**Purpose:** The primary purpose of this trial was to define and describe the toxicities of oral valproic acid (VPA) at doses required to maintain trough concentrations of 100 to 150 mcg/mL or 150 to 200 mcg/mL in children with refractory solid or central nervous system (CNS) tumors. Secondary objectives included assessment of free and total VPA pharmacokinetics (PKs) and histone acetylation in peripheral blood mononuclear cells (PBMC) at steady state.

**Patients and Methods:** Oral VPA, initially administered twice daily and subsequently three times daily, was continued without interruption to maintain trough concentrations of 100 to 150 mcg/mL. First-dose and steady-state PKs were studied. Histone H3 and H4 acetylation in PBMCs was evaluated using an ELISA technique.

**Results:** Twenty-six children, sixteen of whom were evaluable for toxicity, were enrolled. Dose-limiting somnolence and intratumoral hemorrhage were associated with VPA troughs of 100 to 150 mcg/mL. Therefore, the final cohort of six children received VPA to maintain troughs of 75 to 100 mcg/mL and did not experience any dose-limiting toxicity. First-dose and steady-state VPA PK parameters were similar to values previously reported in children with seizures. Increased PBMC histone acetylation was documented in 50% of patients studied. One confirmed partial response (glioblastoma multiforme) and one minor response (brainstem glioma) were observed.

**Conclusions:** VPA administered three times daily to maintain trough concentrations of 75 to 100 mcg/mL was well tolerated in children with refractory solid or CNS tumors. Histone hyperacetylation in PBMCs was observed in half of the patients at steady state. Future trials combining VPA with chemotherapy and/or radiation therapy should be considered, especially for CNS tumors.

**Introduction**

Chromatin remodeling is critical for epigenetic regulation of gene expression. Acetylation of histones’ amino-terminal tails by histone acetyltransferase relaxes the chromatin for transcription (1), and removal of acetyl groups by histone deacetylase (HDAC) compacts the chromatin and represses transcription (2). Histone hypoacetylation and inappropriate transcriptional repression are hypothesized to be key contributors to the development of human cancers (3, 4), and global hypoacetylation of histone H4 has been identified as an abnormal epigenetic signature common to many malignancies (5). HDAC inhibitors (HDACi), which are hypothesized to reactivate expression of critical genes aberrantly silenced during tumorigenesis, have been shown to cause pleiotropic effects on human cancer cells, including apoptosis, cell cycle arrests, and differentiation (3, 4, 6).

Valproic acid (VPA), an anticonvulsant widely used in children for over 30 years (7), was recently discovered to be an HDACi (8, 9) that inhibits growth of human cancers (9–12), including pediatric solid tumors (13–15). Functional studies (8, 9, 11, 16–18) have confirmed that HDAC inhibition and histone hyperacetylation are essential for VPA’s antitumor activity. Several phase 1 and 2 studies of VPA in adults with hematologic (19–22) and solid malignancies (23–29) showed that VPA treatment, either as a monotherapy or combined with other agents, was reasonably well tolerated and resulted in some encouraging tumor responses. Most recently, a clinical trial combining VPA, radiation, and chemotherapy for children with high-grade gliomas was reported (30). In this trial, children with high-grade gliomas received radiation and 6 cycles of chemotherapy, followed by maintenance VPA (trough concentrations of 100–150 mcg/mL) while those whose tumors progressed prior to completing radiation and/or chemotherapy received VPA monotherapy as a salvage therapy.
We report the results of a Children’s Oncology Group Phase I Consortium trial of VPA in children with recurrent or refractory solid tumors, including brain tumors. The primary objective was to define the toxicities of administering oral VPA daily without interruption to maintain trough plasma concentrations of 100 to 150 mcg/mL, and escalate to 150 to 200 mcg/mL in a subsequent cohort if well tolerated. Secondary objectives were to characterize free and total VPA pharmacokinetics (PKs), measure steady-state histone acetylation in peripheral blood mononuclear cell (PBMC), correlate histone acetylation with total or free VPA concentrations, and preliminarily evaluate the antitumor activity of VPA.

**Patients and Methods**

**Patient eligibility**

Eligible patients included children age 2 to 21 years with measurable or evaluable solid tumors [including central nervous system (CNS) tumors] that were recurrent or refractory to standard therapy. Histologic verification of malignancy at initial diagnosis or subsequent relapse was required except for patients with intrinsic brainstem or optic pathway gliomas. Other eligibility criteria included: a Lansky or Karnofsky performance score of 50 or higher, recovery from acute toxicities of prior therapies, adequate bone marrow functions (peripheral absolute neutrophil count ≥1,000/µL, platelet count ≥100,000/µL [transfusion independent], and hemoglobin ≥8 g/dL), adequate renal function (age adjusted normal serum creatinine or a creatinine clearance or glomerular filtration rate 70 mL/min/1.73 m² or higher), and adequate liver function (total bilirubin less than 1.5 times the institutional upper limit of normal; ALT ≤110 unit/L, and albumin ≥2 gm/dL).

The protocol was approved by the Institutional Review Boards at participating institutions. Informed consent and assent, as appropriate, were obtained according to local institutional guidelines.

**Drug administration and study design**

Administration of VPA as a syrup only (250 mg/5 mL) was strongly encouraged in all patients to ensure exact dosing, but a combination of capsules (250 mg) and syrup was allowed if requested by patient/family. The starting dose was 25 mg/kg/d divided b.i.d., with weekly dose escalation by 10 mg/kg until trough concentrations between 100 and 150 mcg/mL were maintained for 4 consecutive weeks, which defined the end of the first cycle. Each subsequent cycle was defined as 28 days, with VPA trough monitored at the start of each cycle and weekly dose adjustments by 10 mg/kg as needed to maintain target concentration within 15% of the upper or lower bound. VPA was to be continued daily without interruption in the absence of toxicity.

Six toxicity evaluable patients were to be enrolled into Cohort 1 (target VPA trough total concentration of 100–150 mcg/mL) and subsequently Cohort 2 (target VPA trough total concentration of 150–200 mcg/mL) if VPA was well tolerated. Enrollment for either Cohort 1 or 2 was to be terminated if 2 or more patients experienced DLTs, and subsequent patients were to be enrolled into Cohort 0 (target VPA trough total concentration of 75–100 mcg/mL). Enrollment for the entire study was to be terminated if 2 or more patients in Cohort 0 experienced DLTs or when 6 patients have been enrolled.

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (version 3.0). Hematologic DLT was defined as any grade-4 thrombocytopenia or neutropenia. Nonhematologic DLT was defined as any grade-4 toxicity, any grade-2 CNS hemorrhage, or any grade-3 toxicity, with specific exclusions of nausea and vomiting of <5 days duration. Asymptomatic elevation of transaminases that returned to levels meeting initial eligibility criteria within 7 days of VPA interruption, and fever or infection <5 days duration. Any grade-2 nonhematologic toxicity that persisted for ≥7 days and was considered sufficiently medically significant or intolerable by patients requiring treatment interruption was also considered dose-limiting. Patients were considered fully evaluable for toxicity after achieving and maintaining targeted VPA concentrations for 4 consecutive weeks.

Weekly trough VPA concentration (total and free), CBC, and liver function tests were obtained during the first cycle, and a history, physical examination, CBC, trough VPA concentration (total and free), liver function tests, renal function tests, electrolytes, and serum albumin were obtained prior to the start of each subsequent cycle. Disease evaluations were obtained at the end of cycle 1 and then after every 2 cycles, thereafter. Tumor response for solid tumors, excluding CNS tumors, was reported using the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0. Tumor response for CNS tumors was reported using WHO bidimensional criteria (product of the greatest tumor diameter and its perpendicular diameter). A maximum...
of 12 cycles of VPA was allowed in the absence of DLT and disease progression.

**Pharmacokinetic studies**

Optional PK studies were performed in consenting patients on day 1, cycle 1 and again during cycle 1 after the targeted VPA concentration had been maintained for 2 consecutive weeks (steady state). Samples were obtained at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after the initial dose, and at −0.5, 0.5, 1, 2, 4, 6, and 8 hours after the steady-state dose. The fluorescent polarization immunoassay technique (31) using the AxSYM or TDx immuno-analyzer from Abbot Laboratory was used to determine VPA concentrations. The known interday coefficient of variation was <5% for the referenced assay. Samples were filtered through a Millipore Centrifree Ultrafiltration Device by spinning at 1,000 to 2,000 × g using a fixed angle centrifuge for determination of free VPA concentrations.

Noncompartmental methods were used to calculate the area under the concentration–time curve (AUC), apparent total body clearance (CL/F), and apparent steady-state volume of distribution (V/F) (F denoting the fraction of bioavailability).

**PBMC histone h3 and h4 acetylation**

Blood samples were drawn prior to starting VPA and at the time of determining steady-state trough VPA concentrations. PBMCs were isolated using Lymphoprep solution (Axis-Shield). Protein lysates were prepared, and histone H3 and H4 acetylation was quantified using PathScan Acetylated Histone H3 and H4 Sandwich ELISA Kit (Cell Signaling). In brief, cell lysates were mixed with sample diluents and incubated at 4°C overnight, followed by sequential incubations with detection antibody, horseradish peroxidase-linked secondary antibody, and 3,3′, 5,5′-tetramethylbenzidine substrate at 37°C. Optical density (O.D.) at 450 nm was determined with a plate reader. Samples from a medulloblastoma cell line D283 treated with 162 mcg/mL of VPA for 24 hours were included as positive controls (32).

**Results**

Twenty-six patients were enrolled, of whom 26 were eligible and 16 were fully evaluable for toxicity. Reasons that patients were ineligible for toxicity included: unable to reach targeted VPA concentration (n = 2), rapidly progressive disease (n = 2), consented but never started therapy (n = 1), withdrawal (n = 3), toxicities unlikely to be related to VPA (n = 1), and VPA concentration exceeding the targeted range (n = 1). The first 2 patients in Cohort 1 failed to reach the targeted VPA concentration range after 6 dose escalations and were removed from study per initial design. As a result, the study was amended to allow continued dose escalation in the absence of DLT. However, because several patients in Cohort 1 required more than 5 (mean 3.8 ± 2.6) dose escalations to achieve VPA concentrations of 100 to 150 mcg/mL, VPA administration was increased from b.i.d. to t.i.d. in an attempt to attain the targeted VPA more promptly. Subsequently, the starting dose was decreased from 25 to 15 mg/kg/d because the first 2 patients receiving t.i.d. dosing experienced dose-limiting somnolence, with 1 patient unexpectedly achieving a trough of 172 mcg/mL after 3 days of VPA. No further amendment was required with a starting dose of 15 mg/kg/d divided t.i.d.

Table 1 summarizes the characteristics of the patients. The median number of administered VPA cycles was 1 (range 1–7).

**Toxicity**

Table 2 summarizes the DLTs in patients enrolled on this trial. One out of 6 evaluable patients in Cohort 1 (VPA troughs 100–150 mcg/mL; b.i.d. dosing) experienced grade-3 somnolence. After the VPA dosing frequency was changed from b.i.d. to t.i.d. and additional patients were enrolled into Cohort 1 to target VPA troughs of 100 to 150 mcg/mL, 2 out of the next 4 patients experienced DLTs (grade-3 somnolence and grade-5 hemorrhage/hemothorax). The patient with the hemothorax had metastatic synovial sarcoma to the lung and was believed to have had an intratumoral hemorrhage. Six subsequent patients
were enrolled into Cohort 0 (VPA troughs 75–100 mcg/mL) as per study design and did not experience any DLT. Enrollment into Cohort 2 (VPA troughs 150–200 mcg/mL) was not pursued because VPA trough concentrations of 100 to 150 mcg/mL were not well tolerated.

Table 3 summarizes non–dose-limiting hematologic and nonhematologic toxicities that were observed in more than 10% of patients. Mild thrombocytopenia was common, but none of the patients required dose reductions or transfusions, except the aforementioned patient with a hemothorax. Anemia was typically mild except for a grade-4 anemia that occurred in the patient with a hemothorax. Study drug interruptions or dose adjustments were not needed for any of the nonhematologic toxicities.

**Tumor response**

One confirmed partial response (PR) was observed in a patient with a biopsy confirmed thalamic GBM who was treated on Cohort 0 (VPA troughs 75–100 mcg/mL) for 7 months. One confirmed minor response (MR, 46% reduction in bidimensional measurements) was observed in another patient with a brainstem glioma who was treated on Cohort 1 (VPA troughs 100–150 mcg/mL) for 5 months. Both responses were confirmed by central review. A third patient with a suprasellar desmoplastic infantile

### Table 2. Summary of dose limiting toxicity (cycle 1)

<table>
<thead>
<tr>
<th>Cohort 1 (trough VPA level 100–150 mcg/mL), b.i.d. dosing</th>
<th>Number of patients enrolled</th>
<th>Number of evaluable patients</th>
<th>Number of patients with DLTs</th>
<th>Description of DLTs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>6</td>
<td>1</td>
<td>Somnolence (1)</td>
</tr>
<tr>
<td>Cohort 1 (trough VPA level 100–150 mcg/mL), t.i.d. dosing</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>Somnolence (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pulmonary hemorrhage (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dyspnea (1)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>Hypoxia (1)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>Hypotension (1)</td>
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<tr>
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<td></td>
<td></td>
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<td>Hypocalcemia (1)</td>
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<td>6</td>
<td>6</td>
<td>0</td>
<td>None</td>
</tr>
</tbody>
</table>

### Table 3. Summary of nondose limiting toxicities observed in 16 evaluable patients

<table>
<thead>
<tr>
<th>Cycle 1 (16 total cycle)</th>
<th>Cycle 2 to 7 (15 total cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum grade across cycle 1</td>
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<tr>
<td></td>
<td>Grade 1</td>
</tr>
<tr>
<td>Hematologic toxicity</td>
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</tr>
<tr>
<td>Anemia</td>
<td>3</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>5</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>4</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>6</td>
</tr>
<tr>
<td>Nonhematologic toxicity*</td>
<td></td>
</tr>
<tr>
<td>Somnolence</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>3</td>
</tr>
<tr>
<td>Nausea</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2</td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td>1</td>
</tr>
<tr>
<td>Dizziness</td>
<td>4</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
</tr>
</tbody>
</table>

*Only nonhematologic toxicities related to protocol therapy occurring in more than 10% of patients during the first cycle of VPA are listed.
than 20 mg/kg/d (Fig. 1A). Concentrations, and their mean final dose of VPA was less required 1 or 2 dose escalations to achieve targeted VPA VPA t.i.d. and at a lower starting dose of 15 mg/kg/d only mean final dose exceeded 50 mg/kg/d. Patients receiving VPA trough concentrations above 100 mcg/mL, and their divided b.i.d. required several dose escalations to achieve patients receiving VPA at a starting dose of 25 mg/kg/d dose and steady-state total VPA concentration (Fig. 1A).

6.5/C6 than total VPA clearance. The steady-state half-life (median 43.9 mL/kg/h, and 0.12 52.2 11.4 15.8%, mean ± SD; 27.7 and 41.3 12.0 11.4 0.157 0.088, respectively. The baseline variability (CV%) for AcH3 and AcH4 measurements were 3.4 14.9 ± 10.5%, respectively. Given the 16.8 6.9%, mean ± SD; 32 measurements) and increased minimally at total VPA concentrations between 100 and 125 mcg/mL (21.3 ± 11.5%, mean ± SD; 17 measurements). The percentage of free VPA increased above 40% (44.9 ± 15.8%, mean ± SD; 8 measurements) when total VPA concentration exceeded 125 mcg/mL (Fig. 1B).

The VPA formulation did not appear to affect the steady-state VPA dose. In patients maintaining VPA concentrations between 100 and 150 mcg/mL, the mean steady-state VPA dose in patients taking syrup only (n = 10) versus syrup and capsules (n = 8) was 36.0 ± 27.7 and 41.3 ± 20.7 mg/kg (P = 0.66 by least-squared means), respectively. Similar, for patients maintaining VPA concentration between 75 and 100 mcg/mL, the mean steady-state VPA doses in patients taking syrup only (n = 4) versus syrup and capsules (n = 2) were 17.5 ± 2.9 mg/kg and 12.5 ± 3.5 mg/kg (P = 0.13), respectively. There were an insufficient number of patients to determine whether VPA formulations had a statistically significant impact on the observed PK parameters or the number of dose escalations required to reach targeted VPA concentrations.

**Pharmacokinetic studies**

Of the 25 patients who received protocol therapy, 14 received VPA syrup only, 1 received capsules only, and 10 received both syrup and capsules. Pharmacokinetic studies were obtained in patients after the first dose of VPA, and the observed median half-life, clearance, and volume of distribution of total VPA were 11.0 ± 2.7 hours, 12.0 ± 5.7 mL/kg/h, and 0.12 ± 0.04 L/kg (median ± SD), respectively. These parameters were consistent with values previously reported in children receiving VPA for seizures (33–35). Steady-state PK was also studied in patients. Steady-state total VPA clearance was 8.0 ± 2.4 mL/kg/h similar to first dose total VPA clearance. Steady-state half-life (median 11.4 ± 3.3 hours) of total VPA was essentially identical to that observed with first dose. First dose peak free VPA levels varied between 4 and 6 mcg/mL and occurred 2 to 4 hours after dosing. First-dose PK analysis could not be performed for free VPA because measured concentrations were very low (near the detection limit of 0.7 mcg/mL). Steady-state free VPA clearance, 52.2 ± 43.9 mL/kg/h (median ± SD), was greater than total VPA clearance. The steady-state half-life (median 6.5 ± 1.3 h) of free VPA was also shorter than that observed with total drug and was similar to what has been previously reported (36, 37). All PK results are summarized in Table 4.

There was no apparent direct correlation between VPA dose and steady-state total VPA concentration (Fig. 1A). Patients who received VPA at a starting dose of 25 mg/kg/d divided b.i.d. required several dose escalations to achieve VPA trough concentrations above 100 mcg/mL, and their mean final dose exceeded 50 mg/kg/d. Patients receiving VPA t.i.d. and at a lower starting dose of 15 mg/kg/d only required 1 or 2 dose escalations to achieve targeted VPA concentrations, and their mean final dose of VPA was less than 20 mg/kg/d (Fig. 1A).

At total VPA concentrations below 100 mcg/mL, free VPA concentrations were less than 20% of total concentrations (16.8 ± 6.9%, mean ± SD; 32 measurements) and increased minimally at total VPA concentrations between 100 and 125 mcg/mL (21.3 ± 11.5%, mean ± SD; 17 measurements). The percentage of free VPA increased above 40% (44.9 ± 15.8%, mean ± SD; 8 measurements) when total VPA concentration exceeded 125 mcg/mL (Fig. 1B).

The following samples were available for assessment of histone acetylation: 7 matching pre- and posttreatment samples; 3 posttreatment samples without matching pretreatment samples; and 9 pretreatment samples without posttreatment samples in patients who progressed or discontinued treatment prior to the end of cycle 1. The mean pretreatment AcH3 and AcH4 values (O.D. at 450 nm) were 0.157 ± 0.039 and 0.208 ± 0.088, respectively. The baseline variability (CV%) for AcH3 and AcH4 measurements were 3.4 ± 2.6%, and 14.9 ± 10.5%, respectively. Given the minimal variability in the pretreatment histone acetylation measurements, we therefore used the mean pretreatment AcH3 and AcH4 values to estimate the changes in AcH3 and AcH4 for the 3 posttreatment samples without matching pretreatment samples. Five patients had reduced posttreatment AcH3 and AcH4 levels in their PBMCs. Five patients showed increased AcH3 and AcH4 levels, which ranged from 18% to 1,100% (AcH3). The patient who received VPA for 7 months and achieved a PR had the most dramatic increase in AcH3 and AcH4 (Table 5).

**Table 4. First-dose and steady-state pharmacokinetics of total and free VPA**

<table>
<thead>
<tr>
<th></th>
<th>t1/2 (hours)</th>
<th>CL/F* (mL/kg/h)</th>
<th>Vd/F† (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First dose, Total VPA (n = 4)</td>
<td>11.0 ± 2.7</td>
<td>12.0 ± 5.7</td>
<td>0.12 ± 0.04</td>
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<tr>
<td>Steady State, Total VPA (n = 4)</td>
<td>11.4 ± 3.3</td>
<td>8.0 ± 2.4</td>
<td>ND‡</td>
</tr>
<tr>
<td>Steady State, Free VPA (n = 4)</td>
<td>6.5 ± 1.3</td>
<td>52.2 ± 43.9</td>
<td>ND‡</td>
</tr>
</tbody>
</table>

*CL/F is the apparent total body clearance.

†Vd/F is the apparent volume of distribution.

‡ND indicates not determined.

**PBMC histone h3 and h4 acetylation**

The following samples were available for assessment of histone acetylation: 7 matching pre- and posttreatment samples; 3 posttreatment samples without matching pretreatment samples; and 9 pretreatment samples without posttreatment samples in patients who progressed or discontinued treatment prior to the end of cycle 1. The mean pretreatment AcH3 and AcH4 values (O.D. at 450 nm) were 0.157 ± 0.039 and 0.208 ± 0.088, respectively. The baseline variability (CV%) for AcH3 and AcH4 measurements were 3.4 ± 2.6%, and 14.9 ± 10.5%, respectively. Given the minimal variability in the pretreatment histone acetylation measurements, we therefore used the mean pretreatment AcH3 and AcH4 values to estimate the changes in AcH3 and AcH4 for the 3 posttreatment samples without matching pretreatment samples. Five patients had reduced posttreatment AcH3 and AcH4 levels in their PBMCs. Five patients showed increased AcH3 and AcH4 levels, which ranged from 18% to 1,100% (AcH3). The patient who received VPA for 7 months and achieved a PR had the most dramatic increase in AcH3 and AcH4 (Table 5).
Discussion

VPA has preclinical activity against many human cancers, and several phase 1 and 2 trials of VPA-containing regimens in adults with hematologic malignancies have response rates of 10\% - 22\% (19, 21). In adults with solid tumors, stable disease has been observed after VPA monotherapy (24, 26, 28), and response rates of 22\% to 64\% have been seen when VPA was combined with chemotherapy (25, 29). VPA-associated antitumor activity has been reported in a child with a glioblastoma multiforme (GBM) who had a CR for 12 months (38) and in 3 additional children with GBMs who received VPA monotherapy for progressive GBM after radiation and chemotherapy (30).

Our study design was based on preclinical studies that suggested the threshold VPA concentration associated with antitumor activity exceeded 100 mcg/mL (10, 14, 32, 39, 40) and on the reports of children with GBM who responded to VPA monotherapy who had VPA concentrations between 100 and 160 mcg/mL (30, 38). The first challenge encountered on our trial, which targeted trough VPA concentrations of 100 to 150 mcg/mL, was the number of intrapatient dose escalations (mean ± SD 3.8 ± 3.6) required to achieve the targeted concentrations when the drug was administered on a b.i.d. schedule. The strategy to move to t.i.d. dosing, which could allow for a higher trough concentration without an overall increase in drug dose or exposure (AUC), appeared successful, with children requiring only 1.3 ± 0.5 dose escalations. Unfortunately, children with refractory cancer did not appear to tolerate drug exposures that maintained trough concentrations between 100 and 150 mcg/mL, as 2 patients developed dose limiting somnolence. Similarly, somnolence, confusion, and other

![Figure 1](image-url)
would have been observed had sampling occurred at the possible that more significant increases in AcH3/AcH4 trough VPA concentration falls below threshold levels (17), it is tone hyperacetylation declines within 1 to 2 hours after peak VPA-induced histone hyperacetylation after VPA treatment appears to bers, could not confirm this observation. However, our study, likely due to insufficient sample num-

CNS toxicities were the most common DLTs observed in adult patients (24–26, 28, 29). In this trial, objective antitumor activity was observed in 2 patients with gliomas, a sustained PR in a patient with a VPA trough concentration of 75–100 mcg/mL and a sustained MR in a patient with a VPA trough concentration of 100–150 mcg/mL. Whether a threshold trough concentration of 100 mcg/mL is required for VPA’s antitumor activity in humans will need to be further examined in future studies. It is possible that the threshold VPA trough concentration required for antitumor activity may be lower when used with adjuvant radiation or chemotherapy.

Pharmacokinetic parameters observed in our patients were similar to previously reported values, suggesting similar drug disposition in children with seizure disorders and cancer. As in adult trials (29, 41), there was no direct correlation between VPA dose and steady-state total VPA concentration (Fig. 1A). The fraction of free VPA remained less than 0.25 until total VPA concentrations exceeded 125 mcg/mL (Fig. 1B). This observation is consistent with results from other studies (29, 42). Recent clinical trials (27, 29) suggest a correlation between VPA concentration and increased histone acetylation in PBMC and tumors; however, our study, likely due to insufficient sample numbers, could not confirm this observation.

Preclinical studies (8, 9, 11, 16–18) have shown that histone hyperacetylation after VPA treatment appears to be critical for its anticaner effect. In our study, 5 out of 10 patients showed increased AcH3 and AcH4 at times of trough VPA drug exposure. Since peak VPA-induced histone hyperacetylation declines within 1 to 2 hours after VPA concentration falls below threshold levels (17), it is possible that more significant increases in AcH3/AcH4 would have been observed had sampling occurred at the time of peak VPA concentrations. A prior pediatric phase 1 study of HDACi, depsipeptide, found histone hyperacetylation in all patients at the time of maximal drug concentration (43). Similarly, in recent adult clinical trials (27, 29), in which PBMC histone acetylation was assessed 4 hours after a dose of VPA, increases in AcH3 and AcH4 were observed in all patients. In our study, 2 out of the 3 patients with antitumor activity showed increased AcH3/AcH4 levels (Table 5), with the highest histone hyperacetylation occurring in a child with a PR. Of note, this patient did not have a matching pretreatment PBMC sample, so the average of 16 pretreatment samples was used for analysis, potentially resulting in an overestimation of the increase in acetylation. However, as the mean pretreatment AcH3 and AcH4 values for the sixteen available samples showed minimal variability, these baseline biological parameters may be fairly uniform in untreated children.

Given that stable disease is the most common response observed with VPA monotherapy in solid tumors, combining this agent with chemotherapy maybe a viable future strategy. Preclinical studies show that VPA enhances cytotoxicity of multiple chemotherapeutic agents (12, 23, 44–46), and combinations of VPA and chemotherapy produced encouraging responses in solid tumor clinical trials (23, 25, 29). Another potentially promising combination, especially for CNS tumors, is VPA and radiation, as this drug inhibits double-stranded DNA repair and enhances malignant glioma’s response to radiation (47, 48). In an ongoing phase II trial of VPA and radiation, followed by maintenance VPA and bevacizumab in children with newly diagnosed high-grade or brainstem gliomas (NCT00879437), we have completed radiation and VPA in 7 children, maintaining VPA trough concentrations of 85 to 115 mcg/mL without encounterng neuro-toxicities or other dose-

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Post-VPA/Pre-VPA AcH3</th>
<th>Post-VPA/Pre-VPA AcH4</th>
<th>Total VPA (mcg/mL)</th>
<th>Free VPA (mcg/mL)</th>
<th>Clinical response</th>
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<td>19.1</td>
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<td>1.38</td>
<td>2.45</td>
<td>77.0</td>
<td>8.1</td>
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<tr>
<td>757318</td>
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<td>1.55</td>
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<td>36.6</td>
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<tr>
<td>734297</td>
<td>1.51</td>
<td>1.11</td>
<td>78.2</td>
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<td>PD</td>
</tr>
<tr>
<td>770059*</td>
<td>12.21*</td>
<td>14.69*</td>
<td>105.7</td>
<td>11</td>
<td>PR</td>
</tr>
</tbody>
</table>

NOTE: Changes in PBMC histone acetylation were determined by dividing posttreatment acetylated-H3 (AcH3) and H4 (AcH4) by pretreatment levels. In the 3 patients with only posttreatment PBMCs available, changes in histone acetylation were estimated by dividing posttreatment AcH3 and AcH4 by the mean AcH3 and AcH4 measurements from 16 pretreatment PBMC samples.

ND = not done; PD = progressive disease; PR = partial response; MR = minor response.
*Only post-VPA PBMCs available.
†Unconfirmed MR (see Subsection "Tumor Response" section under Section "Results").

Table 5. Changes in PBMC H3 and H4 acetylation
limiting toxicities, interruption of radiation, or VPA dose reduction.

In conclusion, VPA deserves continued investigation in pediatric oncology, especially in children with malignant gliomas and possibly other CNS tumors. As VPA has limited myelosuppression, combination with cytotoxic chemotherapy and/or radiation therapy appears feasible, and a number of clinical trials are pursuing such combination strategies (e.g., VPA with temozolomide and radiation therapy in adult brain tumors, NCT00302159; VPA with chemoradiotherapy for nonsmall cell lung cancer, NCT01203735). Our study suggests that a t.i.d. dosing schedule may shorten the time required to reach desired VPA trough concentrations. Close monitoring for somnolence and other CNS toxicities is imperative especially if targeting a VPA concentration above 100 mcg/mL. Whether a threshold trough VPA concentration above 100 mcg/mL for antitumor effect is required in all children remains to be determined. Defining optimal anticancer VPA concentrations and identifying correlative biological parameters to guide therapy and predict clinical responses re challenges for future studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank members of the study committee Junfeng Sun, Rebecca Holmes Johnson, and Renee Klenke for their contributions to the study protocol. We additionally thank Susan Milligan, Dori Triplett, and Tessa Chung for outstanding administrative and data management support throughout the development and conduct of this trial.

Grant Support

National Cancer Institute K12 Pediatric Oncology Clinical Research Training Program K12CA00343-01A1; K23 Career Development Award 1K23CA113721 (J.M. Su).
National Cancer Institute Grant U01 CA97452 and M01 RR00188.

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Received March 23, 2010; revised September 27, 2010; accepted October 28, 2010; published OnlineFirst November 29, 2010.

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