

## Phase I Study of Vorinostat in Combination with Temozolomide in Patients with High-Grade Gliomas: North American Brain Tumor Consortium Study 04-03

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### Abstract

**Purpose:** A phase I, dose-finding study of vorinostat in combination with temozolomide (TMZ) was conducted to determine the maximum tolerated dose (MTD), safety, and pharmacokinetics in patients with high-grade glioma (HGG).

**Experimental Design:** This phase I, dose-finding, investigational study was conducted in two parts. Part 1 was a dose-escalation study of vorinostat in combination with TMZ 150 mg/m<sup>2</sup>/day for 5 days every 28 days. Part 2 was a dose-escalation study of vorinostat in combination with TMZ 150 mg/m<sup>2</sup>/day for 5 days of the first cycle and 200 mg/m<sup>2</sup>/day for 5 days of the subsequent 28-day cycles.

**Results:** In part 1, the MTD of vorinostat administered on days 1 to 7 and 15 to 21 of every 28-day cycle, in combination with TMZ, was 500 mg daily. Dose-limiting toxicities (DLT) included grade 3 anorexia, grade 3 ALT, and grade 5 hemorrhage in the setting of grade 4 thrombocytopenia. In part 2, the MTD of vorinostat on days 1 to 7 and 15 to 21 of every 28-day cycle, combined with TMZ, was 400 mg daily. No DLTs were encountered, but vorinostat dosing could not be escalated further due to thrombocytopenia. The most common serious adverse events were fatigue, lymphopenia, thrombocytopenia, and thromboembolic events. There were no apparent pharmacokinetic interactions between vorinostat and TMZ. Vorinostat treatment resulted in hyperacetylation of histones H3 and H4 in peripheral mononuclear cells.

**Conclusion:** Vorinostat in combination with temozolomide is well tolerated in patients with HGG. A phase I/II trial of vorinostat with radiotherapy and concomitant TMZ in newly diagnosed glioblastoma is underway. *Clin Cancer Res*; 18(21); 6032–9. ©2012 AACR.

### Introduction

Histone proteins organize DNA into nucleosomes, which are regular repeating structures of chromatin (1). The acety-

lation status of histones alter chromatin structure and is regulated by 2 classes of enzymes, histone deacetylases (HDAC) and histone acetyltransferases (HAT). This acetylation affects the regulation of gene expression by rendering certain genes accessible to transcriptional machinery. There is increasing evidence that HDAC or HAT activity is altered in many cancers, including gliomas. HDAC inhibitors (HDACi) can induce growth arrest, differentiation, and/or apoptosis of tumor cells *in vitro* and *in vivo* by altering the transcription of a small number of genes and represent a promising novel therapeutic approach to cancer (1, 2).

Vorinostat is a small-molecule inhibitor of HDAC that binds directly in the enzyme's active site in the presence of a zinc ion and is approved by the United States Federal Drug Administration (FDA) for use in patients with cutaneous T-cell lymphoma who have progressive, persistent, or recurrent disease on or following 2 systemic therapies. Vorinostat has activity against high-grade glioma (HGG) lines *in vitro* and *in vivo* and seems to pass through the blood brain barrier (3–6). In a phase II study of recurrent glioblastoma (GBM), vorinostat was

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### Translational Relevance

Vorinostat, a histone deacetylase inhibitor, has activity against high-grade glioma in preclinical studies and may work synergistically with cytotoxic chemotherapy. In this phase I trial, we evaluated escalating doses of vorinostat in combination with temozolomide. We show that vorinostat can be administered safely on days 1 to 7 and 15 to 21 of every 28-day cycle in doses up to 500 mg daily when combined with temozolomide 150 mg/m<sup>2</sup>/day for 5 days in 28-day cycles and in doses up to 400 mg daily when combined with temozolomide 150 mg/m<sup>2</sup>/day for 5 days for the first 28-day cycle and 200 mg/m<sup>2</sup>/day for 5 days for the subsequent 28-day cycles. Pharmacokinetic analysis revealed no significant interactions between vorinostat and temozolomide. On the basis of these results, a phase I/II trial of vorinostat in combination with temozolomide and radiotherapy for newly diagnosed glioblastoma is in progress.

generally well tolerated and showed modest single-agent activity with a 6-month progression-free survival rate (PFS<sub>6</sub>) of 15.2% (7). In addition, tumor samples of patients with GBM, who received vorinostat before surgery, indicated that this agent was able to penetrate tumors and inhibited histone acetylation (7).

Acetylation of key lysine residues in core histones leads to a more relaxed chromatin configuration. Therefore, HDAC inhibitors, by allowing acetylation, may provide cytotoxic chemotherapy with enhanced access to DNA resulting in synergistic activity (2). Temozolomide (TMZ) is an alkylating agent with efficacy in HGG; as established by the phase III clinical trial showing that the addition of TMZ to radiation extends overall survival in patients with newly diagnosed GBM compared with radiation alone (8, 9). Treatment of U87 glioma cell lines with vorinostat and temozolomide results in a supra-additive effect on growth inhibition (10). Therefore, the North American Brain Tumor Consortium (NABTC) conducted a phase I study to investigate the combination of vorinostat with temozolomide in patients with HGG.

### Materials and Methods

This phase I, open-label, dose-finding study was conducted in 2 parts. Part 1 was a dose-escalation study of vorinostat in combination with TMZ 150 mg/m<sup>2</sup>/day for 5 days every 28 days. Part 2 was a dose-escalation study of vorinostat in combination with TMZ 150 mg/m<sup>2</sup>/day for 5 days of the first 28-day cycle and 200 mg/m<sup>2</sup>/day for 5 days of the subsequent 28-day cycles. The study was approved by the Institutional Review Boards of each participating center and was conducted in accordance with the Declaration of Helsinki. The trial was registered in the NIH clinical trials database (NCT00268385). Informed consent was obtained from each patient before participating in the study.

### Patient selection

Patients were eligible for the study if they had histologically proven intracranial HGG (glioblastoma, gliosarcoma, anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic mixed oligoastrocytoma, and malignant astrocytoma not otherwise specified), were 18 years or older, and had a Karnofsky performance status (KPS) 60 or more, adequate bone marrow function (WBC  $\geq$  3,000/ $\mu$ L, ANC  $\geq$  1,500/mm<sup>3</sup>, platelet count  $\geq$  100,000/mm<sup>3</sup>, and hemoglobin  $\geq$  10 gm/dL), adequate liver function (SGOT and bilirubin  $<$  2 times the upper limit of normal), adequate renal function (creatinine  $<$  1.5 mg/dL), and were 3 or more weeks from completion of radiotherapy. Patients who had previously progressed on TMZ as well as patients who were taking valproic acid (another HDAC inhibitor) within 2 weeks before enrollment were excluded. For part 1, patients with either stable disease after radiotherapy (with or without concurrent TMZ) or following progression on radiotherapy alone (i.e., no concurrent TMZ) were eligible. Any number of prior relapses was allowed. For part 2, only patients with stable disease after radiotherapy were eligible and the only prior therapies permitted were concomitant TMZ with radiotherapy or radiotherapy alone. Central pathology review was conducted by K. Aldape.

### Study design

The primary objectives of the study were to define the maximum tolerated dose (MTD) and to characterize the safety profile of vorinostat in combination with TMZ in patients with HGG. Secondary objectives included pharmacokinetic analysis of vorinostat in combination with TMZ. In part 1 of this study, patients received TMZ 150 mg/m<sup>2</sup>/day on days 1 to 5 of every 28 days in addition to variable doses of vorinostat administered either on days 1 to 14 of every 28 days (group A) or on days 1 to 7 and 15 to 21 of every 28 days (group B; Table 2). The TMZ dose was not escalated during part 1 for safety. In part 2 of the study, all patients received TMZ 150 mg/m<sup>2</sup>/day on days 1 to 5 of the first 28-day cycle, followed by dose escalation to 200 mg/m<sup>2</sup>/day on days 1 to 5 of subsequent 28-day cycles. Variable doses of vorinostat were administered in part 2 on days 1 to 7 and 15 to 21 every 28 days (Table 2). Patients in both parts 1 and 2 of the study were treated for a maximum of 13 cycles of TMZ in combination with vorinostat but had the option to continue vorinostat monotherapy at the discretion of the treating physician. Vorinostat was supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute (Bethesda, MD) under a Cooperative Research and Development Agreement with Merck Sharp and Dohme.

### Safety assessment

Dose limiting toxicities (DLT) based on Common Terminology Criteria for Adverse Events (CTCAE), version 3, included grade 3 or higher thrombocytopenia, grade 4 anemia, grade 4 neutropenia, any nonhematologic grade 3 or higher toxicity (except alopecia and grade 3 deep

venous thrombosis). In addition, grade 3 or 4 nausea, vomiting, or diarrhea not be controlled by medical therapy were considered DLTs.

A standard 3 + 3 dose-escalation design was used for both parts of the study and MTD was defined as the dose at which fewer than one-third of patients experienced a DLT to vorinostat in combination with TMZ. In part 1, the MTD was based on assessment of DLT during the first 28 days of treatment. After determining the MTD in part 1, part 2 opened. The MTD in part 2 was based on assessment of DLT in the first 56 days of treatment. In both parts 1 and 2, additional patients (to a total of 12 patients in each part) were enrolled at the MTD to further characterize the toxicity profile.

### Pharmacokinetic evaluation

**Sample collection.** In cycle 1, blood (6 mL) was collected from a peripheral vein into anticoagulant-free (vorinostat) or heparinized (TMZ) tubes before treatment and 1, 2, 3, 4, 6, and 8 hours after the first dose on day 1. Blood samples were also drawn before the first dose on day 2 and day 8. In part 2 of the study, samples were also collected in cycle 2 using a similar schedule. Blood collected for determination of vorinostat was allowed to clot at 4°C for 20 to 30 minutes. Blood collected for determination of TMZ was stabilized with 1.0 N HCl. Blood was separated by centrifugation into serum (vorinostat) or plasma (TMZ), transferred into plastic cryotubes, and stored at -70°C until analysis.

**Temozolomide assay methodology.** TMZ was assayed using high-performance liquid chromatography with UV detection as previously described (11).

**Vorinostat assay methodology.** Vorinostat concentrations were measured by a validated liquid chromatography–electrospray ionization tandem mass spectrometry method as described by Galanis and colleagues (12).

### Correlative studies

Blood samples for acetylation status of histones in peripheral blood mononuclear cells (PBMC) were collected before the initial dose of vorinostat on cycle 1 and subsequently at 1, 2, 6, 8, and 24 hours after administration of the first dose of vorinostat on day 1 of cycle 1; the 24-hour sample was drawn immediately before the first dose of day 2. Additional samples were drawn immediately before (baseline) and within 4 hours after administration of vorinostat on day 1 of cycle 2. Samples were collected using BD Vacutainer CPT cell preparation tubes with sodium citrate (Cat # 362761, Beckton Dickinson) at each participating institution. The samples were centrifuged according to manufacturer's specified protocol and the tubes shipped at ambient temperature to the laboratory of Vinay Puduvalli at MD Anderson Cancer Center (Houston, TX).

After partially removing the plasma, the mononuclear cell layer was collected with a Pasteur Pipette and transferred to a 15 mL size conical centrifuge tube as per manufacturer's specified method. The cells were then washed in PBS by tube inversion, centrifuged at 300 RCF for 15 minutes, and the

supernatant aspirated. The cell pellet was resuspended, the cells subsequently lysed, and protein extracted. Changes in levels of acetylated histone H3 and H4 in various samples were examined by Western blot analysis as previously reported (13).

### Statistical analysis

Baseline characteristics were summarized across all enrolled patients. Safety variables were summarized by descriptive statistics. Adverse events were described in terms of incidence and severity. Vorinostat and TMZ concentrations and pharmacokinetic parameters are presented in tabular and graphic form. Vorinostat, VA, and VG serum concentration data were analyzed by standard noncompartmental methods using the Program WinNonlin Professional, version 4.1 (Pharsight Corporation). In addition, summary tables depicting individual patient concentrations and individual and mean pharmacokinetic parameters are provided. Normalized OD values of histone H3 and H4 were plotted as a vertical scatter plot and the differences in the means of the values were compared for significance using a 2-way ANOVA (for cycle 1 samples) or a paired *t* test (for cycle 2 and for cycle 1 baseline vs. 24-hour sample comparison).

## Results

### Patient characteristics

Fifty-nine eligible patients were enrolled (Table 1). Pathology included 39 GBM, 2 gliosarcoma, 13 AA, 1 AO, and 4 AOA. Median age was 51 (range 25–81) and median KPS was 90 (range 60–100). Thirty-four were male and 25 were female.

### MTD and safety

The MTD of vorinostat on days 1 to 14 of every 28 days in combination with TMZ 150 mg/m<sup>2</sup>/day for 5 days every 28

**Table 1.** Patient characteristics

Characteristic	Median or No. (N = 59)	% or Range
Age	51	25–81
KPS	90	60–100
Race		
Caucasian	58	98
Unknown/not reported	1	2
Gender		
Male	34	58
Female	25	42
Diagnosis		
Glioblastoma	39	66
Gliosarcoma	2	3
Anaplastic Astrocytoma	13	22
Anaplastic Oligodendroglioma	1	2
Anaplastic Oligoastrocytoma	4	7

**Table 2.** Dose-escalation and dose-limiting toxicities

Dose Level	Vorinostat dose	TMZ dose	DLT
<b>Part 1A</b>			
Level 1 (starting dose)	200 mg BID on days 1–14 of every 28 days	150 mg/m <sup>2</sup> on days 1–5 of every 28 days	2/6 DLTs: grade 3 fatigue in 2 patients
Level 2	300 mg BID on days 1–14 of every 28 days	150 mg/m <sup>2</sup> on days 1–5 of every 28 days	2/3 DLT: 1 grade 3 thrombocytopenia, 1 grade 3 fatigue
Level 2a	200 mg TID on days 1–14 of every 28 days	150 mg/m <sup>2</sup> on days 1–5 of every 28 days	2/3 DLTs: 1 grade 3 nausea, 1 grade 4 thrombocytopenia
Level -1 (MTD)	300 mg QD on days 1–14 of every 28 days	150 mg/m <sup>2</sup> on days 1–5 of every 28 days	No DLTs were encountered (16 patients)
<b>Part 1B</b>			
Level 1 (starting dose)	400 mg QD on days 1–7 and 14–21 of every 28 days	150 mg/m <sup>2</sup> on days 1–5 of every 28 days	No DLTs were encountered (3 patients)
Level 2 (MTD)	500 mg QD on days 1–7 and 14–21 of every 28 days	150 mg/m <sup>2</sup> on days 1–5 of every 28 days	3/12 DLTs: grade 3 anorexia, grade 3 ALT, grade 4 thrombocytopenia + grade 5 hemorrhage
<b>Part 2</b>			
Level 1 (MTD)	400 mg QD on days 1–7 and 14–21 of every 28 days	150 mg/m <sup>2</sup> on days 1–5 of first 28 day cycle and 200 mg/m <sup>2</sup> on days 1–5 of subsequent 28-day cycle	No DLTs (6 patients)
Level 2	500 mg QD on days 1–7 and 14–21 of every 28 days	150 mg/m <sup>2</sup> on days 1–5 of first 28 day cycle and 200 mg/m <sup>2</sup> on days 1–5 of a subsequent 28-day cycle	4 patients treated but unable to escalate to 200 mg/m <sup>2</sup> for subsequent cycles mainly due to thrombocytopenia

days was 300 mg daily (group A). However, the exposure of vorinostat achieved with this dose was low based on pharmacokinetic analysis. To increase the exposure, a schedule of vorinostat administered on days 1 to 7 and 15 to 21 of every 28-day cycle in combination with TMZ was examined (group B). On this schedule, the MTD of vorinostat was 500 mg daily in combination with TMZ. In part 2, the MTD of vorinostat on days 1 to 7 and 15 to 21 of every 28-day cycle combined with TMZ 150 mg/m<sup>2</sup>/day on days 1 to 5 of the first cycle and 200 mg/m<sup>2</sup>/day on days 1 to 5 of subsequent cycles was 400 mg daily.

Table 2 summarizes the DLTs encountered at each dose level. In part 1B, DLTs included grade 3 anorexia, grade 3 ALT, and grade 5 hemorrhage in the setting of grade 4 thrombocytopenia (resulting in the only death on this study). In part 2, no DLTs were encountered at the MTD. Four patients were treated at one dose level above MTD, but none of the patients were able to dose escalate TMZ to 200 mg/m<sup>2</sup> due to thrombocytopenia.

Table 3 lists the grade 3 or higher adverse events related to combination therapy according to dose levels. The most common serious adverse events considered possibly, probably, or definitely related to vorinostat in combination with TMZ were fatigue (15%), lymphopenia (8%), thrombocytopenia (8%), and thromboembolic event (5%).

### Pharmacokinetics

Pharmacokinetic analysis of TMZ was conducted on 16 patients enrolled in the study (Supplementary Table S1). Peak plasma concentrations were achieved 1.95 ± 0.91 hours after the oral dose, and the mean TMZ half-life was 1.95 ± 1.95 hours. These pharmacokinetic findings are comparable with a comparison trial of single-agent TMZ in advanced cancers (14) suggesting no significant interaction with vorinostat.

Pharmacokinetic analysis of vorinostat and the vorinostat metabolites (VG and VA) was conducted on 56 patients enrolled in the study. Results for each vorinostat dose are summarized in Table 4. Peak plasma concentrations of vorinostat were achieved 2.2 ± 1.5 hours after the oral dose. The mean vorinostat half-life and oral clearance values for all patients were 2.1 ± 1.3 hours and 430 ± 150 L/hour, respectively. Peak plasma concentrations of VA and VG were achieved 3.3 and 2.5 hours, respectively, after the oral vorinostat dose. The mean VA and VG half-life values for all patients were 8.9 ± 6.4 hours and 2.1 ± 0.7 hours, respectively. While there was substantial variability in drug disposition at each dose level, C<sub>max</sub> and AUC values seemed to increase in proportion to dose level. Accumulation of vorinostat and its metabolites in serum was assessed following 7 days of treatment with vorinostat by comparing the day 8 pretreatment

**Table 3.** Grade 3 or 4 events with relationship possible or higher to combination therapy (Vorinostat + TMZ)

Dose levels	200 mg BID on days 1-14	200 mg TID on days 1-14	300 mg BID on days 1-14	300 mg QD on days 1-14	400 mg QD on days 1-7 and 15-21	500 mg QD on days 1-7 and 15-21	Total (%) n = 59
Abdominal pain		1			1		3%
ALT elevation						1	2%
Anemia			1				2%
Anorexia			1		1		3%
Constipation					1		2%
Creatinine elevation						1	2%
Fatigue	3	1	3	1		1	15%
Headache						1	2%
Hematoma						1	2%
Hyperglycemia					1		2%
Hyponatremia						1	2%
Intracranial hemorrhage						1	2%
Lymphopenia	1			2	1	1	8%
Nausea		1	1				3%
Neutropenia		1			1		3%
Peripheral sensory neuropathy						1	2%
Thrombocytopenia		1	1	1	1	1	8%
Thromboembolic event					1	2	5%
Vomiting		1	1				3%
Leukopenia					1	1	3%
Wound infection						1	2%

concentration values with the day 1- $C_{8h}$  value for twice a day (BID) and three times a day (TID) dosing or the day 1- $C_{24h}$  value for everyday dosing. No accumulation was observed for vorinostat and VG ( $R < 1$ ). Modest accumulation (BID or TID-  $R = 2.1$ ; QD- $R = 1.6$ ) of 4-anilino-4-oxobutanoic acid was consistent with the 8.9-hour half-life value.

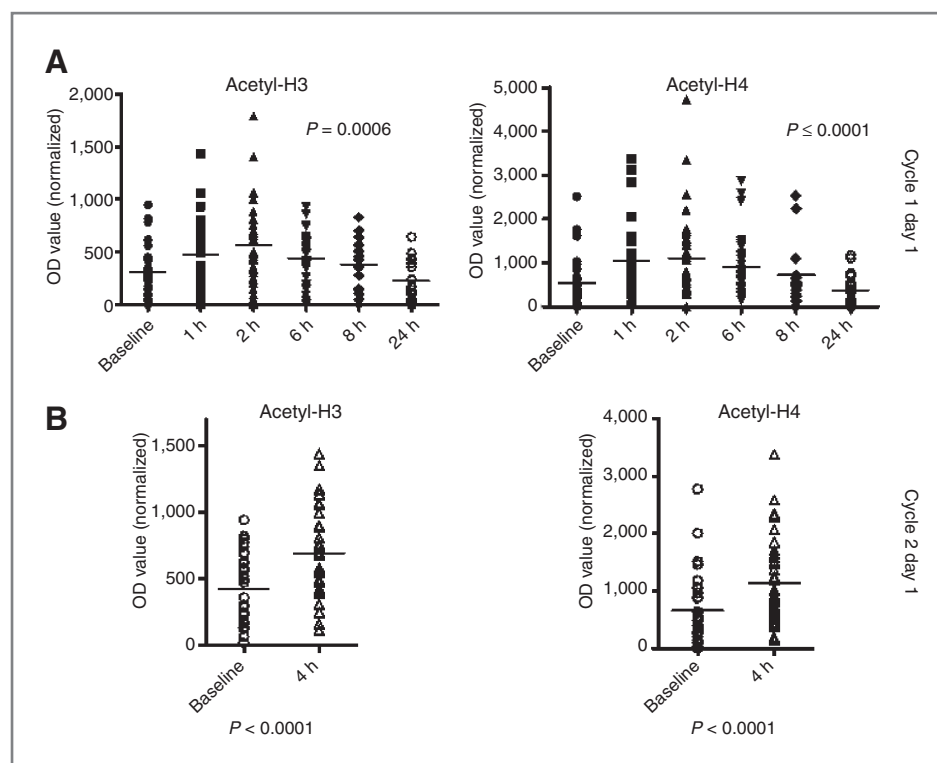
#### Correlative studies

Samples were available from 33 patients for assessment of histone H3 and H4 acetylation for the baseline and at least 3 consecutive posttreatment samples for each patient for cycle 1. Vorinostat treatment resulted in hyperacetylation of both proteins in these samples with the highest average levels being seen at the 2-hour time point as

**Table 4.** Pharmacokinetics of vorinostat and its metabolites

	Dose (mg)	200	300	400	500
	N	9	19	12	16
Vorinostat	$T_{max}$ (h)	2.2 ± 1.2	2.4 ± 1.6	2.4 ± 1.8	1.9 ± 1.3
	$C_{max}$ (ng/mL)	114 ± 56	239 ± 95	292 ± 186	327 ± 107
	$t_{1/2}$ (h)	1.96 ± 0.75	1.86 ± 1.40	1.98 ± 0.94	2.49 ± 1.75
	$AUC_{0-8h}$ (ng/mL·h)	388 ± 160	672 ± 209	933 ± 357	1,240 ± 300
	Cl/F (L/h)	518 ± 174	452 ± 155	417 ± 145	351 ± 94
SAHA-acid	$T_{max}$ (h)	2.9 ± 1.2	2.9 ± 1.9	3.6 ± 1.6	3.8 ± 1.7
	$C_{max}$ (ng/mL)	401 ± 160	788 ± 260	803 ± 454	1,060 ± 420
	$t_{1/2}$ (h)	7.4 ± 9.2	9.4 ± 8.2	9.9 ± 4.4	8.4 ± 2.1
SAHA-glucur-onide	$AUC_{0-8h}$ (ng/mL·h)	1,850 ± 760	3,360 ± 1,120	3360 ± 1280	5,500 ± 2,110
	$T_{max}$ (h)	2.5 ± 1.2	2.5 ± 1.7	2.7 ± 2.0	2.4 ± 1.3
	$C_{max}$ (ng/mL)	933 ± 340	1,690 ± 690	1760 ± 520	2,380 ± 1,410
	$t_{1/2}$ (h)	1.9 ± 0.6	1.7 ± 0.7	2.3 ± 0.7	2.5 ± 0.5
	$AUC_{0-8h}$ (ng/mL·h)	3,360 ± 1,440	5,160 ± 1,640	6340 ± 1400	10,110 ± 6,040

**Figure 1.** A, levels of acetylation of histone H3 ( $N = 33$ ) and H4 ( $N = 37$ ) in PBMCs isolated from patients at baseline and at various time points indicated after cycle 1 dose 1 of vorinostat as determined by Western blot analysis. Differences in means of acetylation levels at various time points were assessed for statistical significance. B, levels of histone H3 ( $N = 30$ ) and H4 ( $N = 34$ ) acetylation in cycle 2 pre- and postdose samples were assessed for treatment-related changes.



shown in Fig. 1A although the highest values in the individual patients occurred at either the 1-hour or 2-hour time point. Of note, there was an apparent decrease in mean of acetylation levels at 24 hours compared with the baseline levels but this was not statistically significant ( $P = 0.35$ ). A similar appearance of histone hyperacetylation was seen during cycle 2 with the posttreatment levels being significantly higher than pretreatment baseline. These findings are consistent with the expected effects of vorinostat in PBMC.

### Clinical outcomes

The median number of cycles of combination therapy was 6 (Table 5), with 2 patients (5%) remaining on study on vorinostat monotherapy as of February 1, 2012, more than one and a half years since initiating treatment on protocol. Of the 57 patients who have stopped treatment on protocol, 29 patients (51%) experienced disease progression and 18 patients (32%) completed treatment per protocol criteria and decided not to continue on vorinostat monotherapy. The remainder stopped early due to adverse events.

### Conclusion

This is the first clinical study to evaluate the safety and tolerability of combining an HDAC inhibitor with TMZ in HGG. We initially tested vorinostat dosing on days 1 to 14 of every 28-day cycles in combination with TMZ. No pharmacokinetic interactions between TMZ and vorinostat were seen. However, the exposure of vorinostat at the MTD

achieved with this schedule was low based on pharmacokinetic analysis. To increase the exposure of vorinostat in combination with TMZ, a schedule of vorinostat administered on days 1 to 7 and 15 to 21 of every 28-day cycle in combination with TMZ was examined. We determined MTDs on the basis of this new dosing schedule.

**Table 5.** Clinical outcomes

	Median or No. (% or range) <b><math>N = 59</math> total patients on study</b>
Median no. of cycles	6 (1-NR <sup>a</sup> )
No. of patients off treatment	57 (97)
Reasons for stopping treatment	$N = 57$ who stopped treatment
Adverse event/side effects/ complications	6 (11)
Disease progression	29 (51)
Patient withdrawal or refusal after beginning protocol therapy	3 (5)
Treatment completed per protocol criteria	18 (32)
Death on study	1 (2)

<sup>a</sup>NR = not reached; 2 patients remain on vorinostat monotherapy after completing treatment per protocol criteria

Hyperacetylation of histones H3 and H4 is the primary effect of vorinostat's HDAC-inhibitory activity. However, assessment of these changes in human glioma tissue is challenging. We showed the feasibility of assessing hyperacetylation in PBMCs as a potential surrogate of tumor tissue. Vorinostat treatment caused histone hyperacetylation, which had its highest average at approximately 2 hours from the initial dose of the agent subsiding to baseline levels over 8 hours later. Given that vorinostat is known to cross the blood–brain barrier, it is possible that a similar duration of effect may be seen in glioma tissue as well. Such assessment may be potentially useful in future studies to determine if the changes correlate with clinical outcome thus providing surrogate markers of clinical benefit.

The combination of vorinostat and TMZ was well tolerated by many patients. The median number of cycles of combination therapy was 6, suggesting that the regimen can be administered for an extended period with appropriate management of side effects. On the basis of the information obtained from this phase I clinical trial, the Alliance in Clinical Trials in Oncology Cooperative Group (ACTION) and the Adult Brain Tumor Consortium (ABTC) are currently conducting an intergroup phase II trial of vorinostat with radiotherapy and concomitant TMZ in newly diagnosed GBM.

#### Disclosure of Potential Conflicts of Interest

E.Q. Lee is on advisory board for Novartis and has royalties from UpToDate and DEMOS Publishers. Vinay K. Puduvalli has a research grant support from Merck, Genentech, and Celgene and is on advisory board for Novartis. Joel M. Reid has a funding from Merck for PK analysis. T.F. Cloughesy is a consultant/advisory member of Merck, Roche, Merck Sorano, Celgene, and Novartis, has honoraria from speakers bureau from Merck, and honoraria from ad board from Genentech and Roche. Susan M. Chang has a research support from Schering. Mark R. Gilbert is on advisory boards of Merck, Genentech and Abbott and has honoraria from Merck, Genentech and Abbott. H. Ian Robins is a consultant/advisory board member of Genentech and Abbott. Andrew B. Lassman is a consultant/advisory board member, is a Speaker, and has research funding from Abbott, Campus Bio, Eisai, Genentech, GSK, Merck/Schering-Plough, Novartis, Kyowa Hakko Kirin Pharma, Keryx, Sigma Tau. R.M. McGovern and Matthew M. Ames

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