

CDK inhibitors in cancer therapy: what is next?

Marcos Malumbres¹, Paolo Pevarello², Mariano Barbacid¹ and James R. Bischoff²

¹ Molecular Oncology Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernandez Almagro 3, 28029 Madrid, Spain

² Experimental Therapeutics Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernandez Almagro 3, 28029 Madrid, Spain

The pursuit for drugs that inhibit cyclin-dependent kinases (CDKs) has been an intense area of research for more than 15 years. The first-generation inhibitors, Flavopiridol and CY-202, are in late-stage clinical trials, but so far have demonstrated only modest activity. Several second-generation inhibitors are now in clinical trials. Future approaches to determine clinical benefit need to incorporate both the lessons learned from these early compounds and information recently obtained from the genetic analysis of CDKs in preclinical models. Here we discuss key concepts that should be considered when validating the clinical utility of CDK inhibitors in cancer therapy.

Introduction

Cyclin-dependent kinases (CDKs) are serine/threonine protein kinases whose activity depends on binding and activation by cyclin partners. These heterodimeric complexes can phosphorylate various substrates involved in the control of transcription and cell-cycle progression in response to different stimuli [1]. Some CDKs, such as CDK1–CDK4, CDK6 and perhaps CDK11, are involved in progression through the cell cycle, whereas CDK7 has dual roles as a CDK-activating kinase (CAK) and a regulator of the transcriptional machinery. CDK8 and CDK9 seem to have key roles in the control of transcription by RNA polymerase II.

Other CDKs and CDK-like proteins function in more specific processes in particular types of cell [1]. CDK activity is increased in proliferative diseases such as cancer, owing to the frequent overexpression of positive regulators (cyclins) and the frequent inactivation of CDK inhibitors [2]. In addition, mutations in CDK4 that render this kinase insensitive to CDK inhibitors of the INK4 family have been observed in hereditary melanoma. These observations have been reproduced in various experimental tumor model systems [1,3]. Abnormalities in CDK activity and regulation have also been found in viral infections, proliferative renal diseases and neurodegenerative disorders such as Alzheimer's, Parkinson's and Nieman-Pick's disease, ischemia and traumatic brain injury, leading to an intensive search for small-molecule CDK inhibitors for therapeutic applications [4–7].

No CDK inhibitor has been approved for commercial use. First-in-class clinical compounds, such as the pan-CDK inhibitors Flavopiridol and CY-202 [which have half-maximal inhibitory concentration (IC₅₀) values in the submicromolar range for CDK1, CDK2, CDK4 (in the case of Flavopiridol), CDK5, CDK7 and CDK9], have undergone numerous phase II and phase III clinical trials. So far, the activity observed does not match the initial expectations for CDK inhibitors, although Flavopiridol recently demonstrated activity in individuals with refractory chronic lymphocytic leukemia. [8]. Second-generation CDK inhibitors that have been publicly disclosed as being either in clinical trials or in advanced preclinical testing are listed in Tables 1 and 2. These compounds are consistently more potent and/or are more drug-like than their forerunners, Flavopiridol and CY-202. In general, these inhibitors can be subdivided into three main classes.

- (i) Compounds with a broad CDK activity profile (e.g. CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9); the inhibitors SNS-032, AG-024322 and R-547 belong to this group.
- (ii) Compounds with exclusive or preferential CDK4/CDK6 or CDK2 activity; the inhibitors AT-7519 (CDK2) and PD-0332991 or P-276-00 (CDK4/CDK6) are examples of this subclass.
- (iii) Compounds with activity against CDKs and additional kinase targets that might be useful to enhance anti-tumor activity; this group features ZK-304709 (with additional VEGFR1–VEGFR3 and PDGFR- β activity), JNJ-7706621 (a CDK1, CDK2 and CDK3 inhibitor with Aurora A/B activity) and GPC-286199 [a CDK1, CDK2, CDK3, CDK5, CDK7 and CDK9 inhibitor with additional activity toward CRK (CDK-related kinase)].

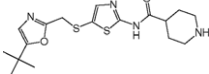
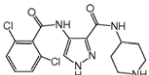
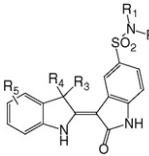
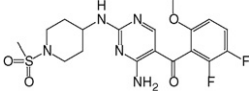
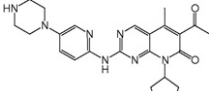
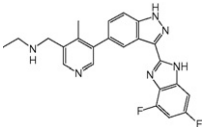
There are several possible explanations for both the modest activity and the toxicity of the CDK inhibitory molecules observed in the clinical setting [9–12], but we will not dwell on these compounds here. Instead, we discuss what we believe are key considerations when evaluating CDK inhibitors that target the cell cycle in order to determine correctly their clinical utility (or lack thereof).

Pharmacological considerations in targeting a cell-cycle-dependent mechanism

The biological role of many of these CDKs is regulation of the cell-division cycle [1]. In simple terms, the cell-division

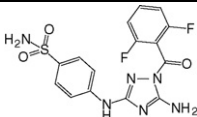
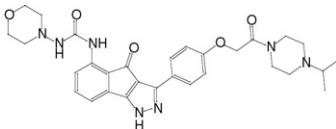
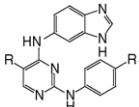
Corresponding author: Bischoff, J.R. (jrbischoff@cnio.es).

Table 1. Second-generation CDK inhibitors in confirmed clinical trials

Company	Code number	Disclosed or <i>bona fide</i> structure or chemotype	Reported CDK activity	Additional kinase activity	Clinical phase (last reported date)	Admin route	Conditions	Refs
Sunesis	SNS-032 (BMS-387032)		1,2,4,7,9	GSK-3	I/II (2007)	i.v.	Advanced breast cancer, melanoma, or non-small cell lung cancer (NSCLC); B-cell malignancies	http://www.cancer.gov/search/ViewClinicalTrials.aspx?cdrid=471893&version=HealthProfessional&protocolsearchid=3121042
Astex	AT-7519		1,2,4,5	GSK-3	I/IIa (2007)	i.v.	Advanced or metastatic solid tumors; refractory non-Hodgkin's lymphoma	http://www.astex-therapeutics.com/products/clinicalcandidates.php#AT7519 ; http://www.clinicaltrials.gov/ct/show/NCT00390117?order=1 [32]; http://www.cancer.gov/search/ViewClinicalTrials.aspx?cdrid=526031&version=HealthProfessional&protocolsearchid=3121002 ; http://www.nicholaspiramal.com/docs/analyst/NPRC_Jan1207.pdf [33–35]
Nicholas Piromal	P276-00	Rohitukine derivative	1,4,9		I/II (2007)	p.o.	Advanced refractory neoplasms; multiple myeloma	
Bayer Schering Pharma AG	ZK 304709		1,2,4,7,9	VEGFR1-3 PDGFR-β Flt-3	I (2006)	p.o.	Relapsed and/or refractory solid tumors	
Hoffmann-LaRoche	R-547 (Ro-4584820)		1,2,4,7		I (2006)	i.v.	Advanced solid tumors	[36]; http://www.clinicaltrials.gov/ct/show/NCT00400296?order=1
Pfizer	PD-0332991		4,6		I (2006)	p.o.	Neoplasms; non-Hodgkin's lymphoma	http://www.clinicaltrials.gov/ct/show/NCT00141297?order=2 ; http://www.pfizer.com/pipeline
Pfizer	AG-24322		1,2,4		I (2007) discontinued July 2007?	i.v.	Advanced solid tumors; non-Hodgkin's lymphoma	http://www.cancer.gov/search/ViewClinicalTrials.aspx?cdrid=447301&version=HealthProfessional&protocolsearchid=3121012 ; http://www.pfizer.com/pipeline

Abbreviations: GSK-3, glycogen synthase kinase 3; i.v., intravenous; PDGFR-β, platelet-derived growth factor receptor β; p.o., oral; VEGFR1-3, vascular endothelial growth factor receptors 1-3.

Table 2. Selected CDK inhibitors in advanced preclinical testing or in unconfirmed clinical trials

Company	Code number	Structure or chemotype	Reported CDK activity	Other kinase Activity	Stage (last reported date)	Admin route	Refs
Johnson and Johnson	JNJ-7706621		1,2,3	Aurora A/B	Selected for clinical trials (2005)	Oral	[37,38]
GPC Biotech	GPC-286199 RGB-286199		1,2,3,5,7,9	CRKs (p42/CCRK, PCTK1/PCTK3, PFTK1)	Preclinical testing (2005)	n.d.	GPC Biotech WO-2006002119; http://www.gpc-biotech.com/de/anticancer_programs/cdk/index.html ; for related compounds, see [39]
Bayer	BAY 80-3000		1,2		Preclinical testing (2005)	n.d.	http://www.bayer.com/de/20051208_rdinvestordaycatino.pdf ; for related compounds, see [40]

Abbreviations: CRKs, CDK-related kinases; n.d., not determined.

cycle is a series of temporally regulated events that require the execution of a previous event before the next event can occur. The dependence on the completion of certain events before the execution of subsequent events allows the cell to proceed through the cell-division cycle in an orderly manner and ultimately to pass an intact genome to each daughter cell. The heterogeneity of cell populations (with regard to the position of the cell in the cell-division cycle) within a single tissue means that an exogenously added reversible inhibitor of the cell cycle should be present for a significant portion of the cell cycle to ensure that most cells are 'hit' at the appropriate point when the target is required in the cell-division cycle. For example, the estimated doubling time of an ovarian carcinoma cell is 5–6 days [13], which implies that ideally the tumor should be exposed to a cell-cycle-dependent targeted inhibitor for 5–6 days to be able to target most cells that are cycling in the tumor.

To cover the whole length of the cell-division cycle pharmacologically, a compound should have a pharmacokinetic and safety profile that takes into account this mechanism of action. In a clinical setting, this profile can be achieved by long intravenous infusions. Although they can be clinically cumbersome, long intravenous infusions would be justified for highly efficacious compounds and/or for compounds with a narrow therapeutic window. Ideally, a CDK inhibitor that is predicted to have a noncytotoxic mechanism of action towards 'normal' tissues should be orally bioavailable and endowed with a pharmacokinetic profile that provides sufficient exposure throughout the whole cell cycle with a reasonable schedule of administration. Clinical trials of the second-generation CDK inhibitors that do not incorporate this salient point into their protocols will not be able to provide the data necessary to conclude whether an observed lack of activity is due to a lack of importance of the target or simply to insufficient exposure of the diseased tissue to the compound. The time required for proper exposure to pan-CDK inhibitors is more difficult to predict because such inhibitors might be acting at multiple points in the cell cycle.

Several preclinical studies have demonstrated synergy when CDK inhibitors are combined with cytotoxic drugs (e.g. cisplatin, 5 fluorouracil, doxorubicin and paclitaxel), especially when administration of the cytotoxic drug precedes that of the CDK inhibitor [14]. These results might suggest that CDK inhibition has a better therapeutic effect when cells are 'synchronized' or arrested in specific cell-cycle phases. To synchronize the cells in the target tissue of an individual, however, the first agent – if reversible – will need to be present for most of the cell cycle of the target tissue to achieve synchronization of the cell population. Such combinations with the first-generation inhibitors have been difficult to evaluate in the clinic because of toxicity, indicating that further molecular understanding is required for to fine-tune the pharmacological inhibition of CDKs in the clinic.

What is genetics telling us about potential therapeutic uses of CDK inhibitors?

Recent studies from genetically engineered mice have failed to support the concept that cell-cycle CDKs play a sequential role in driving the various phases of the cell cycle, at least during interphase [15–22]. It is possible that these observations are due to compensatory functions of the known interphase kinases, CDK2, CDK4 and CDK6. CDK3, another interphase CDK, does not play a role in the cell cycle in these models because it is inactivated owing to a naturally occurring mutation. In addition, these studies have revealed that each of these CDKs has unexpected roles in specialized cell types that cannot be compensated by other CDKs.

For example, genetic ablation of *Cdk2* does not result in obvious abnormalities in cell proliferation and *Cdk2*-null mice are viable [15,16]. These mice are sterile owing to specific arrest in the first meiotic division, however, indicating that CDK2 has a key role in germ cells (Figure 1). Deletion of *Cdk4* results in decreased proliferation of adult pancreatic β cells and pituitary endocrine cells [17,18]. Similarly, deletion of *Cdk6* provokes decreased erythroid cell numbers [19]. Most of the other

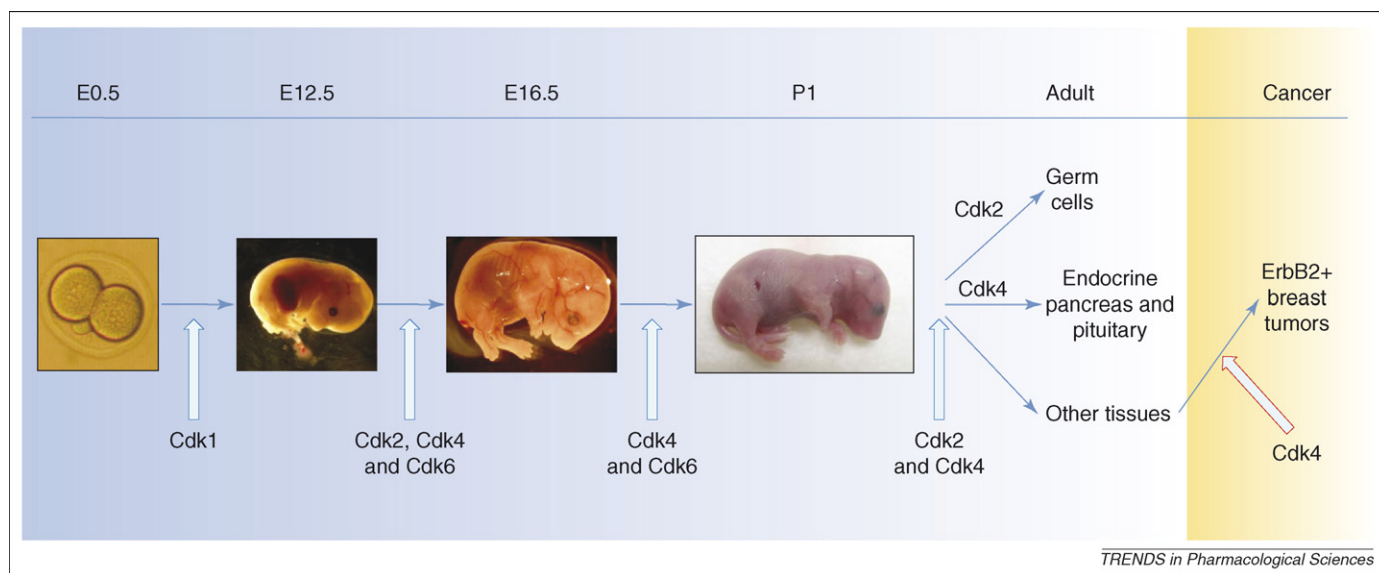


Figure 1. Physiological requirement for different CDK family members in mouse development and tumor cells. It was originally thought that CDK proteins had differential roles throughout the basic cell cycle, but recent work in mouse models suggests that these cell-cycle regulators have cell-type-specific functions *in vivo*. CDK1 is required for the first cell divisions in the embryos, whereas CDK2 and CDK4 are required only for the postnatal division of germ cells and specific endocrine cells, respectively. CDK6 deficiency results in minor defects in erythroid numbers and is not represented here. These interphase CDKs have complementary roles because a combined deficiency in CDK2 and CDK4 results in perinatal lethality, whereas the ablation of both CDK4 and CDK6 blocks development after E16.5. The elimination of all of these interphase CDKs impedes progression after E12.5. Note that these mouse strains also lack CDK3, as do most laboratory mice. Although CDK4 is not required for breast development, it is necessary for ErbB2- and Ras-induced breast tumors, indicating that this CDK might have a significant therapeutic value in these malignancies (red arrow).

cell types are not affected in these mouse models, suggesting that CDK2, CDK4 and CDK6 have cell-type-specific functions and/or that other family members have compensatory roles (Figure 1). Indeed, combined inactivation of *Cdk4* and *Cdk6* provokes a marked decrease in the proliferative potential of hematopoietic precursors, which die shortly after embryonic day 16.5 (E16.5) [19]. Likewise combined loss of *Cdk2* and *Cdk4* results in defective proliferation of embryonic cardiomyocytes [20,21]. These two defects are not observed in single *Cdk* mutants. Lastly, complete ablation of all of the interphase CDKs (CDK2, CDK4 and CDK6, along with CDK3, which is absent in all of these mouse models) is compatible with organogenesis, although the mutant embryos die because of earlier hemamopoietic defects by E12.5 [22].

Importantly, cells that lack different combinations of CDKs or are deficient in all interphase CDKs exit quiescence and proceed through the early phases of the cell cycle accompanied by phosphorylation of the retinoblastoma protein, thereby suggesting that any of the interphase CDKs can drive cells through the G1 and S phases of the cell cycle, at least in mice. These genetic models suggest that specific inhibition of one of these kinases should have a limited toxic effect, but that increasing the number of kinases inhibited will increase toxicity. It should be noted, however, that these genetic experiments involved complete ablation of *Cdk* expression in the germ line. Conditional knockout models in which CDKs are eliminated in adult tissues should provide more relevant information for pharmacological and/or therapeutic purposes.

In this regard, we and our co-workers [21] have recently reported that ablation of *Cdk2* in adult mice lacking *Cdk4* does not result in major defects. Moreover, these mice can regenerate their livers after partial hepatectomy with basically normal kinetics [21]. Thus, most normal cells

in adult mice can proliferate normally without *Cdk4* and *Cdk2*. These results suggest that adult tissues are likely to be less susceptible to toxic effects due to CDK inhibition, provided that the inhibitors do not have off-target effects. Further generation of conditional knockout and/or knockin strains for the various CDKs should provide valuable information for evaluating their roles in somatic tissues and thus should enable us to predict both potential toxic effects and anti-tumor efficacy (see later).

Inhibition of single CDK family members might result in protection from cancer in specific cell types and under particular oncogenic activities. For example, inhibition of CDK4 alone can protect mammary gland cells from Ras- or Her2-, but not Myc-, induced tumorigenesis [23–25]. CDK4 kinase activity is not required for normal development of the mammary gland, indicating that this kinase is essential for Her2- or Ras-induced tumorigenesis without toxic effects in normal tissue. Although similar evidence has not been reported for other CDKs or in different situations, these results suggest that some of these kinases have significant therapeutic value in a genetic-context-specific and cell-type-specific manner. The CDK4/CDK6-selective inhibitor PD-0332991 [26] is a potential tool with which to test this hypothesis in the clinic.

By contrast, genetic ablation of *Cdk1* in the mouse germ line results in very early embryonic lethality [27], indicating that CDK1 is required for cell division during the early stages of embryonic development and thereby suggesting that specific CDK1 inhibitors will have high toxicity. It should be noted, however, that a conditional *Cdk1* knockout strain is not yet available, and it is possible that CDK1 activity might not be essential in at least some adult cells. Preliminary data obtained using CDK inhibitors indicate that CDK1 inhibition might have relevant therapeutic effects in cells transformed by *MYC* but not other oncogenes

[28]. *MYC*-induced lymphomas and hepatoblastomas rapidly undergo apoptosis when treated with purvalanol A, a small-molecule CDK inhibitor with specificity towards CDK1, CDK2 and CDK5, suggesting that CDK1 inhibition might contribute relevant therapeutic benefits in human tumors that overexpress *MYC* [28]. In fact, it had previously been demonstrated that prolonged simultaneous diminution of CDK1 and CDK2 expression by RNA interference provided increased efficacy in blocking proliferation of tumor cell lines, as compared with RNA interference constructs targeting either CDK1 or CDK2 alone [29].

Specific inhibition of CDK7 disrupts assembly and activation of CDK1/cyclin B [30], and ablation of the Mat1 subunit of the CAK complex results in early embryonic lethality [1]. Thus, inhibition of CDK7 might have effects similar to those of CDK1 inhibition. These results imply that CDK1 and CDK7 inhibitors need to be developed as a so-called traditional cytotoxic agent. The therapeutic value of inhibiting CDK1 or CDK7 in experimental genetic models and in specific adult tissues remains, however, to be evaluated. Similarly, the therapeutic value of inhibiting non-cell-cycle CDKs, such as CDK8 or CDK9, has not been properly evaluated, and the pharmacological tools with which to specifically evaluate these targets are lacking.

We should also keep in mind that the crossover from gene knockout and knockin strains to the chemical genetics driven by preclinical and clinic compounds is difficult. Gene knockout and knockin models provide an ideal platform to generate a hypothesis for drug development and target selection under highly controlled conditions. The pharmacological tools can, however, rarely match the selectivity achieved in these genetic experiments. For example, it will be difficult to interpret the mechanism of action of pan-CDK inhibitors in 'genetically defined' situations both preclinically and clinically. As drug developers, therefore, we should not waste clean thoughts on dirty compounds and should be aware of the limitations that the existing drugs have in ability to test the proposed hypothesis properly.

Future perspectives: third-generation CDK inhibitors

A key question that remains unanswered for CDK inhibitors is which CDK or spectrum of CDKs should be

targeted. From the genetic data, it seems clear that this 'specificity versus potency' issue needs to be evaluated in each specific type of cell type, taking into account the possible compensatory roles that exist among different CDKs. First, the toxicity of inhibitory molecules of specific CDK family members might be restricted to some cell types, such as pituitary or pancreatic β cells for CDK4 inhibitors or germ cells for CDK2 inhibitors (Table 3), or might show a more general effect, as in the case of CDK1 inhibitors. Second, the therapeutic value of inhibiting specific CDKs will be highly dependent on the genetic context and the specific activation of signaling pathways that drive proliferation of the tumor cells. For example, inhibition of CDK4 might be highly efficient in *HER2*-positive mammary gland tumors but have no effect on *MYC*-induced breast carcinomas [25]. By contrast, inhibition of CDK1 (and probably other CDKs) with purvalanol A has been shown to have cytotoxic effects in *MYC*-overexpressing cells but not in cells transformed by other oncogenes [28]. In summary, the genetic context within the tumor cell offers a potential window of opportunity for long exposure to drugs that target CDKs.

During the past decade, three developments have made it possible to imagine that highly selective inhibitors of individual protein kinases will be possible in the near future: a subdiscipline of protein-kinase-targeted medicinal chemistry has emerged, the 3D structural database of protein kinases has increased from approximately 10 to more than 90 structures in the public domain [31], and today it is possible to evaluate more than 250 of the 516 protein kinases encoded by the human genome for catalytic inhibition. Of course, the availability of small molecules for these kinases is not, in itself, sufficient to ensure the success of such compounds in clinical settings. The use of highly specific inhibitors will require a balance between knowledge of the therapeutic value of the targeted kinase under the specific genetic alterations present in each primary tumor or metastatic clone and information on the toxicities expected for inhibition of the target. Pre-clinical studies in appropriate genetic mouse models should provide relevant information that will hopefully contribute a hypothesis for the design of more focused clinical studies to evaluate better the utility of these agents.

Table 3. Pharmacological and molecular considerations for hypothetical third-generation CDK inhibitors

Target (selective) ^a	Single-agent cytotoxicity expected	Mechanism of action	Indication
CDK1	Yes, essential gene	Antimitotic; pro-apoptotic	<i>MYC</i> -overexpressing tumors; other types of cancer; proliferative diseases
CDK2	Sterility	Possible inhibition of DNA synthesis; specific inhibition of meiosis	Not clear
CDK4/6	Reduced proliferation of endocrine or erythroid cells	Genetic context dependent; inhibition of DNA synthesis	<i>HER2</i> -positive mammary gland tumors; other types of cancer
CDK5	Nervous system	Inhibition of neurotrophic pathways; anti-apoptotic; protection from neurofibrillary degeneration in Alzheimer disease	Neurodegenerative diseases; pain
CDK7	Yes, essential gene	Inactivation of other CDKs such a CDK1 or general repression of transcription	Cancer; antiviral
CDK8 or CDK9	Not known	General repression of transcription?	Cancer; antiviral
CDK11	Yes, essential gene	Antimitotic; repression of transcription	Not clear

^aNo information is available on the therapeutic effects of inhibiting other CDKs such as CDK3 or CDK10.

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