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A Phase I, Pharmacokinetic, and Pharmacodynamic Study of Two Schedules of Vorinostat in Combination with 5-Fluorouracil and Leucovorin in Patients with Refractory Solid Tumors

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Abstract

Purpose—We conducted a phase I clinical trial to determine the maximum tolerated dose (MTD) of daily or twice daily vorinostat \times 3 days when combined with fixed doses of 5-fluorouracil (FU) and leucovorin every 2 weeks.

Experimental Design—Vorinostat doses were escalated in a standard 3×3 phase I design. FU/leucovorin was started on day 2 of vorinostat and consisted of leucovorin 400 mg/m² i.v. over 2 hours followed by FU 400 mg/m² i.v. bolus and 2,400 mg/m² over 46 hours (sLV5FU2).

Results—Forty-three patients were enrolled. Grade 3 fatigue, and hand and foot syndrome were the dose-limiting toxicities (DLT) at the 2,000 mg vorinostat once-daily dose level. Grade 3 fatigue and mucositis were DLTs at the 800 mg vorinostat twice-daily dose level. None of six patients at the 1,700 mg once daily or six patients at the 600 mg twice daily dose levels had a DLT; those dose levels represent the MTD. Twenty-one of 38 patients with FU-refractory colorectal cancer had stable disease, and one had a partial response. Vorinostat maximum serum concentrations at the MTD exceeded concentrations associated with thymidylate synthase downregulation *in vitro*. No pharmacokinetic interactions were noted between vorinostat and FU.

Conclusions—The MTD of vorinostat in combination with sLV5FU2 is 1,700 mg orally once daily \times 3 or 600 mg orally twice daily \times 3 days every 2 weeks. Clinical activity in refractory colorectal cancer supports further clinical development of this combination.

Histone deacetylases (HDAC) have been identified as potential anticancer targets. Three classes of HDAC have been identified in humans (1–3). Class I includes HDAC 1, 2, 3, and 8, which are related to yeast RPD3 deacetylase. HDAC1, HDAC2, and HDAC3 are

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Disclosure of Potential Conflicts of Interest

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overexpressed in colonic tumors, suggesting that HDAC may be an important target in the treatment of that disease (4–6). The mechanisms of growth inhibition produced by HDAC inhibitors include effects on gene expression, cell cycle progression, and cell death pathways (6–14).

Vorinostat (suberoylanilide hydroxamic acid) is a potent inhibitor of class I and II HDAC and has proven clinical activity against cutaneous T-cell lymphomas (15). Vorinostat modulates several genes implicated in tumor growth (16). *In vitro* and *in vivo* models have shown that vorinostat can downregulate thymidylate synthase expression by as much as a 100-fold when measured by quantitative-PCR analysis, and this downregulation has been confirmed by Western blot after only 24 hours of vorinostat exposure (16,17). The inhibition of thymidylate synthase expression by vorinostat occurs at the transcription level and results in synergistic antitumor activity *in vitro* and *in vivo* when combined with 5-fluorouracil (FU; refs. 17–19). Vorinostat effectively inhibits FU-induced acute thymidylate synthase induction *in vitro* and results in an increase in the FU active metabolite dUTP (17). In that thymidylate synthase overexpression is associated with clinical resistance to FU (20,21), the integration of vorinostat may represent an attractive pathway to improve FU response and delay tumor progression. With that premise, we previously investigated a combination of vorinostat given orally twice daily $\times 7$ days in combination with FU, oxaliplatin, and leucovorin (FOLFOX) in a phase I study in patients with refractory colorectal cancer (22). The maximum tolerated dose (MTD) of vorinostat was 300 mg orally twice daily $\times 1$ week repeated every two weeks. That dose level was associated with maximum vorinostat serum concentrations $<1.5 \mu\text{mol/L}$, which are not optimal for thymidylate synthase downmodulation (22). Interestingly, higher vorinostat dose-levels were associated with lower FU clearance, suggesting a possible pharmacokinetic interaction between vorinostat and FU (22).

Translational Relevance

Vorinostat, a histone deacetylase inhibitor, has been associated with thymidylate synthase downregulation and synergy with 5-fluorouracil (FU) in preclinical studies. In this phase I clinical trial, we evaluated escalating doses of vorinostat in combination with fixed doses of FU and leucovorin. We show that vorinostat can be administered safely up to doses of 1,700 mg orally once daily $\times 3$ or 600 mg orally twice daily $\times 3$ days, every 2 weeks, in combination with full doses of FU/leucovorin. These dose levels produce vorinostat concentrations that downregulate thymidylate synthase preclinically. Clinical activity was noted in patients with FU-refractory colorectal cancer, which supports further clinical development of this combination.

Because FU clearance predominantly reflects FU metabolism by dihydropyrimidine dehydrogenase (DPD), we hypothesized that vorinostat decreases FU clearance through DPD inhibition. In this follow-up phase I study, we investigated two alternative regimens of vorinostat (once daily $\times 3$ days or twice daily $\times 3$ days) in combination with every-2-weeks FU and leucovorin, and investigated pharmacokinetic and pharmacodynamic (thymidylate synthase) end points in dedicated cohorts at the MTD. We show that a short intermittent schedule of vorinostat mitigates its toxicities and results in vorinostat serum concentrations that exceed the threshold for thymidylate synthase downregulation and do not modulate FU pharmacokinetics.

Materials and Methods

This phase I, open-label, dose-escalation study of vorinostat in combination with fixed doses of FU and leucovorin was conducted at Roswell Park Cancer Institute (Buffalo, NY). The

primary objective of the study was to determine the MTD of once-daily and twice-daily oral vorinostat given for 3 days every 2 weeks in combination with FU and leucovorin on days 2 and 3 of vorinostat. Secondary objectives included the description of treatment-related toxicities, the evaluation of vorinostat and FU pharmacokinetics at the MTD and the effects of vorinostat on peripheral blood mononuclear cell (PBMC) DPD activity, and the description of any observed clinical responses. Exploratory end points included the evaluation of vorinostat effects on intratumoral thymidylate synthase expression.

Patient criteria

Patients with advanced solid tumors who had failed standard chemotherapy or for whom no standard chemotherapy existed were eligible for enrollment. Treatment failure was defined as progression on, or within 3 months from last treatment. In addition, patients had to be ≥ 18 years of age, have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, have an expected survival ≥ 12 weeks, and have acceptable organ function defined as: WBC count $\geq 3,000/\mu\text{L}$, absolute neutrophil count $\geq 1,500/\mu\text{L}$, serum creatinine equal to or less than the upper institutional normal level, total bilirubin equal to or less than upper institutional normal level, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 3 \times$ upper institutional normal. Patients could not have received any chemotherapy within 3 weeks from initiation of study treatment with the exception of nitrosureas or mitomycin C, which required a 6-week interval before study treatment. Patients with brain metastases, grade ≥ 2 neuropathy, or other severe intercurrent illnesses were excluded. Patients who were HIV positive and taking antiretroviral medicines were excluded because of potential drug-drug interactions. No other HDAC 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 eligible nor were pregnant or lactating females. All consenting patients having the potential of conceiving agreed to use 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 (DLT), the next dose level was explored. If DLT was seen in one patient, three additional patients were added at that dose level, and if no additional DLT was seen, escalation to the next dose level occurred. If at least two patients at a given dose level had DLT, accrual to that dose level was stopped; and it was defined as the maximally administered dose. Additional 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 < 2 of 6 patients had DLT. This was defined as the MTD. Three additional patients were recruited at the MTD of the once-daily and twice-daily schedules (total of 6 patients) to evaluate potential pharmacokinetic interactions between vorinostat and FU and to explore the effects of the MTD of vorinostat on intratumoral thymidylate synthase expression.

Treatment plan—Patients received vorinostat orally with food. The investigated dose levels (DL) of vorinostat ranged between 600 mg and 2,000 mg on the once-daily schedule and from 500 mg (twice daily) to 800 mg (twice daily) on the twice-daily schedule (Table 1). Vorinostat was administered for 3 consecutive days every 14 days. A simplified fixed-dose FU plus leucovorin regimen (sLV5FU2) was administered on days 2 and 3 of vorinostat treatment. Leucovorin was dosed at $400 \text{ mg}/\text{m}^2$ over 2 hours, followed by FU 400

mg/m² over 5 minutes as an i.v. bolus and 2,400 mg/m² over 46 hours as a continuous i.v. infusion. Patients were premedicated with prochlorperazine 10 mg orally or i.v. prior to sLV5FU2. Other antiemetics, such as dexamethasone and 5'-HT₃ inhibitors were allowed at the investigator's discretion. Each cycle consisted of 2 weeks, starting with day 1 of vorinostat.

The pharmacokinetic cohorts consisted of three patients each at the once-daily and twice-daily MTD levels. These cohorts received only sLV5FU2 on cycle 1 followed by vorinostat plus sLV5FU2 on cycle 2 and beyond.

Clinical evaluation and follow-up

A complete medical history, physical examination, pregnancy test for women with reproductive potential, complete blood count (CBC), and comprehensive chemistry profile were obtained within a week prior to treatment initiation. Baseline computed tomography (CT) scans were obtained within 3 weeks prior to initiation of treatment. CBC and comprehensive chemistry profile were repeated every two weeks, on the day of a scheduled cycle start. 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. CT scans were repeated every four cycles (eight weeks) to assess response. Responses were categorized according to Response Evaluation Criteria in Solid Tumors (23).

DLTs—A DLT was defined as any of the following attributable to study treatment in cycle 1: any nonhematologic toxicity \geq grade 3, with the exception of grade 3 diarrhea lasting $<$ 48 hours or grade 3 vomiting that had not been adequately medicated; any grade 4 thrombocytopenia or any grade 3 thrombocytopenia lasting $>$ 6 days; any grade 4 neutropenia lasting $>$ 6 days or any grade 4 neutropenia associated with fever; or any dose delay secondary to toxicity that lasted $>$ 1 week.

Dose delays and modifications—A new cycle was delayed until grade ≥ 2 nonhematologic treatment-related toxicities resolved and neutrophil and platelet counts recovered to $\geq 1,200/\mu\text{L}$ and $\geq 75,000/\mu\text{L}$, respectively. In the case of any grade ≥ 3 neutropenia or any grade ≥ 2 thrombocytopenia prior to a scheduled vorinostat/sLV5FU2 treatment, sLV5FU2 was reduced by one dose level. No dose reductions were allowed below dose level -3 . Any grade ≥ 3 nonhematologic toxicity (except nausea) attributed to sLV5FU2 resulted in a dose-reduction by one dose level. Prolonged grade 2 toxicities, such as diarrhea and mucositis, allowed for a dose reduction in sLV5FU2 by one dose level, at the treating physician's discretion. Dose level -1 of FU/leucovorin consisted of leucovorin 400 mg/m², FU bolus 300 mg/m², and FU infusion 2,000 mg/m². Dose level -2 consisted of leucovorin 400 mg/m², no FU bolus, and FU infusion 1,800 mg/m². Dose level -3 consisted of leucovorin 400 mg/m², no FU bolus, and FU infusion 1,600 mg/m². No dose reduction below dose level -3 was allowed.

Vorinostat dose reductions were recommended in the event of recurrent \geq grade 2 toxicities with a likely or definitive attribution to vorinostat. In those cases, vorinostat was reduced to the prior dose level. No reductions were allowed on DL1.

Pharmacokinetics

FU—FU pharmacokinetic studies were done in designated 3-patient cohorts at the once-daily and twice-daily MTD. FU pharmacokinetics were studied on cycle 1 (without vorinostat) and cycle 2 (with vorinostat). On cycle 2, the morning vorinostat dose was given immediately prior to the FU bolus.

Heparinized, 7 mL blood samples were collected to determine FU concentrations at 0 (pre-FU bolus), 0.25, 0.5, 1, 2, 4, 8, and 22 hours and at a time between 40 and 46 hours. FU in plasma was measured using a modified validated liquid chromatography/tandem mass spectrometry (LC-MS/MS) method on an Applied Biosystems MDS Sciex API 3000 Triple Quadrupole Mass Spectrometer (Concord) equipped with an Aligent 1100 HPLC system. The measurement and validation techniques have been described previously (22).

Vorinostat—Blood samples (5 mL) were collected in redtopped vacutainers (no anticoagulant) at 0 (pre-FU bolus and pre-vorinostat), 0.5, 1, 2, 4, and 8 hours after the morning vorinostat dose on the first day of FU infusion on cycle 2. Concentrations of vorinostat in serum were quantitated with a validated liquid chromatography electrospray ionization LC-MS/MS method (24).

Pharmacokinetics analysis—Plasma concentration versus time data for FU were analyzed noncompartmentally (25). For determination of steady-state FU plasma concentration, the average of plasma concentrations at 8, 22, and 40 to 46 hours was used. Paired Student's *t*-test was used to evaluate statistical significance between groups (25).

Vorinostat concentration versus time data were modeled noncompartmentally using the LaGrange function (26) as implemented by the LAGRAN computer program (27). Vorinostat C_{max} and the time at which it occurred (T_{max}) were determined by visual inspection of the data.

Tumor biopsies

Pretreatment and on-treatment tumor samples were collected from patients on the pharmacokinetic cohorts. An ultrasound-directed liver metastasis biopsy was done using a 16-gauge needle on day 2 of FU on cycles 1 (without vorinostat) and 2 (with vorinostat). On cycle 2, the biopsy was done 2 to 4 hours after the morning dose of vorinostat. Patients without liver metastases or with liver metastases that were not accessible for biopsy were exempted from these procedures.

Thymidylate synthase gene expression

Gene expression measurements for thymidylate synthase were carried out with a real-time quantitative reverse transcriptase-PCR (RT-PCR) assay using a PE-ABI PRISM 7700 Sequence Detection System (Applied Biosystems Inc.). The assay used β -actin as the endogenous standard and a comparative C_T method of quantitation with $2^{-\Delta\Delta CT}$ (28). 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 thymidylate synthase gene expression methodology has been described previously (28).

DPD assays

During the dose-escalation phase, DPD assays were done on all patients before study treatment and on days 2, 3, and 7 of cycle 1. In the pharmacokinetic cohorts, DPD assays were done on days 2, 3, and 7 of cycle 2. DPD assays were done in Dr. Robert Diasio's Laboratory at the Mayo Clinic (Rochester, MN). The assay has been previously described in detail (29,30).

Results

Demographics

Forty-three patients were entered on study: 24 patients on the once-daily escalation schedule, 12 patients on the twice-daily escalation schedule, and 7 patients on the pharmacokinetic cohorts (Table 2). All patients had failed prior standard chemotherapy. Recruitment occurred predominantly from a population of patients with chemotherapy-refractory colorectal cancer. Specifically, 38 patients with fluoropyrimidine-refractory, and oxaliplatin-, irinotecan-, bevacizumab-, and cetuximab-resistant colorectal tumors were enrolled. The other patients had a variety of solid tumors.

Treatment administration, DLT, and MTD

Once-daily schedule—Three patients were enrolled at each dose level 1 through 6 without any DLT. At DL7 (2,000 mg once daily \times 3), two patients developed a DLT. One DLT consisted of grade 3 hand and foot syndrome in a 51-year-old female with refractory colorectal cancer and a history of hand and foot syndrome with prior chemotherapy. The second DLT consisted of grade 3 fatigue in a 68-year-old male with extensive metastatic disease in the liver and peritoneum. This DLT was complicated by grade 3 gastrointestinal bleeding that was attributed to warfarin. Therefore DL6 (1,700 mg once daily \times 3) was expanded to six patients. Because none of the patients at DL6 developed a DLT, DL6 was declared the MTD. Four additional patients were enrolled at DL6 on the pharmacokinetic cohort, and none of them experienced toxicities compatible with DLT on their first cycle of vorinostat plus sLV5FU2 (cycle 2 of treatment). A median of 8 cycles (range, 1–36) was administered on the once-daily vorinosta schedule.

Twice-daily schedule—Three patients were enrolled on DL1 (500 mg orally twice daily \times 3) and DL2 (600 mg orally twice daily) without any DLT. Three patients were enrolled on DL3 (800 mg orally twice daily \times 3), and two of them developed DLT. One DLT consisted of grade 3 fatigue in a 56-year-old heavily pretreated male with refractory colorectal cancer and both pelvic recurrence and hepatic metastases. The other DLT consisted of a grade 3 mucositis in a heavily pretreated 68-year-old female with metastatic colorectal cancer. Dose level 2 was then expanded to a total of six patients without any DLT and was declared the MTD. Another three patients were enrolled at DL2 on the pharmacokinetic cohort, and none of them experienced toxicities compatible with DLT on their first cycle of vorinostat plus sLV5FU2 (cycle 2 of treatment). The median number of cycles administered was 6 (range, 1–16).

Toxicity

All 43 patients were evaluable for toxicity (Tables 3 and 4). Only data for toxicities \geq grade 2 were collected and reported. Severe neutropenia and thrombocytopenia were uncommon. The most common nonhematologic toxicities included fatigue, nausea or vomiting, anorexia, and mucositis. The frequency and intensity of these toxicities increased with higher dose levels.

Antitumor activity

Only 3 of 43 patients were not evaluable for response. One patient at DL7 on the once-daily arm was taken off study after one cycle due to DLT and associated clinical deterioration. One patient in the twice-daily arm (DL1) withdrew from study after completion of one cycle of treatment so that she could undergo an elective surgical procedure. One patient on the pharmacokinetic cohort of the twice-daily arm was taken off study and replaced due to leucovorin-associated hypersensitivity.

Antitumor activity on the once-daily arm—Twenty-six patients on the once-daily arm were evaluable for response. Sixteen patients had stable disease, and one patient with refractory colorectal cancer had a partial response that is ongoing at 18 months. This patient had evidence of FOLFOX-refractory colorectal peritoneal metastasis that was treated surgically. His peritoneal disease unfortunately recurred. After a prolonged response to irinotecan and irinotecan plus cetuximab, he had evidence of further peritoneal disease progression and was enrolled in this study. Disease control (stable disease or partial response) lasted ≥ 6 months and ≥ 8 months in 9 and 6 evaluable patients, respectively. In an exploratory posthoc analysis, the progression-free survival (PFS) and overall survival (OS) of 24 patients with refractory colorectal cancer enrolled on this arm were analyzed. The median PFS was 4.4 months (95% confidence interval, 2.1–7.7 months), and the median OS was 9.2 months (95% confidence interval, 8.4 months to unreached).

Antitumor activity on the twice-daily arm—Fourteen patients on the twice-daily arm were evaluable for response. Eight patients with refractory colorectal cancer had stable disease. Disease control for ≥ 6 months occurred in two patients.

Pharmacokinetics

FU—Table 5 summarizes the FU steady state concentration, C_{\max} , and area under the curve on the once-daily and twice-daily pharmacokinetic cohorts for cycle 1 (without vorinostat) and cycle 2 (with vorinostat). Changes between cycle 1 and cycle 2 were not statistically significant when compared by a paired Student's *t*-test ($P < 0.05$), except in the case of C_{\max} in the once-daily group, which was 0.82 times lower in the group receiving 1,700 mg vorinostat once daily.

Vorinostat

Vorinostat concentration versus time data varied among the three patients treated with 600 and 1,700 mg on the two pharmacokinetic cohorts (Table 6). T_{\max} varied between 0.5 and 4 hours. Although the 8-hour sampling schedule may have caused underestimation of the true values, vorinostat half-lives in patient treated with 600 mg of vorinostat were estimated to be 1.5, 2.2, and 2.3 hours, and the half-lives in patients treated with 1,700 mg were estimated to be 27.7, 2.4, and 3.2 hours. There was evidence of higher vorinostat doses being associated with higher vorinostat exposure, but there was overlap among the patients treated at the two doses studied. C_{\max} values in patients treated with 600 mg of vorinostat were 4.99, 3.43, and 1.97 $\mu\text{mol/L}$, whereas C_{\max} values in patients treated with 1,700 mg of vorinostat were 0.92, 6.15, and 6.4 $\mu\text{mol/L}$. The vorinostat areas under the concentration versus time curve from 0 to 8 hours were 14.3, 7.8, and 10.2 $\mu\text{mol/L} \times \text{hours}$ in patients treated with 600 mg, and were 2.6, 26.0, and 35.1 $\mu\text{mol/L} \times \text{hours}$ in patients treated with 1,700 mg.

Pharmacodynamics

Tumor thymidylate synthase expression by RT-PCR—Two patients underwent serial biopsies at the 1,700 mg once-daily dose level, and three patients underwent serial biopsies at the 600 mg orally twice-daily dose level. Consistent with prior reports (21,31–33), considerable interpatient thymidylate synthase intratumor expression variability was noted. The method was done in duplicate (two separate aliquots) for all samples and was highly reproducible. No definitive impact of vorinostat was noted on the posttreatment biopsies. Two patients underwent pre- and post-vorinostat biopsies at the 1,700 mg once-daily dose level. Thymidylate synthase expression decreased from 0.23 to 0.13 in one patient. The second patient had a relatively low expression pre- and post-vorinostat (0.02 and 0.05, respectively). Three patients underwent pre- and post-vorinostat biopsies at the 600 mg orally twice-daily dose level. Thymidylate synthase levels were relatively low in all

three patients and ranged from 0.012 to 0.029 pre-vorinostat and from 0.004 to 0.023 post-vorinostat.

DPD activity assay—DPD levels were analyzed for the once-daily and twice-daily MTD cohorts. Several patients were excluded from the analysis due to inadequate pellets for DPD assays. Four patients had evaluable levels at the once-daily MTD, and three patients had evaluable levels on the twice-daily MTD at all end points (baseline, and days 2, 3, and 7 of cycle 1). The mean DPD levels for the once-daily arm were 0.189, 0.142, 0.138, and 0.126 nmol/min/mg protein at baseline, day 2, day 3, and day 7 of cycle 1, respectively. The mean DPD levels for the twice-daily arm were 0.253, 0.199, 0.138, and 0.198 nmol/min/mg protein, at baseline, day 2, day 3, and day 7 of cycle 1, respectively. The differences were not statistically significant within the once-daily or twice-daily cohorts by one way ANOVA ($P > 0.05$ for once-daily arm and twice-daily arm). The lack of significant modulation of DPD by vorinostat at the MTD levels was consistent with the lack of modulation FU pharmacokinetics by vorinostat.

Discussion

Intratumor thymidylate synthase overexpression is associated with colorectal cancer resistance to fluoropyrimidine therapy, preclinically (34,35) and clinically (21,31–33,36). One strategy to overcome such resistance involves the integration of agents that downregulate thymidylate synthase. Vorinostat has been shown to potently downregulate thymidylate synthase preclinically *in vitro* and *in vivo*, leading to a synergistic activity when combined with FU (17–19). However, one of the impediments to the ability of vorinostat to downregulate thymidylate synthase clinically is its pharmacokinetics at the Food and Drug Administration–approved dosing (37). To circumvent the suboptimal pharmacokinetics associated with the 400 mg daily oral dosing, we investigated an intermitted schedule of vorinostat given daily or twice daily \times 3 days concurrently with FU/leucovorin on days 2 and 3, repeated every two weeks. We show that this schedule is associated with favorable pharmacokinetics at the once-daily and twice-daily MTD schedule. At the 1,700 mg orally once daily \times 3 days MTD, vorinostat C_{\max} exceeded 5 $\mu\text{mol/L}$ in 2 of 3 patients. At the 600 mg orally twice daily \times 3 days MTD, vorinostat C_{\max} exceeded 3 $\mu\text{mol/L}$ in 2 of 3 patients. Large interpatient variability was noted on both once-daily and twice-daily MTD levels, suggesting large variations in vorinostat absorption or metabolism. Although vorinostat C_{\max} at the once-daily MTD were considerably higher than those associated with thymidylate synthase downregulation preclinically (16), no clear signs of thymidylate synthase downregulation were noted in our study. The lack of thymidylate synthase downregulation could reflect inadequate vorinostat AUC despite achieving the target C_{\max} . Indeed, preclinical studies supporting major downregulation of thymidylate synthase have involved sustained (24 hours) exposures to 5 $\mu\text{mol/L}$ vorinostat. Given that the vorinostat half-life is approximately 2 hours, it is possible that clinical thymidylate synthase downregulation may not be attainable with daily or twice-daily regimens. Thymidylate synthase assessment in our study could have also been impacted by other factors such as limited sample size, lack of tumor microdissection, sampling time, and intratumor sampling heterogeneity.

Despite the lack of evidence of thymidylate synthase downregulation, encouraging clinical results were noted in our study. The median PFS and OS in metastatic colorectal cancer patients treated on the once-daily vorinostat schedule were 4.4 and 9.2 months, respectively, despite prior refractoriness to fluoropyrimidines. Although these results are considered exploratory, they do compare favorably with a historical median PFS of 1.9 months with second-line infusional FU/leucovorin in patients who previously progressed on first-line bolus FU and irinotecan therapy (38). The clinical benefits observed in our study cannot be

explained by FU pharmacokinetic modulation. We did not observe any decrease in FU clearance with the addition of vorinostat at the once-daily or twice-daily MTD, nor did we observe any meaningful modulation of DPD activity at those levels. It is possible that the clinical benefits seen from the combination of vorinostat and FU could be a result of vorinostat-induced events other than thymidylate synthase modulation. These could include cell-cycle arrest, gene-expression modulation, apoptosis, and angiogenesis (6–14). Given the limitation of our correlative-science sample size, we cannot rule out a modest effect on thymidylate synthase expression downregulation; this may need to be investigated in larger clinical trials.

In view of the clinical benefits observed in this study, we are currently investigating the once-daily vorinostat schedule (in combination with FU) in a randomized phase II clinical trial. The ongoing study is designed to evaluate the activity of moderate- (800 mg) and high-dose (1,400 mg) vorinostat daily \times 3 in combination with FU/leucovorin in metastatic colorectal cancer patients refractory to FU/leucovorin and resistant to other standard therapies. The 1,700 mg dose level was not selected for the phase II study due to the high incidence of grade \geq 2 nausea or vomiting noted in the phase I study. The primary end point of the ongoing phase II study is to assess the efficacy of vorinostat plus FU/leucovorin in refractory colorectal cancer patients and to select an optimal dose level for future clinical development.

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Table 1

Dose levels of vorinostat plus sLV5FU2

| Dose level | Vorinostat (days 1-3 every 2 wk) | Leucovorin (day 2 every 2 wk) | FU bolus (day 2 every 2 wk) | FU infusion (over 46 h after FU bolus) | |
|----------------------|----------------------------------|-------------------------------|-----------------------------|--|-------------------------|
| Once-daily schedule | DL1-once daily | 600 mg once daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |
| | DL2-once daily | 800 mg once daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |
| | DL3-once daily | 1,000 mg once daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |
| | DL4-once daily | 1,200 mg once daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |
| | DL5-once daily | 1,400 mg once daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |
| | DL6-once daily | 1,700 mg once daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |
| | DL7-once daily | 2,000 mg once daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |
| Twice-daily schedule | DL1-twice daily | 500 mg twice daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |
| | DL2-twice daily | 600 mg twice daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |
| | DL3-twice daily | 800 mg twice daily | 400 mg/m ² | 400 mg/m ² | 2,400 mg/m ² |

Table 2

Patients characteristics

| Patient characteristics (n = 43) | |
|---|--------------|
| Sex (male/female) | 22/21 |
| Age, median (range) | 61 y (31–79) |
| ECOG performance status (0/1) | 12/31 |
| Prior radiation therapy | 8 |
| Primary tumor: | |
| Colorectal | 38 |
| Gastric | 1 |
| Esophagus | 2 |
| Anal | 1 |
| Cholangiocarcinoma | 1 |

Table 3

Treatment-related \geq grade 2 toxicities on the once-daily vorinostat arm

| | DL1 600 mg once daily \times 3 | | DL2 800 mg once daily \times 3 | | DL3 1,000 mg once daily \times 3 | | DL4 1,200 mg once daily \times 3 | | DL5 1,400 mg once daily \times 3 | | DL6 1,700 mg once daily \times 3 | | DL7 2,000 mg once daily \times 3 | |
|------------------|--|-----------|--|-----------|--|-----------|--|-----------|--|-----------|--|-----------|--|-----------|
| | $n = 3$ (G ₂ /G ₃ /G ₄) | | $n = 3$ (G ₂ /G ₃ /G ₄) | | $n = 3$ (G ₂ /G ₃ /G ₄) | | $n = 3$ (G ₂ /G ₃ /G ₄) | | $n = 3$ (G ₂ /G ₃ /G ₄) | | $n = 6$ (G ₂ /G ₃ /G ₄) | | $n = 3$ (G ₂ /G ₃ /G ₄) | |
| | Ist cycle | Any cycle | Ist cycle | Any cycle | Ist cycle | Any cycle | Ist cycle | Any cycle | Ist cycle | Any cycle | Ist cycle | Any cycle | Ist cycle | Any cycle |
| Anemia | 1/0/0 | 1/0/0 | 1/0/0 | 1/0/0 | 1/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 0/0/0 | 2/1/0 | 0/2/0 | 0/2/0 |
| Neutropenia | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 0/1/0 | 0/0/0 | 1/0/0 | 1/0/0 | 1/0/0 |
| Thrombocytopenia | 1/0/0 | 1/0/0 | 1/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 0/1/0 | 0/1/0 |
| Nausea | 1/0/0 | 1/0/0 | 1/0/0 | 2/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 2/0/0 | 4/2/0 | 1/0/0 | 2/0/0 |
| Vomiting | 0/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 2/0/0 | 0/0/0 | 1/0/0 | 0/0/0 | 3/3/0 | 0/0/0 | 0/0/0 |
| Diarrhea | 0/0/0 | 0/1/0 | 0/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/1/0 | 0/0/0 | 2/0/0 | 0/1/0 | 3/2/0 | 0/0/0 | 0/0/0 |
| Anorexia | 1/0/0 | 1/0/0 | 1/0/0 | 2/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 0/0/0 | 1/0/0 | 2/0/0 | 5/0/0 | 1/0/0 | 2/0/0 |
| Fatigue | 1/0/0 | 2/0/0 | 1/0/0 | 2/0/0 | 1/0/0 | 2/0/0 | 2/0/0 | 2/0/0 | 0/0/0 | 2/0/0 | 3/0/0 | 6/0/0 | 0/1*/0 | 0/2/0 |
| Dehydration | 0/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 2/1/0 | 1/0/0 | 1/1/0 |
| Creatinine | 0/0/0 | 1/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 |
| GI bleed | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/1/0 | 0/1/0 |
| Hand and Foot | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/1*/0 | 0/1/0 |
| Mucositis | 0/0/0 | 2/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 1/1/0 | 0/0/0 | 0/0/0 |
| DVT/PE | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/1/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/1/0 | 0/0/0 | 0/0/0 |
| Weight Loss | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 0/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 |
| Infection | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/2/0 | 0/0/0 | 0/0/0 |

* Two patients experienced a DLT at DL7.

Abbreviation: DVT/PE, deep vein thrombus/pulmonary event.

Table 4

Treatment-related \geq grade 2 toxicities on the twice-daily vorinostat arm

| | DL1 500 mg twice daily \times 3 <i>n</i> = 3 (G ₂ /G ₃ /G ₄) | | DL2 600 mg twice daily \times 3 <i>n</i> = 6 (G ₂ /G ₃ /G ₄) | | DL3 800 mg twice daily \times 3 <i>n</i> = 3 (G ₂ /G ₃ /G ₄) | |
|------------------|---|-----------|---|-----------|---|-----------|
| | Ist cycle | Any cycle | Ist cycle | Any cycle | Ist cycle | Any cycle |
| Anemia | 0/0/0 | 0/0/0 | 1/0/0 | 1/1/0 | 0/0/0 | 0/2/0 |
| Neutropenia | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 2/0/0 |
| Thrombocytopenia | 0/0/0 | 0/0/0 | 0/1/0 | 0/0/1 | 3/0/0 | 2/1/0 |
| Nausea | 1/0/0 | 2/0/0 | 3/0/0 | 3/0/0 | 0/0/0 | 0/0/0 |
| Vomiting | 1/0/0 | 1/0/0 | 1/0/0 | 1/0/0 | 0/0/0 | 0/0/0 |
| Diarrhea | 1/0/0 | 1/0/0 | 1/0/0 | 1/0/0 | 1/0/0 | 0/1/0 |
| Anorexia | 0/0/0 | 2/0/0 | 2/0/0 | 2/0/0 | 1/0/0 | 2/0/0 |
| Fatigue | 2/0/0 | 3/0/0 | 3/0/0 | 3/1/0 | 2/1*/0 | 1/2/0 |
| Mucositis | 0/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 1/1*/0* | 2/1/1 |
| Chelitis | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 2/0/0 |
| Esophagitis | 0/0/0 | 1/0/0 | 0/0/0 | 0/0/0 | 1/0/0 | 1/0/0 |
| Dehydration | 0/0/0 | 0/0/0 | 0/0/0 | 2/0/0 | 2/0/0 | 2/0/0 |
| Fever | 0/0/0 | 1/0/0 | 0/0/0 | 0/0/1 | 0/0/0 | 0/0/0 |
| Headache | 0/0/0 | 0/0/0 | 0/0/0 | 0/1/0 | 0/0/0 | 0/0/0 |
| VTE | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/1 |
| ATE | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/0 | 0/0/1 |

* Two patients experienced a DLT at DL3.

Table 5Mean \pm SD pharmacokinetic parameters for plasma FU

| FU maintenance dose (mg/m ²) | Vorinostat (mg) | C _{ss} (μg/mL) | C _{max} (μg/mL) | AUC (μg h/mL) |
|--|------------------|-------------------------|--------------------------|-----------------|
| 2,400 | 600 twice daily | 0.271 \pm 0.014 | 13.0 \pm 3.17 | 24.8 \pm 3.51 |
| 2,400 | — | 0.347 \pm 0.092 | 11.2 \pm 5.55 | 21.8 \pm 9.68 |
| 2,400 | 1,700 once daily | 0.398 \pm 0.106 | 14.2 \pm 2.87 | 27.2 \pm 5.11 |
| 2,400 | — | 0.439 \pm 0.172 | 17.3 \pm 2.97 | 29.9 \pm 6.66 |

NOTE: Data were compared by paired Student's *t*-test. The difference in C_{max} pre- and post-vorinostat on the 1,700 once-daily dose level was the only statistically significant finding (*P* < 0.05).

Table 6

Vorinostat pharmacokinetics

| Patients | C _{max} (μmol/L) | T _{max} (h) | T _{1/2} (h) | AUC ₀₋₈ (μmol/L × h) |
|----------------------------|---------------------------|----------------------|----------------------|---------------------------------|
| 600 mg orally twice daily | | | | |
| 1 | 4.99 | 2 | 1.5 | 14.3 |
| 2 | 3.43 | 1 | 2.2 | 7.8 |
| 3 | 1.97 | 4 | 2.3 | 10.2 |
| Mean | 3.46 | 2.3 | 2.0 | 10.8 |
| SD | 1.51 | 1.5 | 0.4 | 3.3 |
| 1,700 mg orally once daily | | | | |
| 1 | 0.92 | 0.5 | 27.7 | 2.6 |
| 2 | 6.15 | 1 | 2.4 | 26 |
| 3 | 6.40 | 4 | 3.2 | 35.1 |
| Mean | 4.49 | 1.8 | 11.1 | 21.2 |
| SD | 3.09 | 1.9 | 14.4 | 16.8 |

Note: T_{1/2}, half-life