A Phase I, Pharmacokinetic and Pharmacodynamic Study on Vorinostat in Combination with 5-Fluorouracil, Leucovorin, and Oxaliplatin in Patients with Refractory Colorectal Cancer

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Abstract

Purpose: We conducted a phase I study to determine the maximum tolerated dose of vorinostat in combination with fixed doses of 5-fluorouracil (FU), leucovorin, and oxaliplatin (FOLFOX).

Experimental Design: Vorinostat was given orally twice daily for 1 week every 2 weeks. FOLFOX was given on days 4 and 5 of vorinostat. The vorinostat starting dose was 100 mg twice daily. Escalation occurred in cohorts of three to six patients. Pharmacokinetics of vorinostat, FU, and oxaliplatin were studied.

Results: Twenty-one patients were enrolled. Thrombocytopenia, neutropenia, gastrointestinal toxicities, and fatigue increased in frequency and severity at higher dose levels of vorinostat. Two of 4 evaluable patients at dose level 4 (vorinostat 400 mg orally twice daily) developed dose-limiting fatigue. One of 10 evaluable patients at dose level 3 (vorinostat 300 mg orally twice daily) had dose-limiting fatigue, anorexia, and dehydration. There were significant relationships between vorinostat dose and the area under the curve on days 1 and 5 (Pearson, < 0.001). The vorinostat area under the curve increased ($P = 0.005$) and clearance decreased ($P = 0.003$) on day 5 compared with day 1. The median C_{max} of FU at each dose level increased significantly with increasing doses of vorinostat, suggesting a pharmacokinetic interaction between FU and vorinostat. Vorinostat-induced thymidylate synthase (TS) modulation was not consistent; only two of six patients had a decrease in intratumoral TS expression by reverse transcription-PCR.

Conclusions: The maximum tolerated dose of vorinostat in combination with FOLFOX is 300 mg orally twice daily $\times$ 1 week every 2 weeks. Alternative vorinostat dosing schedules may be needed for optimal down-regulation of TS expression.

Major advances in the systemic treatment of metastatic colorectal cancer have occurred in the last decade. The addition of oxaliplatin or irinotecan to 5-fluorouracil (FU) chemotherapy in the first-line setting has resulted in significant improvements in progression-free survival and overall survival (1–3). Furthermore, the inhibition of the epidermal growth factor or vascular growth factor receptors has resulted in improvements in progression-free survival in the first-, second-, and third-line treatments of colorectal cancer (4–8). Despite improvements in cytotoxic and targeted therapy, the median overall survival of patients with metastatic colorectal cancer is ≤26 months, and their 5-year overall survival rate remains ~11% (9). Therefore, targeting of novel pathways essential for tumor survival or treatment resistance is essential to ensure further improvement in the outcome of patients with metastatic unresectable colorectal cancer.

Histone deacetylases have been recently identified as potential anticancer targets. Three classes of histone deacetylase have been identified in humans (10–12). Class I includes histone deacetylases 1, 2, 3, and 8, which are related to yeast RPD3 deacetylase. Histone deacetylases 1 and 2 are overexpressed in colonic tumors, suggesting that histone deacetylase may be a potential target in the treatment of that disease (13, 14). It has also been recently shown that histone deacetylase 3 is overexpressed in colorectal cancer and that its inhibition results in antitumor activity that is independent of other individual histone deacetylase (15). The mechanisms of growth inhibition produced by histone deacetylase inhibitors include effects on gene expression, cell cycle progression, and cell death pathways; these have been reviewed elsewhere (15–23).

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Translational Relevance

Vorinostat, a histone deacetylase inhibitor, has been associated with thymidylate synthase down-regulation and synergy with 5-fluorouracil (FU) in preclinical studies. In this phase I clinical trial, we have evaluated escalating doses of vorinostat in combination with a fixed dose of FU, leucovorin, and oxaliplatin (FOLFOX). We show that vorinostat can be administered safely up to doses of 300 mg orally twice daily × 1week every 2 weeks in combination with a full dose of FOLFOX. However, despite this high dose of vorinostat, the pharmacokinetics of vorinostat was not optimal for the downstaging of thymidylate synthase expression by reverse transcription-PCR and immunohistochemistry from serial tumor biopsies. Our data suggest the need of shorter intermittent high doses of vorinostat in combination with FOLFOX or FU/leucovorin. Indeed, such regimens (vorinostat once daily or twice daily × 3 days every 2 weeks with FU/leucovorin or FOLFOX) are currently being investigated in our institute with promising preliminary results.

Vorinostat (suberoylanilide hydroxamic acid) is a potent inhibitor of class I and II histone deacetylase with proven clinical activity against cutaneous T-cell lymphomas (24). Despite its single-agent activity in cutaneous lymphomas, vorinostat has failed to show any notable clinical activity as a single agent against solid tumors (25–27). However, the ability of vorinostat to modulate several genes implicated in tumor growth has triggered ongoing interest in its use when combined with various chemotherapeutic agents (28). *In vitro* and *in vivo* models have shown that vorinostat can down-regulate thymidylate synthase (TS) expression by as much as 100-fold when measured by quantitative PCR analysis, and this down-regulation has been confirmed by Western blot after only 24 hours of vorinostat exposure (28, 29). In that TS overexpression has been associated with clinical resistance to FU, it is feasible that vorinostat may overcome such resistance by down-regulating TS expression (28). Indeed, vorinostat has been shown to potentiate FU antitumor activity in preclinical models (30). Vorinostat has also been shown to potentiate the antitumor activity of platinum-containing agents *in vitro*. This effect is likely secondary to DNA unwinding and increased accessibility of DNA-targeting agents (31).

Given the preclinical data supporting the addition of vorinostat to fluoropyrimidines and platinum-containing agents, we conducted a phase I clinical trial to determine the maximum tolerated dose of vorinostat when administered with a standard fixed dose of folinic acid (leucovorin), FU, and oxaliplatin (FOLFOX) in patients with refractory colorectal cancer.

Materials and Methods

This phase I, open-label, dose-escalation study on vorinostat in combination with a fixed dose of FOLFOX was conducted at Roswell Park Cancer Institute (Buffalo, NY). The primary objective of the study was to determine the maximum tolerated dose of twice-daily oral vorinostat given for 1 wk every 2 wk in combination with FOLFOX on days 4 and 5 of vorinostat. Secondary objectives included evaluation of vorinostat, FU, and oxaliplatin pharmacokinetics; description of treatment-related toxicities; and description of any observed clinical responses. Exploratory endpoints included the evaluation of vorinostat effects on intratumoral TS expression.

Patient criteria

Patients with metastatic colorectal cancer who had failed at least two previous lines of treatment, including oxaliplatin, a fluoropyrimidine, and irinotecan, were eligible for enrollment. Treatment failure was defined as progression on or within 3 mo from last treatment. In addition, patients had to be ≥18 y of age, have an Eastern Cooperative Oncology Group performance status of 0 to 1, have an expected survival of at least 12 wk, and have acceptable organ function defined as: WBC count ≥ 3,000/μL, absolute neutrophil count ≥ 1,500/μL, serum creatinine less than or equal to the upper institutional normal level, total bilirubin less than or equal to upper institutional normal level, and serum aspartate aminotransferase and alanine aminotransferase ≤ 2.5 × upper institutional normal. Patients could not have received any chemotherapy within 4 wk from initiation of study treatment, with the exception of nitrosureas or mitomycin C, which required a 6-wk interval before study treatment. Patients with brain metastases, grade ≥ 2 neuropathy, or other severe intercurrent illness were excluded. Patients who were human immunodeficiency virus–positive and taking antiretroviral medicines were excluded because of potential drug-drug interactions. No other histone deacetylase inhibitors (such as valproic acid) or other investigational agents were allowed while patients were on study. Patients taking drugs with major inhibitory or stimulatory effects on CYP450 enzymes were not allowed to participate because of the potential interaction with vorinostat. Pregnant or lactating females were not allowed on study. All consenting patients having the potential of conceiving agreed to the use of double contraception during the study period. The study and consent form were approved by the Institutional Scientific and Review Committee and the institutional review board before the study was activated. All patients provided signed informed consent before study entry. The study was conducted in accordance with the Good Clinical Practice Guidelines as issued by the International Conference on Harmonization and the Declaration of Helsinki.

Study design and treatment plan

Study design. Three patients were entered at each dose level. In the absence of dose-limiting toxicity, the next dose level was explored. If dose-limiting toxicity was seen in one patient, three additional patients were added at that dose level, and if no additional dose-limiting toxicity was seen, escalation to the next dose level occurred. If at least two patients had dose-limiting toxicity at a given dose level, accrual to that dose level was stopped; this was the maximally administered dose. Further patients were then added as required to the previous dose level (and, if necessary, to lower dose levels) to establish the highest dose at which less than two of six patients had dose-limiting toxicity. This was the maximum tolerated dose. Four additional patients were recruited at the maximum tolerated dose to delineate better the safety of that dose level.

Treatment plan. Patients received vorinostat orally twice daily with food. The investigated dose levels of vorinostat were 100, 200, 300, and 400 mg orally twice daily. Vorinostat was administered for 7 d (consecutive) every 14 d. A modified FOLFOX6 regimen was administered at a fixed dose on days 4 and 5 of vorinostat treatment. Folinic acid was dosed at 400 mg/m^2 over 2 h concurrently with 85 mg/m^2 oxaliplatin, followed by FU 400 mg/m^2 over 5 to 10 min as an i.v. bolus and FU 2,400 mg/m^2 over 46 h as a continuous i.v. infusion. Patients were premedicated with i.v. dexamethasone (10 mg) and i.v. ondasetron (8 mg) or its equivalent. Each cycle consisted of 2 wk, starting with day 1 of vorinostat.

Clinical evaluation and follow-up

A complete medical history, physical examination, pregnancy test for women with reproductive potential, complete blood count, and comprehensive chemistry profile were obtained within a week before treatment initiation. Baseline computed tomographic scans were obtained within 4 wk before initiation of treatment. Complete blood count and comprehensive chemistry profile were repeated on a weekly basis. Medical history, physical examination, and toxicity assessment as per National Cancer Institute Common Toxicity Criteria 3.0 were done weekly during the first cycle and every cycle thereafter. Computed tomographic scans were repeated every 4 cycles (8 wk) to assess response. Responses were categorized according to Response Evaluation Criteria In Solid Tumors (32).

Dose-limiting toxicities. A dose-limiting toxicity was defined as any of the following attributable to study treatment in cycle 1: any nonhematologic toxicity ≥ grade 3, with the exception of grade 3 diarrhea lasting <48 h or grade 3 vomiting that had not been adequately medicated; any grade 4 thrombocytopenia or any grade 3 thrombocytopenia lasting >6 d; any grade 4 neutropenia lasting >6 d or any grade 4 neutropenia associated with fever; and any dose delay secondary to toxicity that lasted >1 wk. Grade 3 hypomagnesemia, grade 3 hypophosphatemia, grade 3 hypokalemia, and sodium concentrations of 128 to 130 mEq/L were not considered dose-limiting toxicities unless they required hospitalization or persistent for >48 h despite medical intervention.

Dose modifications. Up to three dose reductions were allowed in FOLFOX, starting with cycle number 2 (Table 1). In the case of any grade 3 or 4 neutropenia or thrombocytopenia during a cycle or any grade 2 neutropenia or thrombocytopenia before the next scheduled FOLFOX cycle, FOLFOX was reduced by one dose level. No dose reductions were allowed below dose level -3. A new treatment cycle was not started unless the neutrophil and platelet counts were >1,500 and 75,000/μL, respectively. No growth factors other than recombinant erythropoietin were allowed.

Any grade 3 or more nonhematologic toxicity (except neuropathy and nausea) attributed to FOLFOX required a dose reduction by one dose level. Treatment was resumed when the nonhematologic toxicities recovered to grade 1 or less.

Only oxaliplatin was modified for neurologic toxicities. Grade 2 sensory neuropathy required a reduction in oxaliplatin dose to 65 mg/m^2, and grade 3 sensory neuropathy resulted in oxaliplatin discontinuation.

Pharmacokinetics

Sample collection for oxaliplatin and FU pharmacokinetics. Heparinized 7-mL blood samples were collected for determination of platinum in plasma ultrafiltrate and plasma FU concentrations at 0 (predose), 1, 2 (end of oxaliplatin infusion, start of FU bolus), 2.25, 2.5, 3, 4, 6, 8, and 24, and between 44 and 48 h, with the average of the last three samples (8, 24, and 46 h) being used to determine the steady-state FU concentration.

Platinum measurements. Plasma ultrafiltrate was prepared from plasma by centrifugal ultrafiltration using Amicon Centrifree Micropartition Systems (Millipore Corporation), and platinum was measured with a validated flameless atomic absorption spectrophotometric (PE ZL4100, Perkin Elmer) method (33). Briefly, plasma ultrafiltrate was diluted 1:1 in 0.1% nitric acid + 0.2% Triton X-100, and a 20 μL was injected into the atomic absorption spectrophotometer. Platinum standards were prepared in the same manner and in the same matrix. Quality assurance was maintained by assaying quality control samples along with patient samples.

Table 1. Dose reduction levels for FOLFOX

	Oxaliplatin (mg/m^2)	LV (mg/m^2)	FU bolus (mg/m^2)	FU infusion (mg/m^2)
Dose level -1	65	400	300	2,000
Dose level -2	55	400	0	1,800
Dose level -3	0	400	0	1,800

Abbreviation: LV, leucovorin.

FU measurements. FU in plasma was measured using a modified validated liquid chromatography/mass spectrometry/mass spectrometry method on an Applied Biosystems MDS Sciex API 3000 Triple Quadrupole Mass Spectrometer equipped with an Aligent 1100 HPLC system (34). FU and its isotopic internal standard [$^{15}N_2$]FU were obtained from Sigma-Aldrich chemical company. Normal human plasma for calibration standards was obtained from BioMedical Resources. Plasma (200 μL) samples spiked with 20 μL of internal standard (final concentration of 50 ng/mL) were extracted with 2 mL of ice-cold acetonitrile. Samples were centrifuged at 1,500 × *g* for 10 min at 4°C to sediment the precipitated protein. The supernatant was transferred to another tube, dried under vacuum, and rehydrated with 200 μL of mobile phase before a 50-μL injection. Calibration standards (5-100 ng/mL) were processed in a similar manner. A Supelcosil LC-18-S (150 cm × 4.6 mm i.d.) C18 column (Supelco) with a mobile phase consisting of methanol:5 mmol/L ammonium formate (v/v, 15:85) and a flow rate of 600 μL/min was used for the separation. Using electrospray ionization, the molecular ion for FU (with an *m/z* of 129.5) and the daughter ion (with an *m/z* of 42.5) were monitored in negative ion mode with multiple reaction monitoring. The isotopic internal standard [$^{15}N_2$]FU is two mass units higher than FU so that the ion pair of *m/z* 131.5 (parent) and *m/z* 43.5 (daughter) were monitored. Product ions chosen for the analyses were the most intense identified in the product ion scan. Under our separation conditions, a turbo gas with a flow of 8 L/min at 550°C and a nebulizer gas setting of 8 were used to assist the vaporization of the solvent from the mobile phase. A voltage of -4,200 V was used for the ionization. The ratio of the peak area of FU and its internal standard ([$^{15}N_2$]FU) were used for quantitation of FU. Quality control samples (15 and 75 ng/mL) made in bulk and stored at -80°C were assayed during assay validation and along with the patient samples to maintain quality assurance. If the observed quality control sample values were >15% different from the expected value for the 75 mg/mL quality control samples and >20% for the 15 mg/mL quality control samples near the lower limit of quantitation, the assays were typically rerun.

Assay validation consisted of assaying three sets of freshly prepared calibration standards and six sets of quality control samples each day for three separate days by the liquid chromatography/mass spectrometry/mass spectrometry method described above. Assay validation showed the lower limit of quantitation to be 5 ng/mL, the $r^2 \geq 0.999$ for all the curves and the intraday precision measured as percent coefficient of variance to be in the range of 0 to 13.3, and that for interday to be 7.7 to 20.3. Accuracy of the assay, based on percent relative error of the quality control concentrations of the observed to the expected, varied from 1.3 to 4.7.

Sample collection for vorinostat pharmacokinetics. Blood samples (5 mL) were collected in red-topped vacutainers (no anticoagulant) before and at 0.5, 1, 2, 4, and 8 h after the morning vorinostat dose on days 1 and 5 of vorinostat treatment. This allowed evaluation of vorinostat pharmacokinetics with and without FOLFOX. Blood samples were allowed to coagulate at 4°C for 20 to 30 min and were then centrifuged at 2,000 × *g* for 15 min at 4°C. The resulting serum was stored at -70°C until assayed for drug concentrations. Concentrations of vorinostat were quantitated with a validated liquid chromatography electrospray ionization tandem mass spectrometric method (35).

Pharmacokinetics analysis. Plasma concentration versus time data for platinum, FU, and vorinostat were analyzed noncompartmentally (36). Because it was unclear whether pharmacokinetic data were normally distributed or not, relationships between vorinostat dose and pharmacokinetic parameters estimated on days 1 and 5 were assessed

Table 2. Patient characteristics

Patient characteristics (n = 21)	
Sex (male/female)	13/8
Age (median/range; y)	58/36-77
ECOG (0/1)	8/13
Previous radiation therapy	8

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

with Pearson correlation as well as Spearman test. For patients with suitable pharmacokinetic data on days 1 and 5, intrapatient changes in estimated day 1 and day 5 vorinostat pharmacokinetic parameters were assessed with the Wilcoxon exact signed ranks test. Two-sided *P*s are reported, and *P*s $\leq$ 0.05 were considered statistically significant. Statistical evaluation was done with SPSS software, version 15 (SPSS).

Tumor Biopsies. Pretreatment and on-treatment tumor samples were collected from patients with liver metastases accessible to ultrasound-guided biopsies. The same target lesion was biopsied with a 16-gauge needle before and during vorinostat treatment. On-treatment samples were collected 2 h following the morning dose of vorinostat on day 4 of cycle 1 (before initiation of FOLFOX). Tumor biopsy samples were placed immediately after the procedure in RNA later for subsequent *TS* gene expression studies and in formalin for TS immunohistochemistry. Patients without liver metastases or with liver metastases that were not accessible for biopsy were exempted from these procedures.

TS immunohistochemistry. TS expression was evaluated using monoclonal antibody TS106 (Novus Biologicals), as previously described by our group (32). Semiquantitative assessment of immunostaining of a sample was done by comparing it with the appropriate known positive control. Staining intensity was categorized as none (0), weak (1+), moderate (2+), or strong (3+) The immunoassays were developed, characterized, and validated using well-known positive and negative control tissues for the markers (positive controls were the germinal center of lymphoid follicle of human tonsil for TS). All histopathologic and immunohistologic analyses and interpretations were done by a board certified pathologist who was blinded to the time of collection of samples and their relation to treatment.

TS gene expression. The gene expression measurements for TS were carried out with real-time quantitative reverse transcription-PCR assay using a PE-ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Inc.), with β-actin as the endogenous standard using a comparative C_T method of quantitation with $2^{-\Delta\Delta CT}$ (32). For these assays, total RNA was extracted using RNeasy Spin Columns (Qiagen, Inc.), and cDNA was synthesized using Superscript II reverse transcriptase (Life Technologies). Our *TS* gene expression methodology was previously detailed (32).

Results

Demographics

Twenty-one patients were entered on study (Table 2). All patients had failed previous fluoropyrimidine, oxaliplatin, irinotecan, and cetuximab chemotherapy.

Treatment administration

One patient on dose level 1 developed complete bowel obstruction related to peritoneal carcinomatosis and was replaced because he was deemed nonevaluable for potential treatment-related gastrointestinal toxicities. None of the other three patients at dose level 1 developed a dose-limiting toxicity. Three additional patients were treated at dose level 2 (vorinostat 200 mg orally twice daily) and dose level 3 (vorinostat 300 mg orally twice daily) without any dose-limiting toxicity. At dose level 4 (vorinostat 400 mg orally twice daily), two of four patients developed a dose-limiting toxicity; therefore, this dose level was declared as intolerable. Dose level 3 was subsequently expanded to six patients, and one of the three additional patients developed a dose-limiting toxicity, which defined this dose level as the maximum tolerated dose. Dose level 3 was expanded by four additional patients, none of whom developed a dose-limiting toxicity.

A total of 25, 33, 53, and 14 cycles were administered at dose levels 1, 2, 3, and 4, respectively.

Toxicity

All 21 patients were evaluable for toxicity. Only data for toxicities of grade 2 or more were collected and reported.

Dose-limiting toxicities and maximum tolerated dose. Two of four patients at dose level 4 developed dose-limiting toxicities. These consisted of grade 3 diarrhea and fatigue in one patient and grade 3 fatigue in the other. Dose level 3 was expanded to six patients, one of whom developed dose-limiting grade 3 fatigue, anorexia, and dehydration. Dose level 3 (vorinostat 300 mg orally twice daily) was declared the maximum tolerated dose and was expanded to a total of 10 patients without any further dose-limiting toxicity.

Hematologic toxicity. Neutropenia and thrombocytopenia were the predominant hematologic toxicities (Table 3). None of the patients on dose levels 1 and 2 experienced grade 3 or more neutropenia or thrombocytopenia. Two of the 10 patients treated at dose level 3 developed grade 3 neutropenia, and three developed grade 2 thrombocytopenia. Of the four patients treated at dose level 4, two developed grade 3 neutropenia, one developed grade 4 neutropenia, and 2 developed grade 4 thrombocytopenia.

Nonhematologic toxicity. Nonhematologic toxicities such as diarrhea, mucositis, and neuropathy were expected given the cytotoxic components of this regimen (Table 4). However, there was a clear increase in the frequency and severity of nausea/vomiting, anorexia, and fatigue at the higher vorinostat dose levels of 300 and 400 mg orally twice daily. Nausea/vomiting and fatigue seemed to peek during FOLFOX chemotherapy, that is, on days 4 to 5 of each cycle.

Antitumor activity

All 21 patients were assessable for response. No patient developed an objective response. Eleven patients had stable disease on their 2-month staging computed tomographic scan. Stable disease was confirmed in five patients, who remained on treatment for 9, 10, 12, 12, and 16 cycles.

Pharmacokinetics

FU pharmacokinetics. Following a loading dose and institution of the 46-hour continuous infusion, FU plasma concentrations achieved steady-state quickly, with median steady-state concentrations ranging from 0.27 to 0.51 μg/mL (Table 5). The median FU steady-state concentration increased with increasing vorinostat doses; 3, 16, 20, and 23 μg/mL for the 100, 200, 300, and 400 mg twice daily dose groups, respectively. Median FU area under the curve also increased with increasing vorinostat dose level; 14, 26, 35, and 44 μg h/mL across the

Table 3. Hematologic toxicities (grade 2 or more)

	Dose level 1 (4 patients) 1st cycle (all cycles)			Dose level 2 (3 patients) 1st cycle (all cycles)			Dose level 3 (10 patients) 1st cycle (all cycles)			Dose level 4 (4 patients) 1st cycle (all cycles)		
	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4
Neutropenia	0 (1)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	2 (6)	1 (2)	0 (0)	0 (1)	0 (2)	0 (1)
Anemia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Thrombocytopenia	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	2 (1)	0 (3)	0 (0)	0 (1)	0 (0)	0 (2)

NOTE: All cycles toxicities are listed in between parenthesis.
Abbreviation: G, grade.

100- to 400-mg dose groups, respectively. However, only C_{max} differences between the different dose level of vorinostat were statistically significant by ANOVA analysis (P = 0.047), which likely reflects the small population size and the large interpatient variability.

Platinum pharmacokinetics. Ultrafilterable platinum plasma concentrations displayed a biexponential decay following oxaliplatin administration with a median half-life of 16 to 21 hours across the various vorinostat cohorts. Median ultrafilterable platinum C_{max} and area under the curve were 0.543 to 0.846 μg/mL and 4.9 to 6.4 μg h/mL, respectively, and these values were consistent across vorinostat cohorts (data not shown).

Vorinostat pharmacokinetics. Vorinostat pharmacokinetic parameters estimated for patients on days 1 and 5 are shown in Table 6. There were significant relationships between vorinostat dose and area under the curve on day 1 (Pearson, 0.006; Spearman, 0.002) and day 5 (Pearson, <0.001, Spearman 0.002), and as a result, no significant relationship, on either day, between vorinostat doses of 100 to 400 mg twice daily and vorinostat apparent clearance. Suitable pharmacokinetic data were available to compare days 1 and 5 C_{max} and T_{max} for 19 patients and area under the curve, $t_{1/2}$, and apparent clearance for 14 patients. There was a significant increase in vorinostat area under the curve (P = 0.005) and associated significant decrease in vorinostat apparent clearance (P = 0.003) within patients when days 1 and 5 values were compared. There was also a statistically significant increase in vorinostat T_{max} when days 1 and 5 values were compared (P = 0.02).

Pharmacodynamics

TS tumor expression by immunohistochemistry. TS tumor expression was evaluated by immunohistochemistry before study treatment and on the fourth day of vorinostat, before oxaliplatin was administered, on cycle 1. The same liver metastasis was biopsied before and after vorinostat. No complications were seen as a result of tumor biopsies. Of six paired samples, four patients showed no change in their staining pattern (two strong and two moderate staining). One patient had a decreased intensity from strong to weak (dose level 1), and one patient had an increase in intensity from weak to strong (dose level 4).

TS tumor expression by reverse transcription-PCR. Following vorinostat treatment, only two of six patients (dose levels 1 and 4) illustrated down-regulation of TS. The decrease in *TS* gene expression was ~33% (2.27 decreased to 1.58 relative to β-actin) in the patient at dose level 4 and 50% (0.92 decreased to 0.45) in the patient at dose level 1. The other four patients did not show any change in TS expression. Of note, the

Table 4. Nonhematologic toxicity (grade 2 or more)

	Dose level 1 (4 patients) 1st cycle (all cycles)			Dose level 2 (3 patients) 1st cycle (all cycles)			Dose level 3 (10 patients) 1st cycle (all cycles)			Dose level 4 (4 patients) 1st cycle (all cycles)		
	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4
Fatigue	0 (3)	0 (1)	0 (0)	0 (2)	0 (0)	0 (0)	3 (4)	1 (1)	0 (0)	1 (1)	2 (2)*	0 (0)
Anorexia	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (4)	1 (1)	0 (0)	1 (1)	0 (0)	0 (0)
Nausea/vomiting	0 (1)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	4 (4)	1 (1)	0 (0)	3 (3)	0 (0)	0 (2)
Mucositis	1 (3)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Diarrhea	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)	1 (1)*	0 (0)
Dehydration	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	2 (2)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Neuropathy	0 (0)	0 (0)	0 (0)	0 (2)	0 (1)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Infection	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Weight loss	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)
LFT elevation	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Headaches	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)	0 (0)	0 (0)

NOTE: All cycles toxicities are listed in between parenthesis.
Abbreviation: LFT, liver function test.
*Three patients experienced dose-limiting toxicity: one patient on dose level 3 experienced grade 3 nausea/vomiting, fatigue, anorexia, and dehydration; and two patients at dose level 4 experienced grade 3 fatigue, one of who had an associated grade 3 diarrhea.

Table 5. Mean ± SD pharmacokinetic parameters for plasma FU

FU maintenance dose (mg/m^2)	Vorinostat dose (mg)	Css (ηg/mL)	C_{max} (ηg/mL)	AUC (ηg h/mL)
2,400	100	0.287 ± 0.044	5.03 ± 4.39	15.3 ± 5.7
2,400	200	0.274 ± 0.045	13.1 ± 7.96	24 ± 9.11
2,400	300	0.396 ± 0.159	24.5 ± 14.5	68.7 ± 88.1
2,400	400	0.432 ± 0.077	26.8 ± 11.4	46.7 ± 10.6

NOTE: ANOVA analysis was done, and only C_{max} was statistically significant among the vorinostat treatment groups.
Abbreviations: Css, steady-state concentration; AUC, area under the curve.

same patient with a decrease in TS by reverse transcription-PCR had an increase in staining by immunohistochemistry.

Discussion

In this phase I clinical trial, we evaluated the combination of a novel schedule of vorinostat orally twice daily × 1 week repeated every 2 weeks in combination with FOLFOX on days 4 and 5 of vorinostat. We have established vorinostat 300 mg orally twice daily in combination with a standard dose of FOLFOX as the maximum tolerated dose. The dose-limiting toxicities of this regimen were consistent with known side effects of FOLFOX and vorinostat and included fatigue, diarrhea, and dehydration (3, 33, 34). Despite the lack of hematologic dose-limiting toxicities, grade 3 to 4 thrombocytopenia was seen in five patients (24%), which is considerably higher than would be expected with FOLFOX alone (3). This finding is consistent with the known platelet-suppressing effects of oxaliplatin and vorinostat and, therefore, the increased incidence with the combination (3, 33, 34).

The pharmacokinetic parameters estimated for vorinostat on day 1 of treatment are consistent with those previously reported for single-agent vorinostat (27). The dose-related increases in vorinostat area under the curve and lack of dose-related changes in vorinostat apparent clearance are also consistent with previous reports (27). The statistically significant increase in vorinostat area under the curve and associated decrease in vorinostat apparent clearance between days 1 and 5 of treatment is consistent with a previous report of vorinostat pharmacokinetics when administered alone and in combination with carboplatin and paclitaxel after 7 days of vorinostat dosing (35). The clinical relevance of this observation is unclear on whether the change is associated with chronic vorinostat dosing or administration with other drugs.

Steady-state concentrations of FU observed in this study were comparable with historical data for concentrations observed with infusional FU regimens (0.065-0.39 μg/mL; ref. 36). Trends of FU steady-state concentration, C_{max}, and area under the curve increases were observed with higher doses of vorinostat, whereas FU clearance decreased. These observations suggest a potential pharmacokinetic interaction between vorinostat and FU. Dihydropyrimidine dehydrogenase is the predominant enzyme in the degradation of FU (37, 38). We hypothesize that vorinostat decreases dihydropyrimidine dehydrogenase activity either by suppressing dihydropyrimidine dehydrogenase mRNA expression or through posttranslational protein alteration, with a subsequent decrease in dihydropyrimidine dehydrogenase enzymatic activity. We are currently investigating this interaction through the conduct of a variant intermittent vorinostat schedule in combination with FU. This ongoing study is evaluating FU pharmacokinetics with and without vorinostat and the effects of vorinostat on dihydropyrimidine dehydrogenase activity (39).

Despite the preclinical synergy between vorinostat and FU and platinums, no confirmed objective response could be documented in this phase I clinical trial. Five of 21 chemoresistant colorectal cancer patients had confirmed stable disease; however, we cannot confirm if these stabilizations were due to FOLFOX rechallenge or to the addition of vorinostat. The lack of significant activity of this combination may be due to the lack of biological activity of vorinostat on TS expression. In six paired tumor samples, there were no

Table 6. Pharmacokinetic parameters for vorinostat on days 1 and 5

Dose (mg)		Day 1					Day 5				
		C_{max} (μmol/L)	T_{max} (h)	AUC (μmol/L h)	$t_{1/2}$ (h)	CLapp (l/h)	C_{max} (μmol/L)	T_{max} (h)	AUC (μmol/L h)	$t_{1/2}$ (h)	CLapp (l/h)
100	Mean	1.241	2.4	2.5	1.5	189	0.702	3.4	1.9	1.2	202
	SD	1.061	1.9	1.2	0.9	102	0.342	3.4	0.4	0.3	60
200	Mean	0.816	1.5	1.9	1.2	400	0.894	2.7	3.3	1.6	231
	SD	0.245	0.9	0.3	0.3	54	0.540	1.2	0.6	0.7	39
300	Mean	1.458	1.6	4.4	2.3	285	0.950	2.4	6.8	2.3	225
	SD	0.712	1.1	1.5	2.0	83	0.590	1.1	4.3	1.1	118
400	Mean	1.511	1.9	4.5	3.7	345	1.475	2.8	7.4	2.2	211
	SD	0.533	1.5	0.6	3.5	48	0.363	1.5	1.4	1.4	40

Abbreviation: CLapp, apparent clearance.

consistent effects of vorinostat on TS expression assessed by reverse transcription-PCR or immunohistochemistry at any dose level. We hypothesize that the lack of TS down-regulation in our study was likely secondary to inadequate vorinostat exposure. In preclinical studies, effective TS down-regulation requires 24 hours of vorinostat exposure at concentrations ≥ 5 μmol/L (28, 29). In our study, the C_{max} of vorinostat was <2 μmol/L, and the half-life was 1.2 to 2.4 hours at all dose levels. Therefore, vorinostat pharmacokinetics in our study was inadequate for optimal modulation of TS expression. It is possible that modulation of vorinostat schedule with a shorter intermittent dosing may allow for a higher dose administration per day and therefore the achievement of suitable vorinostat concentrations. A single-agent i.v. vorinostat study previously established the feasibility of daily vorinostat at 900 mg/m^2 i.v. over 2 hours × 3 days every 3 weeks (26). The C_{max} of vorinostat at that dose level exceeded 20 μmol/L. We are currently investigating a daily × 3 schedule of vorinostat every 2 weeks in combination with FU/leucovorin on days 2 and 3 of treatment. This ongoing study will evaluate if this shorter intermittent schedule of oral vorinostat achieves more suitable concentrations and results in down-regulation of tumor TS.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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