

Phase I and Pharmacokinetic Study of Vorinostat, A Histone Deacetylase Inhibitor, in Combination with Carboplatin and Paclitaxel for Advanced Solid Malignancies

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Abstract Purpose: The primary objective of this study was to determine the recommended phase II doses of the novel histone deacetylase inhibitor vorinostat when administered in combination with carboplatin and paclitaxel.

Experimental Design: Patients ($N = 28$) with advanced solid malignancies were treated with vorinostat, administered orally once daily for 2 weeks or twice daily for 1 week, every 3 weeks. Carboplatin and paclitaxel were administered i.v. once every 3 weeks. Doses of vorinostat and paclitaxel were escalated in sequential cohorts of three patients. The pharmacokinetics of vorinostat, its metabolites, and paclitaxel were characterized.

Results: Vorinostat was administered safely up to 400 mg qd or 300 mg bd with carboplatin and paclitaxel. Two of 12 patients at the 400 mg qd schedule experienced dose-limiting toxicities of grade 3 emesis and grade 4 neutropenia with fever. Non – dose-limiting toxicity included nausea, diarrhea, fatigue, neuropathy, thrombocytopenia, and anemia. Of 25 patients evaluable for response, partial responses occurred in 11 (10 non – small cell lung cancer and 1 head and neck cancer) and stable disease occurred in 7. Vorinostat pharmacokinetics were linear over the dose range studied. Vorinostat area under the concentration versus time curve and half-life increased when vorinostat was coadministered with carboplatin and paclitaxel, but vorinostat did not alter paclitaxel pharmacokinetics.

Conclusions: Both schedules of vorinostat (400 mg oral qd \times 14 days or 300 mg bd \times 7 days) were tolerated well in combination with carboplatin (area under the concentration versus time curve = 6 mg/mL \times min) and paclitaxel (200 mg/m²). Encouraging anticancer activity was noted in patients with previously untreated non – small cell lung cancer.

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Received 1/19/07; revised 2/28/07; accepted 3/7/07.

Grant support: National Cancer Institute grants U01 CA099168-01 and P30CA 47904, NIH/National Center of Competence in Research/General Clinical Research Center grant 5M01RR 00056, and American Society of Clinical Oncology Foundation Clinical Research Career Development Award (S.S. Ramalingam).

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Note: Presented as a poster at the 42nd Annual Meeting of the American Society of Clinical Oncology, 2006, Atlanta, Georgia.

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doi:10.1158/1078-0432.CCR-07-0162

Histone deacetylases (HDAC) are a family of enzymes that play an important role in regulation of gene transcription (1). DNA wrapped around core histones in their nonacetylated state is transcriptionally inactive. Acetylation of NH₂-terminal lysine residues on histones results in a more open chromatin configuration that is transcriptionally active (2, 3). The acetylation status of histones is determined by the complex interplay between histone acetyltransferases and HDACs. Aberrant transcriptional activation and repression mediated by histone acetyltransferases and HDACs, respectively, occurs in various malignancies (4). Anticancer activity of HDAC inhibitors is also attributed to their effect on several nonhistone proteins, such as tubulin and heat shock protein 90 (5). Therefore, HDAC inhibition has emerged as a therapeutic strategy for cancer.

Vorinostat (Zolinza, suberoylanilide hydroxamic acid, NSC 701852) is a small molecule that inhibits HDAC activity (6). Vorinostat not only results in the induction of genes, including $p21^{CIP1/WAF1}$ and $p27^{KIP1}$, but also causes the repression of several genes, such as *thymidylate synthetase* and *vascular*

endothelial growth factor receptor (7–13). Inhibition of HDAC activity by vorinostat also results in an increase of acetylated nonhistone proteins, such as cytoskeletal proteins, molecular chaperones, and nuclear import factors (14). *In vitro*, vorinostat inhibits proliferation and induces differentiation and apoptosis in various cancer cell lines (15–17). Clinically, vorinostat is tolerated well at a daily oral dose of 400 mg or twice-daily dose of 200 mg as monotherapy (18, 19). The dose-limiting toxicities (DLT) were diarrhea, thrombocytopenia, and fatigue. Objective responses were noted in patients with mesothelioma, thyroid carcinoma, and squamous cell carcinoma of the larynx. Phase II studies of vorinostat monotherapy are ongoing for various solid malignancies. Vorinostat has recently received approval by the Food and Drug Administration as monotherapy for patients with refractory cutaneous T-cell lymphoma based on phase II studies (20, 21).

We conducted a phase I study to determine the recommended phase II doses of vorinostat, administered on two different schedules, with carboplatin and paclitaxel, based on several clinical and preclinical considerations. Combination chemotherapy with carboplatin and paclitaxel is commonly used to treat several solid malignancies (22, 23), and the combination results in less platelet toxicity than that seen with carboplatin alone (24). This combination has a toxicity profile that does not overlap with that of vorinostat. Preclinical studies have shown enhanced cytotoxicity when vorinostat was coadministered with either cisplatin or taxanes (25, 26). Increased platinum adduct formation of the more open DNA configuration facilitated by HDAC inhibition has been proposed as a potential mechanism for the enhanced cisplatin-induced cell kill (27). Enhancement of taxane activity may be related to alterations in α -tubulin acetylation mediated by inhibition of HDAC6 (28).

Materials and Methods

The primary objective of the study was to determine the recommended doses of vorinostat, carboplatin, and paclitaxel that can be administered in combination. Secondary end points included determination of the DLT and definition of non-DLTs and to conduct pharmacokinetic studies to evaluate potential drug-drug interactions.

Patient eligibility

Patients (ages >18 years) with advanced solid malignancies who were candidates for combination therapy with carboplatin and paclitaxel were eligible. Eastern Cooperative Oncology Group performance status of 0, 1, or 2 was required and less than two or two prior chemotherapy regimens were allowed. Qualifying laboratory criteria were the following: leukocytes $\geq 3,000/\mu\text{L}$, absolute neutrophil count $\geq 1,500/\mu\text{L}$, platelets $\geq 100,000/\mu\text{L}$, serum total bilirubin \leq institutional

upper limit of normal, serum transaminases $\leq 2.5 \times$ upper limit of normal, and serum creatinine \leq upper limit of normal. Patients with serum creatinine levels \geq upper limit of normal were eligible if estimated creatinine clearance was $\geq 60 \text{ mL/min/1.73 m}^2$. At least 4 weeks had to have elapsed since prior radiation or chemotherapy. Patients with grade >1 peripheral neuropathy, the inability to take oral medications, prior paclitaxel therapy, use of valproic acid (a HDAC inhibitor) within 4 weeks of enrollment, or uncontrolled severe comorbid disease were excluded. Patients with treated and stable brain metastases were eligible. Pregnant women were excluded, and women with reproductive potential were required to use contraception. All patients provided written informed consent. The study was approved by the University of Pittsburgh Institutional Review Board.

Treatment plan

Vorinostat was provided by the Cancer Therapy Evaluation Program of the National Cancer Institute (Bethesda, MD). Patients in the first four dose levels (schedule A) received oral vorinostat daily for the first 14 days of each 21-day treatment cycle (Table 1). After the first four dose levels were completed, vorinostat was given twice daily on dose level 5 (schedule B) for the first 7 days of each 21-day cycle. Patients were required to maintain a calendar to document their taking vorinostat. Missed doses were not to be replaced.

During cycle 1, vorinostat was initiated 4 days before chemotherapy to obtain single-agent pharmacokinetic data. Carboplatin and paclitaxel were administered on day 5 of cycle 1 and on day 1 for all subsequent cycles. On the days of chemotherapy administration, patients ingested vorinostat in the clinic, after which paclitaxel, diluted in 500 mL of 5% dextrose, was administered as a 3-h i.v. infusion. Premedications for paclitaxel included dexamethasone (20 mg oral doses 12 and 6 h before the paclitaxel), diphenhydramine (50 mg i.v.), and a histamine receptor 2 antagonist (50 mg ranitidine or 300 mg i.v. cimetidine). Following the paclitaxel infusion, carboplatin, dissolved in 100 mL of 5% dextrose/0.9% saline, was administered as a 30-min i.v. infusion. The carboplatin dose was calculated using the Calvert formula (29).

Combination treatment was continued for a maximum of six cycles, following which patients with a response or stable disease were continued on vorinostat monotherapy at the same schedule and dosage that was used with chemotherapy. Treatment was continued until disease progression, unacceptable toxicity, or withdrawal of consent. Granulocyte colony-stimulating factor was not allowed during the first cycle of therapy but was allowed for subsequent cycles according to standard guidelines. Patients experiencing grade 3 or 4 toxicities attributable to vorinostat had dose reduction by 100 mg in subsequent cycles. Paclitaxel and carboplatin doses were reduced by 25 mg/m² and area under the concentration versus time curve (AUC) of 1, respectively, based on adverse effects attributed to either drug. Patients who required more than two dose reductions were removed from the study. Reduction in the dose of two drugs at the same time was considered one dose reduction. All toxicities (except neuropathy and alopecia) were required to resolve to grade ≤ 1 before initiation of the next cycle.

Dose-limiting toxicity

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 3.0. DLT was defined as occurrence

Table 1. Dose escalation scheme

Schedule	Dose level	Vorinostat, days 1-14 (mg)	Paclitaxel, day 1 (mg/m ²)	Carboplatin, day 1 (AUC mg/mL \times min)	No. patients
A	1	200 qd	175	6	4
	2	300 qd	175	6	3
	3	400 qd	175	6	3
	4	400 qd	200	6	12
B	5	300 bd (days 1-7)	200	6	6

Table 2. Baseline patient characteristics

No. patients	28
Median age (y)	64 (range, 30-77)
Sex	
Male	21
Female	7
Tumor type	
NSCLC	19
Head and neck	4
Bladder	2
Mesothelioma	1
Others	2
No. patients with no prior chemotherapy	23

of one or more of the following events during the first cycle of therapy: grade ≥ 3 nonhematologic toxicity except nausea, vomiting, or alopecia; grade ≥ 3 nausea or vomiting that lasted >48 h despite maximal medical therapy; absolute neutrophil count $<1,000/\mu\text{L}$ that lasted >7 days; grade 3 or 4 neutropenia with sepsis or fever $>38^\circ\text{C}$; grade 4 thrombocytopenia; and toxicity-related delay in starting cycle 2 by >2 weeks. Abnormal nonhematologic laboratory criteria (grade ≥ 3) were considered DLT if clinically significant and drug related.

An "up and down" dose escalation scheme with cohorts of three patients was used for schedule A. The first cohort was treated at dose level 1. If one of three patients experienced DLT, three more patients were added at the same level, and if zero of three or one of six experienced DLT, the dose for the next cohort was escalated by one level. Escalation stopped and de-escalation began as soon as two patients at a dose level experienced DLT. If only three patients had been treated at a dose level, three additional patients would be treated. If more than one of six patients experienced DLT, de-escalation continued until less than one or one of six patients at a dose level experienced DLT; this level was defined to be the recommended phase II dose. Schedule B was included to evaluate the feasibility of a twice-daily schedule of vorinostat administered for 7, as opposed to 14 days per cycle. Inpatient dose escalation was not permitted.

Patient evaluation

Pretreatment evaluations were the following: history and physical examination, assessment of performance status, complete blood count, and hepatic and renal function tests. A baseline electrocardiogram was obtained if clinically indicated. Women of reproductive age underwent a serum pregnancy test. Patients were evaluated weekly with complete blood count and hepatic and renal function tests. Radiological studies were done after every two cycles of therapy. History and physical examination and assessment of performance status were done before initiation of each cycle. Responses were assessed with Response Evaluation Criteria in Solid Tumors criteria (30).

Pharmacokinetic studies

Sampling. Pharmacokinetic studies were done during the first cycle. Twenty-three patients had blood samples drawn on day 1 to characterize the pharmacokinetics of single-agent vorinostat. Seventeen of those also had blood samples drawn on day 5 to characterize the pharmacokinetics of vorinostat and paclitaxel given in combination. Six patients did not participate on pharmacokinetic sampling on day 5 due to logistical reasons. On day 1, 5 mL of blood were collected in a red-top vacutainer tube (without anticoagulant; Becton Dickinson) before and at 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after ingestion of vorinostat. On day 5, samples were collected before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 h after ingestion of vorinostat. Blood samples were allowed to coagulate at 4°C for 20 to 30 min and then centrifuged at $2,000 \times g$ for 15 min at 4°C . The resulting serum was stored at -70°C until assayed for drug content.

Analytic chemical methods. Concentrations of vorinostat and two metabolites (vorinostat glucuronide and 4-anilino-4-oxobutanoic acid) were quantitated with a liquid chromatography-electrospray ionization tandem mass spectrometric method that was developed and validated in our laboratory (31). Paclitaxel concentrations were quantitated with a liquid chromatography-mass spectrometric method that was developed and validated in our laboratory (32). Paclitaxel concentrations were only characterized in serum of patients treated at the $200 \text{ mg}/\text{m}^2$ dose level.

Pharmacokinetic modeling. Plasma concentration versus time data for vorinostat and metabolites were analyzed noncompartmentally using the LaGrange function (33) as implemented by the Lagran computer program (34). Paclitaxel concentration versus time data were analyzed compartmentally by fitting a previously described, three-compartment, nonlinear model using Bayesian estimation and the ADAPT II computer program (35, 36). Each patient's paclitaxel AUC and the time that paclitaxel concentrations remained above $0.05 \mu\text{mol}/\text{L}$ were calculated using patient-specific pharmacokinetic variables. The pharmacokinetic/pharmacodynamic relationship for paclitaxel-induced neutropenia was evaluated with a sigmoid Emax model and compared with historical data for single-agent paclitaxel (35).

Statistical methods. The day 1-to-day 5 changes in vorinostat pharmacokinetic variables were assessed via the Wilcoxon signed ranks tests after data were log transformed; two-sided *P* values are reported.

Results

Patients. Twenty-eight patients were accrued (Table 2). All patients had an Eastern Cooperative Oncology Group performance status of 0 or 1. A total of 132 cycles (101 with chemotherapy and 31 as vorinostat monotherapy) of therapy was administered. The median number of cycles was 4 (range, 1-11). Eight patients completed the maximum allowed six cycles of combination therapy and went on to receive vorinostat maintenance therapy, and six (of eight) received ≥ 10 cycles of therapy.

Disease progression was the most common reason for discontinuation of therapy ($n = 13$) during the first six cycles. One patient was removed from study after only 3 days as the diagnosis was revised by pathology review. Two non-small cell lung cancer (NSCLC) patients (1 partial response and 1 stable disease) were discontinued after four cycles of therapy to undergo local therapy. Three with stable disease were discontinued after three, four, and four cycles, respectively. One patient with a partial response discontinued after two cycles because he relocated to another geographic area.

DLT and recommended phase II dose. No DLT was noted after three patients had been accrued to each cohort in schedule A (Table 1). Dose level 4 was expanded to six patients, of which one experienced grade 3 vomiting. Six additional patients were subsequently enrolled to dose level 4 to obtain additional safety and pharmacokinetic data, and one of those patients experienced febrile neutropenia (grade 4) during the first cycle. Therefore, 10 of 12 patients at dose level 4 tolerated therapy without DLT. No DLT was encountered in the six patients treated on schedule B. Consequently, both dose levels 4 and 5 were considered safe. Common non-DLTs are outlined in Table 3. One patient developed neutropenic sepsis following the fifth cycle and was removed from study. A patient with head and neck cancer and a partial response after two cycles of therapy was discontinued because of mechanical complications with his feeding gastrostomy tube.

Table 3. Toxicity

Hematologic toxicity	No. patients (all cycles)		
	Grade 2	Grade 3	Grade 4
Anemia	9	3	—
Neutropenia	2	8	14
Fever with Neutropenia	—	—	2
Thrombocytopenia	5	7	4
Nonhematologic toxicity	No. patients (all cycles)		
	Grade 1	Grade 2	Grade 3
Altered taste	2	—	—
Anorexia	15	—	—
Constipation	5	—	—
Dehydration	2	—	2
Diarrhea	6	1	—
Fatigue	19	6	—
Heartburn	3	1	—
Myalgia	2	—	1
Neuropathy	11	2	1
Nausea	12	4	—
Skin rash	2	1	—
Vomiting	4	1	2

NOTE: By worst grade of toxicity per patient, occurring in at least 10% (three) of the patients.

Antitumor activity. Twenty-five patients were evaluable for response. Eleven had a partial response and 7 had stable disease. Objective responses were noted at all dose levels. Five of 12 patients at dose level 4 and 2 of 6 patients at dose level 5 experienced partial responses. Of 19 patients with NSCLC (18 chemotherapy naive), 10 experienced a partial response and 4 had stable disease (response rate, 53%; 95% confidence interval, 29-76%). None of those 14 patients had received prior chemotherapy. The histologic subtypes for the 10 NSCLC patients who had a partial response were adenocarcinoma ($n = 6$), squamous cell carcinoma ($n = 2$), and large cell carcinoma with neuroendocrine features ($n = 2$). One of four patients with head and neck cancer had a partial response and two had stable disease. A patient with an advanced malignant pleural mesothelioma had prolonged disease stabilization.

Pharmacokinetics

Vorinostat and metabolites. Figure 1 shows representative concentration versus time data for vorinostat and metabolites after the first dose of vorinostat. As shown, vorinostat concentrations were usually less than, and declined more rapidly than, those of its two inactive metabolites. Vorinostat pharmacokinetics were linear across the range of doses used in the study (Table 4). Specifically, maximum serum vorinostat concentrations (C_{max}) and AUC increased proportionally with increasing vorinostat dose, whereas the time to reach C_{max} (T_{max}), half-life, and apparent clearance (Cl_{app}) remained constant within errors. Similar relationships were also noted for the two vorinostat metabolites that were quantitated, although, as might be expected for metabolites, the dose proportionality was somewhat less obvious. When an individual patient's vorinostat pharmacokinetics on day 5 was compared with those on day 1, several differences were noted. In most patients, the AUC and half-life of vorinostat on day 5

were greater than on day 1 and Cl_{app} on day 5 was less than on day 1 (Table 5). In contrast, there were no obvious differences between vorinostat C_{max} or T_{max} on days 1 and 5. Interindividual variation in vorinostat Cl_{app} on day 5 was also less than on day 1. The C_{max} and T_{max} comparisons are based on the data from 17 patients; other variables are based on 16 patients.

Paclitaxel. There was large interpatient variability in paclitaxel AUC at the 200 mg/m² dose. This was consistent with that observed in patients from other studies using a wide range of other paclitaxel doses. When compared with AUCs produced by other doses of paclitaxel, the paclitaxel AUCs estimated for patients in the current study were consistent with those expected from a 200 mg/m² dose. The duration of time that paclitaxel concentrations remained above 0.05 μmol/L ranged from 26 to 70 h, which, according to the sigmoid Emax model relating that paclitaxel pharmacokinetic variable to paclitaxel-induced neutropenia, would be associated with a percentage decrease in absolute neutrophil count ranging from 71% to 96%.

Discussion

This is the first study to report on a vorinostat-containing combination therapy. Although the results of the study may have been favorably influenced by the majority of the patients being chemotherapy naive and having an excellent performance status, the study clearly shows that the combination regimen can be administered safely. The median number of cycles of therapy delivered was 4, suggesting that the regimen can be administered for an extended duration, with appropriate management of side effects. Because carboplatin-paclitaxel-based regimens are likely to be used as front-line therapy in a variety of solid malignancies, the results of our study are germane to a wide patient population.

Vorinostat is given on a continuous daily schedule for the treatment of cutaneous T-cell lymphoma. To achieve maximal anticancer activity, it is unclear whether vorinostat should be given for a brief period with chemotherapy or for a longer duration following chemotherapy. Therefore, we chose to evaluate both the qd × 14 days and the bd × 7 days schedules.

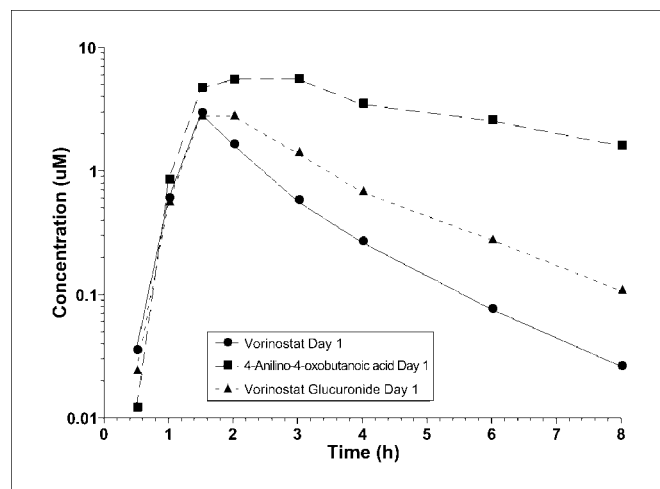


Fig. 1. Representative concentration versus time profiles of vorinostat and metabolites after a patient's first oral 400 mg dose.

Table 4. Pharmacokinetic variables for vorinostat and metabolites on day 1 of treatment

Dose (mg)	No. patients	C _{max} (μmol/L)	T _{max} (h)	AUC (μmol/L × h)	t _{1/2} (h)	Cl _{app} (L/min)
Vorinostat						
200	1	1.45	0.5	2.4	1.8	5.2
300	8	1.36 ± 0.52	1.38 ± 0.52	3.54 ± 1.31	1.37 ± 0.42	5.73 ± 2.04
400	15	1.81 ± 0.70	1.27 ± 0.42	4.67 ± 2.33	1.45 ± 0.56	6.19 ± 1.88
Vorinostat glucuronide						
200	1	2.81	1	6.9	2.1	NA
300	8	3.67 ± 2.09	2.19 ± 1.03	11.7 ± 11.3	1.56 ± 0.21	NA
400	15	3.62 ± 1.22	1.50 ± 0.38	12.8 ± 7.2	2.08 ± 1.36	NA
4-Anilino-4-oxobutanoic acid						
200	1	3.82	1	15.3	5.9	NA
300	8	4.87 ± 1.99	2.75 ± 1.13	27.2 ± 10.8	6.6 ± 5.0	NA
400	15	5.14 ± 1.42	2.23 ± 1.03	23.5 ± 6.9	4.2 ± 1.8	NA

Abbreviations: t_{1/2}, half-life; NA, not applicable.

Further mechanistic and clinical data are necessary to determine the schedule with the optimal therapeutic index. The 14-day regimen of vorinostat was chosen for our phase II study because majority of the patients in our phase I study were treated on this schedule.

We also documented encouraging activity against NSCLC and head and neck cancer. Treatment with carboplatin-paclitaxel of chemo-naïve patients with advanced NSCLC results in response rates of approximately 20% to 30% (22, 37). In our study, 10 of 19 (53%) advanced NSCLC patients experienced a partial response and 4 had stable disease, suggesting that the anticancer activity of carboplatin-paclitaxel may be enhanced by vorinostat. The exact reasons behind the favorable interaction between vorinostat and carboplatin/paclitaxel are not known. To evaluate this further, we are conducting preclinical experiments to evaluate the mechanistic aspects of the positive interaction, including the effect of vorinostat on platinum-DNA adduct formation and acetylation of tubulin. In the clinical setting, we are conducting a phase II study that randomizes advanced NSCLC patients to carboplatin and paclitaxel with either vorinostat or placebo.

The pharmacokinetic variables estimated for vorinostat on day 1 of the current study agree well with data from a previously published study of single-agent vorinostat (19). Although data were also generated for the two major metabolites of vorinostat, the implications and utility of those data are unclear because neither metabolite is active and their toxicities, if any, have not been defined. Potential considerations about the metabolites include whether UGT genotyping would be relevant or useful in patients being treated with vorinostat and whether the relatively long half-life of 4-anilino-4-oxobutanoic acid might allow it to be monitored as an indication of adherence to vorinostat therapy.

Although there was evidence of paclitaxel-associated alterations in vorinostat pharmacokinetics, there are no obvious explanations for this potential interaction. The two drugs have different metabolic pathways. Premedications given with paclitaxel might have contributed to the observed interaction. Furthermore, food-related variations in absorption of vorinostat or the fact that all pharmacokinetic studies were done with the same sequence of administration of vorinostat and chemotherapy might be factors. Therefore,

further studies to evaluate this potential drug-drug interaction are warranted.

Although only 2 patients in the current study experienced febrile neutropenia, 14 patients (50%) experienced grade 4 neutropenia, which is more than expected from carboplatin-paclitaxel chemotherapy alone (38). There was no definite relationship between the dose and schedule of administration of vorinostat and the incidence of grade 3/4 neutropenia. Based on comparison with historical controls, there was no evidence of a vorinostat-related alteration of paclitaxel pharmacokinetics that might explain the increased neutropenia. However, the durations that paclitaxel concentrations remained above 0.5 μmol/L in individual patients were consistent with the degree of neutropenia that was observed. It is possible that enhanced tubulin acetylation, a desired effect on tumor cells, mediated by the combination of vorinostat and paclitaxel might contribute to the higher incidence of neutropenia as well. Carboplatin pharmacokinetics were not studied because of the different matrices required for quantitation of paclitaxel and carboplatin and the logistical constraints associated with sample collection and processing.

Our study did not evaluate the molecular effects of the combination in tumor tissue, which would have required pretreatment and posttreatment biopsies. Initial phase I studies have evaluated the effects of vorinostat on histone targets in peripheral blood mononuclear cells (19). Accumulation of acetylated histones was consistently noted in

Table 5. Comparison of day 1 versus day 5 pharmacokinetic variables of vorinostat

Variable	Median, day 1	Median, day 5	P
C _{max} (μmol/L)	1.7	1.3	0.52
T _{max} (h)	1.5	1.6	0.085
AUC (μmol/L × h)	3.8	5.2	0.011
t _{1/2} (h)	1.3	2.1	0.012
Cl _{app} (L/min)	6.4	4.4	0.0063

NOTE: C_{max} and T_{max} are based on 17 patients; other variables are based on 16 patients.

peripheral blood mononuclear cell at doses between 100 and 600 mg of vorinostat. However, no clinical study, to date, has been conducted to correlate histone acetylation in peripheral blood mononuclear cells with that in tumor tissue. Ideally, such data can be generated in future phase II studies to correlate the molecular effects of vorinostat on the tumor with response to therapy.

In summary, the regimen of vorinostat, carboplatin, and paclitaxel represents a novel strategy for the treatment of

solid tumors. The promising anticancer activity noted in this study provides the rationale to conduct disease-specific phase II studies.

Acknowledgments

We thank Jeremy A. Hedges for providing valuable secretarial assistance with preparation of the manuscript and the University of Pittsburgh Cancer Institute Hematology-Oncology Writing Group for helpful suggestions.

References

- Marks PA, Richon VM, Rifkin RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. *J Natl Cancer Inst* 2000;92:1210–6.
- Workman JL, Kingston RE. Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu Rev Biochem* 1998;67:545–79.
- Arts J, de Schepper S, Van Emelen K. Histone deacetylase inhibitors: from chromatin remodeling to experimental cancer therapeutics. *Curr Med Chem* 2003;10:2343–50.
- Hess-Stumpp H. Histone deacetylase inhibitors and cancer: from cell biology to the clinic. *Eur J Cell Biol* 2005;84:109–21.
- Marks PA, Richon VM, Breslow R, Rifkin RA. Histone deacetylase inhibitors as new cancer drugs. *Curr Opin Oncol* 2001;13:477–83.
- Finnin MS, Donigan JR, Cohen A, et al. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* 1999;401:188–93.
- Richon VM, Sandhoff TW, Rifkin RA, Marks PA. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. *Proc Natl Acad Sci U S A* 2000;97:10014–9.
- Huang L, Pardee AB. Suberoylanilide hydroxamic acid as a potential therapeutic agent for human breast cancer treatment. *Mol Med* 2000;6:849–66.
- Chen X, Singh TR, Shankar S, Srivastava RK. *In vivo* regulation of apoptosis and angiogenesis by histone deacetylase inhibitors and TRAIL in human prostate cancer. *Proc Am Assoc Cancer Res* 2003;44:4305.
- Glaser KB, Staver MJ, Waring JF, Stender J, Ulrich RG, Davidsen SK. Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. *Mol Cancer Ther* 2003;2:151–63.
- Nimmanapalli R, Fuino L, Stobaugh C, Richon V, Bhalla K. Cotreatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) enhances imatinib-induced apoptosis of Bcr-Abl-positive human acute leukemia cells. *Blood* 2003;101:3236–9.
- Deroanne CF, Bonjean K, Servotte S, et al. Histone deacetylase inhibitors as anti-angiogenic agents altering vascular endothelial growth factor signaling. *Oncogene* 2002;21:427–36.
- Tabe Y, Konopleva M, Kondo Y, et al. PML-RAR α and AML1-ETO translocations are rarely associated with methylation of the RAR β 2 promoter. *Ann Hematol* 2006;85:689–704.
- Glozak MA, Sengupta N, Zhang X, Seto E. Acetylation and deacetylation of non-histone proteins. *Gene* 2005;363:15–23.
- Richon VM, Webb Y, Merger R, et al. Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. *Proc Natl Acad Sci U S A* 1996;93:5705–8.
- Richon VM, Emiliani S, Verdin E, et al. A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. *Proc Natl Acad Sci U S A* 1998;95:3003–7.
- 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;16:1331–43.
- 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;9:3578–88.
- 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;23:3923–31.
- Olsen E, Kim YH, Kuzel T, et al. Vorinostat is clinically active in advanced cutaneous T-cell lymphoma (CTCL): results of a phase II B trial [abstract 7500]. *J Clin Oncol* 2006;24:422s.
- 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;109:31–9.
- Schiller J, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002;346:92–8.
- Ozols RF, Bundy BN, Greer BE, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. *J Clin Oncol* 2003;21:3194–200.
- Belani CP, Kearns CM, Zuhowski EG, et al. Phase I trial, including pharmacokinetic and pharmacodynamic correlations, of combination paclitaxel and carboplatin in patients with metastatic non-small-cell lung cancer. *J Clin Oncol* 1999;17:676–84.
- Kim MS, Blake M, Baek JH. Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. *Proc Am Assoc Cancer Res* 2003;44:790.
- Bali P, Pranpat M, Swaby R, et al. Activity of suberoylanilide hydroxamic acid against human breast cancer cells with amplification of her-2. *Clin Cancer Res* 2005;11:6382–9.
- Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F. Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. *Cancer Res* 2003;63:7291–300.
- Hubbert C, Guardiola A, Shao R, et al. HDAC6 is a microtubule-associated deacetylase. *Nature* 2002;417:455–8.
- Calvert AH, Newell DR, Gumbrell LA, et al. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *J Clin Oncol* 1989;7:1748–56.
- Therasse P, Arbuuck 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;92:205–16.
- 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;840:108–15.
- Parise RA, Ramanathan RK, Zamboni WC, Egorin MJ. Sensitive liquid chromatography-mass spectrometry assay for quantitation of docetaxel and paclitaxel in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003;783:231–6.
- Yeh KC, Kwan KC. A comparison of numerical integrating algorithms by trapezoidal, Lagrange, and spline approximation. *J Pharmacokinet Biopharm* 1978;6:79–98.
- Rocci ML, Jr., Jusko WJ. LAGRAN program for area and moments in pharmacokinetic analysis. *Comput Programs Biomed* 1983;16:203–16.
- Kearns CM, Egorin MJ. Considerations regarding the less-than-expected thrombocytopenia encountered with combination paclitaxel/carboplatin chemotherapy. *Semin Oncol* 1997;24:S2-91–S2-96.
- D'Argenio DZ, Schumitzky A. *Adapt II user's guide: pharmacokinetic/pharmacodynamic systems analysis software*. Los Angeles (CA): Biomedical Simulations Resource; 1997.
- Kelly K, Crowley J, Bunn PA, Jr., et al. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small-cell lung cancer: a Southwest Oncology Group trial. *J Clin Oncol* 2001;19:3210–8.
- Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006;355:2542–50.

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Clin Cancer Res 2007;13:3605-3610.

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