Phase I Study of Flavopiridol with Oxaliplatin and Fluorouracil/ Leucovorin in Advanced Solid Tumors

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Abstract

Purpose: Flavopiridol, a cyclin-dependent kinase inhibitor, has promising clinical activity when combined with chemotherapy. Preclinical data indicate that flavopiridol enhances oxaliplatin- and fluorouracil (5FU)–induced apoptosis in a sequence-dependent manner.

Experimental Design: We conducted a phase I trial of flavopiridol + FOLFOX (folinic acid, 5FU, and oxaliplatin) for advanced solid tumors. Flavopiridol was administered every 2 weeks with oxaliplatin before 5FU, based on sequence-dependent growth inhibition. Flavopiridol pharmacokinetics and p53 status were evaluated.

Results: Forty-eight patients were treated on study. With dose escalation of oxaliplatin (85 mg/m^2) and 5FU (2,400 mg/m^2), dose-limiting toxicities included hyponatremia, thrombocytopenia, and neutropenia. 5FU was subsequently reduced to allow for dose escalation of flavopiridol. Dose-limiting toxicities with escalation of flavopiridol were nausea, vomiting, and neutropenia. The maximum tolerated dose was 70 mg/m^2 flavopiridol, 85 mg/m^2 oxaliplatin, and 1,800 mg/m^2 5FU continuous infusion over 48 hours. Clinical activity was noted in platinum-refractory germ cell tumors: 3 of 9 (33%) evaluable patients showed a partial response on imaging and 7 of 10 (70%) had a decline in serum tumor markers. Responses were also observed in pancreatic, gastric, and sweat gland tumors. Flavopiridol pharmacokinetics had significant interpatient variability. At the maximum tolerated dose, tumor samples were p53 mutant (>30% positive cells) for responders and p53 wild-type for nonresponders.

Conclusions: Flavopiridol with FOLFOX is a safe and tolerable regimen. Promising clinical activity was seen across tumor types. Encouraging results in the platinum-refractory germ cell tumor population has prompted a phase II trial that is currently open for accrual. (Clin Cancer Res 2009;15(23):7405–11)

Flavopiridol is a pan-cyclin–dependent kinase inhibitor that promotes cell cycle arrest at nanomolar concentrations and has been associated with the selective induction of apoptosis in DNA-damaged tumor cells (1, 2). In the laboratory, flavopiridol has been shown to potently enhance the effects of a wide range of chemotherapeutic agents, including SN38 and taxane derivatives, in a time- and sequence-dependent manner (3–5). This has been translated into a series of phase I trials in advanced solid tumors with encouraging clinical results, a reasonable safety profile, and pharmacologic levels of the drug that are sufficient to potentiate the effect of chemotherapy *in vivo* (6–8).

Oxaliplatin, a platinum-based agent, has shown antiproliferative activity equivalent to or higher than that of cisplatin in a wide range of experimental tumor models. *In vitro* and *in vivo*, oxaliplatin has exhibited enhanced cytotoxic properties when combined with fluoropyrimidines [fluorouracil (5FU) and gemcitabine], thymidylate synthase inhibitors (AG337), topoisomerase I inhibitors (CPT-11 and SN38), microtubule inhibitors (paclitaxel), and DNA-modifying agents (cisplatin and cyclophosphamide; 9, 10). In the clinic, oxaliplatin has shown antitumor activity as a single agent in a variety of solid tumors, and also in combination with leucovorin [folinic acid (FOL)] and 5FU as part of the FOLFOX regimen for the treatment of metastatic colon cancer (11).

Similar to preclinical data on the effects of flavopiridol with mitomycin C, paclitaxel, and SN38, flavopiridol enhances the

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Translational Relevance

Flavopiridol is a potent cyclin-dependent kinase inhibitor that promotes cell cycle arrest and has been associated with apoptosis in DNA-damaged tumor cells. Based on preclinical data showing that flavopiridol enhances the effect of oxaliplatin in a time- and sequence-dependent manner, we conducted a phase I trial of biweekly flavopiridol administered concurrently with oxaliplatin and leucovorin as a 1-hour bolus infusion, followed by fluorouracil (FOLFOX). This trial in patients with advanced solid tumors showed a tolerable safety profile and pharmacologic levels of flavopiridol sufficient to potentiate the effects of chemotherapy *in vivo*. In contrast to prior studies combining flavopiridol with irinotecan, p53 wild-type status did not correlate with increased sensitivity. Encouraging clinical results were seen, particularly in patients with platinum-refractory germ cell tumors, prompting a phase II trial for this patient population, which will continue to explore the role of p53 and apoptotic markers relative to treatment response.

effect of oxaliplatin in a sequence-dependent manner. However, in HCT-116 colon cancer cells, flavopiridol exhibits its most potent effects when administered concomitantly with oxaliplatin rather than sequentially.[7] This effect is similar to that reported for flavopiridol in combination with cisplatin (12). Therefore, based on our preclinical observations, we elected to add flavopiridol to the FOLFOX regimen for the treatment of patients with advanced solid tumors. Every other week, flavopiridol was administered concurrently with oxaliplatin and leucovorin as a 1-hour bolus infusion, followed by 5FU to maximize the treatment effect.

During the course of this study, the 5FU continuous infusion was de-escalated from 2,400 mg/m^2 over 48 hours to 1,800 mg/m^2 over 48 hours, to facilitate dose escalation of the flavopiridol. At the recommended phase II dose, additional patients were treated to better define the toxicity profile of the combination. Because we had previously reported that the expression of wild-type p53 status at baseline seemed to be predictive of clinical benefit from flavopiridol when combined with irinotecan (7), pretherapy tumor samples were examined for p53 status. Classic pharmacokinetic (PK) analysis with flavopiridol plasma levels was done at all dose levels.

Patients and Methods

Eligibility. Patients >18 years of age with advanced solid tumors refractory to standard therapy, or for which there was no standard therapy, were eligible. Patients had a Karnofsky performance status ≥70% and adequate organ function. Prior chemotherapy, immunotherapy, hormonal therapy, or radiotherapy was allowed, but only if 4 wk had elapsed between the last dose and study entry. The protocol was approved by the institutional review board of Memorial Sloan-Kettering Cancer Center, and all patients signed informed consent forms.

[7] G.K. Schwartz, unpublished data.

Study design. This was a phase I open-label, nonrandomized, dose-escalation study. A minimum of three patients were followed for at least one complete cycle (three treatments in 6 wk) before dose escalation. If one instance of dose-limiting toxicity (DLT) was observed, an additional three patients were treated at that dose level. The maximum tolerated dose (MTD) was defined as the dose one level below the dose at which two or more patients within a cohort experienced DLT.

Toxicity was graded in accordance with the National Cancer Institute (Bethesda, MD) Common Toxicity Criteria (version 3.0). DLT was defined in cycle 1 as the occurrence of any of the following during the first cycle of therapy: grade 4 hematologic toxicity, grade 3 or 4 nonhematologic toxicity including diarrhea despite prophylaxis, or any delay in treatment resulting in fewer than three treatments in 6 wk. If a DLT was observed in the first cohort, the patient would be removed from the study without further dose attenuation. At the discretion of the investigator, patients who experienced toxicity in subsequent cycles could continue to receive study treatment after recovery with appropriate dose modifications defined by protocol. All treatments were administered in the outpatient setting.

Treatment plan. Groups of three to six patients were treated sequentially with flavopiridol (starting dose 40 mg/m^2 over 60 min), concomitant oxaliplatin (starting dose 60 mg/m^2 over 120 min), and leucovorin (fixed dose 400 mg/m^2 over 120 min). This was immediately followed by a bolus of 5FU (fixed dose 400 mg/m^2) and continuous 5FU (starting dose 1,800 mg/m^2 over 48 h; Fig. 1). This regimen was administered i.v. every 2 wk. Due to toxicity before flavopiridol escalation with 5FU at 2,400 mg/m^2 over 48 h, 5FU was de-escalated to the starting dose of 1,800 mg/m^2 over 48 h. Dose escalation with flavopiridol was then pursued in 10 mg/m^2 intervals up to 80 mg/m^2. The MTD of 70 mg/m^2 was then expanded to additional patients.

Treatment assessments. Patients were evaluated by a physician biweekly at the time of treatment for the first two cycles (12 wk) to document toxicities. Following the second cycle, these evaluations were done at the initiation of each cycle or more frequently if necessary. Treatment responses were evaluated after every two cycles. Standard Response Evaluation Criteria in Solid Tumors (RECIST) was used for response assessment and was done by an independent protocol radiologist (13).

Drug supply. Flavopiridol (HMR 1275) was provided by Sanofi-Aventis and distributed by the National Cancer Institute in 10 and 50 mg sterile vials, as previously reported (6). Flavopiridol was reconstituted in 250 mL of 0.9% sodium chloride injection, USP, or 5% dextrose for injection, USP, so that the final concentration recommended by the company ranged from 0.09 to 1 mg/mL to decrease the risk of thrombotic complications.

Statistical design. The main objective of this study was to determine the MTD of biweekly flavopiridol when administered in combination with FOLFOX to patients with advanced solid tumors. The incidence of hematologic and nonhematologic toxicities was summarized separately, by cycle and by flavopiridol cohort. Secondary analyses included a PK analysis of flavopiridol.

Pharmacokinetics. PK studies of flavopiridol were done for each patient during week 1 and compared with historical controls. Blood samples were collected through an indwelling peripheral catheter or through peripheral venipuncture into heparinized coated tubes: before treatment (0 h), completion of flavopiridol (1:00 h), oxaliplatin (2:00 h), 5FU bolus (2:15 h), and 5FU continuous infusion (50:15 h). 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. High-performance liquid chromatography/tandem mass spectrometry (Sciex API 4000, Applied Biosystems) analysis using an electrospray ionization method in the positive ion mode was 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

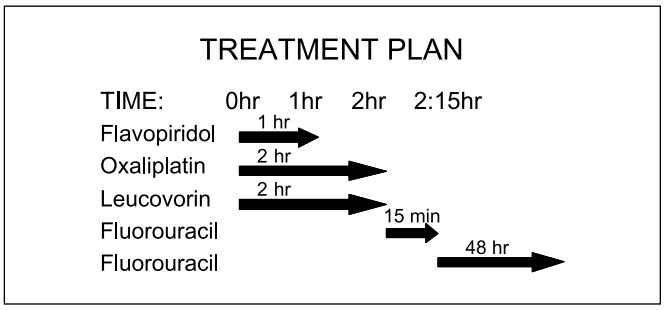

Fig. 1. Patients received flavopiridol as a 1-h infusion ($t = 0$ h), concurrently with oxaliplatin at a starting dose of 60 mg/m^2 and leucovorin at 400 mg/m^2 administered over 2 h ($t = 0$ h). This was followed by 5FU given as a bolus infusion starting at 400 mg/m^2 ($t = 2$ h) and then a 48-h continuous infusion at either 1,800 or 2,400 mg/m^2 over 48 h ($t = 2:15$ h).

tandem MS/MS detector also permitted 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 nmol/L.

Biological assays. Pretreatment tumor samples of patients enrolled in the expanded cohort at the MTD were evaluated for p53 status. The biopsy specimen was fixed in formalin and embedded in paraffin. Five-micrometer sections were cut for H&E and immunohistochemistry staining. The monoclonal antibody for p53 (PAb1801, Calbiochem Immunochemicals, EMD Biosciences North America) was used at a concentration of 0.2 μg/mL. Both positive and negative controls were run at the time of each experiment. Nuclear staining was considered specific reactivity for p53 and percent of positive tumor cells was estimated by examining different fields throughout the entire tissue section. The staining was reviewed by a pathologist. Mutant (or positive) p53 staining was considered if >20% of the nuclei stained positive.

Results

Patient characteristics. Between March 2007 and October 2008, 52 patients with advanced solid tumors were registered to the study. Of the 52 patients enrolled, 4 were not treated and an additional 11 patients did not complete a full cycle of treatment (6 weeks). These patients came off study early due to personal choice (1), intolerability of or hypersensitivity to oxaliplatin (2), hypersensitivity to flavopiridol (1), progression based on early imaging (4), or progression based on symptoms of disease (3). Baseline characteristics for the 48 patients who received at least one treatment with flavopiridol and FOLFOX are outlined in Table 1.

The median age was 51 years (range, 19-77 years) and the Karnofsky performance status was 90% (range, 70-90%). All but one patient with metastatic gastric cancer had received prior chemotherapy. The median number of prior treatment regimens was 3 (range, 0-10); 33 patients (69%) had previously received a platinum agent, of which 16 (33%) had received oxaliplatin. All germ cell tumor (GCT) patients had received prior cisplatin; 1 had also received oxaliplatin.

Dose-limiting toxicity. Table 2 lists the dose levels and most common cumulative toxicities (grades 2 to 4) for the 48 patients treated on study. In total, there were six DLTs noted, including thrombocytopenia in cohort 1, syncope attributed to hyponatremia and neutropenia in cohort 3 (prompting 5FU de-escalation in cohorts 4a through 7a to continue flavopiridol escalation), and febrile neutropenia, nausea and vomiting, and failure to complete three cycles of therapy within 6 weeks in cohort 7a. As a result, the MTD was determined to be cohort 6a with 70 mg/m^2 flavopiridol, 85 mg/m^2 oxaliplatin, and 1,800 mg/m^2 5FU continuous infusion over 48 hours. There were no observed DLTs in the expanded MTD cohort.

Hematologic and nonhematologic toxicity. As shown, the most common ≥grade 3 toxicities were hematologic (leukopenia, lymphopenia, neutropenia). All grade 4 toxicities were hematologic, including neutropenia (2 patients) and thrombocytopenia (1 patient). As would be expected with FOLFOX chemotherapy, nonhematologic toxicities occurring <20% of the time included fatigue, diarrhea, nausea and vomiting, electrolyte abnormalities, sensory neuropathy, and febrile neutropenia.

Pharmacokinetics. Blood samples for PK analysis were obtained from 30 patients. Table 3 summarizes the maximum observed plasma concentration (Cmax) across all subjects in a cohort. Flavopiridol PK showed significant interpatient variability. When evaluated by higher (70-80 mg/m^2) and lower (40 mg/m^2) dose levels, flavopiridol Cmax seemed to increase with dose (1.95 μmol/L ± 0.56 versus 1.23 μmol/L ± 0.56; paired samples t test, $P = 0.002$). In the last cohort (7a), the three patients who experienced a DLT had a higher flavopiridol Cmax than the other patients in the cohort (2.26 μmol/L ± 0.03 versus 1.45 μmol/L ± 0.44; paired samples t test, $P = 0.07$).

Antitumor activity. In total, 42 of 48 treated patients were evaluable for antitumor response. Twenty-two of these patients had progression of disease based on imaging or symptoms as

Table 1. Patient characteristics

Characteristic		No. patients
Total		48
Male		31
Female		17
Age, y		
Median	51	
Range	19-77	
KPS, %		
Median	90	
Range	70-90	
Prior chemotherapy		47
No. prior regimens		
Median	3	
Range	0-10	
Prior platinum	33	
Prior oxaliplatin	16	
Primary sites of disease		
Germ cell tumor		10
Colon		8
Pancreatic		6
Ampullary		3
Melanoma		3
Stomach		3
Gastric		2
Rectal		2
Anal		1
Breast		1
Desmoplastic small round cell tumor		1
Esophageal		1
Head and neck		1
Liposarcoma		1
Lung		1
Neuroendocrine carcinoma		1
Small bowel		1
Sweat gland carcinoma		1
Wilms' tumor		1

Table 2. Cycle 1 nonhematologic and hematologic toxicity (n = 48)

Cohort (patients)	O	F	5FU	Fatigue			Diarrhea			Nausea			Vomiting			Febrile neut		
				2	3	4	2	3	4	2	3	4	2	3	4	2	3	4
Cohort 1 (9)	60	40	1800	2			2			1	1							
Cohort 2 (3)	85	40	1800	1			1	1		1								
Cohort 3 (6)	85	40	2400	4						2			1					
Cohort 4a (4)	85	50	1800	1														
Cohort 5a (3)	85	60	1800															
Cohort 6a (17)	85	70	1800				4											
Cohort 7a (6)	85	80	1800	1							**1****			**1***			**1**	

Cohort (patients)	O	F	5FU	ANC			Platelets			Leukocytes			Lymphopenia			Hyponatremia		
				2	3	4	2	3	4	2	3	4	2	3	4	2	3	4
Cohort 1 (9)	60	40	1800	1					**1**	1	1			2				
Cohort 2 (3)	85	40	1800		1		2				1							
Cohort 3 (6)	85	40	2400	2	1	**1**				2				4			**1**	
Cohort 4a (4)	85	50	1800	3	1					2	2							
Cohort 5a (3)	85	60	1800				1											
Cohort 6a (17)	85	70	1800	1			2			2				2				
Cohort 7a (6)	85	80	1800	1	**4**†	**1**	2	1		3	3			2				

NOTE: DLTs are in boldface and underlined. Grade 2 to 4 toxicities for possibly, probably, or definitely attributable to therapy.
Abbreviations: O, oxaliplatin; F, flavopiridol; ANC, absolute neutrophil count.
*Patient experienced grade 3 nausea and vomiting, as well as grade 3 hypophosphatemia.
†Patient experienced treatment delays due to grade 3 absolute neutrophil count (×2); unable to complete three treatments in 6 weeks.

their best response. Table 4 outlines the 20 patients who had stable disease (SD), a partial response (PR), or a complete response (CR) to the treatment combination. A CR was seen in one patient with pancreatic cancer (2%) who had previously progressed on treatment with gemcitabine. A PR was seen in six patients (14%): three with GCTs, two with gastric, and one with sweat gland carcinoma. An additional 13 patients (31%) showed SD. The median time on study was 20 weeks (range, 8-39 weeks).

Of the 10 patients with platinum-refractory GCTs enrolled on study, 1 patient who had progressed on prior oxaliplatin had a hypersensitivity reaction to oxaliplatin [although the patient's serum α-fetoprotein (AFP) declined by 40%] and was inevaluable for response. Examples of tumor response are shown in Fig. 2. Of the nine evaluable patients, three achieved a PR (33%) and three showed SD. Notably, of the three patients who progressed, one developed new brain metastases despite a 65% reduction in his serum AFP, and the other two patients showed disease progression after only 1 week of treatment, prompting removal from study. Overall, 7 of 10 (70%) patients with GCTs who received at least one cycle of treatment showed a decline in tumor markers.

Correlative studies. All nine patients enrolled in the expanded MTD cohort (6a) were eligible for and underwent computed tomography–guided biopsy of their tumor to assess pretreatment p53 status. All samples showed tumor on H&E staining and were adequate for subsequent immunohistochemical analysis for p53. Based on preclinical studies indicating that flavopiridol enhanced the effect of the DNA-damaging agent irinotecan in a p53-dependent manner, we hypothesized that

Table 3. Dose levels, dose-limiting toxicities, and pharmacokinetics

Cohort	Evaluable for DLT* (n = 37)	Evaluable for PK (n = 30)	Flavo (mg/m^2)	OX (mg/m^2)	LV/5FU (mg/m^2)	5FU CI/48 h	DLT	Mean Cmax (μmol/L)
1	6	6	40	60	400/400	1,800	Yes	1.51 ± 0.45
2	3	3	40	85	400/400	1,800	No	1.52 ± 0.38
3	6	6	40	85	400/400	2,400	Yes	0.66 ± 0.34
4a	3	3	50	85	400/400	1,800	No	1.80 ± 0.40
5a	3	3	60	85	400/400	1,800	No	0.94 ± 0.24
6a	12	5	70	85	400/400	1,800	No	2.23 ± 0.51
7a	4	4	80	85	400/400	1,800	Yes	1.72 ± 0.54

Abbreviations: Flavo, flavopiridol; OX, oxaliplatin; LV, leucovorin.
*Forty-eight patients were treated on study; 37 were evaluable for DLT. The additional 11 patients did not complete one cycle of therapy due to a hypersensitivity reaction (n = 3), disease progression (n = 7), or elective withdrawal of consent (n = 1).

Table 4. Clinical activity by tumor type (n = 20)

Tumor type	Response	Duration (wk)	Prior platinum
Anal	SD	29	Cisplatin
Ampullary	SD	33	No
	SD	14	No
Breast	SD	19	No
Colon	SD	31	No
	SD	29	Oxaliplatin
	SD	14	Oxaliplatin
	SD	12	Oxaliplatin
Esophageal	SD	19	Cisplatin, oxaliplatin
Gastric	PR	27	Cisplatin
	PR	22	No
Germ cell tumor	PR	39	Cisplatin, carboplatin
	PR	36	Cisplatin, carboplatin
	PR	17	Cisplatin, carboplatin
	SD	20	Cisplatin
	SD	12	Cisplatin, carboplatin
	SD	11	Cisplatin
Neuroendocrine carcinoma	SD	9	Oxaliplatin
Pancreatic	CR	34	No
Sweat gland carcinoma	PR	25	No

NOTE: Forty-two patients were evaluable for antitumor response. An additional 22 patients had progression of disease as their best response.

patients with pretreatment wild-type p53 positivity would also respond better than patients who were negative (14). However, this was not borne out in our immunohistochemical analysis for p53. In fact, the two patients (GCT, sweat gland) who achieved a PR at the MTD were p53 mutant (≥30% p53-positive tumor cells), and the four patients (1 gastric, 3 GCT) with SD and three patients (GCT, lung, pancreas) with disease progression were p53 wild-type (0-15% p53 positive tumor cells).

Discussion

Based on the success of oxaliplatin as part of the FOLFOX regimen in colorectal cancer and the preclinical evidence that flavopiridol enhances the cytotoxicity of oxaliplatin, we conducted a phase I trial of flavopiridol plus FOLFOX in patients with advanced solid tumors. The primary end point of the trial was to establish the MTD of the drugs used in this combination; additional end points focused on antitumor activity and biological correlates.

Forty-eight patients were treated on this trial, including 16 who had received prior oxaliplatin. Notably, 11 patients did not complete a full cycle of therapy (three treatments in 6 weeks). Although hypersensitivity reactions and patient choice played a role in early withdrawal from the study, seven patients had disease progression based on imaging or symptoms that prompted discontinuation of flavopiridol and FOLFOX (F-FOLFOX) after only one or two treatments. Given the advanced stage and refractory nature of the tumors treated on this study, the early progression rate of 15% (7 of 48 treated patients) seems to be a reasonable expectation and further underscores the need for safe and effective therapies in this population of heavily pretreated patients.

Overall, treatment with F-FOLFOX was well tolerated in the majority of patients despite a median of three prior chemotherapy regimens (range, 0-10). DLTs included neutropenia, thrombocytopenia, nausea and vomiting, and electrolyte abnormalities (hyponatremia, hyperphosphatemia). De-escalation of the 5FU continuous infusion from 2,400 to 1,800 mg/m^2 took place in favor of dose-escalation of flavopiridol. The MTD was established as 70 mg/m^2 flavopiridol, 85 mg/m^2 oxaliplatin, 400 mg/m^2 leucovorin, 400 mg/m^2 5FU bolus, and 5FU continuous infusion over 48 hours at a dose of 1,800 mg/m^2. In 12 patients who were treated at this dose level, no DLTs occurred.

Previous studies of flavopiridol alone, and in combination with chemotherapy, have confirmed an MTD of 70 mg/m^2 when administered as a 1-hour infusion, with a similar DLT profile consisting of neutropenia, diarrhea, and fatigue (6, 15). At this dose level, PK during cycle 1 seemed to be consistent with other chemotherapy combinations (Cmax 2.23 ± 0.51 μmol/L combined with FOLFOX versus Cmax 2.76 ± 0.54 μmol/L combined with irinotecan; ref. 5). However, in contrast to prior studies combining flavopiridol with chemotherapy, p53 wild-type status did not correlate with increased sensitivity. In fact, the patients who had the major tumor regressions were p53 mutant. This may be related to different mechanisms for a DNA damage response between irinotecan and oxaliplatin, such that only irinotecan is p53 dependent.

Antitumor activity was seen across a variety of tumor types in this phase I study, independent of prior treatment with platinum agents. Seven of 42 evaluable patients (17%) experienced either a CR or PR, including four patients who had previously received platinum-based therapy. Although the results documented with F-FOLFOX in our trial are consistent with data from the GERCOR V308 and EFC4584 trials, which reported overall response rates of 10% and 15% with second-line FOLFOX alone for advanced colon cancer (16–18), promising activity was noted in the subset of patients with platinum-refractory GCT treated on this study.

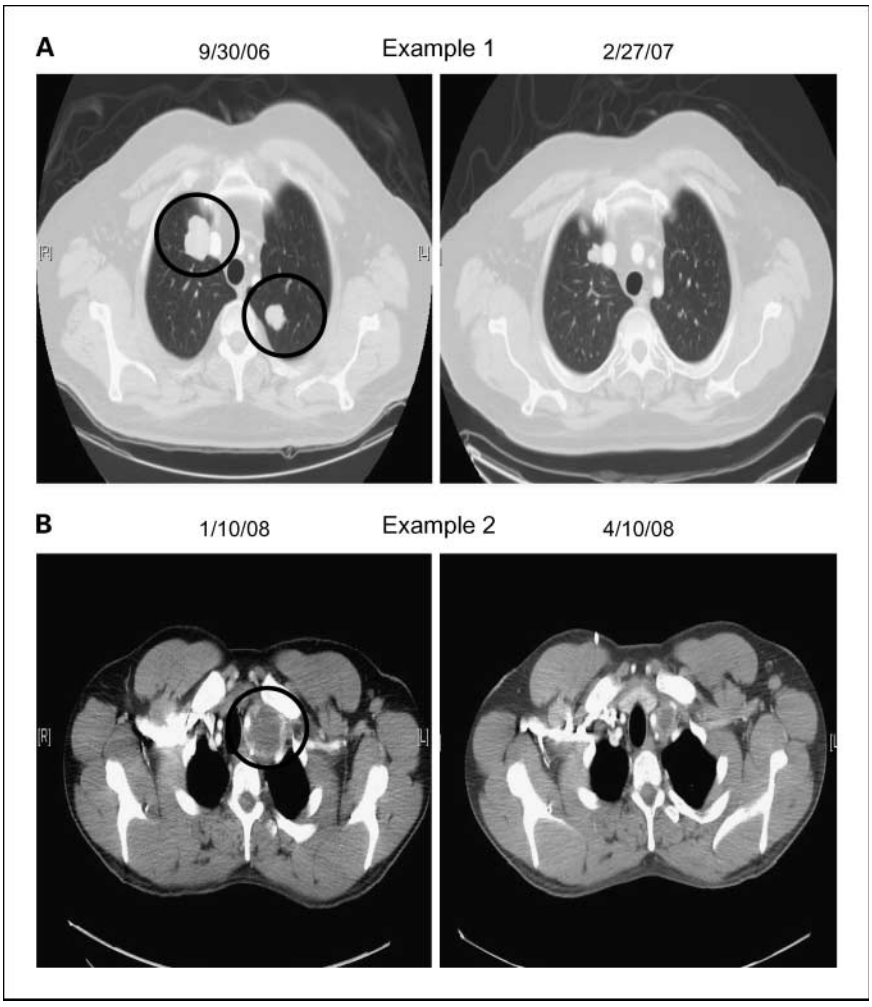

Fig. 2. Radiographic examples of GCT response. Two cisplatin-refractory GCT patients showed areas of disease pretreatment (*circled in black*), which decreased by 65% (*Example 1*) and 43% (*Example 2*) posttreatment.

Ten patients with platinum-refractory GCT were enrolled on the F-FOLFOX trial, five of whom had either a mediastinal primary tumor or late relapse, two features that predict a lack of response to salvage treatments (19). Of the nine patients evaluable for radiographic response by RECIST criteria, three had PR, three had SD, and one patient had progression in the brain despite a 68% reduction in the tumor marker AFP. The two additional GCT patients who showed disease progression on study did so after only one treatment. Another patient who had previously progressed on 130 mg/m^2 oxaliplatin experienced a 40% decline in his AFP after one cycle of F-FOLFOX but came off study due to an oxaliplatin-related hypersensitivity reaction.

For patients with relapsed or refractory GCTs, the optimal treatment regimen has not yet been established. Up to 40% of patients will not be cured after treatment with high-dose therapy in the second-line setting, and specific subgroups of patients, such as those with mediastinal nonseminomatous GCT or primary refractory GCT, have been identified as being particularly unlikely to benefit from this approach (20). Oxaliplatin has previously been studied as a single agent for patients with cisplatin-refractory GCT using two different dosing schedules (21). An initial group of patients was treated with 60 mg/m^2 weekly on days 1, 8, and 15 every 28 days with an overall response rate of 6%. A second cohort was treated with 130 mg/m^2 every 2 weeks with an objective response rate of 19%.

Overall, the combination of FOLFOX plus flavopiridol was well tolerated with activity observed across a range of solid tumors. Taking into account the published response rates to single-agent oxaliplatin, the extent of prior treatment, and the high-risk refractory nature of the GCTs treated on this study, the response in the GCT population is particularly encouraging. A phase II trial of F-FOLFOX for patients with refractory GCTs is currently open to accrual and will continue to explore antitumor activity and the role of p53 relative to treatment response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Kaur G, Stelter-Stevenson M, Sebers S, et al. Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86-8275. J Natl Cancer Inst 1992;84:1736–40.
2. Schwartz GK, Shah MA. Targeting the cell cycle: a new approach to cancer therapy. J Clin Oncol 2005;23:9408–21.
3. Schwartz GK, Farsi K, Maslak P, Kelsen DP, Spriggs D. Potentiation of apoptosis by flavopiridol in mitomycin-C-treated gastric and breast cancer cells. Clin Cancer Res 1997;5:1467–72.
4. Motwani M, Delorhery TM, 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;7:1876–83.

5. Motwani M, Jung C, Sirotnak FM, et al. Augmentation of apoptosis and tumor regression by flavopiridol in the presence of CPT-11 in HCT-116 colon cancer monolayers and xenografts. J Clin Oncol 2001;7:4209–19.

6. Schwartz GK, O'Reilly E, Ilson D, et al. Phase I study of the cyclin-dependent kinase inhibitor flavopiridol in combination with paclitaxel in patients with advanced solid tumors. J Clin Oncol 2002;20:2157–70.

7. Shah MA, 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.

8. Fornier MN, Rathkopf D, Shah M, et al. Phase I dose-finding study of weekly docetaxel followed by flavopiridol for patients with advanced solid tumors. Clin Cancer Res 2007;13:5841–6.

9. Fukuda M, Ohe Y, Kanzawa F, Oka M, Hara K, Saijo N. Evaluation of novel platinum complexes, inhibitors of topoisomerase I and II in non-small cell lung cancer (NSCLC) sublines resistant to cisplatin. Anticancer Res 1995;15: 393–8.

10. Raymond E, Buquet-Fagot C, Djelloul S, et al. Antitumor activity of oxaliplatin in combination with 5-fluorouracil and the thymidylate synthase inhibitor AG337 in human colon, breast and ovarian cancers. Anticancer Drugs 1997;8: 876–85.

11. de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000;18:2938–47.

12. Bible KC, Kaufmann SH. Flavopiridol: a cytotoxic flavone that induces cell death in noncycling A549 human lung carcinoma cells. Cancer Res 1996;56:4856–61.

13. 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.

14. Ambrosini G, Seelman SL, Qin LX, Schwartz GK. The cyclin-dependent kinase inhibitor flavopiridol potentiates the effects of topoisomerase I poisons by suppressing Rad51 expression in a p53-dependent manner. Cancer Res 2008;68: 2312–20.

15. Senderowicz AM, Headlee D, Stinson S, et al. Phase I trial of continuous infusion flavopiridol, a novel cyclic-dependent kinase inhibitor, in patients with refractory neoplasms. J Clin Oncol 1998;16:2986–99.

16. Tournigand C, André T, Achille E, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol 2004;22:229–37.

17. Rothenberg ML, Oza AM, Bigelow RH, et al. Superiority of oxaliplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: interim results of a phase III trial. J Clin Oncol 2003;21: 2059–69.

18. Rothenberg ML, Oza AM, Burger B, et al. Final results of a phase III trial of 5-FU/leucovorin versus oxaliplatin versus the combination in patients with metastatic colorectal cancer following irinotecan, 5-FU, and leucovorin [abstract 1011]. J Clin Oncol 2003;22:252.

19. Motzer RJ, Geller NL, Tan CC, et al. Salvage chemotherapy for patients with germ cell tumors. The Memorial Sloan-Kettering Cancer Center experience (1979-1989). Cancer 1991;67: 1305–10.

20. Feldman DR, Bosl GJ, Sheinfeld J, Motzer RJ. Medical treatment of advanced testicular cancer [review]. JAMA 2008;299:672–84.

21. Kollmannsberger C, Rick O, Derigs HG, et al. Activity of oxaliplatin in patients with relapsed or cisplatin-refractory germ cell cancer: a study of the German Testicular Cancer Study Group. J Clin Oncol 2002;20:2031–7.