

CDK inhibitors in 3D: Problems with the drugs, their development plans or their linkage to disease?

Review Article

Andrew Hughes

AstraZeneca, Alderley Park, Cheshire, UK

***Correspondence:** Dr Andrew Hughes, AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK; Tel: +44 (0)1625 512092; Fax: +44 (0)1625 590913; E-mail: andrew.hughes@astrazeneca.com

Key words: CDK, CDK inhibitor, cell cycle, pRb, flavopiridol, UCN-01, CYC202, E7070, BMS 387032

Abbreviations: area under the curve, (AUC); chronic lymphocytic leukaemia, (CLL); confidence interval, (CI); cyclin-dependent kinase, (CDK); dose-limiting toxicity, (DLT); maximum concentration, (C_{max}); maximum tolerated dose, (MTD); non-small-cell lung cancer, (NSCLC); protein kinase C, (PKC); protein product, (p); Retinoblastoma, (Rb); steady-state concentration, (C_{ss})

Received: 12 May 2005; Accepted: 20 October 2005; electronically published: February 2006

Summary

Targeting the cell cycle is an attractive anticancer strategy, as its dysregulation is a common, if not ubiquitous, occurrence in tumour development. Cell-cycle control is achieved by the interaction of a complex set of enzymes and other proteins, including the family of protein kinases known as cyclin-dependent kinases (CDKs) and the protein product of the tumour suppressor gene Retinoblastoma. CDKs are required for the orderly progression of cells through the cell cycle and hyperactivation of the CDKs in tumour development, as well as mutation and overexpression of these proteins, is common. Several compounds collectively known as 'CDK inhibitors' have been in clinical trials for a number of years. Although often grouped together, they differ in their molecular and cellular modes of action. Several agents in this group, such as flavopiridol, E7070 and UCN-01, have multiple CDK and non-CDK targets or inhibit upstream regulators of the CDKs, whereas other CDK inhibitors, such as CYC-202, appear to target CDK1 and CDK2 more specifically. Most CDK inhibitors are administered intravenously and various schedules have been explored in the clinic. Although generally well tolerated, CDK inhibitors as monotherapy have given mixed efficacy results, possibly due to problems related to specificity or in dosing/scheduling leading to suboptimal exposure. A lack of pharmacodynamic end points, together with the multiple cellular targets of some agents, has made the assessment of 'CDK inhibition' and its contribution to antitumour effect in the clinic difficult. However, recent pharmacokinetic studies are examining dosing and scheduling regimens, and novel markers of drug activity and patient suitability are being developed. In addition, newer, specifically targeted oral agents may prove more effective, less toxic and more amenable to optimisation in clinical trials.

I. The cell cycle

The cell cycle can be divided into four phases: synthesis (S phase) and mitosis (M phase) preceded by two preparatory or gap phases, G_1 and G_2 (Figure 1). DNA is replicated during the S phase and the fully replicated chromosomes are segregated to each of the two daughter nuclei in the M phase. During G_1 and G_2 , the synthesis of cellular constituents needed to support the subsequent phases, and ultimately to complete cell division, occurs. In mammalian cells, the length of the G_1 phase is highly variable and can range from about 6 hours to several days or longer. Cells that persist in G_1 can enter a distinct state called G_0 , in which they are metabolically active but not actively proliferating, and can re-enter the cell cycle or remain in G_0 indefinitely. The major

regulatory checkpoint in the cell cycle is the G_1/S transition and cell-cycle defects at this point are common in cancer. In contrast to G_1 , the length of the S, G_2 and M phases in mammalian cells are relatively constant and transitions between these phases are principally controlled by intracellular regulatory pathways.

A. Cell-cycle regulation

A complex set of enzymes and other proteins, including the family of cyclin-dependent kinases (CDKs) and the protein product (p) of the tumour suppressor Retinoblastoma susceptibility gene (Rb), regulates progression through the cell cycle. To make the transition from G_1 to S phase, normal cells require mitogenic growth signals such as diffusible growth factors, extracellular

matrix components or cell-cell interaction/adhesion molecules. Many growth signalling pathways are channelled through pRb, which, in its unphosphorylated state, sequesters and inactivates E2F, which is required for activation of multiple cell-cycle related transcription factors (Hanahan and Weinberg, 2000; Harbour et al, 1999; Sherr, 2000). When pRb is phosphorylated, E2F is released; thus, pRb plays a central role in G₁/S transition. Phosphorylation of pRb during G₁ requires the activation of CDKs: first CDK4, 6/cyclin D, then by CDK2/cyclin E (Figure 2) (Swanton, 2004). Hyperphosphorylated pRb can no longer repress E2F, which is then able to activate

genes required for the S phase. Once in S phase, cells are able to continue the cell division process without extracellular mitogens, although it has been proposed that regulatory checkpoints are still enforced by the requirement for activated CDK2/cyclin A and CDK1/cyclin B complexes in the S/G₂ and G₂/M transitions, respectively (Figure 2), although the need for activated CDK2 in this process is unclear: tumour cells deficient in CDK2 protein and kinase activity have recently been shown to proliferate normally (Gladden and Diehl, 2003; Hinds, 2003).

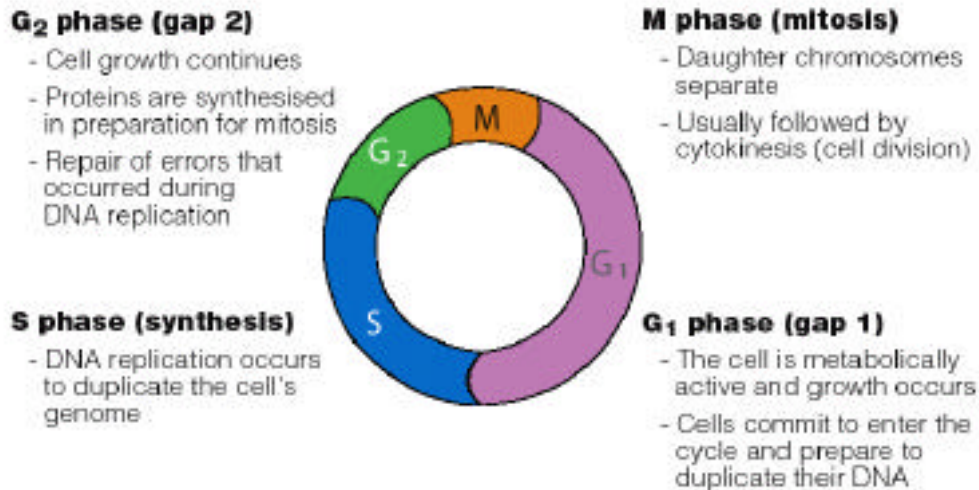


Figure 1. The cell cycle.

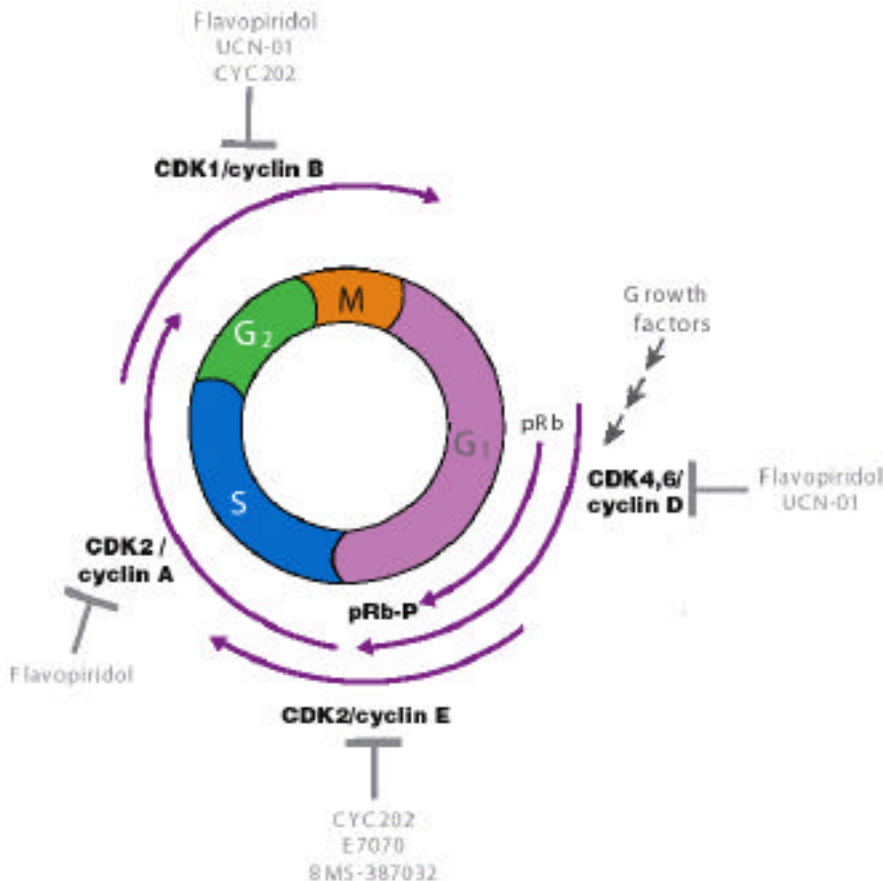


Figure 2. Key drivers of the cell cycle and points at which CDK inhibitors are active. Growth-factor-mediated activation of CDK4, 6/cyclin D and the subsequent phosphorylation of pRb are major events in progressing the cell from G₁ to S, via the restriction checkpoint R.

Synthesis of cyclins occurs at specific times during the cell cycle or, in the case of cyclin D1, in response to certain growth factors. CDKs depend on cyclins for their activity: the active CDK holoenzyme is composed of a catalytic subunit and the cyclin regulatory subunit. Since each cyclin is synthesised at a particular stage of the cell cycle, it in turn directs the appropriate activation of its specific catalytic subunit (Harbour et al, 1999). Another level of regulation is conferred by endogenous 'CDK inhibitors', which can inhibit assembly or activation of the cyclin/CDK complex. Endogenous CDK inhibitors are primarily involved in the control of the G₁ and S phases, and fall into two distinct classes: one class specifically inhibits CDK4/cyclin D or CDK6/cyclin D and includes p16^{INK4A}, p15^{INK4B}, p18^{INK4C} and p19^{INK4D}, and the other class inhibits multiple CDKs and includes p21^{CIP1}, p27^{KIP1} and p57^{KIP2} (Sherr, 2000).

Mutations that disable key regulators of G₁ phase progression are common in cancer, and loss of regulation of the G₁/S transition occurs in most types of human tumour (Sherr, 2000; Senderowicz, 2002). The CDK/pRb pathway, crucial in regulating G₁ progression, can be seen as comprising two oncogenes (cyclins D and E) and two tumour suppressor genes (Rb and p16). Disruption of the CDK/pRb pathway can result from direct mutational inactivation of pRb function, overexpression of CDKs, via mutations in CDKs that render them unresponsive to negative regulators such as p14^{INK4A}, through dysregulation of CDK inhibitors, or through loss or methylation of p16^{INK4B} (Hanahan and Weinberg, 2000; Sherr, 2000). Lesions in the CDK/pRb pathway occur frequently, and increased expression of cyclin E and/or loss of p16 carry a poor prognostic significance in many common forms of cancer (Sherr, 2000).

II. CDK inhibitors in clinical development

The identification of CDKs as targets for cancer therapy has led to the development of many inhibitors of CDK activity (Senderowicz, 2003a; Senderowicz, 2003b; Dai and Grant, 2003), several of which are in clinical trials (Table 1). These agents differ markedly in their modes of action and several agents that are classed as CDK inhibitors have additional effects on other cellular processes. Examples of direct CDK inhibitors that target the adenosine triphosphate binding site of CDKs (among other reported effects) include flavopiridol (NSC-649890) and CYC202 (NSC-701554; roscovitine). In contrast, indirect CDK inhibitors, including UCN-01 and E7070 (Indisulam), affect CDK function due to inhibition of upstream pathways required for CDK activation. Flavopiridol exerts a number of effects on tumour cells. In addition to inhibiting multiple CDKs, principally 4 and 6 or 1 and 2, causing G₁/S or G₂/M arrest, respectively, flavopiridol causes transcriptional inhibition following disruption of P-TEFb (the CDK9/cyclin T complex), induction of apoptosis (possibly a consequence of downregulation of various anti-apoptotic proteins) and anti-angiogenic effects (Senderowicz, 2002). CYC202 is an orally active inhibitor of CDK1 and CDK2/cyclin E,

which leads to inhibition of transcription and the induction of apoptosis (Senderowicz, 2003a; White et al, 2004; McClue et al, 2002). BMS-387032 is an inhibitor of CDK2/cyclin E, with modest potency against CDK1/cyclin E and CDK4/cyclin D (Senderowicz, 2003b; Shapiro et al, 2003).

UCN-01, a hydroxylated derivative of staurosporine, is a non-specific inhibitor of protein kinases with activity against protein kinase C (PKC) and CDKs. UCN-01 causes inhibition or inappropriate activation of CDK1/cdc2, G₁ arrest, abrogation of the G₂ and S checkpoints, inhibition of PDK1/Akt (phosphoinositide-dependent kinase 1/Akt, a pathway that plays a pivotal role in malignant transformation) and PKC-independent induction of apoptosis (Senderowicz, 2003a; Senderowicz, 2003b). E7070 is a synthetic sulphonamide compound that does not directly inhibit CDKs but causes G₁ arrest *in vitro* by inducing depletion of CDK2/cyclin E and repressing transcription of cyclin H, leading to reduced CDK7/CDK activating kinase activity and hypophosphorylation of pRb (Senderowicz, 2003b; Terret et al, 2003; Haddad et al, 2004).

III. CDK inhibitors in the clinic: the data and the drawbacks

Flavopiridol, UCN-01, E7070, CYC202 and BMS-387032 are currently under clinical evaluation.

A. Flavopiridol

Flavopiridol entered clinical trials in 1996 and is the most intensively studied of the CDK inhibitors in the clinic. It is administered intravenously and various schedules have been investigated in clinical trials (Table 2).

1. 72-hour infusion Q14d

Phase I trials identified a maximum tolerated dose (MTD) of 40-50 mg/m²/day when flavopiridol was administered as a 72-hour continuous intravenous infusion every 2 weeks (Table 2) (Thomas et al, 1997; Senderowicz et al, 1998; Thomas et al, 2002). The dose-limiting toxicity (DLT) in these trials was secretory diarrhoea, and some antitumour activity was seen in patients with renal cancer (one partial response) and metastatic gastric cancer (one complete response). Phase II trials of flavopiridol have further defined the safety profile of this schedule but have failed to demonstrate significant single-agent activity in any tumour setting investigated. Trials have explored the 72-hour continuous intravenous infusion regimen using a dose of 50 mg/m²/day in patients with a variety of tumours, including previously untreated stage IV non-small-cell lung cancer (NSCLC), advanced gastric carcinoma and metastatic renal cancer (Stadler et al, 2000; Schwartz et al, 2001; Shapiro et al, 2001). However, response rates were low: objective responses were seen in only 2 of 35 (6%) patients with renal cancer (Stadler et al, 2000), although protracted stable disease (12 weeks) was seen in 6 (30%) of the patients with NSCLC. The median overall survival for the 20 patients who received treatment was 7.5 months

Table 1. CDK inhibitors in clinical development

Agent	Company/ collaborator	Target	Effect	Entered clinical trials	Current development phase
Flavopiridol (NSC-649890)	Aventis/NCI	Multiple CDKs including CDKs 1/2/4/6	Cell-cycle arrest in G ₁ and G ₂	1996	II
UCN-01	Kyowa Hakko Kogyo/NCI	PKC and CDKs including CDK1/cdc2, PDK1/Akt	Abrogation of G ₂ and S checkpoints; apoptosis	1997	II
E7070 (Indisulam)	Eisai/EORTC	Depletion of CDK2/cyclin E; transcriptional repression of cyclin H	G ₁ arrest	1998	II
CYC202 (NSC-701554, roscovitine)	Cyclacel	CDKs 1/2; also activity against CDKs 5/7/9	Cell-cycle stage- independent apoptosis	2000	II
BMS-387032	Bristol Myers Squibb	CDK2/cyclinE; also activity against CDK1/cyclin E + CDK4/cyclin D	G ₁ arrest	2003	I

NCI, National Cancer Institute; EORTC, European Organisation for Research and Treatment of Cancer

Table 2. Results from Phase I dose-escalation trials of flavopiridol

Schedule		Dose, mg/m ² /day	Pts, n	DLT	RD, mg/m ² /day	Reference
72-hour CII	Every 2 weeks	Unknown	76	Secretory diarrhoea	50 78 ^a	Senderowicz et al, 1998; Rudek et al, 2003
72-hour CII	Every 2 weeks	8, 16, 26.6, 40, 50, 56	38	Diarrhoea	40	Thomas et al, 2002
1-hour infusion	Once every 3 weeks	62.5 or 78	12	Neutropenia	62.5	Tan et al, 2002
1-hour infusion	3 days every 3 weeks	50 or 62.5	12	Neutropenia	50	Tan et al, 2002
1-hour infusion	3 days every 3 weeks	50 or 62.5	12	Nausea/vomiting, ^b neutropenia, fatigue, hepatotoxicity	50	Senderowicz et al, 2000
1-hour infusion	5 days every 3 weeks	12-52.5	24	Nausea/vomiting, ^b neutropenia, fatigue	37.5	Senderowicz et al, 2000
1-hour infusion	5 days every 3 weeks	12-52.5	31	Neutropenia, fatigue, hypotension, hypoalbuminaemi a	37.5	Tan et al, 2002
24-hour infusion	1 day/week for 4 weeks	40-100	20	Multiple colon ulcers, abdominal pain, abdominal distention	80	Sasaki et al, 2002
30-minute iv infusion + 4- hour infusion	1 day/week for 4 weeks every 6 weeks	60 (30+30)- 80 (40+40)	21	Acute tumour lysis syndrome	60 (30+30)	Byrd et al, 2004b

^aWith anti-diarrhoeal prophylaxis using loperamide and cholestyramine ^bAvoided subsequently with metoclopramide/granisetron iv, intravenous; RD, recommended dose for Phase II trials; CII, continuous intravenous infusion (Shapiro et al, 2001), which may indicate a possible survival advantage in advanced NSCLC, although further trials are needed to confirm this. An unexpectedly high

frequency of thrombotic events was seen in two of these trials (Schwartz et al, 2001).

In 36 patients with metastatic hormone-refractory prostate cancer, receiving treatment every 14 days at the starting dose of 40 mg/m²/day, dose escalation up to 60 mg/m²/day was permitted if no significant toxicity was observed (Sumitomo et al, 2004). In 22 evaluable patients there were no objective responses and evidence of stable disease in only 4 patients (16-48 weeks). The most common toxicities were diarrhoea (predominantly grades 1 and 2) and nausea. The authors concluded that, in light of disappointing single-agent activity, the use of flavopiridol in prostate cancer should be reserved for evaluation in combination therapies or alternative schedules.

Twenty chemotherapy-naïve patients with previously untreated advanced colorectal cancer received flavopiridol at a dose of 50 mg/m²/day every 14 days (Aklilu et al, 2003). The most common grade 3/4 toxicities were diarrhoea, fatigue and hyperglycaemia, occurring in 21%, 11% and 11% of patients, respectively; other common toxicities included anaemia, anorexia and nausea/vomiting. Again, single-agent activity was disappointing, with no objective responses, a median time to progression of 8 weeks and median survival of 65 weeks, leading the authors to conclude that flavopiridol in this dose and schedule does not have single-agent activity in patients with advanced colorectal cancer.

Cyclin D1 is overexpressed in 95-100% of cases of mantle cell lymphoma. In a small study, 10 patients with relapsed or refractory mantle cell lymphoma were treated with flavopiridol 50 mg/m²/day (Lin et al, 2002). One patient developed grade 3/4 non-haematological toxicity. There were no clinical responses. Three patients maintained stable disease and disease progressed within 2 months in seven patients. The authors concluded that flavopiridol was ineffective as a single agent with this schedule in this setting (Byrd et al, 2004a).

Sequential Phase II studies in chronic lymphocytic leukaemia (CLL) have compared bolus schedules in previously treated patients (Byrd et al, 2004a). Patients received up to six cycles of flavopiridol (50 mg/m² daily) as a continuous intravenous infusion over 72 hours every 2 weeks. Of 15 patients, 6 (40%) had intermediate- (Rai stage I or II) and 9 (60%) had high-risk (Rai stage III and IV) stages. No responses were noted in this group but 27% had stable disease and 73% had progressive disease. The median progression-free survival was 2.1 months (95% confidence interval [CI] 1.8-3.8) and the median overall survival was 27 months (95% CI 20-42). Grade 3 and 4 toxicities were 20% and 27%, respectively. This 72-hour schedule proved to have only moderate efficacy compared with a 1-hour schedule (Byrd et al, 2004a) reported below.

2. 1-hour infusion schedules: daily 1, 3 or 5 Q21d

In an attempt to achieve high peak plasma concentrations, flavopiridol has been given as a 1-hour infusion for 1, 3 or 5 consecutive days every 3 weeks (Table 2) (Senderowicz et al, 2000; Tan et al, 2002). In a Phase I study, 55 patients with advanced cancer were

treated with flavopiridol at doses of 12-52.5 mg/m²/day for 5 days, 50 and 62.5 mg/m²/day for 3 days, and 62.5 and 78 mg/m²/day for 1 day (Tan et al, 2002). Of 50 assessable patients, 12 had stable disease for 3 months with a median duration of 6 months. The recommended doses of flavopiridol as a 1-hour infusion were 37.5 mg/m²/day for 5 days, 50 mg/m²/day for 3 days and 62.5 mg/m²/day for 1 day (Table 2). Another Phase I study with 12-62.5 mg/m²/day for 3 or 5 days every 3 weeks in patients with advanced neoplasms obtained similar results (Table 2); stable disease for >6 months was seen in 3/36 patients (Senderowicz et al, 2000).

On the basis of these data, several Phase II trials (some still ongoing) have been started using a 1-hour infusion for 3 or 5 days every 3 weeks. Seventeen patients with incurable malignant melanoma were administered flavopiridol 50 mg/m² over 1 hour daily for 3 consecutive days every 3 weeks. The schedule was well tolerated, with the most common treatment-related non-haematological toxicities being diarrhoea (82%), nausea (47%) and fatigue (41%) (Burdette-Radoux et al, 2004). Haematological toxicities were minor (grade 1 or 2). However, in 16 evaluable patients, no objective responses were documented, although 7 patients (44%) had stable disease after 2 cycles, with a median duration of 2.8 months (range 1.8-9.2). In another Phase II trial, 18 patients with refractory multiple myeloma were treated on a 3 days/21-day cycle; however, the trial was stopped at interim analysis when 15 patients had progressed and 3 patients had discontinued due to toxicity (Dispenzieri et al, 2004). The authors considered that, on this schedule and in this setting, flavopiridol as a single agent lacked activity. Other trials are ongoing but so far, in solid tumours, 1-hour infusion schedules have failed to improve on the poor efficacy rates seen with the 72-hour continuous intravenous infusion schedules.

Recent data have demonstrated, in a haematological tumour, that scheduling can make a difference to both efficacy and toxicity (Byrd et al, 2004a). Sequential Phase II studies in CLL compared schedules of 72 hours (reported above) versus 1 hour in previously treated patients. Patients in the second trial received up to eight cycles of flavopiridol 50 mg/m² as a 1-hour intravenous infusion daily for 3 days every 3 weeks. Of 36 patients, 13 (36%) had intermediate- and 23 (64%) had high-risk disease. Four patients (11%) had partial responses, 19 (53%) had stable disease and 13 (36%) progressed on treatment. Progression-free survival ranged from 2.9-19.3 months in responders, with a median of 3.2 months (95% CI 2.5-7.4); median overall survival was 24 months (95% CI 18-31). Toxicity was manageable and included mainly myelosuppression (granulocytopenia and thrombocytopenia), infections, diarrhoea and fatigue. Grade 3 and 4 toxicities were 39% and 33%, respectively. The authors concluded that flavopiridol has modest, schedule-dependent clinical activity in relapsed CLL and warrants future investigation using alternative schedules of administration.

3. 24-hour infusion schedules: 24-hour continuous infusion Q7d

Flavopiridol is also being investigated in Phase II trials with a 24-hour infusion schedule. A rising-dose-escalation Phase I study established an MTD of 80 mg/m²/day with abdominal pain and distension as the DLTs (Sasaki et al, 2002). Of 20 evaluable patients, 5 showed durable stable disease (>90 days) and 4 a reduction in tumour markers, although no patient had an objective response. The authors considered these data to be encouraging.

4. Loading dose plus infusion scheduling

Recently reported data have suggested that, at least in a haematological malignancy, a pharmacokinetically modelled schedule in which a dose split equally between a 30-minute loading dose and a following 4-hour continuous intravenous infusion has significant clinical activity (Byrd et al, 2004b). Due to the high plasma protein binding of flavopiridol (approximately 95%), a 30-minute 'loading dose' that achieves saturation of protein binding followed by a 4-hour infusion designed to achieve free drug concentrations above those active *in vitro* is being used. A Phase I study in genetically high-risk patients with refractory CLL reached DLT of tumour lysis syndrome at 80 mg/m² with one treatment-associated death. Twenty-two patients were evaluable for response. Nine patients (41%) achieved a partial response; of these, seven remain in remission (3-11+ months) and two relapsed at 7 and 12 months, respectively; eight of the nine patients were refractory to fludarabine. The authors suggested that their data support the hypothesis that efficacy and toxicity are maximum concentration (C_{max})-, area under the curve (AUC)- and steady-state concentration (C_{ss})-related and that this schedule provides clinically relevant activity. These results support the intriguing hypothesis that the lack of efficacy previously seen with flavopiridol monotherapy is due to inadequate free drug concentrations, rather than CDK inhibition having no effect on tumour growth. Further exploration of this schedule in non-haematological malignancies is eagerly awaited.

5. Exposure to flavopiridol in the various schedules

Rudek and colleagues analysed the clinical pharmacology data for flavopiridol from one of the Phase I trials (Thomas et al, 1997; Senderowicz et al, 1998) in which 76 patients were treated with a 72-hour infusion of flavopiridol at 13 dose levels for a total of 504 cycles of treatment (Rudek et al, 2003). Serial plasma samples were collected and analysed by high-performance liquid chromatography. The average C_{ss} was 26.5 and 253 nM at 4 and 122.5 mg/m², respectively. No clear relationship was identified between dose or concentration of flavopiridol and the DLT, secretory diarrhoea. At 50 and 78 mg/m²/day, the mean plasma C_{ss} was 278 and 390 nM; concentrations that are well above those noted for *in vitro* antiproliferative activity. However, samples were collected for only 2 days post-infusion, and concentration/time

curves show a marked reduction in plasma concentration after the 72-hour infusion period, to <10% of steady-state levels at 120 hours. Thus, for this regimen given every 2 weeks, putative efficacious concentrations (from *in vitro* predictions) of drug are only obtained for approximately 21% of the time.

For the 1-hour infusion regimens, pharmacokinetic assays showed that, at the doses of 37.5 mg/m²/day for 5 days and 50 mg/m²/day for 3 days, mean plasma concentrations reached the micromolar range (Tan et al, 2002). However, following infusion, plasma concentrations fell rapidly and were at the submicromolar level within a further 2 hours. Therefore, efficacious concentrations of drug are only obtained for a fraction of the 3 or 5 days of administration, which, in the 3-week scheduling period, cover 14% and 24% of the time spent on drug, respectively. Thus, exposure to flavopiridol has been markedly transient in all reported trials, which may account for the limited antitumour effects observed. Given the frequency of thrombotic events seen with the 72-hour continuous intravenous infusion regimen at the recommended dose, there would appear to be limited room for improving the therapeutic index for this agent when administered intravenously. In addition, flavopiridol is highly protein-bound in serum (a mean unbound fraction of 6% across the concentration range [622-4977 nM]; data not given for lower concentrations) (Rudek et al, 2003). This factor may further accentuate variability in pharmacokinetic exposure to free concentrations of flavopiridol in patients with altered serum protein concentrations.

B. UCN-01

1. Continuous infusion schedules: 72 hours Q14 days; 36 hours Q28 days

In the first clinical trial with UCN-01, the initial schedule was a 72-hour continuous intravenous infusion every 2 weeks, starting at a dose of 1.8 mg/m²/day. Surprisingly, in the first nine patients, the half-life of UCN-01 appeared to be 100-fold longer than that observed in preclinical models (Sausville et al, 2001). Therefore, the treatment schedule was altered to a 36-hour continuous intravenous infusion of 12 mg/m²/day every 4 weeks, received by a further 38 patients (Sausville et al, 2001). DLTs at 53 mg/m²/day included nausea/vomiting, symptomatic hyperglycaemia and pulmonary toxicity. Findings included a very long mean half-life (approximately 588 hours), a result that was supported in a preliminary report of a Phase I study in Japanese patients (Tamura et al, 1999). A partial response was observed in a patient with melanoma, and a long period of stable disease (>2.5 years) was seen in a patient with refractory anaplastic large-cell lymphoma (Sausville et al, 2001). Target modulation by UCN-01 was assessed by the level of phosphorylation of the PKC substrate adducin in bone marrow and tumour samples taken during UCN-01 treatment: this was significantly reduced compared with pretreatment samples. The authors concluded that UCN-01 can be administered safely and effectively, with a recommended dose of 42.5 mg/m²/day for an initial 72-

hour continuous intravenous infusion with subsequent monthly doses administered as 36-hour infusions.

2. Short infusion schedules: 1 hour Q28 days; 3 hours Q28 days; 3 hours Q21 days

In view of the extended half-life of UCN-01, a Phase I study was conducted to evaluate a short infusion time (1-3 hours) every 28 days. Preliminary data from this trial reported on 6 dose levels ranging from 3 mg/m² over 3 hours to 95 mg/m² over 1 hour in 15 patients with various tumour types (Dees et al, 2000). At doses 68 mg/m² over 1 hour, toxicity was mild and reversible; however, at 95 mg/m² over 1 hour, dose-limiting hypotension with syncope and reversible respiratory arrest occurred in one patient. This was considered to be related to rapid infusion; therefore, the duration of infusion was lengthened to 3 hours at that dose level. There had been no responses at the time of reporting.

In a Phase II trial in metastatic renal-cell carcinoma, patients received UCN-01 intravenously every 3 weeks, and, because of the cytostatic mechanism of UCN-01, time to disease progression was adopted as the primary end point (Shaw et al, 2003). Therapy was well tolerated, with infusional reactions including nausea, headache and hyperglycaemia (all grade 2 or below) observed within 48 hours after treatment. At the time of a preliminary report, 15 patients (median of 1 prior systemic treatment) had been treated; median time to progression was 80 days and no patient had achieved an objective response. Therefore, UCN-01 appears to have limited activity in metastatic renal-cell carcinoma.

A Phase II study of UCN-01 monotherapy (3-hour Q21d) in patients with metastatic melanoma is ongoing, as are several trials in combination with cisplatin and other DNA-damaging agents, seeking to exploit the activity of UCN-01 as an inhibitor of the G2 DNA damage checkpoint kinase Chk1.

The attributes of UCN-01 as an inhibitor of several cellular proteins, including Akt, Chk1, CDKs and PKC, make it an interesting agent for study but lead to difficulties in its clinical optimisation. These additional activities confound the interpretation of clinical trial

results as truly reflecting the effects of CDK inhibition in humans (Sausville et al, 2001). Preclinical models failed to predict the hyperglycaemia seen in patients receiving UCN-01. This may be an effect of Akt or CDK5 blockade and is considered to be related to the development of tissue insulin resistance. In the affected patient, hyperosmolar, non-ketotic rises in blood glucose can often require 2 days of insulin infusion to reverse and an expert endocrinologist is required to monitor the patient.

3. Exposure to UCN-01

Surprisingly, in the first patients exposed over 72 hours, the half-life of UCN-01 (approximately 588 hours) appeared to be 100-fold longer than that observed in preclinical models, and high total drug plasma concentration (30-40 µM) (Sausville et al, 2001). In addition, the initial concentrations achieved in plasma were high (approximately 4-7 mM), well above those found to be lethal in animal models (Fuse et al, 2000). A preliminary pharmacokinetic analysis (n=14) of the 1-3-hour schedule also showed a long mean half-life (approximately 550 hours), and that exposure increased roughly proportionally with dose (Dees et al, 2000). However, the extended half-life and high plasma concentrations are thought to be due largely to strong binding of UCN-01 to plasma 1-acidic glycoprotein, which may render the agent inactive. Indeed, at the end of 72-hour infusions of doses of 25-55 mg/m²/day, plasma concentrations of 20-60 µM were observed; however, saliva concentrations, used as a surrogate measure of effective free drug, were 100-fold lower (Senderowicz, 2002).

C. E7070

Four Phase I dose-escalating clinical trials (summarised in **Table 3**) using different infusion schedules in patients with advanced solid tumours have been appraised.

Table 3. Results from Phase I dose-escalation trials of E7070

Schedule	Dose	Pts, n	DLT	RD	Reference
5-day CII every 3 weeks	6-200 mg/m ² /day	27	Neutropenia, thrombocytopenia	96 mg/m ² /day	Terret et al, 2003
5-day CII every 3 weeks	10-160 mg/m ² /day	33	Febrile neutropenia, thrombocytopenia, diarrhoea, skin folliculitis, asthenia, stomatitis	130 mg/m ² /day	Punt et al, 2001
1 hour/week for 4 consecutive weeks	80-500 mg/m ² /week	46	Neutropenia, thrombocytopenia	400 mg/m ² /week	Dittrich et al, 2003
1 hour every 3 weeks	50-1000 mg/m ² /week	30	Neutropenia, thrombocytopenia, anaemia	700 mg/m ² /week	Raymond et al, 2000

RD, recommended dose for Phase II trials; CII, continuous intravenous infusion

1. Continuous infusion schedule: continuous intravenous infusion 5d Q21d

A Phase I study in 27 patients given doses ranging from 6-200 mg/m²/day by continuous intravenous infusion for 5 days every 3 weeks showed that E7070 has a non-linear pharmacokinetic profile, particularly at dose levels >24 mg/m²/day, with a reduction in clearance and an increase in half-life (Terret et al, 2003). This was associated with toxicity, and the risk of myelosuppression became significant at AUC levels >4000 µg h/mL. DLTs were dose-dependent reversible neutropenia and thrombocytopenia, and the recommended dose for further studies was 96 mg/m²/day when administered in this schedule. However, no objective responses were observed.

2. 1-hour infusion schedules: daily 5 Q21d; weekly 4 Q6weeks; 1 Q21d

E7070 has been administered to 33 patients with advanced cancer as a 1-hour intravenous daily infusion for 5 days once every 3 weeks (dose escalation from 10 mg/m²/day) (Punt et al, 2001). DLTs occurred at doses of 160 and 200 mg/m²/day, consisting of febrile neutropenia, thrombocytopenia, diarrhoea, skin folliculitis, asthenia and stomatitis. A partial response was observed in a patient with heavily pretreated breast cancer, and stable disease and some minor responses were also documented. The recommended dose for further studies at this daily-times-five schedule was 130 mg/m²/day.

In an alternative schedule, two cohorts of patients with different prognoses and different degrees of hepatic involvement received 1-hour intravenous E7070 at weekly intervals for 4 consecutive weeks every 6 weeks (Dittrich et al, 2003). The MTD was 500 mg/m²/week for both groups, with reversible neutropenia and thrombocytopenia being the most common DLTs. The pharmacokinetics of E7070 were non-linear over the dose range 160-500 mg/m². A partial response was observed in a patient with an endometrial adenocarcinoma, and 12 other patients (27%) had stable disease (median duration 5.3 months [range 1.9-30.6]), including one patient with metastatic melanoma. The recommended dose for further study of E7070 using this schedule is 400 mg/m²/week.

A further schedule comprising a 1-hour infusion given every 3 weeks has been reported. In a dose-escalating Phase I study patients (n=30) with advanced cancer received E7070 from a dose of 50 mg/m² (Raymond et al, 2000). Pharmacokinetic parameters were again found to be non-linear. Antitumour activity was seen in patients with adenocarcinoma of unknown origin, renal and breast cancers. The MTD was 800 mg/m², with haematological DLTs. However, this dose was recommended for less heavily pretreated patients with lower tumour burden and hepatic involvement, while 700 mg/m² was recommended for more heavily pretreated patients. The same schedule has been reported in 21 Japanese patients (Yamada et al, 2004). In this study, an MTD of 900 mg/m² was established and DLT was myelosuppression. The severity of myelosuppression correlated with exposure, which was in turn related to mutational status of CYP2C19, commonly polymorphic in the Japanese population. No objective responses were

seen, although there was one durable minor response. The recommended Phase II dose was 800 mg/m².

A Phase II study with this schedule has recently been reported: patients with advanced head-and-neck cancer received 700 mg/m² over 1 hour every 3 weeks (Haddad et al, 2004). Thirty-nine cycles of E7070 were delivered (median 2.6 cycles/patient), six patients had stable disease after two cycles, and two patients each subsequently received one, two and three additional cycles, respectively, before experiencing progression. However, none of the first 15 patients achieved progression-free survival of >4 months and the trial was stopped prematurely. Nevertheless, immunohistochemistry of tumour cell aspirates from three patients demonstrated reduced post-treatment pRb phosphorylation, suggesting that CDK activity can be inhibited by E7070 in tumour cells. The authors suggested that more frequent administration of E7070 may be required to sustain pRb hypophosphorylation and cytostatic growth arrest.

3. Exposure to E7070 in the various schedules

Van Kesteren and colleagues (2002) have summarised population pharmacokinetic analyses of the four Phase I studies described. Data show that, following a 1-hour infusion of 700 mg/m², plasma concentrations of E7070 remain well above 1 mg/L for >100 hours post-dose. With daily-times-five 1-hour infusion or with 120-hour continuous intravenous infusion at 160 mg/m²/day, this also applies, giving a period of 10 days in the 3-week schedule where plasma E7070 is in the mg/L range. Plasma concentration-time data of patients from all four studies (n=143) were best described using a three-compartment model with non-linear distribution to a peripheral compartment and two parallel pathways of elimination from the central compartment: a linear and a saturable pathway. Unusually for an anticancer agent, body-surface area was significantly correlated to both the volume of distribution of the central compartment and to the maximum elimination capacity, and these authors recommend body-surface-area-guided dosing for E7070.

In summary, E7070 has shown some antitumour activity but limited Phase II data in patients with squamous-cell carcinoma of the head and neck have not supported Phase I results. Dosing, typically by 1 hour daily or continuous intravenous infusion over 3-5 days every 3 weeks, has led to plasma concentrations in the µM or mg/L range, but intermittent dosing has led to transient exposure (Van Kesteren et al, 2002), in common with flavopiridol. However, E7070 has a long half-life compared with other agents, and as its activity at low concentrations in preclinical models is dependent on exposure time, it is possible that these concentrations may also be of sufficient duration to be effective in humans. This is supported by the limited data from pRb phosphorylation assays indicating G₁ arrest in tumours. However, like UCN-01, the activity of E7070 may not reflect CDK inhibition since it also has multiple targets and its mode of action remains uncertain.

D. BMS-387032

1. 1-hour infusion schedules: 1 Q21d; 1 Q7d

In a 3-weekly schedule, 1-hour infusion of 9.6-59 mg/m² BMS-387032, the C_{max} and exposure increased with dose, and common adverse events possibly related to BMS-387032 included grade 1/2 fatigue (52%) and various gastrointestinal toxicities (16-32%) (Jones et al, 2003). Transient transaminase elevations (grade 1-3) occurred in six patients receiving doses from 17.5-59 mg/m². Prolonged stable disease has been seen in patients with renal-cell carcinoma (10 and 15+ months), NSCLC (6 months), oral cavity carcinoma (8+ months) and leiomyosarcoma (7 months), and dose escalation was continuing at the time of reporting. Early data from another Phase I dose-escalation study in patients with advanced solid tumours or lymphoma refractory to standard therapy has been reported, with BMS-387032 administered weekly as a 1-hourly infusion (McCormick et al, 2003). Nine patients had been treated at three dose levels (4, 6.7 and 10 mg/m²), and cycle 1 pharmacokinetic data indicated that, at these dose levels, the systemic exposure appeared to be dose proportional. The MTD had not been reached and accrual to the study was ongoing.

2. 24-hour continuous intravenous infusion Q21d

In a third Phase I trial of BMS-387032, with a 24-hour infusion given every 3 weeks, 17 patients have been treated at 5 dose levels ranging from 4.8-17 mg/m² (Shapiro et al, 2003). Diarrhoea was the most common toxicity considered to be treatment related (22%; grades 1-2). One patient with a history of childhood Rb and leiomyosarcoma of the bladder previously treated with doxorubicin, gemcitabine and vinorelbine had a minor response, and dose escalation was continuing.

E. CYC202

1. Bid for 5, 7 or 10d Q21d

CYC202 is orally available. In a Phase I trial of CYC202 in heavily pretreated patients with advanced solid tumours, patients received twice-daily CYC202 for 5 days every 3 weeks (Pierga et al, 2003). In the first part of this dose-escalation study (n=25), MTD was reached at 3200 mg/day, with vomiting as the DLT; other adverse events at this dose included hypokalaemia and increased creatinine. No objective responses were seen, although stable disease for > 6 months was observed in three patients with adrenocortical carcinoma and cylindroma, and the recommended dose for this schedule is 2500 mg/day. In the second part of the study, four patients received 2000 mg/day for 10 days every 3 weeks. DLTs at this dose were hypokalaemia and skin rash (both grade 3). No responses had been noted and exploration of the second schedule at a reduced dose of 1600 mg/day was ongoing at the time of reporting. Another Phase I dose-escalation study has also reported preliminary data: CYC202 was given at a starting dose of 200 mg/day twice daily for 7 days every 3 weeks, with the patient fasting for 2 hours before and after drug administration (Benson et al, 2003). Data from 19 patients showed DLTs (grade 3 skin rash and grade 4

hypokalaemia) at 1600 mg/day. Pharmacokinetic parameters showed an increase in AUC with dose. CYC202 was widely distributed and cleared rapidly from the circulation with a mean half-life of 4.02 hours (95% CI 2.8, 5.2). A third Phase I trial (n=21) has examined CYC202 given twice-daily for 7 days every 3 weeks, with a starting dose of 200 mg/day divided in two doses, in patients with advanced malignancy. At a dose level of 1600 mg/day, DLTs were grade 3 rash and grade 4 hypokalaemia. There was a trend to linear increase in AUC with dose (r²=0.5) (White et al, 2004).

In summary, CYC202 has shown evidence for dose-related exposure, and its oral availability is likely to be advantageous in optimising scheduling. However, response or other efficacy data are poor or lacking in monotherapy. CYC202 is currently being studied in Phase II trials for breast and lung cancer in combination therapy.

IV. Why has monotherapy with CDK inhibitors shown poor clinical efficacy?

The efficacy of CDK inhibitors as monotherapy in the clinic has not been commensurate with expectations based on preclinical data. The factors that have contributed to the disappointing efficacy data, to varying degrees for each agent, are discussed in turn.

A. Trial end points

Trial designs that assess efficacy by tumour response rate may underestimate the value of CDK inhibitors, which, based on preclinical *in vivo* models, are expected to be more likely cytostatic as monotherapy. Relatively higher rates of stable disease than objective response have been a feature of clinical results so far (Shapiro, 2004), although many trials have not been designed to assess prolonged disease stabilisation as an efficacy end point. Randomised controlled designs do use progression end points rather than the objective response end points seen in uncontrolled trials. An exception has been a Phase II study of UCN-01 in patients with metastatic renal-cell carcinoma, which defined time to disease progression as its primary end point (Shaw et al, 2003). This trial reported preliminary data showing a median time to progression of 80 days (n=15), which underestimated the true time to progression, as seven patients were still on therapy at the time of reporting. The design of future trials should take into account the cytostatic mechanism of CDK inhibitors as monotherapy; although if TTP is the primary endpoint, randomized controlled trials are recommended to determine efficacy.

B. Multiple drug targets

Some agents that are classed as CDK inhibitors appear to act primarily by several non-specific pharmacological mechanisms. For instance, flavopiridol's arrest of the cell cycle in G₁ and G₂ could be a result of inhibition of all, or a subset of, its targets, including CDK1, CDK2 and CDK4 (by direct inhibition) and cyclins D1, D3 and B (by downregulation). For CYC202, the effect of inhibiting a range of CDKs, including CDK1 and CDK2, has similar drawbacks. E7070 has the widely

different effects of depleting CDK2/cyclin E and transcriptionally repressing cyclin H. Similarly, the non-specific protein kinase activity of UCN-01 affects a number of cell-cycle associated control proteins in addition to CDKs. On top of this, the relative importance of CDK and cyclin targets may vary between individual tumours and tumour types. Furthermore, the mechanisms responsible for resistance to cell-cycle inhibitors, both innate and acquired, have yet to be determined. Therefore, it may be difficult to infer the cause of tumour control/regression seen in any patient benefiting from treatment, or the cause of treatment failure in non-responding or progressing patients.

C. Dosing schedules and exposure

Of the CDK inhibitors, flavopiridol has been longest in clinical development. However, as trials have shown, intravenous administration has been problematic and studies examining revised schedules have been necessary. Initial schedules of flavopiridol used prolonged continuous intravenous infusions that produced nanomolar levels of drug, based on effective concentrations used in tumour cell lines: exposure to 200-300 nM flavopiridol is sufficient to cause cell-cycle arrest in most tumour cell lines (Shapiro, 2004), and only slightly higher concentrations are needed to induce apoptosis (Dai and Grant, 2003; Shapiro, 2004). However, to achieve effective concentrations in human tumours, micromolar serum concentrations are likely to be effective and the investigation of shorter infusions that achieve a higher C_{max} , and of loading followed by maintenance infusion, have been designed with this in mind. Revised schedules were, initially at least, ineffective in sustaining exposure over the entire period of drug administration, and estimates of the duration of drug coverage (ie, the proportion of time at or above an effective concentration) for flavopiridol are in the range of 14-24%, based on data from Phase I trials (Tan et al, 2002; Rudek et al, 2003). However, recent data in a haematological malignancy have been promising, showing that a pharmacokinetically modelled schedule, comprising a 30-minute bolus followed by a 4-hour continuous intravenous infusion, has significant clinical activity (Byrd et al, 2004b).

For CYC202, which has also displayed limited efficacy in the clinic, suboptimal plasma exposure may have resulted from the 11-fold variation in human AUCs observed.

Several agents have failed to demonstrate efficacy across tumour types at the same dose. For instance, in Phase I and Phase II trials, flavopiridol has shown a modest response rate, at 50 mg/m²/day continuous intravenous infusion every 2 weeks, in metastatic renal cancer but not in NSCLC or gastric cancer (Senderowicz et al, 1998; Stadler et al, 2000; Shapiro et al, 2001; Schwartz et al, 2001). E7070, shown to have antitumour activity in a Phase I trial against adenocarcinoma of unknown origin, renal and breast cancer at a dose of 700 mg/m² in a 1-hour infusion every 3 weeks (Raymond et al, 2000), did not have a disease-stabilising effect in a Phase II trial in squamous-cell carcinoma of the head and neck (Haddad et al, 2004). These data imply that often the dose

identified as having some utility in one tumour type may not be sufficient for a different tumour type, and that each tumour type will require dose/scheduling optimisation in separate Phase I dose-escalation studies.

D. Biomarkers of CDK inhibition

Reliable, validated pharmacodynamic assays to confirm target inhibition are badly needed for trials of CDK inhibitors and the absence of discrete pharmacodynamic end points to confirm target inhibition has hampered clinical development. Several candidate markers that may stratify patients into responders versus non-responders are under investigation. Candidate immunohistochemical markers for testing of tumour samples include phosphorylated/unphosphorylated Rb, p27^{KIP1}, E2F-1 and survivin (Shapiro, 2004). A study has reported reduced tumour Rb phosphorylation in two out of two patients (from whom tumour material was available and fully informative) with squamous-cell carcinoma of the head and neck in a Phase II trial of E7070; however, only one of the patients had stable disease, while the other had progressive disease (Haddad et al, 2004). Other potentially useful markers include p16, p53 and cyclin D. The CDK inhibitor p16 is thought to be responsible for G₁ arrest in senescing cells and overexpression of p16 can lead to G₁ arrest in tumour cells, both effects being due to inhibition of CDK2 activity (Calbó et al, 2004; Stein et al, 1999). Flavopiridol has been shown to mimic, in part, the effect of p16 in that exposure of cells to high flavopiridol concentrations (>100 nM) resulted in decreased expression of genes downstream in the normal p16 cell-cycle control pathway, including Rb and E2F (Robinson et al, 2003). The authors pointed out that p16 is frequently lost or mutated in malignant melanoma, making CDK2 inhibition an ideal candidate for targeted therapy in this disease. Indeed, this principle may be applicable to any tumour deficient in p16 expression. The p53 family (p53, p63 and p73) function as transcription factors that play a major role in regulating responses to stress and damage, in part through the transcriptional activation of genes involved in controlling CDKs, and may therefore also be useful markers of tumours deficient in CDK inhibition.

V. Conclusions

It is recognised that cell-cycle dysregulation is a hallmark of cancer, with G₁/S dysregulation occurring among virtually all types of human tumour, often via overexpression of CDKs, mutations in CDKs or loss of normal CDK inhibitor activity. Therefore, CDK inhibition forms a valid approach to tumour therapy (Sherr, 2000; Senderowicz, 2002, 2003b). However, the novel small-molecule CDK modulators that are being tested in the clinic have given interesting but so far rather disappointing results as monotherapy.

Flavopiridol is the CDK inhibitor furthest into clinical development. However, intravenous administration has proven difficult to optimise and scheduling studies continue to address this problem. A regimen using 72-hour continuous intravenous infusion every 2 weeks has been most extensively applied in

clinical trials but efficacy rates have been poor; 1-hour infusion has also been explored to achieve higher peak concentrations. However, a pharmacokinetically modelled schedule, comprising a 30-minute bolus followed by a 4-hour continuous intravenous infusion, recently showed significant clinical activity in haematological malignancies. For flavopiridol and E7070, there are subsets of patients with prolonged stable disease in several Phase II trials, although few responses have been observed. Further Phase I and II trials are ongoing to determine the effectiveness of these agents as monotherapy and in combination with standard chemotherapeutic regimens and various tumour types. UCN-01 and CYC202 have shown dose-proportional exposure in Phase I trials but efficacy rates have been low and it is unclear from current data whether serum concentrations are adequate or sustained enough to give an antitumour effect. In addition, the multiple molecular effects of UCN-01 are likely to hamper optimisation of its administration, and its association with hyperglycaemia is a significant drawback in the clinic. In comparison, the relative selectivity of CYC202, combined with its oral availability and flexibility in dosing/scheduling, may be advantageous in attempts to improve efficacy rates with this agent.

Three proposals are presented to address the information from the first four CDK inhibitors that have entered clinical trials. Firstly, careful clinical trial design with trial end points that assess disease stability will be needed to evaluate this class of agents fully. For instance, in patients with NSCLC treated with flavopiridol (Shapiro et al, 2001), the objective response rate was low but overall survival was similar to that obtained in a randomised trial of four platinum-based chemotherapy regimens containing platinum analogues in combination with taxanes or gemcitabine (Schiller et al, 2002). However, randomised clinical trials that assess survival as an end point are needed to confirm whether this is a real effect. Secondly, the use of biomarkers that demonstrate the drug has achieved CDK inhibition in humans is warranted to ensure that biologically active free drug concentrations are achieved. Delineation of an optimum schedule based on data from humans, rather than empirically based schedules, will provide confidence that any failure of clinical efficacy is potentially due to relevance of target rather than simply a failure to achieve biologically active concentrations of drug. Candidate markers, including Rb, p16 and p53, are being developed and increasingly introduced into clinical trials. Finally, combining CDK inhibitors with either conventional cytotoxic drugs or novel agents targeting signal transduction pathways has shown promising antitumour activity in preclinical models, particularly in the induction of apoptosis. The introduction of novel combination regimens into clinical trials is progressing even in the absence of proven clinical activity for CDK monotherapy, and a Phase III trial comparing standard combination chemotherapy versus combination chemotherapy plus flavopiridol is currently under investigation (Senderowicz, 2003a). It remains to be seen whether existing CDK inhibitors will be effectively deployed in this way.

Acknowledgement

Editorial assistance was provided by John H Bull with financial support from AstraZeneca.

References

- Aklilu M, Kindler HL, Donehower RC, Mani S and Vokes EE (2003) Phase II study of flavopiridol in patients with advanced colorectal cancer. **Ann Oncol** 14, 1270-1273.
- Benson C, White J, Twelves C, O'Donnell A, Cruickshank C, Tan S, Gianella-Borradori A and Judson I (2003) A phase I trial of the oral cyclin dependent kinase inhibitor CYC202 in patients with advanced malignancy. **Proc Am Soc Clin Oncol** 22, 209, abs 838.
- Burdette-Radoux S, Tozer RG, Lohmann RC, Quirt I, Ernst DS, Walsh W, Wainman N, Colevas AD and Eisenhauer EA (2004) Phase II trial of flavopiridol, a cyclin dependent kinase inhibitor, in untreated metastatic malignant melanoma. **Invest New Drugs** 22, 315-322.
- Byrd JC, Peterson BL, Gabilove JL, Odenike OM, Grever MR, Rai KR, Vardiman JW and Larson RA (2004a) Sequential phase II studies of flavopiridol by 72-hour continuous infusion and 1-hour intravenous bolus for the treatment of relapsed B-cell chronic lymphocytic leukemia: results from CALGB Study 19805. **Blood** 104, abs 3485.
- Byrd JC, Lin TS, Dalton JT, Wu D, Fischer B, Moran ME, Blum KA, Shank RS, Lucas DM, Lucas MS, Suarez J-R, Colevas AD and Grever MR (2004b) Flavopiridol administered as a pharmacologically-derived schedule demonstrates marked clinical activity in refractory, genetically high risk, chronic lymphocytic leukemia (CLL). **Blood** 104, abs 341.
- Calbó J, Serna C, Garriga J, Graña X and Mazo A (2004) The fate of pancreatic tumor cell lines following p16 overexpression depends on the modulation of CDK2 activity. **Cell Death Differ** 11, 1055-1065.
- Dai Y and Grant S (2003) Cyclin-dependent kinase inhibitors. **Curr Opin Pharmacol** 3, 362-370.
- Dees EC, O'Reilly S, Figg WD, Elza-Brown K, Aylesworth C, Carducci M, Byrd J, Grever M and Donehower RC (2000) A phase I and pharmacologic study of UCN-01, a protein kinase C inhibitor. **Proc Am Soc Clin Oncol** 19, 205a, abs 797.
- Dispenzieri A, Bible KC, Lacy MQ, Fitch TR, Geyer SM, Fenton R, Fonseca R, Getman CR, Zeisner SC, Erlichman C and Gertz MA (2004) The lack of clinical efficacy of flavopiridol in patients with relapsed refractory myeloma. **Blood** 104, abs 3461.
- Dittrich C, Dumez H, Calvert H, Hanauske A, Faber M, Wanders J, Yule M, Ravic M and Fumoleau P (2003) Phase I and pharmacokinetic study of E7070, a chloroindolyl-sulfonamide anticancer agent, administered on a weekly schedule to patients with solid tumors. **Clin Cancer Res** 9, 5195-5204.
- Fuse E, Hashimoto A, Sato N, Tani H, Kuwabara T, Kobayashi S and Sugiyama Y (2000) Physiological modeling of altered pharmacokinetics of a novel anticancer drug, UCN-01 (7-hydroxystaurosporine), caused by slow dissociation of UCN-01 from human α 1-acid glycoprotein. **Pharm Res** 17, 553-564.
- Gladden AB and Diehl JA (2003) Cell cycle progression without cyclin E/CDK2: Breaking down the walls of dogma. **Cancer Cell** 4, 160-162.
- Haddad RI, Weinstein LJ, Wiczorek TJ, Bhattacharya N, Raftopoulos H, Oster MW, Zhang X, Latham Jr VM,

- Costello R, Faucher J, DeRosa C, Yule M, Miller LP, Loda M, Posner MR and Shapiro GI (2004) A Phase II clinical and pharmacodynamic study of E7070 in patients with metastatic, recurrent, or refractory squamous cell carcinoma of the head and neck: modulation of retinoblastoma protein phosphorylation by a novel chloroindolyl sulfonamide cell cycle inhibitor. **Clin Cancer Res** 10, 4680-4687.
- Hanahan D and Weinberg RA (2000) The hallmarks of cancer. **Cell** 100, 57-70.
- Harbour JW, Luo RX, Dei Santi A, Postigo AA and Dean DC (1999) Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. **Cell** 98, 859-869.
- Hinds PW (2003) Cdk2 dethroned as master of S phase entry. **Cancer Cell** 3, 305-307.
- Jones SF, Burris HA, Kies M, Willcutt N, Degen P, Bai S, Mauro D, Decillis A, Youssoufian H and Papadimitrakopoulou V (2003) A phase I study to determine the safety and pharmacokinetics (PK) of BMS-387032 given intravenously every three weeks in patients with metastatic refractory solid tumors. **Proc Am Soc Clin Oncol** 22, 199, abs 798.
- Lin TS, Howard OM, Neuberger DS, Kim HH and Shipp MA (2002) Seventy-two hour continuous infusion flavopiridol in relapsed and refractory mantle cell lymphoma. **Leuk Lymphoma** 43, 793-797.
- McClue SJ, Blake D, Clarke R, Cowan A, Cummings L, Fischer PM, MacKenzie M, Melville J, Stewart K, Wang S, Zhelev N, Zheleva D and Lane DP (2002) In vitro and in vivo antitumor properties of the cyclin dependent kinase inhibitor CYC202 (R-roscovitine). **Int J Cancer** 102, 463-468.
- McCormick J, Gadgeel SM, Helmke W, Chaplen R, Van Leeuwen B, Mauro D, Youssoufian H, Decillis A, Bai S and Lorusso PM (2003) Phase I study of BMS-387032, a cyclin dependent kinase (CDK) 2 inhibitor. **Proc Am Soc Clin Oncol** 22, 208, abs 835.
- Pierga J-Y, Faivre S, Vera K, Laurence V, Delbaldo C, Bekradda M, Armand J-P, Gianella-Borradori A, Dieras V and Raymond E (2003) A phase I and pharmacokinetic (PK) trial of CYC202, a novel oral cyclin-dependent kinase (CDK) inhibitor, in patients (pts) with advanced solid tumors. **Proc Am Soc Clin Oncol** 22, 210, abs 840.
- Punt CJ, Fumoleau P, van de Walle B, Faber MN, Ravic M and Campone M (2001) Phase I and pharmacokinetic study of E7070, a novel sulfonamide, given at a daily times five schedule in patients with solid tumors. A study by the EORTC-early clinical studies group (ECSG). **Ann Oncol** 12, 1289-1293.
- Raymond E, Ten Bokkel Huinink WW, Taieb J, Beijnen JH, Mekhaldi S, Kroon K, Wanders J, Ravic M, Fumoleau P, Ducreux M, Escudier B and Armand JP (2000) Phase I and pharmacokinetic (PK) study of E7070, a new chloroindolylsulfonamide, given as a 1-hour infusion every 3 weeks in patients (pts) with advanced solid tumors. **Proc Am Assoc Cancer Res** 41, 611-612.
- Robinson WA, Miller TL, Harrold EA, Bemis LT, Brady BMR and Nelson RP (2003) The effect of flavopiridol on the growth of p16+ and p16- melanoma cell lines. **Melanoma Res** 13, 231-238.
- Rudek MA, Bauer Jr KS, Lush III RM, Stinson SF, Senderowicz AM, Headlee DJ, Arbuck SG, Cox MC, Murgu AJ, Sausville EA and Figg WD (2003) Clinical pharmacology of flavopiridol following a 72-hour continuous infusion. **Ann Pharmacother** 37, 1369-1374.
- Sasaki Y, Sasaki T, Minami H, Kawada K, Fujii H, Igarashi T, Ishizawa K, Ito K, Usubuchi N, Evi H, Saeki T, Shigeoka Y, Hatake K, Aiba K, Ito Y, Takahashi S, Mizunuma N, Irie T, Maeda Y, Kobayashi T, Omuro Y, Okamoto R and Horikoshi N (2002) A phase I pharmacokinetic (PK)-pharmacodynamic (PD) study of flavopiridol by 24 hours continuous infusion (CI) repeating every week. **Proc Am Soc Clin Oncol** 21, 93a, abs 371.
- Sausville EA, Arbuck SG, Messmann R, Headlee D, Bauer KS, Lush RM, Murgu A, Figg WD, Lahusen T, Jaken S, Jing X, Roberge M, Fuse E, Kuwabara T and Senderowicz AM (2001) Phase I trial of 72-hour continuous infusion UCN-01 in patients with refractory neoplasms. **J Clin Oncol** 19, 2319-2333.
- Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH and The Eastern Cooperative Oncology Group (2002) Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. **N Engl J Med** 346, 92-98.
- Schwartz GK, Ilson D, Saltz L, O'Reilly E, Tong W, Maslak P, Werner J, Perkins P, Stoltz M and Kelsen D (2001) Phase II study of the cyclin-dependent kinase inhibitor flavopiridol administered to patients with advanced gastric carcinoma. **J Clin Oncol** 19, 1985-1992.
- Senderowicz AM (2002) The cell cycle as a target for cancer therapy: basic and clinical findings with the small molecule inhibitors flavopiridol and UCN-01. **Oncologist** 7 (Suppl 3), 12-19.
- Senderowicz AM (2003a) Novel small molecule cyclin-dependent kinases modulators in human clinical trials. **Cancer Biol Ther** 2 (Suppl 1), S84-S95.
- Senderowicz AM (2003b) Small-molecule cyclin-dependent kinase modulators. **Oncogene** 22, 6609-6620.
- Senderowicz AM, Headlee D, Stinson SF, Lush RM, Kalil N, Villalba L, Hill K, Steinberg SM, Figg WD, Tompkins A, Arbuck SG and Sausville EA (1998) Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasms. **J Clin Oncol** 16, 2986-2999.
- Senderowicz AM, Messmann R, Arbuck S, Headlee D, Zhai S, Murgu A, Melillo G, Figg WD and Sausville EA (2000) A phase I trial of 1 hour infusion of flavopiridol (FLA), a novel cyclin-dependent kinase inhibitor, in patients with advanced neoplasms. **Proc Am Soc Clin Oncol** 19, 204a, abs 796.
- Shapiro G, Lewis N, Bai S, Van Leeuwen B, Mauro D, Youssoufian H, Decillis A and Cohen RB (2003) A phase I study to determine the safety and pharmacokinetics (PK) of BMS-387032 with a 24-hr infusion given every three weeks in patients with metastatic refractory solid tumors. **Proc Am Soc Clin Oncol** 22, 199, abs 799.
- Shapiro GI (2004) Preclinical and clinical development of the cyclin-dependent kinase inhibitor flavopiridol. **Clin Cancer Res** 10 (Suppl), 4270s-4275s.
- Shapiro GI, Supko JG, Patterson A, Lynch C, Lucca J, Zaccarola PF, Muzikansky A, Wright JJ, Lynch Jr TJ and Rollins BJ (2001) A Phase II trial of the cyclin-dependent kinase inhibitor flavopiridol in patients with previously untreated stage IV non-small cell lung cancer. **Clin Cancer Res** 7, 1590-1599.
- Shaw VA, Rini BI, Park JW and Small EJ (2003) A phase 2 study of UCN-01 in advanced renal cell carcinoma. **Proc Am Soc Clin Oncol** 22, 442, abs 1775.
- Sherr CJ (2000) The Pezcoller Lecture: cancer cell cycles revisited. **Cancer Res** 60, 3689-3695.
- Stadler WM, Vogelzang NJ, Amato R, Sosman J, Taber D, Liebowitz D and Vokes EE (2000) Flavopiridol, a novel cyclin-dependent kinase inhibitor, in metastatic renal cancer: a University of Chicago Phase II Consortium study. **J Clin Oncol** 18, 371-375.
- Stein GH, Drullinger LF, Soulard A and Dulic V (1999) Differential roles for cyclin-dependent kinase inhibitors p21 and p16 in the mechanisms of senescence and differentiation in human fibroblasts. **Mol Cell Biol** 19, 2109-2117.

- Sumitomo M, Asano T, Asakuma J, Asano T, Horiguchi A and Hayakawa M (2004) ZD1839 modulates paclitaxel response in renal cancer by blocking paclitaxel-induced activation of the epidermal growth factor receptor–extracellular signal-regulated kinase pathway. **Clin Cancer Res** 10, 794-801.
- Swanton C (2004) Cell-cycle targeted therapies. **Lancet Oncol** 5, 27-36.
- Tamura T, Sasaki Y, Minami H, Fujii H, Ito K, Igarashi T, Kamiya Y, Kurata T, Ohtsu T, Onozawa Y, Yamamoto N, Yamamoto N, Watanabe Y, Tanigawara Y, Fuse E, Kuwabara T, Kobayashi S and Shimada Y (1999) Phase I study of UCN-01 by 3-hour infusion. **Proc Am Soc Clin Oncol** 18, 159a, abs 611.
- Tan AR, Headlee D, Messmann R, Sausville EA, Arbuck SG, Murgu AJ, Melillo G, Zhai S, Figg WD, Swain SM and Senderowicz AM (2002) Phase I clinical and pharmacokinetic study of flavopiridol administered as a daily 1-hour infusion in patients with advanced neoplasms. **J Clin Oncol** 20, 4074-4082.
- Terret C, Zanetta S, Roche H, Schellens JH, Faber MN, Wanders J, Ravic M, Droz JP and EORTC Early Clinical Study Group (2003) Phase I clinical and pharmacokinetic study of E7070, a novel sulfonamide given as a 5-day continuous infusion repeated every 3 weeks in patients with solid tumours. A study by the EORTC Early Clinical Study Group (ECSG). **Eur J Cancer** 39, 1097-1104.
- Thomas J, Cleary J, Tutsch K, Arzoomanian R, Alberti D, Simon K, Feierabend C, Morgan K and Wilding G (1997) Phase I clinical and pharmacokinetic trial of flavopiridol. **Proc Am Assoc Cancer Res** 38, 222, abs 1496.
- Thomas JP, Tutsch KD, Cleary JF, Bailey HH, Arzoomanian R, Alberti D, Simon K, Feierabend C, Binger K, Marnocha R, Dresen A and Wilding G (2002) Phase I clinical and pharmacokinetic trial of the cyclin-dependent kinase inhibitor flavopiridol. **Cancer Chemother Pharmacol** 50, 465-472.
- Van Kesteren Ch, Mathot RA, Raymond E, Armand JP, Dittrich Ch, Dumez H, Roche H, Droz JP, Punt C, Ravic M, Wanders J, Beijnen JH, Fumoleau P and Schellens JH (2002) Population pharmacokinetics of the novel anticancer agent E7070 during four phase I studies: model building and validation. **J Clin Oncol** 20, 4065-4073.
- White JD, Cassidy J, Twelves C, Benson C, Pacey S, Judson I, McGrath H, Rose F and Frenz L (2004) A phase I trial of the oral cyclin dependent kinase inhibitor CYC202 in patients with advanced malignancy. **Proc Am Soc Clin Oncol** 23, 205, abs 3042.
- Yamada Y, Yamamoto N, Shimoyama T, Horiike A, Fujisaka Y, Takayama K, Sakamoto T, Nishioka Y, Yasuda S and Tamura T (2004) A phase I pharmacokinetic and pharmacogenomic study of E7070 administered once every 21 days. **Proc Am Soc Clin Oncol** 23, 134, abs 2030.



Andrew Hughes

