Cancer Chemother Pharmacol (2012) 69:743–751
DOI 10.1007/s00280-011-1762-1

ORIGINAL ARTICLE

A randomized phase II study of two doses of vorinostat in combination with 5-FU/LV in patients with refractory colorectal cancer

M. G. Fakih · A. Groman · J. McMahon · G. Wilding · J. R. Muindi

Received: 27 August 2011 / Accepted: 5 October 2011 / Published online: 22 October 2011

Abstract
Background Vorinostat is synergistic with 5-FU in vitro and in vivo models. A combination of these two agents was associated with clinical activity in 5-FU refractory colorectal cancer patients in a phase I clinical trial, therefore warranting the conduct of this prospective phase II study.
Patients and methods Patients with refractory metastatic colorectal cancer were randomized in a two-stage design to receive vorinostat at 800 or 1,400 mg/day once a day × 3, every 2 weeks. 5-FU, preceded by leucovorin, was administered as a bolus followed by a 46-h infusion on days 2 and 3 of vorinostat. A pre-specified 2-month progression-free survival (PFS) rate of 27/43 patients per arm was needed to deem an arm interesting for further investigation.
Results The high-dose vorinostat arm did not reach the needed efficacy endpoint at completion of the first stage, with only 8 out of 15 patients being alive and progression free at 2 months. The low-dose vorinostat arm proceeded to accrue 43 patients with a 2-month PFS rate of 53% (23 out 43), including one partial response. The median PFS and overall survival on the low-dose arm were 2.4 and 6.5 months, respectively. Both treatment arms were well tolerated. No differences were noted in the pharmacokinetics of vorinostat at the 800- or 1,400-mg dose-levels, suggesting bioavailability saturation.
Conclusions While the addition of vorinostat to 5-FU resulted in 1 partial response and in some disease stabilizations, the limited activity does not warrant the unselected use of this combination in chemotherapy-refractory colorectal cancer.

Keywords Colorectal · Phase II · 5-Fluorouracil · Vorinostat · Histone deacetylase inhibitor

This work has been presented in part at the ESMO meeting in Milan in 2010.

M. G. Fakih
Department of Medicine, University of Michigan, Ann Arbor, MI, USA

M. G. Fakih (✉)
Division of Hematology and Oncology, Department of Medicine, University of Michigan Cancer Center, 1500 E Medical Drive, Ann Arbor, MI 48109, USA
e-mail: marwanf@med.umich.edu

A. Groman · G. Wilding
Department of Biostatistics, University at Buffalo, Buffalo, NY, USA

J. McMahon
Clinical Research Services, Roswell Park Cancer Institute, Buffalo, NY, USA

J. R. Muindi
Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY, USA

Introduction

Vorinostat, a histone deacetylase (HDAC) inhibitor, exerts its antitumor properties through cell cycle arrest, tubulin acetylation, anti-angiogenesis, gene expression modulation, Wnt signaling modulation, protein acetylation, epidermal growth factor receptor (EGFR) down-regulation, DNA damage, and among other pathways [1–12]. Vorinostat has demonstrated clinical activity in cutaneous T-cell lymphoma and mesothelioma but has no significant single agent activity in other solid tumors [13–21]. The lack of single agent activity in solid tumors did not decrease the enthusiasm in developing this agent in combination with cytotoxic agents, especially in view of its

demonstrated pre-clinical additive or synergistic antitumor activity [12, 22–26]. Unfortunately, this preclinical synergy has not translated into clinically relevant activity in numerous clinical trials, including a randomized phase III trial of carboplatin and paclitaxel in the first-line treatment of non-small-cell lung cancer (NSCLC) [27–29].

We have previously investigated a combination of vorinostat and 5-FU in patients with refractory solid tumors [30, 31]. The rationale for these studies was based on the pre-clinically demonstrated, vorinostat-induced, thymidylate synthase down-regulation in colorectal cancer models as well as the demonstrated synergy between 5-FU and vorinostat in colorectal cancer xenografts [22]. Despite the lack of evidence of thymidylate synthase down-regulation on serial tumor biopsies, we noted interesting clinical activity in patients with refractory colorectal cancer [30, 31]. In a phase I study, 24 patients with chemotherapy-refractory metastatic colorectal cancer were treated with daily vorinostat on days 1, 2, and 3 combined with infusional 5-FU on days 2 and 3 and repeated in 2-week cycles [30]. The median progression-free survival (PFS) and overall survival (OS) in this cohort were 4.4 and 9.2 months, respectively. Prolonged disease stabilizations were noted at vorinostat doses of 800 mg/day × 3 and higher [30].

To better define the activity of vorinostat in combination with 5-FU in chemotherapy-refractory colorectal cancer, we conducted a prospective randomized phase II clinical trial in this population. We selected two doses of vorinostat (800 and 1,400 mg) in combination with a standard dose of infusional 5-FU. The 800-mg dose was the lowest dose associated with prolonged disease stabilization, while 1,400 mg was the highest tolerable dose in combination with 5-FU in our prior phase I study [30]. The goal of this phase II study was to investigate the efficacy and safety of these two doses in patients with chemotherapy-refractory colorectal cancer and to determine whether either arm would be candidate for development into a future randomized phase III study.

Patients and methods

The main endpoint of this prospective randomized phase II clinical trial was to determine the 2-month progression-free survival rate with two doses of vorinostat, in combination with 5-FU and leucovorin in patients with refractory colorectal cancer. Secondary endpoints included PFS, OS, and safety as evaluated by the National Cancer Institute Common Toxicity Criteria Adverse Event version 4 (NCI CTCAE v4). Other secondary endpoints included vorinostat and 5-FU population pharmacokinetics and serial QTc interval assessment on the high-dose vorinostat arm.

Patients' criteria

Patients with advanced metastatic colorectal cancer who had failed standard chemotherapy were eligible for enrollment. To be eligible for study treatment, patients should have documented radiographic progression within 6 months from completion of oxaliplatin-based therapy, irinotecan-based therapy, and cetuximab-based therapy (in the event of a *KRAS* wild-type tumor). In addition, patients should have exhibited refractoriness to fluoropyrimidine therapy. Refractoriness to fluoropyrimidine was defined as radiographic progression within 4 weeks following a minimum of 6 weeks of 5-FU or capecitabine-based therapy. Patients had to be $\geq$18 years of age, have an ECOG performance status of 0–2, and have an acceptable organ function: white blood cell count $\geq 3{,}000/10^{-6}$ L, absolute neutrophil count $\geq 1{,}500/10^{-6}$ L, platelets $\geq 75{,}000/10^{-6}$ L, serum creatinine $\leq 1.5 \times$ upper institutional normal level, total bilirubin $\leq$ upper institutional normal level, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 3 \times$ upper institutional normal in the absence of liver metastases and $\leq 5 \times$ upper institutional normal in the setting of liver metastases. Patients could not have received any chemotherapy within 3 weeks from initiation of study treatment with the exception of nitrosureas or mitomycin C, which required a 6-week interval before study treatment. Patients with brain metastases, grade $\geq$2 neuropathy, grade $\geq$2 QTc prolongation, or other severe intercurrent illnesses were excluded. Patients who were HIV positive and taking antiretroviral medicines were excluded because of potential drug–drug interactions. No other HDAC inhibitors (such as valproic acid) or other investigational agents were allowed while patients were on study. Prior vorinostat therapy was not allowed. Pregnant or lactating women were excluded from participation. All consenting patients having the potential of conceiving agreed to use double contraception during the study period. The study and consent form were approved by the Institutional Scientific and Review Committee and the Institutional Review Board before the study was activated. All patients provided signed informed consent before study entry. The study was IRB approved and conducted in accordance with the Good Clinical Practice Guidelines as issued by the International Conference on Harmonization and the Declaration of Helsinki.

Treatment plan and dose modifications

Patients satisfying the inclusion criteria were randomized to receive vorinostat at 800 or 1,400 mg, given orally once daily for 3 consecutive days in 2-week cycles. Vorinostat (Zolinza®) was supplied by Merck Sharp and Dohme Corp as 100-mg capsules. Patients received all 8

or 14 designated capsules at the same time during the day, preferably with a meal. Leucovorin (LV) was given intravenously at 400 mg/m^2 over 2 h after day 2 dose of vorinostat and was followed by 5-FU intravenous bolus at 400 mg/m^2 and 5-FU infusion at 2,400 mg/m^2 over 46 h (days 2 and 3 of vorinostat). 5-FU and leucovorin were obtained commercially. Patients were pre-medicated with a 5-HT3 antagonist on day 2, prior to 5-FU/LV. Other anti-emetics were used per investigator discretion. Dose modifications were allowed for both vorinostat and 5-FU/LV based on toxicity. Grade $\geq$3 toxicities attributed to vorinostat (such as fatigue and nausea/vomiting) required a dose-reduction starting the subsequent cycle. The first and second dose-level reductions for the 800-mg vorinostat arm were 600 and 400 mg, respectively. Up to four dose-level reductions were allowed on the 1,400-mg arm: 1,100, 800, 600, and 400 mg. No dose-reductions below 400 mg of vorinostat were allowed. Grace $\geq$3 hematological and non-hematological toxicities attributed to 5-FU required a dose-reduction by one dose-level starting the subsequent cycle. The first dose-reduction consisted of a reduction in 5-FU bolus to 300 mg/m^2 and 5-FU infusion to 2,000 mg/m^2. The second dose-reduction resulted in elimination of the 5-FU bolus and further reduction in 5-FU infusion to 1,800 mg/m^2. The third dose-reduction consisted of further reduction in 5-FU infusion to 1,600 mg/m^2. No further dose-reductions in 5-FU were allowed.

Response assessment

Patients underwent a CT scan of the chest, abdomen, and pelvis within 3 weeks before the start of treatment. A CT scan was repeated every 8 weeks (4 cycles). Patients with progressive disease were taken off study. Response status was determined as per RECIST 1.1 criteria [32].The primary endpoint of the study was progression-free survival rate at 2 months from treatment initiation. PFS was defined as lack of progression or death as per RECIST 1.1.

Pharmacokinetics

Vorinostat pharmacokinetics (PK) were evaluated at 0 (pre-vorinostat), 0.5, 1, 2, 3, 4, 6, and 8 h after vorinostat, on day 2 of cycle 1. 5-FU steady-state levels were measured at 6 h after the start of 5-FU infusion on day 2 of cycle 1. Vorinostat PK and 5-FU levels were evaluated on each treatment arm in the first 10 and 20 patients, respectively. The methodology was previously detailed [31].

Non-compartmental analysis of vorinostat plasma PK parameters was performed using WinNonLin Version 5.3 software (Pharsight Corp, Mountain View, CA). The pharmacokinetic parameters for each vorinostat dose were summarized using mean $\pm$ SEM. The parameters estimated for vorinostat and its two major metabolites (glucuronide-conjugate and 4-anilino-4-oxobutanoic acid) were peak levels (C_{max}), time to C_{max}, and area under curve from 0 to 8 h (AUC_{0-8h}); the elimination half-life ($T_{1/2}$) was determined for patients with at least 3 terminal elimination times points with correlation coefficient (r^2) > 0.80.

QTc assessment

Since the effect of vorinostat on QTc interval prolongation had not been previously evaluated at doses exceeding 800 mg [33], we measured the QTc interval in the first 10 patients on the 1,400 mg/day dose-level. QTc interval was measured on a 12-lead EKG before, at 2, 4, and 6 h after the first dose of 1,400-mg vorinostat. In the event 2 or more grade $\geq$3 QTc prolongations were seen, the high-dose vorinostat arm would have been terminated.

Statistical methods

To evaluate the efficacy of the two different doses of vorinostat in combination with 5-FU/LV as measured by 2-month progression-free survival (PFS), a two-stage Simon design was utilized. Since 5-FU re-exposure in the setting of prior 5-FU failure has been associated with a 2-month PFS rate of approximately 50%, we deemed that any of our study arms would be interesting for future investigation if evidence was observed that the PFS rate exceeds 50% at 2 months. The two-stage design consisted of 15 patients on the first stage and 28 patients on the second stage (in each arm). Continuation to the second stage occurred only if $\geq$9 out of 15 patients on the first stage were alive and progression free at 2 months. Otherwise, if the observed count was less than the threshold, the corresponding arm of treatment was deemed not interesting for further investigation and the enrollment on that arm was halted. An arm was considered efficacious after the second stage if 27 or more out of 43 patients were alive and progression free at 2 months. The decision rules are associated with a type I error rate of 5%. The sample size (43 patients per arm) was selected so to provide 80% power in detecting an increase of 20% points as compared to the historical control rate of 50%. Subjects were randomized to either of the two treatment arms in a 1:1 fashion using a permuted block randomization scheme. In order to maintain balance between the two arm with regard to LDH ($\leq$upper normal limit vs. >upper normal limit) and ECOG performance status score (0, 1 vs. 2), randomization was further stratified by these factors.

Table 1 Patients' demographics

	Vorinostat 800 mg ($N = 43$)	Vorinostat 1,400 mg ($N = 15$)	Overall population ($N = 58$)
Age (median, range)	60 (44–84)	62 (41–78)	60 (41–84)
Gender (male/female)	21/22	6/9	27/31
LDH (normal/elevated)	15/27	8/7	23/34
ECOG (0/1/2)	21/17/5	10/3/2	31/20/7
KRAS (WT/MT)	14/29	10/5	24/34

LDH lactate dehydrogenase, *ECOG* eastern cooperative group performance status, *WT* wild type, *MT* mutant

Results

Study enrollment and patient demographics

The study was conducted at Roswell Park Cancer Institute, Buffalo, NY and was registered under ClinicalTrials.gov as NCT00942266. Study enrollment started in August 2009 and was completed in December of 2010. A total of 58 patients were enrolled on study, 43 patients on the low-dose vorinostat arm and 15 patients on the high-dose vorinostat arm. All patients had confirmed 5-FU refractory colorectal cancer as defined in the inclusion criteria, and all had progressed following all standard chemotherapy for colorectal cancer. About 60% of patients had an elevated LDH, a known negative prognostic factor in patients with advanced colorectal cancer. The patient demographics per arm are detailed in Table 1.

Toxicity

Both treatment arms were well tolerated with most common grade ≥2 toxicities being fatigue, nausea, hand and foot syndrome, and diarrhea. Nausea was more prevalent on the 1,400-mg vorinostat dose-level, while other toxicities did not appear to be influenced by dosing. No grade ≥3 neutropenia or thrombocytopenia was recorded, reflecting the non-myelosuppressive nature of this regimen. Grade ≥2 toxicities for the 800- and 1,600-mg vorinostat arms are summarized in Tables 2 and 3, respectively.

Table 2 Non-hematological toxicities on the 800 mg vorinostat arm

Event	Vorinostat 800 mg QD × 3 ($N = 43$)		
	Grade 2	Grade 3	Grade 4/5
Anorexia	8	0	0
Cheiliti+s	2	1	0
DVT	0	1	0
Dehydration	1	0	0
Diarrhea	6	2	0
Dyspepsia	3	0	0
Fatigue	14	3	0
Hand and foot	1	3	0
Mucositis	3	1	0
Nausea	4	2	0
Pneumonitis	0	0	1[a]
Pulmonary embolus	0	0	1[b]
Vomiting	4	0	0
Weight loss	1	0	0

[a] A patient with baseline COPD and oxygen dependence had stable disease for 8 months. After 8 months of treatment, she developed diffuse pulmonary infiltrates and respiratory failure leading to death. Bronchoscopy was non-diagnostic, and an autopsy was not performed. The relationship to treatment remains unclear but cannot be ruled out

[b] Grade 4 Pulmonary Embolus successfully treated with anti-coagulation

Pharmacokinetic

Mean (± SEM) steady-state day 1 plasma 5-FU levels, measured 6 h after 5-FU infusion initiation, were 416.7 ± 30.5 and 433.5 ± 46.3 ng/mL for patients receiving 800 ($N = 19$) and 1,400 ($N = 10$) mg of SAHA, respectively ($P = 0.76$).

A summary of the pharmacokinetic parameters of vorinostat and its two major metabolites is shown in Table 4. No significant PK differences were observed between patients following oral administration of vorinostat at the 800- and 1,400-mg doses. Plasma C_{max} and AUC_{0-8h} values of the two inactive metabolites, vorinostat-glucuronide and 4-anilino-4-oxobutanoic acid, were 3–7 times higher than corresponding values for vorinostat, the parent drug ($P < 0.0001$, one-way ANOVA). These results were consistent with prior reports [34]. There was no difference in pharmacokinetics for the metabolites between both arms, suggesting no increased metabolism on the high-dose arm.

QTc assessment

The first ten patients enrolled at the 1,400-mg vorinostat dose-level underwent serial EKGs after their first dose of vorinostat. Four of the 10 patients had a baseline grade 1 QTc prolongation. No grade ≥3 QTc prolongations were recorded. Four patients had grade 2 QTc prolongations after vorinostat, 3 of whom had a baseline Grade 1 QTc prolongation.

Table 3 Non-hematological toxicities on the 1,600-mg vorinostat arm

Event	Vorinostat 1,600-mg QD × 3 ($N = 15$)		
	Grade 2	Grade 3	Grade 4/5
Anorexia	5	1	0
Dizziness	0	1	0
Dysgeusia	3	0	0
Enteritis	0	2	0
Esophagitis	1	0	0
Fatigue	2	5	0
Mucositis	2	0	0
Nausea	8	2	0
Pneumonia	0	1	0
Vomiting	3	1	0
Weight loss	1	0	0

Efficacy

High-dose (1,400 mg) vorinostat

Fifteen patients were treated on the first stage of the study. Stable disease was documented at 2 months in 8 patients while 7 patients progressed. Since the number of patients without progression at 2 months did not reach the threshold of 9 patients required for the second stage of the study, accrual on this arm was halted. The median PFS and OS were 2.9 (95% CI: 1.6–5.6) and 6.7 months (95% CI: 3.0 to not-reached), respectively.

Low-dose (800 mg) vorinostat

Fifteen patients were treated on the first stage of the study. One patient with pulmonary metastases had a documented partial response (PR), and eight patients had stable disease (SD) for a PFS rate of 9 out of 15, supporting progression into the second stage of the study. An additional 28 patients were enrolled on the second stage for a total of 43 patients. Only 23 out of 43 patients were progression free (1 PR, 22 SD) at 2 months for a PFS rate of 53%. The median PFS and OS of the overall population were 2.4 (95% CI: 1.8–3.6) and 6.5 months (95% CI: 4.8–7.8), respectively (Fig. 1). The PFS rate did not reach the pre-specified threshold of 27 out of 43 patients, and the combination was not deemed interesting enough for further evaluation. Interestingly, lung target lesions appeared to respond better to treatment than hepatic lesions on waterfall plots (Fig. 2). Since KRAS MT tumors are more likely to metastasize to the lungs [35], we performed a multivariate analysis to evaluate the impact of KRAS, LDH, gender, and performance status on PFS and OS. None of these variables resulted in a statistically significant impact on either efficacy outcomes. An elevated LDH resulted in a trend in worsening in PFS (Hazard Ratio of 1.63, $P = 0.197$). Similarly, the waterfall plots did not suggest a better likelihood of response based on KRAS status (Fig. 2).

Discussion

Vorinostat is a potent inhibitor of class I and II HDAC that has proven clinical activity against cutaneous T-cell lymphomas [36].This agent has significant synergy with 5-FU in pre-clinical models [22]. It has been postulated that this synergy may be related to intratumor thymidylate synthase down-regulation [22, 37]. However, we have failed to document such down-regulation in colorectal metastases, even at doses exceeding 800 mg/day, despite interesting preliminary clinical activity [30, 31]. We therefore hypothesized that the combination of 5-FU and vorinostat

Table 4 PK parameters of vorinostat and its major metabolites after oral administration of 800- and 1,400-mg doses

Plasma metabolite measured	PK parameter	Vorinostat dose (mg)		*P* value
		800 ($N = 10$)	1,400 ($N = 10$)	
Vorinostat	$T_{1/2}$ (h)	3.2 ± 0.6[a]	2.2 ± 0.4[a]	0.173
	T_{max} (h)	3.0 ± 0.6	2.8 ± 0.5	0.762
	C_{max} (μmol/L)	3.13 ± 0.71	3.60 ± 0.56	0.606
	AUC_{0-8h} (h μmol/L)	12.8 ± 3.02	14.9 ± 3.27	0.648
Vorinostat-glucuronide	$T_{1/2}$ (h)	4.5 ± 1.3[b]	2.4 ± 0.5[c]	0.150
	T_{max} (h)	4.1 ± 0.8	3.7 ± 0.5	0.696
	C_{max} (μmol/L)	8.47 ± 2.27	8.45 ± 1.05	0.993
	AUC_{0-8h} (h μmol/L)	38.2 ± 10.6	38.1 ± 4.86	0.991
4-Anilino-4-oxobutanoic acid	$T_{1/2}$ (h)	33.4 ± 12.4[b]	7.5 ± 4.1[c]	0.163
	T_{max} (h)	3.3 ± 0.9	4.8 ± 0.7	0.221
	C_{max} (μmol/L)	14.3 ± 4.38	15.1 ± 2.23	0.867
	AUC_{0-8h} (h μmol/L)	85.6 ± 32.6	79.5 ± 11.6	0.860

Data are presented as mean ± SEM, *P* value from unpaired *t* test

[a] $N = 8$

[b] $N = 7$

[c] $N = 4$

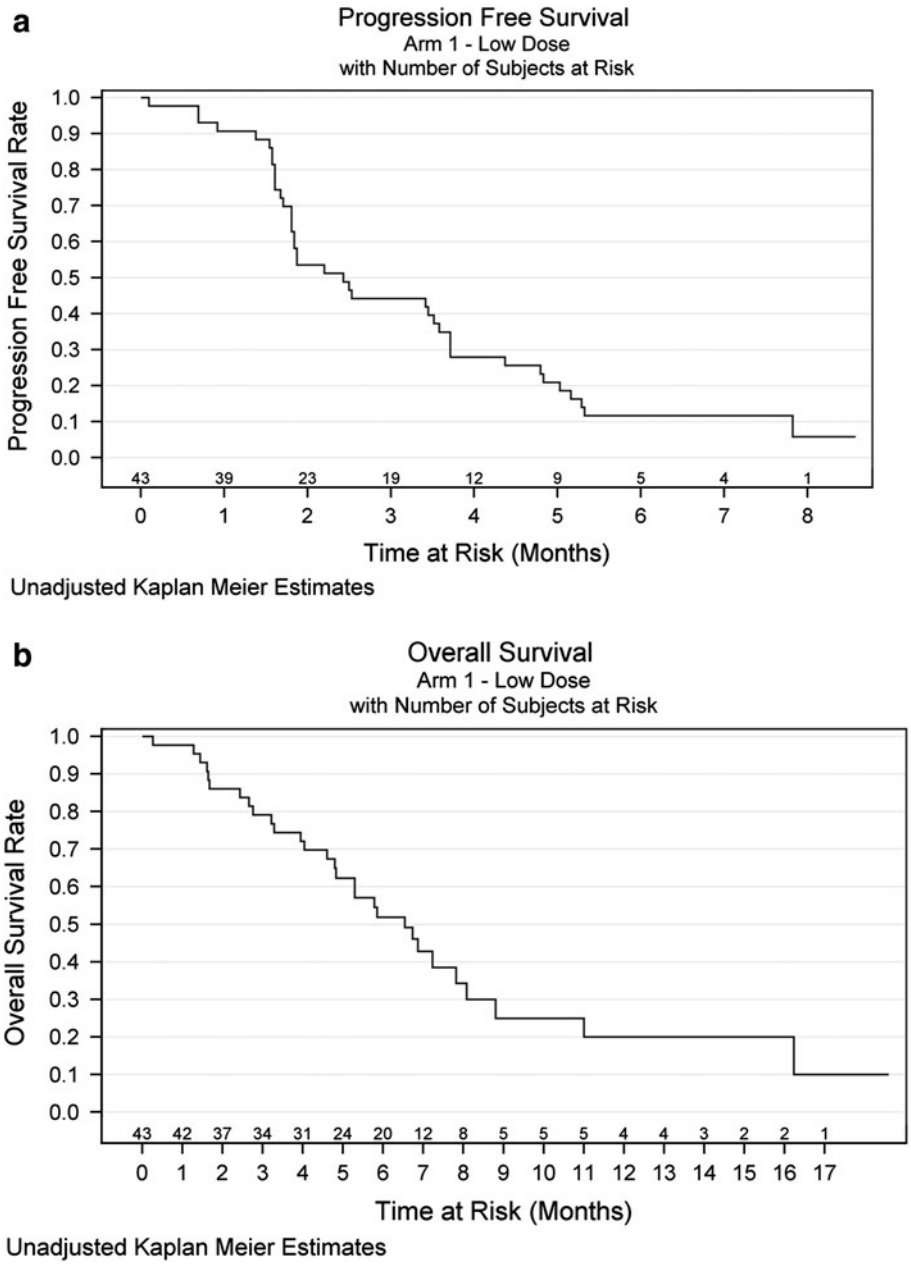

Fig. 1 **a** Progression-free survival curve for the 800-mg vorinostat dose-level. **b** Overall survival curve for the 800-mg vorinostat dose-level

may be an effective combination in colorectal cancer and that its associated activity may not be related to thymidylate synthase down-regulation.

In this phase II randomized clinical trial, we set to investigate whether the combination of relatively high doses of intermittent vorinostat overcomes resistance to 5-FU in refractory metastatic colorectal cancer. Unfortunately, we did not see significant signs of activity on either arms of the study. The 1,400-mg dose-level was closed after the first stage, while the 800-mg dose-level accrued to the pre-planned total of 43 patients. The 2-month PFS rate on both arms was 53%, and only 1 PR was observed among 58 total patients. The OS of 6.5 months compares favorably to a previously reported best supportive care median of 4.6 months [38]. However, this number is inferior to other studies incorporating 5-FU infusional therapy in patients who previously failed 5-FU treatment [39, 40].

The potential explanations for the negative outcome of this study are numerous. At the forefront is the possibility that HDAC inhibition does not improve clinical outcomes when added to cytotoxic therapy. This would not be inconsistent with the clinical body of evidence in other tumor types and with other cytotoxics [27]. Other possibilities include the patient selection on this study. All of our patients were radiographically refractory to fluoropyrimidine. The rationale for this selection was predominantly

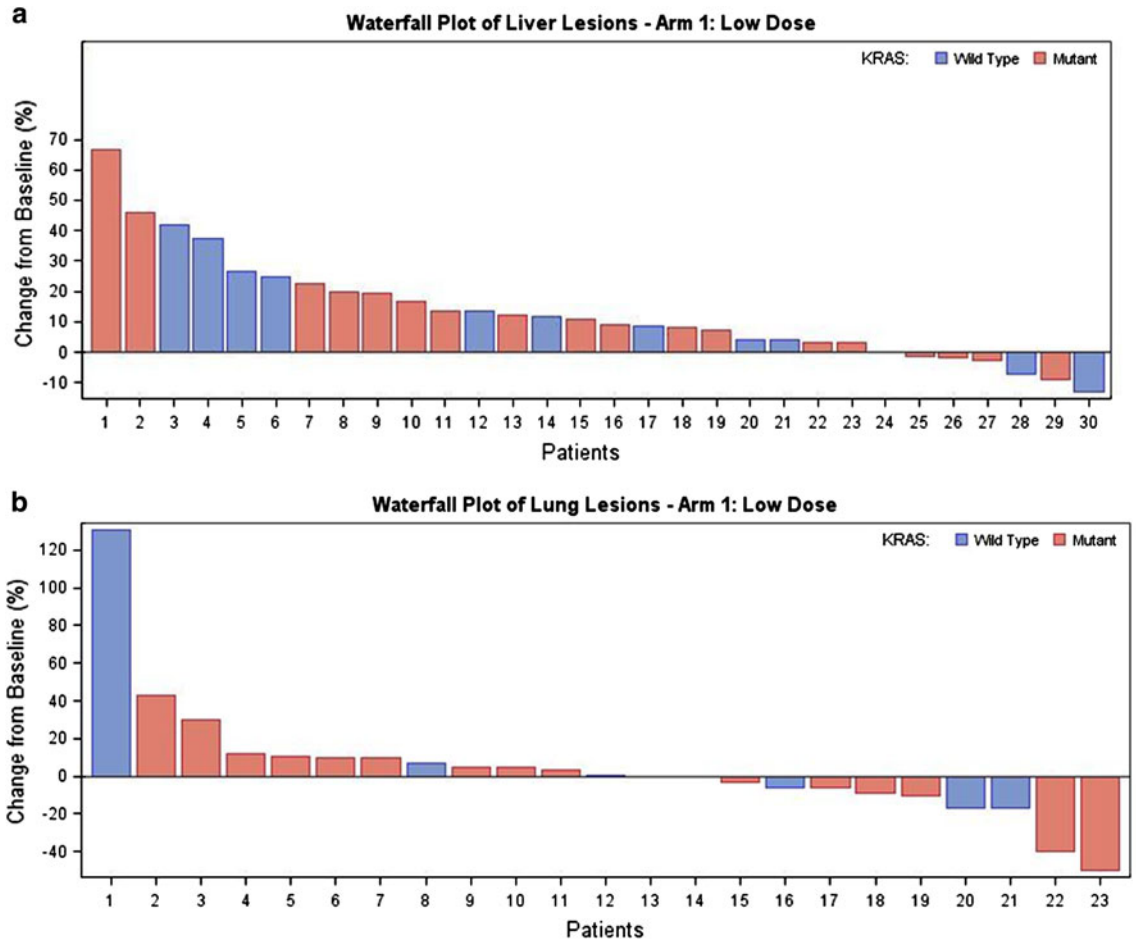

Fig. 2 **a** Waterfall chart for hepatic lesions and by KRAS status on the 800-mg dose-level. **b** Waterfall chart for lung lesions and by KRAS on the 800-mg dose-level

based on a large subgroup analysis from a prior phase I study and not on 5-FU refractory pre-clinical models. Since pre-clinical synergy was demonstrated in 5-FU-sensitive models, a more appropriate clinical investigation would have focused on non-5-FU-resistant colorectal cancer patients. Another potential explanation for the poor outcome is the high percentage of patients with elevated LDH, a known negative prognostic factor in patients with stage IV disease.

We have shown, for the first time, evidence of bioavailability saturation of vorinostat at doses exceeding 800 mg. The 800- and 1,400-mg dose-levels were associated with a median C_{max} of 3.1 and 3.6 μM, and $AUC_{0–8h}$ (h μmol/L) of 12.8 and 14.9, respectively. The lack of dose-PK effect at vorinostat doses exceeding 800 mg is likely due to absorption saturation rather than due to vorinostat self-induced metabolism, especially in view of the similar metabolite pharmacokinetic profile on both arms. Despite achieving concentrations associated with antitumor activity ($C_{max} > 2$ μM), the AUC profile would be considered inferior to the conditions associated with antitumor activity when combined with 5-FU in vitro [22, 41]. When considering concentrations and exposure times similar to what was achieved in our study, vorinostat did not enhance 5-FU antitumor activity in vitro [42]. It has also been recently shown that vorinostat intratumor concentrations are considerably lower than corresponding serum concentrations, stressing further the need for higher and more sustained vorinostat serum concentrations [43]. Such favorable pharmacokinetics can be achieved with an intravenous formulation of vorinostat (no longer available) but are unlikely to occur with the current oral formulation [44].

Finally, we explored the impact of vorinostat on lung metastases following the observation of prolonged clinical control in patients isolated lung metastases. While the waterfall charts do suggest increased activity in lung metastases in comparison with liver metastases, we are unable to confirm whether this is related to selective activity of vorinostat in this subgroup of patients versus differences in tumor kinetics in relationship to the site of metastases. Since HDAC inhibitors have been reported to

have enhanced activity in the presence of KRAS mutation [45], and in view of the increased prevalence of KRAS mutations in lung metastases [35], we investigated the impact of KRAS status on outcome in our study. KRAS status did not significantly impact PFS or OS.

In conclusion, we have failed to show clinically relevant activity for the combination of vorinostat (800 or 1,400 mg) and 5-FU in patients with refractory colorectal cancer. Further clinical development of this combination is not warranted.

Acknowledgments The authors would like to acknowledge the late Dr. Merrill Egorin. Dr. Egorin made major contributions to the earlier vorinostat plus 5-FU phase I clinical trial, which formed the basis for the conduct of this phase II study. Dr. Egorin developed the clinical assay for vorinostat and his laboratory performed all vorinostat pharmacokinetic measurements on this study. This study was supported by a research grant from Merck Sharp and Dohme Corp.

References

1. Wilson AJ et al (2006) Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer. J Biol Chem 281(19):13548–13558
2. Archer SY et al (2005) The histone deacetylase inhibitor butyrate downregulates cyclin B1 gene expression via a p21/WAF-1-dependent mechanism in human colon cancer cells. Am J Physiol Gastrointest Liver Physiol 289(4):G696–G703
3. Nawrocki ST et al (2007) Histone deacetylase inhibitors enhance lexatumumab-induced apoptosis via a p21Cip1-dependent decrease in survivin levels. Cancer Res 67(14):6987–6994
4. Xu WS et al (2005) Induction of polyploidy by histone deacetylase inhibitor: a pathway for antitumor effects. Cancer Res 65(17):7832–7839
5. Place RF, Noonan EJ, Giardina C (2005) HDAC inhibition prevents NF-kappa B activation by suppressing proteasome activity: down-regulation of proteasome subunit expression stabilizes I kappa B alpha. Biochem Pharmacol 70(3):394–406
6. Dokmanovic M, Marks PA (2005) Prospects: histone deacetylase inhibitors. J Cell Biochem 96(2):293–304
7. Kim DH, Kim M, Kwon HJ (2003) Histone deacetylase in carcinogenesis and its inhibitors as anti-cancer agents. J Biochem Mol Biol 36(1):110–119
8. Vigushin DM, Coombes RC (2002) Histone deacetylase inhibitors in cancer treatment. Anticancer Drugs 13(1):1–13
9. Liu T et al (2006) Histone deacetylase inhibitors: multifunctional anticancer agents. Cancer Treat Rev 32(3):157–165
10. Deroanne CF et al (2002) Histone deacetylases inhibitors as anti-angiogenic agents altering vascular endothelial growth factor signaling. Oncogene 21(3):427–436
11. Conti C et al (2010) Inhibition of histone deacetylase in cancer cells slows down replication forks, activates dormant origins, and induces DNA damage. Cancer Res 70(11):4470–4480
12. Owonikoko TK et al (2010) Vorinostat increases carboplatin and paclitaxel activity in non-small-cell lung cancer cells. Int J Cancer 126(3):743–755
13. Kirschbaum M et al (2011) Phase II study of vorinostat for treatment of relapsed or refractory indolent non-Hodgkin's lymphoma and mantle cell lymphoma. J Clin Oncol 29(9):1198–1203
14. Duvic M et al (2007) Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood 109(1):31–39
15. Traynor AM et al (2009) Vorinostat (NSC# 701852) in patients with relapsed non-small cell lung cancer: a Wisconsin Oncology Network phase II study. J Thorac Oncol 4(4):522–526
16. Galanis E et al (2009) Phase II trial of vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study. J Clin Oncol 27(12):2052–2058
17. Luu TH et al (2008) A phase II trial of vorinostat (suberoylanilide hydroxamic acid) in metastatic breast cancer: a California Cancer Consortium study. Clin Cancer Res 14(21):7138–7142
18. Vansteenkiste J et al (2008) Early phase II trial of oral vorinostat in relapsed or refractory breast, colorectal, or non-small cell lung cancer. Invest New Drugs 26(5):483–488
19. Modesitt SC et al (2008) A phase II study of vorinostat in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma: a Gynecologic Oncology Group study. Gynecol Oncol 109(2):182–186
20. Blumenschein GR Jr et al (2008) Phase II trial of the histone deacetylase inhibitor vorinostat (Zolinza, suberoylanilide hydroxamic acid, SAHA) in patients with recurrent and/or metastatic head and neck cancer. Invest New Drugs 26(1):81–87
21. Krug LM et al (2006) Potential role of histone deacetylase inhibitors in mesothelioma: clinical experience with suberoylanilide hydroxamic acid. Clin Lung Cancer 7(4):257–261
22. Fazzone W et al (2009) Histone deacetylase inhibitors suppress thymidylate synthase gene expression and synergize with the fluoropyrimidines in colon cancer cells. Int J Cancer 125(2):463–473
23. Cheriyath V et al (2011) Potentiation of apoptosis by histone deacetylase inhibitors and doxorubicin combination: cytoplasmic cathepsin B as a mediator of apoptosis in multiple myeloma. Br J Cancer 104(6):957–967
24. Arnold NB et al (2007) The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces growth inhibition and enhances gemcitabine-induced cell death in pancreatic cancer. Clin Cancer Res 13(1):18–26
25. Shi YK et al (2010) The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces growth inhibition and enhances taxol-induced cell death in breast cancer. Cancer Chemother Pharmacol 66(6):1131–1140
26. Suzuki M et al (2009) Enhancement of cisplatin cytotoxicity by SAHA involves endoplasmic reticulum stress-mediated apoptosis in oral squamous cell carcinoma cells. Cancer Chemother Pharmacol 64(6):1115–1122
27. Ramalingam SS et al (2010) Carboplatin and Paclitaxel in combination with either vorinostat or placebo for first-line therapy of advanced non-small-cell lung cancer. J Clin Oncol 28(1):56–62
28. Munster PN et al (2009) Phase I trial of vorinostat and doxorubicin in solid tumours: histone deacetylase 2 expression as a predictive marker. Br J Cancer 101(7):1044–1050
29. Wilson PM et al (2010) A phase I/II trial of vorinostat in combination with 5-fluorouracil in patients with metastatic colorectal cancer who previously failed 5-FU-based chemotherapy. Cancer Chemother Pharmacol 65(5):979–988
30. Fakih MG et al (2010) A phase I, pharmacokinetic, and pharmacodynamic study of two schedules of vorinostat in combination with 5-fluorouracil and leucovorin in patients with refractory solid tumors. Clin Cancer Res 16(14):3786–3794
31. Fakih MG et al (2009) 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 15(9):3189–3195
32. Eisenhauer EA et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45(2):228–247

33. Munster PN et al (2009) A single supratherapeutic dose of vorinostat does not prolong the QTc interval in patients with advanced cancer. Clin Cancer Res 15(22):7077–7084
34. Ramalingam SS et al (2007) Phase I and pharmacokinetic study of vorinostat, a histone deacetylase inhibitor, in combination with carboplatin and paclitaxel for advanced solid malignancies. Clin Cancer Res 13(12):3605–3610
35. Tie J et al (2011) KRAS mutation is associated with lung metastasis in patients with curatively resected colorectal cancer. Clin Cancer Res 17(5):1122–1130
36. Olsen EA et al (2007) Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. J Clin Oncol 25(21):3109–3115
37. Glaser KB et al (2003) 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 2(2):151–163
38. Jonker DJ et al (2007) Cetuximab for the treatment of colorectal cancer. N Engl J Med 357(20):2040–2048
39. Rothenberg ML et al (2003) Superiority of oxaliplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: interim results of a phase III trial. J Clin Oncol 21(11):2059–2069
40. Rougier P et al (1998) Randomised trial of irinotecan versus fluorouracil by continuous infusion after fluorouracil failure in patients with metastatic colorectal cancer. Lancet 352(9138): 1407–1412
41. Di Gennaro E et al (2009) Modulation of thymidilate synthase and p53 expression by HDAC inhibitor vorinostat resulted in synergistic antitumor effect in combination with 5FU or raltitrexed. Cancer Biol Ther 8(9):782–791
42. Kim MS et al (2003) Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. Cancer Res 63(21):7291–7300
43. Novotny-Diermayr V et al (2010) SB939, a novel potent and orally active histone deacetylase inhibitor with high tumor exposure and efficacy in mouse models of colorectal cancer. Mol Cancer Ther 9(3):642–652
44. Kelly WK et al (2003) Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously. Clin Cancer Res 9(10 Pt 1):3578–3588
45. Klampfer L et al (2007) Histone deacetylase inhibitors induce cell death selectively in cells that harbor activated kRasV12: the role of signal transducers and activators of transcription 1 and p21. Cancer Res 67(18):8477–8485