

A Phase II Trial of the Cyclin-dependent Kinase Inhibitor Flavopiridol in Patients with Previously Untreated Stage IV Non-Small Cell Lung Cancer¹

Geoffrey I. Shapiro,² Jeffrey G. Supko, Angus Patterson, Cathy Lynch, Joan Lucca, Patrick F. Zaccarola, Alona Muzikansky, John J. Wright, Thomas J. Lynch, Jr., and Barrett J. Rollins

Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115 [G. I. S., C. L., J. L., P. F. Z., B. J. R.]; Massachusetts General Hospital Cancer Center, Boston, Massachusetts 02114 [J. G. S., A. P., A. M., T. J. L.]; and Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Maryland 20892 [J. J. W.]

ABSTRACT

Purpose: Flavopiridol is a potent cyclin-dependent kinase inhibitor with preclinical activity against non-small cell lung cancer (NSCLC), inhibiting tumor growth *in vitro* and *in vivo* by cytostatic and cytotoxic mechanisms. A Phase II trial was conducted to determine the activity and toxicity of flavopiridol in untreated patients with metastatic NSCLC.

Experimental Design: A total of 20 patients were treated with a 72-h continuous infusion of flavopiridol every 14 days at a dose of 50 mg/m²/day and a concentration of 0.1–0.2 mg/ml. Dose escalation to 60 mg/m²/day was permitted if no significant toxicity occurred. Response was initially assessed after every two infusions; patients treated longer than 8 weeks were then assessed after every four infusions. Plasma levels of flavopiridol were measured daily during the first two infusions to determine steady-state concentrations.

Results: This study was designed to evaluate a total of 45 patients in two stages. However, because no objective responses were seen in the first 20 patients, the early-stopping rule was invoked, and patient accrual was halted. In four patients who received eight infusions, progression was documented at 15, 20, 40, and 65 weeks, respectively. The most common toxicities included grade 1 or 2 diarrhea in 11 patients, asthenia in 10 patients, and venous thromboses in 7

patients. The mean \pm SD steady-state concentration of drug during the first infusion was 200 \pm 89.9 nM, sufficient for cytostatic effects in *in vitro* models.

Conclusions: At the current doses and schedule, flavopiridol does not have cytotoxic activity in NSCLC, although protracted periods of disease stability were observed with an acceptable degree of toxicity.

INTRODUCTION

Despite modest improvements in the treatment of NSCLC³ afforded by the taxanes, vinorelbine, gemcitabine, and irinotecan (1, 2), the outcome for metastatic disease remains uniformly fatal. Continued progress will require the discovery of additional agents aimed at novel targets. Cyclin-dependent kinases (Cdks) comprise a promising set of such targets. These enzymes are core components of the cell cycle machinery that govern the transition between phases during cell cycle progression (3). Deregulated cdk activity is a hallmark of malignancy.

Cdks are activated in part by their association with cyclins. NSCLCs frequently overexpress cyclin D1 (4–7), leading to increased activity of cdk4 and cdk6 and loss of growth control. In addition, two groups of inhibitors, known as Cip/Kip and INK4 proteins, also regulate cdk activity. Diminished or absent expression of cdk inhibitors is common in human cancer. For example, in NSCLC, the gene encoding p16^{INK4A} is frequently inactivated by deletion, mutation, or promoter hypermethylation (8, 9). Expression of p21^{Waf1/Cip1}, transcriptionally regulated by a p53-dependent pathway (10), is often decreased in tumors such as NSCLC that harbor p53 mutations. p27^{Kip1}, controlled at the level of ubiquitin-mediated proteolysis, is frequently present only at low levels in NSCLC cells, and reduced expression correlates with poor survival (11).

Ectopic expression of cdk inhibitors in tumor cells often results in cytostatic cell cycle arrest and inhibition of tumor growth, which has translated into therapeutic benefit in animal models with decreased tumor volume, slowed tumor growth, and improved host survival (12, 13). In addition, adenoviruses encoding p27^{Kip1} or p16^{INK4A} have been shown to induce apoptotic cell death *in vitro* (14–18) and tumor regression *in vivo* (19). These observations have prompted great interest in the development of pharmacological cdk inhibitors that could produce similar antitumor effects.

Flavopiridol (L86-8275, HMR 1275) is the first potent cdk inhibitor to enter clinical trials. The drug is an analogue of a

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² To whom requests for reprints should be addressed, at Dana-Farber Cancer Institute, Mayer 422B, 44 Binney Street, Boston, MA 02115. Phone: (617) 632-4942; Fax: (617) 632-5998; E-mail: geoffrey_shapiro@dfci.harvard.edu.

³ The abbreviations used are: NSCLC, non-small cell lung cancer; cdk, cyclin-dependent kinase; TFA, trifluoroacetic acid; NCI, National Cancer Institute; CT, computed tomography; CR, complete response; PR, partial response; DVT, deep vein thrombosis; Rb, retinoblastoma.

naturally occurring flavonoid isolated from the stem bark of *Dysoxylum binectariferum*, a plant native to India. Flavopiridol has been shown to inhibit the kinase activity of multiple cdk's from exponentially growing cells with IC_{50} 's of ~ 100 – 400 nM (20). Structure-activity studies have demonstrated that the aromatic substituent of deschloro-flavopiridol interferes with the binding of ATP to the adenine-binding pocket of cdk2 (21). Whereas other kinases are also inhibited, including protein kinase C, protein kinase A, and epidermal growth factor receptor, the IC_{50} 's are $10 \mu\text{M}$ or greater, suggesting that flavopiridol is relatively specific for cdk's at the nanomolar concentrations achieved *in vivo*. In addition to its direct effects on cdk activity, flavopiridol can prevent the phosphorylation events necessary for activating cdk's (20) and has been shown recently to repress transcription of the *cyclin D1* gene (22).

Preclinical data, both *in vitro* and *in vivo*, have demonstrated antiproliferative activity of flavopiridol against NSCLC. In cell lines, flavopiridol causes arrest at both the G_1 and G_2 phases of the cell cycle (23, 24) consistent with its inhibition of cdk2, cdk4, and cdk1 (25, 26). This occurs within 24 h of drug exposure and requires concentrations of at least 150–200 nM. In addition to these cytostatic effects, cytotoxicity has been observed at concentrations of ~ 300 nM, although it occurs most efficiently at concentrations of 500 nM and above (23, 24). Cell death usually follows or accompanies cell cycle arrest, is maximally observed at 72 h after the initiation of treatment, and is the result of p53-independent apoptosis (24). Flavopiridol also inhibits the growth of lung cancer xenografts in nude mice bearing tumors transplanted into the subrenal capsule or subcutaneously (20).

Although inhibition of cdk's clearly plays a role in cell cycle arrest mediated by flavopiridol, the relative contribution of cdk inhibition to the cytotoxicity of the drug is not yet clarified. In this regard, flavopiridol has also been found to be cytotoxic to noncycling cells (23, 27). In addition, it has been shown recently that flavopiridol can bind to DNA *in vitro*, suggesting the possibility of another cellular target (28). Furthermore, flavopiridol has antiangiogenic and antimetastatic properties that may contribute to its cytotoxic activity, including induction of apoptosis in human umbilical vein endothelial cells (27), inhibition of blood vessel formation in a mouse Matrigel model of angiogenesis (29), the suppression of hypoxic induction of vascular endothelial growth factor expression in monocytes (30), and inhibition of secretion of matrix metalloproteinases (31). Finally, flavopiridol has been found recently to inhibit glycogen phosphorylase and may starve cancer cells of glycolytic intermediates (32).

In xenograft models, the most pronounced antitumor effects were seen after prolonged exposure to flavopiridol, prompting the evaluation of a 72-h i.v. continuous infusion every 2 weeks in Phase I trials (33, 34). The dose-limiting toxicity was secretory diarrhea, and the maximum tolerated dose was $50 \text{ mg/m}^2/\text{day} \times 3$. In the presence of antidiarrheal prophylaxis, including loperamide and cholestyramine, hypotension was the dose-limiting toxicity with a maximum tolerated dose of $78 \text{ mg/m}^2/\text{day}$. However, with efforts to dose escalate, a proinflammatory syndrome characterized by flu-like symptoms, alterations in acute-phase reactants, and local tumor pain limited the ability to administer chronic therapy, resulting in the recommended Phase II dose of $50 \text{ mg/m}^2/\text{day}$ (33). Other less

common toxicities included pleural and pericardial effusions, noted in patients with extensive lung metastases or prior mediastinal radiation, as well as transient hyperbilirubinemia. Importantly, major toxicities observed with conventional cytotoxic agents, including myelosuppression, mucositis, cardiac toxicity, renal toxicity, or neurotoxicity, were not observed.

In Phase I trials, a CR was achieved in a patient with gastric cancer, and PRs occurred in patients with renal cell carcinoma. In addition, minor responses were reported in patients with non-Hodgkin's lymphoma and colorectal cancer. In the trial conducted at the NCI, 10 of 76 patients achieved protracted disease stability (≥ 6 months), most with renal or prostate carcinoma. At the dose of $50 \text{ mg/m}^2/\text{day}$ for 3 days, the mean steady-state plasma concentration (C_{ss}) was 271 nmol/liter, within the range required for inhibition of cdk activity and NSCLC cell proliferation *in vitro* (33). The compelling mechanism of action, as well as the promising preclinical activity and favorable toxicity profile, led to our initiation of a Phase II study to determine the overall response rate of flavopiridol in patients with metastatic NSCLC who had not yet received previous chemotherapy.

PATIENTS AND METHODS

Eligibility

All patients were required to have measurable, pathologically confirmed NSCLC. Patients had to be ambulatory with an Eastern Cooperative Oncology Group performance status of 0–2. Adequate bone marrow reserve was required, including an absolute neutrophil count $\geq 2,000$ cells/ μl and platelet count $\geq 100,000$ cells/ μl . Adequate hepatic and renal function were also required, defined as serum bilirubin ≤ 1.5 mg/dl, aspartate aminotransferase ≤ 2 times the upper limit of normal, and a serum creatinine ≤ 1.5 mg/dl or a 24-h creatinine clearance ≥ 60 ml/min.

Only patients with stage IV disease who had not received chemotherapy previously were enrolled. Patients with brain metastases were ineligible. Patients with large pleural effusions were only enrolled once the fluid was drained, preferably with pleurodesis. Prior treatment with radiation was permitted if measurable disease was present outside of the radiation port and at least 2 weeks had elapsed since its conclusion. All patients were required to have an indwelling central venous catheter. Written informed consent in accordance with federal guidelines and approved by the Dana-Farber/Partners Cancer Care Institutional Review Board was required before therapy could be administered.

Treatment

Flavopiridol was administered via a battery-powered CADD-1 ambulatory infusion pump (SIMS Deltec, Inc., St. Paul, MN) connected to a Port-a-cath as a 72-h continuous infusion at a dose of $50 \text{ mg/m}^2/\text{day}$ and a concentration of 0.1–0.2 mg/ml. The flavopiridol supply was changed daily. Cycles were administered every 14 days until disease progression occurred. Patients who received at least two 72-h infusions without developing grade 2 toxicities (defined by the NCI common toxicity criteria) were dose escalated to $60 \text{ mg/m}^2/\text{day}$. Patients who developed diarrhea were treated with loperamide

(4 mg after the first watery bowel movement, followed by 2 mg every 2 h until there was no diarrhea for 12 h). A dose reduction to 40 mg/m²/day was required if grade 3 diarrhea occurred despite loperamide treatment and considered in most cases when other nonhematological grade 3 toxicities developed or if a decline in performance status occurred. The exception was occurrence of DVT, managed by interruption of the infusion, initiation of appropriate anticoagulation, and subsequent continuation on schedule without dose reduction. If treatment was well tolerated at the reduced dose, re-escalation was permitted at the discretion of the treating physician.

Follow-Up

The end points of this study were response rate and toxicity. Complete blood counts, including platelet count, and serum chemistries were obtained at the initiation of each flavopiridol infusion. During the cycle, potassium and magnesium were repeated at the discretion of the treating physician if diarrhea occurred and were replenished as needed. Orthostatic vital signs were checked each day of the infusion during the first two cycles, as well as each day during the first two dose-escalated cycles, and were discontinued if no significant hemodynamic alterations occurred. Initially, measurable disease was reevaluated by CT scan after every two infusions (1-month intervals); if stable or responding disease was present after four infusions, CT scans were subsequently performed after every additional four infusions (2-month intervals). All patients who received flavopiridol were considered evaluable for toxicity, but only patients completing at least two infusions were considered evaluable for response. A CR was defined as the disappearance of all measurable disease for 4 weeks. A PR was defined as $\geq 50\%$ decrease from baseline in the sum of the products of the maximum perpendicular diameters for indicator lesions, with no progressive disease observed for at least 4 weeks. Stable disease was defined as the absence of CR, PR, or disease progression for at least 8 weeks from study entry. Progression was defined as a $>25\%$ increase in the sum of the products of the maximum perpendicular diameters for indicator lesions or the appearance of new lesions.

Pharmacokinetics

Sample Collection. During the first two cycles of therapy, blood specimens were drawn in heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) before treatment, 15 min before the daily infusion bags were replaced at 24 and 48 h, and 15 min before the end of the 72-h infusion. After centrifugation ($2500 \times g$ for 10 min at 4°C), plasma was separated from blood cells and stored in polypropylene tubes at -70°C until assayed.

Analytical Solutions. An analytical reference sample of flavopiridol hydrochloride (National Service Center 649890) was provided by the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, NCI (Bethesda, MD). A stock solution of the drug in DMSO was diluted with acetonitrile to create a working solution. The internal standard used in the assay, bis(*O*-methyl) flavopiridol, was prepared by the microscale derivatization of flavopiridol with excess methyl iodide and anhy-

drous potassium carbonate in acetone with overnight refluxing and purified by semipreparative reversed-phase high-pressure liquid chromatography, followed by solid phase extraction before dilution in acetonitrile. The working solution and internal standard were highly stable when stored at 0–5°C. A series of six standard solutions were made daily by serially diluting the flavopiridol working solution with human donor plasma.

Sample Preparation. After centrifugation ($10,000 \times g$ for 10 min) of thawed study samples, an aliquot of plasma (250 μl), supplemented with 2 μl of the internal standard, was directly applied onto a 3-ml (60-mg) Oasis HLB solid-phase extraction cartridge (Waters Corp., Milford, MA) that had been conditioned sequentially with 2 ml each of methanol, water, and 10 mM TFA (pH 2.0). The cartridge was washed with 2 ml of 10 mM TFA and 2 ml of water, after which the compounds of interest were eluted with 3 ml of methanol. The extract was evaporated to dryness under a stream of nitrogen at 40–45°C and reconstituted in 300 μl of acetonitrile:10 mM TFA (1:2, v/v).

Liquid Chromatography-Electrospray-Mass Spectrometry Analysis. Analyses were performed using an HP 1100 Series LC/mass selective detector system (Hewlett-Packard, Palo Alto, CA). The reconstituted sample extracts (250 μl) were loaded onto a 15 cm \times 3.9-mm inside diameter Nova-Pak phenyl high-pressure liquid chromatography column (Waters Corp.), preceded by a Brownlee NewGuard phenyl guard cartridge (Perkin-Elmer, Norwalk, CT). Chromatography was performed at ambient temperature using an isocratic mobile phase composed of acetonitrile:10 mM TFA (37:63, v/v), delivered at 1.0 ml/min. Flow from the analytical column was directed into the nebulizer-assisted atmospheric pressure ionization-electrospray interface of the mass selective detector without splitting. The protonated molecular ions of flavopiridol and the internal standard at m/z 402.1 and 430.1, respectively, were measured by selective ion monitoring using a mass width of 0.7 u, a dwell time of 199 ms, and a fragmentor voltage of 130 V. Chromatograms were integrated to provide peak areas using the data analysis functions of the HP ChemStation software. Typical retention times were 5.3 min for the drug and 8.3 min for the internal standard.

Quantitation. All study samples were assayed together with a series of calibration standards on a daily basis. Standard curves were constructed by plotting the flavopiridol to internal standard chromatographic peak area ratio against the known concentration of drug. Linear least squares regression was performed using a weighting factor of $1/y$. Values of the slope and y -intercept of the best-fit line were used to calculate the drug concentration in study samples. Specimens with concentrations exceeding the upper range of the standard curve were reassayed upon dilution with drug-free human plasma. Chromatograms of donor plasma and study specimens acquired from patients before, during, and after treatment with flavopiridol showed no peaks, either of endogenous origin or attributable to a concurrently administered medication, that interfered with the detection of the drug or internal standard. The standard curves exhibited excellent linearity, with correlation coefficients that were 0.991 on average, and the y -intercept values did not differ significantly from the origin. Accuracy and precision of the assay were assessed from the results of 12 standard curves of flavopiridol in plasma run during a 4-week period. The mean

difference between the measured and actual concentration of drug in the six standard solutions ranged from 2.4 to 17.0%. The coefficient of variation for the mean determinations was 8.8% at the highest concentration (249.8 nM) and 18.5% at the lowest concentration (6.16 nM) of flavopiridol.

Statistical Methods

Pharmacokinetic Data Analysis. The C_{ss} of flavopiridol for each patient was calculated as the geometric mean of the daily drug levels determined during the infusion (35). Values were not reported in cases where only a single sample was acquired from a patient during the infusion. SDs for geometric mean values were estimated by the jackknife method (36). The mean C_{ss} for the entire group of patients that received two cycles of therapy at the same dose was compared after logarithmic transformation of the individual values using the paired two-sample t test (35, 37). The highest C_{ss} achieved was compared in patients who were stable and who had progressed at 8 weeks using a two-sample t test.

Study Design. A two-stage design was used to allow the possibility of early stopping for lack of efficacy (38). A total of 20 patients were to be accrued in the first stage. At least three objective responses (CR + PR) were required in the first 20 cases to continue accrual to a final target of 45 patients. The regimen would be considered worthy of further study if 7 or more of 45 patients (>15%) experienced an objective response. This design yields a $\geq 88\%$ probability of rejecting the regimen if the true response rate is $\leq 10\%$, so that the probability of a type 1 error is $< 12\%$. It also yields a $\geq 90\%$ probability of accepting the regimen if the true response rate is at least 25%, so that the probability of a type 2 error is $< 10\%$. In addition, it gives a $\geq 68\%$ probability of early termination if the flavopiridol response rate in this population is $\leq 10\%$. Survival probabilities were estimated by the method of Kaplan and Meier (39).

RESULTS

Patient Characteristics and Treatment. From March to December 1998, 21 patients with previously untreated NSCLC were enrolled in the study. One patient suffered a cerebral vascular accident and a decline in performance status before the initiation of treatment and was cancelled because eligibility criteria were no longer met, resulting in a group of 20 patients who received flavopiridol (Table 1). Half of the patients treated were women, and their median age was 58.5 years. Tumors were predominantly of nonsquamous cell histology.

Flavopiridol was administered as a 72-h continuous infusion every 14 days. The median number of infusions administered was four (range, <1–31). A total of 119 infusions were given. A total of 11 patients received scheduled therapy for 8 weeks (4 infusions), and 4 patients received therapy for 15 weeks or more (8+ infusions), confirming the feasibility of this schedule.

Toxicity. Overall, flavopiridol was well tolerated. In all cases, removal from the protocol occurred because of disease progression and not drug-related toxicity. Toxicities are listed in Table 2. Consistent with previous experience, significant hematological, neurological, cardiac, or renal toxicities were not observed. Mild diarrhea was the most common side effect,

Table 1 Patient characteristics (n = 20)

	No.	%
Sex		
Male	10	50
Female	10	50
Race		
White	17	85
African-American	2	10
Asian	1	5
Age (yr)		
Median	58.5	
Range	47–78	
Performance status		
0	8	40
1	9	45
2	3	15
Histology		
Adenocarcinoma	11	55
Large cell	3	15
Bronchioloalveolar carcinoma	1	5
Squamous	1	5
Poorly differentiated or unspecified	4	20
Metastatic disease sites ^a		
Liver	5	25
Bone	9	45
Adrenal	1	5
Lung/Pleura	14	70

^a All patients enrolled had stage IV disease. Patients with central nervous system metastases were ineligible. Patients with large pleural effusions were not enrolled until the fluid was drained \pm pleurodesis.

occurring in 11 patients. Five patients experienced only grade 1 toxicity, whereas six patients also experienced at least one grade 2 episode. Although grade 1 diarrhea occurred during 51 of 119 (43%) infusions, grade 2 diarrhea only complicated 14 of 119 (12%) infusions, and grade 3 diarrhea was not observed. In two patients, grade 2 diarrhea only occurred when the dose was escalated to 60 mg/m²/day. Diarrhea was most commonly documented during the 72-h infusion and in the first two days after the completion of flavopiridol. Loperamide treatment was almost always successful, and additional cholestyramine was only required on two occasions.

Half of the patients treated with flavopiridol experienced fatigue. Mild to moderate symptoms occurred in seven patients and were not accompanied by the proinflammatory syndrome described in Phase I trials. Symptoms universally improved with a 4-mg dose of dexamethasone administered each day during the 72-h infusion and usually did not persist during the recovery period of the cycle. Three patients had severe asthenia and fatigue. One patient improved significantly with dexamethasone, whereas the other two patients required dose reduction and concomitant steroids. However, in no case did fatigue cause discontinuation of protocol treatment.

Thrombotic events occurred in seven patients (35%) during the course of this study. Two of the four Port-a-cath clots developed within 24 h after completion of the first flavopiridol infusion. Lower extremity DVT occurred during either the first or second infusion in two patients. In one of these patients, clot was documented in the left soleal vein; she had previously undergone pinning of her left hip, followed by radiation therapy that was complicated by chronic left lower extremity edema.

Table 2 Toxicities (n = 20)

Toxicity	No.	%
Diarrhea		
Grade 1 (increase of 1–3 stools/day over pretreatment)	5	25
Grade 2 (increase of 4–6 stools/day over pretreatment)	6	30
Grade 3 (increase of ≥ 7 stools/day over pretreatment)	0	0
Fatigue/Asthenia		
Grade 1 (mild)	2	10
Grade 2 (moderate)	5	25
Grade 3 (severe)	3	15
Thrombotic events		
Port-a-cath clot	4	20
DVT (lower extremity)	2	10
Pulmonary embolus (intermediate probability)	1	5
Tumor pain		
Grade 2	1	5
Orthostatic hypotension		
Grade 2	1	5
Nausea		
Grade 1	10	50
Grade 2	3	15
Grade 3	1	5
Fever		
Grade 2	2	10
Increased pleural effusion (not clearly related to disease)	1	5
Headache		
Grade 3	1	5

Flavopiridol was interrupted, anticoagulation was initiated, and subsequent cycles were administered on schedule. One patient receiving flavopiridol at reduced dose developed pain across the chest wall and dyspnea in the middle of the third infusion. V/Q scan was read as intermediate probability, demonstrating patchy ventilation and the absence of perfusion in the right lower lobe, although this was an area where the tumor had been radiated previously. He was anticoagulated and continued on therapy.

Several other notable toxicities occurred less frequently. Nausea was severe in only one patient. One patient experienced chest wall pain in close proximity to a dominant tumor mass, temporally related to flavopiridol infusions, and improved with narcotics. Increases in pleural effusions were related to disease progression in most, but not all cases. One patient underwent chest tube drainage and sclerosis of a right pleural effusion before therapy. After two cycles, he developed loculated collections of fluid along the right lateral chest wall and in the right major fissure, as well as a small left pleural effusion, despite stability to slight improvement of measurable disease. After an additional two cycles, there was almost complete resolution of the fluid collections. Another patient developed a mild headache shortly after her fourth infusion. This escalated to grade 3 toxicity during the last 24 h of her fifth infusion. Both head CT and magnetic resonance imaging were negative for evidence of metastases, and she responded to narcotic analgesics, which allowed continuation of treatment without dose reduction.

Two patients developed grade 3 dyspnea during the first cycle, prompting interruption of the flavopiridol infusion and hospital admission. One patient experienced significant bron-

Table 3 Response (n = 20)

	No.	%
PR	0	0
SD (weeks)		
8	10	50
12	6	30
15	4 ^a	20
40	2	10
65	1	5
PD ^b (8 weeks)	8	40
Not evaluable ^c	2	10

^a Minor responses noted in three patients, stable for 20, 40, and 65 weeks, respectively.

^b PD, progressive disease.

^c Patients received <1 full 72-h infusion.

chospasm, and the other became hypoxic. In both cases, progressive disease was documented, involving hilar lesions causing bronchial obliteration and postobstructive collapse. Both patients received radiation therapy without significant response, and both died within 2 weeks of the initiation of flavopiridol.

Dose Modifications. Four patients received cycles at the reduced dose of 40 mg/m²/day. In one patient, grade 3 bone pain at a site of known metastases prompted dose reduction, although this was later attributed to a hairline fracture at the sacroiliac joint not first appreciated on radiographic studies; pain was ultimately attributed to tumor progression, rather than flavopiridol, and therapy was discontinued. In a second patient, the dose reduction was instituted because of a combination of grade 2 fever, diarrhea with grade 2 dehydration (*i.e.*, requiring i.v. hydration), as well as DVT. However, he was subsequently re-escalated to the starting dose without incident. Interestingly, this patient had completed radiation therapy to the lumbosacral spine 2 weeks before the initiation of flavopiridol, perhaps predisposing to more severe toxicity to the bowel mucosa. In a third patient, grade 3 fatigue as well as grade 2 diarrhea persisting beyond the 72-h infusion were compromising her ability to work, and cycles 8–12 were administered at 40 mg/m²/day. Subsequently, she received the starting dose of 50 mg/m²/day, which was well tolerated over cycles 13–31. The fourth patient only received 48 h of the first infusion because of grade 3 fatigue, grade 3 nausea, and grade 2 diarrhea, which continued through loperamide and cholestyramine. Seven subsequent cycles at the reduced dose were better tolerated, along with loperamide and dexamethasone, before disease progression.

In six patients, the dose was escalated to 60 mg/m²/day. In four of these patients, this occurred within the two cycles before documentation of disease progression so that prolonged observation at this dose was not possible. In one patient, who received a total of 19 infusions, the escalated dose was only tolerated for three infusions before a combination of grade 2 diarrhea and fatigue began to affect her performance status, prompting a reduction back to the original dose of 50 mg/m²/day. Another attempt to dose escalate later in the course was only possible for two infusions. The fifth patient developed grade 2 fever and severe orthostatic hypotension during his first cycle administered at 60 mg/m²/day. Symptoms improved with an interruption of the infusion, and i.v. hydration was required briefly. Later in his course, he tolerated re-escalation to 60 mg/m²/day

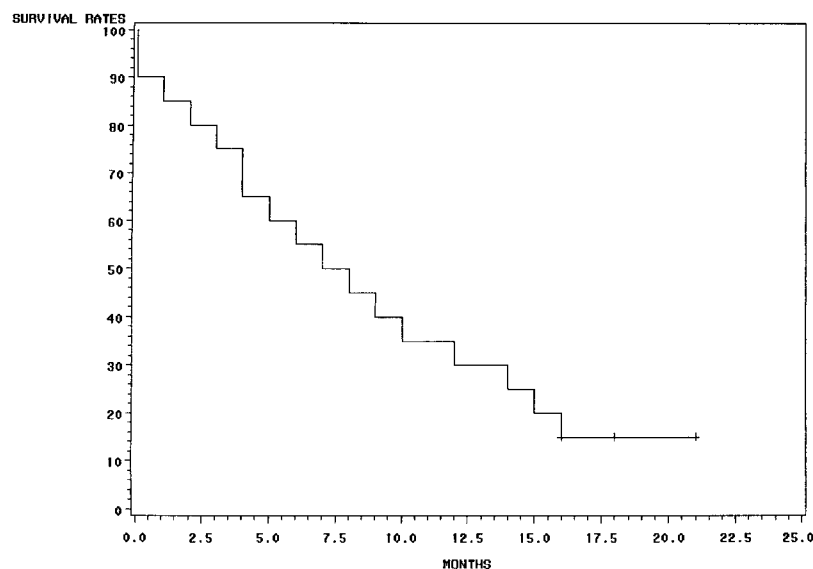


Fig. 1 Overall survival. Tick marks, censored patients.

and was able to tolerate six infusions of flavopiridol at the higher dose with only minimally increased toxicity, including one episode of grade 2 diarrhea.

Efficacy. No objective responses were observed. Minor responses of index lesions were noted in three patients, which were sustained for at least 4 weeks. In two of these patients, tumor regression of 19–43% of index lesions (including primary lung masses and mediastinal adenopathy) was noted over the first four infusions (8 weeks) of therapy, and they remained on treatment for 40 and 65 weeks, respectively. A third patient had a right hilar mass that decreased in area by 42% over the first two infusions. This response was durable over his course of 10 infusions; however, pleural-based progression ultimately necessitated his removal from the protocol. Of note, when the sum of the longest diameter for all target lesions is calculated for these three patients, they had reductions by 10, 16, and 20%, respectively, so that they would have been scored as stable disease by Response Evaluation Criteria in Solid Tumors criteria (40).

At 8 weeks, 18 patients were evaluable for response; 10 patients had stable disease, and 8 had progressed. Among the four patients who received eight infusions of therapy, progression was documented at 15, 20, 40, and 65 weeks (*i.e.*, ~ 4, 5, 10, and 16 months), respectively (Table 3). In several cases, stable disease did appear to represent a cessation of tumor growth; for example, the first of these patients, who initially did not wish to receive any systemic treatment, presented with eight liver metastases. When he returned <1 month later to rediscuss flavopiridol, the eight liver lesions had grown larger, and four new liver lesions were documented. Flavopiridol was initiated shortly after, and his disease remained stable for ~4 months. Median overall survival for the 20 patients who received treatment was 7.5 months (Fig. 1), and median time to progression was 3 months.

Pharmacokinetics. Plasma concentrations of flavopiridol were determined daily over the 3-day course of drug administration and used to estimate C_{ss} . A minimum of two samples

were obtained from 16 patients during the first infusion and 15 patients during the second infusion. During the first infusion, the C_{ss} ranged from 97.5 to 358.6 nM, with a mean (\pm SD) of 200.0 ± 89.9 nM. Similar data were obtained for the second infusion, during which the C_{ss} ranged from 83.7 to 432.2 nM with a mean (\pm SD) of 225.6 ± 124.4 nM. Among the 14 patients for whom determinations were made during both infusions, statistically significant differences in the C_{ss} values between the two infusions were not evident (Fig. 2). No clear correlation emerged between the C_{ss} and the incidence and severity of toxicities, including diarrhea and fatigue.

Importantly, only six patients achieved a C_{ss} exceeding 300 nM during either infusion, and no patient achieved a $C_{ss} > 500$ nM, consistent with the absence of major objective responses. However, the C_{ss} values during either infusion were frequently within the range of 150–300 nM, which is sufficient for cdk inhibition and growth arrest *in vitro*. Plasma concentrations of flavopiridol were determined in 9 of the 10 patients with stable disease at 8 weeks, including 2 with minor response. Among these nine patients, seven achieved a C_{ss} that was 1 SD or more above 150 nM during either the first or second infusion. In contrast, among the eight patients with disease progression at 8 weeks, only two achieved similar plasma concentrations (Fig. 3). Among the two groups of patients, there was a statistically significant difference in the highest C_{ss} of flavopiridol achieved (two-sample *t* test, $P = 0.042$). For three patients who had progressive disease at 8 weeks, only one C_{ss} value was available, from either the first or second infusion. Because there was no systematic omission of pharmacokinetic blood samples, they were included in the analysis.

DISCUSSION

We have conducted a Phase II study of the cdk inhibitor flavopiridol in patients with previously untreated stage IV NSCLC. Overall, flavopiridol was well tolerated at the dose of $50 \text{ mg/m}^2/\text{day} \times 3$ every 2 weeks, and it was possible to deliver

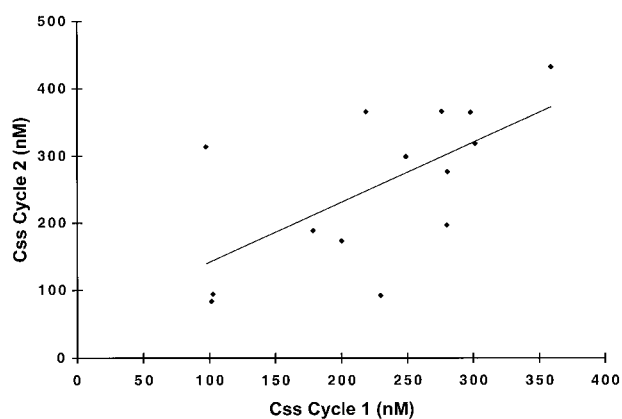


Fig. 2 Comparison of the C_{ss} of flavopiridol during the first two infusions among 14 patients. Among these patients, there were no statistically significant differences between the C_{ss} achieved during the two infusions, as determined by a paired two-sample t test ($P = 0.264$). Points, mean C_{ss} values observed in individual patients; —, generated by linear regression analysis of the data, for which the slope is 0.89 ($r = 0.63$).

the drug for protracted periods, despite the demanding treatment schedule. As reported in both Phase I data and the Phase II trial in metastatic renal cancer, there was no significant hematological, hepatic, renal, or neurotoxicity (33, 41). Prominent toxicities included diarrhea, fatigue/asthenia, and venous thromboses. Although diarrhea occurred in 11 patients, only 6 patients reported grade 2 episodes. Grade 3 diarrhea was not observed, so that the toxicity experienced by patients in our trial was milder than reported in other experiences with flavopiridol (33, 41–43). Interestingly, only 1 patient in our study had received prior radiation to the lumbosacral spine, a port that may have predisposed to subsequent treatment-related bowel toxicity. In contrast, prior radiotherapy was more common among patients in published trials, many of whom had prostate or renal cell cancer (33, 41). In addition, the incidence of diarrhea may be related to the metabolism of flavopiridol, as a recent study suggests that patients who develop diarrhea have lower rates of glucuronidation, which may allow for greater luminal exposure (44).

Fatigue and asthenia have been commonly reported with flavopiridol. In our study, this was ameliorated with a daily dose of dexamethasone each day during the infusion. Symptoms were only severe in 3 patients, and no patient in this study discontinued therapy because of fatigue. In patients who received flavopiridol over protracted periods, episodes of fatigue were erratic and did not occur during every cycle. The use of dexamethasone had no apparent effect on flavopiridol pharmacokinetics; therefore, improvement in fatigue was not associated with reduced drug levels.

Although other studies have also reported flavopiridol-related asthenia, it is important to note that this symptom occurs commonly in the lung cancer population. For example, in a recently randomized trial including two doses of docetaxel in patients treated previously, 47% of patients in the best supportive care arm experienced asthenia, rated at grade 3 or 4 in 28% of patients (45). Therefore, we cannot definitively conclude that the toxicity observed in our trial was entirely drug related.

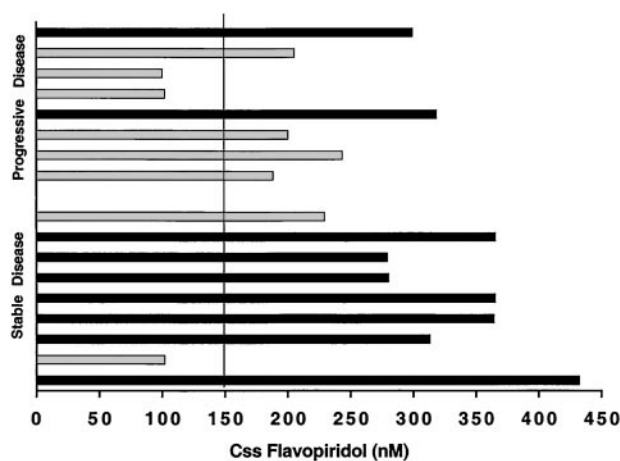


Fig. 3 Comparison of the highest C_{ss} of flavopiridol achieved over the first two infusions among nine patients with stable disease and eight patients with progressive disease at 8 weeks. Black bars, concentrations >1 SD above 150 nM. Gray bars, concentrations that are <1 SD above 150 nM.

Venous thromboses occurred in seven patients, and several events were temporally related to the first flavopiridol infusion. In addition, whereas the majority of these events occurred at the Port-a-cath site, lower extremity DVT and probable pulmonary embolism also occurred. Our results are similar to those reported in Phase II trials in patients with metastatic gastric or renal cell cancer. Five of 13 patients treated who had metastatic gastric cancer developed catheter-related thrombosis (42). Similarly, in the renal cancer trial, 6 patients developed DVTs; two events were catheter-related, two occurred in the lower extremities, and 2 patients suffered probable pulmonary emboli. In addition, in this trial, 3 patients experienced arterial vascular adverse events, including myocardial infarction, transient ischemic attack, and transient vision loss (41). However, the relationship of these events to flavopiridol was unclear.

The incidence of venous thromboses does not appear to be affected by the concentration at which flavopiridol is administered. Although a high concentration of 2.5 mg/ml was used in the gastric cancer trial, the renal cell cancer trial administered the drug at a concentration of 0.1–0.5 mg/ml, and in our trial, all patients received flavopiridol at a maximal concentration of 0.2 mg/ml. Despite the presumed higher volume and flow rate of infusion associated with the administration of lower drug concentrations, catheter-related thromboses were common.

Although patients with metastatic gastric, renal, or lung cancer have high incidences of DVT, the frequency of clotting events was higher than expected compared with other clinical trials in comparable patient cohorts (41, 46). Nonetheless, whether flavopiridol truly causes a clotting diathesis remains unclear. For example, in the Phase I trial, only five instances of catheter-related thrombosis occurred among 76 patients, and spontaneous thrombosis in the absence of foreign bodies was not observed, despite alterations in plasma fibrinogen levels (33). Similarly, in a preliminary report of the Phase II trial in patients with metastatic colorectal cancer, only 1 of 14 patients treated developed a catheter-related subclavian thrombosis (43).

Measurement of multiple clotting parameters in future trials may help assess the etiology of these events.

Despite encouraging preclinical data, flavopiridol does not have cytotoxic activity in NSCLC at the dose and schedule used in this trial. In NSCLC cell lines, cytotoxicity is only achieved at concentrations of at least 300 nM, and in many cell lines, concentrations ≥ 500 nM are required (23, 24). In addition, in a concentration range of 300–500 nM, extended exposure times are necessary to observe significant cell death (24). The mean C_{ss} of flavopiridol during the first infusion among 16 patients in this study was 200 ± 89.9 (SD) nM, similar to the 271 nM observed in the Phase I study at the same dose. However, over the first two infusions, 11 of the 17 patients for whom pharmacokinetic data were obtained had a $C_{ss} < 300$ nM, well below the concentrations required for cytotoxicity *in vitro*. Therefore, in this study, major objective responses were unlikely. Apoptosis within 24 h of exposure to low concentrations of flavopiridol has only been noted in a small number of cell types including hematopoietic (47), head and neck (48), and small cell lung cancer cell lines (49), which may be more appropriate tumor types for testing of the current dose and schedule. Alternative schedules may result in higher peak plasma concentrations that are potentially capable of producing cytotoxic effects. *In vitro*, at concentrations closer to 1 μ M, prolonged exposure times are not required to observe apoptosis, so that higher doses of shorter infusions may be more efficacious. A Phase I trial, in which flavopiridol is administered as a daily 1-h bolus for 3 or 5 consecutive days, has been completed recently. Pharmacokinetic studies demonstrated that micromolar peak flavopiridol concentrations were achieved (50, 51); therefore, it may be reasonable to examine this schedule in NSCLC.

At the C_{ss} values achieved in this study, flavopiridol is capable of inducing cell cycle arrest *in vitro*, a transient effect that is reversible after removal of drug. Therefore, it is possible that such an effect could translate into slowing of tumor growth *in vivo*. This trial was not designed to determine whether flavopiridol has cytostatic effects. Although 4, 5, 10, and 16 months of disease stability are provocative in stage IV NSCLC, it is possible that these patients' tumors had biological characteristics predicting a more indolent growth rate. Nonetheless, clear demonstration of a cytostatic effect will require Phase III trials, perhaps best evaluated after a cytotoxic response from standard chemotherapy.

Although concentrations of flavopiridol observed in this and other studies in which the drug was given as a continuous IV infusion should be capable of achieving cdk inhibition in cycling tumor cells, this has not yet been confirmed in clinical tumor specimens. The importance of this is underscored by the recent report that immobilized flavopiridol can bind to cytosolic aldehyde dehydrogenase 1 in NSCLC cell lines, which may reduce its ability to compete with cellular ATP for cdk binding and compromise its cytostatic and cytotoxic effects (52). Attempts to document cell cycle perturbations in peripheral blood mononuclear cells stimulated *in vitro* have been unsuccessful, largely because they are no longer exposed to flavopiridol during *ex vivo* growth (41). During the G_1 -S transition, the Rb-susceptibility gene product is an important target of cdk2, cdk4, and cdk6. Inhibition of these cdk6 should cause decreased phosphorylation of Rb at specific sites. The availability of

phospho-specific Rb antibodies may allow confirmation of cdk inhibition in selected biopsies in future studies. Similarly, determination of cyclin D1 levels by immunohistochemistry may also confirm the biological activity of flavopiridol in primary tumor specimens.

Our study contained a large percentage of patients with adenocarcinomas, and only 1 patient entered clearly had squamous cell histology. However, it is noteworthy that abnormalities in expression of cell cycle-regulatory proteins are similar among NSCLC histological subtypes. Although loss of expression of p16^{INKA} is somewhat more common in resected squamous cell carcinomas compared with adenocarcinomas (~60% versus 40%; Refs. 7, 53, and 54), genetic alterations are more frequent in advanced stage tumors, such that all histologies demonstrate significant rates of p16^{INKA} inactivation (55–58). In addition, no significant differences in p27^{Kip1} expression have been found between adenocarcinomas and squamous carcinomas (11). Furthermore, the preclinical data reveal similar effects of flavopiridol on adenocarcinoma and squamous cell carcinoma NSCLC cell lines (24). Therefore, there is little reason to expect that the outcome of this trial would have been different had a higher percentage of patients with squamous histology been enrolled.

Although flavopiridol appears to have limited activity when used alone, it would be premature to abandon its use in NSCLC. Even with this suboptimal dose and schedule, minor responses were observed. Although caution must be exercised in comparing this selected group of patients without central nervous system metastases to larger groups of patients with advanced disease, it is noteworthy that the median survival of 7.5 months achieved with flavopiridol is similar to the range of 7.4–8.2 months reported recently in a randomized trial of four chemotherapy regimens containing platinum analogues in combination with taxanes or gemcitabine (59). Furthermore, flavopiridol may be particularly useful in combination with standard chemotherapy agents because it can augment cell death mediated by several drugs used commonly in lung cancer treatment, including cisplatin, etoposide, and paclitaxel (60, 61). In general, effects are greatest when exposure to flavopiridol follows treatment with the other agents. In addition, synergism with established anticancer drugs appears to occur with shorter exposure to flavopiridol concentrations that are more easily achievable *in vivo* (49, 61). Although the precise mechanisms of these synergistic interactions have not been determined, it is possible that cdk inhibition after cell cycle disruption by standard chemotherapy drugs drives cells down apoptotic pathways (62). Flavopiridol may also affect the accumulation of standard chemotherapy drugs in tumor cells (63), in some cases by its interaction with multidrug resistance protein 1 (64). Alternatively, other effects of flavopiridol on angiogenic factors may add to the efficacy of existing cytotoxic agents. Combinations of flavopiridol with platinum analogues, paclitaxel (65), and gemcitabine are now being evaluated in Phase I studies and may help define a role for this cdk inhibitor in the treatment of solid tumors.

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