Minireview
Clinical anticancer drug development: targeting the cyclin-dependent kinases

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Cell division involves a cyclical biochemical process composed of several step-wise reactions that have to occur once per cell cycle. Dysregulation of cell division is a hallmark of all cancers. Genetic and epigenetic mechanisms frequently result in deranged expression and/or activity of cell-cycle proteins including the cyclins, cyclin-dependent kinases (Cdks), Cdk inhibitors and checkpoint control proteins. The critical nature of these proteins in cell cycling raises hope that targeting them may result in selective cytotoxicity and valuable anticancer activity.

Keywords: cell cycle; targeted therapy

STRATEGIES FOR TARGETING THE CELL CYCLE
Dysregulation of the cell cycle is a hallmark of malignancy (Table 1). Many different strategies for targeting the cell cycle have been described. Attention has focused primarily on targeting mitosis (by targeting tubulin and more recently mitotic kinases including KSP/Eg5, Polo-like kinase 1 and Aurora kinases) as well as chemical inhibitors of cyclin-dependent kinase (Cdk) catalytic activity. Other potential strategies include therapies that can inhibit the interaction between cyclins and Cdks; decrease cyclin expression; promote the degradation of cyclins by increasing their phosphorylation; and restore endogenous Cdk inhibitor function. Other possibilities include the inhibition of the ‘noncycling’ cyclin-activating kinase complex of Cdk7/cyclin H, and the indirect targeting of the late G2 to M checkpoint by inhibiting CDC25 or by activating WEE1 or MYT1. This review will, however, focus on the chemical Cdk inhibitors evaluated in clinical trials to date and will highlight the main trial results reported to date.

PHARMACOLOGIC CDK INHIBITORS
Considerable progress has been made in the identification of pharmacologic agents targeting the Cdks (Senderowitz, 2003). The first generation of Cdk inhibitors lacked specificity, with flavopiridol, staurosporine and its analogue UCN-01 and E7070 being nonselective inhibitors of not only Cdks but many other targets. Second-generation inhibitors are more selective, with many of these compounds specifically developed to target selected Cdks (Table 2).

Flavopiridol
Flavopiridol (Alvocidib™) has several mechanisms of anticancer activity (Senderowicz and Sausville, 2000) and is a broad-spectrum Cdk inhibitor targeting Cdks 1, 2, 4, 6 and 7, interacting with the adenosine triphosphate (ATP) binding site. Flavopiridol also inhibits the Cdk9/cyclin T complex, broadly repressing transcription and decreasing cyclin D1 mRNA expression (Carlson et al., 1999). It induces cell-cycle arrest in G1 and is cytotoxic to cells undergoing DNA synthesis. It also inhibits other kinases including PKC and PKA at higher concentrations, inducing apoptosis, (Patel et al., 1998) and is active in many xenograft models. The first Phase I trial of flavopiridol utilised a 72-h continuous infusion every 2 weeks, a schedule supported by preclinical models (Senderowicz et al., 1998). The maximum-tolerated dose (MTD) achieved in this clinical trial was 50 mg m⁻² day⁻¹, the initial dose-limiting toxicity being secretory diarrhoea. The MTD with antidiarrheal prophylaxis was 78 mg m⁻² day⁻¹ (Kahn et al., 2001; Messmann et al., 2003) The achieved plasma levels were sufficient for Cdk inhibition (200–400 nM), with one partial response in renal cell cancer and minor responses in renal, colorectal carcinomas and non-Hodgkin’s lymphoma.

This 72-h schedule was subsequently investigated in further Phase I and Phase II trials in patients with mantle cell lymphoma, renal cancer, melanoma, gastric cancer, non-small-cell lung cancer (NSCLC) and colorectal cancers (Bennett et al, 1999; Stadler et al., 2000; Schwartz et al., 2001; Shapiro et al., 2001; Lin et al., 2002; Thomas et al., 2002). An unusually high incidence of arterial and venous thromboembolic events was documented in the majority of these trials, including deep venous thrombosis, pulmonary thromboembolism, myocardial infarction and transient neurological ischaemic events. This serious drug-related toxicity was not appreciated in the initial studies but was reported in more than 30% of patients in later trials and is of unknown aetiology.
A study of flavopiridol administered as a 1-h infusion was then pursued supported by data indicating that higher plasma concentrations than those achieved in the 72-h infusion studies were required to induce apoptosis (Arguello et al, 1998; Tan et al, 2002). Initially, a 1-h infusion administered for 5 days every 3 weeks was investigated. The recommended Phase II dose for this schedule was 37.5 mg m$^{-2}$ day$^{-1}$, with grade 4 neutropenia and grade 3 fatigue being dose limiting. The three- and one-day schedules administered every 3 weeks were also evaluated with the aim of further increasing peak serum levels of drug. Grade 4 neutropenia was also dose limiting in these studies, with the recommended Phase II dose of these schedules being 50 mg m$^{-2}$ day$^{-1}$ and 62.5 mg m$^{-2}$, respectively. The peak plasma concentrations, however, remained lower than the concentrations required in preclinical studies to induce apoptosis (5 – 7 μmol l$^{-1}$), with no objective tumour responses being observed. Phase II studies of flavopiridol administered by 1-h infusion daily for 3 days every 3 weeks have been reported. No responses were observed in advanced melanoma (Burdette-Radoux et al, 2002); however, in patients with advanced mantle cell lymphoma, three responses out of 28 evaluable patients (11%) were reported, with 20 patients having stable disease (71%) for a median duration of 3.4 months (Kouroukis et al, 2003).

An alternative administration schedule has been recently evaluated for flavopiridol in patients with chronic lymphatic leukaemia. Previous Phase I/II studies had shown that, while flavopiridol induces apoptosis in CLL cells in a p53-independent manner in vitro, the drug is inactive using a 24- to 72-h CIVI schedule. Based on pharmacokinetic modelling data demonstrating high drug binding to human plasma proteins, an optimised dosing schedule of 30-min i.v. bolus (IVB) followed by 4-h CIVI has been pursued. This alternative schedule has demonstrated promising activity including flavopiridol-induced tumour lysis syndrome, suggesting that this agent may warrant further evaluation using this novel schedule (Lin et al, 2004).

The use of flavopiridol in combination studies has also been pursued (Bible and Kaufmann, 1997; Motwani et al, 2001). Treatment scheduling appears to be important in ensuring that flavopiridol augments the effects of other agents (Schwartz et al, 2002; Wittmann et al, 2003).

**UCN-01**

The second chemical Cdk inhibitor to be evaluated was UCN-01 (7-hydroxyxystaurosporine) (Senderowicz and Sausville, 2000), which also has several mechanisms of action including Cdk1 (cdc2) and Cdk2 inhibition (IC$_{50}$ of 300–600 nm). UCN-01 causes cell-cycle arrest and apoptosis at concentrations above 100 nm (Akiyama et al, 1997), abrogating the G$_2$ checkpoint in response to DNA damage and promoting the induction of p53-independent apoptosis by inhibiting Chk1 and Chk2 (IC$_{50}$ for both approximately 10–30 nm). (Wang et al, 1996; Busby et al, 2000; Yu et al, 2002). UCN-01 also abrogates S-phase arrest in CHO cells treated with cisplatin, promoting the induction of apoptosis (Bunch and Eastman, 1997), and may target Akt signalling by inhibiting PDK1 (IC$_{50}$ of 33 nm) (Sato et al, 2002). Frequent dosing is required to optimise the antitumour activity of UCN-01 (Bunch and Eastman, 1997), and may target Akt signalling by inhibiting PDK1 (IC$_{50}$ of 33 nm) (Sato et al, 2002). Frequent dosing is required to optimise the antitumour activity of UCN-01 (Bunch and Eastman, 1997), and may target Akt signalling by inhibiting PDK1 (IC$_{50}$ of 33 nm) (Sato et al, 2002). Frequent dosing is required to optimise the antitumour activity of UCN-01 (Bunch and Eastman, 1997), and may target Akt signalling by inhibiting PDK1 (IC$_{50}$ of 33 nm) (Sato et al, 2002).

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**Table 1**

(a) Deregulated cyclins and Cdk’s and associated tumours and (b) deregulated endogenous Cdk inhibitors and associated tumours

| Target | Oncogenic changes                                      | Associated tumours                  |
|--------|--------------------------------------------------------|-------------------------------------|
| Cyclin D1 | Gene amplification Overexpression Translocation | 40–80% Breast carcinoma 70% Familial polyposis 50% B-cell lymphoma 50% NSCLC 35% Head and neck carcinoma 25–50% Oesophageal carcinoma 25% Bladder carcinoma |
| Cyclin E | Gene amplification Overexpression | 90% Colorectal carcinoma 30–80% Breast carcinoma 70% Prostate carcinoma 18% Ovarian carcinoma Gastric carcinoma Cervical carcinoma |
| Cyclin E2 | Overexpression                                    | Breast carcinoma Small-cell lung carcinoma Cervical carcinoma |
| Cyclin B1 | Overexpression                                      | 90% Colorectal carcinoma |
| Cyclin A | Amplification or overexpression                     | Hepatocellular carcinoma Colorctal carcinoma |
| CDK2   | Overexpression                                      | Sarcomas, gliomas |
| CDK4   | Amplification                                      | |
| p16INK4a Mutations (5% of human cancers) | Deletion (14% of human cancers) Epigenetic (19% of human cancers) | Pancreatic cancer Melanoma Gliomas Bladder cancer Head and neck cancer NSCLC Lymphoma/leukaemia |
| p21$^{CDK4}$ Mutation/deletion rare Downregulation rare Intracellular mislocalisation? | Oral (rare mutations) Oesophageal (rare mutations) Breast (rare mutations) |
| p27$^{CDK2}$ Mutations/deletions rare Downregulation (increased degradation)$^*$ | Breast$^*$ Colon$^*$ Prostate$^*$ |

NSCLC = non-small-cell lung cancer; $^*$Increased degradation.
apoptosis (Ozawa et al, 2001). Its potency is enhanced by longer drug exposures (Ozawa et al, 2001). E7070 has a broad spectrum of in vitro and in vivo preclinical antitumour activity, inducing regression of established tumours. This efficacy is schedule dependent, with a daily-for-8-days schedule being more efficacious than 4- and 1-day schedules.

A number of Phase I trials of this agent have been performed investigating different dosing regimens. When given daily by intravenous infusion for 5 days every 3 weeks, the DLTs consisted of neutropenia, thrombocytopenia, diarrhoea and stomatitis, with a recommended Phase II dose of 130 mg m\(^2\) day\(^{-1}\). One partial response was seen in a patient with heavily pretreated breast cancer (Punt et al, 2002). A second trial investigated an alternative schedule – a 1-h infusion administered every 3 weeks (Raymond et al, 2002). Toxicities were similar to the 5-day regimen and comprised myelosuppression, acne-like skin rash, alopecia, mucositis, conjunctivitis, hypoglycaemia, nausea and fatigue. Recommended doses were 700 mg m\(^{-2}\) for the heavily pretreated group and 800 mg m\(^{-2}\) for the lightly pretreated group. No partial responses were observed. A weekly 1-h infusion schedule for 4 consecutive weeks and a continuous 120-h infusion have also been evaluated with similar toxicities being observed. The recommended doses for the weekly and continuous infusion schedules were 400 and 96 mg m\(^{-2}\), respectively. Pharmacokinetic studies indicated that the clearance and volume of distribution at steady state of E7070 decrease with increasing dose.

Phase II single-agent studies are under way in a number of different tumour types investigating the once-every-3-weeks and daily-for-5-weeks schedules. In patients with fluorouracil refractory colorectal cancer, two of 21 patients receiving the once-every-4-weeks schedule, and two of 23 patients receiving the daily-for-5-days schedule, had objective responses, with 10 and 13% of patients, respectively, having stable disease at 6 months (Mainwaring et al, 2002). In a Phase II study of patients with NSCLC, who had previously received one chemotherapy regimen, only one of 44 patients had an objective response (Talbot et al, 2002). No objective responses were observed in patients with metastatic melanoma receiving a dose of 700 mg m\(^{-2}\) for over 1 h every 3 weeks (Aamdal et al, 2002).

**Table 2** Chemical Cdk inhibitors and their targets

| Cdk Inhibitors in the Clinic | Flavopiridol | R-Roscovitine (CYC-202) | BMS 387032 | UCN01 |
|-------------------------------|-------------|-------------------------|-----------|-------|
| Cdk1/B IC\(_{50}\) = 0.03 \(\mu\)M | Cdk1/B IC\(_{50}\) = 2.69 \(\mu\)M | Cdk1/B IC\(_{50}\) = 0.481 \(\mu\)M | Cdk1/B IC\(_{50}\) = 0.3 \(\mu\)M |
| Cdk2/E IC\(_{50}\) = 0.17 \(\mu\)M | Cdk2/E IC\(_{50}\) = 0.10 \(\mu\)M | Cdk2/E IC\(_{50}\) = 0.3 \(\mu\)M |
| Cdk4/D IC\(_{50}\) = 0.10 \(\mu\)M | Cdk4/D IC\(_{50}\) = 14.21 \(\mu\)M | Cdk4/D IC\(_{50}\) = 0.03 \(\mu\)M |
| Cdk9/T IC\(_{50}\) < 0.010 \(\mu\)M\(^{a}\) | Cdk9/T IC\(_{50}\) = 0.8 \(\mu\)M | Cdk9/T IC\(_{50}\) = 0.11 \(\mu\)M |

*Roscovitine (CYC202)

The purine analogue R-roscovitine (CYC202) is a highly selective, orally bioavailable, small molecule inhibitor of several Cdks competing with their ATP binding sites: it is a relatively potent inhibitor of human Cdk2/cyclin E, Cdk7/cyclin H, Cdk9/cyclin T1 with IC\(_{50}\) of 0.1, 0.5 and 0.8 \(\mu\)M, respectively (Meijer et al, 1997), but inhibits Cdk4/cyclin D1 with an IC\(_{50}\) of 14.2 \(\mu\)M. It has an average IC\(_{50}\) against the NCI cell-line panel of 16 \(\mu\)M. It also blocks the degradation of p53 through the inhibition of MDM2 expression (Lu et al, 2001). CYC202 induces G1 and G2/M arrest and cell death from all compartments of the cell cycle (Schutte et al, 1997; McClue et al, 2002). The antitumour efficacy of CYC202 has also been tested in a panel of human tumour xenografts (McClue et al, 2002). Continuous exposure to CYC202 at dosages ranging from 0.3 to 100 \(\mu\)M demonstrated dose-dependent antitumour activity.

Recent molecular pharmacology studies have shown that treatment of colorectal cancer cells with CYC202 results in a decrease in pRB phosphorylation (serines 780, 608, 807, 811 and Thr-821), indicative of direct Cdk2 inhibition (Whittaker et al, 2004). In addition, CYC202 causes a downregulation of various cyclins, including cyclin D1; this is likely to lead to a secondary inhibition of various cyclins, which would explain the reduced phosphorylation at multiple sites on RB that is seen at later time points.

CYC202 has been evaluated in Phase I clinical trials. Two such trials have recently been reported. In the first trial, CYC202 was administered orally, twice daily for 7 days out of every 21. In all, 19 patients were treated for a total of 36 cycles of CYC202. At 800 mg BD, DLTs comprising grade 3 skin rash and grade 4 hypokalaemia were observed. Other toxicities seen included reversible renal impairment, mild reversible transaminitis and emesis. MAG-3 studies indicated that the renal impairment was related to altered renal blood flow. No evidence of renal tubular damage was detected. The aetiology of these renal changes is unknown but could be related to the effects of CYC202 on adenosine receptors. The pharmacokinetics of the compound were dose proportional, with CYC202 being widely distributed (720.8 l, 95% CI 384.9 – 1056.8) and rapidly cleared (142.6 l/h, 95% CI 80.5 – 204.9) with a mean terminal elimination half-life of 4.02 h (95% CI 2.8 – 5.2).

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"*In* increased degradation downregulate transcription, for example, \(\downarrow\) cyclin D1 expression."
This trial is still recruiting patients, but no objective responses have yet been seen (Benson et al, 2003; White et al, 2004).

In the second trial, a twice-daily-for-5-days schedule, administered every 3 weeks was evaluated. The maximum twice-daily dose achieved was 1600 mg BD with a recommended dose of 1250 mg BD. Toxicities reported have included grade 4 emesis, grade 3 asthenia and skin rash. No objective responses were seen but stable disease was recorded in three out of 29 patients treated. (Pierga et al, 2003) Exploration of a 10-day schedule is now under way.

**BMS-387032**

High-throughput screening followed by lead optimisation has resulted in the identification of the 2-aminothiazole BMS-387032 as a potent, selective and competitive small molecule inhibitor of the Cdk2/cyclin E complex, with an IC$_{50}$ of 48 nM. The 2-aminothiazoles have been reported to be 10- and 30-fold more potent against Cdk2 than Cdk1 and Cdk4, and three to five orders of magnitude less potent against all other tested non-Cdk kinases. The X-ray crystal structure of this compound with Cdk2 has been reported, and has revealed the mechanisms by which this compound interacts with the Cdk2 ATP binding site (Misra et al, 2004). In vitro, BMS-387032 inhibits Cdk2 phosphorylation in the A2780 ovarian carcinoma cell line, inhibiting the phosphorylation of downstream targets of Cdk2 including pRB, histone H1 and DNA polymerase-α-β.

The compound displays potent in vitro cytotoxicity against the A2780 cell line with an IC$_{50}$ of 50 nM, and is active against a broad array of cell lines. In vivo studies have confirmed oral bioavailability and activity against a variety of cell lines, including P388 murine leukaemia, A2780 ovarian and A431 human squamous carcinoma. Combination studies indicate that BMS-387032 is synergistic with cisplatin in SV-1 colon carcinoma cells, this synergy being dependent on the drug sequence (Lane et al, 2003). Phase I clinical trials with BMS-387032 are ongoing utilising different schedules (Jones et al, 2003; McCormick et al, 2003; Shapiro et al, 2003).

**PHARMACODYNAMIC STUDIES**

As with other molecularly targeted therapeutics under investigation, the clinical development of the Cdk inhibitors in early trials requires the study of their biological effects in tumour cells acquired through serial tumour biopsies. These studies are required to select optimal biological dosing and schedule, and identify the patient population most likely to benefit from these agents. Potential pharmacodynamic parameters under investigation include the inhibition of pRB phosphorylation; the depletion of cyclins and Cdk5; increases in Cdk inhibitor protein expression; and the suppression of mdm2 expression and induction of p53 expression. Pharmacodynamic studies have been reported for flavopiridol (Lam et al, 2001) and CYC202 (Whittaker et al, 2004). More recent studies indicate that $^{18}$F-labelled 3'-deoxy-3'-fluorothymidine may be a useful imaging modality for the selective Cdk2 inhibitor BMS-387032 (Fischman et al, 2004). These translational studies are critically important in the optimal development of these agents.

**CONCLUDING REMARKS**

Significant progress has been made in the clinical targeting of the Cdk.s. Newer and more specific Cdk inhibitors are envisioned to result in decreased toxicity and more selective cytotoxicity. Increased specificity may not, however, spare noncycling cells since recent data have implicated the Cdk5, 7, 8 and 9 in cellular functions that do not involve the cell cycle. Cdk5, 7, 8 and 9 have been reported to regulate RNA transcription through the phosphorylation of RNA polymerase II, while Cdk5 has been shown to be involved in regulating insulin secretion, synaptic vesicle recycling, neuronal survival and tau (microtubule associated protein) phosphorylation and aggregation (Sausville, 2002). These findings may explain why hyperglycaemia has been observed with many chemical Cdk inhibitors. The RNA polymerase II regulatory activity of Cdk 7, 8 and 9 may, however, enhance the antitumour effect of these agents, and it has indeed been suggested that the antitumor activity of flavopiridol may be in part related to the inhibition of RNA transcription. It remains to date unclear whether the inhibition of Cdk 7, 8 and 9 activity is desirable for an anticancer agent, or whether the toxicity associated with inhibiting these noncycling Cdks will substantially decrease cytotoxic selectivity and the therapeutic index.

Overall, these data suggest that highly selective small molecule inhibitors of specific Cdk5s may be preferable in order to decrease toxicity. Generating this specificity, however, not only remains a significant challenge to chemists but may also decrease antitumor efficacy in view of the inherent functional redundancy of this family of kinases. A broader-spectrum inhibitor that can, for example, selectively inhibit Cdk1, Cdk2, Cdk4 and Cdk6 at low nanomolar IC$_{50}$ concentrations may therefore be preferable. The recent observations that selectively inhibiting Cdk2 in certain cell lines is not sufficient for antitumour activity would support this view (Tetsu and McCormick, 2003), as would the demonstration that the Cdk2 knockout mouse shows no major abnormalities and in particular no effects on proliferation (Ortega et al, 2003). The high sequence homology of the ATP binding sites of these and the noncycling kinases Cdk 5, 7, 8 and 9, as well as that of glycogen synthase kinase-3β and that of the adenosine receptors makes this a difficult task. However, much has already been achieved and while many questions need to be answered we are moving closer to the Holy Grail: the development of compounds selectively cytotoxic to tumour cells, yet sparing normal cells.

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