Neuro-Oncology

24(4), 655–664, 2022 | https://doi.org/10.1093/neuonc/noab188 | Advance Access date 4 August 2021

Phase I/II trial of vorinostat and radiation and maintenance vorinostat in children with diffuse intrinsic pontine glioma: A Children's Oncology Group report

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Abstract

Background. A phase I/II trial of vorinostat (suberoylanilide hydroxamic acid), an oral histone deacetylase inhibitor, was conducted in children with newly diagnosed diffuse intrinsic pontine glioma (DIPG) through the Children's Oncology Group (COG) to: 1) determine the recommended phase II dose (RP2D) of vorinostat given concurrently with radiation therapy; 2) document the toxicities of continuing vorinostat as maintenance therapy after radiation; and 3) to determine the efficacy of this regimen by comparing the risk of progression or death with a historical model from past COG trials.

Methods. Vorinostat was given once daily, Monday through Friday, during radiation therapy (54 Gy in 30 fractions), and then continued at 230 mg/m^2 daily for a maximum of twelve 28-day cycles.

Results. Twelve patients enrolled in the phase I study; the RP2D of vorinostat given concurrently with radiation was 230 mg/m^2/day, Monday through Friday weekly. The six patients enrolled at the RP2D and an additional 64 patients enrolled in the phase II study contributed to the efficacy assessment. Although vorinostat was well-tolerated, did not interrupt radiation therapy, and was permanently discontinued in only 8.6% of patients due to toxicities, risk for EFS-event was not significantly reduced compared with the target risk derived from historical COG data ($P = 0.32$; 1-sided). The 1-year EFS was 5.85% (95% CI 1.89–13.1%) and 1-year OS was 39.2% (27.8–50.5%).

Conclusions. Vorinostat given concurrently with radiation followed by vorinostat monotherapy was well tolerated in children with newly diagnosed DIPG but failed to improve outcome.

Key Points

1. Vorinostat and concurrent radiation, followed by maintenance vorinostat, were well tolerated in children with newly diagnosed diffuse intrinsic pontine glioma.
2. Vorinostat and concurrent radiation, followed by maintenance vorinostat did not improve outcome in children with newly diagnosed intrinsic pontine glioma.

Importance of the Study

Vorinostat is an oral histone deacetylase (HDAC) inhibitor that inhibits high-grade glioma growth and enhances radiation efficacy in preclinical studies. The Children's Oncology Group conducted a phase I/II clinical trial of vorinostat and radiation, followed by maintenance vorinostat in children with newly diagnosed intrinsic pontine glioma. The recommended phase II dose of vorinostat and concurrent radiation was 230 mg/m^2/day, Monday through Friday throughout radiation therapy. Although the novel combination was well tolerated, this regimen did not improve the survival of patients compared to historical series. Our results suggest that monotherapy with vorinostat, an HDAC inhibitor, in children with diffuse intrinsic pontine gliomas is of limited clinical value, and therefore future trials of similar agents should explore combination therapies instead.

Diffuse intrinsic pontine gliomas (DIPG) in children remain essentially incurable. Recent reviews of contemporary trials of pediatric DIPG showed only approximately 10% survival beyond 2 years,[1,2] with essentially identical event-free (EFS) or overall survival (OS) in recent trials[3,4] compared to older series. Clinical trials of hyper-fractionated radiation, delivering up to 72 Gy radiation, showed no benefit over conventional (54–58 Gy) radiation.[5–11] Chemotherapy or biologic agents, whether given pre-irradiation,[5,11–13] with radiation,[3,4,14] and/or after radiation,[6–10,12,13,15–22] including myeloablative regimen with stem cell rescue,[22–24] have also failed to improve EFS or OS. Collectively, numerous clinical trials over the last two decades have shown similar and dismal median EFS (5 to 8.8 months), median OS (8 to 12 months), and 2-year survival (5 to 15%).

Because radiation often induces a temporary response and symptomatic improvement for children with DIPG, concurrent administration of an agent with both an antiglioma and a radiosensitizing effect may potentially improve outcome. Vorinostat (suberoylanilide hydroxamic acid, SAHA), an orally bioavailable histone deacetylase (HDAC) inhibitor[25] that had initially shown encouraging responses in adult solid tumors and hematologic malignancies[26–28], was ultimately approved by the FDA for treatment of refractory cutaneous T-cell lymphoma. Vorinostat inhibited growth of malignant gliomas pre-clinically,[29–32] enhanced radiation sensitivity of malignant glioma,[30] and induced increased levels of acetylated histones in normal mouse brain,[33] intracranial tumor xenografts,[34] and adult glioblastoma tumors,[35] suggesting effective crossing of the blood-brain barrier. After a Children's Oncology Group (COG) phase I trial of vorinostat in children with refractory solid tumors (ADVL0416) was completed and established the maximum-tolerate dose (MTD) as 230 mg/m^2/day continuously,[36] we conducted a clinical trial of vorinostat in children with newly diagnosed DIPG (ACNS0927, NCT01189266).

ACNS0927 was a phase I/II study to determine the tolerability and efficacy of vorinostat given concurrently with radiation, and as maintenance therapy postradiation, in children with newly diagnosed DIPG. Part A was the phase I dose-finding component of the trial to define the recommended phase II dose (RP2D) of vorinostat in combination with radiation therapy, and Part B was the phase II portion of the trial to assess the efficacy of vorinostat concurrently with radiotherapy and as maintenance therapy after radiation, for a maximum of twelve 28-day cycles. After determination of the RP2D of vorinostat in combination with radiation therapy from Part A, conducted through the COG Phase I Consortium, Part B opened to enrollment at all COG institutions, with all patients receiving vorinostat and radiation at the RP2D. In part A and B, all patients received vorinostat as maintenance therapy at 230 mg/m^2/day, the MTD of vorinostat monotherapy defined in a preceding COG ADVL0416 trial.

The primary endpoints for toxicity and safety monitoring of vorinostat in combination with radiation therapy included dose-limiting toxicities (DLTs) and toxic death, and toxicities for vorinostat maintenance therapy were also documented. Toxicities were graded according to NCI's Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0. The primary endpoint for the evaluation of treatment efficacy was risk for EFS-event and a secondary endpoint was risk of death.

Patients and Methods

Patient Eligibility

Children age 3 to 21 years with newly diagnosed DIPG, radiographically defined as tumors with a pontine epicenter and diffuse involvement of at least two-thirds of the pons, were eligible without histological confirmation. Patients with brainstem tumors that did not meet the above definition of "typical" DIPG were only eligible if tumors were biopsied and proven to be an anaplastic astrocytoma, glioblastoma, gliosarcoma, or anaplastic mixed glioma. Patients with disseminated DIPG were not eligible. Other eligibility criteria included: a Lansky or Karnofsky performance score of 50 or higher, no prior therapy except for surgery and/or dexamethasone, 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), 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), ability to swallow capsules or liquid, and enrollment within 28 days of diagnosis.

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.

Radiation Therapy

Radiation therapy was initiated within 7 days after enrollment. Computed tomography (CT)-based treatment planning with slice thickness of <5 mm was required. Radiation therapy treatment technique was either three-dimensional conformal (3DCRT), or intensity-modulated (IMRT). Proton therapy was not permitted. Planning CT and diagnostic MRI registration were required for target volume delineation. The gross tumor volume (GTV) was defined by a combination of the T1 contrast, T2, and FLAIR abnormality. The clinical target volume (CTV) included the GTV with a geometric expansion of 1 cm but was limited by natural anatomic barriers such as bony calvarium and tentorium. An institution-specific planning target volume (PTV) expansion was added to the CTV to account for setup uncertainty. This margin was typically 5 mm, but 3 mm was allowed if daily image guidance was utilized. Prescription dose was 54 Gy in 1.8 Gy daily fractions with 95% of the prescription dose required to cover 100% of the PTV. No modification of dose or fractionation was allowed based on patient age.

At the completion of treatment, data for all patients were compiled at the Imaging and Radiation Oncology Core (IROC) in Providence, Rhode Island (formerly the Quality Assurance Review Center, QARC). Clinical information and detailed radiation treatment data including diagnostic imaging, digital treatment plan, dose-volume distributions for all targets and organs at risk, and other supportive data were submitted. All data were reviewed at IROC RI by the dosimetry and the RT study chair (DBM).

Vorinostat Dosing and Administration

Vorinostat was supplied by industrial sponsor Merck and given either as 100-mg capsules or a 50 mg/ml pediatric suspension. The pediatric suspension was mandated for patients with BSA < 1.25 m^2, and remaining patients had options of taking either capsules or the suspension. Capsule-dosing was rounded to the nearest 100-mg using a nomogram to minimize deviation in dosing below 15%, suspension was rounded to the nearest 5-mg, and maximum daily dose was not to exceed 500 mg.

Vorinostat and Concurrent Radiation

Vorinostat was started on the first day of radiation and given Monday through Friday for the duration of radiation treatment. Vorinostat was given orally once daily 60 to 120 minutes prior to start of daily radiation or at bedtime for young children who required daily anesthesia. For Part A, initial patients started vorinostat at 180 mg/m^2/day, Monday through Friday weekly during radiation, with the design for one dose escalation to 230 mg/m^2/day, Monday through Friday weekly, and one dose de-escalation to 180 mg/m^2/day, Monday, Wednesday, and Friday weekly, if needed (Table 1). The rolling six phase I trial design[37] was used for the determination of RP2D. In order to be considered evaluable for the dose-escalation decision, a patient must have been eligible and received at least 85% of the planned dose of radiation and vorinostat, or have received at least one dose of vorinostat and experienced a DLT during combination therapy. The study chair reviewed incidents of DLT reported by institutional investigators and those verified were used for the dose-escalation rule. Once the RP2D of vorinostat given concurrently with radiation was determined, all patients in Part B received vorinostat at the RP2D concurrently with radiation.

Vorinostat Maintenance Therapy After Radiation

In both Part A and Part B, after completion of vorinostat with concurrent radiation, patients continued vorinostat without interruption, at 230 mg/m^2 once daily, for a maximum of 12 cycles, in the absence of disease progression or intolerable toxicities. Each maintenance cycle of vorinostat consisted of 28 days and commenced when ANC was ≥ 1,000/μL and platelet count was ≥ 100,000/μL. Dose reductions to 230 mg/m^2/day, Monday through Friday weekly, and to 230 mg/m^2/day, Monday, Wednesday, and Friday weekly, if needed, were allowed for DLTs during maintenance therapy.

Toxicity Definition, Monitoring, and Dose Modification of Vorinostat

DLTs were defined for the two parts of planned protocol therapy: concurrent radiation therapy and vorinostat; and maintenance therapy with vorinostat only. During concurrent radiation therapy and vorinostat, DLT was defined as the following: 1) any vorinostat-related toxicity that necessitated interruption of radiation for 5 consecutive days or 10 cumulative days, 2) any grade 4 non-hematologic toxicity, 3) any grade 3 non-hematologic toxicity, excluding nausea and vomiting of <5 days duration, asymptomatic elevation of transaminases that returned to levels meeting initial eligibility criteria within 7 days of vorinostat interruption and did not recur upon drug re-challenge, fever or infection < 5 days duration, electrolyte deficiency that responded to oral supplementation, and diarrhea that improved to grade 1 or better within 48 hours of starting anti-diarrhea treatment, 4) any grade 2 non-hematologic toxicity that persisted

Table 1 Dose-Limiting Toxicities (DLT) During Vorinostat and Radiation, Part A (Phase I Component)

Dose Level	Vorinostat (mg/m^2/day)	Number of Patients Enrolled	Number of Patients Evaluable	Number of Patients with DLT
0	180, M, W, F	0	0	NA
1 (starting dose)	180, M-F weekly	6	6	0
2	230, M-F weekly	6	6	0

Abbreviations: M, Monday; W, Wednesday; F, Friday.

for ≥ 7 days and was considered sufficiently medically significant or intolerable by patients requiring treatment interruption, 5) grade 3 thrombocytopenia, and 6) grade 4 neutropenia.

During maintenance vorinostat, DLTs were defined as stated above, except with removal of radiation interruption, and addition of any delay of >14 days in starting the subsequent cycle of vorinostat due to ANC < 1,000/μL and/or platelet < 100,000/μL. DLTs during maintenance vorinostat were reported as the maximum grade of an individual toxicity across all cycles per patient.

During vorinostat and radiation, patients experiencing non-hematologic DLTs discontinued vorinostat until the toxicity resolved to meet on-study parameters and resumed vorinostat at one dose level lower (Table 1). Patients who experienced hematologic DLTs received platelet transfusion(s) and/or myeloid growth factor support, if clinically indicated, and resumed vorinostat at one dose level lower upon recovery of ANC ≥ 1,000/μL and platelet ≥ 100,000/μL. Radiation was not interrupted despite vorinostat DLTs unless clinically indicated. Patients who experienced the identical DLT during vorinostat and radiation treatment despite dose reduction or any DLT at the lowest dose level would discontinue vorinostat completely for the remainder of radiation treatment. Dose modification and drug discontinuation guidelines during vorinostat maintenance therapy were identical as stated above.

Patient Monitoring and Disease Evaluation

A history and physical examination, CBC, liver function tests, electrolyte, and renal function tests were obtained weekly during vorinostat and concurrent radiation treatment and at the start of each cycle of vorinostat maintenance treatment. Weekly CBCs were obtained for the duration of the entire treatment period. Disease evaluations were obtained at the end of vorinostat maintenance cycle 1, 3, and 5, and then every 3 cycles afterward until completion of study. Tumor response was determined using WHO bi-dimensional criteria (product of the greatest tumor diameter and its perpendicular diameter), using either T1 or T2 weighted images (whichever gives the best estimate of tumor size), to allow comparison to the historical studies, CCG-9941, and ACNS0126. Radiographic progressive disease (PD) was defined as a 25% or more increase in the product of the perpendicular diameters of the tumor. Protocol therapy was discontinued and a patient was considered to have disease progression when he/she showed radiographic PD and/or clinical evidence of disease progression that could not be attributed to other causes unrelated to tumor progression.

Determination of Efficacy Endpoints

A patient was considered evaluable for the primary outcome measure if he/she was eligible and received at least one dose of vorinostat during radiation therapy. Patients enrolled at the RP2D during Part A were included in the outcome analyses for Part B of the trial. EFS was calculated as the time from enrollment to disease progression, diagnosis of a second malignant neoplasm, death, or last contact without any of the aforementioned events, whichever occurred first. Patients who experienced disease progression, second malignant neoplasm or death from any cause were considered to have experienced an EFS-event; otherwise the patient was considered censored at last contact. Median follow-up for EFS was estimated by the reverse Kaplan-Meier method.[38]

OS was defined to be the time from enrollment to death or last patient contact alive. Patients who died, regardless of cause, were considered to have experienced an OS-event; otherwise the patient was censored at last contact.

In order to assess the efficacy of vorinostat administered at the RP2D in combination with radiation therapy, we conducted a Woolson 1-sample log-rank test[39] with an exponential reference distribution with hazard rate of 1.54 per year. This model was obtained by fitting a cohort of 124 patients enrolled on CCG-9941 and ACNS0126 who had the same diagnosis as patients enrolled in our current study. This historical model, for example, predicts 1-year EFS will be 21%.

The proportion of patients event-free, EFS, and the proportion of patients alive at last contact, OS, as a function of time since enrollment were estimated by the method of Kaplan and Meier.[40] In addition to the primary analysis, risk for EFS-event and death were compared to historical data from ACNS0126[4] and CCG-9941.[11] The definition of EFS and OS provided above were applied to the data from ACNS0126 and CCG-9941. The log-rank test[41] was used to calculate *P*-values for the test of homogeneity of risk for EFS-event and death between the three studies. Data current as of June 2005 was used for CCG-9941 and data current to June 2008 was used for ACNS0126.

During the period of study accrual, interim monitoring was performed after one year of enrollment and every six months subsequently until 2.5 years after enrollment to Part B started. A spending function approach[42] with αt^2 was used with the one sample log-rank statistic used for the primary comparison. The times were selected to correspond to when 37%, 69%, 90%, and 98% of the information was expected to be available. Monitoring for inefficacy was not utilized in this study.

All eligible and evaluable patients enrolled at the RP2D of 230 mg/m^2/day were considered in the analysis of the occurrence of DLT. All incidents of CTCAE codeable adverse events (AEs) reported by institutional investigators were reviewed by the study chair and vice chair (JMS, LK) and assessed as to whether they met the criteria for DLTs. Each patient was followed for the occurrence of DLT until the first reporting period where a DLT was observed or until the patient was removed from protocol therapy. The non-parametric estimate of the probability of remaining free of DLT through a particular reporting period was calculated using the non-parametric method[41] where the observation of a DLT during a reporting period is considered an event.

Results

ACNS0927 opened in August 2010 and closed in February 2014. Data current to June 30, 2017 are used in this analysis.

Twelve patients were enrolled onto Part A, with six patients each at dose level 1 and 2 (Table 1 and Figure 1). All twelve patients were eligible and evaluable, and no DLT was observed, so vorinostat at 230 mg/m^2/day, Monday through Friday weekly during radiation therapy, was declared the RP2D for Part B, which opened to COG group-wide enrollment on February 6, 2012. Sixty-seven patients were enrolled onto Part B (Figure 1), with one ineligible patient (not a typical DIPG on MRI, and tumor not biopsied) and two inevaluable patients (one patient lost ability to swallow capsule/liquid after enrollment, and one patient deteriorated and never started protocol therapy). The six patients from Part A who received vorinostat at RP2D were included with eligible and evaluable patients from Part B in the efficacy analysis, and these 70 patients' characteristics are shown in Table 2. Tumor biopsies of nine patients with "atypical" appearing brainstem tumors showed glioblastomas ($n = 5$) and anaplastic astrocytomas ($n = 4$).

In the 70 patients who received vorinostat at RP2D with concurrent radiation, hematologic DLTs (Table 3) included grade 3 thrombocytopenia ($n = 3$) and grade 4 neutropenia ($n = 1$), necessitating withholding of vorinostat and dose reduction upon count recovery. Additional non-hematologic DLTs are detailed in Table 3. One patient developed grade 3 confusion and agitation during vorinostat-radiotherapy, accompanied by other signs of neurologic deterioration (aspiration, dysarthria, fatigue, and somnolence). Although radiation edema/injury was suspected, imaging studies at

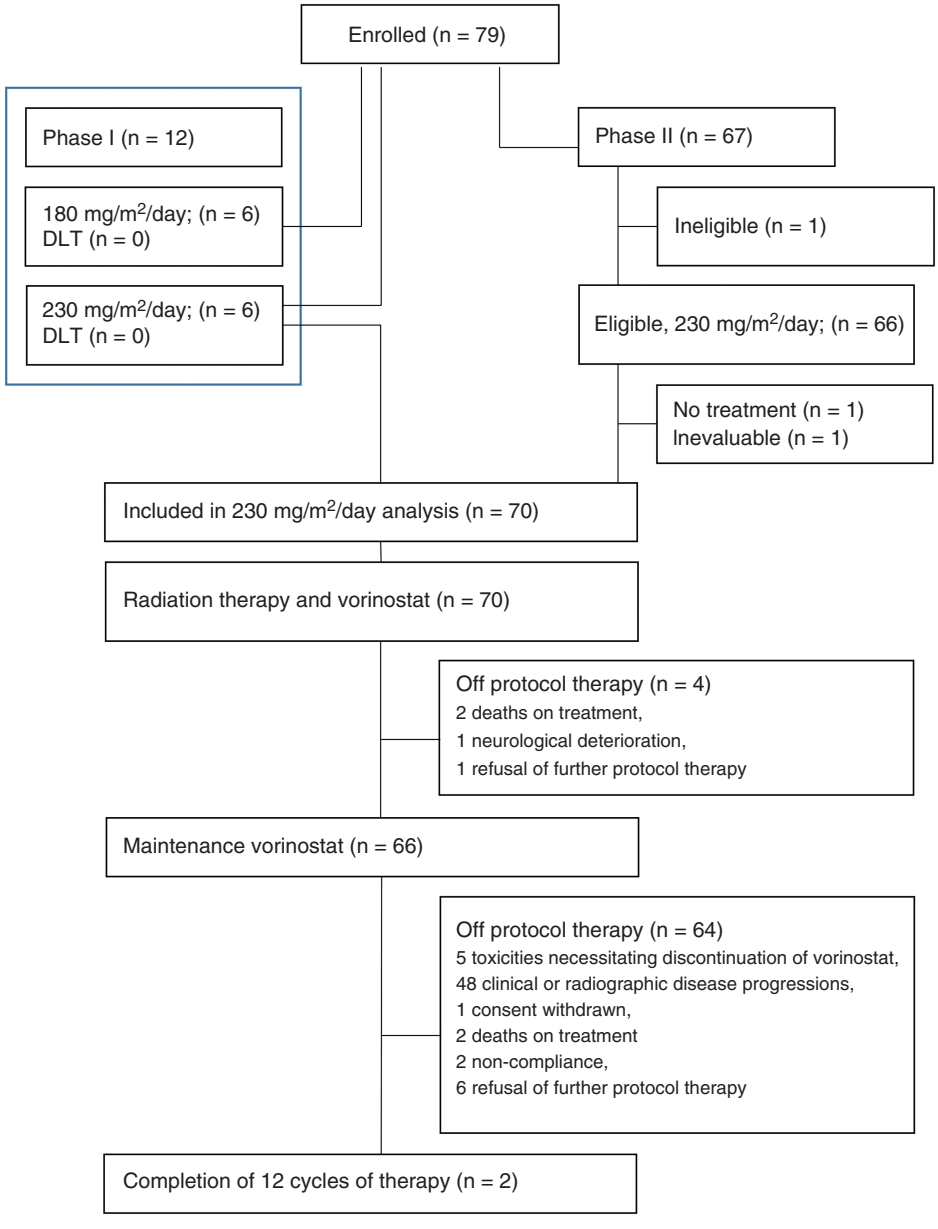

Fig. 1 ACNS0927 patient consort.

Table 2 Characteristics of All Eligible and Efficacy Evaluable Patients ($n = 70$)

Characteristic	Number (%)
Age (years)	
Median	7.1
Range	3.3–19.4
Sex	
Male	32 (45.7)
Female	38 (54.3)
Race	
White	49 (70)
Asian	3 (4.3)
American Indian or Alaska Native	0 (0)
Black or African American	8 (11.4)
Unknown	10 (14.3)
Ethnicity	
Non-Hispanic	54 (77.1)
Hispanic	14 (20)
Unknown	2 (2.9)
Diagnoses	
"Typical" diffuse intrinsic pontine glioma	61 (87.1)
Glioblastoma	5 (7.1)
Anaplastic astrocytoma	4 (5.7)

the peak of these clinical symptoms did not demonstrate radiographic evidences of edema/necrosis (reviewed by study radiation-oncologist DBM). Two patients developed grade 2 intra-tumoral hemorrhage during vorinostat-radiation therapy, and while the severity of toxicity did not meet definition of a DLT, vorinostat was discontinued to ensure patient safety. Collectively, radiation was not interrupted for any patient for vorinostat-related toxicity except for the patient who developed neurological deterioration/presumed radiation edema.

During vorinostat maintenance therapy, hematologic DLTs (grade 4 neutropenia and grade 3 or higher thrombocytopenia) were the most common toxicities requiring dose modifications (Table 3). Additional notable non-hematologicDLTs requiring vorinostat dose modifications included grade 3 anorexia ($n = 1$), grade 3 dehydration ($n = 1$), grade 3 creatinine elevation ($n = 1$), grade 3 hypermagnesemia ($n = 4$), and grade 3 thromboembolism ($n = 1$). Overall, it was predicted that 60% of patients would develop at least 1 DLT after completing 12 cycles of maintenance vorinostat (Table 4); however, of these patients who developed DLTs during vorinostat maintenance therapy, only 4 patients developed recurring toxicities despite dose reductions (2 patients with recurring grade 4 neutropenia, 1 patient with recurring grade 3 thrombocytopenia, and 1 patient with recurring grade 3 creatinine elevation), requiring permanent discontinuation of vorinostat. One patient with grade 3 thromboembolism required immediate discontinuation of protocol therapy. Overall, 6 out of 70 (8.6%) patients required permanent discontinuation of vorinostat due to defined DLTs, either during radiation or maintenance vorinostat.

Table 3 Summary of Instances of DLT During Radiation and Vorinostat and Subsequent Maintenance Vorinostat that are Considered in the Analysis of Time to First DLT Event

Reporting Period	DLTs Observed in at Least One Patient	Number of Patients With DLT Types
Radiation Therapy and Vorinostat	Grade 2 creatinine elevation	1
	Grade 3 agitation; Grade 3 confusion; Grade 3 hyperglycemia	1
	Grade 3 anorexia	1
	Grade 3 hypertension	1
	Grade 3 hyponatremia	1
	Grade 3 mucositis; Grade 3 thrombocytopenia	1
	Grade 3 thrombocytopenia	1
	Grade 3 maculopapular rash	1
	Grade 4 hyperglycemia	1
	Grade 4 neutropenia; Grade 3 thrombocytopenia	1
Maintenance Vorinostat Cycles 1 and 2	Grade 2 anorexia; Grade 2 weight loss	1
	Grade 2 creatinine elevation	1
	Grade 3 creatinine elevation	1
	Grade 3 hypermagnesemia	1[a]
	Grade 3 thrombocytopenia	3
	Grade 3 thrombocytopenia; Grade 4 neutropenia	2
	Grade 4 thrombocytopenia	1
	Grade 4 thrombocytopenia; Grade 3 hypermagnesemia	1
	Grade 4 thrombocytopenia; Grade 4 neutropenia	1
Maintenance Vorinostat Cycles 3 and 4	Grade 3 alkaline phosphatase elevation	1
	Grade 3 dehydration	1
	Grade 3 thrombocytopenia	1
	Grade 4 neutropenia	2
Maintenance Vorinostat Cycles 5 and 6	Grade 3 depressed level of consciousness	1
	Grade 4 intracranial hemorrhage	1
Maintenance Vorinostat Cycles 7 and 8	Grade 3 weight loss; Grade 2 creatinine elevation	1
Maintenance Vorinostat Cycles 9 and 10	Grade 3 weight loss	1
Total		31

For patients experiencing more than 1 DLTs, only the first DLT and associated reporting period are included in this table.
[a]This patient also developed grade 3 thromboembolism during cycle 5 and 6 reporting period.

There were no toxic deaths related to vorinostat and protocol therapy, either during vorinostat-radiation therapy or maintenance therapy. Radiation treatment

Table 4 Non-Parametric Estimate of the Probability of Remaining Free of Experiencing DLT According to Cycle of Therapy Received

Reporting Period	Number of Patients Who Received Therapy	Number of Patients Who Experienced DLT	Estimated Probability of Remaining Free of DLT from The Start of Therapy Through the Noted Reporting Period
Radiation therapy and Vorinostat	70	10	0.8571
Maintenance Vorinostat Cycles 1 and 2	59	12	0.6828
Maintenance Vorinostat Cycles 3 and 4	39	5	0.5953
Maintenance Vorinostat Cycles 5 and 6	26	2	0.5495
Maintenance Vorinostat Cycles 7 and 8	14	1	0.5102
Maintenance Vorinostat Cycles 9 and 10	5	1	0.4082
Maintenance Vorinostat Cycles 11 and 12	3	0	0.4082

For example, it is estimated that 40.8% (100 x 0.4082) of patients will complete maintenance vorinostat through cycle 9 and 10 without experiencing any DLT.

compliance with protocol guidelines was excellent. Only one major deviation was recorded which was due to dose heterogeneity.

The trial was not stopped on the basis of interim monitoring and accrued all patients as specified in the study design. The median follow-up for EFS was 3.8 years (95% CI 3.6 to 3.9 years).[38] Risk for EFS-event was compared to historical COG trials using a Woolson 1-sample log-rank test,[39] and the observed value of the one sample log-rank test is 0.23 and the 1-sided *P*-value is 0.32 (Figure 2A). We conclude that there is no evidence to indicate vorinostat, given according to the ACNS0927 protocol, reduces the risk of EFS-events in this population. The 1-year EFS ACNS0927 is 5.85% (95% CI 1.89 to 13.1%), and 1-year OS is 39.2% (27.8 to 50.5%). The risk for EFS-event and the risk for death did not differ significantly when the three studies were compared using the log-rank test (Figure 2B and C; $P = 0.33$ and $P = 0.79$, respectively).

Discussion

Our failures in achieving even minor incremental improvement in survival for children with DIPG over the last two decades highlights the ineffectiveness of radiation and chemotherapy for this dreadful disease and the urgent need to improve our insights into tumor biology in order to identify promising novel drugs and treatment strategies. Based on pre-clinical growth inhibition of malignant gliomas and enhancement of radiation sensitivity, the HDAC inhibitor, vorinostat, was considered a promising agent in children with newly diagnosed DIPG. The RP2D of vorinostat given concurrently with radiation therapy was 230 mg/m^2/day, Monday through Friday weekly. Although vorinostat was well tolerated, this regimen failed to improve outcome in children with newly diagnosed DIPG according to the primary analysis comparing EFS outcome with the exponential reference distribution. The risk for EFS-event and OS were not significantly different when compared with two historical COG DIPG trials (CCG-9941 and ACNS0126) using a log-rank test. Observed 1-year OS of 39.2% is nearly identical to results from contemporary DIPG trials.[1–4]

As vorinostat may potentially enhance radiation sensitivity of tumor and normal brainstem tissue, increased incidences of radiation edema/injury was a theoretical concern but not observed on this trial. Only one patient required temporary interruption of radiation treatment due to neurological toxicity but showed no radiographic evidence of radiation injury. Two patients had grade 2 intra-tumoral hemorrhage that did not meet definition of DLTs, but vorinostat was discontinued to ensure patient safety. The rarity of these events (in <5% of patients) suggests that they were more likely related to the evolution of DIPG during/after radiation treatment and less likely secondary to concurrent vorinostat administration. All three patients ultimately completed radiation treatment without vorinostat and did not experience further worsening of their toxicities.

During vorinostat maintenance therapy, hematologic DLTs were common, occurring in 18 out of the 66-patient cohort (Table 3). Other notable DLTs during maintenance therapy included anorexia, weight loss, dehydration, creatinine elevation, and thromboembolism, which have been reported in previous adult and pediatric trials and should be monitored closely in future trials employing continuous vorinostat administration.

Lack of vorinostat efficacy in our clinical trial is likely multi-factorial. Although early pre-clinical and human studies[33–35] indirectly confirmed vorinostat's CNS entry, and subsequent investigations, including a human study[43] documenting mean steady-state vorinostat concentration of 75.4 nM in 2 pediatric patients with Ommaya reservoirs and a study confirming vorinostat's biological effect in mice brainstems,[44] ratio of mice brain to plasma vorinostat concentrations appears to be low, ranging from 1 to 12%.[44,45] Therefore, poor vorinostat entry into pons of children with DIPG is a possible factor for vorinostat's failure in our trial. A substantial number of our young patients who require daily anesthesia for radiation therapy also received vorinostat at bedtime instead of within 2 hours of receiving radiation, and given the drug's short half-life of 2 hours, this suboptimal timing may also have contributed to lack of efficacy. Lastly, in accordance with our hypothesis that vorinostat may enhance radiation efficacy in patients with DIPG, and also with the aim of minimizing potential toxicity, vorinostat was given Monday through Friday during concurrent radiation. In retrospect,

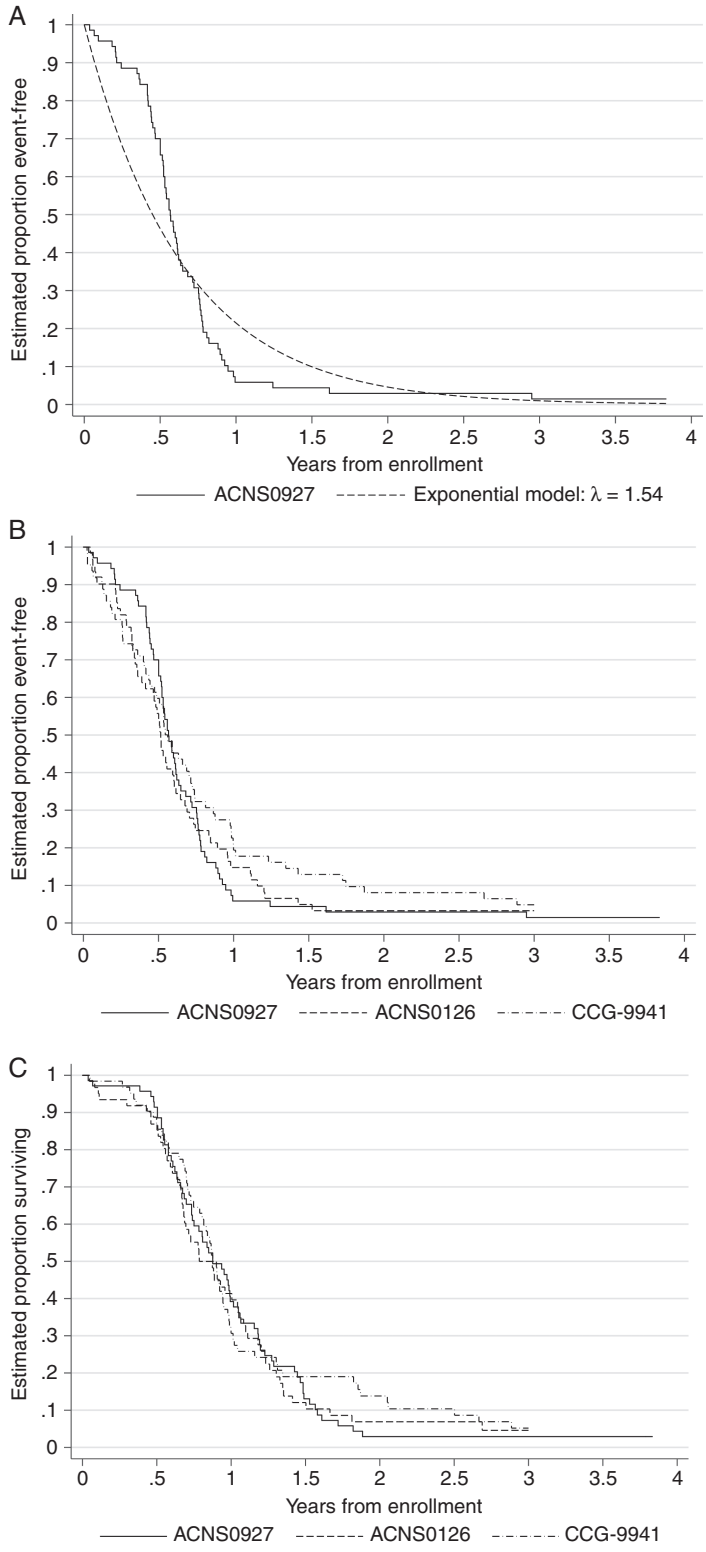

Fig. 2 (A) Event-free survival (EFS) of ACNS0927 ($n = 70$) versus exponential model with lambda = 1.54, Woolson 0.32 (1-sided). Woolson 1-sample log-rank test with an exponential reference distribution with hazard rate of 1.54 per year was used to generate this exponential model. This model predicts 1-year EFS will be 21% and was obtained by fitting a cohort of 124 patients enrolled on CCG-9941 ($n = 61$) and ACNS0126 ($n = 63$) who had the same diagnosis as patients enrolled on ACNS0927. (B) One-year EFS for the current study is 5.85% (95% CI 1.9 to 13.1%). The risk for EFS-event did not differ significantly when the three studies were compared using the log-rank test; $P = 0.33$. (C) One-year OS for the current study is 39.2% (95% CI 27.8 to 50.5%). The risk for death did not differ significantly when the three studies were compared using the log-rank test; $P = 0.79$.

since concurrent vorinostat and radiation were well tolerated, it might have been a wiser study design to administer vorinostat daily instead of only Monday through Friday during radiation therapy.

Recent genomic analyses of pediatric DIPG have shown potential driver mutations/alterations in H3F3A and ACVR1[46,47] and revealed possible subgroups with distinct genomic signatures and differing outcomes.[47,48] Lack of proven vorinostat inhibition of these newly discovered pathways and other molecular heterogeneity in tumor biology may partially explain our failure to demonstrate vorinostat efficacy in children with newly diagnosed DIPG, and in future trials, documenting status of H3F3A and other biomarkers through tumor biopsy and correlating with response to targeted therapies should be strongly considered. Another HDAC inhibitor, panobinostat, has demonstrated promising pre-clinical activity in DIPG models[49] and is currently undergoing testing in children with refractory DIPG through the Pediatric Brain Tumor Consortium (PBTC-047, NCT02717455). Based on results of ACNS0927, continuation of an HDAC inhibitor monotherapy after radiation is of limited clinical value in children with DIPG, and exploration of combination therapy may be more effective in future trials.[50]

Keywords

children | diffuse intrinsic pontine glioma | phase I/II clinical trials | suberoylanilide hydroxamic acid | Vorinostat

Funding

This work was supported by National Institute of Health/National Cancer Institute (IROC grant, U24CA180803), National Cancer Institute, National Clinical Trials Network (Operations Center Grant, U10CA180886), National Cancer Institute, National Clinical Trials Network (Statistics & Data Center Grant, U10CA180899) and St. Baldrick's Foundation Consortium Grant.

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Health.

Conflict of interest. None of the authors declare a conflict of interest.

Authorship statement. Experimental design and implementation: J.M.S., L.B.K., D.B.M., M.K., A.B., A.A., A.G., P.C.A., B.W., E.F., S.M.B., M.F. Data analysis and interpretation: J.M.S., L.B.K., D.B.M., M.K., A.B., A.A., S.M.B. Writing of manuscript: J.M.S., L.B.K., D.B.M., M.K., A.B., A.A., A.G., P.C.A., B.W., E.F., S.M.B., M.F. Final read and approval of manuscript: J.M.S., L.B.K., D.B.M., M.K., A.B., A.A., A.G., P.C.A., B.W., E.F., S.M.B., M.F.

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