both preclinical and clinical studies, it has become increasingly apparent that the effectiveness of such therapies is dictated by genetic context and by the activation status of specific cellular pathways. For example, early studies showed that sensitivity to PD0332991, the CDK4/6 inhibitor, in various xenograft models was dependent on the presence of functional RB protein.[52] However, it has since been shown even RB competent cells display a range of sensitivities to the inhibitor due to the varied expression of other key components of the cell cycle.[55,57,62–64]

In a study in which 40 human ovarian cancer cell lines were treated with PD0332991, responses were considerably varied, with approximately half demonstrating high sensitivity to CDK4/6 inhibition. Of the 18 cell lines that were insensitive to the anti-proliferative effect of PD0332991, 9 were RB proficient, and in 2 of the 22 sensitive cell lines, RB was deleted; thus it was clear that RB status alone was not an accurate predictor of response. It was later determined that RB-competent cell lines with low p16 expression were most responsive to PD0332991.[57] This is concordant with other studies in which high levels of p16 (the endogenous inhibitor for CDK4/6 activity) was associated with acquired independence from CDK4/6 signaling, and thus insensitivity to CDK4/6 inhibition.[65–67] In addition, a gain of cyclin E1 or cyclin D1 gene copy number, or the presence of p53 mutations with subsequent low expression of p21, could also confer resistance to CDK4/6 inhibition in ovarian cancer cells.[57] Within a cohort of 292 ovarian cancer patient samples, 92 were RB proficient with low p16 expression.[57] This suggests that only just over a third of ovarian cancer patients would benefit from CDK4/6 inhibition, despite normal RB expression in 96% of these tumors,[57] indicating that the use of PD0332991, and related therapeutic agents, should be directed selectively to avoid such resistance mechanisms.

Acquired resistance to PD0332991 has also been reported in acute myeloid leukemia (AML) and breast cancer cell lines, with emerging populations featuring elevated CDK2 protein levels and attenuation of endogenous CDK2 inhibitors. The resistant cells also featured increased levels of E2F-target genes, cyclins A and E, which are essential for CDK2 function, thus facilitating CDK4/6 independence and a proliferative cell cycle driven by CDK2.[63–64]

Numerous knockdown and knockout (KO) experiments have provided valuable insights into the specific roles of individual CDKs and highlighted redundancy issues and compensatory mechanisms that could represent a key determinant of therapeutic failure.[68] The mammalian cell cycle can progress in the absence of one or more non-mitotic CDKs,[69–71] and strikingly, triple ablation of CDK2, 4, and 6 can be compensated by CDK1, raising doubts over inhibition of these CDKs as a therapeutic strategy.[72] However, the relevance of such KO models to the pharmacological inhibition of individual CDKs can be questioned. One major issue arises from the differences in mechanism of action: while the target CDK is absent in KO models, CDK inhibitors impair kinase activity but not the formation of complexes between the target CDK and cognate cyclins, various substrates, or inhibitors. Furthermore, because KO models are deficient in the gene(s) of interest through development, there is potentially a greater opportunity to develop compensatory mechanisms. Finally, chemical intervention is generally less specific than the genetic depletion of a specific CDK.

D. CDK Inhibitors: Recent Advances

1. Combination Therapies

Recent data suggest that the future for CDK inhibitors in cancer therapy maybe in combinatory

strategies. Both preclinical studies and clinical trials have demonstrated that CDK inhibitors can act in synergy with cytotoxic drugs, suggesting that CDK inhibitors work better when cells are synchronized or arrested in specific cell phases.[48] For example, PHA-848125, a second-generation CDK inhibitor, showed anti-tumor effects in several cancer models, including prostate, pancreas, and lung, as well as showing more than an additive effect when used in combination with docetaxel and bevacizumab.[73] This idea is supported by encouraging data derived from a phase II study of AML, where the combination of cytarabine and mitoxantrone with Flavopiridol gave a complete response in 75% of patients.[74]

The potential for CDK inhibitors to reverse resistance to radiotherapy and chemotherapy is also being investigated. SNS-032, another second-generation CDK2/7/9 inhibitor, which has recently been shown to exhibit significant single-agent activity in primary AML cells, as well as remarkable synergism with cytarabine,[75] sensitizes radiotherapy-resistant NSCLC cells to ionizing radiation.[76]

CDK inhibitors may also be used successfully to enhance the efficiency of other drugs, such as the proteasome inhibitor bortezomib or dexamethasone. Co-treatment with the CDK4/6 inhibitor PD0332991 enhances multiple myeloma cell death, and is currently undergoing phase I and II clinical trials.[77–78] This CDK inhibitor also enhanced the activity of a FLT3 inhibitor in AML cell lines that expressed a mutant form of the FLT3 receptor tyrosine kinase,[63] and acted synergistically with the BCR-ABL kinase inhibitor imatinib in leukemia cell lines.[79] In addition, PD0332991 demonstrated synergy with the anti-estrogen tamoxifen and the HER2-targeted therapy Trastuzumab in ER-positive breast cancer cell lines, and was also effective in anti-estrogen-resistant cell lines.[55] A phase I/II trial using PD0332991 in combination with the anti-estrogen letrozole is in progress (Table 1).

2. CDK Inhibitors with Multiple Kinase Targets

Recent reports have described CDK inhibitors with activity against additional kinase targets that may serve to enhance anti-tumor activity. These include TG02, an inhibitor of CDK 1, 2, 7, 9, and JAK2, and FLT3, which inhibits the proliferation of a broad range of tumor cell lines, including primary cultures of progenitor cells derived from AML and polycythemia vera patients. It also caused tumor regression in murine models of leukemia and is currently undergoing phase I trials (Table 1).[80] ZK-304709, which inhibits VEGFR 1-3 and PDGFRβ as well as CDK 1, 2, 4, 7, and 9, demonstrated superior efficacy compared to standard chemotherapeutic compounds both in subcutaneous human tumor xenografts and orthotopic human pancreatic carcinoma models,[81] and is currently being assessed for tolerability in phase I trials.[82] RGB-286638 also inhibits other kinases in addition to CDKs, including several receptor (e.g., FLT1, FLT3, FLT4) and nonreceptor (e.g., Abl, Jak, c-Src family members) tyrosine kinases as well as serine/threonine kinases such as AMPK, GSK3, PIM1, HIPK1-3, and MAPK.[83] Increased survival and tumor regression were observed in several preclinical models of both solid and hematological malignancies following RGB-286638 treatment, and as a result, it was due to undergo phase I clinical evaluation in late 2011, however it was withdrawn prior to enrollment (Table 1). JNJ-7706621, a CDK inhibitor that also inhibits aurora kinases, selectively blocked proliferation of tumor cells of various origins *in vitro*, as well as in xenograft models; however, to date, it has not been assessed clinically.[84]

3. Non-ATP-Competitive CDK Inhibitors

The off-target effects and subsequent dose limitations associated with ATP-competitive inhibitors have led to interest in designing non-ATP-competitive CDK inhibitor compounds,[85] for example inhibitors that target kinase substrates and regulatory binding sites. Examples of these are summarized below.

a. Inhibitors of the Cyclin Binding Groove

Cyclins contain an exposed hydrophobic groove when bound in an active complex, acting as a recruitment point for numerous substrates and endogenous inhibitory proteins. Cell permeable peptides containing the appropriate Arg/Lys-X-Leu ("RXL" or "KXL")

binding motif have been reported to induce apoptosis in tumor cells and inhibit tumor growth *in vivo*.[86–87]

b. Inhibitors of CDK-Cyclin Association

In order to inhibit the active protein-protein complexes being formed between different cyclins and CDKs, peptides, such as NBI1 (an all d-amino acid hexapeptide) have been developed to selectively bind to cyclin A and prevent the kinase activity of the CDK2-cyclin A complex. A cell-permeable derivative of NBI1 induces apoptosis and inhibits proliferation of tumor cell lines, and thus the NBI1-binding site on cyclin A may represent a new target for the selective inhibition of CDK2 activity.[88]

c. Inhibitor Derivatives from CDK Substrates

Derivatives from the inhibitory CDK substrates p53 and pRB have been used to block CDK2 activity *in vitro* and *in vivo*. For example, CIP is a 20-mer peptide designed to mimic p53 that prevents CDK2-mediated p53 phosphorylation and induces cell death in A375 melanoma cells.[89] Spa310 (derived from the spacer domain of pRb2/p130) is able to inhibit the activity of CDK2 and induce cell cycle arrest at G_0-G_1 in NIH3T3 cells and also inhibit tumor development in an A549 xenograft mouse model by mimicking RBL2 tumor suppressor function.[90]

Although these routes to CDK inhibition show promise in preclinical models, much more extensive testing in experimental models will be required before they reach clinical testing. In addition, the significant challenges in drug delivery for peptide-based therapeutics will need to be addressed.[91–92]

4. Patient Selection

Technologies that allow genomewide transcriptome, methylome, and sequencing analyses of cancer have resulted in unprecedented insights into the heterogeneity of the disease. It has become apparent that cancers that might seem to be similar on the basis of tissue of origin and histological appearance can, in fact, be very different at the molecular level.[93] Consequently, testing novel targeted therapeutic strategies in unselected patients may underestimate the efficacy that could be achieved in appropriately selected subgroups. Although many trials are still being carried out in unselected patient cohorts, clinical trials in which patients are selected on the basis of a common somatic aberration that can be targeted are becoming more prevalent and achieving greater success. For example, clinical trials in the development of Trastuzumab (Herceptin®), the therapeutic monoclonal antibody against HER2, recruited only those patients with overexpression/amplification of the target molecule, which substantially decreased the number of patients required to demonstrate efficacy, accelerating its implementation and subsequent extension to gastric cancer.[94] This rational design strategy is slowly being implemented in CDK inhibitor trials. For example, P276-00, the potent inhibitor of CDK4, is in phase II trials in subjects with malignant melanoma whose tumors are positive for cyclin D1, i.e., the cyclin that activates CDK4. Also, the CDK4/6 inhibitor PD0332991 is in phase II trials in patients with recurrent RB-positive glioblastoma (Table 1). The advent of these "tailored" clinical trials will no doubt give these compounds a better chance of success and, more importantly, the patients receiving the agents a higher likelihood of response. However, although some progress has been made in identifying biomarkers of response to CDK inhibition, more comprehensive signatures of response are likely to be required to avoid drug resistance and clinical failure.

IV. INHIBITORS OF MITOTIC KINASES

Cells are more sensitive to apoptotic cell death during mitosis than in any other phase of the cell cycle. Indeed, some commonly used first-line therapies (i.e., taxanes and vinca alkaloids) are anti-mitotic agents. These drugs act as microtubule toxins and so activate the spindle assembly checkpoint (SAC), causing prolonged mitosis and subsequent cell death.[4,46] However, because microtubules have vital functions in both dividing and nondividing cells, microtubule toxins cause a range of side effects. New mitotic targets that specifically block spindle assembly in dividing cells have been intensively pursued over recent years, with aurora kinases and PLKs becoming the lead targets.[23–24] Numerous inhibitors are now in

clinical development and offer promising therapeutic strategies for the near future (Tables 2 and 3).

The three aurora kinases (aurora kinases A, B, and C) are essential to ensure error-free cell division, and their overexpression appears to be intimately linked to centrosome amplification, malignant transformation, and resistance to microtubule poisons.[95] Aurora A localizes to centrosomes and spindle poles during mitosis and is overexpressed in many epithelial cancers.[32–36,96] Inhibition of aurora A by RNA interference or by small-molecule inhibitors causes SAC-induced mitotic arrest and the formation of unipolar spindles, which can eventually lead to apoptosis.[97] Aurora B is a chromosomal passenger implicated in SAC whose localization to the mitotic apparatus varies depending upon the stage of the cell cycle.[96] It too is overexpressed in various tumors, including those of the breast, colon, rectum, kidney, lung, and prostate.[98] Although Aurora B overexpression alone cannot induce malignant transformation of cells, its overexpression is involved in Ras-mediated transformation and is correlated with increased genomic instability.[99–100] 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.[97] Aurora C is structurally and functionally related to aurora B, although its expression is restricted to certain cell lineages.[96]

Although the impact of off-target effects and the prevalence of drug resistance have yet to be fully explored in preclinical models, there are many clinical trials underway using inhibitors of aurora kinases (both pan and specific inhibitors), which are summarized in Table 2. Despite many phase I trials being terminated following concerns over efficacy and safety, preliminary data would suggest that aurora inhibitors are generally well tolerated.[101] Furthermore, in a recent phase I/II trial testing AZD1152, a highly selective aurora B inhibitor, 25% of patients with advanced AML demonstrated a hematological response with manageable toxicity, highlighting their potential use as therapeutics.[102]

The most well characterized member of the mammalian PLK family is PLK1. PLK1 specifically localizes to centrosomes, the spindle midzone, and the post-mitotic bridge, and it participates in both mitotic entry and mitotic progression.[103] Constitutive high expression of PLK1 is associated with numerous types of solid tumors.[37–45] Depletion of PLK1 by RNA interference results in metaphase arrest and formation of abnormal chromatin structures. Knockdown of PLK1 also reduces cell survival and elevates drug sensitivity of tumor, but not normal, cells.[104–105] Four other PLKs (PLK2–5) have been identified, though their roles in tumorigenesis have not been well classified.

Clinical trials underway using inhibitors of PLK1 are summarized in Table 3. As with the aurora kinase inhibitors, most PLK1 inhibitors are in phase I trials and their clinical efficacy remains unclear. However, the results from phase II testing of BI2536 in patients with various solid tumors have recently been published, each describing modest responses at best, in contrast to promising preclinical studies.[106] Further clinical evaluation of PLK inhibitors is therefore required to determine how they will compare with current antimitotic agents.

V. NOVEL CELL CYCLE KINASE TARGETS

In addition to CDKs, aurora kinases, and PLK1, other cell cycle kinases that have recently been identified as deregulated in cancer are being explored as potential new therapeutic targets.

Wee1 kinase regulates M-phase entry by inactivating CDK1 through the phosphorylation of Y15, preventing the formation of the cyclin B-CDK1 complex.[107] The newly identified Wee1 inhibitor MK-1775 potently sensitizes p53-defective human tumor cells derived from lung, breast, and prostate cancers to radiation at nanomolar concentrations,[108] and enhances the anti-tumor efficacy of various DNA-damaging agents in p53-deficient human colon cancer cells.[109] This sensitization appears to involve a drug-induced, premature acceleration of G2 phase cells into mitosis. Such cells harbor unrepaired DNA lesions that lead to abnormal cell divisions and cell death. These findings support the continued phase I/

TABLE 2. Aurora Kinase Inhibitors Undergoing Clinical Investigation (http://clinicaltrials.gov)

Compound	Primary Aurora Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
AMG900	Aurora A (5 nM)	p38α (53 nM)	I	NCT01380756	Acute Leukemias and Related Disorders	Recruiting
	Aurora B (4 nM)	TYK2 (220 nM)	I	NCT00858377	Advanced Solid Tumors	Recruiting
	Aurora C (1 nM)	JNK2 (520 nM)				
		MET (550 nM)				
		TIE2 (650 nM)				
AZD1152	Aurora A (1.4 μM)	n/a	I	NCT01019161	Acute Myeloid Leukaemia	Completed
	Aurora B (0.37 nM)		I	NCT00530699	Relapsed Acute Myeloid Leukaemia	Completed
	Aurora C (17 nM)		I	NCT00497991	Relapsed Acute Myeloid Leukemia	Completed
			II/III	NCT00952588	With and Without Cytosine Arabinoside in Patients with Acute Myeloid Leukaemia	Ongoing
			I	NCT00926731	With Arabinoside in Acute Myeloid Leukaemia	Completed
			I	NCT01354392	Diffuse Large B-cell Lymphoma	Recruiting
			I	NCT00338182	Advanced Solid Malignancies	Ongoing
			I	NCT00497679	Advanced Solid Malignancies	Terminated
			I	NCT00497731	Advanced Solid Malignancies	Terminated
AT9283	Aurora A (3 nM)	JAK2	I	NCT00443976	Advanced or Metastatic Solid Tumors or Non-Hodgkin's Lymphoma	Completed
	Aurora B (3 nM)	Flt3 (1–30 nM)	I	NCT01431664	Relapsed or Refractory Acute Leukemia	Not yet open
	Aurora C n/a	Abl	II	NCT01145989	Relapsed or Refractory Multiple Myeloma	Recruiting
			I	NCT00985868	Relapsed or Refractory Solid Tumors	Recruiting
			I/II	NCT00522990	Leukemias	Terminated
BI811283	Aurora A n/a	n/a	II	NCT00632749	With Cytarabine in Previously Untreated AML Ineligible for Intensive Treatment	Recruiting
	Aurora B (9 nM)		I	NCT00701324	Various Solid Tumors	Completed
	Aurora C n/a					
CYC116	Aurora A (44 nM)	VEGFR2 (69 nM)	I	NCT00560716	Advanced Solid Tumors	Terminated
	Aurora B (19 nM)					
	Aurora C (65 nM)					

TABLE 2. (Continued)

Compound	Primary Aurora Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
ENMD2076	Aurora A (14 nM)	Flt3 (3 nM)	I	NCT00904787	Relapsed or Refractory Hematological Malignancies	Completed
	Aurora B (350 nM)	Src (23 nM)	I	NCT00806065	Multiple Myeloma	Recruiting
	Aurora C n/a	KDR (40 nM)	I	NCT00658671	Advanced Cancer	Not yet open
		VEGFR2 (93 nM)	II	NCT01104675	Ovarian Cancer	Not yet open
		FGFR1 (120 nM)				
GSK1070916	Aurora A (490 nM)	n/a	I	NCT01118611	Advanced Solid Tumors	Recruiting
	Aurora B (0.38 nM)					
	Aurora C (1.5 nM)					
MLN8054	Aurora A (4 nM)	n/a	I	NCT00249301	Advanced Solid Tumors	Terminated
	Aurora B (172 nM)		I	NCT00652158	Advanced Malignancies	Terminated
	Aurora C n/a					
MLN8237	Aurora A (1 nM)	n/a	I/II	NCT01045421	Lung, Breast, Head, and Neck or Gastroesophageal Malignancies	Recruiting
	Aurora B (>200 nM)		I/II	NCT01091428	Ovarian, Fallopian Tube, or Peritoneal Cancer	Recruiting
	Aurora C n/a		I	NCT00962091	Advanced Solid Tumors	Recruiting
			I	NCT00500903	Advanced Solid Tumors	Completed
			I	NCT00697346	Advanced Hematological Malignancies	Recruiting
			I	NCT00651664	Advanced Malignancies	Completed
			I/II	NCT01397825	Relapsed or Refractory Aggressive B-Cell Lymphoma Treated with Rituximab & Vincristine	Recruiting
			II	NCT00830518	Acute Myelogenous Leukemia and High-Grade Myelodysplastic Syndrome	Completed
			I/II	NCT01034553	With Bortezomib in Patients with Relapsed or Refractory Multiple Myeloma	Recruiting
			II	NCT01316692	Unresectable Stage III–IV Melanoma	Not yet open
			I	NCT01094288	Advanced Solid Tumors Including Castration-Resistant Prostate Cancer	Recruiting

TABLE 2. (Continued)

Compound	Primary Aurora Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
			I/II	NCT00739427	Relapsed or Refractory Solid Tumors or Acute Lymphoblastic Leukemia	Ongoing
			II	NCT00853307	Ovarian, Fallopian Tube, or Peritoneal Carcinoma	Ongoing
			II	NCT00807495	Aggressive Non-Hodgkin's Lymphoma	Ongoing
			II	NCT01154816	Recurrent or Refractory Solid Tumors or Leukemia	Recruiting
PF03814735	Aurora A (5 nM)	n/a	I	NCT00424632	Advanced Solid Tumors	Completed
	Aurora B (0.8 nM)					
	Aurora C n/a					
PHA739358	Aurora A (13 nM)	FGFR1 (47 nM)	II	NCT00766324	Hormone Refractory Prostate Cancer	Completed
	Aurora B (79 nM)	Abl (25 nM)	II	NCT00335868	Chronic Myelogenous Leukemia That Relapsed after Imatinib Mesylate or c-ABL Therapy	Unknown
	Aurora C (61 nM)	Ret (31 nM)	II	NCT00872300	Multiple Myeloma	Terminated
		TRKA (31 nM)				
R763/ AS703569	Aurora A (4 nM)	n/a	I	NCT01097512	Gemcitabine Combination in Advanced Malignancies	Completed
	Aurora B (4.8 nM)		I	NCT01080664	Hematological Malignancies	Ongoing
	Aurora C (6.8 nM)		I	NCT00391521	Solid Tumors	Completed
SNS314	Aurora A (9 nM)	TRKB (5 nM)	I	NCT00519662	Advanced Solid Tumors	Completed
	Aurora B (31 nM)	TRKA (12 nM)				
	Aurora C (3.4 nM)	Flt4 (14 nM)				
		Fms (15 nM)				
		DDR2 (82 nM)				
		Axl (84 nM)				
		c-Raf (100 nM)				
VX680/ MK0457	Aurora A (0.7 nM)	n/a	I	NCT00104351	Advanced Cancer	Terminated
	Aurora B (18 nM)		I	NCT00111683	Leukemia	Completed

TABLE 2. (Continued)

Compound	Primary Aurora Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
	Aurora C (4.6 nM)		II	NCT00290550	Lung Cancer	Terminated
			I	NCT00099346	Advanced Colorectal Cancer and Other Advanced Solid Tumors	Terminated
			II	NCT00405054	T315I Mutant CML	Terminated
			I	NCT00500006	Chronic Myelogenous Leukemia and Ph-Positive Acute Lymphoblastic Leukemia	Terminated
VX689/ MK5108	Aurora A (0.064 nM)	n/a	I	NCT00543387	Advanced and/or Refractory Solid Tumors	Completed
	Aurora B (14 nM)					
	Aurora C (12 nM)					
XL228	Aurora A (3 nM)	Src (5 nM)	I	NCT00526838	Advanced Malignancies	Terminated
	Aurora B (0.6 nM)	IGF1R (2 nM)	I	NCT00464113	Chronic Myeloid Leukemia or Ph-Positive Acute Lymphocytic Leukemia	Terminated
	Aurora C n/a	FGFR1 (8 nM)				
		FGFR2 (2 nM)				
		FGFR3 (3 nM)				
		Abl (7 nM)				

TABLE 3. Polo-Like Kinase Inhibitors Undergoing Clinical Investigation (http://clinicaltrials.gov)

Compound	Primary PLK Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
BI2536	PLK1 (0.8 nM)	ERBB4	II	NCT00701766	Refractory or Relapsed Acute Myeloid Leukaemia	Completed
	PLK2 (3.5 nM)	HGFR (<10 nM)	II	NCT00376623	Advanced or Metastatic Non-Small Cell Lung Cancer	Completed
	PLK3 (39 nM)	PI3Kα	I	NCT00243087	Refractory or Relapsed Advanced Non-Hodgkin's Lymphoma	Unknown
		TIE2	II	NCT00526149	Recurrent or Metastatic Solid Tumors	Ongoing
			II	NCT00710710	Advanced, Unresectable Pancreatic Cancer	Completed
			II	NCT00412880	Small Cell Lung Cancer	Completed
			II	NCT00706498	Prostate Cancer	Completed
BI6727	PLK1 (0.87 nM)	n/a	II	NCT00804856	Monotherapy or in Combination with Cytarabine in Acute Myeloid Leukaemia	Recruiting
	PLK2 (5 nM)		II	NCT00824408	With and Without Pemetrexed Compared to Pemetrexed in Advanced NSCLC	Ongoing
	PLK3 (56 nM)		I	NCT00969761	With Cisplatin or Carboplatin in Patients with Advanced or Metastatic Solid Tumor	Recruiting
			I	NCT01145885	Various Solid Tumors	Completed
			I	NCT01022853	Combination with BIBF1120 in Solid Tumors	Recruiting
			I	NCT01206816	Combination with Oral BIBW 2992 (Afatinib) in Patients with Advanced Solid Tumors	Recruiting
			II	NCT01121406	Ovarian Cancer	Ongoing
			II	NCT01023958	Urothelial Cancer	Ongoing
			I	NCT00969553	Various Solid Cancers	Ongoing
			I	NCT01348347	Advanced Solid Tumors	Recruiting
GSK461364	PLK1 (2.2 nM)	Aurora A (4.8 nM)	I	NCT00536835	Non-Hodgkins Lymphoma	Completed
	PLK2 n/a	CDK2 (7.6 nM)				
	PLK3 (9.1 nM)					
NMS1286937	PLK1 n/a	n/a	I	NCT01014429	Advanced/Metastatic Solid Tumors	Recruiting
	PLK2 n/a					
	PLK3 n/a					

TABLE 3. (Continued)

Compound	Primary PLK Targets (IC50)	Activity Against Other Kinases (IC50)	Clinical Information			
			Phase	Trial	Condition(s)	Status
ON01910	PLK1 (9 nM)	PDGFR (18 nM)	I	NCT00861783	With Irinotecan or Oxaliplatin in Patients with Hepatoma	Completed
	PLK2 (260 nM)	Bcr-Abl (32 nM)	I	NCT00861328	With Irinotecan or Oxaliplatin in Patients with Advanced Solid Tumors	Completed
	PLK3 (>10 μM)	Flt1 (42 nM)	I	NCT01125891	With Gemcitabine in Solid Tumors	Completed
		Src (155 nM)	I	NCT01048619	Myelodysplastic Syndrome	Ongoing
		Fyn (182 nM)	I	NCT00854646	Refractory Leukemia or Myelodysplastic Syndrome	Recruiting
		CDK1 (260 nM)	II/III	NCT01360853	Untreated Metastatic Pancreatic Cancer	Recruiting
			I/II	NCT00854945	Myelodysplastic Syndrome or Acute Myeloid Leukaemia	Recruiting
			I	NCT00861510	Relapsed Mantle Cell Lymphoma, Multiple Myeloma, Chronic Lymphocytic Leukemia	Recruiting
			III	NCT01241500	Refractory Myelodysplastic Syndrome Patients with Excess Blasts	Recruiting
			I	NCT01168011	Solid Tumors	Recruiting
			II	NCT00856791	Ovarian Cancer	Completed
			I	NCT01165905	With Gemcitabine in Advanced Solid Tumors	Completed
			I/II	NCT01167166	Acute Myeloid Leukemia and Acute Lymphoid Leukemia	Recruiting
			II	NCT00906334	Myelodysplastic Syndrome	Ongoing
			I	NCT00867061	Intermediate-1, -2, or High Risk Myelodysplastic Syndrome	Withdrawn
			I	NCT00533416	Myelodysplasia	Recruiting
			II	NCT01326377	Intermediate-1, -2, or High Risk Trisomy 8 Myelodysplastic Syndrome	Ongoing
			II	NCT00987584	Myelodysplastic Syndrome	Ongoing
TAK960	PLK1 n/a PLK2 n/a PLK3 n/a	n/a	I	NCT01179399	Advanced Nonhematologic Malignancies	Recruiting

II clinical assessments of MK-1775 in combination with DNA-damaging agents including radiation in ovarian, cervical and other solid tumors (http://clinicaltrials.gov).

Haspin is a protein kinase that specifically phosphorylates histone H3 at threonine 3, a process critical to mitotic progression.[110] Small-molecule inhibitors of Haspin, such as CHR-6494, have recently been identified and possess powerful anti-tumor activity *in vitro*, *ex vivo*, and *in vivo* but have yet to be tested in a clinical setting. [111]

Cdc7 is a serine-threonine kinase that is necessary to initiate S phase.[112] Although originally discovered in budding yeast, Cdc7 and its protein regulator Dbf4 are overexpressed in human cancer cell lines and in many primary tumors compared with matched normal tissues.[113] Inhibition of Cdc7 in cancer cells by RNAi inhibits S-phase progression, causing p53-independent cell death.[114–115] Two inhibitors of Cdc7 activity are undergoing clinical trials, BMS-863233 and NMS-1116354 (http://clinicaltrials.gov), results for which have yet to be released.

VI. CONCLUSION

Despite promising results from targeting cell cycle kinases in preclinical models, further experimental and clinical data are necessary before they can be considered for routine clinical application. Areas of unmet need include: understanding the role of individual CDK activities in specific tumor subtypes; rational drug design that takes into account the pharmacokinetic complexities of targeting the cell cycle machinery; and clinically useful predictors of therapeutic benefit to ensure that drugs are used in the appropriate patient subgroups and if relevant, used in appropriate combinations with other compounds to provide the best therapeutic strategies. The latest generation of cell cycle kinase inhibitors suggests that there is the potential for eventual translation into the clinic, although much work remains to be done to determine which combination of CDKs or CDKs plus other kinases will prove most effective as targets for cancer therapy, and how to best match specific cell cycle kinase inhibitors to the CDK/tyrosine kinase signature of a specific cancer, or indeed a specific patient profile.

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