A Phase I Clinical Trial of the Sequential Combination of Irinotecan Followed by Flavopiridol

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Abstract

Purpose: Flavopiridol potently enhances the effect of irinotecan with cures in colorectal cancer xenografts, and is associated with modulation of several molecular targets, including p21, Differentiation-related gene 1 (Drg1), and p53. We initiated a phase I trial of the sequential combination of irinotecan followed by flavopiridol to determine the maximal tolerated dose of this combination therapy.

Patients and Methods: Forty-five patients with advanced solid tumors were enrolled. Irinotecan was administered first (100 or 125 mg/m²) followed 7 hours later by escalating flavopiridol (10-70 mg/m²) given weekly over 1 hour for 4 of 6 weeks. At the maximal tolerated dose, the pharmacokinetic analysis was expanded and pre- and posttreatment tumor biopsies were done.

Results: At irinotecan 100 mg/m², dose-limiting diarrhea and myelosuppression were observed with flavopiridol 70 mg/m². At irinotecan 125 mg/m², we observed dose-limiting hyperbilirubinemia, fatigue, and myelosuppression at flavopiridol 60 mg/m². Peak flavopiridol concentrations of ≥2 μmol/L were achieved above flavopiridol 50 mg/m². No significant pharmacokinetic interactions with irinotecan were noted. Baseline serum bilirubin significantly predicted cycle 1 dose-limiting toxicity and neutropenia. We observed partial responses in three patients and prolonged stable disease (i.e., >6 months) in 36% of patients including adrenocortical cancer and hepatocellular cancer. Patients with wild-type p53 and either no change or low posttreatment biopsy p21 and a decrease in Drg1 expression showed stable or responsive disease to the combination therapy.

Conclusions: The recommended phase II dose with irinotecan 100 mg/m² is flavopiridol 60 mg/m² and with irinotecan 125 mg/m² is flavopiridol 50 mg/m². Toxicity can be predicted by baseline bilirubin. Clinical activity is encouraging and may correlate to changes in p21 and Drg1 levels in patients with wild type p53 tumors following therapy.

The rationale for targeting the cell cycle, and in particular cyclin-dependent kinases (CDK), in anticancer therapy lies in the high frequency of their perturbations in human malignancy and the observation that their inhibition, leading to cell cycle arrest, would induce apoptosis (1, 2). Targeting CDKs recapitulate aberrant cell cycle checkpoints, thereby limiting the ability of a tumor cell to cycle and thus facilitating the induction of apoptosis (3). This led to the development of CDK inhibitors as novel antitumor agents (4), with flavopiridol serving as an early model drug in this new class. Flavopiridol inhibits multiple CDKs (5–8), as well as attacks other molecular targets including inhibition of angiogenesis, survivin, IκB kinase and nuclear factor κB (5–11). Although single agent phase I studies (4, 12) showed safety and encouraging clinical activity, single agent phase II trials have shown disappointing activity (13–18), perhaps due to an inability to achieve adequate free drug concentrations due to high protein binding.

There is a growing body of data suggesting that flavopiridol may improve the efficacy of cytotoxic chemotherapy, including irinotecan (19–24). In our xenograft studies, maximal tumor regressions and pathologic cures were shown without significant toxicity in HCT-116 tumor-bearing mice treated with irinotecan followed by flavopiridol at 7 or 16 hours. We also noted the importance of p21 and Differentiation-related gene 1 (Drg1) in these experiments. Specifically, irinotecan administration was associated with the inhibition of cell proliferation, the induction of p53, p21, and Drg1, and a failure to undergo...
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Apoptosis. However, the subsequent administration of flavopiridol was associated with decreases in p21 and Drg1, inhibition of X-linked inhibitor of apoptosis (an inhibitor of caspase 3), and an induction of apoptosis (21, 25).

In these xenograft studies, we administered flavopiridol as an intraperitoneal injection. The rapid i.p. absorption of this drug approximates a shortened i.v. infusion of flavopiridol. This implied that a prolonged continuous infusion of flavopiridol would not be necessary to potentiate the effect of irinotecan. Because prolonged infusions of flavopiridol had been associated with considerable clinical toxicity, we felt it would be possible to avoid these toxicities with the drug administered over a shorter time interval, thereby allowing for potentiation of chemotherapy. On the basis of these laboratory and preclinical studies, we initiated a phase I study of irinotecan at a fixed dose followed 7 hours later by escalating doses of flavopiridol given over 1 hour for 4 of 6 weeks.

Patients and Methods

Adult patients (≥ 18 years old) with a histologically confirmed solid tumor that was refractory to standard therapy (or for which there was no standard therapy) were eligible. Eligibility and exclusion criteria were as previously reported (26). Prior irinotecan therapy was allowed. The protocol was reviewed and approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center. Written informed consent was obtained from each patient.

Treatment plan. This was an open-label, nonrandomized dose-escalation study. Groups of three to six patients received sequentially a fixed dose of irinotecan i.v. over 30 minutes followed 7 hours later by increasing dosages of flavopiridol administered over 60 minutes on a schedule of 4 weeks of therapy followed by a 2-week break. At the initiation of this study, a weekly schedule of flavopiridol had never previously been tested. Therefore, we chose the starting dose for weekly flavopiridol to be 10 mg/m², representing a total dose of 40 mg/m² over the 6-week cycle, which is well below the safe dose of flavopiridol when administered on an every-21-day schedule. Irinotecan was first fixed at 100 mg/m² and flavopiridol was escalated in successive cohorts as follows: 10–30–50–60–70 mg/m². Once the maximal tolerated dose (MTD) of flavopiridol with irinotecan 100 mg/m² was defined, we then determined the MTD of flavopiridol with irinotecan 125 mg/m². Flavopiridol was decreased to one dose level below its MTD with irinotecan 100 mg/m², and then we continued flavopiridol dose escalation in successive cohorts with irinotecan 125 mg/m².

All treatments were administered in the outpatient setting, and once assigned to a dose level, dose escalation was not permitted. For the first two cycles, patients were evaluated by a physician weekly before chemotherapy administration and then at the initiation of every cycle thereafter. Treatment responses were evaluated after every two cycles.

Response Evaluation Criteria in Solid Tumors were used for response assessment and done by an independent protocol radiologist.

Toxicity was graded in accordance with the Common Toxicity Criteria version 2.0. Dose-limiting toxicity (DLT) was defined in cycle 1 with any occurrence of grade 4 hematologic toxicity, grade 3 or 4 nonhematologic toxicity including diarrhea despite antiarrheal prophylaxis. At the onset, we realized that, independent of the flavopiridol administration, some patients would be unable to tolerate 4 weekly treatments of irinotecan followed by a 2-week break. These patients often tolerate intermittent dosing, such as irinotecan for 2 weeks followed by a 1-week break, thereby still receiving 4 of 6 weeks of therapy. Therefore, DLT was also defined as any delay in treatment resulting in less than 4 treatments in a 6-week cycle. 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 (ANC) was ≥1,500/mm³, and platelet count ≥100,000/mm³.

If counts were below these levels, then therapy was delayed until the blood counts recovered. Treatment was also held for incompletely resolved diarrhea or stomatitis. A minimum of three patients were treated for at least one complete cycle (6 weeks) before dose escalation continued. If one instance of DLT was observed, an additional three patients were treated at that dose level. Dose escalation proceeded if these additional patients did not experience DLT. The MTD was defined as the dose one level below the dose at which two or more patients experienced DLT.

At the final MTD, 10 additional patients were enrolled to better define the toxicity profile. We planned to choose the recommended phase II dose based on the cumulative experience of all patients treated at a particular dose level, including the patients in the expanded cohorts, with the use of a confidence interval for toxicity. If 12 patients were treated at a particular dose level, the occurrence of 4 DLTs would define an unacceptable dose for further study.

Drug supply. Flavopiridol (HMR 1275) was supplied by the National Cancer Institute (Bethesda, MD) in 10 and 50 mg sterile vials, as previously reported (26). Flavopiridol was reconstituted in 250 mL of 0.9% Sodium Chloride Injection, USP, or 5% Dextrose for Injection, USP, so that the final concentration did not exceed 0.5 mg/mL. Irinotecan (Campotosar, UpJohn/Pharmacia, New Brunswick, NJ) was commercially available in two forms: 2 mL vials containing 40 mg of drug and 5 mL vials containing 100 mg of drug. The appropriate dose was diluted in 250 mL of 5% dextrose (D5W) solution.

Pharmacokinetics. We examined the plasma levels of flavopiridol and irinotecan and its metabolites, SN-38 and SN-38G, in all patients the first week of the first two cycles of chemotherapy. During week 1 of cycle 1, blood draws were done at the following time points, relative to flavopiridol administration: before flavopiridol (t = 0 hour), at flavopiridol completion (t = 1 hour), 2 hours, and 20 hours. During week 1 of cycle 2 only, the flavopiridol and irinotecan order was reversed so as to obtain pharmacologic data on flavopiridol without the influence of irinotecan. Flavopiridol was given alone on day 1 and irinotecan was given on day 2, 24 hours later. Relative to flavopiridol, blood draws during the first week of cycle 2 were done at the following time points: flavopiridol completion (t = 1 hour), 1.5, 2, 2.5, 3, 7, 9, 10, 24 hours before irinotecan administration). For the next 3 weeks of cycle 2, and for subsequent cycles, irinotecan was given 7 hours before flavopiridol as usual.

At the expanded cohort, the blood draws were expanded to better characterize both irinotecan and flavopiridol pharmacology. During week 1 of cycle 1, blood draws occurred (relative to flavopiridol) at the following times: at irinotecan completion (t = 6.5 hours), before flavopiridol (t = 0 hour), 1, 1.5, 2, 2.5, 3, 17, and 20 hours. During cycle 2, week 1, blood draws occurred at the following times: flavopiridol completion (t = 1 hour), 1.5, 2, 2.5, 3, 7, 9, 10, 24 hours before irinotecan), 24.5 hours (irinotecan completion), 25.5, 26, and 48 hours. Irinotecan, SN-38, and SN-38G pharmacokinetics were done by high-performance liquid chromatography by published methods (27). Flavopiridol pharmacokinetics were done at Quintiles Laboratory (Kansas City, MO) by published methods (26).

Biological assays. Patients enrolled at the expanded cohort at the MTD underwent computed tomography–guided biopsies before initiation of protocol treatment and again after 2 weeks of treatment. All posttherapy computed tomography–guided biopsies occurred within 48 hours of the week 2 treatment. Eighteen-gauge core needle biopsies were done for liver or lymph node biopsies and fine needle aspirates for lung lesions to minimize complications. The biopsy specimen was split in half when possible, fixed in formalin, and embedded in paraffin. Five-micrometer thin sections were cut for hematoxylin and eosin (H&E) and heat-induced epitope retrieval with 0.01 mol/L citric acid (pH 6) were applied.
Drg1 rabbit polyclonal antibody (courtesy of Therese Commes, UPR Center National de la Recherche Scientifique, Universite Montpellier II, France) was used at 1:8,000 final dilution. Sections were immersed in 0.01 mol/L citric acid (pH 6) and boiled in a microwave oven for 15 minutes. After cooling to room temperature, sections were incubated with 10% normal goat blocking serum for 30 minutes, followed by the primary antibody and overnight incubation at 4°C. After washing, biotinylated goat anti-rabbit immunoglobulin G (Vector Laboratories, Burlingame, CA) was applied at 1:1,000 dilution for 30 minutes, followed by avidin-biotin complex (Vector Laboratories) at 1:25 dilution. Diaminobenzidine was used as the final chromogen and hematoxylin as the nuclear counterstain.

Both positive and negative controls were run at the time of each experiment. Nuclear staining was considered specific reaction for both p53 and p21 antibodies and percent of positive tumor cells was estimated examining different fields throughout the entire tissue section. For Drg1, cytoplasmic/plasma membrane staining was considered specific staining and results were recorded as percent of tumor cells reactive with the antibody, as estimated from the examination of different fields throughout the entire tissue section. Data were recorded in a continuum as the percentage of tumor cells stained. The staining was reviewed by two pathologists (M.D. and C.C.) for a consensus assessment for each antigen. Mutant (or positive) p53 staining was considered if greater than 20% of the nuclei stained positive. A change in p21 staining was considered significant if it was greater than 30%. Because the SD of Drg1 expression in 131 patients used to characterize the antigen was ±20% (28), the posttreatment biopsy was considered significantly changed if it was at least 20% different, either increased or decreased, from the pretreatment biopsy for Drg1. This analysis was done without knowledge of patient outcomes.

Statistics. All statistical analyses were done with SAS version 9.0. The probability of DLT was estimated using the observed proportion and 95% confidence intervals were formed by inverting the exact binomial test. The difference between SN-38 levels before and after the administration of flavopiridol was compared using the signed ranks test (paired Wilcoxon test). The area under the curve (AUC) was determined by a one-compartment model for all patients. We noted that at equivalent flavopiridol dosing, the AUCs calculated in patients with expanded pharmacology blood draws were similar with the AUCs calculated with limited pharmacology blood draws, suggesting appropriate AUC estimates. The terminal half-life was determined in the expanded cohorts for which additional pharmacology blood draws were done using a two-compartmental method. Calculation of clearance requires the knowledge of the initial dose which is not available to us for irinotecan metabolites. For this reason, clearance of the metabolites is presented in terms of the formation fraction, which is computed using the irinotecan dose in the calculations (29). The association of AUC and ANC levels with cycle, cohort, and toxicity was computed using the irinotecan dose in the calculations (29).

Results

Patient characteristics. From May 30, 2001 to May 6, 2003, 51 patients with advanced solid tumors were enrolled on protocol. Six of these patients were invaluable for the primary end point of toxicity assessment due to rapid progression of disease (four patients who received only one or two doses of therapy), medical noncompliance (one patient), and patient withdrawal (one patient). Table 1 provides the patient characteristics for the remaining 45 patients who were assessable for toxicity, of whom 43 were assessable for response to the combination therapy. The median age was 55 (range 35-80 years), and the median Karnofsky performance status was 90% (range 70-90%). There were 29 men and 16 women. The cancers treated and frequency included colorectal (28), pancreas and cholangiocarcinoma (three each), breast, hepatocellular, gastric, and adenocarcinoid (two each), esophageal, germ cell, and neuroendocrine (one each). Forty-three patients (96%) had received prior chemotherapy, and 35 (78%) had received prior irinotecan (33 patients) or the related camptothecin, DX-8951F (2 patients), with one patient having received both drugs before participation in this trial. The median number of prior regimens for metastatic disease was 2 (range 0 to 6), with 38 (84%) having received between 1 and 4 prior treatment regimens.

Toxicity. Table 2 provides common cycle 1 nonhematologic and hematologic grades 2 to 4 toxicity that was possibly, probably, or definitely attributable to chemotherapy. All toxicities shown were either DLTs or occurred more than once in each cohort. Patients enrolled in the first four cohorts completed cycle 1 without DLT. However, in cohort 5, we observed 3 DLTs. One patient developed grade 3 diarrhea and two other patients were unable to receive 4 weekly treatments within the 6-week cycle due to modest (grade 2) but sustained myelosuppression. As shown in Table 3, each of these patients had previously received and tolerated weekly doses of irinotecan at 125 mg/m², thereby suggesting the toxicity observed was due to the combination therapy.

We then continued enrollment by reducing flavopiridol by two dose levels (to 50 mg/m²) and increasing irinotecan to 125 mg/m² in cohort 6. In this cohort, one of six patients developed dose-limiting grade 3 fatigue and diarrhea. In retrospect, this...
toxicity may have been predicted in this patient because he previously experienced similar toxicity with irinotecan, fluorouracil, and leucovorin (see Table 3). However, as this was the only patient with DLT at this dose level, dose escalation continued.

In the first three patients treated in cohort 7, one patient with metastatic cholangiocarcinoma developed dose-limiting hyperbilirubinemia and fatigue. After the first dose of irinotecan and flavopiridol, he presented with grade 2 fatigue and a bilirubin that had increased from 1.3 to 3.4 mg/dL. Week 2 was held, and the patient presented on week 3 with a bilirubin back to baseline, however, with grade 3 fatigue. A computed tomography scan at that point showed disease progression. Although this toxicity was unusual, we felt it was possibly attributed to the combination therapy, and the cohort was expanded to six patients. In the expanded cohort, another patient developed grade 3 fatigue. This patient was subsequently retreated at a reduced dose of irinotecan 100 mg/m² and did not develop subsequent significant toxicity.

Although we observed two of six patients with DLT, it was unclear whether we had exceeded our MTD because one of the patients also had evidence of disease progression. Therefore, to better evaluate the tolerability of this dose level, we subsequently expanded this dose level with six additional patients (cohort 7b) for a total of 12 treated at this dose level. We observed two additional patients developing DLT, both unable to receive 4 weeks of therapy within the 6-week cycle due to sustained modest myelosuppression. Thus, a total of 4 of 12 patients treated at this dose level developed DLT in cohort 7. Three of these patients had received prior weekly irinotecan-based therapy (Table 3), which they had tolerated without significant toxicity, thereby suggesting intolerance to the combination therapy rather than to irinotecan alone.

Thus, the MTD of flavopiridol with irinotecan 100 mg/m² is 60 mg/m², and with irinotecan 125 mg/m² is 50 mg/m². We next treated 10 patients in an expanded cohort at the MTD (cohort 8) to better establish the recommended phase II doses of the combination therapy. Based on the patient’s number of prior regimens and the potential for increased myelosuppression, it was left to the discretion of the treating physician whether to treat with flavopiridol at full-dose irinotecan (125 mg/m²) or a lower dose of irinotecan (100 mg/m²). In the nine additional patients treated at irinotecan 100 mg/m² and flavopiridol 60 mg/m², we observed three patients with DLT; one patient was unable to receive 4 treatments in 6 weeks, and the other two with grade 4 hematologic toxicity. Two of these patients were previously heavily treated (four and six previous therapies, respectively), and the third DLT occurred in a patient who previously developed neutropenia with gemcitabine therapy alone. Of note, one patient in the expanded cohort was treated at irinotecan 125 mg/m² and flavopiridol 50 mg/m², and no DLT was observed.

Cumulative toxicity was not significantly different from cycle 1 toxicity (data not shown). The most common cumulative nonhematologic toxicities included grade 2 diarrhea (46%), fatigue (31%), nausea (20%), and vomiting (22%). Grade 3 diarrhea, nausea, and vomiting occurred in 5 (11%), 4 (9%), and 2 (4%) patients, respectively. Common cumulative hematologic toxicities included grade 2 leukopenia (42%), grade 2 neutropenia (36%), and grade 2 anemia (22%). Grades 3 to 4 neutropenia occurred in 12 patients (27%), and was complicated by fever and neutropenia in 4 patients (9%). There does not seem to be cumulative hematologic toxicity in these patients, as the mean hematologic values for leukocytes, neutrophils, platelets, and hemoglobin are similar for cycle 1 and for all cycles. There were no treatment-related deaths on this study.

**Toxicity and its relationship with baseline bilirubin.** In one patient, we observed an interesting relation between a fluctuating bilirubin and myelosuppression. This patient had a baseline bilirubin of 1.6 mg/dL and ranged from 0.9 to 2.4 mg/dL. This patient also developed grade III neutropenia.

### Table 2. Cycle 1 nonhematologic and hematologic toxicity

| Cohort (patients) | CPT | CPT F | Fatigue | Diarrhea | Nausea | Vomiting | Bilirubin | Anemia | Leukocytes | ANC | Neutropenia | Fever/ |
|-------------------|-----|-------|---------|----------|--------|----------|-----------|--------|------------|-----|-------------| 4 of 6 |
| Cohort 1 (3)      | 100 | 10    | 1       | 1        | 1      | 1        | 1         | 1      | 1          |     |             |       |
| Cohort 2 (3)      | 100 | 30    | 2       |          |        |          | 1         | 1      | 1          |     |             |       |
| Cohort 3 (3)      | 100 | 50    | 1       |          |        |          | 1         | 1      | 1          |     |             |       |
| Cohort 4 (3)      | 100 | 60    | 1       | 2        | 1      |          | 1         | 1      | 1          |     |             |       |
| Cohort 5 (5)      | 100 | 70    | 1       | 1        | 1      | 1        | 1         | 4      | 4          | 2   |             | 4 of 6 |
| Cohort 6 (6)      | 125 | 50    | 2       | 1        | 1      | 2        | 3         | 3      | 3          |     |             |       |
| Cohort 7a (6)     | 125 | 60    | 1       | 1        | 1      | 1        | 1         | 1      | 1          | 1   |             |       |
| Cohort 7b (6)     | 125 | 60    | 1       | 2        | 1      | 1        | 1         | 2      | 3          | 2   |             |       |
| Cohort 8 (9)      | 100 | 60    | 2       | 3        | 2      | 1        | 2         | 5      | 2          | 1   | 5           |       |
| (1)               | 125 | 50    | 1       |          |        |          |           |        |             |     | 1           |       |

**NOTE:** DLTs are in boldface. Grades 2-4, possibly, probably, or definitely attributable to chemotherapy.

*Six patients were initially treated in this cohort, but then the cohort was expanded with six additional patients to better evaluate toxicity.

*One of these patients also had grade 3 bilirubinemia. Additional toxicity includes one patient in cohort 6 who developed grade 2 hiccups, one patient in cohort 7 who developed grade 2 alopecia, and one patient with grade 2 weight loss in the expanded cohort (irinotecan 100 mg/m², flavopiridol 60 mg/m²).
with therapy, requiring dose delays, which seemingly correlated with high bilirubin levels during the period of neutropenia. We note that SN-38, flavopiridol, and bilirubin are all glucuronidated by a common pathway involving the family of UGT1A glucuronidation enzymes (30, 31). Polymorphisms in the UGT1A1 promoter affect the efficiency of this enzyme, which can be reflected in a patient’s baseline bilirubin such that an elevated serum bilirubin would suggest slow bilirubin glucuronidation, and therefore possibly reduced UGT1A enzymatic efficiency (32). To examine the hypothesis that slow glucuronidation may be associated with increased active drug levels, and consequently to increased toxicity of the combination regimen, we retrospectively explored the relationship between baseline serum bilirubin (as a marker of UGT1A1 activity) and toxicity. The median baseline serum bilirubin across all dose levels on this study was 0.7 mg/dL. Using this level as a cutoff, the probability of experiencing DLT with a bilirubin of <0.7 mg/dL was significantly less than if the bilirubin was ≥0.7 mg/dL. Specifically, we observed 1 DLT of 18 patients (5.8%) with a bilirubin <0.7 mg/dL, whereas there were 10 DLTs of 27 patients (37%) with a baseline bilirubin of ≥0.7 mg/dL, \( P = 0.03 \). The baseline serum bilirubin also correlated with neutropenia in cycle 1 such that patients with a baseline serum bilirubin of <0.7 mg/dL had a significantly higher mean cycle 1 ANC as well as a significantly higher cycle 1 ANC nadir than if the serum bilirubin at baseline was ≥0.7 mg/dL. (Wilcoxon \( P = 0.01 \) for both mean and nadir ANC). Note that of the 33 patients who had previously received irinotecan-based therapy, we were able to obtain the baseline bilirubin (before their previous irinotecan treatment) in 20 patients. We noted no significant differences between their previous bilirubin baseline and the baseline bilirubin before initiation of this study, suggesting that the association of bilirubin and toxicity is related to the combination therapy.

**Pharmacokinetics and pharmacodynamics.** Plasma samples for pharmacokinetic analyses were obtained from all 45 patients, although cohort 1, cycle 1 pharmacology is not available. The one-compartment modeled flavopiridol plasma concentration-time profile (flavopiridol 60 mg/m²) for cycle 1 (following irinotecan) and cycle 2 (preceding irinotecan) is presented in Fig. 1. Table 4 summarizes the flavopiridol pharmacokinetic variables for each dose level examined for cycles 1 and 2. Flavopiridol AUC increased significantly with dose \( (P < 0.01, \text{using local regression}) \), however, the rate of increase is dampened above flavopiridol 60 mg/m². There was no significant difference between flavopiridol AUC in cycles 1 and 2. Furthermore, at equivalent flavopiridol dosing, there was no significant difference in flavopiridol AUC when given with irinotecan 100 or 125 mg/m². At the flavopiridol MTD range (50-60 mg/m²), the mean flavopiridol pharmacokinetic variables are as follows: AUC \( (7.36-14.04 \text{ A} \text{mol/L/h}) \), clearance \( (12.8-26.1 \text{ L/h/m²}) \), and terminal half-life \( (5.1-7.7 \text{ hours}) \). Flavopiridol \( C_{\text{max}} \) or AUC in cycle 1 did not correlate with toxicity of the combination regimen, including cycle 1 DLT, ANC nadir, and mean ANC in cycle 1.

We expanded the pharmacology blood draws in cohorts 7b and 8 to further characterize irinotecan pharmacokinetic. The cycles 1 and 2 irinotecan, SN-38, and SN-38G pharmacokinetic variables are presented in Table 5. The terminal half-lives for irinotecan, SN-38, and SN-38G in cycle 1 are 7.6, 16, and 18.1 hours, respectively. There was no significant difference between the pharmacokinetic variables for irinotecan and its metabolites between cycles 1 and 2.

### Table 3. Summary of DLTs and previous irinotecan-based therapy

<table>
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<th>Cohort (patients)</th>
<th>Irinotecan</th>
<th>Flavopiridol</th>
<th>Pts with DLT</th>
<th>Toxicity</th>
<th>Prior irinotecan therapy (irinotecan dose)</th>
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<td>Cohort 5 (5)</td>
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<td>IFL (125 mg/m² weekly)</td>
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<td></td>
<td></td>
<td></td>
<td>4 of 6</td>
<td>IFL (125 mg/m² weekly)</td>
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<td>125</td>
<td>50</td>
<td>1</td>
<td>G 3 fatigues and diarrhea</td>
<td>IFL (100 mg/m² weekly)</td>
<td>Grade 3 fatigue and diarrhea</td>
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<td>Cohort 7b (6)</td>
<td>125</td>
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<td></td>
<td>G 4 neutropenia + fever</td>
<td>IFL 1 SN-38 80 mg/m² twice weekly</td>
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</table>

*IFL: irinotecan 100-125 mg/m², fluorouracil 500 mg/m², leucovorin 20 mg/m² weekly for 4 weeks followed by a 2-week break.

† IHA FUDR: intrahepatic pump chemotherapy with flouxuridine.
**Antitumor activity.** Forty-three patients were assessable for response assessment. We observed three patients with a partial response (two colorectal, one gastric), and 20 patients with stable disease, many of whom remained on study for prolonged periods of time, including 16 patients who remained on study for more than 6 months (36% of all enrolled patients) and 4 patients who remained on study for over 1 year. The median time on study for patients with partial responses or stable disease was 7.6 months with a range of 3.7 to 16.5 months. Notable durable stable responses included two patients with hepatocellular carcinoma who remained on study for 13.8 and 16.5 months, and two patients with adenocarcinoma who remained on study with stable disease for 8.1 and 15.4 months. There was no association between the prior use of camptothecin and response to therapy.

Among the 27 evaluable patients with colorectal cancer, patients had a median of 2 prior treatment regimens with a range of 1 to 6 prior regimens. Twenty-three patients had received prior irinotecan, and oxaliplatin before initiation of this clinical trial. We observed 14 patients (52%) with stable disease, one partial response, and 12 patients (44%) with disease progression as their best response. Patients with stable or responsive disease had durable periods of disease control with a median of 6.8 months (range 3.6-12.7 months).

**Correlative studies.** All 10 of the patients enrolled in the expanded cohort at the MTD (cohort 8) were eligible for and underwent computed tomography–guided biopsy of their tumor. These included six patients with colorectal cancer, and one each with gastric, pancreas, hepatocellular, and breast cancer. We planned pre- and posttreatment biopsies on each patient for a total of 20 biopsies. We were able to perform 19 biopsies (10 pretreatment and 9 posttreatment), with one patient not receiving a posttreatment biopsy because of a negative pretreatment biopsy. Seventeen biopsies (89%) showed tumor on H&E staining, and 15 biopsies (78%) were adequate for subsequent immunohistochemical analysis. Even following two treatments of irinotecan and flavopiridol, there was pathologic evidence of antitumor activity with tumor necrosis and apoptosis identified in H&E staining in one patient with radiographically stable disease (Fig. 2). The induction of apoptosis was confirmed by terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (not shown). Two biopsies had no tumor on further sectioning. No biopsy-related complications were observed.

Tumors were evaluated for staining by immunohistochemistry for p53, p21, and Drg1, both at pretreatment and within 24 to 48 hours following the week 2 therapy (Table 6). As expected, p53 expression did not change when comparing the pre- and posttreatment biopsies. Therefore, a single result is reported. Matched samples for p21 staining are available for five patients. In the six patients who were p53 wild-type (negative p53 staining), p21 remained stable or was non-detectable on the posttreatment biopsy in three patients (patients 6, 8, and 9), all of whom had stable disease or a partial response. The change in p21 is uninterpretable in two patients (patients 2 and 7), both of whom also had stable disease. Matched samples for Drg1 staining are available for six patients. Figure 2 shows one patient with pre- and posttreatment biopsies stained for Drg1. We observed in five of six patients (84%) that the modulation of Drg1 correctly predicted outcome. Patients 1 and 3 (increase and no change) had disease progression, and patients 6, 7, and 8 (all with decreases in Drg1 following irinotecan and flavopiridol therapy) each had radiographic stable disease and remained on study for more than 7 months.

The patients who progressed were either p53 mutant (patients 3, 4, and 5) or were p53 wild-type, but with a rising

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**Table 4.** Flavopiridol pharmacokinetic variables for cycle 1 and cycle 2 for each dose level

<table>
<thead>
<tr>
<th>Dose levels (patients)</th>
<th>Cycle 1</th>
<th></th>
<th></th>
<th>Cycle 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irinotecan</td>
<td>Flavopiridol</td>
<td>Cmax (µmol/L)</td>
<td>SD</td>
<td>AUC (µmol/L h)</td>
<td>SD</td>
</tr>
<tr>
<td>1 (3)</td>
<td>100</td>
<td>10</td>
<td>1.247 (0.244)</td>
<td>6.37 (1.82)</td>
<td>11.31 (2.80)</td>
<td>11.67 (0.48)</td>
</tr>
<tr>
<td>2 (3)</td>
<td>100</td>
<td>30</td>
<td>2.044 (0.600)</td>
<td>10.02 (0.69)</td>
<td>11.67 (0.48)</td>
<td>1.921 (0.282)</td>
</tr>
<tr>
<td>3 (3)</td>
<td>100</td>
<td>50</td>
<td>3.078 (1.72)</td>
<td>7.12 (8.48)</td>
<td>9.34 (9.34)</td>
<td>3.145 (1.829)</td>
</tr>
<tr>
<td>4 (12)</td>
<td>100</td>
<td>60</td>
<td>2.768 (0.545)</td>
<td>16.24 (5.46)</td>
<td>10.14 (1.95)</td>
<td>2.512 (1.213)</td>
</tr>
<tr>
<td>5 (5)</td>
<td>100</td>
<td>70</td>
<td>2.137 (0.677)</td>
<td>12.79 (6.16)</td>
<td>9.59 (3.07)</td>
<td>1.888 (0.756)</td>
</tr>
<tr>
<td>6 (7)</td>
<td>125</td>
<td>50</td>
<td>3.861 (2.017)</td>
<td>18.49 (9.27)</td>
<td>8.59 (2.63)</td>
<td>3.375 (1.128)</td>
</tr>
<tr>
<td>7 (12)</td>
<td>125</td>
<td>60</td>
<td>4.000 (2.017)</td>
<td>18.49 (9.27)</td>
<td>8.59 (2.63)</td>
<td>3.375 (1.128)</td>
</tr>
</tbody>
</table>
p21 and Drg1 following therapy (patient 1). Although the numbers are small, these data support the preclinical model that baseline wild-type p53 and pharmacodynamic changes in p21 and Drg1 may be predictive of response.

**Table 5.** Pharmacokinetic variables for cycle 1 and cycle 2 for the expanded cohorts for which additional pharmacology blood draws were done for CPT and its metabolites

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (mol/L)</td>
<td>SD</td>
</tr>
<tr>
<td>Irinotecan 100</td>
<td>1,928.39</td>
<td>412.80</td>
</tr>
<tr>
<td>125</td>
<td>2,024.96</td>
<td>575.22</td>
</tr>
<tr>
<td>SN-38 100</td>
<td>74.52</td>
<td>58.71</td>
</tr>
<tr>
<td>125</td>
<td>88.21</td>
<td>361.8</td>
</tr>
<tr>
<td>SN-38G 100</td>
<td>178.35</td>
<td>208.21</td>
</tr>
<tr>
<td>125</td>
<td>195.86</td>
<td>173.30</td>
</tr>
</tbody>
</table>

*Clearance/F<sub>M</sub> is the clearance divided by the formation fraction.

This represents the first study in which flavopiridol was administered on a 1-hour weekly schedule, either as a single agent or in combination therapy. On this schedule, we achieved micromolar concentrations of flavopiridol, which is significantly higher than reported with continuous infusion schedules of 24 to 72 hours (26, 33). Assuming 10% free drug concentrations, the 2 to 3 µmol/L levels we achieved at the flavopiridol MTDs should result in free flavopiridol concentrations of 200 to 300 nmol/L, which have been shown in vitro to significantly potentiate the effects of chemotherapy (34).

The only other flavopiridol trial in which the 1-hour infusion of flavopiridol was tested clinically was reported by Tan and colleagues. This group reported on three different 1-hour infusion schedules of flavopiridol, including daily for 5 days, daily for 3 days, and over 1 hour for a single treatment but repeated every 3 weeks (35). The recommended phase II

**Fig. 2.** Pre- and posttreatment biopsy H&E and Drg1 stains at 400× magnification. A and C, H&E and Drg1 stains, respectively, of the pretreatment baseline biopsy obtained within 2 weeks of study entry. B and D, H&E and Drg1 stains, respectively, of the posttreatment biopsy that was obtained within 48 hours of the week 2 treatment of CPT and flavopiridol. B and D, significant treatment effects including vacuolization, chromatin clumping, and apoptotic bodies.
dose for flavopiridol administered 1 hour on an every-3-week schedule was 62.5 mg/m². We observed similar DLTs, including neutropenia, diarrhea, and fatigue, with the more frequent weekly dosing. However, we did not observe several of the nonhematologic toxicities previously reported, such as anorexia, hypotension, hypophosphatemia, and hypalbuminemia (35). In both Tan’s study and our study, flavopiridol doses above 70 mg/m² were associated with significant and unacceptable toxicity. At the MTD in our study, the pharmacokinetic variables of flavopiridol, including \( C_{\text{max}} \), AUC, clearance, and terminal half-life, are consistent with previous reports of similar short infusion flavopiridol schedules (35, 36). Thus, our study suggests that patients are able to receive another flavopiridol dose 1 week later, presumably because of having recovered from the previous dose 1 week earlier. Furthermore, the rate of increase in AUC with flavopiridol doses above 60 mg/m² was less pronounced, suggesting an apparent threshold for flavopiridol dosing above which clearance of the drug is possibly increased. This will need further confirmatory evaluation in larger pharmacology studies with this drug in this schedule. Interestingly, thrombotic events, which were a cause for concern with flavopiridol when administered as a 3-day continuous infusion, were rarely observed with flavopiridol administered over 1 hour in both studies.

It remains to be determined what is the best schedule to administer irinotecan with flavopiridol. Even though we could not administer the combination for 4 consecutive weeks in all patients, we still believe the weekly schedule of irinotecan with flavopiridol is superior to an every-3-week irinotecan schedule as it provides more frequent opportunity to potentiate chemotherapy with this CDK inhibitor. Notably, we did not observe a significant pharmacokinetic interaction between irinotecan and flavopiridol.

We found a significant correlation between baseline serum bilirubin and the probability of toxicity of the combination therapy, in particular myelosuppression. Transient hyperbilirubinemia was previously observed with 1-hour flavopiridol administration as well, suggesting a shared pathway with bilirubin and flavopiridol metabolism (35, 37, 31). Similarly, bilirubin and irinotecan share this metabolic pathway as well (30, 38, 39). In one recent study, there was a significant association between an elevated baseline serum bilirubin and the occurrence of grades 3 or 4 neutropenia in patients receiving a weekly schedule of irinotecan (40). In our study, patients with a baseline serum bilirubin below the median were significantly less likely to have DLT or neutropenia. Although a significant portion of the toxicity predicted by the baseline serum bilirubin may have been attributable to irinotecan, virtually all of the patients enrolled on this study previously received irinotecan-based therapy and tolerated it, and their previous baseline bilirubin value was not significantly different than when they initiated this study. This suggests that the serum bilirubin may be a sensitive indicator of toxicity of the combination of irinotecan and flavopiridol because both active moieties are metabolized and inactivated by the same pathway, the activity of which can be assayed by the serum bilirubin level. Such data place into question the current definitions of DLT with the combination regimen, and indicate that, perhaps, the optimal dose of the combination for individual patients may be defined by their baseline serum bilirubin. This hypothesis would require prospective confirmatory evaluation.

Notably, the UGT1A1 polymorphisms were examined with regard to flavopiridol toxicity previously (36). In that study, UGT1A1 polymorphisms were not associated with flavopiridol pharmacokinetics or flavopiridol-induced diarrhea. It is possible that UGT1A9 is the primary enzyme responsible for flavopiridol glucuronidation (41). Examination of polymorphisms in the promoter region of UGT1A1 and UGT1A9 is planned in future studies examining irinotecan and flavopiridol combinations.

Our preclinical model suggested that the potentiation of irinotecan by flavopiridol is related to suppression of p21 and Drg1 following irinotecan therapy (21, 25). p21 is both an inhibitor of CDKs, causing both G1 and G2 cell cycle arrests, as well as an inhibitor of apoptosis by binding to and inhibiting caspase 3. It has recently been reported that a decrease in p21
expression following preoperative chemoradiotherapy for rectal cancer was associated with improved disease-free survival (42). Thus, induction of p21 seems to be a novel mechanism for chemotherapy resistance. Interestingly, irinotecan induces p21, especially in tumors with an intact (or wild-type) p53 pathway (21). Drg1 is a recently identified gene product that is upregulated in differentiation and may be a metastatic suppressor in colon cancer (43) and in breast and prostate cancers (44, 45). With regard to resistance to irinotecan, similarly, Drg1 is transcriptionally induced by irinotecan treatment and is associated with resistance to irinotecan-based therapy. Subsequent administration of flavopiridol is associated with a decrease in Drg1 and subsequent sensitivity to irinotecan (25). We elected to explore this hypothesis in this phase I study by conducting pre- and posttreatment biopsies at the expanded MTD. We observed that patients who showed disease control indeed were p53 wild-type and either had a low p21 level that did not increase or a decrease in Drg1 expression. Patients with disease progression had an increase in p21 and Drg1 or were p53 mutants. Although preliminary, these data support the preclinical model that sensitivity to the combination is related to the p53-p21 DNA damage/repair axis. These preliminary studies have led to ongoing translational laboratory research aimed at better defining the role of these proteins in increasing irinotecan sensitivity, and suggest that flavopiridol achieves this by suppressing discrete molecular events that prevent apoptosis in irinotecan-treated tumor cells. Confirmatory prospective evaluation is again necessary for definitive conclusions.

This combination has proven to be safe and tolerable, and is associated with interesting clinical activity with patients having stable or responsive disease and remaining on study for prolonged periods of time. There are several patients who have remained on study for over 12 months without cumulative toxicity. Overlapping glucuronidation pathways may be important in drug metabolism and consequent toxicity of the combination therapy. Finally, our correlative studies support the biological basis for the combination. This combination is being evaluated in a phase II clinical trial in hepatocellular carcinoma, and in combination with cisplatin and with 5-fluorouracil/leucovorin in separate phase I studies. Laboratory efforts to better understand resistance and sensitivity pathways are ongoing.

References


Clinical Cancer Research

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