

A phase 1 and pharmacokinetic study of enzastaurin in pediatric patients with refractory primary central nervous system tumors: a pediatric brain tumor consortium study

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Background. We sought to estimate the maximum tolerated or recommended phase 2 dose and describe the pharmacokinetics and toxicities of enzastaurin, an oral inhibitor of protein kinase C β , in children with recurrent central nervous system malignancies.

Methods. Enzastaurin was administered continuously once daily at 3 dose levels (260, 340, and 440 mg/m²) and twice daily at 440 mg/m²/day. Plasma pharmacokinetics were evaluated following a single dose and at steady state. Inhibition of protein kinase C and Akt cell signaling in peripheral blood mononuclear cells was evaluated. Akt pathway activity was measured in pretreatment tumor samples.

Results. Thirty-three patients enrolled; 1 was ineligible, and 3 were nonevaluable secondary to early progressive disease. There were no dose-limiting toxicities during the dose-finding phase. Two participants receiving 440 mg/m² given twice daily experienced dose-limiting toxicities of grade 3 thrombocytopenia resulting in delayed start of course 2 and grade 3 alanine transaminase elevation that did not recover within 5 days. There were no grade 4 toxicities during treatment. The concentration of enzastaurin increased with increasing dose and with continuous dosing; however, there was not a significant difference at the 440 mg/m² dosing level when enzastaurin was administered once daily versus twice daily. There were no objective responses; however, 11 participants had stable disease >3 cycles, 7 with glioma, 2 with ependymoma, and 2 with brainstem glioma.

Conclusion. Enzastaurin was well tolerated in children with recurrent CNS malignancies, with chromaturia, fatigue, anemia, thrombocytopenia, and nausea being the most common toxicities. The recommended phase 2 dose is 440 mg/m²/day administered once daily.

Keywords: brain tumor, enzastaurin, pediatric, pharmacokinetic, phase 1.

Central nervous system (CNS) tumors are the most common solid malignancy of childhood. In spite of significant improvements in therapy for pediatric malignancies over the past decades, pediatric CNS malignancy remains a leading cause of

death due to cancer.¹ Furthermore, acute and long-term morbidities from current therapies are significant.² Thus, novel therapeutic agents that target molecular abnormalities in these cancers are greatly needed.

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Enzastaurin is a potent oral serine/threonine kinase inhibitor of protein kinase C β , (PKC β) and the PI3K/Akt pathways.^{3,4} Activation of PKC β has been implicated in cell survival, growth, and invasiveness in solid and lymphoid malignancies.^{4,5} PKC β lies in the signal cascade of vascular endothelial growth factor (VEGF). By blocking these pathways, enzastaurin and its metabolites (LY326020 and LY485912) have been shown in a number of tumor models, including glioblastoma, to suppress tumor cell proliferation, induce tumor cell death, and inhibit tumor-induced angiogenesis.^{3,6-8} Enzastaurin has also been shown to enhance the antiangiogenic effects of radiation, making it an appealing agent for development in pediatric CNS malignancies.⁹⁻¹¹

Enzastaurin has been studied in adult clinical trials following once- or twice-daily dosing schedules at doses ranging from 20 to 900 mg/day.^{4,12-31} Oral doses of 500–525 mg have attained plasma exposures that exceeded a target concentration (1400 nM) associated with biologic activity.³⁰ More recently, twice-daily dosing has been shown to further increase enzastaurin exposure.^{20,23}

Initial phase 1 and 2 trials of enzastaurin in participants demonstrated encouraging antitumor activity in adults with glioblastoma.^{20,21} However, a subsequent phase 3 study in adults with recurrent glioblastoma failed to meet the endpoint of superiority over lomustine (CCNU). Nevertheless, enzastaurin was reasonably well tolerated compared with lomustine and had similar efficacy.¹² Further studies of enzastaurin in combination with radiation, cytotoxics, and anti-angiogenic agents are ongoing.

We report the results of a phase 1 study to evaluate once-daily dosing of enzastaurin in children with recurrent or refractory CNS tumors. The primary objectives were to estimate the maximum tolerated dose (MTD) and/or recommend a phase 2 dose of enzastaurin and to describe the toxicities and pharmacokinetics of the recommended dose of enzastaurin when administered twice daily. The secondary objectives were to characterize the toxicities, pharmacokinetics, antitumor activity, and impact of enzastaurin on protein kinase C and Akt cell signaling in peripheral blood mononuclear cells.

Participants and Methods

Eligibility

Children younger than age 22 years with histologically verified recurrent, progressive, or refractory primary CNS tumors (histology was not required for intrinsic brainstem or optic pathway tumors) and a Karnofsky (age >16 y) or Lansky (age \leq 16 y) performance status of \geq 60% were eligible for this study. Participants were required to have recovered from the acute toxic effects of prior therapy and could not to have received myelosuppressive chemotherapy within 3 weeks (6 weeks if prior nitrosurea), biologic antineoplastic agents within 7 days, hematopoietic growth factors within 7 days (14 day for long-acting formulations), craniospinal or radiation therapy (RT) to 50% or more of the pelvis within 6 months, other substantial bone marrow RT within 6 weeks, local small port RT within 2 weeks, or autologous bone marrow transplant (BMT) within 3 months. Patients receiving dexamethasone had to be on a stable or decreasing dose. Other eligibility criteria included adequate hematologic function (absolute neutrophil count \geq 1000/mm³,

platelets \geq 100 000/mm³, hemoglobin \geq 8 g/dL), age-appropriate renal function, and adequate hepatic function (albumin \geq 2.5 g/dL, bilirubin \leq 1.5 \times and alanine transaminase \leq 5 \times institutional upper limit of normal for age): Because the potential to prolong the corrected QT interval (QTc) was noted in some preclinical toxicology studies, patients were required to have a normal QTc for age and no clinically significant arrhythmias on electrocardiogram prior to registration. Exclusion criteria included receipt of other anticancer or experimental therapy, enzyme-inducing anti-convulsants or potent inducers, inhibitors or substrates of CYP3A4 or substrates of CYP2C8 or CYP2C9, known sensitivity to enzastaurin or its components, and any clinically significant unrelated systemic illness that would compromise their ability to tolerate protocol therapy. Patients who were pregnant or lactating were also excluded. Children with a body surface area less than 0.5 m² were excluded because of inability to deliver the prescribed dose based on the fixed increment of the formulation. The institutional review boards of each Pediatric Brain Tumor Consortium (PBTC) institution approved the protocol prior to initial participant enrollment, and continuing approval was maintained throughout the study. Patients or their legal guardians signed written informed consent, and assent was obtained as appropriate at the time of enrollment.

Drug Administration and Study Design

Enzastaurin was supplied as enzastaurin monohydrochloride tablets containing 125 mg of active drug by Eli Lilly. Enzastaurin was administered orally once daily within 30 minutes after the ingestion of food, at the same time each day in courses of 28 days duration. A dosing nomogram was used to minimize inter-participant dosing variability with tablets rounded to the nearest increment of a whole or half tablet. Tablets could be swallowed whole or crushed and administered with a small quantity of food.

The starting enzastaurin dose was 260 mg/m²/day, which was 80% of the adult recommended dose, with dose escalations to 340 and 440 mg/m². Dose escalation decisions between participant cohorts were based on toxicity observations during the first course of enzastaurin therapy. The 440 mg/m² dose level was expanded to further evaluate the toxicity and the pharmacokinetics of daily (7 participants) and twice-daily (220 mg/m² BID) dosing (14 participants). Participants consenting to pharmacokinetic studies received a single dose of enzastaurin on day –2 followed by pharmacokinetic sampling through day 1, when continuous daily enzastaurin dosing was initiated. In the absence of progressive disease or dose-limiting toxicity (DLT), enzastaurin dosing was continued without interruption for up to 26 courses.

Monitoring

Pretreatment evaluations included history and physical examination, assessment of performance status, MRI for disease evaluation, and basic laboratory studies including a pregnancy test for females of childbearing age. Participants were monitored with history, physical examination, and laboratory studies weekly during the first course and then at the start of each subsequent course, with weekly complete blood count continued through course 2. An electrocardiogram was obtained

pretherapy and at the end of course 1. Disease evaluations with MRI including perfusion, diffusion, and gradient echo pulse sequences were obtained at baseline, after courses 1, 3, and 5, and then at the end of every third course.

Trial Design

Eligible participants receiving at least one dose of therapy were evaluable for toxicity and efficacy. In the absence of toxicity, participants were required to complete the first course of therapy to be considered fully evaluable for estimating the MTD. The modified continual reassessment method was used to estimate the MTD.³² Since enzastaurin is an oral drug available in fixed dosage strengths, which result in variability in the dose deliverable compared with the target dose, the continual reassessment method allows for use of the actual delivered dose rather than the assigned dose level in modeling the dose-toxicity relationship.

Toxicities were graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE version 3.0)³³ DLTs were defined as any of the following drug related adverse events: any grade 4 neutropenia or thrombocytopenia; any grade 3 or 4 nonhematologic toxicity with the specific exclusion of grade 3 nausea or emesis of <4 days duration; grade 3 aspartate aminotransferase or grade 3 alanine transaminase elevation that returned to meet eligibility criteria within 5 days of drug interruption; grade 3 fever or infection ≤5 days duration; grade 3 hypophosphatemia, hypomagnesemia, or hypokalemia responsive to oral supplementation; asymptomatic elevations in amylase or lipase resolving to grade 1 within 5 days of drug interruption; any grade 2 nonhematologic toxicity that persisted for >5 days and was sufficiently medically significant or intolerable that it required treatment interruption; or any enzastaurin-related adverse event that resulted in delay of 8 days between courses or results in need for cessation of therapy for >5 days during the first course.

Standard 2-dimensional imaging criteria were used for response assessment. Response findings for stable disease or complete or partial response had to be maintained for at least 4 weeks and be accompanied by a stable or decreasing dexamethasone dose and a stable or improving neurological exam. Because enzastaurin is a potential cytostatic agent, participants could continue to receive enzastaurin despite a radiographic increase in tumor size (25%–49%) if the investigator and patient/family thought that the participant was benefiting from therapy.

Pharmacokinetics

Plasma samples for pharmacokinetic studies were obtained from consenting participants on days –2 and 28 of course 1. Starting on day –2, samples were obtained prior to and at 1, 2, 4, 6, 8, 24, 48, and 72 hours following a single enzastaurin dose. Samples were also obtained on day 28 of therapy, prior to and at 2, 4, 8, and 10–12 and 24 hours after the dose. In participants receiving twice-daily dosing, pharmacokinetic sampling was required, and the evening dose of enzastaurin was held on days –2 and 28. Blood samples (1 mL) were collected in EDTA, and the plasma was separated and stored at –20°C or lower. Plasma samples were analyzed at Advion BioServices Inc

for enzastaurin (LY317615) and its active metabolite (LY326020) using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS).⁴ Concentration versus time data were analyzed for pharmacokinetic parameters by noncompartmental methods using WinNonlin® Enterprise Version 5.3.

After a single dose, the maximum observed drug concentration (C_{max}), time of maximal observed concentration (t_{max}), area under the concentration versus time curve from zero to infinity ($AUC_{0-\infty}$), half-life associated with the terminal rate constant (λ_z) in noncompartmental analysis ($t_{1/2}$), apparent volume of distribution during the terminal phase after extravascular administration (V_z/F), and apparent total body clearance of drug calculated after extravascular administration (CL/F) were estimated for enzastaurin. C_{max} , t_{max} , $AUC_{0-\infty}$, $t_{1/2}$, and metabolic ratio (MR; $AUC_{0-\infty}$, LY326020/ $AUC_{0-\infty}$, enzastaurin) were estimated for LY326020. C_{max} , t_{max} and $AUC_{0-\infty}$ were also estimated for the total analyte (enzastaurin + LY326020). Similar parameters were also assessed at steady state.

Pharmacodynamic Studies

Correlative molecular biology studies were conducted to investigate the relationship between enzastaurin exposure and in vivo inhibition of Akt signaling. Blood samples (2 mL) were collected from consenting participants in heparinized tubes before enzastaurin and at days 14 and 28 in course 1. Peripheral blood mononuclear cells (PBMCs) were isolated using standard methods. Protein was then extracted from PBMC by direct lysis in protein lysis buffer, and total protein levels were quantified using a colorimetric assay (Pierce). The expression of total and phosphorylated versions of 7 Akt-pathway proteins was assessed by a multiplexed, Luminex bead-based assay. All reagents were purchased as a kit from Life Technologies. Proteins assayed, along with their respective sites of phosphorylation, were as follows: IR[pYpY1162?1163], IGF-1R[pYpY1135?1136], IRS-1[pS312], AKT[pS473], PRAS40[pT246], P70S6K[pTpS421?424], and GSK-3β[pS9]. The assay was performed according to the manufacturer's instructions, and beads were counted using a BioPlex 200 flow system (Bio-Rad). Relative phosphorylation scores were generated as a ratio of the mean fluorescent intensity for each phosphorylated protein versus the mean fluorescent intensity of its total counterpart.

We also assessed prestudy tumor samples for evidence of activation of the Akt pathway. Formalin-fixed, paraffin-embedded tumor sections (5 μm) were dewaxed and rehydrated prior to undergoing antigen retrieval in a rice steam-cooker (30 min; Target Retrieval Solution, Dako). Standard procedures were used to probe for pAkt and pS6 proteins (antibodies #3787 and #2211, Cell Signaling Technology). Primary antibodies were then detected (Vector Stain Elite kit, Vector Labs) and visualized by DAB staining (Dako), according to the manufacturers' instructions. Immunoreactivity in each section was scored quantitatively as a percentage of positive cells (0–100) and semiquantitatively as a measure of signal intensity (1–3, low to high).

Results

A total of 33 participants were enrolled in the study, and 29 were fully evaluable for toxicity. Three participants experienced

Table 1. Patient Characteristics (n = 33)

Characteristic	No. of Patients
Total patients enrolled	33
Eligible	32
Evaluable	29
Inevaluable	3
Ineligible	1
Diagnosis	
Ependymoma, anaplastic	6
Embryonal	
Medulloblastoma	1
Atypical teratoid/rhabdoid tumor	1
Pineoblastoma	1
Primitive neuroectodermal tumor	1
Glial tumors	
Pilocytic astrocytoma	2
Anaplastic astrocytoma	3
Glioblastoma	5
Astrocytoma, NOS (no grade provided)	4
Glioma, NOS (no grade provided)	3
Brainstem glioma	5
Meningioma	1
Sex	
Male	16
Female	17
Age (years, at study entry)	
Median	12
Range	3–21
Race	
Black	3
White, non-Hispanic	29
Multiple race (White/Asian)	1

Abbreviation: NOS, not otherwise specified.

disease progression prior to development of toxicity or completion of the first course and were therefore not fully evaluable. The treatment-related toxicities experienced by these participants while on therapy are, however, included in Table 2. One participant was determined to be ineligible due to initiation of therapy within insufficient time (<3 half-lives) after completion of bevacizumab therapy. This participant experienced rapid disease progression, developed an intratumoral hemorrhage on day 12 of enzastaurin therapy, and died the following day.

Demographics and Baseline Characteristics

Patient characteristics for all enrolled participants are outlined in Table 1. Overall, the median age was 11.6 years (range, 3–21 years). Males and females were nearly equally represented (48.5% and 51.5%, respectively). Performance status (Lansky or Karnofsky) was 100% in 51.5% of participants (n = 17) and ≥80% in 87.9% of participants (n = 29). In this study, anaplastic ependymoma was the most common disease type (18.2%), followed by brain stem glioma (15.2%), and glioblastoma multiforme (15.2%).

Adverse events

Enzastaurin was generally well tolerated with mostly low-grade toxicities, the most common treatment-related toxicities being urine color change (34%), anemia (34%), fatigue (25%), thrombocytopenia (25%), diarrhea (22%), and nausea (25%). Treatment-related toxicities grade 2 and higher are presented in Table 2. There were no DLTs observed in any of the 10 evaluable participants enrolled in the dose-escalation phase or in the expanded cohort of 7 participants receiving once-daily dosing at the maximum dose level of 440 mg/m²/day. All toxicities observed in eligible participants with once-daily dosing were grade 2 or lower with the exception of one participant with grade 3 lymphopenia. With twice-daily dosing at 440 mg/m²/day, 2 of the 12 evaluable participants experienced DLTs, which were grade 3 thrombocytopenia (decreased in grade within 1 day but resulted in a delay in the start of the next course) and grade 3 ALT elevation (decreased in grade but did not return to baseline within the protocol-defined time period). Although still well tolerated, there appeared to be a slight increase in toxicities observed with the twice-daily dosing schedule. Five deaths (15.2% overall) occurred within 30 days of discontinuation of enzastaurin: 1 at 260 mg/m/day, 2 at 440 mg/m/day daily, and 2 at 440 mg/m/day twice-daily dose. All deaths were related to tumor progression.

Response

No objective antitumor responses were reported. However, 11 (34%) participants had stable disease (>3 cycles): 5 with astrocytoma, 2 with ependymoma, 2 with brainstem glioma, and 2 with glioma (not otherwise specified). Two participants (glioma and brainstem glioma) were on treatment for longer than 12 months. The median time on therapy was 1.9 months (range, 0.4–17.5 months).

Pharmacokinetics

Pharmacokinetic data are available from 16 participants, 10 of whom received twice-daily dosing (see Table 3). There was significant interpatient variability in all variables evaluated.

There was accumulation of enzastaurin over time, with the C_{max} approximately doubling from day –2 to day 28. The concentration at steady state of enzastaurin increased with increasing dose; however, there was no significant difference at the 440 mg/m² dosing level when enzastaurin was administered once daily versus twice daily. The total analyte C_{avg,ss} for participants taking enzastaurin 440 mg/m² as a single daily dose or twice-daily dose (220 mg/m²/dose) were 2220 and 2110 nmol/L, respectively. The half-life of enzastaurin ranged from 8.7 to 15.3 hours. There were insufficient participants in each dose group to assess dose proportionality for C_{max} and AUC.

Correlative Biology

Sufficient archival tumor samples were available from 18 participants. Some degree of p-AKT expression was detected in 11 of 18 tumors, with 4 of the 18 samples having >50% of cells demonstrating at least moderately intense expression of the

Table 2. Adverse events attributed to enzastaurin during any course including the 30 days after discontinuation of treatment

Adverse Event	Grade 2				Grade 3/4	
	260 mg/m ² (n = 3)	340 mg/m ² (n = 3)	440 mg/m ² (n = 13)	440 mg/m ² bid (n = 13)	440 mg/m ² (n = 12)	440 mg/m ² bid (n = 12)
Anemia				1		1*
Leukopenia			1			
Lymphopenia			1	2	2	
Thrombocytopenia			1			2
Neutropenia			1	1		
Fatigue		1	1			
Hypotension			1			
Prolonged QTc				1		
Dyspnea						1
Diarrhea			1			
Vomiting				2		
Hypoalbuminemia	1			1		
Pancreatitis			1			
ALT(SGPT) Elevation						1
AST(SGOT) elevation				1		
Bilirubin increase	1					
GGT increase				1		
Proteinuria				2		

Abbreviations: ALT (SGPT), alanine transaminase; AST (SGOT), aspartate aminotransferase; bid, twice a day; GGT, gamma-glutamyl transpeptidase.

phosphorylated protein within the nucleus and cytoplasm (Figure 1). Immunohistochemical localization of p-S6 kinase was associated with the membrane in cells present in all 18 tumor samples, and 6 of 18 samples demonstrated that more than half of cells within the tumor had at least moderately intense staining. Thus, the Akt signaling pathway appears to be broadly expressed and is presumably active in several types of relapsed pediatric brain tumors.

To address whether enzastaurin had any effect on AKT pathway activation in PBMCs, we evaluated phosphorylation of Akt, p70S6K, and GSK-3 β in PBMCs collected from participants prior to enzastaurin and at 14 and 28 days of therapy by immunodetection. Out of 33 participants enrolled and treated, 18 had a baseline biology sample, 13 had both baseline and day 14 samples, 15 had both baseline and day 28 samples, and 10 had the biology sample at all 3 time points. Unfortunately, only 4 of these participants also had pharmacokinetics available for analysis, and it was not possible to show any statistically significant relationship between the pharmacokinetic parameters of AUC or C_{max} of enzastaurin and the levels of protein phosphorylation. When the relative phosphorylation of day 14 and 28 samples was analyzed, there was also no significant difference in the phosphorylation level of any of the proteins from baseline.

Discussion

This is the first study that evaluated enzastaurin in children and established the recommended phase 2 dose for enzastaurin in children as 440 mg/m²/day given once daily. This is about 50%

higher than the approximately 500 mg/day (~280 mg/m²/day) dosing used in most adult studies. Similar to findings in adult studies, enzastaurin was very well tolerated as a single agent.^{4,12,14,17-21,25} Most toxicities were grade 1 and grade 2, with chromaturia and fatigue being the most commonly reported toxicities. Also similar to previous reports in adults, twice-daily dosing resulted in an increase of toxicity, with the only DLTs noted during the study occurring on the twice-daily dosing schedule.

Extended pharmacokinetic sampling was performed in consenting participants, and an additional cohort of patients were enrolled with required pharmacokinetic studies at the recommended phase 2 dose following daily and twice-daily dosing. The mean total analytes C_{av,ss} for participants taking 440 mg/m²/day as single or twice-daily doses were similar and reached target efficacious concentrations of 1400 nM. In contrast to other studies, the increase in toxicity noted with twice-daily dosing was not explained by an increase in average concentration at steady state or other pharmacokinetic parameters at the dose level studied.^{13,21,23} Pharmacokinetic parameters were otherwise similar to those described in adult studies. Since the twice-daily dosing was associated with increased toxicity without increased exposure, we recommend the once-daily dosing for the pediatric patient population.

Immunohistochemical analysis of protein phosphorylation of Akt and P6s confirms that there is evidence of Akt pathway activation in most of the tumor samples analyzed and strong immunolocalization in more than half of the samples. However, it is not known whether this presumed activity is promoting tumor growth and if it is a pathway whose activity is essential

Table 3. Pharmacokinetic parameters following enzastaurin administration

	Single Dose (day -2), Geometric mean (CV%)				Steady State (day 28), mean (SD)		
	260 mg/m ² daily (n = 1)	340 mg/m ² daily (n = 1)	440 mg/m ² daily (n = 4)	440 mg/m ² bid (n = 10)	340 mg/m ² daily (n = 1)	440 mg/m ² daily (n = 4)	440 mg/m ² bid (n = 7)
Enzastaurin							
C _{max} (nmol/L)	790 (NC)	1418 (NC)	1020 (60)	1020 (74)	1406 (NC)	1920 (90)	1610 (111)
C _{avg, ss} (nmol/L)	(NC)	(NC)	(NC)	(NC)	680 (NC)	1060 (142)	11 150 (162) ^d
T _{max} (h) ^a	1.00 (NC)	8.00 (NC)	5.04 (2.00–6.00)	4.00 (1.00–8.00)	2.00 (NC)	3.00 (2.00–8.00)	2.05 (1.92–4.00)
t _{1/2} (h)	13.7 (NC)	8.68 (NC)	15.3 (37)	12.1 (43) ^c	(NC)	(NC)	(NC)
Cl/F (L/h)	112 (NC)	37.3 (NC)	73.2 (59)	32.2 (80) ^c	52.0 (NC)	51.3 (125)	43.7 (90) ^d
Vz/F (L)	2230 (NC)	466 (NC)	1620 (35)	564 (132) ^c	(NC)	(NC)	(NC)
AUC ^b (nmol · h/L)	5390 (NC)	22 800 (NC)	16 600 (88)	16 100 (79) ^c	16 300 (NC)	25 400 (142)	13 800 (162) ^d
LY326020							
C _{max} (nmol/L)	432	672	549 (23)	334 (30)	823	1210 (11)	922 (54)
C _{avg, ss} (nmol/L)	(NC)	(NC)	(NC)	(NC)	628	1000 (19)	832 (60) ^d
T _{max} (h)	2.00	8.00	5.0 4 (4.00–6.00)	6.00 (2.00–8.00)	4.00	3.00 (2.00–8.00)	2.00 (0.00–7.95)
t _{1/2} (h)	22.2	24.8	41.5 (78)	29.2 (32) ^c	(NC)	(NC)	(NC)
AUC ^b (nmol · h/L)	12 400	26 900	29 600 (55)	16 300 (36) ^c	15 100	24 000 (19)	9970 (60) ^d
Total Analyte							
C _{max} (nmol/l)	1116	2090	1570 (41)	1370 (55)	2195	3210 (54)	2590 (78)
C _{avg, ss} (nmol/l)	(NC)	(NC)	(NC)	(NC)	1310	2220 (76)	2110 (90)
T _{max} (h)	2.00	8.00	5.04 (2.00–6.00)	6.00 (1.00–8.00)	2.00	3.00 (2.00–8.00)	2.00 (0.00–4.00)
AUC ^b (nmol · h/L)	17 800	49 600	46 600 (66)	33 400 (50) ^c	31 400	53 300 (76)	25 300 (90)

^aMedian (Min-Max).^bReported AUC represents AUC(0–∞) for single dose and AUC_{τ,ss} at steady state (Tau = 12 for BID and 24 for QD).^cN = 9.^dN = 6.AUC (area under the curve); C_{avg, ss} (average concentration at steady state); C_{max} (maximum concentration); Cl/F (apparent clearance); %CV (coefficient of variability); NC (not calculated); SD (standard deviation); t_{1/2} (half-life); T_{max} (time to C_{max}); Vz/F (apparent volume of distribution).

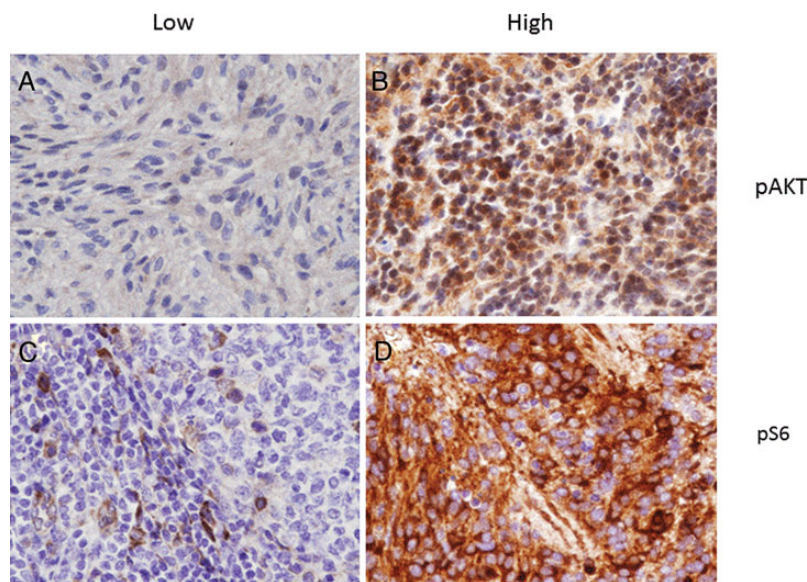


Fig. 1. Prominent expression of AKT signaling pathway components. Representative immunohistochemical localization of low and high expression of p-AKT within the nuclei of tumor cells from patients with meningioma (A) and medulloblastoma (B) respectively (magnification 100 \times). Also note, membranous immunolocalization of p-S6 kinase was present within the cells in all 18 tumor samples (representative samples from 2 patients with ependymoma are shown here, C and D). Variability of staining was noted within the tumor and demonstrated that more than half of cells within the tumor had at least moderate intensity of staining. Thus, the AKT signaling pathway appears to be broadly expressed and is presumably active in several types of relapsed pediatric brain tumors.

for tumor growth. Unfortunately, the sample numbers were so small that it was not possible to determine whether phosphorylation of these Akt pathway components in PBMCs would be useful for detecting biological activity of enzastaurin.

Although no objective responses were reported, 11 participants had stable disease, including a number of patients with gliomas (similar to prior experience in adults). Although enzastaurin demonstrated encouraging preliminary activity in adults with glioblastoma, results of the phase 3 study in recurrent glioblastoma were not impressive enough to continue development as a single agent in this patient population.^{12,20} However, preclinical studies have shown that enzastaurin enhances the activity of both cytotoxic and targeted agents in a variety of tumor types,^{34,35} and clinical trials evaluating its use in various combinations studies have been initiated.³⁶⁻⁴⁰

Preclinical studies of enzastaurin have also demonstrated that it enhances the effect of radiation in glioblastoma and other tumor models. Agents that sensitize tumors to RT are particularly appealing for the treatment of CNS malignancies because RT remains an important component of the treatment for most CNS malignancies. Early phase Clinical trials have been completed in adults with glioblastoma evaluating the use of enzastaurin in combination with RT.^{31,36,41}

The results of our study, combined with available preclinical data and information from adult studies, suggest that future clinical trials of enzastaurin in children should combine it with other agents or radiotherapy. Its favorable toxicity profile, availability as an oral agent, and novel mechanism of action make it a promising agent for combination therapy with RT, antiangiogenic, or traditional chemotherapy.

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