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A Phase I, Pharmacokinetic, and Pharmacodynamic Study of 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 (MTD) of vorinostat in combination with fixed doses of 5-Fluorouracil (5-FU), leucovorin, and oxaliplatin (FOLFOX).

Experimental Design—Vorinostat was given PO BID for 1 week every 2 weeks. FOLFOX was given on days 4 and 5 of vorinostat. The vorinostat starting dose was 100 mg BID. Escalation occurred in cohorts of 3–6 patients. Pharmacokinetics of vorinostat, 5-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 (DL) of vorinostat. Two of 4 evaluable patients at DL 4 (vorinostat 400 mg PO BID) developed dose-limiting fatigue. One of 10 evaluable patients at DL3 (vorinostat 300 mg PO BID) had dose-limiting fatigue, anorexia, and dehydration. There were significant relationships between vorinostat dose and AUC on days 1 and 5 (Pearson, < 0.001). Vorinostat AUC increased ($p = 0.005$) and clearance decreased ($p = 0.003$) on day 5 compared to day 1. The median C_{max} of 5-FU at each DL increased significantly with increasing doses of vorinostat, suggesting a pharmacokinetic interaction between 5-FU and vorinostat. Vorinostat-induced thymidylate synthase modulation

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was not consistent; only two of six patients had a decrease in intra-tumoral thymidylate synthase expression by RT-PCR.

Conclusions—The MTD of vorinostat in combination with FOLFOX is 300 mg PO BID x 1 week every two weeks. Alternative vorinostat dosing schedules may be needed for optimal down-regulation of thymidylate synthase expression.

Keywords

vorinostat; 5-fluorouracil; oxaliplatin; pharmacokinetics; phase I; colon cancer; thymidylate synthase

INTRODUCTION

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 (5-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 approximately 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 (HDAC) have been recently identified as potential anticancer targets. Three classes of HDAC have been identified in humans.[10–12] Class I includes HDAC 1, 2, 3 and 8, which are related to yeast RPD3 deacetylase. HDAC1 and HDAC2 are overexpressed in colonic tumors, suggesting that HDAC may be a potential target in the treatment of that disease.[13, 14] It has also been recently shown that HDAC 3 is over-expressed in colorectal cancer and that its inhibition results in antitumor activity that is independent of other individual HDAC.[15] The mechanisms of growth inhibition produced by HDAC inhibitors include effects on gene expression, cell cycle progression, and cell death pathways; these have been reviewed elsewhere. [15–23]

Vorinostat (Suberoylanilide Hydroxamic Acid, SAHA) is a potent inhibitor of class I and II HDAC 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 demonstrated that vorinostat can down-regulate thymidylate synthase (TS) expression by as much as a 100-fold when measured by Q-PCR analysis, and this down-regulation has been confirmed by western blot after only 24 hours of vorinostat exposure.[28, 29] In that TS over-expression has been associated with clinical resistance to 5-FU, it is feasible that vorinostat may overcome such resistance by down-regulating TS

expression.[28] Indeed, vorinostat has been shown to potentiate 5-FU antitumor activity in pre-clinical 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 pre-clinical 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 (MTD) of vorinostat when administered with a standard, fixed-dose of folinic acid (leucovorin), 5-FU, and oxaliplatin (FOLFOX) in patients with refractory colorectal cancer.

MATERIALS AND METHODS

This phase I, open-label, dose-escalation study of 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 MTD of twice-daily oral vorinostat given for 1 week every 2 weeks in combination with FOLFOX on Days 4 and 5 of vorinostat. Secondary objectives included evaluation of vorinostat, 5-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 intra-tumoral TS expression.

Patient Criteria

Patients with metastatic colorectal cancer who had failed at least two prior lines of treatment including oxaliplatin, a fluoropyrimidine, and irinotecan 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 ECOG performance status of 0–1, have an expected survival of at least 12 weeks, and have acceptable organ function defined as: white blood cell count ≥ 3,000/μl, absolute neutrophil count ≥ 1500/μl, serum creatinine ≤ upper institutional normal level, total bilirubin ≤ upper institutional normal level, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 x upper institutional normal. Patients could not have received any chemotherapy within 4 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 illness were excluded. Patients who were HIV-positive and taking anti-retroviral 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 allowed to participate due to 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 (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 had DLT 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 < 2/6 patients had DLT. This was the MTD. Four additional patients were recruited at the MTD to delineate better the safety of that dose-level.

Treatment Plan—Patients received vorinostat PO BID with food. The investigated dose-levels of vorinostat were 100, 200, 300, and 400 mg PO BID. Vorinostat was administered for 7 consecutive days every 14 days. 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 hours concurrently with 85 mg/m^{2}oxaliplatin, followed by 5-FU 400 mg/m^2 over 5–10 minutes as an intravenous (i.v.) bolus and 5-FU 2400 mg/m^2 over 46 hours as a continuous i.v. infusion. Patients were pre-medicated with i.v. dexamethasone (10 mg) and i.v. ondasetron (8 mg) or its equivalent. Each cycle consisted of 2 weeks, 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 (CBC), and comprehensive chemistry profile were obtained within a week prior to treatment initiation. Baseline CT scans were obtained within 4 weeks prior to initiation of treatment. CBC and comprehensive chemistry profile were repeated on a weekly basis. Medical history, physical examination, and toxicity assessment as per NCI CTC 3.0 were performed weekly during the first cycle and every cycle thereafter. CT scans were repeated every 4 cycles (8 weeks) to assess response. Responses were categorized according to RECIST. [32]

Dose Limiting Toxicities (DLT)—A DLT was defined as any of the following attributable to study treatment in cycle 1: any non-hematological toxicity ≥ 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; any dose delay secondary to toxicity that lasted >1 week. Grade 3 hypomagnesemia, grade 3 hypophosphatemia, grade 3 hypokalemia, and sodium concentrations of 128 – 130 mEq/l were not considered DLTs unless they required hospitalization or persistent for >48 hours 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 prior to 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 >1500 and 75,000/μl, respectively. No growth factors, other than recombinant erythropoietin, were allowed.

Any ≥ grade 3 non-hematological toxicity (except neuropathy and nausea) attributed to FOLFOX required a dose-reduction by one dose level. Treatment was resumed when the non-hematological toxicities recovered to ≤ grade 1.

Only oxaliplatin was modified for neurological 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 5-FU pharmacokinetics—Heparinized, 7-ml blood samples were collected for determination of platinum in plasma ultrafiltrate (PUF) and plasma 5-FU concentrations at 0 (pre-dose), 1, 2 (end of oxaliplatin infusion, start of 5-FU bolus), 2.25, 2.5, 3, 4, 6, 8, 24, and between 44–48 hours, with the average of the last three samples (8, 24, and 46 hours) being used to determine the steady-state 5-FU concentration.

Platinum measurements—Plasma ultrafiltrate (PUF) was prepared from plasma by centrifugal ultrafiltration using Amicon Centrifree Micropartition Systems (Millipore Corporation, Billerica, MA) and platinum was measured with a validated flameless atomic absorption spectrophotometric (PE ZL4100; Perkin Elmer, Shelton, CT) method.[33] Briefly, PUF was diluted 1:1 in 0.1% nitric acid + 0.2% triton X-100, and a 20 μl was injected into the AA. 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.

5-FU measurements—5-FU in plasma was measured using a modified validated LC/MS/MS method on an Applied Biosystems MDS Sciex API 3000 Triple Quadrupole Mass Spectrometer (Concord, Ontario, CA) equipped with an Aligent 1100 HPLC system (Palo Alto, CA).[34] 5-FU and its isotopic internal standard [$^{15}N_2$] 5-FU were obtained from Sigma-Aldrich chemical company (St. Louis, MO). Normal human plasma for calibration standards was obtained from BioMedical Resources (Hatboro, PA). 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 x 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 prior to a 50 μl injection. Calibration standards (5 ng/ml to 100 ng/ml) were processed in a similar manner. A Supelcosil LC-18-S (150 cm x 4.6 mm i.d.) C18 column (Supelco; St. Louis, MO), with a mobile phase consisting of methanol: 5mM ammonium formate (15:85, v/v) and a flow rate

of 600 μl/min was used for the separation. Using electrospray ionization, the molecular ion for 5-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 [$^{15}N_2$] 5-FU is two mass units higher than 5-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 −4200 V was used for the ionization. The ratio of the peak area of 5-FU and its internal standard ([$^{15}N_2$] 5-FU) were used for quantitation of 5-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 LC/MS/MS 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 (CV%) to be in the range of 0 to 13.3 and that for inter-day 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 hours 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 20–30 minutes and were then centrifuged at 2,000 x g for 15 minutes 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, 5-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 with Pearson's correlation as well as Spearman's 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 values are reported, and P values ≤0.05 were considered statistically significant. Statistical evaluation was performed with SPSS software, version 15 (SPSS, Chicago, IL).

Tumor Biopsies

Pre-treatment 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 hours 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, Littleton, CO), 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, kupfer cells of human liver for TP, and cytoplasm of human hepatocytes for DPD). 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 RT-PCR assay using a PE-ABI PRISM 7700 Sequence Detection System (Applied Biosystems Inc., Foster City, CA) 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, Valencia, CA), and cDNA was synthesized using Superscript II reverse transcriptase (Life Technologies, Grand Island, NY). Our TS gene expression methodology was previously detailed.[32]

RESULTS

Demographics

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

Treatment Administration

One patient on DL 1 developed complete bowel obstruction related to peritoneal carcinomatosis and was replaced because he was deemed non-evaluable for potential treatment-related gastrointestinal toxicities. None of the other 3 patients at DL1 developed a DLT. Three additional patients were treated at DL2 (vorinostat 200 mg PO BID) and DL3 (vorinostat 300 mg PO BID) without any DLT. At DL4 (vorinostat 400 mg PO BID), 2 of 4 patients developed a DLT; therefore, this dose-level was declared as intolerable. DL3 was

subsequently expanded to 6 patients, and one of the 3 additional patients developed a DLT, which defined this dose-level as the MTD. DL3 was expanded by 4 additional patients, none of whom developed a DLT. A total of 25, 33, 53, and 14 cycles were administered at DL1, DL2, DL3, and DL4, respectively.

Toxicity

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

Dose Limiting Toxicities and Maximum Tolerated Dose—Two of 4 patients at DL4 developed DLTs. These consisted of grade 3 diarrhea and fatigue in one patient and grade 3 fatigue in the other. DL3 was expanded to 6 patients, one of whom developed dose-limiting grade 3 fatigue, anorexia, and dehydration. DL3 (vorinostat 300 mg PO BID) was declared the MTD and was expanded to a total of 10 patients without any further DLT.

Hematological Toxicity—Neutropenia and thrombocytopenia were the predominant hematological toxicities (Table 3). None of the patients on DL1 and 2 experienced ≥ grade 3 neutropenia or thrombocytopenia. Two of the 10 patients treated at DL3 developed grade 3 neutropenia, and 3 developed grade 2 thrombocytopenia. Of the 4 patients treated at DL4, 2 developed grade 3 neutropenia, one developed grade 4 neutropenia, and 2 developed grade 4 thrombocytopenia.

Non-hematological Toxicity—Non-hematological 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 mg and 400 mg PO BID. Nausea/ vomiting and fatigue seemed to peek during FOLFOX chemotherapy, i.e. on days 4–5 of each cycle.

Antitumor Activity—All 21 patients were assessable for response. No patient developed an objective response. Eleven patients had stable disease (SD) on their 2-month staging CT scan. SD was confirmed in 5 patients, who remained on treatment for 9, 10, 12, 12, and 16 cycles.

Pharmacokinetics

5-FU Pharmacokinetics—Following a loading dose and institution of the 46-hour continuous infusion, 5-FU plasma concentrations achieved steady-state quickly, with median steady-state concentrations (Css) ranging from 0.27–0.51 μg/ml (Table 5). The median 5-FU Css increased with increasing vorinostat doses; 3, 16, 20 and 23 μg/ml for the 100, 200, 300, and 400 mg BID dose groups, respectively. Median 5-FU AUC also increased with increasing vorinostat dose level; 14, 26, 35, and 44 μg*hr/ml across the 100–400 mg dose groups, respectively. However, only C_{max} differences between the different DL of vorinostat were statistically significant by ANOVA analysis ($p = 0.047$), which likely reflects the small population size and the large inter-patient variability.

Platinum Pharmacokinetics—Ultrafilterable platinum plasma concentrations displayed a biexponential decay following oxaliplatin administration with a median half life of 16–21 hours across the various vorinostat cohorts. Median ultrafilterable platinum C_{max} and AUC were 0.543–0.846 μg/ml and 4.9 – 6.4 μg*hr/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 AUC on both 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 100–400 mg BID and vorinostat apparent clearance (Clapp). Suitable pharmacokinetic data were available to compare days 1 and 5 Cmax and Tmax for 19 patients and AUC, t1/2, and Clapp for 14 patients. There was a significant increase in vorinostat AUC (p=0.005) and associated significant decrease in vorinostat Clapp (p=0.003) within patients when days 1 and 5 values were compared. There was also a statistically significant increase in vorinostat Tmax when days 1 and 5 values were compared (p=0.02).

Pharmacodynamics

TS tumor expression by IHC—TS tumor expression was evaluated by immunohistochemistry before study treatment and on the 4th 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. Out of 6 paired samples, 4 patients showed no change in their staining pattern (2 strong and 2 moderate staining). One patient had a decreased intensity from strong to weak (DL1), and one patient had an increase in intensity from weak to strong (DL4).

TS tumor expression by RT-PCR—Following vorinostat treatment, only 2 of 6 patients (DL1 and DL4) illustrated down-regulation of TS. The decrease in TS gene expression was approximately 33% (2.27 decreased to 1.58 relative to β-actin) in the patient at DL4 and 50% (0.92 decreased to 0.45) in the patient at DL1. The other four patients did not show any change in TS expression. Of note, the same patient with a decrease in TS by RT-PCR had an increase in staining by IHC.

DISCUSSION

In this phase I clinical trial, we evaluated the combination of a novel schedule of vorinostat PO BID x 1 week repeated every 2 weeks in combination with FOLFOX on days 4 and 5 of vorinostat. We have established vorinostat 300 mg PO BID in combination with a standard dose of FOLFOX as the MTD. The DLTs 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 hematological DLTs, grade 3–4 thrombocytopenia was seen in 5 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 AUC and lack of dose-related changes in vorinostat Clapp are also consistent with previous reports.[27] The statistically significant increase in vorinostat AUC and associated decrease in vorinostat Clapp 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 as is whether the change is associated with chronic vorinostat dosing or administration with other drugs.

Steady-state concentrations of 5-FU observed in this study were comparable to historical data for concentrations observed with infusional 5-FU regimens (0.065 – 0.39 μg/mL).[36] Trends of 5-FU Css, C_{max}, and AUC increases were observed with higher doses of vorinostat while 5-FU clearance decreased. These observations suggest a potential pharmacokinetic interaction between vorinostat and 5-FU. Dihydropyrimidine dehydrogenase (DPD) is the predominant enzyme in the degradation of 5-FU.[37, 38] We hypothesize that vorinostat decreases DPD activity either by suppressing DPD mRNA expression or through post-translational protein alteration, with a subsequent decrease in DPD-enzymatic activity. We are currently investigating this interaction through the conduct of a variant intermittent vorinostat schedule in combination with 5-FU. This ongoing study is evaluating 5-FU pharmacokinetics with and without vorinostat and the effects of vorinostat on DPD activity.[39]

Despite the pre-clinical synergy between vorinostat and 5-FU and platinums, no confirmed objective response could be documented in this phase I clinical trial. Five of 21 chemo-resistant colorectal cancer patients had confirmed stable disease; however, we cannot confirm if these stabilizations were due to FOLFOX re-challenge 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 6 paired tumor samples, there were no consistent effects of vorinostat on TS expression, assessed by RT-PCR or IHC, at any dose-level. We hypothesize that the lack of TS down-regulation in our study was likely secondary to inadequate vorinostat exposure. In pre-clinical studies, effective TS down-regulation requires 24 hours of vorinostat exposure at concentrations ≥ 5 μM.[28, 29] In our study, the C_{max} of vorinostat was < 2 μM, and the half-life was 1.2 – 2.4 hours, at all dose levels. Therefore, vorinostat pharmacokinetics in our study were 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/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 x 3 days every 3 weeks.[26] The C_{max} of vorinostat at that dose level exceeded 20 μM. We are currently investigating a daily x 3 schedule of vorinostat every 2 weeks in combination with 5-FU/LV 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.

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Statement of Relevance

Vorinostat, a histone deacetylase inhibitor has been associated with thymidylate synthase downregulation and synergy with 5-FU in pre-clinical studies. In this phase I clinical trial, we have evaluated escalating doses of vorinostat in combination with a fixed dose of 5-FU, leucovorin, and oxaliplatin (FOLFOX). We show that vorinostat can be administered safely up to doses of 300 mg PO BID x 1 week every 2 weeks, in combination with a full dose of FOLFOX. However, despite this high dose of vorinostat, the pharmacokinetics (PK) of vorinostat were not optimal for the down-staging of thymidylate synthase expression by RT-PCR and IHC from serial tumor biopsies. Our data suggest the need of shorter intermittent high doses of vorinostat in combination with FOLFOX or 5-FU/LV. Indeed, such regimens (vorinostat QD or BID x 3 days every 2 weeks with 5-FU/LV or FOLFOX) are currently being investigated in our institute with promising preliminary results.

Table 1

Dose reduction levels for FOLFOX

	Oxaliplatin	LV	5-FU Bolus	5-FU Infusion
Dose level -1	65mg/m^2	400mg/m^2	300mg/m^2	2000mg/m^2
Dose level -2	55mg/m^2	400mg/m^2	0	1800mg/m^2
Dose level -3	0	400mg/m^2	0	1800mg/m^2

Table 2

Patient Characteristics

Patient Characteristics (n = 21)	
Sex (male/female)	13/8
Age (median/range)	58/ 36–77 years
ECOG (0/1)	8/13
Prior Radiation Therapy	8

Table 3

Hematological Toxicities (≥ Grade 2)

	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)

All cycles toxicities are listed in between parenthesis

Table 4

Non-Hematological Toxicity (≥ Grade 2)

	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)

All cycles toxicities are listed in between parenthesis

* Three patients experienced DLT: one patient on DL-3 experienced G3 nausea/vomiting, fatigue, anorexia, and dehydration; two patients at DL-4 experienced G3 fatigue one of who and an associated G3 diarrhea

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Table 5

Mean ± SD Pharmacokinetic Parameters for Plasma 5-FU*

5-FU Maintenance Dose (mg/m^2)	Vorinostat Dose (mg)	Css (ηg/mL)	Cmax (ηg/mL)	AUC (ηg* hr/mL)
2400	100	0.287 ± 0.044	5.03 ± 4.39	15.3 ± 5.7
2400	200	0.274 ± 0.045	13.1 ± 7.96	24 ± 9.11
2400	300	0.396 ± 0.159	24.5 ± 14.5	68.7 ± 88.1
2400	400	0.432 ± 0.077	26.8 ± 11.4	46.7 ± 10.6

*ANOVA analysis was performed and only C_{max} was statistically significant among the vorinostat treatment groups.

Table 6

Pharmacokinetic Parameters for Vorinostat on Days 1 and 5

		Day 1					Day 5				
Dose (mg)		**Cmax (μM)**	**Tmax (h)**	**AUC (μM*h)**	**t1/2 (h)**	**CLapp (l/h)**	**Cmax (μM)**	**Tmax (h)**	**AUC (μM*h)**	**t1/2 (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