Received: 30 January 2020 | Revised: 2 March 2020 | Accepted: 9 March 2020

DOI: 10.1002/pbc.28283

Pediatric Blood & Cancer | SIOP International Society of Paediatric Oncology | aspho The American Society of Pediatric Hematology/Oncology | WILEY

ONCOLOGY: RESEARCH ARTICLE

A phase 2 study of valproic acid and radiation, followed by maintenance valproic acid and bevacizumab in children with newly diagnosed diffuse intrinsic pontine glioma or high-grade glioma

Jack Meng-Fen Su[1] | Jeffrey C. Murray[2] | Rene Y. McNall-Knapp[3] | Daniel C. Bowers[4] | Shafqat Shah[5] | Adekunle M. Adesina[6] | Arnold C. Paulino[7] | Eunji Jo[8] | Qianxing Mo[8] | Patricia A. Baxter[1] | Susan M. Blaney[1]

[1]Texas Children's Cancer Center, Baylor College of Medicine, Houston, Texas

[2]Cook Children's Medical Center, Fort Worth, Texas

[3]Department of Pediatrics, The University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma

[4]Children's Medical Center/The University of Texas Southwestern Medical Center, Dallas, Texas

[5]The University of Texas Health Science Center, Department of Pediatric Hematology-Oncology, San Antonio, Texas

[6]Texas Children's Hospital, Department of Pathology, Houston, Texas

[7]The University of Texas MD Anderson Cancer Center, Houston, Texas

[8]Dan L Duncan Cancer Center, Department of Medicine, Biostatistics and Bioinformatics, Houston, Texas

Correspondence
Jack Meng-Fen Su, Texas Children's Cancer Center, 6701 Fannin Street, CC1510, Houston, TX 77030.
Email: jmsu@txch.org

Funding information
Genentech

Abstract

Purpose: To study the efficacy and tolerability of valproic acid (VPA) and radiation, followed by VPA and bevacizumab in children with newly diagnosed diffuse intrinsic pontine glioma (DIPG) or high-grade glioma (HGG).

Methods: Children 3 to 21 years of age received radiation therapy and VPA at 15 mg/kg/day and dose adjusted to maintain a trough range of 85 to 115 μg/mL. VPA was continued post-radiation, and bevacizumab was started at 10 mg/kg intravenously biweekly, four weeks after completing radiation therapy.

Results: From September 2009 through August 2015, 20 DIPG and 18 HGG patients were enrolled (NCT00879437). During radiation and VPA, grade 3 or higher toxicities requiring discontinuation or modification of VPA dosing included grade 3 thrombocytopenia (1), grade 3 weight gain (1), and grade 3 pancreatitis (1). During VPA and bevacizumab, the most common grade 3 or higher toxicities were grade 3 neutropenia (3), grade 3 thrombocytopenia (3), grade 3 fatigue (3), and grade 3 hypertension (4). Two patients discontinued protocol therapy prior to disease progression (one grade 4 thrombosis and one grade 1 intratumoral hemorrhage). Median event-free survival (EFS) and overall survival (OS) for DIPG were 7.8 (95% CI 5.6-8.2) and 10.3 (7.4-13.4) months, and estimated one-year EFS was 12% (2%-31%). Median EFS and OS for HGG were 9.1 (6.4-11) and 12.1 (10-22.1) months, and estimated one-year EFS was 24% (7%-45%). Four patients with glioblastoma and mismatch-repair deficiency syndrome had EFS of 28.5, 16.7, 10.4, and 9 months.

Conclusion: Addition of VPA and bevacizumab to radiation was well tolerated but did not appear to improve EFS or OS in children with DIPG or HGG.

KEYWORDS

bevacizumab, children, diffuse intrinsic pontine glioma, high-grade glioma, valproic acid

Abbreviations: AA, anaplastic astrocytoma; COG, Children's Oncology Group; CR, complete response; CTCAE, Common Terminology Criteria for Adverse Events; DIPG, diffuse intrinsic pontine glioma; EFS, event-free survival; GBM, glioblastoma; HDAC, histone deacetylase; HGG, high-grade glioma; MMRD, mismatch-repair deficiency; MR, minor response; OS, overall survival; PBTC, Pediatric Brain Tumor Consortium; PD, progressive disease; PR, partial response; SD, stable disease; tid, three times a day; ULN, upper limit of normal; VPA, valproic acid.

1 | INTRODUCTION

Children with high-grade glioma (HGG) and diffuse intrinsic pontine glioma (DIPG) continue to have dismal prognosis, with no improvement in outcome over the last two decades. A recent pediatric national collaborative trial[1] showed a disappointing three-year event-free survival (EFS) of 7% ± 4% for glioblastoma (GBM), which was unimproved compared with the preceding trial.[2] Similarly, recent national collaborative trials in children with DIPG[3-5] also failed to improve survival compared with a historical trial.[6] These disappointing outcomes highlight the urgent need to identify novel therapeutic agents and strategies for children with HGG and DIPG.

Because radiation often induces a temporary response and symptomatic improvement for children with HGGs and DIPGs, concurrent administration of an agent with both an antiglioma and a radiosensitizing effect should theoretically improve outcome. Valproic acid (VPA), an anticonvulsant used in children for over 30 years, was discovered to inhibit histone deacetylase (HDAC).[7-8] HDAC inhibition by drugs such as VPA may reactivate critical pathways silenced during tumorigenesis and therefore inhibit tumor growth.[8-9] VPA has been shown to inhibit malignant glioma cell line growth *in vitro*[10-12] and to enhance radiation efficacy against malignant glioma.[13-15] In addition, a Children's Oncology Group (COG) phase I trial of single-agent VPA in children with recurrent solid tumors showed a partial response in a child with GBM and a minor response in a child with DIPG.[16]

Bevacizumab, a humanized monoclonal IgG antibody against the human vascular endothelial growth factor-A isoform, has been approved as a treatment, either as monotherapy or in combination with irinotecan, for adult GBM based on improved response rate and progression-free survival.[17-19] At the time of the conception of our clinical trial, bevacizumab was being studied in two randomized trials in adults with newly diagnosed GBM[20-21] and in a Pediatric Brain Tumor Consortium (PBTC) trial for recurrent CNS tumors, including HGG.[22]

We therefore initiated a multi-institution, phase 2 clinical trial of radiation and VPA, followed by maintenance VPA and bevacizumab, in children with newly diagnosed DIPG or HGG. The primary aims of this study were to determine the efficacy of this treatment strategy, as measured by one-year EFS for children with HGG or DIPG, and to determine the toxicities of VPA administered concurrently with radiation as well as for coadministration of VPA and bevacizumab in the post-radiation maintenance phase. Secondary aims were to determine the one-year overall survival (OS) for patients with HGG or DIPG and to assess tumor response.

2 | PATIENTS AND METHODS

2.1 | Patient eligibility

Children age 3 to 21 years, with histological confirmation of a HGG (glioblastoma multiforme [GBM], anaplastic astrocytoma [AA], or gliosarcoma) or a MRI evidence of a DIPG, defined as a tumor with a pontine epicenter and diffuse rather than focal involvement of the pons, were eligible. Patients with brainstem tumors who did not meet these radiographic criteria but with biopsy evidence of AA, GBM, or gliosarcoma were also eligible. Other eligibility criteria include no prior therapy with the exception of surgery and/or corticosteroids; a Karnofsky (age > 16 years) or Lansky (age ≤ 16 years) performance status of ≥ 50; absolute neutrophil count ≥ 1000/mm^3, platelets ≥ 100 000/mm^3, hemoglobin ≥ 8 g/dL; age-appropriate renal function; adequate hepatic function (albumin ≥ 2 g/dL, bilirubin ≤ 1.5× and SGPT/ ALT ≤ 2.5× institutional upper limit of normal [ULN] for age); normal amylase and lipase (< 2× ULN); normal coagulation parameters (PT and PTT < 1.2 × ULN, INR < 1.5). Patients with any intratumoral or intracranial hemorrhage, at diagnosis, after surgery, or before study entry, were eligible as long as they were asymptomatic and the widest diameter of any hemorrhage was < 1 cm on MRI ECHO gradient sequences. Specific exclusion criteria included prior or current treatment with VPA, pregnancy, cardiac disease, evidence of prior ischemia or infarction, coagulopathy/bleeding disorder, hypertension (SBP and/or DBP > 95 percentile for age and height), significant vascular or gastrointestinal disease, a known urea cycle disorder, metaphyseal growth plate abnormality, or anon-healing wound/ulcer. Children who required other anticonvulsants for seizures were excluded from study participation because of the known, but rare, risk of fatal hepatic toxicity, encephalopathy, and pancreatitis with concomitant administration of VPA and other anticonvulsants.[23-26]

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.

2.2 | Radiation therapy

Radiation therapy was to begin within 30 days of surgery or radiographic diagnosis, whichever occurred latest. Photon radiation was administered in 30 to 33 fractions over 6 to 7 weeks at a total dose of 54.0 Gy for completely resected HGG and brainstem gliomas and 59.4 Gy for incompletely resected non-brainstem HGGs. For primary spinal cord HGG, radiation was administered in 28 to 30 fractions over 5 to 6 weeks, based on the treating radiation oncologist's preference, to a total dose between 50.4 and 54 Gy.

2.3 | Valproic acid dosing and monitoring

VPA therapy was initiated a few days prior to or on the same day as radiation therapy. VPA, 15 mg/kg/day divided three times a day (tid) and escalated by 5 mg/kg/day every three to five days, was given with the goal of maintaining a trough concentration range of 85 to 115 μg/mL, as per the dosing strategy of the preceding COG phase 1 trial.[16] VPA was administered tid continuously throughout the entire duration of protocol therapy, for a maximum of 104 weeks (two years), in the absence of toxicities requiring interruption of therapy or disease progression. VPA concentrations were monitored every 4 weeks, following attainment of a VPA trough in the targeted range.

A history and physical examination, complete blood count, liver function tests, electrolyte, and renal function tests were

obtained weekly during concurrent radiation and VPA, and then every four weeks starting week 11 of protocol therapy.

2.4 | Bevacizumab dosing and monitoring

Bevacizumab was started 4 weeks after completion of XRT (approximately week 11), at 10 mg/kg/dose intravenously every 2 weeks. Bevacizumab was continued through 104 weeks of protocol therapy, in the absence of toxicities requiring interruption of therapy or disease progression.

Blood pressure, monitored every 2 weeks after initiation of bevacizumab therapy, was maintained below the 95 percentile for sex, age, and height for each patient. The urine protein to creatinine ratio was monitored every 4 weeks while on bevacizumab. Tibial radiographs were obtained prior to bevacizumab and every 24 weeks while receiving bevacizumab to monitor for metaphyseal plate dysplasia.

2.5 | Toxicity definition and dose modification for VPA

Toxicities were graded according to the NCI Common Terminology Criteria for Adverse Events v3.0 (CTCAE). During concurrent radiation therapy and VPA, VPA was withheld for grade 4 neutropenia, ≥ grade 3 thrombocytopenia, and ≥ grade 3 nonhematologic toxicities (except for nausea/vomiting or infection < 5 days in duration, transient transaminitis < 7-day duration, and electrolyte abnormalities responsive to oral supplementation). Upon improvement of toxicities to meet on-study criteria, VPA was restarted with a decrement of 5 mg/kg/day and to target a trough of 50 to 85 μg/mL. VPA during radiation therapy was to be discontinued permanently for interruption of radiation therapy for five consecutive days or 10 cumulative days or recurrence of a toxicity despite a VPA trough of 50 to 85 μg/mL. Radiation was continued without interruption, if deemed safe to proceed.

VPA treatment was reinitiated at week 11 of protocol therapy (initiation of bevacizumab) in those who required discontinuation of VPA during XRT, if the VPA-related toxicities improved to meet on-study criteria. VPA was restarted at 10 mg/kg/day divided tid to target a trough concentration range of 50 to 85 μg/mL.

VPA was permanently discontinued at any phase of protocol therapy if any of the following occurred: new or progressive CNS hemorrhage (except for punctate lesions without clinical symptoms); ≥ grade 2 CNS hemorrhage; ≥ grade 3 systemic bleeding or encephalopathy; evidence of bone marrow aplasia or myelodysplastic syndrome; need for anticoagulation; pregnancy, or symptomatic pancreatitis.

2.6 | Toxicity definition and dose modification for bevacizumab

Toxicities were graded according to the CTCAE v3.0. Bevacizumab was permanently discontinued in the event of any of the following toxicities: CNS or systemic hemorrhage, thrombosis/ischemia, significantly delayed wound healing, poorly controlled hypertension, metaphyseal dysplasia, severe proteinuria, or pregnancy.

2.7 | Disease monitoring

MRI for evaluation of disease was obtained at study entry, week 10, and then every 12 weeks afterward for the duration of protocol therapy. If patients had evidence of disease progression between weeks 10 and 34, protocol therapy could continue with repeat imaging in 8 weeks, due to the potential for radiation enhancement from VPA and resultant pseudoprogression. If the repeat study showed stable to improving disease, then disease evaluations were resumed at 12-week intervals.

2.8 | Definition of evaluable patients

All patients were considered evaluable for toxicity provided that they have received at least one day of radiation and VPA. Patients were considered evaluable for response and survival after they had achieved and maintained the targeted VPA trough of 85 to 115 μg/mL.

2.9 | Response evaluation

Tumor response was determined using WHO bidimensional criteria (product of the greatest tumor diameter and its perpendicular diameter), with progressive disease (PD) defined as a more than 25% increase in tumor size or the emergence of new lesions, stable disease (SD) as less than 25% increase in size, minor response (MR) as less than 50% reduction in tumor size, partial response (PR) as 51% to 99% reduction in tumor size, and complete response (CR) as complete disappearance of all measurable lesion(s). A response was considered sustained if observed on two consecutive MRIs.

2.10 | EFS and OS comparison with historical trials

Using a one-year EFS of 36% for GBM from the COG trial ACNS0126,[1] and 17% for DIPG from Children's Cancer Group trial, CCG-9941[6] as historical comparisons, the trial was designed to enroll 21 evaluable patients with HGG and 19 with DIPG to provide 80% power for detecting a 20% improvement in one-year EFS using the one-sample log-rank test.[27] The Kaplan-Meier method was used to estimate the one-year EFS and OS for each cohort with 95% confidence intervals. EFS was defined as the interval of time between study entry and documentation of disease progression (clinical or radiographic), secondary malignancy, death from other causes, or date of last follow-up. OS was defined as interval of time between diagnosis and death due to any cause, or date of last follow-up. All analyses were performed in SAS version 9.4 statistical software (SAS Institute Inc) and R (https://cran.r-project.org/). All reported P values are two-sided with $P < 0.05$ considered as statistically significant.

2.11 | Data safety monitoring committee

All adverse events, regardless of perceived relationship to study treatment, were reported to and reviewed by study chair (JMS) on a monthly basis. Any serious or immediately life-threatening adverse event was reported to study chair and the site's institutional review

TABLE 1 Characteristics of eligible and evaluable DIPG patients

Age (yrs)	Sex	Ethnicity	EFS (mos)	OS (mos)	Best response	Notable clinical information
10.6	M	Black	14.3	22.1	PR	Fragile-X syndrome
11.7	M	Caucasian	12.9	14.9	MR	
6.8	F	Hispanic	9.2	9.2	MR	
3.5	F	Hispanic	8.5	12.0	MR	
7.7	M	Hispanic	8.2	10.7	MR	
10.3	F	Caucasian	8.2	18.2	PR	
5	M	Hispanic	8.1	12.6	PR	
3.2	F	Hispanic	8.0	20.7	PR	
7.4	F	Caucasian	7.9	13.4	PR	
7.1	M	Caucasian	7.8	10.3	MR	
8.1	F	Caucasian	6.9	7.1	MR	Fanconi anemia
5.3	F	Caucasian	6.8	7.3	PR	
6.4	M	Caucasian	6.2	16.9	PR	
7.4	F	Mixed	6[a]	10.0	PR	[a] Parents refused bevacizumab after VPA + XRT
10.3	M	Caucasian	5.7	8.8	MR	
5	F	Hispanic	5.6	6.2	MR	Pseudoprogression at week 10
17.9	M	Hispanic	5.6[b]	8.8	MR	Pseudoprogression and radiation necrosis at week 10; cessation of protocol therapy after DVT and pulmonary emboli during week 23
3.4	M	American Indian	2.1	2.4	SD	Disease progression/death before receiving bevacizumab
7.2	M	Caucasian	1.4[c]	1.4	NA	Died before receiving bevacizumab
9.5	F	Black				Eligible but not evaluable; never achieved targeted VPA trough; died from tracheostomy complications

DIPG, diffuse intrinsic pontine glioma; EFS, event-free survival; F, female; M, male; mos, months; MR, minor response; NA, not available; OS, overall survival; PR, partial response; SD, stable disease; yrs, years.
[a] Parents declined bevacizumab therapy despite PR after radiation and valproic acid.
[b] Developed DVT and pulmonary embolism, necessitating cessation of protocol therapy.
[c] Patient died of respiratory depression while receiving radiation and valproic acid, and parents declined imaging study or autopsy.

board within two working days. The Dan L. Duncan Cancer Center Pediatric Safety Monitoring Committee at Baylor College of Medicine monitored the adverse events for this clinical trial.

3 | RESULTS

Between September 2009 and August 2015, a total of 38 eligible patients (20 DIPG and 18 HGG) were enrolled from five institutions (Texas Children's Hospital, Cook Children's Medical Center, University of Oklahoma Health Sciences Center, University of Texas Southwestern Medical Center, and University of Texas Health Science Center). Patient demographics, survival, and response data are detailed in Tables 1 (DIPG) and 2 (HGG). There were 20 males and 18 females, who had a median age of 7.9 years at the time of enrollment (range, 3.2-19.9 years).

3.1 | DIPG cohort

One patient was eligible but not evaluable as she deteriorated quickly after diagnosis, requiring intubation and ultimately a tracheostomy. She received two weeks of VPA and radiation, never achieved targeted VPA trough of 85 to 110 μg/mL, and died of a surgical complication unrelated to protocol therapy or disease. Three of the 19 remaining evaluable patients did not receive bevacizumab: one died of severe respiratory depression during week 6 of VPA and XRT and the family declined resuscitation, MRI imaging, or autopsy, so the exact cause of death was uncertain; one had disease progression before week 11; and parents of one patient declined bevacizumab or further protocol therapy, despite a PR after radiation and VPA.

The median EFS and OS for the DIPG cohort were 7.8 months (95% CI 5.6-8.2) and 10.3 months (95% CI 7.4-13.4; Figure 1A), respectively. The estimated one-year EFS was 12% (95% CI 2%-31%). Eight of 18 evaluable DIPG patients had sustained PRs beyond week 22 of protocol therapy, and two patients had pseudoprogression at week 10 with eventual demonstration of a sustained MR on subsequent MRIs.

3.2 | HGG cohort

Of the 18 patients with HGG, 12 had GBM and 6 had AA. There were seven hemispheric, four midline (two thalamic, two brainstem),

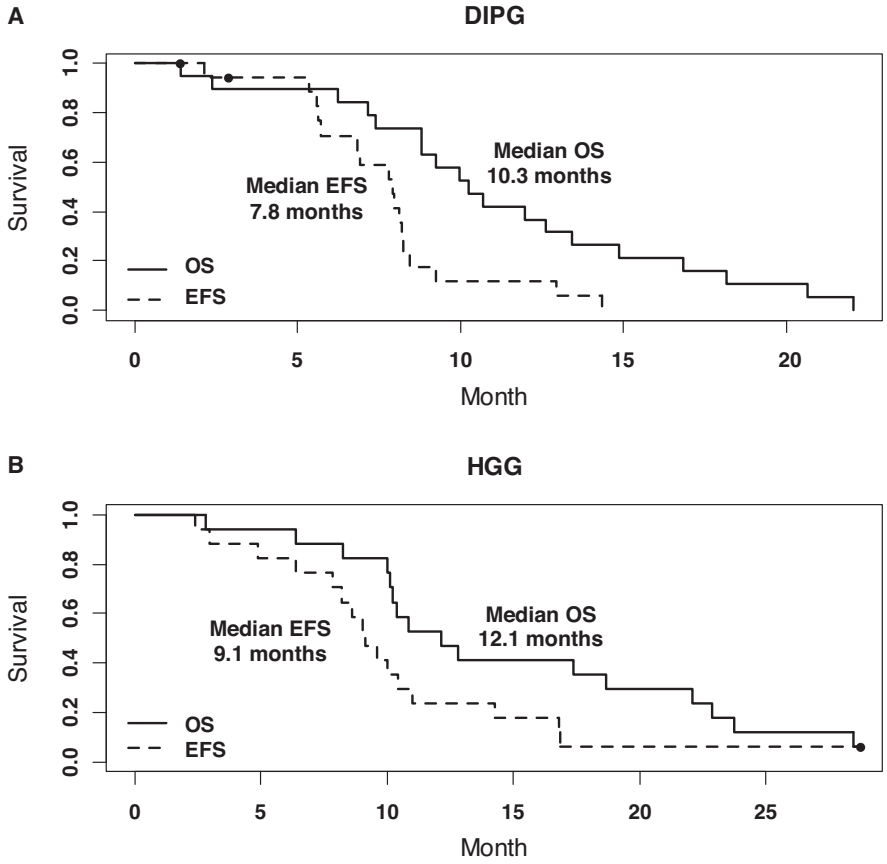

FIGURE 1 Kaplan-Meier plot of EFS and OS in evaluable patients. (A) EFS and OS in patients with DIPG. (B) EFS and OS in patients with HGG

two cerebellar, and one spinal cord HGG, and the remaining four patients had gliomatosis cerebri. One patient was inevaluable because of noncompliance with protocol therapy (confirmed by VPA trough repeatedly below the targeted range of 85 to 115 μg/mL), and she was removed from study prior to completion of radiation and VPA. Two of the 17 remaining evaluable patients did not receive bevacizumab due to PD before week 11 of protocol therapy. Four patients had constitutional mismatch-repair deficiency (MMRD) and one had neurofibromatosis-1.

The median EFS and OS for HGG patients were 9.1 (95% CI 6.4-11) and 12.1 (10-22.1) months (Figure 1B), respectively. The estimated one-year EFS was 24% (7%-45%). Interestingly, the four patients with GBM and MMRD syndrome had EFSs of 28.5, 16.7, 10.4, and 9 months, at or exceeding the median EFS for the HGG cohort. One of these patients completed protocol therapy and sustained a CR at 24 months. Six of 14 patients with measurable HGGs had objective responses, consisting of five PRs and one CR. Five HGG patients had evidence of pseudoprogression. Eight patients with HGG showed tumor dissemination at the time of disease recurrence/progression, although two of whom had not yet received bevacizumab.

3.3 | Toxicity during VPA and radiation therapy

Grade 3 or higher hematologic toxicities and ≥ grade 2 nonhematologic toxicities during concurrent VPA and radiation therapy, reported as maximum grade per each patient, are detailed inTable3. Two patients required VPA interruption and dose modification during radiation therapy: one patient with known Fanconi anemia had grade 3 thrombocytopenia and one patient had grade 3 weight gain, and toxicities improved to grade 2 after dose modification. Radiation therapy was only interrupted in the patient with severe respiratory depression who died during week 6 of protocol therapy. One patient had radiation necrosis on his week 10 MRI, but clinical symptoms and MRI improved after starting bevacizumab. One patient had a grade 3 amylase/lipase elevation and pancreatitis noted at the time of tumor progression (week 10) and was taken off protocol therapy.

3.4 | Toxicity during VPA and bevacizumab maintenance

Grade 3 or higher hematologic toxicities and ≥ grade 2 nonhematologic toxicities during VPA and bevacizumab maintenance therapy, reported as maximum grade per each patient, are detailed in Table 4. Protocol therapy was discontinued due to toxicities in two patients: one who developed multiple thrombi and a pulmonary embolism (week 23) and one who achieved a PR but had an asymptomatic intratumoral hemorrhage > 1 cm in widest dimension (week 24). Intratumoral hemorrhage was observed at the time of tumor progression in three other patients: one with grade 2 intratumoral hemorrhage at week 23 and two with grade 1 intratumoral hemorrhage at weeks 24 and 33, respectively.

TABLE 2 Characteristics of eligible and evaluable HGG patients

Age (yrs)	Sex	Ethnicity	Hist	Surgery	EFS (mos)	OS (mos)	Best response	Notable clinical information
14.8	F	Caucasian	GBM	Near GTR	28.5	28.5	CR	Constitutional MLH1 deficiency; pseudoprogression at week 10
6.1	M	Caucasian	AA	Biopsy	16.9	28.5	SD	Gliomatosis cerebri; pseudoprogression at week 25
13.6	F	Black	GBM	GTR	16.7	22.9	NM	Constitutional MSH2 deficiency; disseminated recurrence
4.5	M	Caucasian	GBM	STR	14.3	22.1	PR	Brainstem
8.2	F	Black	GBM	Biopsy	10.4	17.4	MR	Constitutional MSH6 deficiency; gliomatosis cerebri
12.6	M	Caucasian	AA	STR	10.0	10.0	SD	
10.7	F	Caucasian	AA	STR	9.6	10.4	PR	Disseminated recurrence
7.5	M	Caucasian	GBM	STR	9.1	10.1	SD	Neurofibromatosis-1; thalamic tumor; pseudoprogression at week 10
19.9	F	Caucasian	GBM	STR	9.0	12.1	PR	Constitutional MLH1 deficiency; gliomatosis cerebri; pseudoprogression at week 10; disseminated recurrence
8.2	M	Caucasian	AA	GTR	8.3	23.7	NM	Thalamic tumor
10.1	F	Hispanic	GBM	STR	8.2	12.8	PR	Disseminated recurrence
7.6	F	Black	AA	Biopsy	7.8	10.9	SD	Gliomatosis cerebri; pseudoprogression at week 10; disseminated recurrence
16.4	M	Mixed	GBM	STR	6.4	10.2	SD	
13.4	M	Hispanic	GBM	Biopsy	5.8	18.7	PR	
6.7	M	Hispanic	GBM	GTR	4.9	6.4	NM	Disseminated recurrence
17.2	M	Caucasian	AA	GTR	3.0	8.2	PD	Disseminated recurrence[a]
9.6	M	Caucasian	GBM	STR	2.4	2.8	PD	Spinal cord; disseminated recurrence[a]
8.8	F	American Indian	GBM					Eligible but not evaluable; noncompliant with therapy

AA, anaplastic astrocytoma; CR, complete response; EFS, event-free survival; F, female; GBM, glioblastoma; GTR, gross total resection; HGG, High-grade glioma; hist, histology; M, male; mos, months; MR, minor response; NM, no measurable disease; OS, overall survival; PD, progressive disease; PR, partial response; SD, stable disease; STR, subtotal resection; yrs, years.
[a]Disease disseminated before receiving bevacizumab.

Hypertension, the most commonly observed adverse event attributable to bevacizumab, was managed per protocol guidelines and did not require discontinuation of protocol therapy in any patient. Fatigue was the next most common nonhematologic toxicity during this phase of therapy. One patient was noted to have a grade 3 subacute bone infarction, possibly attributed to bevacizumab, at the time of tumor progression.

Nine of 31 patients required VPA dose modifications for the following toxicities: grade 3 thrombocytopenia (3), grade 3 somnolence (1), grade 3 fatigue (3), and grade 3 weight gain (2); all toxicities improved after dose modifications, and none of them required discontinuation of VPA.

4 | DISCUSSION

The combination of VPA, an HDAC inhibitor with potential for radiation sensitization, with radiation and subsequently with bevacizumab, an angiogenesis inhibitor, for post-radiation maintenance therapy, was generally well tolerated. None of the 38 patients who received concurrent VPA and radiation treatment required interruption of radiation therapy. Only two of the 31 patients (6.5%) receiving VPA and bevacizumab required cessation of treatment due to toxicities, compared with the discontinuation rates of 22% and 24% observed in children with CNS tumors receiving bevacizumab and irinotecan[22] or bevacizumab and temozolomide,[28] respectively. Disappointingly, the survival data for our DIPG and HGG patient cohorts were not improved compared with historical series. For DIPG patients, median EFS of 7.8 and OS of 10.3 months were comparable to other recent collaborative trials (Table 5), but not statistically superior. The heterogeneous nature of HGG patients (degree of resection, location, histology, etc.) precludes a meaningful conclusion from our small cohort, but our median EFS of 9.1 and median OS of 12.1 months are also comparable to historical survival figures. Similarly, adult trials[20-21] of up-front bevacizumab combined with temozolomide and radiation showed only a minor improvement in EFS but no impact on OS, and a recent pediatric trial[28] also failed to demonstrate improvement in survival. Our HGG cohort showed a high rate of disseminated progression (six of 15 HGG patients who received bevacizumab), in agreement with another retrospective review in children with HGG

TABLE 3 Summary of toxicities during valproic acid and radiation, weeks 1 to 10 of therapy ($n = 38$ eligible patients)[a]

Hematologic toxicities	Grade 3	Grade 4
Thrombocytopenia	1[b]	0
Neutropenia	3	0
Lymphopenia	5	0
Leukopenia	1	0
Anemia	0	0

Nonhematologic toxicities	Grade 2	Grade 3	Grade 4	Grade 5
Somnolence	1	0	0	0
Fatigue	3	0	0	0
Weight gain	4	1[b]	0	0
Hypoglycemia	1	0	0	0
Lipase and amylase elevation[c]	0	1	0	0
Pancreatitis[c]	1	0	0	0
Dehydration[c]	0	1	0	0
Radiation necrosis[d]	0	0	1	0
Abdominal pain	1	0	0	0
AST elevation	1	0	0	0
Cystitis	1	0	0	0

[a]Toxicities listed are ≥ grade 3 hematologic and ≥ grade 2 nonhematologic, maximum grade per patient.
[b]Required lowering of targeted valproic acid trough to 50–85 μg/mL.
[c]Occurred in association with disease progression, and therefore protocol therapy stopped.
[d]Occurred during week 10 of protocol therapy and showed clinical and radiographic improvement after bevacizumab started.

TABLE 4 Summary of toxicities during valproic acid and bevacizumab maintenance therapy ($n = 31$ patients who received therapy beyond week 11)[a]

Hematologic toxicities	Grade 3	Grade 4
Thrombocytopenia	3[b]	0
Neutropenia	3	0
Lymphopenia	3	0
Leukopenia	0	0
Anemia[b]	0	0

Nonhematologic toxicities	Grade 2	Grade 3	Grade 4
Intratumoral/intracranial hemorrhage	1[c]	0	0
Somnolence	2	1[b]	0
Fatigue	7	3[b]	0
Weight gain	1	2[b]	0
Hypertension	8	4	0
Hypoglycemia	1	0	0
Subacute bone infarction	0	1[d]	0
Cellulitis	0	1[d]	0
Proteinuria	2	0	0
Deep vein thrombosis, pulmonary embolism	0	0	1[e]
Ocular keratitis	1	0	0
Urinary tract infection	1	0	0
Cough	1	0	0
Anorexia	3	0	0
Hypoalbuminemia	3	0	0
Abdominal pain	1	0	0

[a]Toxicities listed are ≥ grade 3 hematologic and ≥ grade 2 nonhematologic, maximum grade per patient.
[b]Required lowering of targeted valproic acid trough to 50–85 μg/mL.
[c]One patient had a grade 1 intratumoral hemorrhage despite showing a partial tumor response and had protocol therapy discontinued for safety reason; two additional patients had grade 1 intratumoral hemorrhage observed at time of tumor progression.
[d]Occurred in association with disease progression, and therefore protocol therapy was stopped.
[e]Required discontinuation of protocol therapy.

or DIPG that also documented diffuse/distant progression in excess of 40% of patients after bevacizumab treatment.[29] This relatively high rate of pediatric HGG dissemination suggests that there may be altered tumor biology after bevacizumab-containing regimens and underscores the need for novel therapies targeting HGG metastasis.

Despite lack of improvement in EFS and OS for patients in our trial, there were objective tumor responses in both cohorts; eight PRs were observed in 16 evaluable DIPG patients (50% response rate) and five PRs and one CR were observed in 14 evaluable HGG patients (42% response rate). In light of the encouraging tumor responses observed in our trial but subsequent high rate of tumor dissemination while receiving post-radiation maintenance therapy, survival may be improved in future trials employing similar drugs by adding new agents inhibiting tumor dissemination. In addition, we observed a high rate of pseudoprogression (19.4%; two in DIPG, five in HGG, out of 36 evaluable patients), most commonly between weeks 10 and 25 of protocol therapy, compared with < 10% in adult trials incorporating up-front bevacizumab,[30] suggesting the potential of VPA's radiation enhancement. For future trials incorporating VPA or other similar HDAC inhibitors in combination with radiation in children with CNS

TABLE 5 Comparison of pediatric DIPG clinical trials and associated survival data

Study	Chemo	Targeted drug	XRT	1-yr EFS	Median EFS	Median OS
Our trial		VPA, BEV	54 Gy	12%	7.8 months	10.3 months
ACNS0126	TMZ		54 Gy	14% ± 4.5%	6.1 months	9.6 months
PBTC014		Tipifarnib	54 Gy	12.9% ± 5%	6.8 months	8.3 months
PBTC07		Gefitinib	54 Gy	20.9% ± 5.6%	7.4 months	
CCG-941	Various		54 Gy	17%		

BEV, bevacizumab; EFS, event-free survival; OS, overall survival; TMZ, temozolomide; VPA, valproic acid; XRT, radiation therapy.

tumors, early clinical and/or radiographic progression 4 to 12 weeks after completing radiation therapy should prompt treatment for radiation edema/injury instead of a premature cessation of protocol therapy.

Of note, our treatment strategy led to relatively prolonged EFS and OS in four patients with GBM and MMRD syndrome, including a patient with Lynch syndrome/MLH1 deficiency and gliomatosis cerebri, who completed protocol therapy and sustained a CR at week 104. As HGGs in children with MMRD syndrome have been reported to progress rapidly despite radiation and chemotherapy,[31-33] our alternative strategy of an HDAC and an angiogenesis inhibitor may warrant further investigation in this subpopulation. Effect of VPA on reducing expression of DNA-repair proteins and checkpoint kinase 1[34] is a plausible explanation of the favorable survival observed in our cohort with GBM and MMRD syndrome, similar to another case report of a dramatic response to checkpoint inhibiton.[35]

In summary, our data suggest that an HDAC inhibitor such as VPA may have radiation enhancement potential, and thus early clinical and/or radiographic progression should alert clinicians to consider treatment for pseudoprogression. The combination of VPA and bevacizumab appears to be better tolerated compared with bevacizumab and chemotherapy, and although encouraging tumor responses were observed in children with DIPG and HGG, including those with MMRD syndrome, our treatment strategy did not improve survival.

CONFLICTS OF INTEREST

None of the authors reported any conflict of interest.

ACKNOWLEDGMENTS

We wish to recognize the expert assistance and dedication of research nurse Suzanne Wheeler in the conduct and monitoring of this clinical trial.

FUNDING INFORMATION

Genentech, Inc. supplied study drug bevacizumab and funding support for the conduct of this clinical trial.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jack Meng-Fen Su https://orcid.org/0000-0003-2125-3659
Arnold C. Paulino https://orcid.org/0000-0002-0269-3045

REFERENCES

1. Cohen K, Pollack IF, Zhou T, et al. Temozolomide in the treatment of high-grade gliomas in children: a report from the Children's Oncology Group. *Neuro Oncol*. 2011;13(3):317-323.
2. Pollack IF, Boyett JM, Yates AJ, et al. The influence of central review on outcome associations in childhood malignant gliomas: results from the CCG-945 experience. *Neuro Oncol*. 2003;5(3):197-207.
3. Hass-Kogan DA, Banerjee A, Poussaint TY, et al. Phase II trial of tipifarnib and radiation in children with newly diagnosed diffuse intrinsic pontine glioma. *Neuro Oncol*. 2011;13(3):298-306.
4. Pollack IF, Stewart CF, Kocak M, et al. A phase II study of gefitinib and irradiation in children with newly diagnosed brainstem gliomas: a report from the Pediatric Brain Tumor Consortium. *Neuro Oncol*. 2011;13(3):290-297.
5. Cohen KJ, Heideman RL, Zhou T, et al. Temozolomide in the treatment of children with newly diagnosed diffuse intrinsic pontine gliomas: a report from the Children's Oncology Group. *Neuro Oncol*. 2011;13(4):410-416.
6. Jennings MT, Sposto R, Boyett JM, et al. Preradiation chemotherapy in primary high-risk brainstem tumors: phase II study CCG-9941 of the Children's Cancer Group. *J Clin Oncol*. 2002;20(16):3431-3437.
7. Blaheta RA, Michaelis M, Driever PH. Evolving anticancer drug valproic acid: insights into the mechanism and clinical studies. *Med Res Rev*. 2005;25(4):383-397.
8. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. *Nat Rev Cancer*. 2001;1(3):194-202.
9. Marks PA, Dokmanovic M. Histone deacetylase inhibitors: discovery and development as anticancer agents. *Expert Opin Investig Drugs*. 2005;14(12):1497-1511.
10. Knupfer MM, Hernaiz-Driever P, Poppenborg H, Wolff JE, Cinatl J. Valproic acid inhibits proliferation and changes expression of CD44 and CD56 of malignant glioma cells in vitro. *Anticancer Res*. 1998;18(5A):3585-3589.
11. Knupfer MM, Pulzer F, Schindler I, Hernaiz Driever P, Knupfer H, Keller E. Different effects of valproic acid on proliferation and migration of malignant glioma cells in vitro. *Anticancer Res*. 2001;21(1A):347-351.
12. Chavez-Blanco A, Perez-Plasencia C, Perez-Cardenas E, et al. Antineoplastic effects of the DNA methylation inhibitor hydralazine and the histone deacetylase inhibitor valproic acid in cancer cell lines. *Cancer Cell Int*. 2006;6:2.
13. Chinnaiyan P, Cerna D, Burgan WE, et al. Postradiation sensitization of the histone deacetylase inhibitor valproic acid. *Clin Cancer Res*. 2008;14(17):5410-5415.
14. Camphausen K, Cerna D, Scott T, et al. Enhancement of in vitro and in vivo tumor cell radiosensitivity by valproic acid. *Int J Cancer*. 2005;114(3):380-386.
15. Karagiannis TC, Kn H, El-Osta A. The epigenetic modifier, valproic acid, enhances radiation sensitivity. *Epigenetics*. 2006;1(3):131-137.
16. Su JM, Li XN, Thompson P, etal. Phase 1 study of valproic acid in pediatric patients with refractory solid or tumors: a Children's Oncology Group report. *Clin Cancer Res*. 2011; ;17(3):589-597.
17. Vredenburgh JJ, Desjardins A. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. *Clin Cancer Res*. 2007;13(4):1253-1259.
18. Vredenburgh JJ, Desjardins A. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. *J Clin Oncol*. 2007;25(30):4722-4729.
19. Cloughesy TF, Prados MD, Wen PY, et al. A phase II, randomized, noncomparative clinical trial of the effect of bevacizumab (BV) alone or in combination with irinotecan (CPT) on 6-month progression free survival (PFS6) in recurrent, treatment-refractory glioblastoma (GBM). *J Clin Oncol*. 2008;26(May 20 suppl; abstr 2010b).
20. Chinot OL, Wick W, Mason W, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N Engl J Med*. 2014;370(8):709-722.
21. Gilbert MR, Dignam JJ, Armstrong TS, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med*. 2014;370(8):699-708.

22. Fangusaro J, Gururangan S, Poussaint TY, et al. Bevacizumab (BVZ)-associated toxicities in children with recurrent central nervous system tumors treated with BVZ and irinotecan (CPT-11): a Pediatric Brain Tumor Consortium Study (PBTC-022). *Cancer*. 2013;119(23):4180-4187.
23. Bryant AE, 3rd Dreifuss FE. Valproic acid hepatic fatalities. III. U.S. experience since 1986. *Neurology*. 1996;46(2):465-469.
24. Koenig SA, Buesing D, Longin E, et al. Valproic acid-induced hepatopathy: nine new fatalities in Germany from 1994 to 2003. *Epilepsia*. 2006;47(12):2027-2031.
25. Verrotti A, Trotta D, Morgese G, Chiarelli F. Valproate-induced hyperammonemic encephalopathy. *Metab Brain Dis*. 2002;17(4):367-373.
26. Grauso-Eby NL, Goldfarb O, Feldman-Winter LB, McAbee GN. Acute pancreatitis in children from valproic acid: case series and review. *Pediatr Neurol*. 2003;28(2):145-148.
27. Woolson RF. Rank tests and a one-sample logrank test for comparing observed data to a standard population. *Biometrics*. 1981;37:687-696.
28. Grill J, Massimino M, Bouffet E, et al. Phase II, open-label randomized, multicenter trial (HERBY) of bevacizumab in pediatric patients with newly diagnosed high-grade glioma. *J Clin Oncol*. 2018;36(10):951-958.
29. Salloum R, DeWire M, Lane A, et al. Patterns of progression in pediatric patients with high-grade glioma of diffuse intrinsic pontine glioma treated with bevacizumab-based therapy at diagnosis. *J Neurooncol*. 2015;121(3):591-598.
30. Wick W, Chinot OL, Bendszus W, et al. Evaluation of pseudoprogression rates and tumor progression patterns in a phase III trial of bevacizumab plus radiotherapy/temozolomide for newly diagnosed glioblastoma. *Neuro Oncol*. 2016;8(10):1434-1441.
31. Ilencikova D, Sejnova D, Jindrova J, Babal P. High-grade brain tumors in siblings with biallelic MSH6 mutations. *Pediatr Blood Cancer*. 2011;57(6):1067-1070.
32. Amayiri N, Al-Hussaini M, Swaiden M, et al. Synchronous glioblastoma and medulloblastoma in a child with mismatch repair mutation. *Childs Nerv Syst*. 2016;32(3):553-557.
33. Walter AW, Ennis S, Best H, et al. Constitutional mismatch repair deficiency presenting in childhood as three simultaneous malignancies. *Pediatr Blood Cancer*. 2013;60(11):E135-136.
34. Cornago M, Garcia-Alberich C, Blasco-Angulo N, et al. Histone deacetylase inhibitors promote glioma cell death by G2 checkpoint abrogation leading to mitotic catastrophe. *Cell Death Dis*. 2014;5:e1435.
35. Bouffet E, Larouche V, Campbell BB, et al. Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. *J Clin Oncol*. 2016;34(19):2206-2211.

How to cite this article: Su JM-F, Murray JC, McNall-Knapp RY, et al. A phase 2 study of valproic acid and radiation, followed by maintenance valproic acid and bevacizumab in children with newly diagnosed diffuse intrinsic pontine glioma or high-grade glioma. *Pediatr Blood Cancer*. 2020;e28283. https://doi.org/10.1002/pbc.28283