Pediatric Blood & Cancer

ONCOLOGY: RESEARCH ARTICLE

A POETIC Phase II study of continuous oral everolimus in recurrent, radiographically progressive pediatric low-grade glioma

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Abstract

Background: To evaluate efficacy, pharmacokinetics (PK) and pharmacodynamics of single-agent everolimus in pediatric patients with radiographically progressive low-grade glioma (LGG).

Methods: Everolimus was administered at 5 mg/m^2 once daily as a tablet or liquid for a planned 48-week duration or until unacceptable toxicity or disease progression. Patients with neurofibromatosis type 1 were excluded. PK and pharmacodynamic endpoints were assessed in consenting patients.

Results: Twenty-three eligible patients (median age 9.2 years) were enrolled. All patients received prior chemotherapy (median number of prior regimens two) and/or

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radiotherapy (two patients). By week 48, two patients had a partial response, 10 stable disease, and 11 clinical or radiographic progression; two discontinued study prior to 1 year (toxicity: 1, physician determination: 1). With a median follow up of 1.8 years (range 0.2-6.7 years), the 2-, 3-, and 5-year progression-free survivals (PFS) were $39 \pm 11\%$, $26 \pm 11\%$, and $26 \pm 11\%$, respectively; two patients died of disease. The 2-, 3-, and 5-year overall survival (OS) were all $93 \pm 6\%$. Grade 1 and 2 toxicities predominated; two definitively related grade 3 toxicities (mucositis and neutropenia) occurred. Grade 4 elevation of liver enzymes was possibly related in one patient. Predose blood levels showed substantial variability between patients with 45.5% below and 18.2% above the target range of 5-15 ng/mL. Pharmacodynamic analysis demonstrated significant inhibition in phospho-S6, 4E-BP1, and modulation of c-Myc expression.

Conclusion: Daily oral everolimus provides a well-tolerated, alternative treatment for multiple recurrent, radiographically progressive pediatric LGG. Based on these results, everolimus is being investigated further for this patient population.

KEYWORDS

brain tumor, clinical trial, everolimus, low-grade glioma, mTOR, phase 2

1 | INTRODUCTION

Low-grade gliomas (LGGs) are the most common tumor of the central nervous system (CNS) in children, and comprise a number of histologic subtypes.[1] While pediatric patients with LGGs have longer survival compared to patients with other CNS tumors,[2] the unique biology of these lesions results in numerous recurrences or progressions for many patients, necessitating additional therapies and consequently cumulative toxicities.[3] Complete surgical resection can be curative, but the tumor's location or infiltrative nature often makes this infeasible. For incompletely resected LGGs, radiation therapy was previously considered standard treatment but fell out of favor due to significant long-term toxicity with respect to neurocognitive impairment, endocrine dysfunction, secondary tumor risk, and stroke.[2] Thus, many centers attempt to defer irradiation with the use of chemotherapy, even for older children and adolescents.[3–6] Moreover, no standard therapy regimen exists for relapsed disease, although multiple regimens have been evaluated.[7–12]

The mammalian target of rapamycin (mTOR) serves as a pivotal signaling pathway regulating key cellular processes, including metabolism, protein synthesis, cell cycle progression, angiogenesis, and apoptosis and autophagy.[13,14] Patients with tuberous sclerosis (TS) have genetic alterations in *Tsc1/2* and exhibit dysregulation of mTOR/S6 kinase signaling, resulting in the development of subependymal giant cell astrocytomas. Everolimus, a macrolide derivative of rapamycin that selectively inhibits mTOR, is highly active in inducing regression of TS-associated SEGA and had been previously approved for that therapeutic indication.[15–17] Both neurofibromatosis type 1 (NF1)-associated and sporadic pediatric LGGs have demonstrated abnormal signaling upstream of mTOR through mutations in receptor tyrosine kinases, or more commonly for sporadic LGG, through alterations in *BRAF*.[18–21]

Given the well-tolerated toxicity profile of everolimus and central role of the Ras/Raf/mTOR pathways in pediatric LGGs,[22] we evaluated its activity in patients with radiographically progressive disease (PD).

2 | PATIENTS AND METHODS

Patients, ages 3-21 years, without NF1 or TS and with confirmed LGG histologies defined as a World Health Organization grade 1 pilocytic astrocytoma, grade 2 pilomyxoid, fibrillary, protoplasmic, or mixed astrocytoma, pleomorphic xanthoastrocytoma, infantile desmoplastic astrocytoma, ganglioglioma, oligodendroglioma, or mixed oligo-astrocytoma were eligible. Separate trials for NF1-associated LGG or TS-associated SEGA were available and thus those patients were excluded. Evidence of radiographically PD with at least one measurable lesion after at least one prior cancer-directed regimen (irradiation or chemotherapy with no upper limit of prior therapies) was required. Clinical symptom progression alone, such as deterioration of vision, was not adequate for trial entry. Lansky or Karnofsky performance score ≥50% was required. Patients had to have recovered from prior therapies and could not be taking strong inducers or inhibitors of cytochrome P450, family 3, subfamily A (CYP3A) to avoid potential confounding factors based on prior conflicting studies suggesting patients with certain genetic polymorphisms may or may not require higher doses of drug to achieve adequate trough concentrations with rapamycin-based therapies.[23,24] At least 4 weeks from prior chemotherapy (6 weeks if it included a nitrosourea), 6 months from radiation therapy, and at least 2 weeks or five half-lives, whichever was longer, for biologic agents were required for eligibility. Physiologic steroid and nonenzyme-inducing anticonvulsants were permitted. Patients were required to have adequate organ function defined as an

absolute neutrophil count (ANC) $\geq$1000/μL, platelets $\geq$100 000/μL, serum creatinine no more than the upper limit of normal for age (ULNFA), bilirubin $\leq$1.5 $\times$ ULNFA, transaminases $\leq$2.5 $\times$ ULNFA, serum albumin >2 g/dL, and an international normalized ratio of <1.3. Patients were also required to have a fasting low-density lipoproteins (LDL) cholesterol $\leq$ULNFA, a fasting serum cholesterol $\leq$300 mg/dL or $\leq$7.75 mmol/L, and a fasting triglyceride of $\leq$2.5 $\times$ ULNFA. Exclusion criteria included chronic, systemic corticosteroids or other immunosuppressive agents (topical or inhaled corticosteroids allowed), severe and uncontrolled underlying medical conditions, pregnant or breast-feeding females, patients with gastrointestinal malabsorption conditions, patients previously treated with another mTOR inhibitor, and patients with prior documented hepatitis B or C infection.

This protocol (NCT00782626), conducted under IND 104003, was approved by Novartis, the POETIC Consortium operations center at Memorial Sloan Kettering Cancer Center and the Dana-Farber/Boston Children's Hospital Institutional review board, as well as those of all participating sites. All patients or their legal guardians provided written informed consent and/or assent as appropriate at enrollment.

Everolimus was administered as a tablet or oral liquid at a standard dose of 5 mg/m^2 once daily, either fasting or after a light, fat-free meal, in 28-day cycles for a planned duration of 12 cycles (48 weeks). Dose modifications were based on the common terminology criteria for adverse events (CTCAE) v3.0 criteria (http://ctep.cancer.gov/forms/CTCAEv3.pdf), except for hyperlipidemia and pneumonitis. Grade 2 toxicities permitted dose interruption followed by retreatment at full dose, while grade 3 toxicities resulted in treatment interruption followed by a reduction to a lower dose level (dose level 1: 3 mg/m^2; dose level 2: 2 mg/m^2). Hyperlipidemia was treated using diet and medical management with lipid-lowering agents rather than discontinuing treatment. Grade 2 noninfectious pneumonitis required dose interruption and possible addition of systemic steroids with reinstitution of therapy at a lower dose level, while grade 3 noninfectious pneumonitis required therapy discontinuation. The management algorithms for stomatitis and noninfectious pneumonitis are provided in the supplemental data (see Table S1). For hematologic toxicities, the following criteria were used: (a) platelets $\geq$50 000/μL and <75 000/μL required a dose interruption until recovery to $\leq$grade 1, at which point everolimus was restarted without dose reduction; if the toxicity recurred, subsequent therapy was reduced by one dose level; (b) platelets $\geq$25 000/μL and <50 000/μL required dose interruption until $\leq$grade 1, and everolimus was resumed at one dose level below; recurrent grade 3 thrombocytopenia resulted in therapy discontinuation, and grade 4 thrombocytopenia required discontinuation of treatment; (c) for an ANC between $\geq$500/μL and <1000/μL, everolimus was held until ANC recovery to $\leq$grade 2, at which point everolimus was restarted at full dose. If toxicity recurred, everolimus was held until recovery to $\leq$grade 2, and restarted at one dose level below. If toxicity recurred, everolimus was discontinued. For grade 4 ANC, everolimus was held until recovery to $\leq$grade 2, then restarted at a lower dose, and if it recurred, everolimus was discontinued. For febrile neutropenia with ANC grade 3, everolimus was held until afebrile and ANC recovery to $\leq$grade 2. The dose was then reduced by one level. If febrile neutropenia recurred, everolimus was discontinued. For grade 4 febrile neutropenia, everolimus was discontinued.

To assess the pharmacokinetics (PK) profile of everolimus in children with LGG, everolimus whole blood concentrations were centrally determined by a validated high performance liquid chromatography tandem mass spectroscopy assay with stable-isotope dilution analysis. The interassay imprecision of the method was 15.4%, 10.7%, 6.9%, and 6.4% expressed as % coefficient of variation for the quality control samples at the lower limit of quantification (1 ng/mL), the low, medium, and high concentrations, respectively. Correspondingly, the intra-assay imprecision was 3.6%, 11.1%, 3.96%, and 4.1%, respectively. Blood everolimus concentrations were determined predose, and at 2 and 5 h postdose. PK analysis was performed using MW/Pharm clinical software (version 3.82, Mediware, Prague, Czech Republic). PK parameter estimates were generated using a previously published two-compartment pediatric PK model as the Bayesian prior.[25] PK parameter estimates such as clearance and volume of distribution were allometrically scaled to body weight to account for body size differences among patients.[26,27] True trough concentration at 24 h postdose and area under the concentration-time curve (AUC) for 24 h of dosing interval were generated for each individual patient.

Pharmacodynamic analysis was also undertaken in consenting patients. Whole blood (2 mL) was obtained from study patients in ethylenediaminetetraacetic acid (EDTA) tubes prior to initiating therapy on day 7 ($\pm$3) and day 14 ($\pm$3) of course 1, prior to start of courses 3, 5, 7, 9, and at completion of treatment (end of course 12). A total of eight samples were drawn when possible from each patient before everolimus administration and on treatment days 15, 28, and 62. Peripheral blood mononuclear cells (PBMCs) were isolated from each sample using the Ficoll reagent and stored at $-80°$C prior to analysis. Total protein was analyzed by Western blotting for levels of phospho-p70 S6 kinase (Thr389), p70 S6 kinase, phospho-S6 ribosomal protein (Ser235/236), S6 ribosomal protein, phospho-4E-BP1 (Thr70), and c-Myc (9E10): sc-40. Antibodies were obtained from Cell Signaling Technology, Inc., and Santa Cruz Biotechnology. Preliminary correlations of PK with changes in pharmacodynamic parameters including p70s6 kinase activity in PBMCs were evaluated as previously reported in other populations treated with mTOR inhibitors.[25,28,29]

2.1 | Response evaluation

All patients were evaluated by magnetic resonance imaging (MRI) within 21 days prior to treatment initiation; PD was defined based on comparing scans from baseline to that demonstrating best response. On-therapy imaging was performed after course 1, every three courses thereafter, and at completion of therapy. All imaging underwent blinded central radiographic review. Due to the complexity of pediatric LGG appearance on MRI and in keeping with the guidelines set forth by the POETIC Consortium, tumor response was not strictly determined by change in enhancement on postcontrast T1 images; measurable change in size and extent of the target lesion(s) was required on fluid-attenuated inversion recovery/T2 sequences or on

TABLE 1 Patient characteristics (n = 23)

Characteristic	Median (range)
Age at enrollment (years)	9.2 (3.8-17.1)
Age at diagnosis (years)	4.3 (0.3-11.8)
Age at treatment (years)	9.2 (3.8-17.1)
Weight at enrollment (kg)	28 (13-61)
Body height (cm)	129 (99-163)
Body surface area (m^2)	1 (0.6-1.62)
	N (%)
Race	
White	20/22 (91)
Black	1/22 (5)
Other	1/22 (5)
Unknown	1
Tumor location	
Brainstem	1 (4)
Frontal lobe, spinal cord	2 (9)
Hypothalamus	2 (9)
Hypothalamus, basal ganglia	1 (4)
Midbrain, thalamus	1 (4)
Optic chiasm	3 (13)
Optic chiasm, thalamus	1 (4)
Optic nerve	1 (4)
Optic pathway	1 (4)
Posterior fossa	2 (9)
Right thalamus/brain stem	1 (4)
Suprasellar	2 (9)
Suprasellar cisternae	1 (4)
Temporal lobe	1 (4)
Temporal lobe, cerebellar peduncle, posterior cranial fossa	1 (4)
Thalamus	2 (9)
Number of prior chemotherapy regimens	
One	2 (9)
Two	8 (35)
More than two	13 (56)
Gender	
Male	6 (26)
Female	17 (74)

precontrast T1 images. The decision to evaluate stable disease (SD) within the response criteria reflects the resultant tumor stabilization for pediatric LGGs that may occur with inhibition of the AKT pathway, as had been observed with other chemotherapy regimens. Response for target lesions was based on three dimensions with an elliptical model volume used (0.5 L*W*T; L: tumor extent in plane perpendicular to the selected plane; W: longest measurement of the tumor width;

T: transverse measurement perpendicular to the width). Complete responses (CR) represented disappearance of all target lesions and no new lesions. Partial responses (PR)correlated to ≥65% decrease in sum of the products from baseline. PD correlated to 40% or more increase in any target lesion (referent smallest product observed on therapy). SD was neither sufficient decrease nor increase to meet other criteria. While radiographic PD was required for study entry (symptom progression was not sufficient), development of clinical progression even in the context of SD on MRI resulted in removal of patient from protocol therapy and was considered PD. Off-therapy scans were performed as per institutional standard.

2.2 | Statistics

The primary objective of the protocol was to determine if treatment demonstrated a response rate ≥25%, which would be considered promising for further study. A response rate <5% was considered evidence of an inactive regimen. A minimum of 20 patients with evaluable radiographic progressive LGG were required. Assuming 15% of patients might not be evaluable or eligible, 23 patients were required for enrollment. Centrally reviewed response assessment was based on the presence of a CR, PR, or SD after completion of therapy, and included patients' responses for those who came off treatment early for toxicity. A patient's best response was utilized; patients who demonstrated PD within the 12 cycles of protocol therapy were counted as PD for statistical analysis, even if they had initially responded. A one-stage design was selected assuming the response rate was likely to occur slowly given the biologic nature of these tumors. If at least three responders were present among the 20, then everolimus would be considered promising for future studies. If the true response rate was 25%, the chance of concluding that the treatment was active would be 0.91 (power), with type I error rate 0.08.

Bioanalytical analysis of PK samples was conducted using validated assays with samples from 22 of 23 patients. Plasma concentrations were summarized by descriptive statistics, including mean, standard deviation, coefficient of variation, minimum, maximum, and median. PK parameters, including clearance, volume of distribution, predose trough (C_{trough}), and AUC were evaluated. Correlations of response with changes in pharmacodynamic parameters including inhibition of p70s6 kinase activity, 4E-PB1, and c-Myc in PBMCs were performed. Toxicity of everolimus was descriptive. Tumor tissue assessment of mTOR targets included assessment of pS6, p4EPB1, and pEIF4G expression. A companion biology protocol (PI Karajannis) was developed to obtain tumor material, if available, using immunohistochemical staining intensity (graded 0-3) as previously described.[30] Blinded analysis was then performed on the samples.

3 | RESULTS

This protocol accrued 23 eligible and evaluable patients between September 2009 and September 2011. Characteristics of the patients

TABLE 2 Number and proportion of patients, by response* based on 2D radiographic imaging (n = 23)

Response	N (%)	Median (range) time to best response (months)	Time to best response (if CR/PR/SD) or time to PD (if PD) (months)
CR	0 (0)	NA	NA
PR	2 (13)	2.3 (0.9-3.7)	0.9, 3.7
SD	10 (48)	0.9 (0.7-3.6)	0.7, 0.8, 0.8, 0.9, 0.9, 0.9, 0.9, 0.9, 1.1, 3.6
PD within 48 weeks**	11 (39)	0.95 (0.8-22.2) (n = 10)	0.8, 0.9, 0.9, 0.9, 0.9, 1.0, 2.5, 3.6, 7.3, 22.2

Abbreviations: CR, complete response; NA, not applicable; PD, progressive disease; PR, partial response; SD, stable disease.

*Patients who demonstrated PD within 48 weeks of protocol therapy were counted as nonresponders.

**One patient with best response of SD at week 17 but less than 48 weeks follow up (went off study for toxicity) was classified as having PD (nonresponder), because it was not possible to rule out PD by week 48. The time to PD is unknown for this patient.

are provided in Table 1 with 17 females (74%), six males (26%), and an age range of 3.8-17.1 years (median age 9.2 years). The median age at initial diagnosis was 4.3 years (range 0.3-11.8 years). Median body surface area at study entry was 1 m^2 (range 0.6-1.62 m^2). All patients had previously received a chemotherapy-containing regimen (median number of prior treatment regimens two), and two had received prior radiation therapy. Performance status at trial entry ranged from 60% to 100% (median 90%). Patients received 2-12 cycles (median 10) of everolimus. Fifteen patients completed 12 cycles.

The response rate was 52.2% (12/23) with two PRs and 10 SD by end of cycle 12. Ten patients progressed prior to cycle 12 despite SD at an earlier time in the trial. One additional patient with SD at week 17 was classified as a nonresponder (PD) due to less than 48 weeks of follow-up (Table 2). For the 12 responders, the median time to best response was 0.9 months (range 0.7-3.7 months). For the 10 patients reporting PD, progression occurred at a median of 0.95 months (range 0.8-22.2 months). Ultimately, 14 patients developed PD. Applying the one-stage rule, with 10 responders of the first 20 evaluable patients, there is evidence to support continued study of everolimus. Assessment of response by the institutional radiologist agreed with the blinded central review performed by a single pediatric neuroradiologist. There were 14 events, including two patients who died. The 2-, 3-, and 5-year progression-free survivals (PFS) were 39 ± 11%, 26 ± 11%, and 26 ± 11%, respectively. The 2-, 3-, and 5-year overall survival (OS) were each 93 ± 6% (Figure 1). The median follow-up time of patients without an event was 1.8 years (range 0.2-6.7 years).

Overall, treatment was well tolerated by the majority of patients (see Table S2). Seventeen patients had at least one grade 3 (n = 15) or grade 4 (n = 5) toxicity; six of these 17 experienced at least one grade 3 or 4 toxicity attributed to the therapy. Grade 4 elevation in liver enzymes was deemed possibly related to everolimus; no grade 5 events occurred. There was one episode of grade 3 unrelated pneumonitis. Three grade 3 toxicities definitely attributed to everolimus therapy included mucositis and neutropenia in one patient and mucositis in another patient.

PK data were available for 22 of 23 patients. Everolimus PK profiles exhibited substantial interpatient variability (Figure 2 and Table 3). Comparably, the observed trough concentrations in patients with TS were below 5 ng/mL in 45.5% of patients with 18.2% of concentrations above 15 ng/mL.[15] Estimates of oral clearance and oral volume
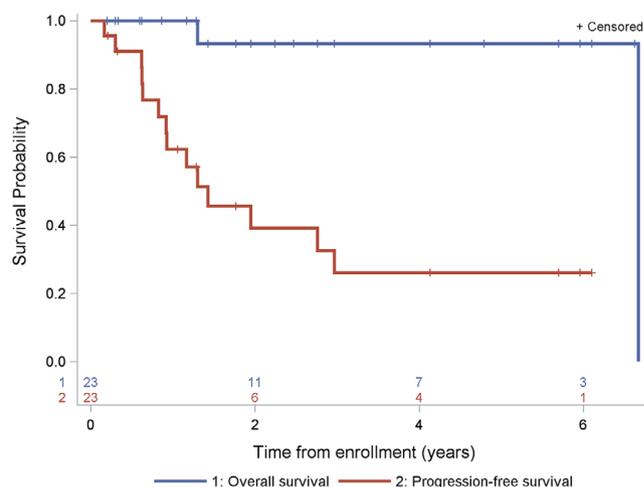

FIGURE 1 Kaplan-Meier curves of progression-free survival and overall survival (n = 23)

of distribution of the central compartment (Vc/F: 36.7 ± 20.2 L) were comparable with findings in the previous Phase I study of everolimus in pediatric patients with refractory solid tumors (Table 3).[25]

The pharmacodynamic activity of everolimus was assessed by analysis of inhibition of phosphorylation of S6, p70S6K, and eukaryotic translation initiation factor 4E binding protein 1 (p-4E-BP1), through IHC and expression of c-Myc (Figure 3). Pharmacodynamic analysis of tumor samples obtained prior to enrollment on this study was performed in eight cases (35%). Significantly decreased levels of phosphorylated 4E-BP1 and S6K were observed at the end of the first week of therapy; inhibition persisted through the duration of the study. While analysis of tumor mTOR inhibition, which would require biopsy of these brain tumors, could not be justified due to the risks involved in this patient population, the notable response rate suggests that the PK levels achieved were clinically relevant and similar to those observed in patients with TS responsive to everolimus.[15]

4 | DISCUSSION

Everolimus, a once daily oral mTOR inhibitor, demonstrated single agent activity in pediatric patients with radiographically progressive
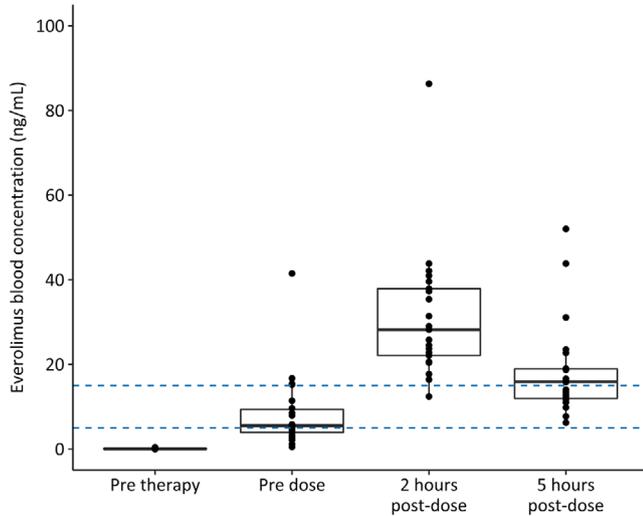
FIGURE 2 Effective RAD001 (everolimus) systemic exposure/pharmacokinetics (mean ± standard error of the mean) (n = 22)

TABLE 3 Everolimus pharmacokinetic parameter estimates

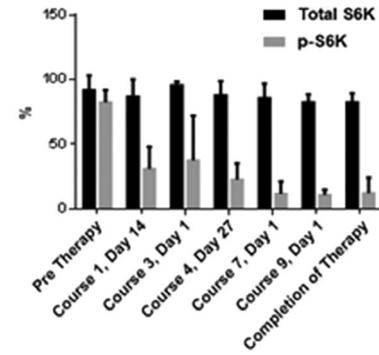| Parameters	Mean	SD
C_{trough} (ng/mL)	7.9	8.8
AUC_{0-24h} (ng h/mL)	400	256
CL/F (L/h/70 kg)	29.6	14.0
Vc/F (L/70 kg)	84.1	30.3
Q/F (L/h/70 kg)	67.5	24.4
Vp/F (L/70 kg)	473	187
K_a (per h)	2.00	0.61

Abbreviations: AUC_{0-24h}, area under the concentration-time curve for time 0-24 h; C_{trough}, estimated trough blood concentration at 24 h postdose; CL/F, oral clearance; K_a, absorption rate constant; Q/F, oral intercompartmental clearance; Vc/F, oral volume of distribution of the central compartment; Vp/F, oral volume of distribution of the peripheral compartment.

FIGURE 3 Pharmacodynamic markers of everolimus. A, Sustained modulation of S6K phosphorylation. B, c-MYC expression. C, Progressive modulation of 4E-BP1 phosphorylation. Data were normalized to highest value within each patient group using two-way repeated measures ANOVA (n = 8)

LGGs after standard chemotherapy and/or radiotherapy. After up to 1 year of treatment, 74% of patients maintained tumor growth arrest or shrinkage, and showed no clinical progression. The therapy was easily administered as capsules or liquid, allowing for accurate dosing in all patients, did not require central venous access for administration, and was well tolerated in most patients.
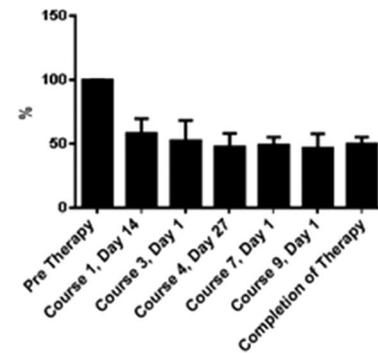
Chemotherapy is standard treatment for pediatric patients with unresectable progressive LGGs.[3] Variable response rates have been observed among differing drug regimens, yet the majority of patients do experience periods of tumor growth arrest interspersed with periods of progression requiring treatment. Despite periods of tumor progression, the majority of patients will be long-term survivors,[2] reinforcing the importance of developing well-tolerated regimens without severe lifelong toxicities.[6]

Our initial understanding of pediatric LGGs was derived from two common genetic diseases, NF1 and TS, for which affected patients have a unique clinical course related to mutations in neurofibromin
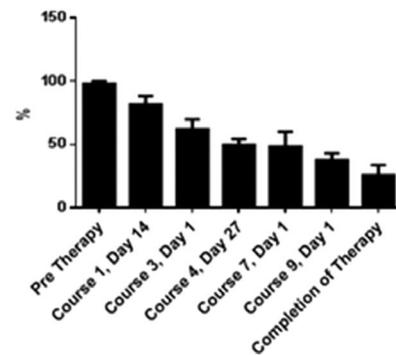
and TSC1/TSC2, respectively.[31-34] Sporadic pediatric LGGs rarely have mutations in these genes; rather, their mutations are found predominantly within BRAF in one of the two common forms. Highly enriched in posterior fossa pilocytic astrocytomas, but identified in all pediatric LGG variants, is the truncated fusion event of BRAF, resulting in loss of its inhibitory domain with translocation to the KIAA1549 gene.[35-37] A less frequent abnormality found across the different subtypes of pediatric LGGs is the BRAF V600E point mutation. Based on these data, pediatric LGG is considered a RAS/RAF/mTOR pathway disease,[38] and consequently mTOR inhibitors, having shown significant activity in TS

patients (for which they are FDA approved), were suggested as treatment for those with sporadic progressive LGG.

mTOR is a downstream component of the PI3/AKT pathway and has two primary substrates, e1F-4E binding protein (4E-BP1) and p70 S6 kinase 1 (S6K1), which regulate translation of important messages, including those encoding the HIF-1 proteins, C-MYC, ornithine decarboxylase, cyclin D1, and the ribosomal proteins themselves. The drug is effective at nanomolar concentrations, and extensive pharmacologic testing in adults and children undergoing organ transplantation has demonstrated overall stable AUCs, supporting the use of standardized dosing. Based on body size, the pharmacology of everolimus is similar in adults and children,[39] and efficacy in oncology trials is evident at clinically relevant doses.[40,41] Real-time PK analysis has been validated, and the range of 5-15 ng/mL is considered optimal.[42] Specifically, PK-pharmacodynamic modeling based on inhibition of a peripheral molecular marker (S6 K1 activity in PBMCs) suggests that 5-10 mg daily in adults should be an adequate dose to produce a high degree of sustained target inhibition. Moreover, pharmacodynamic studies investigating changes in the molecular pathology of biopsied tumor by immunohistochemistry in treated patients at 5 and 10 mg daily are associated with dephosphorylation of protein effectors known to be immediately downstream of mTOR, such as S6 and 4EBP1. Pharmacodynamic analysis of tumor samples in our eight pediatric cases at a dose of 5 mg/m^2 was consistent with sustained modulation of S6K phosphorylation and c-Myc expression and progressive modulation of 4EBP1. This near-total S6 and partial 4EBP1 inhibition observed in patients mirrors the results observed preclinically in the in vivo models in which everolimus demonstrated clear antitumor activity.

It is difficult to compare response rates between LGG studies for a number of reasons. Generally speaking, tumor histologies are heterogeneous. The spontaneous cessation of growth by LGGs over time also complicates the analysis of this patient population; however, we opted to evaluate SD within the response criteria unlike other studies. All patients were required to have evidence of radiographic progression at time of enrollment to better measure the drug's true effect on tumor growth. The relationship to BRAF was not yet known at the time of this trial and therefore not incorporated into the analysis or outcomes, further complicating comparisons to more recent studies. Therefore, from a statistical point of view, we cannot make a valid comparison of outcomes between trials, given the number of variables among the studies.

Certainly, clinicians may ask how these results fit into the treatment of LGG in the era of MAPK/MEK/BRAF inhibition. Taking into consideration the 5-year OS and PFS, this drug compares similarly to other standard chemotherapy trials for refractory disease utilizing vinblastine or upfront trials utilizing carboplatin/vincristine.[6,9,43–45] It is difficult to make comparative statements regarding other targeted agents like selumetinib and trametinib, however, for whom the early data are descriptive in terms of sustained PRs or includes combined cohorts of low-grade patients treated with different BRAF/MEK inhibitors.[46–48]

In conclusion, everolimus does demonstrate activity in pediatric patients with radiographically progressive LGGs and provides another potential option for patients with recurrent disease. Its limited, reversible toxicity and administration as a pill or liquid preparation obviates the need for intravenous access. Furthermore, demonstration of synergy between everolimus and carboplatin in pediatric LGG cell lines and slowed tumor growth in the in vivo pediatric LGG models support its potential utility in future multiagent protocols.[49] Lastly, a separate recently completed Phase II study of everolimus by the Pacific Neuro-Oncology Consortium requiring tissue at enrollment may provide further insight into its relevance for molecular subtypes of pediatric LGG while addressing quality-of-life measures and functional outcomes, which are now recognized as paramount to pediatric LGG assessments of response.

CONFLICT OF INTEREST

Mark W. Kieran served as a paid scientific advisory board member and received travel support from Novartis Pharmaceutical.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Furthermore, results are available at www.clinicaltrials.gov, NCT00782626.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.