Cancer Chemother Pharmacol (2002) 50: 465–472
DOI 10.1007/s00280-002-0527-2

ORIGINAL ARTICLE

**James P. Thomas · Kendra D. Tutsch · James F. Cleary
Howard H. Bailey · Rhoda Arzoomanian · Dona Alberti
Kris Simon · Chris Feierabend · Kimberly Binger
Rebecca Marnocha · Amy Dresen · George Wilding**

Phase I clinical and pharmacokinetic trial of the cyclin-dependent kinase inhibitor flavopiridol

Received: 4 March 2002 / Accepted: 23 August 2002 / Published online: 2 October 2002

Abstract *Purpose*: Flavopiridol (NSC 649890) is a synthetic flavone possessing significant antitumor activity in preclinical models. Flavopiridol is capable of inducing cell cycle arrest and apoptosis, presumably through its potent, specific inhibition of cyclin-dependent kinases. We conducted a phase I trial and pharmacokinetic study of flavopiridol given as a 72-h continuous intravenous infusion repeated every 2 weeks. *Methods*: A total of 38 patients were treated at dose levels of 8, 16, 26.6, 40, 50 and 56 mg/m^2/24 h. During the first infusion, plasma was sampled at 24, 48 and 72 h to determine steady-state concentrations, and peripheral blood lymphocytes were assessed by flow cytometry for evidence of apoptosis. Additional postinfusion pharmacokinetic sampling was done at the 40 and 50 mg/m^2/24 h dose levels. *Results*: Gastrointestinal toxicity was dose limiting, with diarrhea being the predominant symptom. Symptomatic orthostatic hypotension was also frequently noted. Several patients experienced tumor-specific pain during their infusions. The maximum tolerated dose (MTD) was determined to be 40 mg/m^2/24 h. A patient with metastatic gastric cancer at this dose level had a complete response and remained disease-free for more than 48 months after completing therapy. Plasma concentrations at 24 h into the infusion were 94% of those achieved at steady state. Steady-state plasma flavopiridol concentrations at the MTD were 416.6 ± 98.9 μM. These concentrations are at or above those needed to see cell cycle arrest and apoptosis in vitro. The mean clearance of flavopiridol over the dose range was 11.3 ± 3.9 l/h per m^2, similar to values obtained preclinically. Elimination was biphasic. The terminal half-life at the MTD was 26.0 h. No significant differences in pharmacokinetic parameters were noted between males and females. Patients taking cholestyramine to ameliorate flavopiridol-induced diarrhea had lower steady-state plasma concentrations. There was no significant change in the cell cycle parameters of peripheral blood lymphocytes analyzed by flow cytometry. *Conclusions*: The MTD and recommended phase II dose of flavopiridol given by this schedule is 40 mg/m^2/24 h. The manageable gastroinestinal toxicity, early signs of clinical activity and lack of hematologic toxicity make further exploration in combination trials warranted.

Keywords Flavopiridol · Phase I · Pharmacokinetics

This work was supported by grants UO1-CA62491, MO1-RR03186 and P30CA14520 from the National Institutes of Health.

J.P. Thomas · K.D. Tutsch (✉) · J.F. Cleary · H.H. Bailey
R. Arzoomanian · D. Alberti · K. Simon · C. Feierabend
K. Binger · R. Marnocha · A. Dresen · G. Wilding
University of Wisconsin Comprehensive Cancer Center,
University of Wisconsin Medical School,
600 Highland Ave, Madison, WI 53792, USA
E-mail: kdtutsch@facstaff.wisc.edu
Tel.: +1-608-2635369
Fax: +1-608-2658133

Introduction

Recent progress in molecular biology and biochemistry has begun to elucidate the complex machinery involved in the control of cellular entry and progression through the cell cycle. One of the characteristic features of neoplastic tissue is a dysregulated cell cycle, allowing for continued expansion of cancer cells. Cyclin-dependent kinases (cdk) are enzymes responsible for the orderly progression through the cell cycle. Cdk are activated by association with cyclins and then can phosphorylate key regulatory substrates such as the Rb protein [1]. The central role of cdk in cell cycle regulation makes this class of kinases an attractive target in the development of antiproliferative strategies.

Flavopiridol (NSC 649890) is a synthetic flavone whose parent structure was purified from an Indian plant *Dysoxylum binectariferum* [2]. Flavopiridol was initially shown to induce cell cycle arrest in a variety of cultured neoplastic cells [3]. Synchronized cells could be blocked in either G_2 or G_1 of the cell cycle. Subsequent

investigations have also shown that flavopiridol can induce apoptosis in several tumor cell lines in vitro [4, 5, 6, 7]. The antiproliferative effects of flavopiridol are thought to be secondary to its potent inhibition of cdk including cdk1, cdk2, cdk4 and cdk6 [8, 9, 10]. Flavopiridol is capable of binding to the ATP-binding site of these cdk where it competitively inhibits ATP binding [11]. Flavopiridol is also capable of inhibiting the phosphorylation of cdk1, 2, 4 and 6, possibly by inhibiting cdk-activating kinase. Inhibition of cdk is potent and relatively specific with other kinases such as serine/threonine and tyrosine protein kinases inhibited only at much higher concentrations [12].

The mechanism by which flavopiridol induces apoptosis is at present unclear. Lethality is not significantly affected by inhibition of DNA synthesis, while RNA or protein synthesis inhibitors substantially reduce toxicity [6]. Drees et al. have shown a marked sensitivity of prostatic and melanoma cancer cells to flavopiridol [13]. The IC_{50} of 14 out of 23 cell lines was less than 10 ng/ml (25 n*M*). Flavopiridol at a dose of 10 mg/kg per day was able to induce tumor regression in a nude mouse xenograft prostate model. Bible and Kaufman have shown that flavopiridol exhibits cytotoxic synergy in A549 human non-small-cell lung cancer cells when combined with such agents as paclitaxel, cytarabine, topotecan and doxorubicin [14]. Synergy was highly schedule dependent, with flavopiridol more active when given after the other cytotoxic agent.

Toxicity of flavopiridol has been evaluated in preclinical models and is primarily gastrointestinal in nature with diarrhea, vomiting and gastrointestinal hemorrhage. Some myelosuppression is seen at higher doses. The maximum tolerated dose (MTD) for flavopiridol in dogs is 1.5 mg/kg per day when given as a 72-h infusion. A prolonged, continuous infusion schedule was chosen for initial clinical development since preclinical studies suggested improved efficacy with longer drug exposure [3, 15]. We performed a phase I dose escalation trial with flavopiridol given as a 72-h continuous intravenous infusion every 2 weeks.

Materials and methods

Patient eligibility

The study was approved by the Human Subjects Committee at the University of Wisconsin Hospital. Patients with microscopic confirmation of malignancy (solid tumor or lymphoma) were eligible for enrollment. Other eligibility requirements included: ECOG performance status of 0 or 1, life expectancy of >12 weeks, age >18 years, and ability to provide informed consent. Patients must not have received chemotherapy and/or radiation therapy in the 4 weeks prior to enrollment and must have recovered completely from any toxicity. Adequate organ function was required, defined as: WBC $>4000/mm^3$, platelets $>100{,}000/mm^3$, ANC $>1500/mm^3$, serum bilirubin <1.5 mg/dl, AST less than twice normal, serum creatinine <1.5 mg/dl, and calcium <11.0 mg%. Patients were ineligible if they had a previous history of brain metastasis, or seizure disorder. Pregnant or nursing females were ineligible.

Evaluation during therapy

Patients were monitored with clinical assessment prior to each infusion and with weekly laboratory evaluation for toxicity. Toxicities were graded using the National Cancer Institute Common Toxicity Criteria (NCI CTC). Evaluation of measurable or evaluable disease was done at least every 6 weeks. Complete response was defined as disappearance of all clinical evidence of active tumor and symptoms for at least 1 month. Partial responses required $>50\%$ decrease in the sum of the products of the perpendicular tumor diameters of all measurable lesions for more than 1 month, without simultaneous increases in the size of any lesion or appearance of any new lesions. Progressive disease was defined as a greater than 25% increase in the size of any measurable lesion. Stable disease represented responses less than a partial response or growth less than that of progressive disease.

Study design

Dose levels for the 72-h continuous infusion of flavopiridol were established using a modified Fibonnaci dose escalation scheme as follows: 8, 16, 26.6, 40, and 56 mg/m^2/24 h. Subsequently, further patients underwent treatment at 50 mg/m^2/24 h. Infusions were repeated every 2 weeks. No dose escalations were performed within an individual patient. Dose-limiting toxicity (DLT) was defined as any grade 3 or greater toxicity (excluding nausea and vomiting) that occurred during the first two courses of flavopiridol. Patients were enrolled in cohorts of three with the following scheme: if none of three patients experienced DLT, the new cohort of three patients were treated at the next higher level; if one of three patients experienced DLT, an additional three patients were added to the cohort; if two of three patients experienced DLT, the new cohort of three was treated at the next lower dose level. Patients were considered evaluable for toxicity if they had received two courses of flavopiridol.

Flavopiridol administration

Flavopiridol was provided by the NCI in sterile vials which contained 10.9 mg of lyophilized flavopiridol equivalent to 10 mg of free base along with 19 mg citric acid, 300 mg hydroxypropyl-B-cylcodextrin and sodium hydroxide to adjust to pH 3.5–5.5. Flavopiridol was reconstituted and diluted with 0.9% sodium chloride. Flavopiridol was administered as a 72-h continuous intravenous infusion via a central venous catheter. Patients received flavopiridol in an inpatient setting. If all toxicities had resolved, patients were eligible to receive further courses every 2 weeks. The criteria for removal from study included: patients wishes, changes in the patient's overall condition which rendered the patient ineligible for further treatment according to the judgment of the investigator, evidence of progressive disease or delay in the interval of chemotherapy of greater than 2 weeks for unresolved toxicity.

Pharmacokinetic sampling

Pharmacokinetic sampling was performed during the first 72-h infusion on all patients at 0, 24, 48 and 72 h. Selected patients at the 40 and 50 mg/m^2/24 h dose levels had additional pharmacokinetic sampling at 15, 30, 45, 60 and 90 min and at 2, 4, 24, 48 and 72 h after the end of the infusion. Blood samples were collected into heparinized tubes and the plasma separated by centrifugation and stored at –70°C until analyzed. For each patient the mean steady-state plasma concentration (C_{ss}) was determined as the average of the 48- and 72-h concentrations. For patients with postinfusion sampling, pharmacokinetic parameters were determined by noncompartmental methods using the PKAnalyst program (MicroMath, Salt Lake City, Utah). The terminal elimination half-life was determined by log-linear extrapolation of the last three or

four concentration time-points and the total area under the concentration-time curve (AUC) from the start of the infusion was determined by the trapezoidal rule extrapolated to ∞. The apparent total plasma clearance (Cl_{tb}) was determined as infusion rate/C_{ss} and was also determined by dose/AUC.

Flavopiridol assay

Flavopiridol in plasma was assayed by a high-performance liquid chromatography (HPLC) method with spectrophotometric detection, which was adapted from several published procedures [16, 17]. Flavopiridol for standards was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI (Bethesda, Md.). Briefly, the HPLC system consisted of a Thermo Separations P4000 pump, UV2000 detector and AS3000 autosampler (ThermoQuest, San Jose, Calif.) and a Shimadzu 4271 Integrator (Shimadzu, Columbia, Md.). Chromatographic separations were achieved on a Nova-Pak phenyl RCM 8×10 cartridge (Waters/Millipore, Milford, Mass.) using an isocratic solvent system of 4% acetonitrile/15% tetrahydrofuran/30% ammonium acetate buffer (100 m*M*, pH 3.8)/51% methanol pumped at 0.7 ml/min. The retention time for flavopiridol in this system was 11.8 min.

Patient samples (300 µl) were extracted with methanol and 20 µl 10% tricloroacetic acid in a siliconized microfuge tube. Internal standard (100 ng biochanin A) was added. Samples were quantitated from standard curves of the ratio of chromatography peak height of flavopiridol to that for the internal standard. Absolute recoveries of flavopiridol and the internal standard in plasma extracts were similar (83 ± 3% and 87 ± 1%, respectively, $n = 7$) and did not vary over the concentration range. Recovery of flavopiridol in patient samples by standard addition averaged 103 ± 4% ($n = 6$). The standard curve was linear in the range 50–500 ng/ml flavopiridol in plasma ($r^2 = 0.996 \pm 0.003$ for eight curves over 5 months) and the limit of quantitation was 20 ng/ml plasma concentration (0.6 ng injected). The intraday variability of four replicate determinations of plasma standards over the concentration range was less than 5%. Duplicate determinations of patient samples were within 6%. Six repeated assays of a control patient sample over a 5-month period showed a variability of 6.2%.

Flow cytometric analysis of peripheral blood lymphocytes

Blood samples before treatment and at 48 h into the infusion were drawn into heparinized tubes and analyzed immediately. Peripheral blood lymphocytes (PBLs) were separated from 15 ml whole blood using Histopaque 1077 (ACCUSPIN tubes, Sigma Chemicals, St. Louis, Mo.). The tubes were spun for 12 min at 1000 *g*. The band of mononuclear cells was removed and then washed twice with cold Hank's CMF medium. Cells were resuspended in 100 µl medium and then 900 µl 100% ethanol (–20°C) was added to fix the cells. Cells were stained with propidium iodide and then analyzed with a Becton Dickinson FACScan flow cytometer.

Results

Toxicity data

A total of 38 patients were enrolled in this trial. All were eligible and evaluable for toxicity data. Demographic information is listed in Table 1. The DLTs observed during trial progression are listed in Table 2. One episode of dose-limiting diarrhea and two other DLTs were observed at the 56 mg/m^2/24 h dose level during the initial two courses. The MTD and recommended phase II dose was declared to be 40 mg/m^2/24 h and additional patients were enrolled at this level to more fully define the MTD and to achieve gender equity for the pharmacokinetic study. An additional intermediate level, 50 mg/m^2/24 h, was also examined. Although dose-limiting diarrhea was not seen at this level, it was felt in general by the investigators to be poorly tolerated. Patients enrolled at the 50 mg/m^2/24 h dose received loperamide during their first course of therapy and cholestyramine during their second course to examine the relative effects each agent had on diarrhea and steady-state pharmacokinetics.

Table 1 Patient demographic data

	No. of patients (except age in years)
Female	15
Male	23
Performance status	
0	11
1	23
2	4
Age (years)	
Median	59.4
Range	32–83
Tumor type	
Prostate	10
Breast	4
Renal	4
Colon	3
Ovary	3
Gallbladder	3
Stomach	2
Esophagus	2
Lung	1
Melanoma	1
Sarcoma	1
Liver	1
Cervix	1
Head and neck	1
Unknown primary	1
Previous therapy[a]	
No prior chemotherapy	4
One prior chemotherapy	7
Two prior chemotherapies	8
Three or more prior chemotherapies	19

[a]Includes hormonal and/or biologic therapies

The predominant toxicity seen in this trial was gastrointestinal which was as predicted by the preclinical evaluation. Table 3 lists the gastrointestinal toxicities observed in each patient over the entire duration of their flavopiridol treatment, along with the highest NCI CTC grade observed. Diarrhea was the most common toxicity seen, with 24 out of 38 patients experiencing at least some level of diarrhea. Somewhat surprisingly, constipation was also seen in this study, with seven patients noting some difficulty with this during the course of their treatment. Diarrhea usually began towards the end of the 72-h infusion and persisted for about 2–3 days after completing treatment. Nausea and vomiting were also

Table 2 DLT data by cohort for purpose of MTD determination

Cohort	Dose level (mg/m^2/24 h)	No. of patients per dose level	DLT		
			No. of patients	Description	NCI CTC grade
1	8	4	0		
2	16	3	0		
3	26.6	4	1	Alkaline phosphatase	3
4	40	13	1	Diarrhea	3
5	56	7	1	Diarrhea	4
			1	Pulmonary	3
			1	Constipation	3
6	50	7	0		

Table 3 Gastrointestinal toxicity (highest grade observed while on study, number of patients)

Cohort	Dose level (mg/m^2/24 h)	Diarrhea				Nausea				Vomiting				Constipation			
		Grade				Grade				Grade				Grade			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	8					1	1				1						
2	16																
3	26		2			2	2				1				1		
4	40	2	7	2		1	6			2	2			2			
5	56	1	2		2		5			2	1				1	1	
6	50	3	3			1	3	1		2	1			1	1		
Totals		6	14	2	2	5	17	1		6	6			3	3	1	

frequently noted. At dose level 6 (50 mg/m^2/24 h), a high-dose loperamide regimen was instituted to ameliorate flavopiridol-induced diarrhea. This regimen was similar to that utilized with irinotecan, with 2 mg of loperamide given at the first loose stool and repeated every 2 h until diarrhea subsided for 24 h. During course 2 of level 6, patients were given cholestyramine to prevent diarrhea. No measurement of stool electrolytes was performed to determine whether this was a secretory or osmotic in nature and no endoscopies were performed. Steady-state flavopiridol levels were compared between the course with loperamide and the course with cholestyramine to determine whether there were any dramatic differences in flavopiridol pharmacokinetics.

Orthostatic hypotension (grade 1-2) was also frequently observed during this study. In general this toxicity was well tolerated and was not associated with significant tachycardia. Orthostatic hypotension was often seen even early during the infusion and in patients who did not experience diarrhea. For these reasons it was felt that the observed orthostatic hypotension was not the result of volume depletion associated with diarrhea. Hypotension quickly resolved at the completion of drug administration. Another, unexpected toxicity seen in this study was increased tumor pain. Patients with pain secondary to their cancers would often report an increase in this specific pain during their infusions. Other patients without a history of painful tumors would sometimes develop pain at their disease sites during the course of the flavopiridol infusions. Several patients developed low-grade fevers during flavopiridol administration.

Fatigue and malaise were also a frequent complaint in patients on this study but did not have a grade on the version of the common toxicity criteria used for this study. Mild to moderate fatigue was reported by 3 of 13 patients at 40 mg/m^2/24 h, 4 of 7 patients at 50 mg/m^2/24 h and 3 of 7 patients at 56 mg/m^2/24 h. Although no dose-limiting diarrhea or other DLT was observed at the 50 mg/m^2/24 h dose level, it was felt by the investigators to be poorly tolerated due to the constitutional symptoms which significantly affected the quality of life of a majority of the patients at this dose level.

One patient with pulmonary toxicity (grade 3) was a patient with a head and neck cancer who developed acute airway compromise secondary to tumor obstruction during the course of his infusion that required emergent tracheostomy. One patient at the 26.6 mg/m^2/24 h dose level had an elevated (grade 3) alkaline phosphatase that was later determined to be probably disease-related. Other toxicities included an episode of atrial fibrillation in a patient with a history of malignant pericardial effusions and prior atrial fibrillation.

Cumulative toxicity was difficult to assess in this trial since few patients received more than 1 or 2 months of therapy. The median number of 2-week courses received was three, with only 13 of 38 patients receiving more than three courses. Most patients were removed from study due to progression after their 6-week disease evaluation. Of note, one patient who went on to receive 14 courses of flavopiridol did not experience any fatigue or gastrointestinal symptoms.

Response data

One patient with a history of gastric carcinoma and liver metastasis achieved a complete response with flavopiridol administration. This patient received a total of 14 courses before a decision was made to discontinue therapy. At the time of this report, he continued to exhibit no evidence of tumor reoccurence 48 months after stopping flavopiridol. This particular patient developed Beau's lines in his fingernails while on flavopiridol (Fig. 1), the significance of which is unknown.

Pharmacokinetic analysis

Steady-state plasma concentrations of flavopiridol were nearly achieved by the 24th hour of administration and there were no significant differences between the plasma concentrations at 24, 48, and 72 h. A typical concentration-time curve is shown in Fig. 2. Greater than 85% of the AUC for flavopiridol was time during the actual infusion. Mean steady-state concentrations increased as a function of increased flavopiridol dose (Fig. 3). The steady-state plasma concentration of flavopiridol (C_{ss}) at the MTD dose of 40 mg/m^2/24 h was 416.6 ± 98.9 n*M* (mean ± SD). The pharmacokinetic parameters at all dose levels are shown in Table 4. Flavopiridol exhibited significant interpatient variability, with steady-state flavopiridol concentrations varying two- to threefold at each dose level. Flavopiridol exhibited biphasic elimination with a terminal (β) half-life of 27 ± 18 h. In 9 of 14 patients analyzed after the end of infusion, significant plateauing occurred, suggestive of possible enterohepatic recirculation and supporting the earlier results seen by Senderowicz et al. [18]. In those patients with postinfusion data where the initial (α) half-life could be calculated, the mean was 1.1 h.

A cohort of patients at the 50 mg/m^2/24 h dose level were treated with cholestyramine during the second course of flavopiridol to investigate the possible effect on plasma concentrations of flavopiridol. Many patients did not tolerate cholestyramine for the entire 72-h infusion period. However, the mean concentration of flavopiridol at 24 h into the infusion given with cholestyramine was 65% of the mean during the first course without cholestyramine (375 ± 116 n*M* vs 578 ± 421 n*M*), indicating that cholestyramine substantially decreased flavopiridol plasma concentrations. Statistical significance was not reached due to the small number of subjects.

The mean clearance of flavopiridol was 11.3 ± 3.9 l/h per m^2. In patients with postinfusion data, clearance calculated from the total AUC was the same as clearance calculated from the steady-state data. A total of six males and seven females were evaluated at the MTD, and there was no significant difference in mean steady-state flavopiridol plasma concentrations or in flavopiridol clearance in these patients.

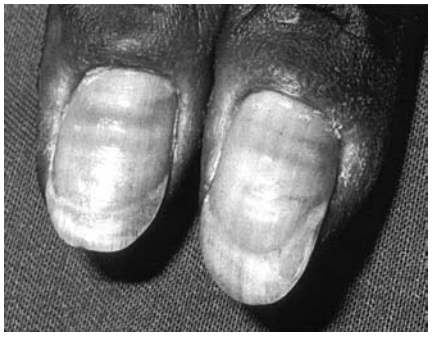

Fig. 1 Fingernails of a patient receiving 14 courses of flavopiridol

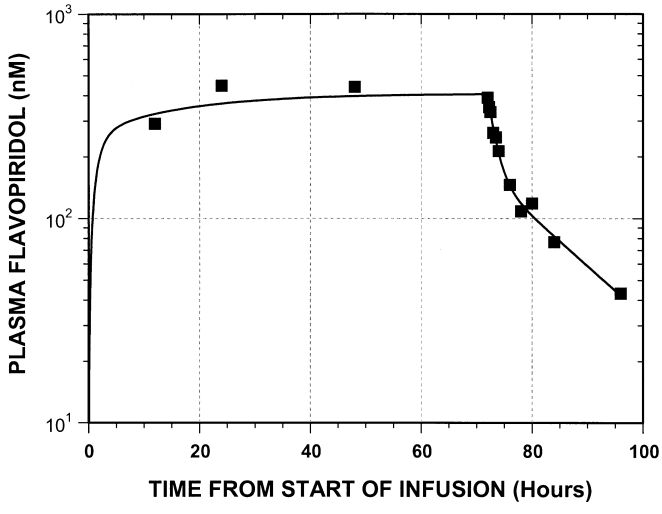

Fig. 2 Plasma concentration of flavopiridol vs time in a single patient at the 50 mg/m^2/24 h dose level

Flow cytometry of peripheral blood lymphocytes

PBLs were isolated from patients at the 40 mg/m^2/24 h dose level at 0, 24 and 72 h into the infusion and 24 h after completion of infusion. PBLs were stained with propidium iodide and then analyzed by flow cytometry. No difference was observed in the relative numbers of PBLs in different phases of the cell cycle (data not shown) before, during, or after flavopiridol. This may have been secondary to the lack of actively cycling cells seen with peripheral PBLs. Similarly there was no increase in the number of apoptotic cells with flavopiridol.

Discussion

Flavones are an interesting group of compounds, many of which possess intrinsic kinase-inhibitory activity. Flavopiridol is unique in that it exhibits considerable selectivity for cdk. Cdk2 is inhibited by flavopiridol with a K_i of about 100 n*M* [10], which is similar to the plasma levels achieved in this study. Consistent with its

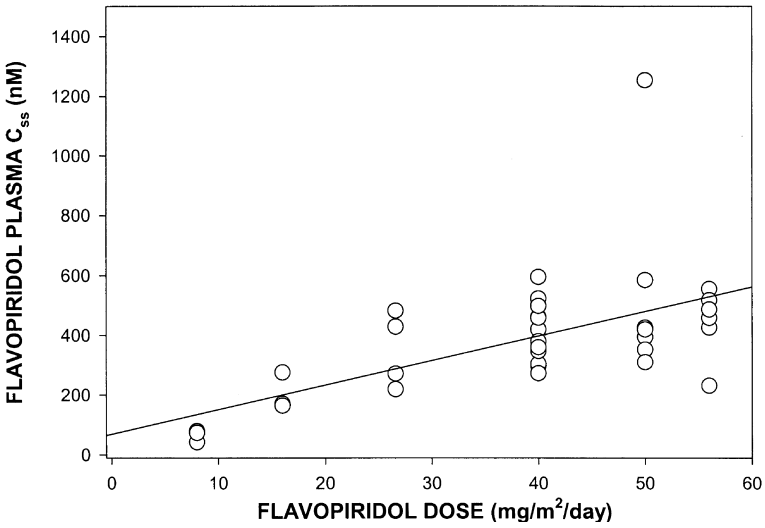

Fig. 3 Flavopiridol plasma concentration at 48 h vs dose administered for 37 patients ($r = 0.625$, $P < 0.001$)

Table 4 Flavopiridol pharmacokinetic parameters. Values are means ± SD (*NA* not available)

Dose level (mg/m^2/24 h)	C_{ss} (n*M*) (range)	Cl_{tb} (l/h/m^2)	AUC (μ*M*·h)	$t_{1/2}\beta$ (h)
8.0 ($n = 3$)	65.2 ± 20.0 (42.3–79.7)	13.8 ± 5.1	NA	NA
16.0 ($n = 3$)	203.4 ± 62.3 (164.4–275.2	8.6 ± 2.3	NA	NA
26.6 ($n = 4$)	350.2 ± 124.9 (219.2–481.9)	8.7 ± 3.2	NA	NA
40.0 ($n = 13$)	416.6 ± 98.9 (272.7–595.3)	10.5 ± 2.6	29.0 ± 13.0	26.0 ± 16.7
50.0 ($n = 7$)	534.6 ± 328.7 (310.1–1254.0)	11.7 ± 4.1	33.3 ± 11.9	29.5 ± 22.5
56.0 ($n = 7$)	443.2 ± 104.6 (231.6–555.4)	14.1 ± 5.0	NA	NA
Mean ± SD	NA	11.3 ± 3.9	NA	27.3 ± 18.3

cdk-inhibitory activity, preclinical studies have shown cell cycle arrest of synchronized cultured cells in both the G_1 and G_2 phases of the cell cycle [3, 10]. Recent preclinical investigations have also demonstrated that flavopiridol is capable of inducing apoptosis in cultured cancer cells [4, 5, 6, 7]. Flavopiridol has also been shown to synergize with a number of chemotherapeutic agents such as mitomycin [5] and topotecan [14]. This synergy is remarkably schedule-dependent, with flavopiridol augmenting toxicity to a much greater extent when given after the other agent. One of the fundamental flaws in many neoplastic cells is control over the cell cycle machinery. Agents that selectively target the cell cycle apparatus may represent a new class of potential antineoplastic compounds. Recently, the role of cdk inhibition in the anticancer effect of flavopiridol has been questioned [19].

A phase I study of flavopiridol given as a continuous 72-h infusion every 2 weeks was undertaken with the goal of obtaining the MTD and DLT profile of this agent. The most frequent toxicities seen in this trial were gastrointestinal, as predicted by the preclinical models. Diarrhea was the most frequently encountered symptom, usually starting on the 2nd or 3rd day of the infusion and lasting 1–3 days after the infusion was completed. There was no cumulative effect of repeated courses on gastrointestinal toxicities. Nausea and loss of appetite were also frequently noted. Orthostatic hypotension was also frequently seen on this study. In general this toxicity was well tolerated and often asymptomatic. Orthostatic hypotension was seen early during the flavopiridol infusion and in patients who did not exhibit diarrhea or other fluid losses. Thus, orthostatic hypotension was thought to be a direct result of the flavopiridol infusion and not the result of diarrheal fluid losses. Flavopiridol has been shown to affect chloride secretion by human colonic epithelial cells [20]. Innocenti et al. have suggested that patients with low flavopiridol glucuronidation rates have increased diarrhea [21].

A limited study of measures to ameliorate flavopiridol-induced diarrhea was attempted. During course 1 at the 50 mg/m^2/24 h dose level, patients experiencing diarrhea received high-dose loperamide in a regimen similar to that employed with irinotecan. Although this measure did decrease the amount of expected diarrhea at this level, it did not appear to improve the overall tolerability of flavopiridol. During their second course at this dose level, patients received prophylactic treatment with cholestyramine. Somewhat unexpectedly, the combination of flavopiridol and cholestryramine was poorly tolerated. Patients complained of nausea, bloating and were often unwilling or unable to take all of the prescribed cholestyramine dose. For this reason, we recommend loperamide over cholestyramine for flavopiridol-induced diarrhea. We examined the steady-state levels of flavopiridol during course 1 and course 2 at this level to see if there was any pronounced, relative effect on flavopiridol pharmacokinetics. Steady-state flavopiridol concentrations were lower during the second course while on

cholestyramine although this difference did not reach statistical significance. This difference may have been secondary to an inhibition of hepatobiliary recirculation or possible altered pharmacokinetics during the second cycle unrelated to the antidiarrheal measure employed. The MTD determined for this trial is lower than that determined by Senderowicz et al. [18] who reported an MTD of 50 mg/m^2 daily for 3 days. As mentioned above, our experience at this dose level, while not generating predescribed DLTs, was not tolerable in an expanded group of patients.

Quite unexpectedly, an increase in tumor-associated pain was observed during many flavopiridol infusions. Several patients who did not have tumor pain prior to this study developed pain at their disease sites during flavopiridol treatment. We are uncertain of the mechanism behind this phenomenon. One possibility is that flavopiridol induces edema specifically in the cancerous tissue. One patient at the 26.6 mg/m^2/24 h level with a head and neck tumor experienced acute tracheal obstruction by his tumor during the flavopiridol infusion. This patient required emergent tracheostomy. Another patient with extensive liver metastasis developed biliary obstruction during the flavopiridol infusion that required stenting.

The pharmacokinetics of flavopiridol were examined in this study. The steady-state concentrations were dose-dependent. At the MTD dose of 40 mg/m^2/24 h, the mean steady-state flavopiridol concentration was 416.6 n*M*. This concentration compares favorably with the levels used preclinically to inhibit cdk2 [8, 9], cause cell cycle arrest [3, 10] and to induce apoptosis [4, 5, 6, 14, 15]. Greater than 85% of the AUC of flavopiridol represented time during the actual infusion, underscoring the relatively rapid elimination of this compound. The mean clearance determined in our study was 11.3 l/h per m^2, similar to that observed in preclinical animal studies. Flavopiridol concentrations measured in our study are in the range of those reported by Senderowicz et al. [18]. Wide interpatient variability was found in that study at the 50 mg/m^2/24 h dose level, with a median steady-state plasma concentration of 271 n*M* and a range of 174 to 2943 n*M*. Our comparable range was 310 to 1254 n*M*, with a mean steady-state plasma concentration of 535 ± 329 n*M*. While in the same range, our values trend higher, possibly due to differences between the sampling techniques. Senderowicz et al. stopped the infusion to draw the pharmacokinetic samples, which could have led to some loss of flavopiridol. Our samples were drawn peripherally from the opposite arm with the infusion running.

One patient with metastatic gastric cancer and liver metastasis had a partial response after two courses of flavopiridol (40 mg/m^2/24 h) and subsequently went on to have a complete response after his fourth course. The patient's steady-state flavopiridol concentration was similar to that of other patients in this cohort. He received a total of 14 courses of flavopiridol. At the time of this report, this patient had remained disease-free for more than 48 months after finishing flavopiridol. Flavopiridol has been examined in a phase II trial of patients with advanced gastric cancer and found to be inactive [22]. We are uncertain of any differences between this patient and the phase II study patients to explain why this patient experienced such a dramatic response.

In summary, our investigation suggests a MTD of 40 mg/m^2/24 h for flavopiridol given as a 72-h continuous infusion, and is our recommended phase II dose. While the 50 mg/m^2/24 h dose was largely devoid of DLTs (as defined by the protocol), it was relatively poorly tolerated by our patients, with significant fatigue, malaise and protracted grade 2 gastrointestinal toxicity. Early signs of clinical activity coupled with a manageable toxicity profile make this a promising agent for further investigation. The absence of hematologic toxicity makes the combination of flavopiridol with other agents an interesting area for future study. Clinical trials of flavopiridol in combination with cytotoxics such as cisplatin, docetaxel, irinotecan and gemcitabine are currently underway.

References

1. Rao RN (1996) Targets for cancer therapy in the cell cycle pathway. Curr Opin Oncol 8:516–524
2. Maik RG, Kattice SL, Bhat SV, Alreja B, de Souza NJ, Rupp RH (1988) An anti-inflammatory cum immunomodulatory piperidinylbenzopyranone from *Dysoxylum binectariferum*: isolation, structure and total synthesis. Tetrahedron 44:2081–2086
3. Kaur G, Stetler-Stevenson M, Sebers S, Worland P, Sedlacek H, Myers C, Czech J, Naik R, Sausville E (1992) Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86-8275. J Natl Cancer Inst 84:1736–1740
4. Parker BW, Kaur G, Nieves-Neira W, Taimi M, Kohlhagen G, Shimizu T, Losiewicz MD, Pommier Y, Sausville EA, Senderowicz AM (1998) Early induction of apoptosis in hematopoietic cell lines after exposure to flavopiridol. Blood 91:458–465
5. Schwartz GK, Farsi K, Maslak P, Kelsen DP, Spriggs D (1997) Potentiation of apoptosis by flavopiridol in mitomycin-C-treated gastric and breast cancer cells. Clin Cancer Res 3:1467–1472
6. Bible KC, Kaufman SH (1996) Flavopiridol: a cytotoxic flavone that induces cell death in noncycling A549 human lung carcinoma cells. Cancer Res 56:4856–4861
7. Arguello F, Alexander M, Sterry JA, Tudor G, Smith EM, Kalavar NT, Greene JF, Koss W, Morgan CD, Stinson SF, Siford TJ, Alvord WG, Klabansky RL, Sausville EA (1998) Flavopiridol induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity in vivo against human leukemia and lymphoma xenografts. Blood 91:2482–2490
8. Worland PJ, Kaur G, Stetler-Stevenson M (1993) Alteration of the phosphorylation state or p34cdc2 kinase by the flavone L86-8275 in breast carcinoma cells. Biochem Pharmacol 46:1831–1840
9. Losiewicz MD, Carlson BA, Kaur G, Sausville E, Worland PJ (1994) Potent Inhibition of CDC2 kinase activity by the flavonoid L86-8275. Biochem Biophys Res Commun 201:589–595
10. Carlson BA, Dubay MM, Sausville EA, Brizuela L, Worland PJ (1996) Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. Cancer Res 56:2973–2978

11. Filgueira de Azevedo W, Mueller-Dieckmann HJ, Schulze-Gahmen U, et al (1996) Structural basis for specificity and potency of a flavonoid inhibitor of human CDK2, a cell cycle kinase. Proc Natl Acad Sci U S A 93:2735–2740
12. Hagiwara M, Inoue S, Tanaka T, Nunoki K, Ito M, Hidaka H (1988) Differential effects of flavonoids as inhibitors of tyrosine protein kinases and serine/threonine protein kinases. Biochem Pharmacol 37:2987–2992
13. Drees M, Dengler WA, Roth T, Labonte H, Mayo J, Malspeis L, Grever M, Sausville EA, Fiebig HH (1997) Flavopiridol (L86-8275): selective antitumor activity in vitro and activity in vivo for prostate carcinoma cells. Clin Cancer Res 3:273–279
14. Bible KC, Kaufman SH (1997) Cytotoxic synergy between flavopiridol (NSC 649890, L86-8275) and various antineoplastic agents – the importance of sequence of administration. Cancer Res 57:3375–3380
15. Sedlacek H, Czech J, Naik R, Worland P, Losiewicz M, Parker B, Carlson B, Smith A, Senderowicz AM, Sausville EA (1996) Flavopiridol (L86-8275; NSC 649890) a new kinase inhibitor for tumor therapy. Int J Oncol 9:1143–1168
16. Wang-Weigun A, Vincent DL, Early RJ, Weems CW (1994) The quantification of phytoestrogens in fresh plant materials by reversed-phase HPLC. Acta Botanica Yunnanica 16:424–430
17. Stinson SF, Hill K, Silford TJ, Phillips LR, Daw TW (1998) Determination of flavopiridol (L86 8275; NSC 649890) in human plasma by reversed-phase liquid chromatography with electrochemical detection. Cancer Chemother Pharmacol 42:261–265
18. Senderowicz AM, Headlee D, Stinson SF, Lush RM, Kalil N, Villaba L, Hill K, Steinberg SM, Figg WD, Tompkins A, Arbuck SG, 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
19. Kelland LR (2000) Flavopiridol, the first cyclin-dependent kinase inhibitor to enter the clinic: current status. Expert Opin Investig Drugs 9:2903–2911
20. Kahn ME, Senderowicz A, Sausville EA, Barrett KE (2001) Possible mechanisms of diarrheal side effects associated with the use of a novel chemotherapeutic agent, flavopiridol. Clin Cancer Res 7:343–349
21. Innocenti F, Stadler WM, Iyer L, Ramirez J, Vokes EE, Ratain MJ (2000) Flavopiridol metabolism in cancer patients is associated with the occurrence of diarrhea. Clin Cancer Res 6:3400–3405
22. Schwartz GK, Ilson D, Saltz L, O'Reilly E, Tong W, Maslak P, Werner J, Perkins P, Stoltz M, 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