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ORIGINAL ARTICLE

A phase I clinical trial of FOLFIRI in combination with the pan-cyclin-dependent kinase (CDK) inhibitor flavopiridol

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Abstract
Background The cyclin-dependent kinase inhibitor flavopiridol increases irinotecan- and fluorouracil-induced apoptosis. We conducted a phase I trial of FOLFIRI + flavopiridol in patients with advanced solid tumors.
Design FOLFIRI + flavopiridol were administered every 2 weeks. Based on sequence-dependent inhibition, flavopiridol was given 3 h after irinotecan but before 5-FU. Two maximum tolerated doses were determined, one with flavopiridol administered over 1 h, and one with flavopiridol split as a 30-min bolus followed by a 4-h infusion.
Results A total of 74 patients were enrolled and 63 were evaluable. The MTD with FOLFIRI was flavopiridol 80 mg/m^2 over 1 h or 35 mg/m^2 bolus + 35 mg/m^2 over 4 h. Dose-limiting toxicities were diarrhea, fatigue, neutropenia, and neuropathy. Clinical activity included 2 partial responses in small bowel cancer and bladder cancer and 1 complete response in mucosal melanoma. Stable disease was seen in 22 patients. Pharmacokinetic studies showed increasing C_{max} with increasing flavopiridol dose. Clinical benefit was correlated with the presence of wild-type p53. Of 25 patients with colorectal cancer, 11 had as best response SD for >3 m (median 6 m, range 4.2–15.4 m), despite failing ≥1 irinotecan-containing regimen.
Conclusions Treatment with flavopiridol and FOLFIRI is a safe and effective regimen. Concentrations of flavopiridol that enhance the effects of FOLFIRI can be achieved. Clinical activity is encouraging and includes prolonged stable disease in patients with irinotecan-refractory colorectal cancer.

Keywords CDKs and CDK inhibitors · Phase I trials · Gastrointestinal cancers: colorectal · Combination chemotherapy · Pharmacokinetics and pharmacodynamics · Novel antitumor agents

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Introduction

Progression through the phases of the cell cycle is driven by the coordinated activation of the cyclin-dependent kinases (CDK). Deregulated CDK activity is frequently present in malignant cells and inhibition of CDKs can lead to cell cycle arrest and subsequent apoptosis [1, 2]. Modulation of the cell cycle also contributes to chemotherapy resistance. This rationale led to the development of CDK inhibitors as novel antitumor agents.

Flavopiridol is one of the first CDK inhibitors to enter clinical trials. It is prepared by total synthesis and is identical to a compound derived from *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, 5].

Although phase I studies showed safety and some encouraging clinical activity, single agent phase II trials in solid tumors have been disappointing [6–15]. In contrast, robust significant single agent activity with tumor lysis syndrome has been observed in chronic lymphocytic leukemia, especially when the schedule of administration is altered to improve the drug's pharmacokinetics [6].

There is evidence that flavopiridol may improve the efficacy of cytotoxic chemotherapy, including irinotecan [16–20]. In our xenograft studies, tumor regressions and pathologic cures were shown without significant toxicity in HCT-116 tumor–bearing mice treated with irinotecan followed by flavopiridol. We have previously reported a phase I trial of the sequential combination of irinotecan and flavopiridol [12]. There was encouraging clinical activity in patients with refractory colorectal cancer. Of 27 evaluable patients with colorectal cancer, there was one partial response and 14 patients with stable disease. Median disease control duration for those patients was 6.8 months. Remarkably, the majority of the evaluable patients had received prior irinotecan, thus suggesting that flavopiridol can modulate the effect of chemotherapy and overcome tumor resistance.

In addition to encouraging clinical activity, we have also reported a proposed mechanism of action: By inhibiting CDK9, flavopiridol suppresses expression of Rad51, a DNA repair protein involved in homologous recombination, and thus sensitizes cells to p53-dependent induction of apoptosis by topo I poisons [21].

To build on the encouraging results of the irinotecan-flavopiridol study, we chose the FOLFIRI regimen as the backbone for this clinical trial. The FOLFIRI regimen is used widely as a regimen for metastatic colon cancer [22]. We studied the addition of flavopiridol to the combination of irinotecan, fluorouracil, and leucovorin in a rational approach to improve on the three-drug combination. Based on pre-clinical data showing sequence-dependent antitumor effects, flavopiridol was given 3–7 h after irinotecan but before 5-FU [21].

At the recommended phase II dose, additional patients were treated to better define the toxicity profile of the combination. In addition, we have previously reported that the expression of wild-type p53 status at baseline appeared to be predicative of clinical benefit to flavopiridol combined with irinotecan [12]. Therefore, pre-therapy tumor samples were examined for p53 status.

The primary objective of this trial was to determine the maximum tolerated dose (MTD) of flavopiridol, as either a 1-h bolus or a 30-min bolus followed by a 4-h infusion, when administered in combination with FOLFIRI in patients with advanced solid tumors. Secondary objectives were to investigate the clinical pharmacokinetics of flavopiridol in combination with FOLFIRI, 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 had to have a Karnofsky performance status ≥60%, total WBC count ≥3,500/mm^3, absolute neutrophil count ≥1,500/mm^3, platelet count ≥100,000/mm^3, and adequate hepatic, renal and cardiac function. Patients could have received prior chemotherapy (including irinotecan and 5-FU), 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 with 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, non-randomized dose escalation study to determine the maximum tolerated dose (MTD) of FOLFIRI in combination with flavopiridol. Groups of three to six patients were treated sequentially according to the dose escalation in Table 1. Treatment was every 2 weeks, provided the absolute neutrophil count (ANC) was ≥ 1,500/mm^3 and platelet count ≥100,000/mm^3. One cycle consisted of 3 treatments in 6 weeks. Patients received irinotecan as a 90-min (cohorts 1–6) or 30-min infusion (cohorts 7–12) based on changing institutional guidelines for irinotecan administration. Seven hours (cohorts 1–6) or 3 h (cohorts 7–12) after the completion of the irinotecan, patients received flavopiridol as either a 1-h bolus (cohorts 1–10) or 30-min bolus followed by a 4-h infusion (cohorts 11 and 12). Immediately following flavopiridol, patients received leucovorin over 30 min followed by fluorouracil as a bolus infusion, and the fluorouracil as a 48-h continuous infusion. Because of concerns for tumor lysis syndrome

Table 1 Dose escalation

Cohort	Irinotecan (mg/m^2)	Flavopiridol (mg/m^2)	Leucovorin (mg/m^2)	Fluorouracil (mg/m^2)	Fluorouracil (mg/m^2)	*N*
	30-min infusion	1-h infusion (3-h post-irinotecan)	30-min infusion (5-h post- irinotecan)	Bolus (after flavopiridol)	48-h infusion (after bolus fluorouracil)	[number evaluable for response]
1	180	40	400	400	1,800	3
2	180	50	400	400	1,800	3
3	125	50	400	400	1,800	3
4	125	50	400	400	2,400	3
5	150	50	400	400	2,400	6
6	150	60	400	400	2,400	3
7	180	60	400	400	2,400	3
8	180	70	400	400	2,400	6
9	180	80	400	400	2,400	6
10	180	90	400	400	2,400	6
11	180	35 → 35	400	400	2,400	16
12	180	40 → 40	400	400	2,400	5

Note: In cohorts 11 and 12, flavopiridol was administered as a 30-min bolus followed by a 4-h infusion

with the split-dose schedule, the day following therapy, tumor lysis bloods were obtained including LDH, calcium, magnesium, and phosphorous.

The starting dose was based on an earlier study that determined the MTD of flavopiridol to be 50 mg/m^2 when combined with irinotecan at 125 mg/m^2 given weekly for 4 of every 6 weeks [12]. Flavopiridol was reduced to 40 mg/m^2 and FOLFIRI was attenuated with a reduction of fluorouracil to 1,800 mg/m^2 over 48 h (Table 1). The starting dose of irinotecan was 180 mg/m^2. Due to excessive toxicity (neutropenic fever), irinotecan was reduced in cohort 3. In addition, entry criteria were changed such that no more than two prior therapies were allowed and patients who had documented grade 4 neutropenia with prior irinotecan in the absence of flavopiridol were excluded from the trial. Fluorouracil was escalated next to the full dose of 2,400 mg/m^2 in cohort 4. Escalation of irinotecan alternated with escalation of flavopiridol until the full irinotecan dose of 180 mg/m^2 was reached (cohort 7). With the fixed full-dose FOLFIRI, flavopiridol escalation continued (cohort 8, 9 and 10) until the MTD was determined.

At the MTD with bolus flavopiridol, the study was expanded to evaluate the combination of FOLFIRI with divided-dose flavopiridol, a pharmacokinetically derived schedule shown to be active in patients with refractory chronic lymphocytic leukemia [6]. Flavopiridol was reduced by one dose level from the MTD and half the dose was administered as a bolus over 30 min immediately followed by the remaining half as a continuous infusion given over 4 h (cohort 12). Flavopiridol was further escalated (cohort 13). The final MTD cohort was expanded with additional patients to assess preliminary efficacy.

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 3.0 [23]. Dose-limiting toxicity (DLT) was defined as the occurrence during the first cycle of Grade 4 hematologic toxicity, Grade 3 or 4 non-hematologic toxicity including diarrhea despite antidiarrheal prophylaxis, nausea despite maximum anti-emetic therapy, or any delay in treatment resulting in fewer than 3 treatments in the first 6-week cycle. The maximum tolerated dose (MTD) was defined as the dose one level below the dose at which two or more of the patients in the initial dose level experience DLT during the first treatment course. 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.

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. 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) was supplied by Sanofi Aventis Pharmaceuticals and distributed by CTEP. Irinotecan was supplied by Pharmacia and Upjohn, Inc. Fluorouracil and leucovorin are commercially available.

Statistical design

The main objective of this study was to determine the MTD of flavopiridol when administered in combination with a FOLFIRI. Standard 3 + 3 design was used for dose escalation. The incidence of hematologic and non-hematologic toxicities was summarized separately by flavopiridol cohort. Secondary analyses included a pharmacokinetic analysis of flavopiridol by non-compartmental methods.

Pharmacokinetics

For each patient, blood samples for pharmacokinetics were collected into heparin-coated tubes. Flavopiridol levels were measured on the first day of treatment at the following times—For patients treated with bolus flavopiridol—Immediately before and at the end of the 1 h bolus infusion. For patients treated with divided-dose flavopiridol—Immediately before treatment, after the 30-min bolus, and at the end of the 4 h infusion.

To assess the level of flavopiridol, the compound was first isolated from plasma with a liquid–liquid phase extraction and followed by a high-performance liquid chromatography (HPLC)/tandem mass spectrometry (Sciex API 4000, Applied Biosystems, Foster City, CA, USA) analysis using an electrospray ionization method in the positive ion mode. Prior to liquid–liquid phase extraction, frozen plasma samples were thawed at ambient temperature. The liquid–liquid phase extraction was done in a solvent mixture of acetonitrile and methanol (4/1, v/v). The supernatant was injected onto a C18 column. HPLC was then used to separate the compound from any potential interference and measured by the MS/MS detection method, calibration curves were determined for the compound (402 [M + H] +) to permit conversion of peak areas to compound amounts against external reference standards. The tandem MS/MS detector also permits verification of peak identity as well as a quantitative assessment of the compounds in the samples. The limit of quantitation for flavopiridol was less than 0.01 nM.

Biological assays

Pre-treatment tumor samples of patients enrolled in the expanded cohort at the MTD were evaluated for p53 status. The biopsy specimens were fixed in formalin and embedded in paraffin. Five-micrometer-thin sections were cut for H&E and immunohistochemistry staining. Monoclonal antibody for p53 (PAb1801, Oncogene Calbiochem, Cambridge, MA, USA) was used at a concentration of 0.2 μg/ml as previously described [12]. Both positive and negative controls were run at the time of each experiment. Nuclear staining was considered specific reactivity for p53, and the percent of positive tumor cells was estimated examining different fields throughout the entire tissue section. The staining was reviewed by a pathologist. Mutant (or positive) p53 staining was considered if greater than 20% of the nuclei stained positive.

Results

Patient characteristics

From 10/8/02 to 6/19/08, 74 patients with advanced solid tumors were registered and treated. Eleven of these were not evaluable for determining DLT because they did not complete one cycle (6 weeks) of treatment. The reasons were clinical deterioration (3 patients), withdrawal of consent (3 patients), adverse effects of 5-FU/leucovorin (3 patients), and protocol non-adherence (2 patients). A further seven patients were not evaluable for determining response because they did not continue treatment until the first restaging scan. The reasons were withdrawal of consent (6 patients) and clinical deterioration (1 patient). Table 2 lists the patient characteristics of the 74 patients who were treated. 63 were evaluable for toxicity and 56 for response.

The median age was 60 years (range, 19–83 years) and the median Karnofsky performance status was 90% (range, 70–90%). There were 43 men and 31 women. The cancers treated and patient numbers were colorectal (31), pancreas (7), melanoma (6), bladder (4), gastric (4), breast (3), cholangiocarcinoma (2), GE junction (2), lung (2), small bowel (2), and others (11). All patients had received prior chemotherapy; 39 (53%) patients had received prior irinotecan.

Cycle 1 toxicities

Table 3 lists the most common grade 2–4 hematologic and non-hematologic toxicities for the first cycle of therapy. In the second cohort, two patients had DLT with grade 4 neutropenic fever. Both of these patients were heavily pretreated and had had neutropenia from prior irinotecan, making it impossible to distinguish neutropenia due to irinotecan alone from that of combination therapy. Therefore, this resulted in a protocol initiated amendment to limit the number of prior regimens to two and to exclude patients with documented grade 4 neutropenia from prior irinotecan

Table 2 Patient characteristics

Characteristic		No. patients
Total		74
Assessable for response		56 (76%)
Male		43 (58%)
Female		31 (42%)
Age (year)		
Median	60	
Range	19–83	
KPS (%)		
Median	90	
Range	70–90	
Prior chemotherapy		74 (100%)
No. prior regimens		
Median	3	
Range	1–10	
Prior Irinotecan		39 (53%)
Primary sites of disease		
Colorectal		31
Pancreas		7
Melanoma		6
Urothelial		6
Breast		3
Cholangiocarcinoma		3
Esophagus/GE junction		3
Gastric		3
Lung		2
Small bowel		2
Anal		1
Basal cell carcinoma		1
Dedifferentiated liposarcoma		1
Head and Neck		1
Hepatocellular carcinoma		1
Ovarian		1
Prostate		1
Thymus		1

therapy. This also led to the dose reduction of irinotecan in the next cohort. The next two cohorts completed cycle 1 without a DLT. In cohort 5, one of three patients had a DLT with grade 3 fatigue. The cohort was expanded to include six evaluable patients with no further DLT observed. Dose escalation continued. In cohort 8, one patient had DLT with grade 4 neuropathy. The cohort was expanded with no further DLT in six evaluable patients. In cohort 9, one patient had protocol-defined DLT since mild thrombocytopenia ($<100{,}000/mm^3$) resulted in less than 3 treatments in 6 weeks. No further DLT was seen in six evaluable patients. In cohort 10, one patient had grade 4 diarrhea, one patient had grade 3 diarrhea (both despite maximal antidiarrheal therapy) and one patient again had a protocol-defined DLT since treatment had to be delayed for thrombocytopenia. Thus, cohort 9 (flavopiridol 80 mg/m^2) was declared the MTD for the bolus schedule.

In the split-dose flavopiridol cohort 11, one of the first three patients had a DLT with grade 4 neutropenia. There was no further DLT in the next three patients treated at that dose. In cohort 12, three of six patients had DLT with grade 3 diarrhea, grade 4 leukopenia, and treatment delay due to neutropenia. Cohort 11 was thus declared the MTD for the split-dose flavopiridol schedule. This cohort was expanded with ten additional patients. Three of those had DLT with grade 3 diarrhea (two patients) and grade 3 fatigue. The DLT rate for the whole MTD cohort was 25% (4/16). This was considered acceptable using pre-specified guidelines since the upper limit of the 95% posterior interval was 40% [24]. Tumor lysis was not observed in any patient.

Cumulative toxicities

Table 4 lists the most common grade 2–4 cumulative hematologic and non-hematologic toxicities. The cumulative pattern of toxicity was similar to that in cycle 1 of treatment and principally included gastrointestinal and myelosuppressive effects. The most common grade 3/4 toxicities were neutropenia (29%), lymphopenia (24%), leukopenia (24%), diarrhea (17%), thrombosis or embolism (8%), fatigue (8%), and nausea/vomiting (7%).

Table 4 lists the most common grade 2–4 cumulative toxicities for patients overall and grouped by flavopiridol schedule (bolus vs. split-dose). Grade 3/4 diarrhea occurred in 24% of patients on the split-dose schedule vs. 14% of those on the bolus schedule. Similarly, grade 3/4 neutropenia and lymphopenia each occurred in 43% of patients on the split-dose schedule. In the bolus schedule, the rate was 14% (lymphopenia) and 21% (neutropenia). In contrast, neutropenic fever occurred in 7% of the patients on the bolus schedule, but none of the patients on split-dose schedule.

One patient with pancreas cancer died of pulmonary embolism and pneumonia 38 days after receiving the first and only treatment with FOLFIRI and flavopiridol. This event was considered to be due to disease progression rather than to toxicity from treatment.

Pharmacokinetics

Blood samples for pharmacokinetic analyses were obtained for 71 patients.

Table 5 summarizes maximum observed plasma concentration (C_{max}) across all subjects in a cohort. C_{max} for flavopiridol ranged from 1.65 (SD 0.34) μM (at flavopiridol dose 40 mg/m^2) up to a maximum of 4.89 (SD 1.22) μM

Table 3 Cycle 1 non-hematologic and hematologic toxicity

Cohort (patients)	Irinotecan	F	5FU	Fatigue			Diarrhea			Nausea			Vomiting			Leukocytes			Lymphopenia			ANC			Febrile neut			Hemoglobin		
				2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4
1 (3)	180	40	1,800					1			1						1					2						1		
2 (3)	180	50	1,800				1			1								1		1			2		2					
3 (3)	125	50	1,800	1			1						2																	
4 (3)	125	50	2,400																											
5 (6)	150	50	2,400		1																									
6 (3)	150	60	2,400	1																										
7 (3)	180	60	2,400	2			1									2						1								
8 (6)	180	70	2,400	2						2							1					1								
9 (6)	180	80	2,400				1	1								3						1								
10 (6)	180	90	2,400		1			1		1						2				1		1	1							
11 (16)	180	35/35	2,400	4	1		2	3*		2			1			5	3			7		2	4	1						
12 (5)	180	40/40	2,400	1			1	2								1	1	1		1		1**	3					1		

* 2 of 3 were DLTs; 3rd patient was not maximally premedicated

** 1 DLT; patient experienced persistent treatment delays due to grade 2 & 3 ANC and was unable to complete 3 treatments in 6 weeks

Other DLTs: 1 patient on cohort 8 experienced dose-limiting grade 4 motor neuropathy

Another patient on cohort 9 experienced dose-limiting grade 2 platelets; patient was unable to complete 3 treatments in 6 weeks

Table 4 Cumulative toxicities

	All patients ($N = 63$)				Bolus schedule ($N = 42$)				Split-dose schedule ($N = 21$)			
	Grade				Grade				Grade			
Toxicity	2	3	4	3 & 4 (%)	2	3	4	3 & 4 (%)	2	3	4	3 & 4 (%)
Diarrhea	10	10	1	11 (17)	7	5	1	6 (14)	3	5	0	5 (24)
Fatigue	21	5	0	5 (8)	14	3	0	3 (7)	7	2	0	2 (10)
Nausea	9	3	0	3 (5)	6	3	0	3 (7)	3	0	0	0 (0)
Vomiting	5	1	0	1 (2)	4	1	0	1 (2)	1	0	0	0 (0)
Thrombosis/Embolism	0	2	3	5 (8)	0	2	2	4 (10)	0	0	1	1 (5)
Leukopenia	16	11	4	15 (24)	8	6	3	9 (21)	8	5	1	6 (29)
Lymphopenia	0	15	0	15 (24)	0	6	0	6 (14)	0	9	0	9 (43)
Neutropenia	11	11	7	18 (29)	8	3	6	9 (21)	3	8	1	9 (43)
Neutropenic fever	0	2	1	3 (5)	0	2	1	3 (7)	0	0	0	0 (0)
Anemia	2	1	1	2 (3)	1	1	1	3 (7)	1	0	0	0 (0)

(at flavopiridol dose 90 mg/m^2). In the setting of varying irinotecan dose with constant flavopiridol dose (cohorts 2–5; cohorts 6 and 7), there was no significant change in flavopiridol C_{max}, suggesting no interaction between irinotecan dose and flavopiridol levels. There was significant interpatient variability. At MTDs, there was higher C_{max} in patients who experienced DLT (3.48 μM) vs those who did not (2.21 μM), but the trend did not meet statistical significance.

Antitumor activity

Fifty-six patients were evaluable for response assessment (Table 6). There were two partial responses (small bowel cancer; bladder cancer) and one complete response (mucosal melanoma). Twenty-two patients had stable disease (median 5.9 m; range 1.5–25.7 m). The clinical benefit rate (CR + PR + SD for >3 m) was 39% (22/56).

Of 25 patients with colorectal cancer, 11 had as best response SD for >3 m (median 6 m, range 4.2–15.4 m). Although prior treatment with irinotecan was neither required nor prohibited for enrollment on this trial, it happened that these 11 had all received and failed at least one prior irinotecan-containing regimen. The median time since prior irinotecan treatment was 2.1 m (range 0.7–11.0 m). In addition to prolonged stable disease, 6 of those 11 patients had marked decreases (36–78%) in CEA.

Table 5 Flavopiridol cycle 1 pharmacokinetic parameters by dose level

Cohort	Irinotecan (mg/m^2)	Flavopiridol (mg/m^2)	*n*	Mean C_{max} (μM)	SD
1	180	40	3	1.65	0.34
2	180	50	3	2.45	0.83
3	125	50	4	2.88	0.37
4	125	50	3	3.10	0.95
5	150	50	9	3.22	1.68
6	150	60	3	3.01	0.35
7	180	60	3	1.68	0.18
8	180	70	7	2.05	0.31
9	180	80	6	2.69	1.48
10	180	90	6	4.89	1.22
11	180	35 → 35	19	2.46	1.36
12	180	40 → 40	5	2.15	0.60

Table 6 Clinical activity by tumor type

Tumor type	Response	Duration (months)	Prior irinotecan
Gastric	SD	10.1	Y
	SD	25.7	N
Melanoma	SD	6.3	N
	CR	10.3	N
Cholangiocarcinoma	SD	1.5	N
GE junction	SD	3.2	N
	SD	8.9	Y
Colorectal	SD	5.8	Y
	SD	5.3	Y
	SD	6.5	Y
	SD	13.5	Y
	SD	4.7	Y
	SD	15.4	Y
	SD	13.3	Y
	SD	4.2	Y
	SD	6.0	Y
	SD	1.8	N
	SD	6.1	Y
	SD	4.9	Y
Small bowel	PR	10.3	N
Urothelial	PR	10.0	N
Thymic	SD	9.3	N
Breast	SD	8.1	N
Basal cell carcinoma	SD	3.7	N
Lung	SD	2.3	N

The patient who achieved a complete response had a history of anal mucosal melanoma that had been resected. The patient subsequently developed metastases to thoracic, abdominal, and pelvic lymph nodes. Metastatic melanoma was confirmed by needle biopsy and therapy with FOLFIRI and flavopiridol began. After 10 months of treatment on protocol, the patient achieved a complete response with resolution of all hypermetabolic lesions. The patient was then observed off treatment. A follow-up scan 6 months later detected a hypermetabolic inguinal lymph node. This was resected and showed nodal melanosis and hemorrhage with no evidence of viable melanoma. The patient remains alive and without evidence of recurrence now 17 months after starting treatment with FOLFIRI and flavopiridol.

Correlative studies

All 9 patients enrolled in the expanded cohort at the MTD (cohort 12) had tumor assessed for p53 status. These included 4 patients with melanoma, 2 with colorectal cancer, and 1 each with esophagus, lung, and basal cell carcinoma. All samples showed tumor on H & E staining and were adequate for subsequent immunohistochemical analysis for p53. Based on pre-clinical studies indicating that flavopiridol enhanced the effect of the DNA-damaging agent irinotecan in a p53-dependent manner, we hypothesized that patients with pre-treatment wild-type p53 would respond better than patients with mutant p53. Supporting this hypothesis, 2 of 3 patients with wild-type p53 had a best response of SD (6.3 m and 11.0 m), whereas 4 of 5 patients with mutant p53 had a best response of PD. The fifth patient with mutant p53 had SD of only brief duration (2.3 m).

Discussion

Based on the pre-clinical evidence that flavopiridol enhances the effect of irinotecan, we conducted a phase I trial of flavopiridol plus FOLFIRI in patients with advanced solid tumors.

The established MTD was irinotecan 180 mg/m^2, LV 400 mg/m^2, and 5FU 400 mg/m^2 bolus plus 2,400 mg/m^2 over 48 h in combination with flavopiridol as either 80 mg/m^2 bolus (MTD_1) or 35 mg/m^2 bolus + 35 mg/m^2 over 4 h (MTD_2). These doses of flavopiridol are comparable to those achieved in other combinations with chemotherapy, and the PK at the MTDs was in the active range based on pre-clinical data [12, 16]. DLTs including diarrhea, fatigue, and neutropenia were similar to other reports and were generally tolerable and manageable. We conclude that flavopiridol can be combined with FOLFIRI chemotherapy safely.

The eligibility criteria were modified during the course of the trial so that patients with prior severe myelosuppression due to irinotecan were excluded. Thus, this may have selected patients with less genetic predisposition to irinotecan toxicity. Therefore, the MTDs only strictly apply to those patients.

In terms of safety, it is not possible to make a definite determination whether the bolus schedule or the split-dose schedule is preferred for future clinical development of this combination therapy. Neutropenia, which was an early issue with the bolus schedule, could be ameliorated with careful patient selection. Though dose-limiting diarrhea was observed at both MTDs, it appeared more common with the split-dose schedule. Neutropenia and lymphopenia also appeared somewhat more common with the split-dose schedule. In addition, though interpatient variability was considerable at all dose levels, both schedules resulted in mean flavopiridol C_{max} that tended to exceed 2 μM. As clinical benefit was observed with both schedules, it also does not appear that maintaining constant exposure to flavopiridol for an additional 4 h resulted in a dramatic improvement in response rate. The only exception to this could be the patient with mucosal melanoma who achieved the CR on the split-dose schedule. However, whether this was due to the schedule or simply to the combination therapy itself is unknown. Furthermore, unlike the studies that utilize the split-dose schedule in the treatment of CLL, we observed no evidence of tumor lysis syndrome in any one patient. Therefore, based on simply patient convenience, the bolus schedule would seem to be preferred for future development of flavopiridol with FOLFIRI.

Correlative studies support the hypothesis that flavopiridol efficacy depends on an intact p53 axis. Of patients treated at the expanded MTD cohort, most with wild-type p53 had clinical benefit from treatment in the form of prolonged stable disease, whereas most with mutant p53 had no benefit. This hypothesis is about to be tested in a clinical trial where patients with metastatic gastric cancer whose tumors are wild-type for p53 will be randomized to irinotecan and flavopiridol or irinotecan alone.

Encouraging periods of prolonged stable disease were noted in several patients with colorectal cancer who had previously had progressive disease on irinotecan-based therapy. Patients with colorectal cancer treated on this trial had progression-free survival (PFS) rates of 44% (11/25) at 3 months and 24% (6/25) at 6 months. This suggests that flavopiridol may modulate the effectiveness of chemotherapy and overcome drug resistance. However, irinotecan-resistance was not strictly defined as it was, for example, in the trial showing that cetuximab can overcome irinotecan-resistance [25]. Thus, the hypothesis that flavopiridol can modulate irinotecan-resistance would need to be formally tested in a prospective trial with a strict definition of irinotecan-resistance in the eligibility criteria.

Partial responses in bladder cancer and small bowel cancer were also seen, although whether the effect was predominantly due to flavopiridol or to FOLFIRI cannot be determined. More striking, however, is a complete response in mucosal melanoma, a notoriously chemotherapy-resistant disease. Although irinotecan, both alone and in combination with the fluoropyrimidine capecitabine, has previously been reported to have some activity in melanoma [26, 27], there is reason to believe a cell cycle inhibitor such as flavopiridol may be active in this disease.

Among the genetic pathways involved in the development of melanoma, both CDK4 and CCND1 (cyclin D1) are essential. In an examination for genome-wide alterations among 126 melanomas, cyclin D1 resided in the most common genomic region affected by focused amplification [28]. CDK4, whose protein is one of the binding partners of Cyclin D1 and which is located on chromosome 12q14, was also subject to recurrent amplifications. Interestingly, there was a striking inverse correlation between BRAF mutations and increases in the copy numbers for both CDK4 and Cyclin D1. Thus, CDK4 and Cyclin D1 appear to be independent oncogenes in melanoma and they are amplified most commonly in melanomas that are wild type for B-RAF. For cyclin D1, this association was independent of the type of melanoma (acral, mucosal, chronic sun induced, and non-chronic sun induced), though for CDK4 the amplifications were more commonly observed in the acral or mucosal melanoma subtypes. Thus, melanomas of the mucosal subtype may be more sensitive to an inhibitor such as flavopiridol. Although a previous phase II study of single agent flavopiridol found no objective responses in 16 patients, there were likely not enough patients with mucosal melanoma to detect activity [8]. Therefore, further study of flavopiridol in combination with chemotherapy for mucosal melanoma is warranted.

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