ELSEVIER

Progress in the evaluation of CDK inhibitors as anti-tumor agents

Campbell McInnes

Pharmaceutical and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC 29208, USA

The increase in understanding of the events of cell growth and division has enabled the development of pharmacological agents inhibiting key regulatory proteins, with the cyclin dependent kinases (CDKs) representing a major area of interest. Owing to multiple CDK variants having cell cycle and transcriptional regulatory roles and difficulties in generating selective inhibitors, the prospects for drug discovery and development are complex. Numerous CDK inhibitors with differing mechanistic profiles are currently being preclinically and clinically evaluated but have not as of yet resulted in a drug approval. The major issues of CDK inhibition related to current understanding from genetic studies and also from observed anti-tumor efficacy of representative compounds are discussed in this review.

Introduction

Since the molecular mechanisms governing the mammalian cell cycle were first elucidated in the 1970s and 1980s by Hartwell, Hunt and Nurse (awarded the Nobel Prize for Medicine in 2001) [1], it has been recognized that synthetic modulators of cell growth and division have significant potential as anti-tumor therapeutics. The cyclin dependent kinases (CDKs) established as master regulators of cell cycle checkpoints have since been extensively pursued as oncology drug targets among many other protein kinases with roles in cell cycle regulation. The inhibition of protein kinase induced phosphorylation events is a principal method by which cell cycle events can be targeted, and has been invigorated by the successful approval of a number of tyrosine kinase inhibitors involved in cell signaling and angiogenesis in oncology. Progress in the development of potential therapeutics targeting the CDKs has been greatly facilitated by the growing body of structural information relating to activation, regulation and small molecule inhibition. Despite more than a decade of investigation, proof of concept for CDK inhibition in terms of an FDA approved cancer therapeutic has not yet been achieved. The status of promising candidates, focusing on the current classes of mechanistically different compounds obtained through selective CDK inhibition, is discussed.

E-mail address: mcinnes@sccp.sc.edu.

Timing is not everything

Subsequent to the first identification of the CDKs in yeast, at least 11 variants of the catalytic subunit have been identified that are dependent upon a cyclin for activation [2]. The cyclins involved in cell cycle progression are transiently expressed in response to growth signals, leading to precise control of the timing of CDK activity. The catalytic subunit of the cell cycle CDKs is infrequently mutated in human tumors, however deregulation of kinase activity has been demonstrated to result in cellular transformation [3]. Such events include cyclin over-expression, mutation of endogenous CDK inhibitors including $p16^{INK4a}$, $p21^{WAF}$ and $p27^{Kip}$ in addition to deregulatory phosphorylation events through activating and deactivating kinases/phosphatases.

Currently, cell cycle roles have been implicated for only CDKs 1–4, 6 and 7 (CDK activating kinase) although CDK11 may play a part in mitotic progression [4]. Sequential phosphorylation of the retinoblastoma protein (pRb) by CDK4,6/cyclin D and CDK2/cyclin E relieves suppression of the activity of members of the E2F family of transcription factors allowing transition through the restriction point prior to the G1/S boundary (Figure 1). Other targets of CDK2/E include p27Kip1, where phosphorylation results in Skp2 mediated destruction [5], as well as p220 nuclear protein and nucleophosmin involved in histone gene transcription and centrosome duplication, respectively [6,7]. As cells prepare to exit from S phase, timed neutralization of E2F transcriptional function by CDK2/cyclinA2 phosphorylation is required to prevent apoptosis

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2008.06.012

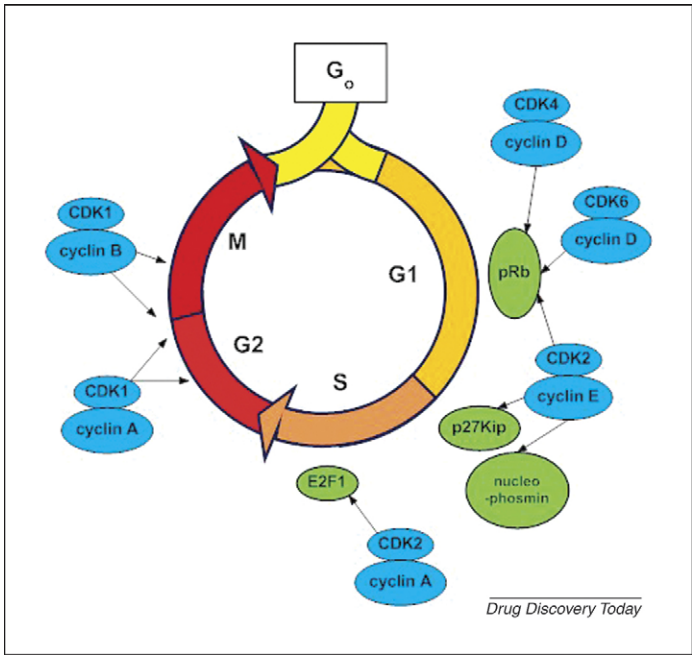


FIGURE 1

Roles of the CDK variants and major CDK substrates involved in regulating the stages of the mammalian cell cycle. Selective inhibition of the illustrated CDKs would be expected to be, and in some cases has been, shown to produce a cellular phenotype consistent with their control of the specific cell cycle phase.

triggered by persistent E2F activity [8]. CDK1 in complex with cyclin A1 or B1 has defined roles in regulating the G2/M checkpoint and progression through mitosis (Figure 1). Numerous CDK1 substrates have been identified and have been implicated in centrosome duplication, DNA replication, spindle assembly and chromosome condensation, among others [9].

In addition to cell cycle regulation, alternate roles have been described for CDKs 2, 7, 8 and 9 in the regulation of transcription initiation and elongation (Figure 2) through phosphorylation of the C-terminal domain of RNA polymerase II (RNAP II). Inhibition of the major players, CDK7/cyclin H and CDK9/cyclin T, provides anti-tumor rationale in that reduction in the mRNA levels (CDK mediated transcriptional control) of anti-apoptotic proteins such as Mcl-1 and Bcl-2 triggers programmed cell death [10]. Transcripts for the anti-apoptotic proteins most sensitive to CDK inhibition are believed to be unstable and rapid degradation of mRNA levels occurs when transcription is attenuated through blocking of CDK mediated phosphorylation events.

Does genetics tell us which CDK to knockout?

In recent years, the conventional understanding of how CDKs function and their roles in the cell cycle has been challenged through a number of studies [11]. First, various cancer cell lines have been shown to continue to proliferate after CDK2 depletion, suggesting that other CDK paralogs substitute in G1 and S [11]. CDK4 was able to phosphorylate pRb at CDK2 specific sites, thus providing support for this hypothesis [12]. Subsequent results indicated that CDK2 is non-essential in that embryonic fibroblasts lacking CDK2 proliferate normally and also that CDK2 knockout mice were viable [12,13]. The redundancy of CDK4 and 6 was also suggested by null mammalian cells that enter the cell cycle and enter S phase normally [14]. Recently, it has been demonstrated that mouse embryos deficient in all of the interphase CDKs develop to mid-gestation as CDK1 can form complexes with all cyclins and resultantly phosphorylate pRb, thus activating E2F mediated transcription of proliferation factors [15]. Elimination of CDKs 2,3,4 and 6 in these investigations showed that expression levels of CDK1, and the cell cycle cyclins, appear to be unchanged, that catalytic activity of Cdk1 is sufficient for cell cycle progression

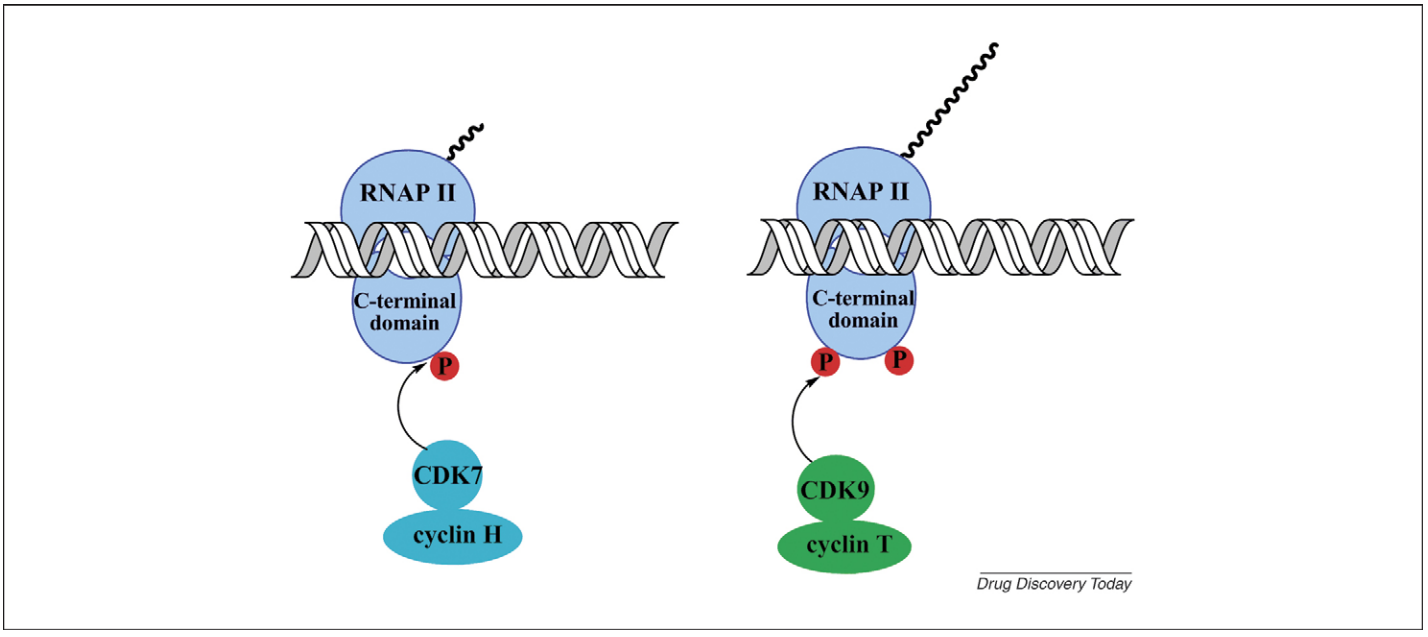


FIGURE 2

An antitumor hypothesis is provided by the role of CDK7 and CDK9 in transcription initiation and elongation. Downregulation of antiapoptotic genes at the mRNA level leads to cell death in tumors that depend on these for survival. Shown on the left is the role of CDK7 in initiating transcription through phosphorylation of the C-terminal domain of RNA polymerase II. Further phosphorylation of the CTD by CDK9 results in elongation of the mRNA (right).

and also that, in the absence of interphase Cdks, Cdk1 can execute all the necessary events for cell division.

The significance of these studies into the role of individual and multiple CDKs for cell cycle progression and survival of cancer cells and implications for anti-tumor drug development are still uncertain. While CDK knockdown and knockout experiments provide key insights into the roles of individual isoforms and redundancy issues, small molecule mediated inhibition of CDK activity will probably result in a significantly different phenotype, when the functional kinase is present. Under 'normal' conditions, transient cyclin expression and CDK binding drives proliferation and is therefore dependant on which cyclin is present at the cell cycle stage. As individual CDKs preferentially form complexes with available cyclins, these will predominate over other CDKs that are present and will be inhibited by the drug treatment. Knockout and knockdown experiments therefore do not diminish justification for pursuing individual CDK isotypes in anti-tumor drug therapy and 'selective' inhibitors should provide specific insights into the biology of the cell cycle and transcriptional inhibition and validate mechanism driven target hypotheses for the CDKs. To this end, the following discussion illustrates key compounds in development, illustrating these concepts while updating clinical progress where available.

Cell cycle CDK inhibitors

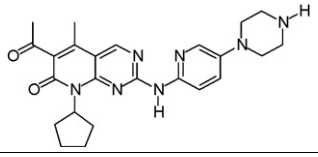
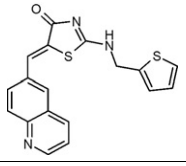
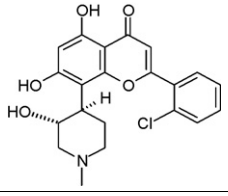
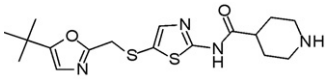
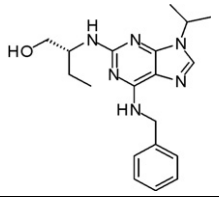
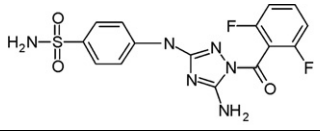
PD0332991

The discovery of PD0332991 (Table 1), is a significant step in the development of specific CDK inhibitors. A particular substitution of the pyrido[2,3-d]pyrimidine-7-one core structure resulted in an ATP competitor that is completely selective for CDK4/cyclin D1 and CDK6/cyclin D2,3 versus studied members of the CDK family and against a variety of protein kinases [16]. As the pathway involving CDK4/6, cyclin D and its regulators and substrates is very frequently disrupted in tumorigenesis, and the observations that ablation of cyclin D1 or CDK4 can confer resistance to breast tumors [17,18], PD0332991 represents at the minimum a useful tool compound in oncology.

This compound (CDK4/D1 IC_{50}, 0.011 μmol/L; CDK6 IC_{50}, 0.016 μmol/L) is a potent antiproliferative agent with reported studies indicating a mechanism consistent with selective CDK4/6 inhibition. Treatment of retinoblastoma (Rb)-positive tumor cells results in induction of G1 arrest accompanied by reduction of phospho-Ser780/Ser795 on the pRb [19]. Evaluation of PD0332991 in a panel of 8 Rb-positive tumors indicated potent anti-proliferative activity against all lines and, conversely, was inactive against the Rb-negative tumors including the MDA-MB-468 breast carcinoma [20]. These data strongly suggest that inhibition of

TABLE 1

Status of *in vivo* anti-tumor activity for described CDK inhibitors

CDK inhibitor	Chemical structure	CDK specificity	Phenotype	Clinical stage
PD0332991 (Pfizer)		CDK4, 6	G1 Arrest	Phase I (Lymphomas; Multiple Myeloma with Bortemizib)
RO3306 (Hoffman-LaRoche)		CDK1	G2/M	Preclinical
Flavopiridol (Aventis)		CDK9	G1/S; G2/M; transcription inhibition	Numerous phase I and II; Phase I (CLL)
SNS-032 (Sunesis)		CDK2, 7,9	Cell cycle/ transcription inhibition	Phase I (advanced solid tumors; B-lympoid malignancies)
R-Roscovitine (Cyclacel)		CDK2, 7,9	G2/M, Transcription Inhibition	Phase I (various tumors; response in metastatic ovarian cancer) Phase II, lung and breast
JNJ-7706621 (Johnson and Johnson)		CDK1,2 (Aurora A and B)	G1 Delay, G2/M arrest, Endoreduplication	Preclinical

CDK4/6 is the major determinant of anti-tumor activity. Subsequent oral administration to mice bearing Colo-205 xenografts resulted in potent tumor regression and, although tumors regrew after treatment was removed, reimplantation into naïve mice resulted in similar activity of the compound, thereby indicating that no resistance had occurred. The hypothesis that selective blocking of CDK4/6 activity is a viable therapeutic strategy in Rb-positive tumors is suggested by these and further ongoing data in the clinical evaluation of PD0332991 in Mantle-Cell and non-Hodkin lymphomas (MCL and NHL, respectively). It has been shown that MCL cell lines displayed diminished expression of the cyclin D1 inhibitor p16 and that PD0332991 profoundly suppressed Rb phosphorylation, proliferation and cell cycle progression at the G0/G1 phase of MCL cells [21]. Currently, recruitment is ongoing for Phase I trials involving PD0332991 in MCL, NHL and as a combination treatment with the proteasome inhibitor, bortemizib, in multiple myeloma [22].

RO-3306

Recent data proposing a less dominant role for CDK2 has stimulated renewed interest in investigating and developing CDK1 inhibitors. The synthesis, characterization and mechanistic study of R0-3306 (Table 1) has recently been undertaken [23–25]. This compound, described as a selective CDK1 inhibitor, belongs to a series of thiazolinones, the most specific of which are more than 100-fold selective versus CDK2. RO-3306 has a CDK1/cyclin B1 IC_{50} of 35 nM, giving it 10-fold and 50-fold selectivity against CDK2/cyclin E and CDK4/cyclin D, respectively. CDK1 is believed to be an appropriate target for anti-cancer drug development due its key role in regulating mitotic entry and progression. CDK1 is activated through cyclin B accumulation and binding and after removal of inhibitory phosphates blocking the catalytic site. A CDK1 specific inhibitor should therefore induce pre-mitotic growth arrest while leaving the G1/S transition unaffected.

The anti-oncogenic potential of CDK1 inhibition was also recently suggested by a study where tumors overexpressing Myc were sensitized to apoptosis through CDK1 inhibitors that led to downregulation of Survivin [26]. RO-3306 indeed led to complete block of the cell cycle in the G2/M phases. In contrast to PD0332991, RO-3306 did not inhibit the phosphorylation of Ser795 and Ser811, the known CDK2 and CDK4 specific sites in the Rb protein, and treated cells passed through the G1/S transition as expected; however a delay in S phase was observed consistent with the mild potency of this compound on CDK2. The authors of this study conclude since G1/S progression occurs in compound treated cells, in the presence of normal levels of CDK2/4, CDK1 does not play a significant part outside of mitosis. Further experiments with RO-3306 on HeLa cells synchronized in prometaphase delineated insights into CDK1 functions in mitosis. Treated cells undergo dramatic changes in morphology resulting from rapid chromosome condensation, loss-of phospho H3 and cyclin B1 and inducing rapid mitotic exit into a G1-like state. Additional data showed that this observation could be attributed solely to loss of CDK1 activity and suggested that catalytic activity of CDK1 is sufficient for maintenance of mitosis in human cells. More complete validation for CDK1 as a drug target was provided by further data where, in contrast to the short duration of treatment in the above studies, HCT116 and SW480, when exposed to RO-3306 for up to 96 h, showed significant pro-apoptotic response as indicated by a substantial sub-G1 fraction in cell cycle analysis and by Annexin V staining compared to non-transformed cell lines. The totality of data from cellular treatment with RO-3306, in conjunction with the recent study indicating that in the absence of all interphase CDKs, CDK1 is sufficient to drive the cell cycle, providing additional target validation for inhibiting CDK1 as an anti-tumor therapeutic strategy. Further preclinical and clinical data is needed to validate this statement however.

Transcriptional CDK inhibitors

Flavopiridol (alvocidib, HMR1275)

Flavopiridol (Table 1) was the first CDK inhibitor to enter clinical trials and has been in development in oncology for a number of years with more than 50 clinical trials undertaken or currently in progress. Since Flavopiridol is selective for CDK9 relative to other CDKs, (IC50, 3 nM versus 30 nM and 100 nM for CDK1 and CDK2, respectively), its mechanism of action is primarily believed to be through inhibition of transcription (Figure 2) [27]. This flavonoid inhibitor has been reported to inhibit the CDKs selectively over other protein kinases involved in signal transduction and cell cycle regulation. A majority of tumor cells exposed *in vitro* to Flavopiridol arrest at G1/S; however significant fractions of cells are found in sub-G1 and at the G2/M transition indicating significant levels of apoptosis and mitotic arrest are induced. Western blot analysis of treated cells demonstrates that proteins under transcriptional control of CDK phosphorylation are downregulated upon treatment including the cell cycle components cyclin D1 and D3, the antiapoptotic factor MCL-1 and the angiogenesis promoter vascular endothelial growth factor receptor (VEGFR) [3]. The mRNAs resulting from CDK mediated transcription initiation and elongation (Figure 2) are thought to be metastable and therefore further abrogation of phosphorylation of RNAPII and additional transcription of these genes leads to a rapid decline in mRNAs for Bcl-2, XIAP, c-Myc and transcripts contributing to the p53 pathway. p53 activation can occur in Flavopiridol treated cells through downregulation of mdm2. This events leads to p53 accumulation and has been shown to contribute to apoptosis in cells containing wild-type p53 [28].

Flavopiridol is highly active against chronic lymphocytic leukemia (CLL), demonstrated *in vitro* and recently *in vivo*, where the survival of tumor cells results from the continuous expression of antiapoptotic proteins and which themselves are dependent on CDK9 activity [29–31]. While completed Phase II trials with Flavopiridol have only revealed marginal anti-tumor activity [32–35], recent findings suggest that the inhibitor is significantly more potent when an optimized dosing regime was used in the treatment of CLL patients [29,30]. A 42 patient Phase I study was conducted using a 30-min loading dose, followed by 4–6 weeks of 4 h infusions in fludaribine refractory CLL patients. The observed dose-limiting toxicity in this trial was acute tumor lysis syndrome resulting in fatal hyperkalemia. Flavopiridol could be administered safely however if careful monitoring and intervention for hyperkalemia was used. Anti-leukemic activity of this drug was indicated by a 45% partial response rate with lasting efficacy evidenced by the median response duration of 12 months. The dramatic responses observed were additionally highlighted by the observation that durable remissions were observed in a significant

proportion of patients with high risk genetic abnormalities and who respond poorly to other treatments. Further clinical trials in CLL patients, as well as expanding the use of this novel dosing schedule to other forms of Leukemia and also to solid tumors, should continue to validate the consequences of pan-CDK inhibition with potent modulation of CDK9 activity playing a significant part.

In addition to investigations of CDK inhibitors as single agents, numerous preclinical and clinical studies have been conducted involving combinations with DNA damaging and other cytotoxic drugs. When administered subsequent to taxane therapy, Flavopiridol potentiates the apoptotic effect of these antimitotic drugs. A recent Phase I trial in which docetaxal was administered at least 4 h before Flavopiridol treatment gave promising results. A fixed dose of docetaxel, followed by a dose range of 20–70 mg/m^2 of Flavopiridol, indicated an effective and safe regimen at all levels, with stable disease observed in 10 patients, 1 complete and 4 partial responses among the 27 patients enrolled in the trial.

SNS-032 (BMS-387032)

SNS-032 (Table 1) is an aminothiazole CDK inhibitor in licensed by Sunesis Pharmaceuticals after discovery and preclinical development by Bristol Myers Squibb. It was initially reported as a CDK2 selective inhibitor (CDK2/cyclinE IC_{50} = 48 nM, 10- and 20-fold selectivity against CDK1 and 4) however further characterization revealed that it is a CDK9 specific compound (IC_{50} = 4 nM). Consistent with the reported activities, SNS-032 has been shown to inhibit the cell cycle and transcription. Comparison of *in vivo* anti-tumor activity of SNS-032 against Flavopiridol suggested that it was significantly more potent in P388 murine models and A2780 human ovarian tumor models [36]. The observed efficacy combined with animal pharmacology and toxicology experiments showed good oral bioavailability, dose-dependant and reversible toxicities and a plasma half-life of 5–7 h, therefore warranting further investigation in human clinical trials. A Phase I dose-escalation trial has been conducted in 21 patients with advanced solid tumors, intravenously administered in three weekly doses over 21 days [37]. Dose escalation was conservative as steep mortality versus dose was observed at higher doses. A starting dose of 4 mg/m^2 was followed by increases of 67, 50, 33, 25, 20 and 15% and doses were administered weekly as a 1-h infusion. This study indicated a half-life of 5–10 h in humans with an average oral bioavailability of 19%. Generally speaking, SNS-032 in this trial was well tolerated with 15% of patients experiencing stable disease. These data suggest that this compound warrants further investigation as an anti-tumor agent although more convincing evidence of *in vivo* activity will be required. SNS-032 is also planned to be evaluated in a Phase I multi-center dose-escalation study in patients with advanced β-lymphoid malignancies via intravenous administration, although no details as of yet are available.

Seliciclib (CYC-202, R-roscovotine)

This is a trisubstituted purine analog (Table 1), selective for CDK2/cyclin E and possessing sub-micromolar activity on CDK7/cyclin H and CDK9/cyclin T. Similar to Flavopiridol and SNS-032, Seliciclib has been demonstrated to downregulate MCL-1, prior to the induction of apoptosis and therefore is consistent with the transcription inhibition mechanism [38,39]. A Phase I trial has been completed and at least one Phase II trial is currently ongoing [40]. The Phase I trial was performed with a 7 day b.i.d. schedule, with Seliciclib being administered orally to 21 patients and who were treated with doses of 100, 200 and 800 mg, respectively. Dose-limiting toxicities observed at 800 mg b.i.d included fatigue, skin rash and hypokalaemia. While no dramatic tumor responses occurred, disease stabilization was recorded in eight patients with one patient lasting for 18 weeks on Seliciclib therapy.

Multi-targeted CDK inhibitors

JNJ-7706621

The dual CDK and Aurora Kinase inhibitor JNJ-7706621 (Table 1) has advanced through preclinical testing although, as of yet, has not reached investigational new drug status [41]. The Aurora Kinases are crucial regulators of progression through mitosis, having key roles in chromosome movement and organization, and in ensuring proper assembly of the mitotic spindle during chromosome segregation. The *in vitro* and *in vivo* activity of this compound has recently been reported indicating significant potential as an anti-tumor agent [42]. JNJ-7706621 is a pan-CDK and Aurora inhibitor with low nanomolar inhibition of CDKs 1, 2 and 3, Auroras A and B and also sub-micromolar activity against CDKs 4 and 6. When this compound was tested against a large panel of various protein kinases little evidence of inhibitory action was observed. The compound shows significant potency against all tumor types evaluated regardless of p53, pRb or P-glycoprotein status – of a similar magnitude to that of Flavopiridol and SNS-032. In this study, high levels of inhibitory phosphorylation and low levels of activating phosphorylation were observed as a result of the inactivation of CDC25C. Described effects of JNJ-7706621 are therefore consistent with its primary action as a CDK inhibitor including the CDK1/cyclin B mitotic arrest phenotype of treated compounds.

Anti-tumor effects mediated by Aurora inhibition were demonstrated in nocodazole synchronized cells, where JNJ-7706621 induced endoreduplication, decreased phosphohistone H3 and compromised spindle checkpoint function. The *in vivo* anti-tumor efficacy of the inhibitor was evaluated in an A375 melanoma xenograft and showed highly potent activity for a 100 mg/kg 7 day on/7 day off dosing schedule. In this study, 93% tumor growth inhibition was achieved with all animals surviving to the end of the study, thus suggesting significant potential of this compound in oncology. Potential mechanisms of resistance to this inhibitor were anticipated by incrementally treating a HeLa cell line with increasing concentrations of JNJ-7706621 after which a 16-fold resistance was observed [43]. Use of the ABCG2 transporter inhibitor fumitremorgin C restored sensitivity to the drug, therefore providing insights for its clinical uses.

Potential alternatives and advances in CDK inhibition

Cyclin groove inhibitors (CGI)

The positive regulatory subunit of the G1 and S phase CDKs – CDK4,6/cyclin D1CDK2/cyclin A and CDK2/cyclin E contains a shallow hydrophobic groove involved in substrate recruitment recognized by a number of cell cycle substrates and endogenous inhibitory proteins. Cell permeable CGI peptides have been shown to block CDK2 activity and to selectively kill tumor cells in an E2F dependant fashion and also to be active *in vivo* against mouse

tumor models [8,44]. Since phosphorylation by the transcriptional CDKs is not dependent on substrate recruitment, CGI molecules offer the potential of selective inhibition of cell cycle CDKs while retaining high specificity against other protein kinases [45]. As this site involves protein–protein recognition, development of drug-like inhibitors has been challenging, thus explaining the slow progress and relative lack of success. Recently, however, novel approaches have led to advances suggesting that the cyclin groove is a druggable binding site. A compound optimized through peptidomimetic development (IC_{50}, 8 nM) has been developed, consisting of only one natural amino acid residue and significantly improving its drug-likeness.

In addition, a drug discovery strategy for protein–protein interactions (REPLACE) was applied to the development of CGI molecules with more pharmaceutically relevant properties [46], replacing components of a highly charged pentapeptide with more drug-like moieties. In another strategy, cyclic peptidomimetic compounds targeting the cyclin groove showed potency enhancements while increasing logP values [47].

Inhibition of CDK–cyclin association

Since activation of all CDKs requires cyclin binding, another alternate strategy would be to abrogate formation of the CDK–cyclin complex. Recent studies have identified peptides [48] that bind to a surface pocket in cyclin A and disrupt its interaction with CDK2. The hexapeptide NBI1 was shown to be non-competitive with respect to ATP and CDK2 substrates, indicating that it is a promising template for further inhibitor design based on blocking this interaction. Each of these studies suggests that the alternative methods for CDK inhibition have potential, although these compounds remain in early discovery and have yet to move to preclinical and clinical stages.

Prognosis for CDK inhibitors in oncology

As of yet, proof of concept for CDK inhibition in the clinical setting has not been conclusively obtained. The latest generation of compounds suggests that there is potential for a drug approval, although much work remains to be done. As described here, depending on the balance of CDK selectivity, the mechanistic profiles and resultant anti-tumor effect of each compound class will be different. The pressing question remains to find out which profile, if any, of those described above, will result in a successful therapeutic outcome. Ongoing studies at the molecular level, in terms of genetics of CDK knockout and knockdown, and with respect to continued learning with current and future inhibitors both as single agents and in combination with conventional chemotherapeutics, should inform and advance clinical development and provide insights into feasibility of drug approvals.

References

1 Balter, M. and Vogel, G. (2001) Nobel prize in physiology or medicine. Cycling toward Stockholm. *Science* 294, 502–503
2 Malumbres, M. and Barbacid, M. (2005) Mammalian cyclin-dependent kinases. *Trends Biochem. Sci.* 30, 630–641
3 Shapiro, G.I. (2006) Cyclin-dependent kinase pathways as targets for cancer treatment. *J. Clin. Oncol.* 24, 1770–1783
4 Hu, D. *et al.* (2007) CDK11(p58) is required for the maintenance of sister chromatid cohesion. *J. Cell Sci.* 120 (Pt 14), 2424–2434
5 Carrano, A.C. *et al.* (1999) SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat. Cell Biol.* 1, 193–199
6 Okuda, M. (2002) The role of nucleophosmin in centrosome duplication. *Oncogene* 21, 6170–6174
7 Ma, T. *et al.* (2000) Cell cycle-regulated phosphorylation of p220(NPAT) by cyclin E/Cdk2 in Cajal bodies promotes histone gene transcription. *Genes Dev.* 14, 2298–2313
8 Chen, Y.N. *et al.* (1999) Selective killing of transformed cells by cyclin/cyclin-dependent kinase 2 antagonists. *Proc. Natl. Acad. Sci. U.S.A.* 96, 4325–4329
9 Ubersax, J.A. *et al.* (2003) Targets of the cyclin-dependent kinase Cdk1. *Nature* 425, 859–864
10 Loyer, P. *et al.* (2005) Role of CDK/cyclin complexes in transcription and RNA splicing. *Cell Signal* 17, 1033–1051
11 Malumbres, M. *et al.* (2008) CDK inhibitors in cancer therapy: what is next? *Trends Pharmacol. Sci.* 29, 16–21
12 Ortega, S. *et al.* (2003) Cyclin-dependant kinase 2 is essential for meiosis but not for mitotic cell division in mice. *Nat. Genet.* 35, 25
13 Berthet, C. *et al.* (2003) Cdk2 knockout mice are viable. *Curr. Biol.* 13, 1775–1785
14 Malumbres, M. *et al.* (2004) Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. *Cell* 118, 493–504
15 Santamaria, D. *et al.* (2007) Cdk1 is sufficient to drive the mammalian cell cycle. *Nature* 448, 811–815
16 Toogood, P.L. *et al.* (2005) Discovery of a potent and selective inhibitor of cyclin-dependent kinase 4/6. *J. Med. Chem.* 48, 2388–2406
17 Landis, M.W. *et al.* (2006) Cyclin D1-dependent kinase activity in murine development and mammary tumorigenesis. *Cancer Cell* 9, 13–22
18 Yu, Q. *et al.* (2006) Requirement for CDK4 kinase function in breast cancer. *Cancer Cell* 9, 23–32
19 Baughn, L.B. *et al.* (2006) 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
20 Fry, D.W. *et al.* (2004) Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. *Mol. Cancer Therap.* 3, 1427–1438
21 Marzec, M. *et al.* (2006) Mantle cell lymphoma cells express predominantly cyclin D1a isoform and are highly sensitive to selective inhibition of CDK4 kinase activity. *Blood* 108, 1744–1750
22 Anon., . www.clinicaltrials.gov/ct2/results?term=pd0332991
23 Vassilev, L.T. *et al.* (2006) Selective small-molecule inhibitor reveals critical mitotic functions of human CDK1. *Proc. Natl. Acad. Sci. U.S.A.* 103, 10660–10665
24 Vassilev, L.T. (2006) Cell cycle synchronization at the G2/M phase border by reversible inhibition of CDK1. *Cell Cycle* 5, 2555–2556
25 Chen, S. *et al.* (2007) Synthesis and activity of quinolinyl-methylene-thiazolinones as potent and selective cyclin-dependent kinase 1 inhibitors. *Bioorg. Med. Chem. Lett.* 17, 2134–2138
26 Goga, A. *et al.* (2007) Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. *Nat. Med.* 13, 820–827
27 Fischer, P.M. and Gianella-Borradori, A. (2005) Recent progress in the discovery and development of cyclin-dependent kinase inhibitors. *Expert Opin. Investig. Drugs* 14, 457–477
28 Demidenko, Z.N. and Blagosklonny, M.V. (2004) Flavopiridol induces p53 via initial inhibition of Mdm2 and p21 and, independently of p53, sensitizes apoptosis-reluctant cells to tumor necrosis factor. *Cancer Res.* 64, 3653–3660
29 Christian, B.A. *et al.* (2007) Flavopiridol in the treatment of chronic lymphocytic leukemia. *Curr. Opin. Oncol.* 19, 573–578
30 Byrd, J.C. *et al.* (2007) 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
31 Chen, R. *et al.* (2005) Flavopiridol induces apoptosis in chronic lymphocytic leukemia (CLL) cells through the transcriptional downregulation of anti-apoptotic proteins. *Proc. Am. Assoc. Cancer Res.* 46, Abs. 3266.
32 Aklilu, M. *et al.* (2003) Phase II study of flavopiridol in patients with advanced colorectal cancer. *Ann. Oncol.* 14, 1270–1273
33 Morris, D.G. *et al.* (2006) A Phase II study of flavopiridol in patients with previously untreated advanced soft tissue sarcoma. *Sarcoma* 64374
34 Van Veldhuizen, P.J. *et al.* (2005) A phase II study of flavopiridol in patients with advanced renal cell carcinoma: results of Southwest Oncology Group Trial 0109. *Cancer Chemother. Pharmacol.* 56, 39–45

35 Liu, G. *et al.* (2004) A Phase II trial of flavopiridol (NSC #649890) in patients with previously untreated metastatic androgen-independent prostate cancer. *Clin. Cancer Res.* 10, 924–928
36 Misra, R.N. *et al.* (2004) *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
37 Heath, E.I. *et al.* (2008) A phase 1 study of SNS-032 (formerly BMS-387032), a potent inhibitor of cyclin-dependent kinases 2, 7 and 9 administered as a single oral dose and weekly infusion in patients with metastatic refractory solid tumors. *Invest. New Drugs* 26, 59–65
38 Raje, N. *et al.* (2005) Seliciclib (CYC202 or R-roscovitine), a small-molecule cyclin-dependent kinase inhibitor, mediates activity via downregulation of Mcl-1 in multiple myeloma. *Blood* 106, 1042–1047
39 MacCallum, D.E. *et al.* (2005) Seliciclib (CYC202, R-Roscovitine) induces cell death in multiple myeloma cells by inhibition of RNA polymerase II-dependent transcription and downregulation of Mcl-1. *Cancer Res.* 65, 5399–5407
40 Benson, C. *et al.* (2007) 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
41 Huang, S. *et al.* (2006) Synthesis and evaluation of *N*-acyl sulfonamides as potential prodrugs of cyclin-dependent kinase inhibitor JNJ-7706621. *Bioorg. Med. Chem. Lett.* 16, 3639–3641
42 Emanuel, S. *et al.* (2005) 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
43 Seamon, J.A. *et al.* (2006) 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
44 Mendoza, N. *et al.* (2003) Selective cyclin-dependent kinase 2/cyclin A antagonists that differ from ATP site inhibitors block tumor growth. *Cancer Res.* 63, 1020–1024
45 Biondi, R.M. and Nebreda, A.R. (2003) Signalling specificity of Ser/Thr protein kinases through docking-site-mediated interactions. *Biochem. J.* 372 (Pt 1), 1–13
46 Andrews, M.J. *et al.* (2006) REPLACE: a strategy for iterative design of cyclin-binding groove inhibitors. *Chem. Biochem.* 7, 1909–1915
47 Andrews, M.J.I. *et al.* (2004) Design, synthesis, biological activity and structural analysis of cyclic peptide inhibitors targeting the substrate recruitment site of cyclin-dependent kinase complexes. *Org. Biomol. Chem.* 2, 2341–2735
48 Canela, N. *et al.* (2006) 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. Vol. 281, pp. 35942–35953