Published in final edited form as:

Invest New Drugs. 2011 October ; 29(5): 1004–1012. doi:10.1007/s10637-010-9447-x.

A phase I pharmacokinetic study of pulse-dose vorinostat with flavopiridol in solid tumors

Mark Andrew Dickson,

Melanoma and Sarcoma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

Dana E. Rathkopf,

Genitourinary Oncology Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Richard D. Carvajal,

Melanoma and Sarcoma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

Steven Grant,

Hematology/Oncology, Department of Medicine, Virginia Commonwealth University Health Sciences Center and the Massey Cancer Center, Richmond, VA, USA

John D. Roberts,

Hematology/Oncology, Department of Medicine, Virginia Commonwealth University Health Sciences Center and the Massey Cancer Center, Richmond, VA, USA

Joel M. Reid,

Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA

Matthew M. Ames,

Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA

Renee M. McGovern,

Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA

Robert A. Lefkowitz,

Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Mithat Gonen,

Biostatistics Service, Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Lauren M. Cane,

Melanoma and Sarcoma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

Heather J. Dials, and

Melanoma and Sarcoma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

Gary K. Schwartz

Melanoma and Sarcoma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA

Gary K. Schwartz: schwartg@mskcc.org

Summary

Purpose—Vorinostat (V) at levels $>2.5 \mu\text{M}$ enhances chemotherapy in vitro. Yet the approved oral dose of 400 mg inconsistently achieves this level in patients. We developed an intermittent oral pulse-dose schedule of V to increase serum levels. We combined V with the cyclin dependent kinase inhibitor flavopiridol (F) which increases V-induced apoptosis.

Experimental Design—One week before combination treatment, V alone was given daily for 3d (cycle –1). Then V was given on d1-3 and d8-10, and F on d2 and d9, every 21-d. Due to neutropenia, this was modified to V on d1-3 and d15–17, and F on d2 and d16, every 28-d. Bolus and split-dose F schedules were studied.

Results—34 patients were treated. On the 21-d schedule, the maximum tolerated dose (MTD) was V 600 mg/d and F 60 mg/m² bolus. On the 28-d schedule, the MTD was V 800 mg/d and F 30 mg/m² over 30 min and 30 mg/m² over 4 h. V C_{max} at the 800 mg dose was $4.8 \mu\text{M}$ (± 2.8). V C_{max} $2.5 \mu\text{M}$ was achieved in 86% of patients at the MTD. F increased the C_{max} of V by 27% (95% CI 11%–43%). F C_{max} of $2 \mu\text{M}$ was achieved in 90% of patients. 8 patients had stable disease for on average 5.5 m (range 1.6–13.2 m).

Conclusions—Intermittent high dose oral V in combination with F is feasible and achieves target serum levels $>2.5 \mu\text{M}$. V concentrations higher than previously reported with oral dosing were achieved.

Keywords

CDKs and CDK inhibitors; Histone deacetylase inhibitors; Phase I trials; Combination chemotherapy; Pharmacokinetics

Introduction

Histone acetylation, a key element of transcription control, is regulated by histone acetyltransferase and histone deacetylase (HDAC) enzymes [1–3]. HDACs act on nucleosomal histones causing coiling of chromatin and silencing of genes implicated in the regulation of cell survival, proliferation, differentiation, and apoptosis [4]. HDACs are also involved in recruiting transcription factors to the promoter region of tumor suppressor genes and alter the acetylation cell cycle regulatory proteins [5].

Because aberrant HDAC activity has been implicated in a variety of cancers, development of HDAC inhibitors is a rational approach to the anticancer therapy. Vorinostat (suberoylanilide hydroxamic acid; Zolinza) is a small molecule HDAC inhibitor that binds enzyme's active site [6]. Vorinostat was approved in 2006 by the U.S. Food and Drug Administration for the treatment of cutaneous T-cell lymphoma.

Preclinical data show that the vorinostat has in vitro activity against many different cancer types and also enhances the effect of agents such as cisplatin and gemcitabine [7, 8]. However, this potentiation generally occurs at vorinostat concentrations of $>2 \mu\text{M}$, which is inconsistently achieved in patients at the approved oral dose of 400 mg/d [9].

Thus, we sought to develop an intermittent oral pulse-dose schedule of vorinostat to increase serum drug levels. We combined vorinostat with the pan-cyclin dependent kinase inhibitor flavopiridol based on our pre-clinical data that sequential treatment with flavopiridol increases vorinostat-induced apoptosis [10].

Flavopiridol (Alvocidib) is one of the first CDK inhibitors to enter clinical trials. It is a semi-synthetic alkaloid that inhibits CDKs including CDK1, CDK2, CDK4, and CDK6 [11]. It inhibits tumor cell growth in vitro through blockade of cell cycle progression at the G₁-S or G₂-M interfaces [12, 13]. Flavopiridol also represses gene transcription by inhibiting CDK9 and downregulating RNA polymerase II [14].

Flavopiridol and vorinostat act synergistically in vitro to induce mitochondrial damage and apoptosis [15–17]. This is thought to be mediated by transcriptional downregulation of the antiapoptotic proteins MCL-1 and XIAP and disruption of NF-kappaB [18, 19]. Our preclinical data show that flavopiridol enhances vorinostat-induced apoptosis in a time- and sequence-dependent manner. In vitro synergy is seen only when vorinostat is given first, followed by flavopiridol. The effect is maximal when flavopiridol is given 24 h after vorinostat, and when vorinostat concentration is 2.5 μ M [10]. This information was used to select the schedule and dose escalation strategy.

The primary objective of this trial was to determine the maximum tolerated dose (MTD) of intermittent pulse-dose oral vorinostat when administered in combination with flavopiridol in patients with advanced solid tumors. Secondary objectives were to investigate the clinical pharmacokinetics of vorinostat and flavopiridol, and to obtain preliminary data on the therapeutic activity of this regimen.

Patients and methods

Eligibility

Patients 18 years of age with a diagnosis of pathologically confirmed measurable or evaluable advanced solid tumor, with disease that was refractory to standard therapy or for which there was no standard therapy, were eligible. Patients had to have an Eastern Cooperative Oncology Group (ECOG) Performance Status score of 2, a total white blood cell count $> 3,000/\text{mm}^3$, an absolute neutrophil count $> 1,500/\text{mm}^3$, a platelet count of $> 100,000/\text{mm}^3$, and adequate hepatic and renal function. Patients may have received prior chemotherapy (including HDAC inhibitors), immunotherapy, hormonal therapy, or radiotherapy, but 2 weeks from last dose had to elapse before study entry (6 weeks for nitrosoureas and mitomycin C). Patients with central nervous system metastases or a primary central nervous system neoplasm were not eligible.

The protocol was approved by the Institutional Review Board of each institution and all patients provided written informed consent.

Treatment plan

This was an open-label, non-randomized, dose escalation study to determine the MTD of vorinostat in combination with flavopiridol. The starting dose of intermittent pulse-dose vorinostat was based on earlier studies which determined that vorinostat can be safely given at 400 mg daily continuously [20]. Prior studies with flavopiridol have shown doses of 60–80 mg/m^2 to be safe and tolerable when combined with other agents [21–23].

Groups of three to six patients were treated sequentially according to the dose escalation in Table 1. One week before beginning combination therapy, vorinostat was administered daily by mouth for 3 days as a single agent (cycle –1). Combination treatment began with cycle 1.

In part 1, vorinostat was initially given on days 1–3 and 8–10 of each 21-day cycle. Flavopiridol was administered by vein on day 2 and 9 of each cycle, following that day's dose of vorinostat. Dose escalation of vorinostat was initially performed with flavopiridol fixed at 60 mg/m² over 1 h. In part 2, a split-dose schedule of flavopiridol 30 mg/m² over 30 min followed by 30 mg/m² over 4 h was investigated. Due to dose-limiting neutropenia, the schedule was modified in part 3 to the following: vorinostat on days 1–3 and 15–17, and flavopiridol on day 2 and 16, of each 28-day cycle.

Because of concerns for tumor lysis syndrome with the split dose schedule, the day following therapy blood samples were obtained for creatinine, LDH, calcium, and phosphorous.

All treatments were administered in the outpatient setting and, once assigned to a dose level, intra-patient dose escalation was not permitted.

Toxicity was graded in accordance with the Common Toxicity Criteria version 3.0 [24]. Dose Limiting Toxicity (DLT) was defined as the occurrence of Grade 4 hematologic toxicity, Grade 3 or 4 non-hematologic toxicity including diarrhea despite antidiarrheal prophylaxis, or any delay in treatment resulting in fewer than 2 treatments in the first 3 weeks of a cycle. The MTD was defined as one level below the dose at which two or more of the patients experience DLT during the first cycle. Patients who experienced a DLT, or toxicity attributed to study medication, could continue to receive study treatment after recovery with appropriate dose modifications as defined per protocol.

To be evaluable for response and to be assessable for determination of MTD, patients had to have received at least one full cycle of therapy. Responses were evaluated after every two cycles with computed tomography scans or other diagnostic tests, as appropriate. Response Evaluation Criteria in Solid Tumors (RECIST) were used by an independent protocol radiologist [25].

Drug supply

Flavopiridol (also known as alvocidib) and vorinostat were supplied by Sanofi Aventis Pharmaceuticals and Merck & Co., Inc., respectively, through the National Cancer Institute, Cancer Therapy Evaluation Program, which supported the trial. Patients were treated at two centers: Memorial Sloan-Kettering Cancer Center ($n=27$) and Medical College of Virginia ($n=7$).

Statistical design

The main objective of this study was to determine the MTD of flavopiridol when administered in combination with vorinostat. Standard 3+3 design was used for dose escalation. The incidence of toxicities was summarized separately by flavopiridol cohort. Secondary analyses included pharmacokinetic analyses of vorinostat and of flavopiridol by non-compartmental methods.

Pharmacokinetics

For each patient, blood samples for vorinostat pharmacokinetics were collected during cycle –1, day 2 (treatment with vorinostat alone), and during cycle 1, day 2 (first combination treatment). Blood was collected into tubes without anticoagulant before and 0.5, 1, 2, 2.5, 3, 4, 6.5, and 7 h after oral administration of vorinostat. The 2, 2.5, and 6.5 h time points corresponded with the beginning and end of flavopiridol bolus and infusion periods. Blood samples were allowed to clot at 4°C for 20–30 min and then centrifuged at 2,000 *g* for 15 min. Serum was stored at –70°C. Vorinostat concentration was measured by a validated

liquid chromatography-electrospray ionization tandem mass spectrometry method [26]. Appropriate quality control samples were used. Area under the curve (AUC) from 0 to 7 h was calculated using a non-compartmental model.

During cycle 1, day 2, samples were also collected to determine flavopiridol pharmacokinetics. Flavopiridol was isolated from plasma by liquid-liquid phase extraction in a mixture of acetonitrile and methanol (4/1, v/v), followed by high-performance liquid chromatography and tandem mass spectrometry (Sciex API 4000, Applied Biosystems, Foster City, California) analysis using an electrospray ionization method in the positive ion mode.

Results

Patient characteristics

From 3/15/06 to 1/15/09, 34 patients with advanced solid tumors were enrolled and treated. Table 2 lists the characteristics of these patients. Three patients did not complete a full cycle of treatment and are therefore not assessable for response.

The median age was 59 years (range: 31–85 years) and the median ECOG status was 1 (range: 0–1). There were 23 men and 11 women. The cancers treated and patient numbers were colorectal (10), sarcoma (7), prostate (3), biliary (2), and others (12). Patients were in general heavily pre-treated with a mean of 4 prior therapies (range: 1–11).

Safety

Table 3 lists the most common grade 2 to 4 toxicities for the first cycle of therapy. In cohorts 1–3, flavopiridol was fixed at 60 mg/m² on day 2 and 9 and vorinostat was escalated from 400 mg to 800 mg on days 1–3 and 8–10. In cohort 2, one patient had a DLT with grade 3 mucositis. The cohort was expanded with no further DLT in six evaluable patients. Of the 4 patients treated at Cohort 3, three experienced DLTs: one patient developed a grade 4 pulmonary embolus; the second developed pancytopenia with grade 4 neutropenia, grade 3 anemia, and grade 4 thrombocytopenia, and the third developed grade 3 lower gastrointestinal bleeding following cycle –1 of therapy with vorinostat alone. Thus the MTD of vorinostat with bolus flavopiridol was determined to be dose level 2, vorinostat 600 mg/day on days 1–3 and 8–10 and flavopiridol 60 mg/m² over 1 hr on days 2 and 9, every 21 days.

Starting then in cohort 4, the study was expanded to evaluate the combination of vorinostat with divided-dose flavopiridol given as 30 mg/m² over 30 min and 30 mg/m² over 4 h. This pharmacokinetically-derived flavopiridol schedule was shown to be active in patients with refractory chronic lymphocytic leukemia [27]. The first patient treated in cohort 4 with vorinostat 600 mg in combination with split-dose flavopiridol experienced dose-limiting neutropenia on day 8, preventing re-treatment on the day 1–3/day 8–10 schedule. The dose of vorinostat therefore was reduced to 400 mg in cohort 5, but again both patients treated at this dose experienced dose-limiting neutropenia on day 8.

In an effort to identify a tolerable dose of vorinostat in combination with split-dose flavopiridol, the treatment schedule was changed in part 3 from a 21-day cycle (2 weeks of treatment followed by 1 week off) to a 28-day cycle (treatment every other week). Thus, starting in cohort 6, vorinostat was given on days 1–3 and 15–17 with split-dose flavopiridol (30 mg/m² over 30 min and 30 mg/m² over 4 h) on days 2 and 16, every 28 days. Three patients in cohort 6 completed cycle 1 with no DLT. In cohort 7, with vorinostat escalated to 600 mg, one of three patients had a DLT with grade 4 neutropenia. The cohort was expanded to include six evaluable patients with no further DLT observed. In cohort 8, at 800

mg of vorinostat, the first three patients experienced no DLT. Dose escalation continued. Of the three patients treated in cohort 9 with 1,000 mg of vorinostat, one had a DLT in the form of grade 4 neutropenia and a second patient developed neutropenic fever, renal and respiratory failure, and ultimately expired. This was considered possibly related to treatment. With two DLTs in three patients, cohort 9 was the maximum administered dose and was considered to exceed the MTD. Therefore the reduced dose of 800 mg of vorinostat was re-explored to better define the MTD. Cohort 8 was expanded with three additional patients. Only one of these patients experienced DLT with grade 4 neutropenia. Thus the MTD was determined to be vorinostat 800 mg given on days 1–3 and 15–17 with split-dose flavopiridol on days 2 and 16, every 28 days. The DLT rate for the MTD cohort was 17% (1/6). This was considered acceptable using pre-specified guidelines since the upper limit of the 95% posterior interval was 40% [28].

The most common grade 3 and 4 cumulative toxicities for all patients are listed in Table 4. The most common drug-related effect was myelosuppression, with leukopenia seen in 44% of patients and neutropenia in 47%. Significant fatigue, often a DLT in other studies of vorinostat, was not seen. Only one patient developed grade 3 fatigue.

Responses

Table 5 summarizes responses. Of 31 evaluable patients, eight had as best response stable disease for an average of 5.5 m (range: 1.6–13.2 m), including a patient with extra-medullary chondrosarcoma who was on study for 13.2 m. There were no objective responses.

Vorinostat pharmacokinetics

Pharmacokinetic data are presented for patients grouped by cohort (Table 6) and by dose of vorinostat (Table 7). Overall, vorinostat C_{max} and AUC increased with vorinostat dose. Mean vorinostat C_{max} for patients treated at the 800 mg dose was 4.8 μM (± 2.8). Mean vorinostat C_{max} for pts treated at the 1,000 mg dose was 12.7 μM (± 5.4). These numbers easily exceeded the goal C_{max} of 2.5 μM .

Cycle –1 and cycle 1 vorinostat concentration for patients grouped by vorinostat dose are shown in Table 8. For each patient, vorinostat C_{max} and AUC generally increased from cycle –1 (when vorinostat was administered alone) to cycle 1 (when administered with flavopiridol). This effect was seen with both the bolus and split-dose flavopiridol schedules. Overall, the average ratio of cycle 1 C_{max} to cycle –1 C_{max} was 1.27 (95% CI 1.11–1.43) and the average ratio of cycle 1 AUC to cycle –1 AUC was 1.14 (95% CI 0.98–1.30). Between cohort 8 (800 mg) and cohort 9 (1,000 mg), mean vorinostat PK levels increased substantially, although there was significant inter-patient variability.

Flavopiridol pharmacokinetics

Samples to measure flavopiridol concentration were available for 20 patients. The mean flavopiridol C_{max} was 3.92 μM (SD 2.12). There was no significant interaction with vorinostat dose or flavopiridol administration schedule. 90% of the patients tested achieved flavopiridol C_{max} of $\geq 2 \mu\text{M}$, a level shown in prior trials to be consistent with synergy with cytotoxic chemotherapy and with vorinostat [10, 22, 29].

Discussions

This clinical trial describes the safety and pharmacokinetics of intermittent pulse-dose oral vorinostat. It is the first combination of an HDAC inhibitor, vorinostat, with a CDK inhibitor, flavopiridol, in patients with solid tumors. The MTD of oral vorinostat was

determined to be 800 mg once daily for 3 days (days 1–3) with flavopiridol on day 2, every 14 days. The flavopiridol schedule is 30 mg/m² over 30 min followed by 30 mg/m² over 4 h. This split-dose schedule is considered preferable for further investigation than the bolus flavopiridol schedule given the promising results seen with split-dose flavopiridol in other studies [30].

Intermittent pulse-dose oral vorinostat has previously been reported in combination with infusional fluorouracil and leucovorin. In that phase I study, the MTD of vorinostat was 1,700 mg PO daily × 3 days, every 14 days [31]. In this study of vorinostat with flavopiridol, the dose of vorinostat could not be escalating to such high levels, likely due to overlapping toxicity of vorinostat and flavopiridol. However, both studies confirm that intermittent treatment with vorinostat at doses higher than the approved 400 mg level is tolerable.

The goal of this study was to achieve vorinostat $C_{max} > 2.5 \mu M$ consistently by intermittent oral pulse dosing. Previously, high serum levels of vorinostat have been achieved with intravenous dosing. In the first phase I trial, vorinostat doses as high as 900 mg/m² IV daily × 3 days every 3 weeks were tolerated with no DLT, achieving C_{max} of well over 30 μM [32]. However, oral dosing generally achieves much lower serum levels. The standard dose of 400 PO mg daily continuously can achieve C_{max} in the 2.5 μM range but, given the large inter-patient variability, C_{max} is significant below 2.5 μM for many patients [9]. Moreover, trials of vorinostat in combination with other agents such as carboplatin and paclitaxel or FOLFOX generally achieve average C_{max} significantly less than 2 μM [33, 34].

Here, vorinostat concentrations higher than previously reported with oral dosing were achieved. The mean vorinostat C_{max} at the 600 mg, 800 mg, and 1,000 mg dose levels were 3.1, 4.8, and 12.7 μM , respectively. Vorinostat PK parameters at the 1,000 mg dose level appear disproportionately high (Table 8), however the number of patients treated at that level was small and the inter-patient variability was large. At the 800 mg dose level, vorinostat $C_{max} > 2.5 \mu M$ was achieved in 86% of patients. Vorinostat levels may have been increased compared to prior studies due to drug accumulation since PK assessments were conducted on the second day of administration. However, this effect is not expected to be significant due to the drug's relatively short half-life.

Vorinostat pharmacokinetics appear to have been altered by concomitant administration of flavopiridol in cycle 1 versus cycle –1. There was a trend to increased vorinostat C_{max} and AUC when flavopiridol was given concurrently. This may simply have been due to variation in drug absorption from meal content, for example [35]. In this trial, patients were not required to be fed or fasting prior to drug administration. Alternatively, this may represent a real drug-drug interaction with flavopiridol. The mechanism is for this potential interaction is not clear. Vorinostat is metabolized by glucuronidation and β -oxidation while flavopiridol is principally metabolized by glucuronidation. Neither agent is significantly metabolized through the cytochrome P450 system. However, it does illustrate the importance of detailed PK monitoring when vorinostat is combined with other small molecules.

The principal toxicity of this combination was myelo-suppression. Although myelosuppression due to vorinostat at standard doses is generally uncommon and mild, it may have been more severe in this trial due to the high serum drug levels. In contrast, significant fatigue often seen with continuous dosing of vorinostat was less often seen here. Overall, this novel schedule may have altered the toxicity profile of vorinostat.

Flavopiridol, with its known myelosuppressive effect, likely contributed to the observed toxicity. This is supported by the observation that even higher doses of vorinostat are tolerable when administered with fluorouracil instead of flavopiridol [31]. Interestingly,

although diarrhea was a major adverse effect in prior trials, especially those combining flavopiridol with irinotecan, here it was not a significant problem, and could be prevented with adequate premedication. Flavopiridol pharmacokinetics were consistent with the dose and schedule of administration and adequate blood levels were achieved.

Although no objective tumor responses were seen in this heavily pre-treated group of solid tumor patients, this combination is appropriate for further testing. Vorinostat is already an approved therapy for cutaneous T-cell lymphoma. Encouraging activity has also been reported in single-agent studies in acute myeloid leukemia, multiple myeloma, and diffuse large B-cell lymphoma [36–38]. Flavopiridol has significant activity in chronic lymphocytic leukemia [27]. The combination of the two agents thus has the potential to be highly active, and the schedule developed here may maximize dose-response benefit. Although continuous dosing vorinostat is also under investigation in combination with intermittent flavopiridol, [39] we have shown here that intermittent administration of high dose vorinostat in combination with flavopiridol is feasible. This novel combination of an HDAC inhibitor and a CDK inhibitor is a rational strategy to pursue in phase 2 trials. These results also support the strategy of combining intermittent high dose vorinostat with other anti-cancer agents where higher vorinostat levels may enhance efficacy.

Acknowledgments

Supported by NCI R01CA67819 to GKS.

References

1. Grunstein M. Histone acetylation in chromatin structure and transcription. *Nature*. 1997; 389:349–352. [PubMed: 9311776]
2. Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat Rev Drug Discov*. 2002; 1:287–299. [PubMed: 12120280]
3. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. *Nat Rev Cancer*. 2001; 1:194–202. [PubMed: 11902574]
4. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet*. 2002; 3:415–428. [PubMed: 12042769]
5. Arts J, de Schepper S, Van Emelen K. Histone deacetylase inhibitors: from chromatin remodeling to experimental cancer therapeutics. *Curr Med Chem*. 2003; 10:2343–2350. [PubMed: 14529477]
6. Finnin MS, Donigian JR, Cohen A, et al. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature*. 1999; 401:188–193. [PubMed: 10490031]
7. Arnold NB, Arkus N, Gunn J, Korc M. The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces growth inhibition and enhances gemcitabine-induced cell death in pancreatic cancer. *Clin Cancer Res*. 2007; 13:18–26. [PubMed: 17200334]
8. Shen J, Huang C, Jiang L, et al. Enhancement of cisplatin induced apoptosis by suberoylanilide hydroxamic acid in human oral squamous cell carcinoma cell lines. *Biochem Pharmacol*. 2007; 73:1901–1909. [PubMed: 17445779]
9. Kelly WK, O'Connor OA, Krug LM, et al. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J Clin Oncol*. 2005; 23:3923–3931. [PubMed: 15897550]
10. Cheema HS, Motwani MV, Schwartz GK. Suberoylanilide Hydroxamic Acid (SAHA), a histone deacetylase (HDAC) inhibitor, induces a unique mitotic effect and potentiates flavopiridol (F) induced apoptosis [abstract]. *Proc Am Assoc Cancer Res*. 2004; 45:Abstract #2443.
11. Naik RG, Kattige SL, Bhat SV, Alreja B, de Souza NJ, Rupp RH. An antiinflammatory cum immunomodulatory piperidiny-benzopyranone from *dysoxylum binectariferum*: isolation, structure and total synthesis. *Tetrahedron*. 1988; 44:2081–2086.

12. Carlson BA, Dubay MM, Sausville EA, Brizuela L, Worland PJ. Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. *Cancer Res.* 1996; 56:2973–2978. [PubMed: 8674031]
13. Losiewicz MD, Carlson BA, Kaur G, Sausville EA, Worland PJ. Potent inhibition of CDC2 kinase activity by the flavonoid L86-8275. *Biochem Biophys Res Commun.* 1994; 201:589–595. [PubMed: 8002990]
14. Shapiro GL. Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol.* 2006; 24:1770–1783. [PubMed: 16603719]
15. Almenara J, Rosato R, Grant S. Synergistic induction of mitochondrial damage and apoptosis in human leukemia cells by flavopiridol and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). *Leukemia.* 2002; 16:1331–1343. [PubMed: 12094258]
16. Mitchell C, Park MA, Zhang G, et al. Extrinsic pathway-and cathepsin-dependent induction of mitochondrial dysfunction are essential for synergistic flavopiridol and vorinostat lethality in breast cancer cells. *Mol Cancer Ther.* 2007; 6:3101–3112. [PubMed: 18065490]
17. Dasmahapatra G, Almenara JA, Grant S. Flavopiridol and histone deacetylase inhibitors promote mitochondrial injury and cell death in human leukemia cells that overexpress Bcl-2. *Mol Pharmacol.* 2006; 69:288–298. [PubMed: 16219908]
18. Gao N, Dai Y, Rahmani M, Dent P, Grant S. Contribution of disruption of the nuclear factor-kappaB pathway to induction of apoptosis in human leukemia cells by histone deacetylase inhibitors and flavopiridol. *Mol Pharmacol.* 2004; 66:956–963. [PubMed: 15235103]
19. Dai Y, Rahmani M, Dent P, Grant S. Blockade of histone deacetylase inhibitor-induced RelA/p65 acetylation and NF-kappaB activation potentiates apoptosis in leukemia cells through a process mediated by oxidative damage, XIAP downregulation, and c-Jun N-terminal kinase 1 activation. *Mol Cell Biol.* 2005; 25:5429–5444. [PubMed: 15964800]
20. Duvic M, Talpur R, Ni X, et al. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). *Blood.* 2007; 109:31–39. [PubMed: 16960145]
21. George S, Kasimis BS, Cogswell J, et al. Phase I study of flavopiridol in combination with Paclitaxel and Carboplatin in patients with non-small-cell lung cancer. *Clin Lung Cancer.* 2008; 9:160–165. [PubMed: 18621626]
22. Fornier MN, Rathkopf D, Shah M, et al. Phase I dose-finding study of weekly docetaxel followed by flavopiridol for patients with advanced solid tumors. *Clin Cancer Res.* 2007; 13:5841–5846. [PubMed: 17908977]
23. Schwartz GK, O'Reilly E, Ilson D, et al. Phase I study of the cyclin-dependent kinase inhibitor flavopiridol in combination with paclitaxel in patients with advanced solid tumors. *J Clin Oncol.* 2002; 20:2157–2170. [PubMed: 11956278]
24. Trotti A, Colevas AD, Setser A, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol.* 2003; 13:176–181. [PubMed: 12903007]
25. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst.* 2000; 92:205–216. [PubMed: 10655437]
26. Parise RA, Holleran JL, Beumer JH, Ramalingam S, Egorin MJ. A liquid chromatography-electrospray ionization tandem mass spectrometric assay for quantitation of the histone deacetylase inhibitor, vorinostat (suberoylanilide hydroxamic acid, SAHA), and its metabolites in human serum. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2006; 840:108–115.
27. Byrd JC, Lin TS, Dalton JT, et al. Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. *Blood.* 2007; 109:399–404. [PubMed: 17003373]
28. Gonen M. A Bayesian evaluation of enrolling additional patients at the maximum tolerated dose in Phase I trials. *Contemp Clin Trials.* 2005; 26:131–140. [PubMed: 15837436]

29. Shah MA, Kortmansky J, Motwani M, et al. A phase I clinical trial of the sequential combination of irinotecan followed by flavopiridol. *Clin Cancer Res.* 2005; 11:3836–3845. [PubMed: 15897584]
30. Lin TS, Ruppert AS, Johnson AJ, et al. Phase II study of flavopiridol in relapsed chronic lymphocytic leukemia demonstrating high response rates in genetically high-risk disease. *J Clin Oncol.* 2009; 27:6012–6018. [PubMed: 19826119]
31. Fakih, MG.; Egorin, MJ.; Fetterly, G., et al. A phase I study of QD and BID vorinostat $\times$ 3 days and sFULV2 in refractory colorectal cancer and other solid tumors. ASCO Gastrointestinal Cancers Symposium; 2010. p. Abstract 334
32. Kelly WK, Richon VM, O'Connor O, et al. Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously. *Clin Cancer Res.* 2003; 9:3578–3588. [PubMed: 14506144]
33. Ramalingam SS, Parise RA, Ramanathan RK, et al. Phase I and pharmacokinetic study of vorinostat, a histone deacetylase inhibitor, in combination with carboplatin and paclitaxel for advanced solid malignancies. *Clin Cancer Res.* 2007; 13:3605–3610. [PubMed: 17510206]
34. Fakih MG, Pendyala L, Fetterly G, et al. A phase I, pharmacokinetic and pharmacodynamic study on vorinostat in combination with 5-fluorouracil, leucovorin, and oxaliplatin in patients with refractory colorectal cancer. *Clin Cancer Res.* 2009; 15:3189–3195. [PubMed: 19383814]
35. Rubin EH, Agrawal NG, Friedman EJ, et al. A study to determine the effects of food and multiple dosing on the pharmacokinetics of vorinostat given orally to patients with advanced cancer. *Clin Cancer Res.* 2006; 12:7039–7045. [PubMed: 17145826]
36. Garcia-Manero G, Yang H, Bueso-Ramos C, et al. Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes. *Blood.* 2008; 111:1060–1066. [PubMed: 17962510]
37. Richardson P, Mitsiades C, Colson K, et al. Phase I trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) in patients with advanced multiple myeloma. *Leuk Lymphoma.* 2008; 49:502–507. [PubMed: 18297527]
38. Crump M, Coiffier B, Jacobsen ED, et al. Phase II trial of oral vorinostat (suberoylanilide hydroxamic acid) in relapsed diffuse large-B-cell lymphoma. *Ann Oncol.* 2008; 19:964–969. [PubMed: 18296419]
39. Grant S, Kolla S, Sirulnik L, et al. Phase I trial of vorinostat (SAHA) in combination with alvocidib (Flavopiridol) in patients with relapsed, refractory or (selected) poor prognosis acute leukemia or refractory anemia with excess blasts-2 (RAEB-2). *Blood (ASH Annual Meeting Abstracts).* 2008; 112:Abstract #2986.

\$watermark-text

\$watermark-text

\$watermark-text

Dose escalation

Table 1

Cohort	Dose (mg)	Vorinostat Schedule	Dose ^a (mg/m ²)	Flavopiridol Schedule	n
Part 1	1 400	D1-3 and D8-10 Q21D	60	D2 and D9 Q21D	3
	2 600	D1-3 and D8-10 Q21D	60	D2 and D9 Q21D	6
	3 800	D1-3 and D8-10 Q21D	60	D2 and D9 Q21D	4
Part 2	4 600	D1-3 and D8-10 Q21D	30 → 30	D2 and D9 Q21D	1
	5 400	D1-3 and D8-10 Q21D	30 → 30	D2 and D9 Q21D	2
Part 3	6 400	D1-3 and D15-17 Q28D	30 → 30	D2 and D16 Q28D	3
	7 600	D1-3 and D15-17 Q28D	30 → 30	D2 and D16 Q28D	6
	8 800	D1-3 and D15-17 Q28D	30 → 30	D2 and D16 Q28D	6
	9 1000	D1-3 and D15-17 Q28D	30 → 30	D2 and D16 Q28D	3

^aIn cohorts 1–3, flavopiridol was administered as a 1 h infusion
In cohorts 4–9, flavopiridol was administered as a 30 min bolus followed by a 4 h infusion

Table 2

Patient characteristics

Characteristic	No. patients
Total	34
Assessable for response	31
Male	23
Female	11
Age, y	
Median	59
Range	31–85
ECOG,	
Median	1
Range	0–1
No. prior regimens	
Median	4
Range	1–11
Primary Sites of Disease	
Colorectal	10
Sarcoma	7
Prostate	3
Biliary	2
Anal	1
Bladder	1
Adenoid Cystic	1
Esophageal	1
GIST	1
Laryngeal	1
Mesothelioma	1
Pancreatic	1
Small Bowel	1
Sweat Gland	1
Thyroid	1

Table 3

Cycle -1 to cycle 1 toxicity

Cohort	vorinostat	flavopiridol	<u>Fatigue</u>				<u>Diarrhea</u>				<u>Nausea</u>				<u>Vomiting</u>				<u>Leukocytes</u>				<u>Lymphopenia</u>				<u>Neutrophils</u>				<u>Platelets</u>																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
			2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
1	400	60														1																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									

Table 4Cumulative toxicities ($n=34$)

Toxicity	3	4	3 & 4 (%)
Anemia	2	2	4 (12%)
Thrombosis/Embolism		3	3 (9%)
Infection	2		2 (6%)
Leukocytes (WBC)	10	5	15 (44%)
Lymphopenia	5	3	8 (24%)
Neutropenia	3	13	16 (47%)
Thrombocytopenia	1	1	2 (6%)

Table 5

Clinical activity by tumor type

Tumor type	Response	Duration (months)
Sarcoma	SD	13.2
	SD	7.3
Colorectal	SD	5.1
	SD	4.0
	SD	3.0
Granulosa Cell	SD	6.1
Prostate	SD	3.5
Thyroid	SD	1.6

Table 6

Vorinostat pharmacokinetic parameters by cohort

Cohort	Vorinostat (mg)	Flavopiridol (mg/m ²)	n	Cycle -1		Cycle 1		Cycle -1		Cycle 1	
				mean Cmax (μM)	SD	mean Cmax (μM)	SD	mean AUC (μM ^a hr)	SD	mean AUC (μM ^a hr)	SD
1	400	60	3	2.69	0.56	2.68	1.22	8.34	1.84	8.99	6.37
2	600	60	6	1.62	0.65	1.58	0.85	4.72	2.45	4.33	2.28
3	800	60	3 ^a	2.34	1.04	2.69	0.71	10.17	5.69	9.47	2.83
4	600	30 → 30	1	1.25	NA	1.16	NA	5.78	NA	4.35	NA
5	400	30 → 30	2	2.64	0.28	3.48	0.62	9.24	3.18	11.09	1.42
6	400	30 → 30	3	1.81	0.13	2.11	0.30	7.00	0.78	5.82	1.62
7	600	30 → 30	6	3.35	1.41	4.87	1.89	12.77	7.53	15.20	9.55
8	800	30 → 30	4 ^a	4.53	2.00	6.43	2.79	16.63	8.30	24.44	8.30
9	1000	30 → 30	3	8.74	1.45	12.74	5.39	37.52	4.82	40.24	16.88

^a complete PK data are available for only 3 of 4 patients in cohort 3 and only 4 of 6 patients in cohort 8

Table 7

Vorinostat pharmacokinetic parameters by dose level

Vorinostat (mg)	n	Cycle -1		Cycle 1		Cycle -1		Cycle 1	
		mean Cmax (μM)	SD	mean Cmax (μM)	SD	mean AUC (μM ^a hr)	SD	mean AUC (μM ^a hr)	SD
400	8	2.35	0.55	2.67	0.91	8.06	1.87	8.32	4.21
600	13	2.39	1.37	3.07	2.20	8.52	6.56	9.35	8.48
800	7	3.59	1.93	4.83	2.84	13.86	7.56	18.02	10.06
1000	3	8.74	1.45	12.74	5.39	37.52	4.82	40.24	16.88

^a complete PK data are available for only 3 of 4 patients in cohort 3 and only 4 of 6 patients in cohort 8

Table 8

Vorinostat pharmacokinetics. Mean vorinostat serum concentrations over time for patients grouped by vorinostat dose. Error bars show standard deviations. In cycle –1, vorinostat is given alone (top). In cycle 1, the same dose of vorinostat is given with flavopiridol (bottom). As shown, co-administration of flavopiridol tends to increase the vorinostat C_{max} and AUC, especially at higher administered doses (600–800 mg) of vorinostat