Phase I Dose-Finding Study of Weekly Docetaxel Followed by Flavopiridol for Patients with Advanced Solid Tumors

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Abstract

Purpose: Flavopiridol is a cyclin-dependent kinase inhibitor that enhances docetaxel-induced apoptosis in a sequence-specific manner. *In vivo*, docetaxel must precede flavopiridol by at least 4 h to induce this effect. We conducted a phase I trial of weekly, sequential docetaxel followed 4 h later by flavopiridol in patients with advanced solid tumors.

Experimental Design: Docetaxel at a fixed dose of 35 mg/m^2 was administered over 30 min, followed 4 h later by escalating doses of flavopiridol, ranging from 20 to 80 mg/m^2 in successive cohorts, administered weekly over 1 h. This schedule was repeated for 3 weeks of each 4-week cycle.

Results: Twenty-seven evaluable patients were enrolled. The combination was well tolerated, with one dose-limiting toxicity occurring at flavopiridol 70 mg/m^2 (grade 3 mucositis) and one dose-limiting toxicity at 80 mg/m^2 (grade 4 neutropenia). We observed 1 complete response in a patient with pancreatic carcinoma and 4 partial responses in pancreatic (1), breast (2), and ovarian (1) cancer patients. Stable disease was seen in 10 patients. Pharmacokinetic studies showed C_{max} ranging from 1.49 $\pm$ 0.69 μmol/L (flavopiridol 20 mg/m^2) to 4.54 $\pm$ 0.08 μmol/L (flavopiridol 60 mg/m^2) in cycle 1.

Conclusions: Treatment with weekly, sequential docetaxel followed by flavopiridol is an effective and safe regimen at all flavopiridol dose levels. The pharmacokinetic data indicate that concentrations of flavopiridol that enhance the effects of docetaxel both *in vitro* and *in vivo* can be achieved. Clinical activity is encouraging, even in patients who have received a prior taxane and in patients with gemcitabine-refractory metastatic pancreatic cancer.

Progression through the different phases of the cell cycle is driven by the coordinated activation of cyclin-dependent kinases (CDK): CDKs are essential enzymes for the cell cycle, and when activated, they allow the transit of the cell between the different phases of the cell cycle, whether it is G_1 to S or G_2 to M. Deregulated CDK activity is frequently present in malignant cells. Inhibition of CDKs can lead to cell cycle arrest and subsequent apoptosis (1, 2). This rationale led to the development of CDK inhibitors as novel antitumor agents. Flavopiridol is among the first potent CDK inhibitors to enter clinical trials. Flavopiridol is prepared by total synthesis and is identical to a compound obtained by derivation from a natural product obtained by *Dysoxylum binectariferum*, a plant indigenous to India (3). Flavopiridol inhibits CDKs including CDK1, CDK2, CDK4, and CDK6, and it inhibits tumor cell growth *in vitro* through blockade of cell cycle progression at the G_1-S or G_2-M interfaces (4–7).

Flavopiridol therefore causes cell cycle arrest, induces apoptosis, inhibits angiogenesis, and potentiates the effects of chemotherapy (5–7). Single-agent antitumor activity has been observed in a variety of preclinical models (8, 9). We have shown that flavopiridol enhances paclitaxel-mediated apoptosis of MKN-74 gastric cancer cells and MCF-7 breast cancer cells by activation of caspase-3 (10). For both docetaxel and paclitaxel, the enhancement of apoptosis by flavopiridol is highly sequence dependent such that the taxane must precede flavopiridol to induce this effect. Furthermore, *in vivo*, the effect is time dependent such that docetaxel must be administered at least 4 h before the flavopiridol (11). Flavopiridol has been evaluated in phase I clinical trials with chemotherapy with promising clinical activity (12, 13).

Based on these preclinical and clinical observations, we conducted a phase I trial of weekly, sequential docetaxel followed by flavopiridol in patients with advanced solid tumors such that docetaxel was administered 4 h before the flavopiridol. Because prolonged infusions of flavopiridol had been associated with considerable clinical toxicity, and because prior phase I experience with weekly flavopiridol in combination

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with irinotecan showed tolerability and encouraging activity, we chose a weekly 1-h infusion of flavopiridol. This schedule would also allow us to achieve higher peak flavopiridol levels (13).

The primary objective of this trial was to determine the maximum tolerated dose (MTD) of weekly flavopiridol when administered in combination with a fixed dose of weekly docetaxel to patients with advanced solid tumors. Secondary objectives were to investigate the clinical pharmacokinetics of weekly i.v. 1-h infusion of flavopiridol in combination with docetaxel, and to obtain preliminary data on the therapeutic activity of this regimen.

Patients and Methods

Eligibility. Male and female patients ≥18 years of age with a diagnosis of pathologically confirmed measurable or evaluable advanced solid tumor, with disease that was refractory to standard therapy or for which there was no standard therapy, were eligible. Patients needed to have a Karnofsky performance status ≥70%, total WBC count ≥3,500/mm^3, an absolute neutrophil count of at least 1,500/mm^3, a platelet count of at least 100,000/mm^3, hemoglobin ≥8 g/dL, and adequate liver, renal, and cardiac function. Patients could have received prior chemotherapy, immunotherapy, hormonal therapy, or radiotherapy for their disease, but 4 weeks from last dose had to elapse before study entry (6 weeks for nitrosoureas and mitomycin C). Patients who had received prior therapy with taxanes were eligible. Patients with symptomatic or untreated central nervous system metastasis or a primary central nervous system neoplasm were not eligible.

The protocol was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center, and all patients provided written informed consent.

Treatment plan. This was an open-label, nonrandomized dose escalation study. Groups of three to six patients were treated sequentially with a fixed dose of docetaxel at 35 mg/m^2 administered i.v. as a 30-min infusion on days 1, 8, and 15 of each 28-day cycle; for each cohort, flavopiridol was administered at increasing dosages i.v. over 1 h via a peristaltic infusion pump, 4 h after the completion of the docetaxel infusion on days 1, 8, and 15 of each 28-day cycle. All patients were premedicated with dexamethasone 4 mg orally twice daily × 3 doses, beginning at ~12 h before the time of the docetaxel infusion. If there was no hypersensitivity reaction after the first full cycle (3 doses of docetaxel), the investigator could change the premedication to i.v. or oral dexamethasone 10 mg, ~30 min before every docetaxel infusion in all subsequent cycles.

The dose escalation of flavopiridol is detailed in Table 1. We chose the starting dose of flavopiridol to be 20 mg/m^2 because this dose was proved in a prior experience (13) to be safe when administered weekly over 1 h with 100 mg/m^2 of irinotecan. Initially, the study defined the highest dose level of flavopiridol at 70 mg/m^2. Once the MTD of flavopiridol with docetaxel 35 mg/m^2 had been defined, the original plan was to reduce the dose of docetaxel to 25 mg/m^2 to allow for further flavopiridol dose escalation. However, the MTD was not reached at the dose level of 70 mg/m^2, and therefore, the study was later amended to add an additional cohort to allow increasing the flavopiridol dose to 80 mg/m^2. At the same time, the additional portion of the study that would have sought to define the MTD of flavopiridol when in combination with docetaxel at 25 mg/m^2 was removed.

Table 1. Dose escalation of flavopiridol

Cohort	Docetaxel (mg/m^2)	Flavopiridol (mg/m^2)	*N*
1	35	20	3
2	35	30	3
3	35	40	3
4	35	50	3
5	35	60	3
6	35	70	6
7	35	80	6

All treatments were administered in the outpatient setting, and once assigned to a dose level, dose escalation within a patient was not permitted.

Toxicity was graded in accordance with the Common Toxicity Criteria version 2.0.[7] Dose-limiting toxicity (DLT) was defined in cycle 1 as the occurrence of any of the following during the first cycle (4 weeks) of therapy: grade 4 hematologic toxicity, grade 4 nonhematologic toxicity, or grade 3 nonhematologic toxicity not controlled by medications. Patients who experienced a DLT, or toxicity attributed to study medication, could continue to receive study treatment after recovery with appropriate dose modifications as defined per protocol. Patients were allowed to proceed with treatment at the same dose in the absence of DLT if, on the day of the scheduled treatment, the absolute neutrophil count was 1,000/mm^3 and platelet count was 100,000/mm^3. If counts were below these levels, therapy was delayed until the blood counts recovered.

To be evaluable for response and to be assessable for determination of MTD, patients had to have received at least one full cycle of therapy. Otherwise, treatment responses were evaluated after every two cycles with computed tomography scans or other diagnostic tests, as appropriate. Response Evaluation Criteria in Solid Tumors were used for response assessment and done by an independent protocol radiologist (14). Complete or partial responses were confirmed by repeat studies 4 weeks after the criteria for response were first met.

Drug supply. Flavopiridol (also known as alvocidib, HMR 1275) and docetaxel were supplied by Sanofi Aventis Pharmaceuticals.

Statistical design. The main objective of this study was to determine the MTD of weekly flavopiridol when administered in combination with a fixed dose of weekly docetaxel. The incidence of hematologic and nonhematologic toxicities was summarized separately by cycle and by flavopiridol cohort. Secondary analyses included a pharmacokinetic analysis of flavopiridol. The maximum observed plasma concentration (C_{max}) and the area under the curve (AUC) were calculated by noncompartmental methods in WinNonlin Professional for each cycle (WinNonlin software, version 2.1, Scientific Consulting, Inc.).

Pharmacokinetics. For each patient on days 1 and 2 of week 1 of cycles 1 and 2, blood samples for pharmacokinetics were collected into heparinized coated tubes for flavopiridol and docetaxel at the following approximate times:

Docetaxel—immediately before drug administration (pretreatment) and 2 h after the start of the flavopiridol infusion (i.e., 2 samples per cycle; 4 samples total for the entire study).

Flavopiridol—immediately before drug administration (pretreatment) and at 60 min (end of infusion), 90 min, 2 h, and 24 h after the start of flavopiridol infusion (i.e., 5 samples for each cycle and 10 samples for the entire study).

Plasma concentrations of flavopiridol were measured by liquid chromatography-tandem mass spectrometry by Aventis Pharma. The lower limit of quantitation of the assay is 5.00 ng/mL. Assessments of plasma flavopiridol concentrations for pharmacokinetic studies were done according to published methods and prior reports (12). Plasma concentrations of docetaxel (RP56976) were done using a liquid chromatography-tandem mass spectrometry method by Aventis Pharma. The lower limit of quantitation is 5.00 ng/mL.

[7] Common Terminology Criteria for Adverse Events (CTCAE). Available at http://ctep.cancer.gov/forms/CTCAEv3.pdf.

Table 2. Patient characteristics

Characteristics	No. patients
Total	31
Evaluable for toxicity	30
Evaluable for response	27
Male	13
Female	18
Age (y)	
Median	56
Range	36-77
Karnofsky performance status (%)	
Mean	80
Range	70-90
Prior chemotherapy	28
Prior taxanes	11
No. prior regimens	
Median	3
Range	0-10
Primary sites of disease	
Pancreas	12
Breast	7
Esophagus	3
Colon	2
Melanoma	2
Lung	1
Ovarian	1
Sarcoma	1
Carcinoid	1
Stomach	1

Results

Patient characteristics. Table 2 lists the patient characteristics. From 11/19/2002 to 12/9/2004, 31 patients with advanced solid tumors were registered to the study. One patient was registered but never treated. Three patients are not evaluable for response: one received two weekly doses of docetaxel and flavopiridol, but was then found to be ineligible due to her prior cardiac history; one was found to have brain metastases after one dose of therapy; and another patient only received one dose of therapy and withdrew consent. Thus, 30 patients were evaluable for toxicity and 27 patients were assessable for MTD determination and evaluable for response.

The median age was 56 years (range, 36-77 years) and the median Karnofsky performance status was 90% (range, 70-90%). There were 13 men and 18 women. The cancers treated and patient numbers were pancreas (11), breast (7), esophagus (3), colon (2), melanoma (2), ovarian (1), sarcoma (1), stomach (1), lung (1), and carcinoid (1). Twenty-eight (93%) patients had received prior chemotherapy; 11 (37%) patients had received prior taxanes.

Hematologic toxicities. Table 3 lists the most common grade 2 to 4 hematologic toxicities for the first cycle of therapy. The first six cohorts of patients completed cycle 1 without a hematologic DLT; however, in cohort 7, with docetaxel at 35 mg/m^2 and flavopiridol at 80 mg/m^2, there was a DLT with an uncomplicated grade 4 neutropenia. Subsequently, three additional patients were enrolled to that cohort, with no further DLT observed.

Nonhematologic toxicities. Table 3 lists the most common grade 2 to 4 nonhematologic toxicities for the first cycle of therapy. As shown, the most common included grade 2 fatigue, diarrhea, vomiting, nausea, and mucositis. There was one nonhematologic DLT in cohort 6, with docetaxel 35 mg/m^2 and flavopiridol 70 mg/m^2: grade 3 mucositis. Subsequently, three additional patients were enrolled to that cohort, with no further DLT observed.

One patient developed pulmonary embolism (grade 4 thrombosis) concomitantly with frank progression of disease after one cycle of therapy; he was removed from the study. This event was not felt to be related to study drugs but rather to his rapidly progressing disease.

Table 3. Cycle 1 hematologic and nonhematologic toxicities

Cohort (patients)	D	F	Anemia			Leukocytes			ANC			Hyperglycemia			Alkalinephosphatase		
	mg/m^2		G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4
Cohort 1 (3)	35	20	2			1	1		1	1		2			1		
Cohort 2 (3)	35	30	2				1			1		1		1	2		
Cohort 3 (3)	35	40					**1**					1	2				
Cohort 4 (3)	35	50	2			1		1		1		3					
Cohort 5 (3)	35	60				1	1			1		1	1				
Cohort 6 (6)	35	70					1					1	1				
Cohort 7 (6)	35	80				2	1		1		**1**	1	2				

Cohort (patients)	D	F	Fatigue			Diarrhea			Vomiting			Mucositis			Dyspnea			Thrombosis			Nausea			ST		
	mg/m^2		G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4
Cohort 1 (3)	35	20	1						1												1					
Cohort 2 (3)	35	30	1			1																				
Cohort 3 (3)	35	40	1																						1	
Cohort 4 (3)	35	50	1			1			1						1						1					
Cohort 5 (3)	35	60				1			1												1					
Cohort 6 (6)	35	70	3				1		1	1			**1**							1	1	1				
Cohort 7 (6)	35	80	1			2			1			1														

NOTE: DLT is in boldface and underlined. Grade 2 to 4 toxicities for possibly, probably, or definitely attributable to therapy.
Abbreviations: D, docetaxel; F, flavopiridol; ANC, absolute neutrophil count; ST, sinus tachicardia.

Table 4. Cumulative nonhematologic toxicities

Cohort (patients)	D	F	Fatigue			Diarrhea			Vomiting			Mucositis			Edema			PPE			ST		
	mg/m²		G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4
Cohort 1 (3)	35	20		1					1						1								
Cohort 2 (3)	35	30	2			1												1					
Cohort 3 (3)	35	40	1																			1	
Cohort 4 (3)	35	50	2			2			1						1								
Cohort 5 (3)	35	60	1			1			1						1								
Cohort 6 (6)	35	70	3				1		1	1			**1**										
Cohort 7 (6)	35	80	1			3						1											

Cohort (patients)	D	F	Dyspnea			Lacrimation			Arthralgia			Thrombosis			Nausea			Paresthesia			FN
	mg/m²		G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G3
Cohort 1 (3)	35	20	1												1			1			
Cohort 2 (3)	35	30	1		1	1			1												
Cohort 3 (3)	35	40																			
Cohort 4 (3)	35	50	1										1		1						1
Cohort 5 (3)	35	60	1												1						
Cohort 6 (6)	35	70							1					1	1	1					
Cohort 7 (6)	35	80													1						

NOTE: Grade 2 to 4 toxicities for possibly, probably or definitely attributable to therapy.
Abbreviation: PPE, palmar-plantar erythrodysesthesia; FN, febrile neutropenia.

Table 4 lists the most common grade 2 to 4 cumulative toxicities. The pattern of toxicities was similar to that which was observed with only one cycle of combination therapy. The most common grade 3 toxicities included diarrhea (4%), fatigue (4%), and vomiting (4%). One (4%) patient developed grade 4 dyspnea.

Dose-limiting toxicities. As stated above, there were two DLTs. The first one was a grade 3 mucositis in cohort 6, at 70 mg/m^2 dose level of flavopiridol; the cohort was expanded to 6 patients, and no further DLTs were observed. The second DLT was a grade 4 neutropenia in cohort 7, at 80 mg/m^2 dose level of flavopiridol. No further DLTs were observed. The dose escalation was stopped at 80 mg/m^2 of flavopiridol because the pharmaceutical sponsor decided to discontinue supply and further investigation of the agent in solid tumors. Thus, MTD was not reached in this phase I experience.

Flavopiridol dose reductions. Between December 2004 and January 2005, patients 3, 4, 5, and 6 of cohort 7 received a reduced dose of flavopiridol at 50 mg/m^2 (rather than the due 80 mg/m^2) for two consecutive weeks (patients 3, 5, and 6) and three consecutive weeks (patient 4) because of a delay in drug supply by the pharmaceutical sponsor. This dose reduction was necessary to treat all patients on study with the drug, and it was approved by our Institutional Review Board. This dose reduction did not affect the determination of DLTs because all patients were beyond cycle 1 of protocol therapy. All patients resumed the regular dosing schedule of 80 mg/m^2 once the drug supply of flavopiridol was received.

Pharmacokinetics. Blood samples for pharmacokinetic analyses were obtained for 21 patients. Pharmacokinetic data were not collected for the six patients in cohort 7 (flavopiridol dose 80 mg/m^2) and were not included in the pharmacokinetic analysis. Table 5 summarizes two pharmacokinetic variables: maximum observed plasma concentration (C_{max}) and AUC across all subjects in a cohort. AUC is not reported for cycle 2 of cohort 2, 4, and 6 due to insufficient sample collection. In this study, for cycle 1, C_{max} for flavopiridol ranged from 1.49 $\pm$ 0.69 μmol/L (at flavopiridol dose 20 mg/m^2) up to a

Table 5. Flavopiridol pharmacokinetic parameters for cycle 1 and cycle 2 by dose level (flavopiridol in micromolar)

Cohort	Flavopiridol	Docetaxel	Cycle 1				Cycle 2			
	Dose (mg/m²)	Dose (mg/m²)	C_{max}	SD	AUC	SD	C_{max}	SD	AUC	SD
1	20	35	1.49	0.69	6.25	3.94	1.94	0.34	12.05	4.41
2	30	35	2.61	0.88	9.12	4.98	2.23	1.09	N.D.	1.34
3	40	35	2.40	0.82	13.54	8.70	2.13	0.91	14.91	3.78
4	50	35	2.45	1.58	15.83	8.49	2.64	0.37	N.D.	17.65
5	60	35	4.54	0.02	33.60	5.28	5.01	3.29	28.76	35.10
6	70	35	4.33	0.92	35.65	21.93	3.95	0.44	N.D.	0.73

Abbreviation: N.D., not determined.

maximum of 4.54 ± 0.08 μmol/L (at flavopiridol dose 60 mg/m^2). For cycle 2, C_{max} ranged from 1.94 ± 0.34 μmol/L (at flavopiridol dose 20 mg/m^2) up to a maximum of 5.01 ± 3.29 μmol/L (at flavopiridol dose 60 mg/m^2). For C_{max}, there was little observed difference in the patient-specific flavopiridol pharmacokinetic profiles for cycle 1 versus cycle 2. For cycle 1, the average concentration of docetaxel 2 h after the administration of flavopiridol was 16 ± 7.5 ng/mL. For cycle 2, the average concentration of docetaxel 2 h after the administration of flavopiridol was 14.4 ± 6.3 ng/mL.

Antitumor activity. Twenty-seven patients were evaluable for response assessment (Table 6). We observed 1 complete response in a patient with metastatic pancreatic cancer and 4 partial responses in pancreatic (1), breast (2), and ovarian (1) cancer patients; the latter three were pretreated with a taxane. All the patients with pancreatic cancer treated on the study had previously progressed on gemcitabine therapy. The median duration of response was 35 weeks (range, 17-108 weeks).

The patient with metastatic pancreatic cancer who developed a complete response on treatment had received one prior dose of gemcitabine and developed a life-threatening allergic reaction that necessitated stopping the drug before entering this clinical trial. He developed a dramatic partial response after two cycles of study therapy with docetaxel and flavopiridol, and then continued to respond to therapy, achieving a complete response of disease. He remained on the study for 26 weeks. He still remains free of disease at the most recent follow-up of 6/21/2007, with negative positron emission tomography and computed tomography scans and without any further therapy for 25 months.

Clinical activity was also noted in two patients with metastatic breast carcinoma and one patient with ovarian carcinoma, who had all been previously treated with a taxane and multiple other chemotherapeutic agents. All three of them achieved a partial response after two cycles of study therapy with docetaxel and flavopiridol.

Stable disease was seen in 10 patients including 4 with gemcitabine-refractory pancreatic cancer. Many of these patients remained on study for prolonged periods of time, as can be seen in Table 6.

Discussion

Flavopiridol is a CDK inhibitor that enhances taxane-induced apoptosis (10). Data from our laboratory indicate that flavopiridol enhances caspase-3–dependent apoptosis in docetaxel-treated cells in both a sequence- and time-dependent manner (10, 11). Promising clinical activity was shown in a prior phase I experience by our group, with paclitaxel administered either as a 24-h or a 3-h infusion on day 1, followed by a 24-h infusion of flavopiridol on day 2 (12). The 24-h infusion of flavopiridol is, however, cumbersome for the patients and is also associated with clinical toxicities including myelosuppression and diarrhea. Prior data showed that weekly flavopiridol is feasible in combination with irinotecan (13). These observations led us to conduct a phase I trial of weekly sequential docetaxel followed by flavopiridol in patients with advanced solid tumors.

The regimen was well tolerated with one DLT occurring in cohort 6 with flavopiridol 70 mg/m^2 (grade 3 mucositis) and one DLT in cohort 7 with flavopiridol at 80 mg/m^2 (grade 4 neutropenia). Other common toxicities included fatigue, diarrhea, nausea, and vomiting. One patient developed pulmonary embolism (grade 4 thrombosis) concomitantly with frank progression of disease after one cycle of therapy: this event was not felt to be related to study drugs but rather to his rapidly progressing disease. However, there have been prior reports of treatment-induced thromboembolic events with flavopiridol, including in combination with carboplatin (15). Therefore, the possibility of this being a known treatment effect of flavopiridol cannot be excluded.

MTD was not reached and dose escalation of flavopiridol had to be discontinued because the pharmaceutical sponsor elected to discontinue further investigation of the agent in solid tumors.

Up to a dose of 50 mg/m^2, the magnitude of C_{max} and AUC found in this study is consistent with that reported in an earlier report of sequential combination of irinotecan followed by weekly 1-h flavopiridol (13). Similar to the data reported by Shah et al., these increases seemed to plateau with flavopiridol doses of 60 mg/m^2 or greater. The C_{max} for flavopiridol at 60 and 70 mg/m^2 in this study exceeds 4 μmol/L in the plasma. In preclinical models, the enhancement of chemotherapy-induced apoptosis requires flavopiridol concentrations of 150 to 300 nmol/L. However, flavopiridol is 90% protein-bound in human plasma, and it is the free flavopiridol fraction that is clinically active. Therefore, these peak flavopiridol concentrations should result in free flavopiridol concentrations of at least 300 nmol/L, which should be sufficient to enhance the effect of docetaxel *in vivo*. Although the MTD was not reached in this experience due to limited drug supply, the pharmacologic level of interest for flavopiridol was indeed achieved; for this reason, we do not feel there would be a need to escalate above the dose level of 80 mg/m^2.

Clinical activity with this regimen was encouraging, with one patient with essentially gemcitabine-naive metastatic pancreatic carcinoma achieving a complete response of disease, which has been maintained for 19 months after discontinuing therapy

Table 6. Clinical activity by tumor type

Tumor type	Response	Duration (wk)	Prior taxane
Ovarian	PR	35	Yes
Breast	PR	17	Yes
	PR	57	Yes
	SD	33	Yes
	SD	18	Yes
	SD	19	Yes
Pancreas	PR	18	No
	CR	134*	No
	SD	25	No
	SD	17	No
	SD	15	No
	SD	25	No
Gastric	SD	21	No
Carcinoid	SD	15	No
Esophageal	SD	11 †	No

Abbreviations: PR, partial response; SD, stable disease; CR, complete response.
*Patient removed from study due to lack of study drug.
† Patient withdrew from study.

and without any further intervening therapy. Four partial responses were observed in pancreatic (1), breast (2), and ovarian (1) cancer patients; the latter three patients were pretreated with taxane. Stable disease was seen in 10 patients including 4 with gemcitabine-refractory pancreatic cancer. Many of these patients remained on study for prolonged periods of time, as can be seen in Table 6.

Other authors have evaluated this drug combination, although with very different dosing schedules. Tan et al. (16) reported a phase I experience with docetaxel and flavopiridol in patients with metastatic breast carcinoma. Five patients received docetaxel 60 mg/m^2 followed after 24 h by a 72-h infusion of flavopiridol at 50 mg/m^2/d every 3 weeks. Because of dose-limiting myelosuppression, the schedule was amended to docetaxel 50 mg/m^2, followed by escalating doses of flavopiridol as a 1-h infusion daily for 3 days, and six patients were enrolled; the DLT was grade 3 hypotension. These schedules were therefore deemed to be not feasible.

El-Rayes et al. (17) recently published their phase I experience with docetaxel followed 24 h later by flavopiridol given via continuous i.v. infusion over a 24-h period. Neutropenia complicated by infection was the major DLT.

The toxicity of flavopiridol in combination with taxanes thus seems to be highly sequence dependent; our data with weekly sequential administration of docetaxel and flavopiridol are indeed promising, showing a good tolerability for this dose-scheduling of the regimen and remarkable clinical activity in this heavily pretreated patient population. Activity was observed even in patients who were previously treated with a taxane, and it was particularly impressive for patients with metastatic pancreatic carcinoma, even for those who had gemcitabine-refractory disease.

The promising results from this study led us to open an ongoing phase II study at our institution of weekly sequential docetaxel and flavopiridol at a dose of 80 mg/m^2 for patients with metastatic, refractory pancreatic carcinoma.

References

1. Hartwell LH, Kastan MB. Cell cycle control and cancer. Science 1994;266:1821–8.
2. Harper J, Elledge SJ. Cdk inhibitors in development and cancer. Curr Opin Genet Dev 1996;6:56–64.
3. Maik RG, Kattice SL, Bhat SV, Alreja B, DeSouza NJ, Rupp RH. An anti-inflammatory cum immunomodulatory piperidinylbenzopyranone from dysoloxylum binectiferum: isolation, structure and total synthesis. Tetrahedron 1988;44:2081–6.
4. Schwartz GK, Shah MA. Targeting the cell cycle: a new approach to cancer therapy. J Clin Oncol 2005; 3:9408–21.
5. Carlson BA, Dubay MM, Sausville EA, et al. Flavopiridol induces G_1 arrest with inhibition of cyclin-dependent kinase CDK2 and CDK4 in human breast carcinoma cells. Cancer Res 1996;56:2973–8.
6. Losiewicz MD, Carlson BA, Kaur G, et al. Potent inhibition of cdc2 kinase activity by the flavonoid L86-8275. Biochem Biophys Res Commun 1994;201: 589–95.
7. Schwartz GK. Development of cell cycle active drugs for the treatment of gastrointestinal cancers: a new approach to cancer therapy. J Clin Oncol 2005;23: 4499–508.
8. Sambol EB, Ambrosini G, Geha RC, et al. Flavopiridol targets c-KIT transcription and induces apoptosis in gastrointestinal stromal tumor cells. Cancer Res 2006;66:5858–66.
9. Wirger A, Perabo FG, Burgemeister S, et al. Flavopiridol, an inhibitor of cyclin-dependent kinases, induces growth inhibition and apoptosis in bladder cancer cells *in vitro* and *in vivo*. Anticancer Res 2005;25:4341–7.
10. Motwani M, DeloheryTM, Schwartz GK. Sequential dependent enhancement of caspase activation and apoptosis by flavopiridol on paclitaxel-treated human gastric and breast cancer cells. Clin Cancer Res 1999; 5:1876–83.
11. Motwani M, Rizzo C, Sirotnak F, et al. Flavopiridol enhances the effect of docetaxel *in vitro* and *in vivo* in human gastric cancer cells. Mol CancerTher 2003; 6:549–55.
12. Schwartz GK, O'Reilly E, Ilson D, et al. A phase I study of cyclin dependent kinase inhibitor flavopiridol in combination with paclitaxel in patients with advanced solid tumors. J Clin Oncol 2002;20:2157–70.
13. Shah M, Kortmansky J, Motwani M, et al. A phase I clinical trial of the sequential combination of irinotecan followed by flavopiridol. Clin Cancer Res 2005;11: 3836–45.
14. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
15. Bible KC, Lensing JL, Nelson SA, et al. Phase 1 trial of flavopiridol combined with cisplatin or carboplatin in patients with advanced malignancies with the assessment of pharmacokinetic and pharmacodynamic end points. Clin Cancer Res 2005;11:5935–41.
16. Tan A, Yang X, Berman A, et al. Phase I trial of the cyclin-dependent kinase inhibitor flavopiridol in combination with docetaxel in patients with metastatic breast cancer. Clin Cancer Res 2004;10: 5038–47.
17. El-Rayes BF, Gadgeel S, Parchment R, et al. A phase I study of flavopiridol and docetaxel. Invest New Drugs 2006;24:305–10.