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A Phase I and surgical study of ribociclib and everolimus in children with recurrent or refractory malignant brain tumors: A Pediatric Brain Tumor Consortium Study

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Abstract

Purpose: Genomic aberrations in cell cycle and PI3K pathways are commonly observed in pediatric brain tumors. This study determined the maximum tolerated dose (MTD)/Recommended phase 2 Dose (RP2D) of ribociclib and everolimus and characterized single-agent ribociclib concentrations in plasma and tumor in children undergoing resection.

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Conflict of Interest: Ira J. Dunkel is a consultant or member of an advisory board for Apexigen, Astra-Zeneca, Bristol-Myers Squibb/Celgene, Fennec, QED and Roche. The remainder of the authors declare no potential conflicts of interest.

Experimental Design: Patients enrolled in the Phase I study according to a rolling 6-design and received ribociclib and everolimus daily for 21 and 28 days, respectively. Surgical patients received ribociclib at the pediatric RP2D (350 mg/m²) for 7–10 days pre-operatively followed by enrollment on the phase I study. Pharmacokinetics were analyzed for both cohorts.

Results: Sixteen patients enrolled on the phase I study (median age 10.3 years; range: 3.9–20.4) and 6 on the surgical cohort (median age 11.4 years; range: 7.2–17.1). Thirteen patients enrolled at dose level 1 without dose-limiting toxicities (DLT). Two of the three patients at dose level 2 experienced DLT (grade 3 hypertension and grade 4 ALT). The most common grade 3/4 toxicities were lymphopenia, neutropenia, and leucopenia. The RP2D of ribociclib and everolimus was 120 mg/m² and 1.2 mg/m² for 21 and 28 days, respectively. Steady state everolimus exposures with ribociclib were 2.5-fold higher than everolimus administered alone. Ribociclib plasma, tumor concentrations and CSF samples were collected. The mean tumor-to-plasma ratio of ribociclib was 19.8 (range: 2.22–53.4).

Conclusions: Ribociclib and everolimus were well tolerated and demonstrated similar pharmacokinetic properties as in adults. Potential therapeutic ribociclib concentrations could be achieved in CSF and tumor tissue, although interpatient variability was observed.

Introduction

Brain tumors are the leading cause of cancer-related death in children. Outcome for recurrent or refractory malignant brain tumors remains poor, despite aggressive multimodal therapy. Improved understanding of the genomic landscape of brain tumors has spurred development of novel therapeutics aimed at targeted inhibition of cancer-promoting pathways.¹

High-throughput sequencing studies in many tumor types have identified focal gains of CDK4/6 and Cyclin D1–3 and/or homozygous loss of CDKN2A, implicating cell cycle regulation in tumor pathogenesis.² Cell cycle dysregulation has been detected in approximately 60% of diffuse intrinsic pontine glioma (DIPG) and high grade glioma (HGG)³ with wide variation in reports about Rb mutation or deletion, ranging from 0–31%.^{4–6} CDK6 overexpression and CDKN2A loss have been detected in 25–30% embryonal tumors with rare incidence of Rb mutations⁷ and may serve as independent prognostic markers of poor overall survival.^{8–10} *p16 loss* has been reported in 25% of ependymomas yet is more prevalent in infants and associated with poor prognosis.^{11,12}

The PI3K pathway is commonly activated in human cancer^{13–15} and downstream of Akt is the mammalian target of rapamycin (mTOR), a ubiquitous serine threonine kinase involved in cell cycle, angiogenesis, and apoptosis.^{16,17} Activation of PI3K pathway has been detected in 26–79% DIPG and HGG,^{3,18–20} 72% of ependymomas,²¹ and *PTEN* loss has been identified in medulloblastomas.^{22,23}

Ribociclib (LEE011; Novartis Pharmaceuticals) is an orally bioavailable CDK 4/6 inhibitor and induces G1 cell-cycle arrest by hypo-phosphorylation of Rb demonstrating dependence on a functional Rb protein for efficacy.^{24,25} Ribociclib is FDA-approved for the treatment of advanced breast cancer in combination with letrozole.²⁶ A pediatric phase I study in

neuroblastoma, malignant rhabdoid tumors, and other tumors with cell cycle aberrations reported the recommended phase 2 dose (RP2D) to be 350 mg/m² daily for 21 days every 28 days with best response as stable disease. The most common adverse event (AE) was myelosuppression.²⁷

Everolimus (RAD001; Novartis Pharmaceuticals) is an inhibitor of the mammalian target of rapamycin (mTOR), specifically targeting the mTOR-raptor signal transduction complex 1 (mTORC1) reducing tumor cell proliferation, glycolysis and angiogenesis. Everolimus is FDA-approved for advanced renal cancer,²⁸ subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis,²⁹ and in combination with exemestane for advanced hormone-positive, HER2-negative breast cancer.³⁰ The pediatric maximum tolerated dose (MTD), as a single agent, was 5 mg/m² continuous daily and resulted in prolonged stable disease in 1/3 of subjects including those with gliomatosis cerebri, osteosarcoma, DIPG, peripheral PNET, anaplastic astrocytoma, and ependymoma.³¹ The DLTs included hypokalemia, hypophosphatemia, elevated ALT, mucositis, and diarrhea, similar to adult studies.^{28,30}

Everolimus-associated stomatitis results in dose delays, reductions or discontinuation of the therapy resulting in compromise of duration and intensity of therapy.³² Topical steroids may improve healing of everolimus-associated aphthous ulcers^{33,34} and resulted in the SWISH trial.³⁵ After 8 weeks of receiving dexamethasone oral solution four times daily in addition to everolimus and exemestane, the incidence of grade 2 or worse stomatitis was 2% vs 33% in the historical control group.³⁰ As a secondary objective, we assessed dexamethasone-based mouthwash for prevention of stomatitis in pediatric patients who received ribociclib and everolimus in combination.

When combined with everolimus, ribociclib induced synergistic growth inhibition in multiple tumor models (RD-2012–50383) providing the justification for an adult Phase I study with ribociclib, everolimus and exemestane (CLEE011X2106/[NCT01857193](#)). The RP2D for the combination was defined as 300 mg/day ribociclib (3-weeks-on/1-week-off) + 2.5 mg/day everolimus (continuous) + 25 mg/day exemestane (continuous) with food³⁶ with reports of 51% stable disease. A dose-dependent drug interaction was observed between ribociclib and everolimus based on clinical pharmacokinetic analyses, where everolimus exposure increased 2- to 4-fold in the presence of ribociclib^{37,38} suggesting that lower doses of both drugs may also be used in the pediatric population.

Here, we report a phase I trial assessing safety and define the MTD/ RP2D of ribociclib and everolimus in combination and a surgical study evaluating ribociclib intra-tumoral PK at the single agent RP2D dose 350 mg/m² in children with recurrent malignant brain tumors (PBTC-50).

Methods

Experimental Design

The primary objectives of the phase I study (PBTC-050, [NCT03387020](#)) were to determine the MTD and/or RP2D of ribociclib and everolimus in children, describe toxicities,

characterize ribociclib and everolimus pharmacokinetics and evaluate for dose-dependent drug interaction. The primary objective of the surgical study was to characterize plasma and intra-tumoral ribociclib concentrations in children with recurrent or refractory brain tumors undergoing neurosurgical resection. Secondary objectives were to describe the response rate of relapsed and refractory malignant brain tumors to ribociclib and everolimus in the context of a phase I study and descriptively assess dexamethasone-based mouthwash for prevention of stomatitis in pediatric patients receiving everolimus therapy.

Eligibility

Common eligibility criteria for the phase I and surgical study included patients 1 but 21 years of age with recurrent, progressive, or refractory brain tumors. All tumors, except radiographically typical DIPG (defined as a tumor with a pontine epicenter and diffuse involvement of more than 2/3 of the pons) were required to have histologic verification of high grade glioma (HGG), medulloblastoma, CNS embryonal tumor (NOS), Ependymoma, or ATRT.³⁹ Patients with low-grade gliomas were excluded. Pre-trial tumor tissue from all patients (except typical DIPGs) were screened for intact Rb by immunohistochemistry (IHC) in a CLIA-certified laboratory. Molecular genomics were not required for eligibility.

For the surgical study, eligible patients included those for whom surgical resection was clinically indicated and were amenable to receiving 7–10 days of ribociclib before surgery. Surgical patients who met treatment initiation criteria for the Phase 1 component post-surgical recovery were enrolled on the current dose level of enrolled non-surgical patients. Additional eligibility criteria for both phase I and surgical studies included: Lansky (< 16 years) or Karnofsky (> 16 years), performance scores at least 50%; recovery from acute toxicities of all prior therapy; > 3 months since craniospinal radiotherapy (>24Gy), > 2 weeks since local palliative radiotherapy, > 3 months since autologous stem cell transplantation; adequate laboratory data, including bone marrow function (hemoglobin > 8 g/dL, absolute neutrophil count > 1,000/mm³, platelets > 100,000/mm³ transfusion-independent, defined as no platelet transfusion within a 7-day period prior to enrollment), renal function (age-adjusted normal serum creatinine or glomerular filtration > 70 ml/min/1.73 m²), liver function (total bilirubin < 1.5 times institutional upper limit of normal for age, ALT [SGPT] and AST [SGOT] < 3 x institutional upper limit of normal for age, albumin > 2g/dL), and on stable or decreasing steroid dosing for at least one week prior to enrollment. Neurological deficits must have been stable for a minimum of 1 week prior to enrollment. Patients with controlled seizures on non-enzyme inducing anticonvulsants were eligible. Patients of childbearing or child fathering potential must have agreed to use medically acceptable form of birth control while on study.

Patients were excluded for the following: pregnancy; concurrent illness that would compromise the patient's ability to tolerate therapy, other current malignancy, investigational agent; on potent CYP3A4 inducers/inhibitors; significant active cardiac disease, hypertension, uncontrolled heart disease, history of cardiac dysfunction, cardiomyopathy, left ventricular ejection fraction < 50%, QTc > 450 ms; on warfarin or other coumadin-derived anticoagulant.

Ethics

The study was designed and conducted in accordance with U.S. Common Rule, Belmont report and US Code of Federal Regulations Title 21. Ethical principles were in accordance with the Declaration of Helsinki. The study protocol, informed consent and assent were approved by institutional review board of participating institutions according to institutional guidelines. All patients (or their guardians) provided written informed consent prior to study entry.

Treatment Regimen, Drug Administration, and Dose Escalation

Ribociclib and everolimus were supplied by Novartis Pharmaceuticals. Ribociclib was provided as 50mg and 200mg capsules or liquid formulation as 30mg/mL and everolimus as 2mg-oral dissolving tablet. The ribociclib liquid formulation was administered via gastric tube or nasogastric tube. Ribociclib capsule dosing was rounded to the nearest 50 mg and liquid formulation (30mg/mL) was rounded to the nearest 50 mg (1.5 mL). Everolimus was provided as 2mg dispersible tablet and dosing was rounded to the nearest 0.1mg. On the phase I study, ribociclib was administered daily for 21 days followed by a 7-day break and everolimus was administered daily for a 28 day cycle. Patients were encouraged to take ribociclib and everolimus at the same time each day

The dose levels were determined in order to accommodate the smallest capsule sizes of 50 mg. While the increments between ribociclib dose levels were larger than 30% which is commonly used, this was necessary to avoid overlaps between the dosages. No intra-patient dose escalation was permitted. The starting dosage for dose level 1 for ribociclib was 120 mg/m² and everolimus 1.2mg/m² with a BSA 0.75 m². Dosage escalation was governed by the Rolling-6 statistical design.⁴⁰ Only DLTs observed during the dose-finding period (cycle 1) of therapy were used to guide dose escalation/de-escalation. In the absence of disease progression or unacceptable toxicity, patients could receive up to 2 years (26 cycles) of treatment.

Surgical patients received ribociclib at the pediatric RP2D (350mg/m²)²⁷ daily for 7–10 days prior to tumor resection. Patients were re-assessed for the Phase I study 2–4 weeks post-surgery and enrolled on the dose level that was concurrently open to enrollment in Phase I.

Dexamethasone mouthwash was incorporated in the treatment plan for at least the first 2 courses of therapy. All patients received alcohol-free, 5–10mL (0.5 mg/5 mL) dexamethasone steroid mouthwash swish and spit 3 times daily (TID) starting on cycle 1, day 3 (initiation of combination therapy). Patient remained NPO (nothing in mouth) for at least an hour after administering mouthwash, with the exception of Nystatin (or another topical antifungal). Subsequent cycles did not mandate mouthwash, yet patients could continue as tolerated. Saltwater mouth rinse (0.9%) as 10mL swish and spit TID prior to the dexamethasone mouthwash (10–15 minutes) was administered once the onset of Grade 1 stomatitis was noted. If the patient was unable to swish and spit mouthwash, a swab sponge was used. Prevention of stomatitis with dexamethasone-based mouthwash during cycle 1 and 2 was descriptively defined by reporting the frequency and grade of stomatitis events.

Toxicity Assessment and Monitoring

Toxicity monitoring included weekly physical examination and laboratory evaluations (complete blood count, serum chemistries, and renal and hepatic functions) during course 1 and prior to each subsequent course. Lipid profiles were monitored every other course. EKG monitoring at baseline, day 15, course 1 and prior to courses 2, 3 and then prior to every 3rd cycle. Echo evaluation was obtained at baseline, prior to course 3 and as clinically indicated thereafter. Disease evaluations with MRI were obtained at baseline and every 8 weeks for the first 6 courses and every 12 weeks thereafter through treatment or progression. Central review of MRI studies was planned if objective responses were observed.

Definition of Dose Limiting Toxicity and Maximum Tolerated Dose

DLTs were graded according to version 5.0 of the NCI Common Terminology Criteria for Adverse Events unless specified otherwise in the protocol (e.g. Pediatric hypertension, mucositis, QTc prolongation, hepatic toxicities). A hematological DLT attributable to ribociclib and/or everolimus that required dose reduction was defined as grade-3 thrombocytopenia or grade-4 neutropenia lasting > 7 consecutive days or myelosuppression that causes a delay > 14 days between treatment cycles. A non-hematologic DLT was defined as grade-3 AST/ALT and/or bilirubin, grade 2 QTc, mucositis, pneumonitis; and any grade-2 non-hematologic toxicity persisting for 7 days and considered medically significant or sufficiently intolerable. The following grade-3 non-hematologic toxicities were not considered DLTs: nausea, vomiting, fever, or infection lasting < 5 days; hypophosphatemia, hypokalemia, hypocalcemia, or hypomagnesemia responsive to oral supplementation; hyperlipidemia, and anorexia.

The MTD was defined based on the Rolling-6 design⁴⁰ as the highest dose level at which six patients have been treated with at most one patient experiencing a DLT and the next higher dose level has been determined to be intolerable. Once the MTD was estimated or the RP2D determined, 6 additional patients were treated at that dose level to better describe the toxicity profile of the agent. Per protocol, moving surgical patients to Phase I treatment were not subject to slot limits.

Patients were evaluable for estimating the MTD if they received at least one dose of ribociclib and everolimus and were taken off treatment for toxicity during the first cycle (dose finding period). In the absence of toxicity, patients must have received approximately 85% (18/21 doses of ribociclib and 22/26 doses of everolimus) of prescribed therapy during the dose-finding period. Patients must have completed all of clinical and laboratory monitoring requirements specified by the protocol for them to be considered evaluable for MTD.

Response Criteria

All eligible patients who had measurable disease present at initiation of study treatment, received at least one dose of the study drugs, and had disease re-evaluated were evaluable for response assessment using the standard 2-dimensional imaging criteria as previously reported.⁴¹ A complete response (CR) or partial response (PR) had to be maintained for at

least 8 weeks, accompanied by a stable or improving neurologic examination and a stable or decreasing dose of corticosteroid

Pharmacokinetics (Phase I and Surgical Study)

Pharmacokinetic sampling in Phase I study—Pharmacokinetic studies were conducted in all patients enrolled on the Phase I study to characterize the disposition of ribociclib in plasma and everolimus in blood, administered alone and in combination. On Day 1 of Course 1, serial ribociclib single-dose blood samples were collected for up to 48 hours post-dose. The ribociclib dose on day 2 was held for the pharmacokinetic studies. On Day 17, serial ribociclib and everolimus steady state blood samples were collected for up to 24 hours post-dose. On Day 1 of Course 2, serial everolimus blood samples were collected for up to 24 hours post-dose. Optional single or serial pharmacokinetic studies in cerebrospinal fluid (CSF) were performed on consenting patients with access to CSF.

Pharmacokinetic sampling in Surgical study—Pharmacokinetic studies were performed on all patients enrolled on the Surgical study to measure ribociclib concentrations in plasma, brain tumor tissue, and CSF. Ribociclib blood samples were collected before the surgery: prior to starting ribociclib therapy (i.e., Days –7 to –10 days), prior to ribociclib doses on Day –5 (± 1 day), and Day –2 (± 1 day), and on the morning day of surgery (Day 0). During the surgery, a blood sample, ~50 to 100 mg of fresh flash-frozen tumor tissue sample, and an optional CSF sample were collected at the time of tumor resection.

Sample processing, bioanalysis, and pharmacokinetic analysis—Ribociclib and everolimus blood samples were collected in K₂-EDTA vacutainer tubes. Ribociclib blood samples were centrifuged at 10,000 rpm for 2 minutes to extract plasma. All samples were stored at –80°C until analysis. Ribociclib total concentrations in plasma, tumor, and CSF, and everolimus total concentrations in blood were determined using validated LC-MS/MS methods^{42,43}. The lower limit of quantification (LLOQ) for plasma ribociclib was 0.058 μ M then reduced to 0.023 μ M. The LLOQ values for ribociclib CSF and tumor samples were both 0.23 nM, and the LLOQ value for everolimus blood samples was 1.04 nM. Single and steady-state plasma ribociclib and blood everolimus concentration-time data were analyzed using a non-compartmental pharmacokinetic approach with Phoenix WinNonlin (v.8.2) software package. Additional details are provided in Supplementary Materials.

Pharmacodynamics (surgical patients)

Pharmacodynamics were evaluated in tumor tissue of consented patients for evaluations of ribociclib treatment on Ki-67 (marker of cellular proliferation) by IHC comparing archival and post-treatment (after 7–10 days of ribociclib) tumor tissue Ki-67 was evaluated as previously described.⁴⁴

Immunohistochemistry for Rb

All diagnostic tumor samples were evaluated for Rb protein by IHC using FFPE samples as previously described.⁴⁵ in a CLIA certified lab – either at Cincinnati Children's Hospital Medical Center (CCHMC) or locally. Unstained slides containing at least 50% tumor cells and a corresponding H & E stained slide were analyzed and tumor Rb protein status was

denoted as “positive” if 20% of tumor cells had positive nuclear staining in at least three 20x-fields evaluated per case. Rb+ endothelial cells served as an internal positive control. All slides were centrally reviewed by the Neuropathologist (C.F.).

Results

Patients

Between January 2018-April 2020, 17 patients were screened and 13 were eligible and enrolled on the study. Additionally, 4 patients with progressive DIPG and 5 patients with prior RB test results were enrolled. Sixteen eligible patients enrolled on the phase I study (median age 10.3 years; range: 3.9–20.4) and 6 patients were enrolled on the surgical cohort (median age 11.4 years; range: 7.2–17.1) (Table 1). Five patients enrolled on the phase I study were in-evaluable for DLT (4 received < required study drugs and 1 patient withdrew prior to beginning study therapy). One of the 6 surgical patients was deemed ineligible due to no evidence of recurrent tumor in tissue after surgery. The remaining 5 surgical patients had clinical genomics on primary and/or recurrent tumor. Two patients had evidence of CKDN2A/B loss. Two patients had BCOR aberrations and 1 patient did not have analyses completed due to specimen quality issues.

Toxicities

Twenty patients started treatment and were evaluable for toxicity, and sixteen patients were evaluable for DLT assessment (11 enrolled in the phase I, 5 enrolled in the surgical cohort). All 5 surgical patients who initiated treatment on the Phase 1 trial were treated at dose level 1. The observed DLTs are summarized in Table 2. Initially, at dose level 1 (ribociclib 120mg/m² × 21 days every 28 days; everolimus 1.2 mg/m² days 1–28), 0/5 patients experienced a DLT prompting dose escalation. However, 2 of 3 patients enrolled on dose level 2 (ribociclib 170mg/m² × 21 days every 28 days; everolimus 1.2 mg/m² days 1–28) developed dose limiting toxicities including grade 3 hypertension and grade 3 hepatotoxicity (elevated ALT). The patient with grade 3 hypertension was also on dexamethasone. The MTD was exceeded on dose level 2 and 8 additional patients were treated under dose level 1 as an expansion cohort and none experienced a DLT establishing the MTD for ribociclib as 120mg/m² × 21 days every 28 days and everolimus 1.2 mg/m² days 1–28. The expansion cohort included one additional subject resulting in 13 total patients treated at dose level 1 due to one patient having moved from the surgical cohort. The median number of cycles at dose level 1 was 2 (range: 1–24). All AEs are outlined in Table 3. Grade 3/4 AE at least possibly related to ribociclib and/or everolimus related occurred in 9 (45%) patients and are outlined in Table 4. The majority of the grade 3/4 AEs were at least possibly related to both study drugs with the exception of two grade 3 elevated cholesterol attributed to everolimus and one grade 3 hypokalemia attributed to ribociclib.

Similar to the toxicity profile in adults,⁴⁶ grade 3/4 AEs at least possibly related to ribociclib and/or everolimus in 20 patients who received both drugs, related to myelosuppression with leukopenia (20%), lymphopenia (30%), neutropenia (25%) the most common. Neither grade 3 nor grade 4 thrombocytopenia was observed. Grade 3 or higher mucositis was not observed. No patient died due to toxicities.

Treatment Response

Eighteen patients were evaluable for treatment response as 2 patients came off therapy before follow-up imaging was obtained. No patients had an objective response to ribociclib and everolimus and 16 patients developed disease progression (clinical and/or imaging) on therapy. One patient removed from study due to a DLT had stable disease. One patient had a partial response in course 21, yet not sustained and developed progression of disease in subsequent course 24: this patient was diagnosed with recurrent anaplastic ependymoma and was treated at dose level 1 for 24 cycles. Given the patient's BSA, the actual dose received (ribociclib 300 mg; everolimus 2.8 mg) was more aligned with the adult RP2D (ribociclib 300 mg; everolimus 2.5 mg). Six patients died due to tumor progression.

Pharmacokinetics

Phase I pharmacokinetic studies—Ribociclib pharmacokinetic studies were performed for 20 patients. The single dose and steady state profiles of one patient were considered unevaluable due to excessive data below the LLOQ. For logistical reasons, steady state profiles were incomplete, and thus, removed for two patients. Everolimus samples collected on Course 1 Day 17 and Course 2 Day 1 were inadvertently processed to plasma for two patients and thus, were excluded. Therefore, everolimus Course 1 Day 17 pharmacokinetic data were available for 18 patients, of which two profiles with missing time-points were unevaluable. Everolimus Course 2 Day 1 data were only available for 12 patients as 6 patients went off treatment before Course 2. No drug interruption was noted between Courses 1 and 2 for 4 patients, whom pharmacokinetic data were all considered at steady state. However, for the remaining patients, the start of Course 2 was delayed, mainly due to toxicities, from 2 days to one month. Thus, the associated everolimus pharmacokinetic data were not considered at steady state.

A summary of the single-dose and steady-state pharmacokinetic parameters for plasma ribociclib and blood everolimus is shown in Tables 5a and 5b, and the concentration-time profiles are depicted in Figures 1 and 2. Large inter-individual variability was observed for both drugs. The mean $\pm$ sd accumulation of ribociclib from single dose alone to steady state in combination, assessed by AUC_{0–24h} ratios, was 2.1 $\pm$ 1.0-fold across dose levels. For everolimus, the slope of the terminal elimination phase was not ideally characterized, as the extrapolated AUC from 24 hours represented >20% of total AUC_{0– ∞} for twelve patients. The mean $\pm$ sd accumulation of everolimus from single dose alone (Course 2 Day 1 delayed) to steady state in combination was 2.8 $\pm$ 0.9-fold across dose levels. Steady state everolimus AUC_{0–24h} when given in combination with ribociclib were about 2.5-fold higher than steady state everolimus AUC_{0–24h} administered alone (Course 2 Day 1 not delayed).

Surgical pharmacokinetic studies—Ribociclib pharmacokinetic studies were performed in 6 patients, of which one has tissue that did not exhibit tumor. On the day of surgery, ribociclib plasma and tumor concentrations were available for 5 patients, and ribociclib CSF samples were collected in 4 patients. These samples were all collected between 19.6 and 23.4 hours after the last ribociclib dose (350 mg/m²) prior to surgery. Large variability was observed among patients for plasma, CSF, and tumor ribociclib

concentrations (Table 6). At the time of surgery, the mean tumor-to-plasma ratio of total ribociclib (Kp) was 19.8 (ranged from 2.22 to 53.4).

Surgical pharmacodynamic studies—Ki-67 was performed in 4 consented patients. After 7–10 days of ribociclib therapy, the most drastic changes in Ki-67 post treatment with ribociclib were 5% in recurrent tumor tissue compared to 40% in archival tissue. The overall range of Ki-67 in tumor tissue noted on IHC was 1–30% compared to 2–40% in archival tissue.

Discussion

Aberrant cell cycle regulation and activation of the PI3K pathway represent important mechanisms of malignant potential in pediatric brain tumors. Joint inhibition of these pathways with ribociclib and everolimus represents a strong candidate combination due to biologic rationale, non-overlapping single-agent toxicities, and clinical experience in adults that suggested tolerability of this combination.³⁶ We report the first study to define the MTD of ribociclib and everolimus in children. The MTD of ribociclib in combination with everolimus was 120 mg/m² days 1–21 and 1.2 mg/m² days 1–28 respectively. This dosage was approximately 70% ribociclib and 80% everolimus of the adult RP2D combination.⁴⁶ The DLTs were elevated ALT and hypertension. The majority of toxicities were myelosuppression in this pre-treated patient population, consistent with the experience in adult patients.³⁶

Everolimus associated mucositis may require dose modifications and dexamethasone mouthwash may minimize the associated toxicity.³² During the first 2 courses, dexamethasone mouthwash was required and no grade 3 or 4 mucositis was observed. Since very few patients proceeded beyond course 2, no conclusion can be drawn regarding decreasing risk of mucositis; yet including a mouthwash as a supportive agent is reasonable.

No sustained objective response to ribociclib and everolimus was reported on this study. Notably, a patient with multiply recurrent anaplastic ependymoma had a partial response and prolonged stable disease for 24 courses. This patient was initially diagnosed with posterior fossa ependymoma (group B, RELA negative) status post gross total resection followed by radiation and chemotherapy and developed recurrent disease 4 years later outside of initial radiation field (right suprasellar cistern around the MCA and a drop metastasis to T7-T8). The patient received radiotherapy to suprasellar cistern followed by ribociclib and everolimus for 24 courses. Both lesions remained stable on therapy and then developed progression in the metastatic spinal lesion. Patient discontinued study drugs and proceeded with surgery followed by focal radiotherapy to the spine. Prolonged stable disease may be confounded by radiotherapy, yet since the spinal lesion was not initially irradiated, ribociclib and everolimus may have contributed to prolonged stabilization.

This is the first report of pharmacokinetic parameters for the combination of ribociclib and everolimus in children with brain tumors. The pediatric population exhibited high interpatient pharmacokinetic variability for both drugs. Compared to the previous pediatric phase I study evaluating ribociclib alone as 280 to 470 mg/m²/day, ribociclib exhibited

similar dose-dependent pharmacokinetic behavior with rapid absorption ($T_{MAX} \sim 2$ hours).²⁷ The range of ribociclib steady state half-lives (7.3 to 17.3 hours) was lower than that reported by Geoerger and colleagues (30 to 41 hours), which may result from differences in the steady-state pharmacokinetic sampling strategy; however, the accumulation of ribociclib from day 1 alone to steady state in combination with everolimus ($\sim 2.1 \pm 1.0$ -fold) agreed with previous studies in children and adults receiving ribociclib alone^{27,47}. Overall, there was no observed evidence of an impact of everolimus on ribociclib pharmacokinetics. When administered alone, everolimus pharmacokinetic profiles in this pediatric population were comparable with those reported in children and adults^{42,48,49}. Median single dose and steady state half-lives for everolimus alone (11.4 to 18.6 hours) agreed with the median values reported in pediatric patients with refractory solid tumors (15.9 to 22.2 hours).^{42,48} Steady state exposures of everolimus combined with ribociclib were about 2.5-fold higher than that of everolimus alone. This may suggest an impact of ribociclib on everolimus pharmacokinetics, although results should be interpreted with caution due to the large interpatient variability. This potential drug-drug interaction was previously observed in a Phase Ib trial evaluating the same drug combination in women with metastatic breast cancer, where everolimus exposure following 2.5 mg daily increased 2 to 4-fold in the presence of 300 mg ribociclib.⁴⁶ This drug-drug interaction is likely to be related to the CYP3A4 enzyme, which plays a role into the metabolism of both drugs.

In vitro IC_{50} values for inhibition of CDK4/6 were determined at 0.01 to 0.039 μM for ribociclib.⁵⁰ No previous pharmacokinetic parameters have been reported for ribociclib in the CSF among children with cancer. Our results showed that following ribociclib treatment at 350 $mg/m^2/day$, 7–10 days prior to surgery, potential therapeutic ribociclib concentrations could be achieved in CSF (range 0.0096 to 0.139 μM) and tumor tissues (range 0.678 to 4.81 nmol/g or μM), although results were variable among patients. Ribociclib brain tumor concentrations were in agreement with those reported in two other studies in adult patients with recurrent glioblastoma.^{51,52} When quantified 4–8 hours after the last steady state dose of 600 mg ribociclib daily prior to surgery, total ribociclib tumor concentrations ranged from 5.8 to 48.9 nmol/g with a mean of 23.4 nmol/g.⁵¹ Following 24 hours after five days of ribociclib dosing at 900 mg daily, mean unbound ribociclib concentrations were found at 0.35 and 1.22 nmol/g in non-enhancing and enhancing tumor, corresponding to total concentrations of ~ 6.4 and 22.1 nmol/g considering a mean unbound fraction of 5.5%.⁵² In those two adult studies, ribociclib CSF concentrations ranged from 0.043 to 0.63 μM . In a recent study, ribociclib CSF concentrations quantified in an 8.4-year-old patient with epithelial sarcoma receiving 3.33 mg/kg ribociclib daily ($\sim 120 mg/m^2$) ranged from 0.042 to 0.045 μM .⁵³ It should be noted that in our study, the ribociclib CSF concentrations do not correlate with the total concentrations measured in tumor tissue, which might result from differences in the extent of tumor protein binding between the patients. Based upon the results of these phase I and surgical studies, and the lack of everolimus influence on ribociclib pharmacokinetics, we could expect at the lower ribociclib dosage (120 mg/m^2) to still achieve ribociclib concentrations above the IC_{50} in tumor tissue. In the absence of everolimus CSF or tumor tissue concentrations, we cannot assess if mTOR inhibition is completely achieved at the tumor site at the everolimus 1.2 mg/m^2 dosage used in this study.

However, we observed that everolimus blood concentrations were overall above the in vitro IC50 values for mTOR inhibition (1 to 5 nM) for at least 12 hours post-dose (Figure 2).

The results from this study define the MTD, toxicity, pharmacokinetics of ribociclib and everolimus and CSF pharmacokinetics of ribociclib in pediatric patients with recurrent or refractory malignant brain tumors. The MTD of this combination may inform future studies evaluating efficacy in patients with tumors harboring specific mutations. This combination is also being evaluated in newly-diagnosed diffuse intrinsic pontine gliomas (DIPG) and high-grade gliomas (HGG) following radiotherapy ([NCT03355794](#)).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of Translational Relevance:

Joint inhibition of CDK4/6 and mTOR has potential to benefit children and adolescents with aggressive brain tumors. Non-overlapping single-agent toxicities and clinical experience in adults suggested tolerability of ribociclib and everolimus. The present study defines the maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) of ribociclib and everolimus in pediatric patients with recurrent or refractory brain tumors and evaluates the pharmacokinetics and drug-drug interactions. A surgical cohort is included evaluating ribociclib concentrations in tumor and plasma in children with recurrent or refractory brain tumors undergoing neurosurgical procedures and confirms potential therapeutic ribociclib concentrations could be achieved in CSF and tumor tissues. The current study will inform future studies evaluating efficacy in patients with tumors harboring specific mutations and may serve as a basis for future combinations of ribociclib and everolimus or ribociclib in combination with other agents.

Figure 1. Ribociclib plasma concentration-time data after single dose on Course 1 Day 1 (green) and at steady-state on Course 1 Day 17 (black). Each circle represents individual concentrations. Dotted lines represent the lower limit of quantification (0.023 μM).

Figure 2.

Everolimus blood concentration-time data at steady-state on Course 1 Day 17 (black), and on Course 2 Day 1 after drug interruption (light blue) or at steady state (dark blue). Each circle represents individual concentrations. Dotted lines represent the lower limit of quantification (1.04 nM)

Table 1:

Patient Characteristics

	<i>Phase I (N=16)</i>		<i>Surgery (N=6)</i>	
AGE (Years)	At Initial Diagnosis	At Study Entry	At Initial Diagnosis	At Study Entry
Median	9.3	10.3	9.6	11.4
Minimum	1.3	3.9	6.1	7.2
Maximum	19.3	20.4	15.0	17.1
	<i>N</i>	<i>%</i>	<i>N</i>	<i>%</i>
GENDER				
Female	6	37.50	1	16.7
Male	10	62.5	5	83.3
ETHNICITY				
Hispanic or Latino	1	6.3	1	16.7
Not Hispanic or Latino	10	62.5	3	50.0
Unknown	5	31.3	2	33.3
RACE				
Asian	0	0	1	16.7
Black or African American	2	12.5	0	0
White	10	62.5	4	66.7
Not Reported/Unknown	4	25.0	1	16.7
DIAGNOSIS	N	%	N	%
Anaplastic astrocytoma	2	12.5	2	33.3
CNS primary tumor, NOS	5	31.3	1	16.7
Diffuse Intrinsic Pontine Glioma *	4	25.00	0	0
Ependymoma, NOS	2	12.50	1	16.7
Glioblastoma multiforme	3	18.8	1	16.7
Medulloblastoma	0	0	1	16.7

* Tumor type did not require immunohistochemistry for Rb; all other tumor types were screened and tested positive for Rb expression based on immunohistochemistry.

NOS, not otherwise specified.

Table 2:

DLT Summary

Dose Level (mg/m ² /dose)	Number of Treated Patients *	Number of Evaluable Patients	Number of Patients with DLTs	Description of DLTs
Ribociclib 120; Everolimus 1.2	5	5	0	
Ribociclib 170; Everolimus 1.2	3	3	2	Grade 3 Pediatric Hypertension (n=1) Grade 3 Alanine aminotransferase increased (n=1)
Ribociclib 120; Everolimus 1.2	12	8	0	

* Enrolled 22 patients, 20 patients were treated. One patient enrolled on surgical cohort deemed ineligible and 1 patient withdrew prior to receiving investigational agents.

Table 3:

All Adverse Events Observed at Dose Level 1 (ribociclib 120mg/m² × 21 days every 28 days; everolimus 1.2 mg/m² days 1–28) which occurred in 10% patients; regardless of attribution

All Patients (N = 17)						
Category/Event Description	Grade					
	1	2	3	4	5	All grades (% patients)
Blood and lymphatic system disorders						
Anemia	6	3	0	0	0	9 (53%)
Gastrointestinal disorders						
Abdominal pain	2	0	0	0	0	2 (12%)
Mucositis oral	2	2	0	0	0	4 (24%)
Nausea	5	0	0	0	0	5 (29%)
Vomiting	4	0	1	0	0	5 (29%)
General disorders and administration site conditions						
Disease progression	0	0	0	0	2	2 (12%)
Fatigue	4	4	0	0	0	8 (47%)
Gait disturbance	0	3	1	0	0	4 (24%)
Pain	0	1	1	0	0	2 (12%)
Investigations						
Alanine aminotransferase increased	5	1	0	0	0	6 (35%)
Aspartate aminotransferase increased	3	0	0	0	0	3 (18%)
Cholesterol high	4	0	0	1	0	5 (29%)
Electrocardiogram QT corrected interval prolonged	2	0	0	0	0	2 (12%)
Lymphocyte count decreased (Lymphopenia)	4	4	2	1	0	11 (65%)
Neutrophil count decreased (Neutropenia)	3	4	3	0	0	10 (59%)
Platelet count decreased (Thrombocytopenia)	3	2	0	0	0	5 (29%)
Weight loss	1	1	0	0	0	2 (12%)
White blood cell decreased (Leukopenia)	9	0	3	0	0	12 (71%)
Metabolism and nutrition disorders						
Hyperglycemia	2	0	0	0	0	2 (12%)
Hypertriglyceridemia	2	0	0	0	0	2 (12%)
Hypokalemia	1	0	2	0	0	3 (18%)
Hyponatremia	3	0	0	0	0	3 (18%)
Hypophosphatemia	4	0	0	0	0	4 (24%)
Musculoskeletal and connective tissue disorders						
Muscle weakness left-sided	0	0	2	0	0	2 (12%)
Nervous system disorders						
Ataxia	0	0	2	0	0	2 (12%)
Depressed level of consciousness	0	0	2	0	0	2 (12%)

All Patients (N = 17)						
Category/Event Description	Grade					
	1	2	3	4	5	All grades (% patients)
Dysgeusia	0	2	0	0	0	2 (12%)
Headache	3	0	0	0	0	3 (18%)
Muscle weakness left-sided	0	0	2	0	0	2 (12%)
Seizure	0	1	2	0	0	3 (18%)
Vascular disorders						
Hypertension	1	3	0	0	0	4 (24%)
Maximum Grade Any Adverse Event						
Number of Patients	1	5	7	2	2	17 (100%)

Each cell represents the **number of patients** with highest grade for that adverse event.

Table 4.

Grade 3/4 Adverse Events at least Possibly related ribociclib and/or everolimus among all treated patients

All Patients (N = 20)			
Category/Event Description	Grade		
	3	4	Grade 3 and 4 (%patients)
Investigations			
Alanine aminotransferase increased	1	0	1 (5%)
Cholesterol high *	2	0	2 (10%)
Lymphocyte count decreased (Lymphopenia)	5	1	6 (30%)
Neutrophil count decreased (Neutropenia)	5	0	5 (25%)
White blood cell decreased (Leukopenia)	4	0	4 (20%)
Metabolism and nutrition disorders			
Hypokalemia	2 [^]	0	2 (10%)
Vascular disorders			
Hypertension	1	0	1 (5%)
Maximum Grade 3 and 4 Adverse Event			
Number of Patients	7	2	9 (45%)

* attributed to everolimus only

[^] one patient attributed to ribociclib only

Table 5a.

Ribociclib plasma pharmacokinetic parameters for patients enrolled in Phase I study

Dose level	Course Day	N	T _{MAX} (h)	C _{MAX} (μM)	AUC _{0–24h} (h·μM)	Half-life (h)	CL/F (L/h/m ²)
Level 1 120 mg/m ²	C1D1 SD	16	2 (1–8)	0.55 (0.17–1.4)	3.96 (2.6–11.2)	11.7 (8.0–18.0)	47.9 (14.5–96.8)
	C1D17 SS	15	2 (1–4)	0.93 (0.45–1.94)	10.3 (5.3–18.7)	8.9 (7.3–17.3)	27.3 (10.2–59.2)
Level 2 170 mg/m ²	C1D1 SD	3	2 (1–2)	2.21 (0.47–2.35)	12.0 (5.4–13.4)	8.5 (8.4–13.9)	26.0 (20.8–62.7)
	C1D17 SS	2	2	1.25 – 1.48	9.6 – 10.7	5.5 – 9.5	37.6 – 39.1

On Course 1 Day 1 (C1D1), ribociclib was administered alone. On Course 1 Day 17 (C1D17), ribociclib was given in combination with everolimus.

Table 5b.

Everolimus blood pharmacokinetic parameters for patients enrolled in Phase I study

Dose level	Course Day	N	T _{MAX} (h)	C _{MAX} (nM)	AUC _{0–24h} (h·nM)	Half-life (h)	CL/F (L/h/m ²)
Level 1 1.2 mg/m ²	C1D17 SS	14	4 (1–8)	15.9 (3.6–41.0)	201 (52.7–399)	13.5 (8.5–23.1)	7.7 (3.1–24.3)
	C2D1 SS	4	2 (1–2)	7.8 (4.5–10.7)	83.0 (29.8–127)	18.6 (12.3–20.4)	15.5 (9.9–42.0)
	C2D1 SD	6	4 (1–8)	9.1 (3.4–19.8)	75.4 (46.8–146)	11.4 (6.9–23.9)	9.2 (5.9–23.3)
Level 2 1.2 mg/m ²	C1D17 SS	2	2	11.3 – 14.8	119 – 176	9.0 – 13.3	7.2 – 10.1
	C2D1 SD	2	0.5	4.6 – 15.6	42.5 – 75.5	7.3 – 15.6	12.6 – 26.4

On Course 2 Day 1 (C2D1), everolimus was administered alone. On Course 1 Day 17 (C1D17), ribociclib was given in combination with everolimus.

Data are reported as median (range)

SD single dose, SS steady state

Table 6.

Total ribociclib concentrations in plasma, CSF, and tumor tissue samples at time of surgery

Patient	Age (yo) BSA (m ²)	Anatomic location and sample weight	Plasma (μM)	CSF (μM)	Tumor (nmol/g)	^a Tumor-to-plasma ratio
P1	8.8 1.11	Spinal cord 2.5 mg	0.129	0.0096	0.678	5.26
P2	17 1.63	Left front parietal 58.2 mg	0.307	-	4.49	14.6
P3	10.8 1.15	Right sylvian fissure 71.1 mg	1.021	0.139	2.27	2.22
P4	7.3 0.98	Right front temporal 85.7 mg	0.090	0.0108	4.81	53.4
P5	12.1 1.33	Frontal 91.8 mg	0.139	0.0596	3.24	23.3

^aThe total tumor-to-plasma ratio was calculated as the ratio of total ribociclib concentration in tumor to that in plasma, assuming a brain tumor tissue density ~ 1.00, such that 1 nmol/g = 1 μmol/L.