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TITLE: A Phase I and Surgical Study of Ribociclib and Everolimus (RAD001) in Children with Recurrent or Refractory Malignant Brain Tumors

Coordinating Center: Operations, Biostatistics and Data Management Core (OBDMC) for the Pediatric Brain Tumor Consortium (PBTC), St. Jude Children's Research Hospital

Principal Investigator: *Mariko DeWire-Schottmiller, M.D.*
[redacted]

Co-Investigator: *Maryam Fouladi, M.D.*
[redacted]

PBTC Biostatistician	**PBTC Biostatistician**	**PBTC Protocol Coordinator**
Haitao Pan, Ph.D.	*Tong Lin, PhD*	Stacye Richardson, MSHS
[redacted]	[redacted]	[redacted]

PBTC Neuroimaging Center *Tina Young Poussaint, M.D.* [redacted]	**Pathology and Pharmacodynamics Correlates Laboratory** *Christine Fuller, MD* [redacted]	**Pharmacokinetics Laboratory** *Clinton Stewart, PharmD* [redacted]
Genomics Correlates Laboratory *Donald W.(Will) Parsons, M.D, PhD* [redacted]	**Investigational Pharmacist** ***Denise Lagory, PharmD*** [redacted]	**Nursing Committee Representative** *Michele Anton, RN BSN CPN* [redacted]
CRA Committee Representative *Emily Sparks, MS* [redacted]		

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- **Ribociclib, NSC # 794613, supplied by Novartis**
- **Everolimus (RAD001), NSC # 733504, supplied by Novartis**

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IND Sponsor: Mariko DeWire-Schottmiller, MD, [redacted]

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Pediatric Brain Tumor Consortium Institutions and Principal Investigators

Arzu Onar-Thomas, Ph.D.*

Ira Dunkel, M.D.

Patricia Baxter, M.D.

Girish Dhall, MD

Kathleen Dorris, MD

Michelle Monje, M.D PhD

Maryam Fouladi, M.D.

Stewart Goldman, M.D.

Eugene Hwang, M.D.

Tobey MacDonald, MD

Giles Robinson, MD

Kathy Warren, M.D.

Ian Pollack, M.D.

[redacted]

Does not have patient care responsibilities

Abstract and Schema

This is Phase I and Surgical study for children with retinoblastoma protein 1 (Rb1) positive recurrent or refractory central nervous system tumors.

Brain tumors are the leading cause of cancer-related death in children. Though cures for some are possible using conventional therapeutic approaches, outcome for highly malignant and recurrent brain tumors remains poor, despite aggressive multimodal therapy. Improved understanding of the inherent tumor biology and genomic landscape of brain tumors has spurred development of novel therapeutics aimed at targeted inhibition of important cancer-promoting pathways.

Aberrant cell cycle regulation and activation of the PI3K/Akt/mTOR pathway represent two highly important mechanisms of malignant potential in pediatric brain tumors. Joint inhibition of CDK4/6 and mTOR is promising due to strong biologic rationale, non-overlapping single-agent toxicities, and preliminary clinical experience in adults that suggests tolerability of this combination. Additionally, increased exposure of RAD001 in the presence of ribociclib, as observed in pharmacokinetic profiling of samples from adults receiving the combination, suggest that lower doses of both drugs may also be used in the pediatric population.

Ribociclib is an orally available cyclin-dependent kinase (CDK) inhibitor targeting cyclin D1/CDK4 and cyclin D3/CDK6 cell cycle pathway, with potential antineoplastic activity. Overexpression of CDK4/6, as seen in certain types of cancer, causes cell cycle deregulation. Ribociclib specifically inhibits CDK4 and 6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation. Inhibition of Rb phosphorylation prevents CDK-mediated G1-S phase transition, thereby arresting the cell cycle in the G1 phase, suppressing DNA synthesis and inhibiting cancer cell growth.

Everolimus is an inhibitor of selective mammalian target of rapamycin (mTOR), the effects of which specifically target the mTOR-raptor signal transduction complex 1 (mTORC1). The PI3K/AKT/mTOR pathway is dysregulated in the majority of human cancers and is the object of many targeted agents. Inhibition of mTORC1, an essential regulator of global protein synthesis downstream on the PI3K/AKT/mTOR pathway, reduces tumor cell proliferation, glycolysis and angiogenesis.

Both ribociclib and everolimus will be supplied by Novartis for PBTC-050 study.

Schema:
This is Phase I and Surgical Study of ribociclib and everolimus given in combination in children with recurrent or refractory malignant brain tumors.

Phase I
The primary objective of the Phase I component is to estimate the MTD and/or the recommended phase II dose (RP2D) of ribociclib and everolimus in children. Ribociclib and everolimus will be given in combination either in oral with drug-in-capsule versus liquid formulation or via gastric tube (liquid formulation). Ribociclib will be administered once a day for 21 days on/7 days off on a 28-day cycle and everolimus will be administered once a day for 28 days. One course is therefore equivalent to 28 days. Therapy may continue for up to a year (13 courses) in the absence of disease progression or unacceptable toxicity. Patients who have at least clinically and radiographically stable disease at the end of course 13

may be able to participate in the extended therapy for up to an additional 13 courses (total maximum duration of treatment is 26 courses).

Dose Escalation Schedule Table

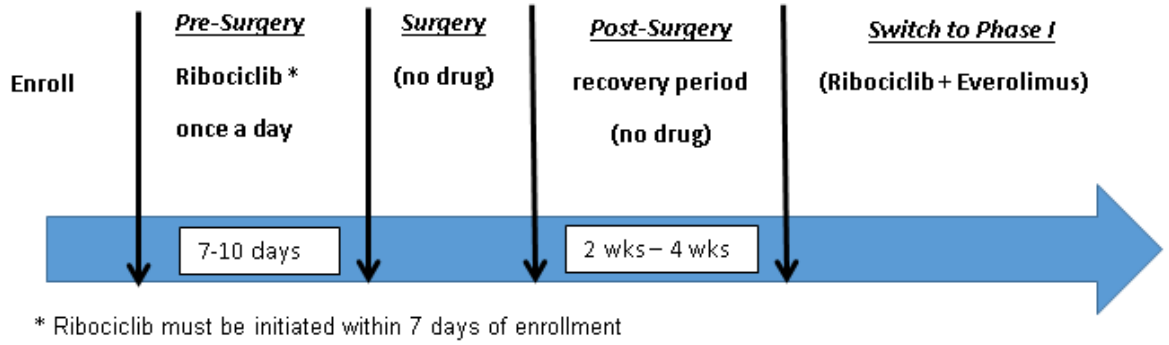
Dose Level	**Ribociclib (mg/m^2/day PO days 1- 21)**	**Everolimus (mg/m^2/day PO days 1-28)**	**BSA Restriction**
-1	75 mg/m^2/day	1 mg/m^2/day	BSA≥0.55m^2
-0.5*	120 mg/m^2/day	1 mg/m^2/day	BSA≥0.75m^2
0	75 mg/m^2/day	1.2 mg/m^2/day	BSA≥0.55m^2
1(starting dose)	**120 mg/m^2/day**	**1.2 mg/m^2/day**	**BSA≥0.75m^2**
2	170 mg/m^2/day	1.2 mg/m^2/day	BSA≥0.45m^2
3	170 mg/m^2/day	1.5 mg/m^2/day	BSA≥0.45m^2

*Dose reduction if toxicities clearly attributable to Everolimus

Surgical Cohort

The primary objective of the Surgical Cohort is to characterize ribociclib concentrations in tumor and plasma in children with refractory or recurrent CNS tumors undergoing neurosurgical procedures. Patients will take ribociclib alone at the pediatric MTD (350 mg/m^2/day) for 7 – 10 days prior to surgery. Following tumor resection and within 2-4 weeks of post-surgery period, patients enrolled on the surgical study will be switched over to the phase I study and be treated with ribociclib and everolimus combination at the assigned dose level.

If the starting dose of ribociclib is intolerable or if DLTs of the starting dose of ribociclib are clearly attributable to the study drug, the dose will be dose de-escalated to 280 mg/m^2/day.



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1 OBJECTIVES

1.1 Primary Objectives

Phase I

1.1.1 To estimate the MTD and/or the recommended phase II dose (RP2D) of ribociclib and everolimus in children with refractory or recurrent CNS tumors

1.1.2 To describe the toxicity profile and define the dose-limiting toxicities (DLTs) of ribociclib and everolimus in children with refractory or recurrent CNS tumors

1.1.3 To characterize the pharmacokinetics of ribociclib and everolimus in children with refractory or recurrent CNS tumors, and the potential for drug-drug interactions between the two compounds in this population

Surgical Study

1.1.4 To characterize ribociclib concentrations in tumor, and plasma in children with refractory or recurrent CNS malignancies undergoing neurosurgical procedures

1.2 Secondary Objectives

Phase I

1.2.1 To describe the response rate of relapsed and refractory malignant brain tumors to ribociclib and everolimus in the context of a phase I study

1.2.2 To assess dexamethasone-based mouthwash for prevention of stomatitis in pediatric patients receiving everolimus therapy

Surgical Study

1.2.3 To explore the effect of ribociclib treatment on Ki-67 by IHC by comparing archival and post-treatment tumor tissue

1.3 Exploratory Objective

1.3.1 To increase knowledge of the genomic landscape of treatment-refractory pediatric CNS tumors, including mechanisms of resistance and response

2 BACKGROUND

2.1 Study Disease

Brain tumors are the leading cause of cancer-related death in children. Though cures for some are possible using conventional therapeutic approaches, outcome for highly malignant and recurrent brain tumors remains poor, despite aggressive multimodal therapy. Improved understanding of the inherent tumor biology and genomic landscape of brain tumors has spurred development of novel therapeutics aimed at targeted inhibition of important cancer-promoting pathways.

Cell Cycle Regulation and Cancer

Tumor cell proliferation requires repetitive cell divisions involving doubling of DNA content (S phase) and halving of the genome during mitosis (M phase) that results in two identical cells. Between the S and M phase, the G1 (Gap 1) phase occurs during which signals either allow continuation to S phase or prompt cell cycle exit into the quiescence phase (G0 phase).[1] During G1, cells grow and prepare for chromosomal replication during the S phase. Following S phase, the cell enters another gap phase (G2 phase), during which the cell prepares for equal division of DNA into two identical daughter cells (M phase). Checkpoints are transitions between various phases of the cell cycle that are tightly regulated by cyclins and cyclin-dependent kinases (CDKs), which are aberrant in the majority of human cancers.[2]

In mammalian cells, cyclins and CDKs are associated with various cell cycle phases, including G1 phase (Cyclins D1-3, E and CDKs 4, 6, 2), S phase (Cyclins A, E and CDK2), and mitosis (Cyclin A, E and CDK 1, 2).[1,2] The "brakes" of the cell cycle are regulated by CDK phosphorylation/ dephosphorylation, cyclin synthesis/degradation, and availability of naturally occurring protein inhibitors, as well as subcellular localization of these regulatory components. Cell cycle inhibition is mediated by two families of proteins: INK4 and CDK inhibitor protein (CIP)/kinase inhibitor protein (KIP).[1] Whereas the INK4 inhibitors ($p16^{INK4A}$ and $p14^{ARF}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$) specifically inhibit CDK4/6 during G1, CIP/KIP ($p21^{CIP1}$, $p27^{KIP1}$, $p57^{KIP2}$) inhibitors work against kinases and cyclins in all phases of the cell cycle.[1]

G1→S phase transition is mediated by activation of the CDK4/Cyclin D complex following release of INK4 from CDK4/6.[1] This results in hypophosphorylation of the retinoblastoma protein (Rb) and partial release of the E2F-DP1 transcription complex. In late G1, release of Cyclin E/CDK2 from the inhibitory effect of $p27^{KIP1}$ results in Rb1 hyperphosphorylation and complete release of E2F-DPI complex, which then binds to promoter regions of genes required for S phase entry and progression (Figure 1).[1]

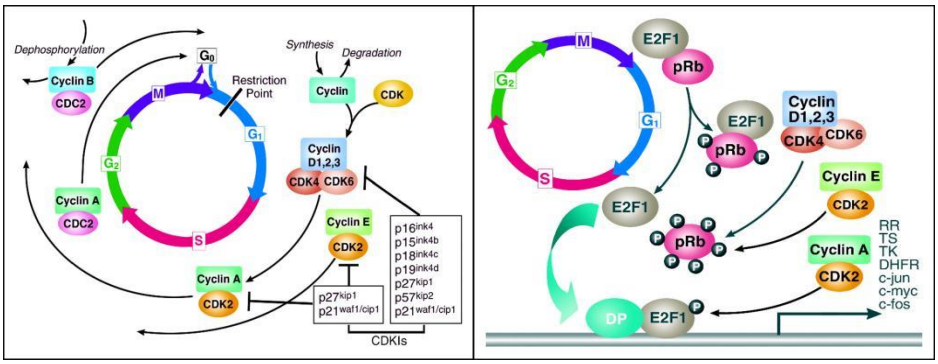


Figure 1: The CDK4/6, p16, Rb-E2F pathway

Cell Cycle Dysregulation in Pediatric Brain Tumors

As in most human cancers, cell cycle dysregulation is common in malignant pediatric brain tumors. High-throughput sequencing studies in many tumor types have identified focal gains of CDK4, CDK6, and Cyclin D1-3 and/or homozygous loss of INK4a-ARF, implicating cell cycle regulation in tumor pathogenesis. CDK4/6 inhibitors bind CDK4/6 and inhibit Rb phosphorylation, which induces G1 cell cycle arrest. Importantly, since the mechanism of CDK4/6 inhibitors is mediated through Rb, functional Rb protein is critical for efficacy.

High-Grade Glioma and DIPG

Cell cycle regulation has been strongly implicated in pediatric high-grade glioma (HGG) and diffuse intrinsic pontine glioma (DIPG). DIPG harbors focal gains of CDK4, CDK6, or cyclin D1-3 and *p16* loss in 35% and 20% of tumors, respectively.[3,4] Paugh et al. also reported focal gains of genes encoding receptor tyrosine kinases (RTKs) or cell cycle regulatory genes in 24/43 (56%) of DIPG.[3] Gain of CDK4, CDK6, or cyclin D2 has less reported incidence in non-brainstem HGG (6%), though *p16* loss is observed at a similar frequency of 20%.[5,6] Mutations of *PTEN* and *TP53*, key tumor suppressors involved in cell cycle regulation, have also been reported commonly in DIPG and HGG at rates of 25% and 42-70%, respectively.[7-10] Collectively, Wu *et al*. implicated mutations predicted to affect cell cycle regulation in 59% of their recently reported cohort of 127 patients with DIPG and non-brainstem HGG.[8] There is wide variation in reports about Rb mutation or deletion in HGG and DIPG, ranging from 0-58%.[6-11] Specifically, Schartzentruber et al. reported Rb1 mutation in 5/49 (10%) HGG[6], and Taylor et al. and Buczkowicz et al. reported Rb1 loss in 6/26 (20%) and 15/48 (31%) of DIPG, respectively.[7,10]

Barton *et al*. demonstrated the efficacy of CDK4/6 inhibitor PD-0332991 in a genetically- engineered brainstem glioma mouse model driven by *PDGF* overexpression and *Ink4-ARF* loss. In a proliferation assay, the IC_{50} of cells derived from the *Ink4a-ARF*-deficient, *PDGF*-driven brainstem glioma model was 2μM, compared to > 5μM for cells derived from a *p53*-deficient *PDGF*-driven brainstem glioma. Two daily doses of PD-0332991 given by gavage to brainstem glioma-bearing mice significantly inhibited tumor cell proliferation as measured by pH3, demonstrating good drug delivery across the blood-brain-barrier.[12] Aoki *et al*. showed that treatment of a brainstem glioma xenograft model with PD-0332991 provides significant survival benefit relative to vehicle-treated mice.[13]

Medulloblastoma and PNET

In medulloblastoma, focal amplification of loci containing CDK and cyclin genes was reported by Li *et al.* in 1-5% of medulloblastoma and 25% of supratentorial primitive neuroectodermal tumor (PNET).[14] A separate study reported *CDK6* overexpression in 30% of medulloblastomas, which served as an independent prognostic marker of poor overall survival.[15] Pfister *et al*. also separately reported *CDKN2A* loss in 7/21 (30%) of PNETs, including 4 with homozygous loss.[16] Neither *Rb1* mutation, nor loss were reported in a large sequencing study of medulloblastoma[17] and have not been reported in PNET.[18]

Dr. James Olson at the Fred Hutchinson Cancer Center in Seattle, WA has treated mice implanted with patient derived medulloblastoma flank xenografts with PD-0332991 at 150 mg/kg/day for 14 days.[19] Significant tumor regressions were observed associated with decrease in proliferation rate (), Rb1 phosphorylation (Figure 2) and improvement in animal survival (Figure 3) compared to vehicle alone. Maintenance of therapy past 38 days of initial treatment resulted in persistent tumor control suggesting that tumors do not develop drug resistance. Decreasing dose to 75 mg/kg/day did not result in loss of efficacy (Figure 4).

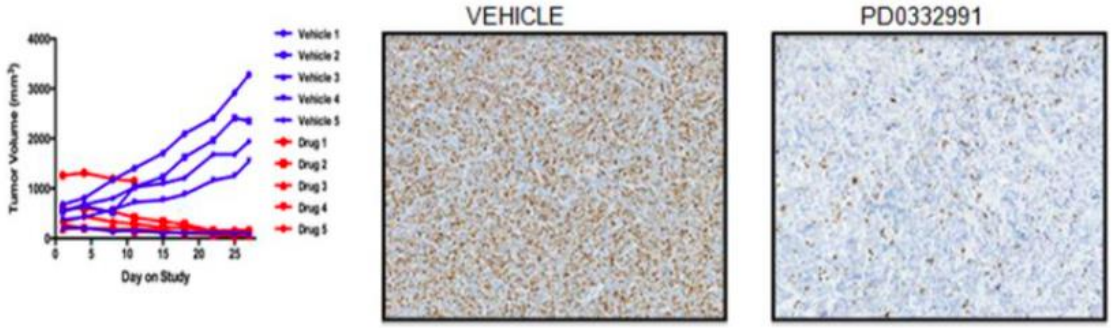


Figure 2: Medulloblastoma flank xenografts in mice treated with vehicle or PD0332991 (150 mg/kg/day for 14 days)

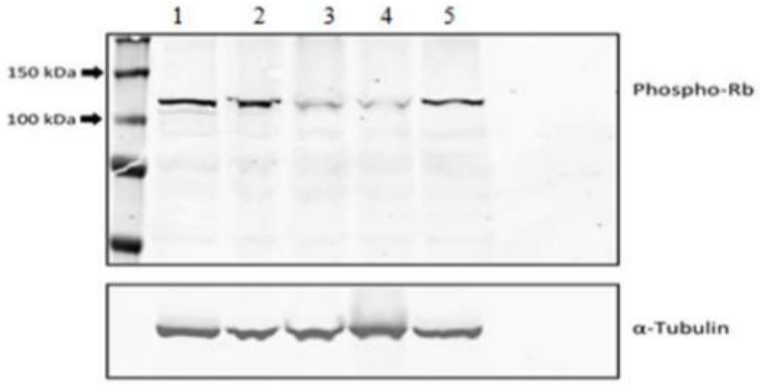


Figure 3: Western blot to demonstrate decrease in RB phosphorylation following treatment with PD-0332991 (Lanes 3, 4, and 5) compared to vehicle (lanes 1 and 2). Tumor from mouse in lane 5 was sacrificed at 24 hours as compared to 4 hours for those on lane 3 and 4.

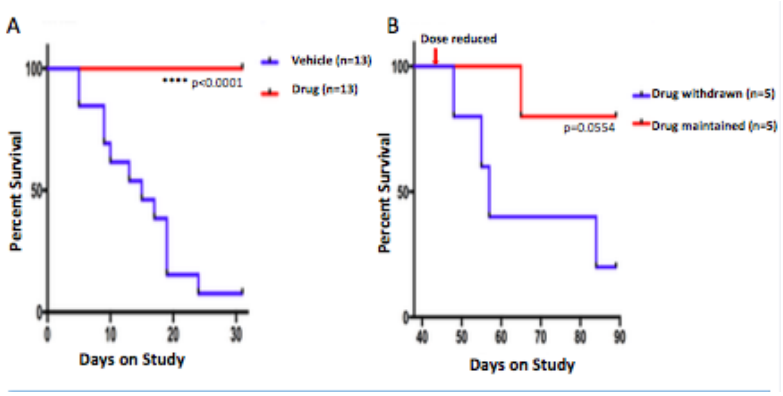


Figure 4: Survival of mice with Medulloblastoma flank tumors treated with vehicle or PD 0332991

<u>Ependymoma</u>

Loss of *p16* has been reported in 22/89 (25%) of ependymomas and is associated with poor prognosis.[20,21] Rb1 deletions have been reported in 22/92 (24%) of ependymomas.[21]

ATRT

Cell cycle regulation plays an important role in atypical teratoid rhabdoid tumor (ATRT). SMARCB1 directly inhibits cyclin D1 transcription through recruitment of histone deacetylase complexes to the cyclin D1 promoter[22], and SMARCB1 loss leads to cyclin D1 overexpression and cell proliferation.[23,24] Tsikitis et al. demonstrated rhabdoid tumor dependence on cyclin D1 overexpression for tumor formation and that showed that reintroduction of SMARCB1 represses cyclin D1 and activates tumor suppressor p16INK4A, preventing cell cycle progression.[25] Betz et al. similarly showed that SMARCB1 reintroduction into deficient cell lines resulted in G0/G1 arrest through strong induction of p16INK4A.[26]

Katsumi et al. reported efficacy of CDK4/6 inhibitor PD-0332991 in malignant rhabdoid tumor, the pediatric SMARCB1-deficient tumor equivalent to ATRT outside of the CNS.[27] In 4 of 5 MRT cell lines, PD-0332991 inhibited cell proliferation > 50 %, (IC_{50} values 0.01 to 0.6 μM) by WST-8 assay, and induced G1-phase cell cycle arrest, as shown by flow cytometry and bromodeoxyuridine (BrdU) incorporation assay.

Dr. Olson also used an ATRT flank xenograft to demonstrate the efficacy of CDK4/6 inhibitor PD-0332991 in this tumor type.[19] Treatment with PD-0332991 at a dose of 150 mg/kg/day for 14 days resulted in excellent tumor regression (Figure 5A) and prolongation of survival of animals receiving the drug as compared to vehicle controls. Tumors treated with PD-0332991 showed decreased proliferation rate and Rb1 phosphorylation (Figure 5B:).

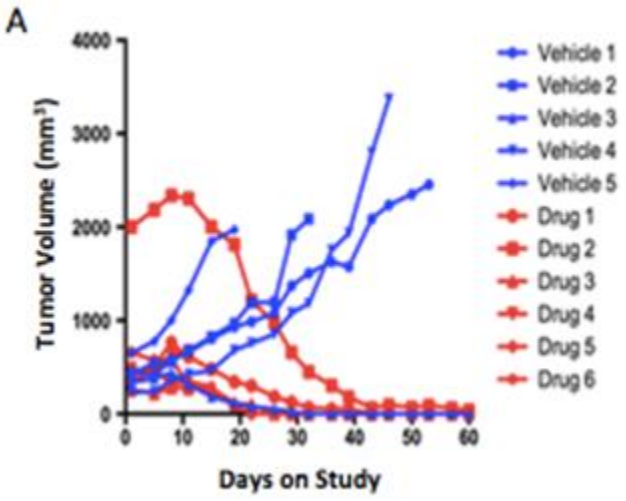


Figure 5A: Palbociclib causes complete regression of ATRT

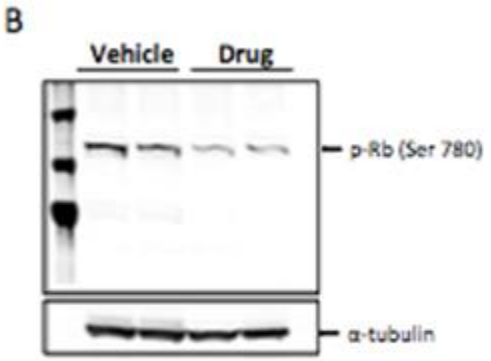


Figure 5B: Inhibition of CDK4/6 by Palbociclib results in reduced phosphorylation of Rb.

Role of the PI3K/Akt/mTOR Pathway in Cancer

The PI3K/Akt/mTOR pathway is one of the most commonly activated pathways in human cancer and has been found to be a crucial driver of tumorigenesis.[28-30] Phosphatidylinositol 3-kinase (PI3K) is activated by a wide range of RTKs, including EGFR, ErbB2, PDGFR and MET. Amplification and activating mutations of RTK genes are frequently reported in many solid and hematologic malignancies. PI3K ligand binding leads to receptor dimerization, transphosphorylation, recruitment of PI3K, and generation of secondary messenger PIP3, which localizes downstream PH proteins to the inner surface of the plasma membrane. PI3K can be activated through direct mutation of PIK3CA[31-33] or inactivating mutation or

somatic loss of PTEN, an endogenous negative regulator of PI3K signaling. Akt, the "master-switch", is activated through phosphorylation at Threonine 308 and Serine 473 and has a myriad of downstream effectors, which promote cell survival, inhibition of apoptosis and autophagy, proliferation, motility, and migration. Amplification or mutation of Akt has been described in human cancer.[34] Downstream of Akt is the mammalian target of rapamycin (mTOR), a ubiquitous serine threonine kinase involved in cell cycle, angiogenesis, and apoptosis.[35,36] The mTOR protein is incorporated into two multiprotein complexes, mTORC1 (regulates growth through S6K1 and 4E-binding protein 1) and mTORC2 (regulates cell survival through phosphorylation of serine 473 on Akt).[37]

PI3K/Akt/mTOR Activation in Pediatric Brain Tumors

High-Grade Glioma and DIPG

A recent analysis of 53 pHGGs revealed activation of the PI3K/AKT/mTOR pathway in 42 tumors (79%).[38] Moreover, tumor grade positively correlated with expression of p-S6 and p-4EBP1 (a surrogate marker of mTOR activation) in 16 pediatric HGGs, in which all grade IV tumors in the study staining positive for p-4EBP1 by immunohistochemistry (IHC).[39] Wu *et al*. also described recurrent fusions involving neutrophin receptor genes *NTRK1*, *NTRK2*, and *NTRK3* in 40% of infant non-brainstem HGGs, which resulted in elevated levels of phosphorylated AKT and MAPK proteins, indicating activation of the PI3K and MAPK pathways.[8] (RTK)-PI3K-MAPK pathway activation was also seen in 69% (39/57) of DIPGs and 67% (47/70) of non-brainstem HGG in their cohort. Findings by Taylor *et al*. and Fontebasso *et al*. support the important role of PI3K/Akt/mTOR in pediatric HGG, as 46% (12/26) of DIPG and 26% (10/39) of DIPG or midline non-brainstem HGG in their respective studies exhibited mutations predicted to activate the (RTK)-PI3K-MAPK pathway.[9,10]

Medulloblastoma

Several growth factor receptors are upregulated across medulloblastoma subgroups and converge on the PI3K/Akt/mTOR pathway, including IGF-1R, PDGFRB, ErbB2, FGFR and c-KIT. *PTEN* loss has also been identified in medulloblastomas and associated with a more aggressive clinical course.[40,41] Castellino *et al*. showed that PTEN loss and resultant PI3K activation is sufficient to cause medulloblastoma tumor formation in granule neuron precursor cells.[42] In human medulloblastoma samples, Hartmann et al. demonstrated phospho-AKT expression in all 22 samples evaluated, indicating prevalent PI3K/Akt/mTOR pathway activation in medulloblastoma.[43]

Ependymoma

Johnson *et al*. demonstrated common PI3K/Akt/mTOR pathway signaling in ependymoma, as 72% of 172 ependymomas exhibited aberrant phospho-Akt, which also predicted poor outcome.[44]

Atypical Teratoid Rhabdoid Tumor

Akt/mTOR pathway activation is common in ATRT.[45] Darr et al. demonstrated elevated phospho-Akt in SMARCB1-deficient MRT xenografts that abated with reintroduction of SMARCB1.[46] Igfbp7, an insulin-like growth factor with proposed effects on angiogenesis, was also upregulated[47], implying a normally suppressive effect of SMARCB1 on this important paracrine signaling molecule. Reintroduction of Igfbp7 in SMARCB1-deficient cells significantly reduced phospho-Akt and inhibited tumor growth, suggesting transformative capacity of both Igfbp7 and PI3K/Akt/mTOR signaling ATRT *in vitro*.

2.2 Ribociclib (LEE011)

Ribociclib is an orally bio-available, highly selective small molecule inhibitor of CDK4/6 that induces G1-arrest at an IC_{50} of 0.1 μM in pRB-positive cancer cells *in vitro*. Ribociclib inhibits the CDK4/CCND1

and CDK6/cyclin-D3 enzyme complexes with IC_{50} values of 0.01 and 0.039 μM, respectively. This inhibition is selective for CDK4/6, as complexes involving CDKs 1, 2, 5 and 9 were roughly 100 to 10,000-fold less sensitive to inhibition by ribociclib (RD-2008-50549). Extended profiling was also carried out against an internal kinase selectivity panel, which contains an additional 38 serine/threonine and tyrosine kinases (RD- 2009-00240). In these studies ribociclib was weakly active against four kinases, Aurora A, HER1, PKCμ and LCK with IC_{50} values of 2.0, 9.0, 7.8 and 7.7 mM, respectively.

In Jeko-1 cells, ribociclib inhibits CDK4/6-dependent pRb phosphorylation with an average IC_{50} of 0.06 μM. Consistent with the observed inhibition of pRb phosphorylation, ribociclib also inhibited G1-to-S phase cell cycle progression in Jeko-1 cells, as judged by inhibition of bromodeoxyuridine (BrdU) uptake (IC_{50} of 0.1 μM) and fluorescence activated cell sorting (FACS) analysis (half-maximal increase in cells in G1 at 0.11 μM). The effect of ribociclib on pRb phosphorylation, BrdU uptake, and cell cycle progression has been assessed in > 40 cell lines derived from hematological, esophageal, liposarcoma, and breast cancers. In pRb+ cell lines, ribociclib inhibits pRb phosphorylation with a median IC_{50} value of 0.275 μM (range: 0.06 to 8.8 μM). Similarly, ribociclib interferes with G1-to-S phase cell cycle progression in these cells, as determined by either BrdU uptake or FACS analysis with a median IC_{50} value of 0.46 μM. In contrast, in lineage-matched pRb negative cell lines, no effect of ribociclib on either pRb phosphorylation or cell cycle progression is observed. Thus, ribociclib is able to impact cell cycle progression in cell lines derived from a variety of tumor types that harbor a diversity of genetic alterations in a manner dependent on intact pRb.

2.2.1 Preclinical Studies

2.2.1.1 Anti-tumor activity

Ribociclib has demonstrated *in vivo* anti-tumor activity in subsets of tumor xenograft models. Consistent with the compound's mechanism of action, efficacy was only observed in tumors expressing pRb. Tumor types in which ribociclib has demonstrated robust anti-tumor activities include, but are not limited to, breast, melanoma, neuroblastoma, malignant rhabdoid, lung, pancreas and hematological malignancies. In addition, ribociclib has shown anti-tumor activity when combined with targeted agents that inhibit signaling pathways known to regulate D-cyclin levels, including inhibitors of the RAF/mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase (PIK3), and mammalian target of rapamycin (mTOR) pathways (Figure 6).

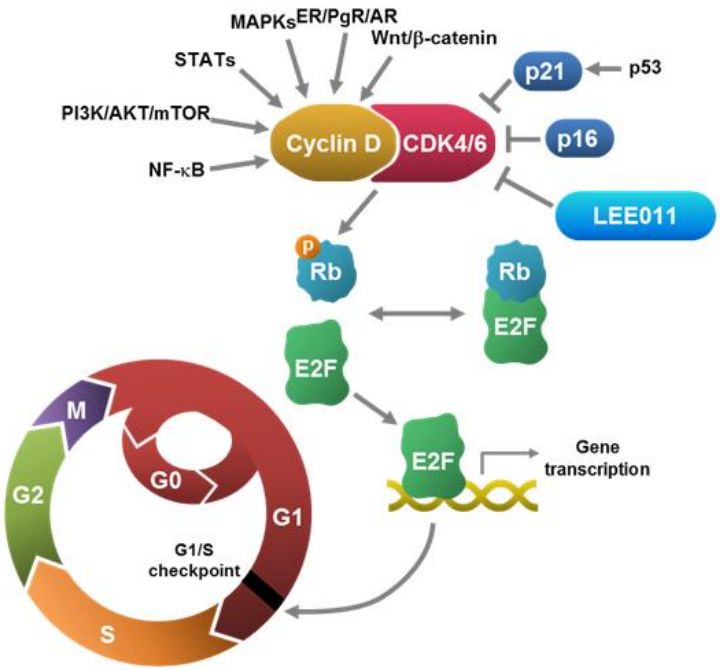


Figure 6: The Cyclin D–CDK4/6–INK4–Rb Pathway

Ribociclib *In Vitro* Studies

Hematological malignancies and solid tumors that are pRb-positive and dependent on D-cyclins for proliferation are expected to be sensitive to ribociclib. To test this hypothesis, a variety of cancer cell lines were examined for their sensitivity to ribociclib, including but not limited to tumor cells derived from acute myeloid leukemia, breast cancer, colon cancer, liposarcoma, MCL, multiple myeloma, melanoma, neuroblastoma, NSCLC, rhabdoid cancer, pancreatic cancer and squamous cell esophageal cancer [(RD-2009-50759), (RD-2012-50290), (RD-2012-50369)]. Cell lines harboring genetic aberrations in the CCND1 and CDK4/6 genes, as well cancers containing genetic/epigenetic changes that indirectly activate CDK4/6-pRb pathway, were sensitive to ribociclib as judged by the inhibition of phosphorylation of pRb, BrdU uptake and/or cell cycle distribution. For example, mantle cell lymphoma cells harboring cyclin D1 translocations and liposarcoma cells with CDK4 amplifications were highly sensitive to ribociclib, exhibiting ~100 nM sensitivity both in terms of pRb phosphorylation and cell cycle arrest in the G1 phase. Similarly, the majority of breast cancer and melanoma-derived cells tested were also sensitive to ribociclib in terms of pRb-phosphorylation and cell cycle progression, often with IC_{50} values less than 1μM. Neuroblastoma and malignant rhabdoid cells were also very sensitive to ribociclib, which underwent robust growth arrest upon compound treatment [(RD-2012-50290), (RD-2012- 50369)]. Lastly, clones of the HCC827 NSCLC cell line which harbor the activating E746_A750 indel allele of EGFR, but which have been selected for resistance to the EGFR inhibitor gefitinib, are also sensitive to ribociclib. Thus, ribociclib may have therapeutic utility in tumors that harbor a wide array of oncogenic drivers, including those that have acquired resistance to other targeted therapies.

<u>*In vitro* CDK4/6 and mTOR inhibitor combination</u>

When combined with the mTOR inhibitor RAD001 (everolimus), ribociclib induced synergistic growth inhibitions in multiple tumor models, including cell lines derived from MCL, ER+ breast cancer, and MRTs (RD-2012-50383). Table 1 shows synergy scores, which demonstrate that co-treatment of

ribociclib and everolimus leads to synergistic growth inhibition.

Cell Line	Type	Synergy Score
T-47D	ER+ breast	7.6
MCF-7	ER+ breast	8.0
A-204	Rhabdoid	7.8
G-401	Rhabdoid	5.6

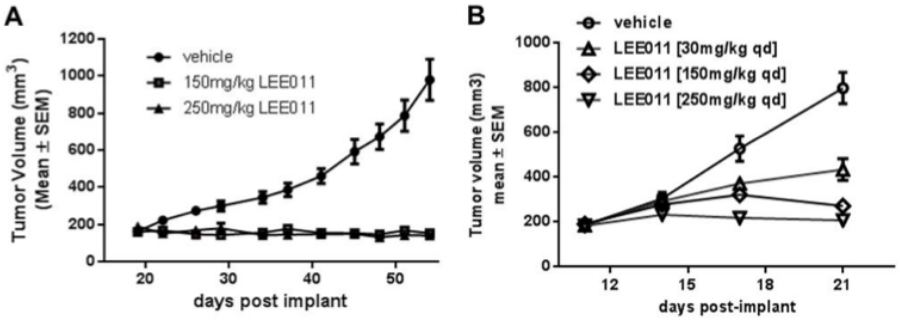
Shown are synergy scores for the combination of LEE011 and everolimus (RAD001, Afinitor) in ER+ and rhabdoid-derived cell lines. Synergy scores are described in Lehar et al (2009) with scores >2 representing real synergy. Both breast cancer derived cell lines harbor activating mutations in the PIK3CA gene, and both rhabdoid derived cell lines have bi-allelic inactivation of SMARCB1.

Table 1: Synergy scores for ribociclib /Everolimus combination in cancer cells

Ribociclib *In Vivo* Studies

Activity in neuroblastoma and rhabdoid xenograft models

Ribociclib inhibited the *in vitro* growth of cell lines derived from MRTs, as well as subsets of neuroblastoma-derived cell lines, at concentrations below 1 µM [(RD-2012-50290), (RD- 2012-50369)]. This *in vitro* sensitivity was also observed *in vivo* as ribociclib induced either regressions or complete stasis at doses ≥150mg/kg in the CHP212 neuroblastoma and G401 rhabdoid tumor models, respectively Figure 7; (RD-2012-50368), (RD-2012-50371).



In vivo activities of LEE011 in CHP212 neuroblastoma (panel A) and G401 rhabdoid tumor (panel B) models. At 250 mg/kg dose, complete growth inhibition was observed in both models.

Figure 7: *In vivo* activity of ribociclib in neuroblastoma and rhabdoid xenograft models

Ribociclib in combination with PIK3CA inhibitor BYL719 and mTOR inhibitor Everolimus in ER positive/PIK3CA-mutant breast cancer xenograft model

Activating mutations in the PIK3CA gene are oncogenic drivers in a variety of cancer types. Notable amongst these cancers is ER+ breast cancer, where such lesions are found in approximately 40% of all instances of the disease. The CDK4/6, in turn, are critical mediators of at least one key effector pathway downstream of the PIK3C/Protein Kinase B (AKT)/mTOR axis as activation of this pathway results in increased levels of CCND1 via both increased mRNA expression and protein stability. The effect of combining ribociclib with either the alpha-selective PIK3C inhibitor BYL719 or the mTORC1-inhibitor everolimus in the ER+, PIK3CA-mutant MCF-7 xenograft model was examined (RD-2013-50141). As demonstrated in Figure 8 (panels A and B), single agent treatments with ribociclib, BYL719, and everolimus resulted in modest tumor regressions of -9%, -15% and -21%, respectively. When ribociclib was combined with everolimus, tumor regressions increased to 49%. Moreover, single agent and

combination treatments were well tolerated as judged by body weight loss (not shown). These data suggest that combining inhibitors that target the PIK3C/AKT/mTOR pathway with ribociclib may be an effective therapeutic strategy.

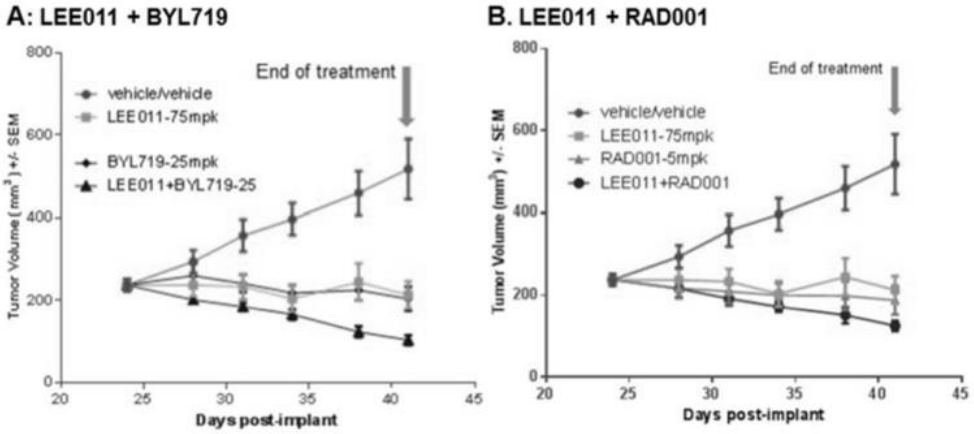


Figure 8: Ribociclib /BYL719 and Ribociclib/RAD001 combinations leads to improved tumor growth delays in ER+/PIK3CA mutant breast cancer model MCF-7 in vivo.

2.2.1.2 Animal Toxicity

In vivo cardiac safety studies demonstrated a signal for QT prolongation with the potential to induce incidences of premature ventricular contractions (PVCs) at higher exposure levels. The effects of ribociclib on the bone marrow (hypocellularity), lymphoid system (lymphoid depletion), intestinal mucosa (atrophy), skin (atrophy), bone (decreased bone formation) and testes (atrophy) are considered to be related to the pharmacological inhibition of cell replication in these tissues due to CDK4/6 inhibition. An increased number of ovarian corpora lutea was observed in a single female dog in the 4-week toxicity study at the highest dose tested (20 mg/kg/day) and this effect could also be related to the pharmacology of ribociclib (arrest of estrous cycle). The liver, bile system and gall bladder (proliferative changes, cholestasis, sand-like gallbladder calculi and inspissated bile) and the kidney (concurrent degeneration and regeneration of tubular epithelial cells) were identified as additional target organs of toxicity which are not likely related to the primary pharmacology of ribociclib. Inflammatory changes in the lungs of dogs were considered secondary to aspiration of test article and are indicative of the irritant potential of the formulated test-article in the respiratory tract. Correlating hematological and/or biochemistry changes were seen for the effects described in the bone marrow, lymphoid system and liver. Generally all changes demonstrated either reversibility or a clear tendency towards reversibility. It can be expected that after a prolonged recovery time incompletely recovered findings would have totally reversed in all species.

Ribociclib was well-tolerated in mice and rats with body weight loss not exceeding 12.5% at doses up to 250 mg/kg qday po or 150 mg/kg qday po, respectively, for up to 28 days. However, myelosuppression was observed and correlated with pRb phosphorylation inhibition. Treatment with ribociclib resulted in tumor regression in the Jeko-1 MCL xenograft model at doses ≥75 mg/kg qday po. *In vivo* pharmacokinetic (PK)/pharmacodynamic (PD) studies demonstrated dose-related inhibition of pRb phosphorylation in tumors, with continuous dosing over at least 3-5 days being required to achieve optimal target inhibition. In male nude rats, a PK/PD/efficacy study indicated that plasma levels corresponding to approximately 0.5 - 4 μM over a 24 h dose interval are sufficient to obtain near complete inhibition of pRb phosphorylation and complete regression in the Jeko-1 MCL xenograft model.

Non-clinical reproductive studies have demonstrated ribociclib induced embryotoxicity and fetotoxicity in rats and rabbits and teratogenicity in rabbits. As of December 22, 2015, a rabbit embryo-fetal development study was performed. Ribociclib was administered at dose levels of 0, 10, 30, or 60 mg/kg/day. The study was conducted at Charles River Laboratories Montreal, ULC (CR-MTL), 22022 Transcanadienne, Senneville, Quebec, Canada, H9X 3R3. *In vitro and in vivo*, ribociclib did not show an indication for a genotoxic potential or phototoxic potential. In the rat, there was no evidence of teratogenicity; however ribociclib was embryofetotoxic in the rabbit. In rabbits, ribociclib was teratogenic at ≥ 30 mg/kg/day (1.5 times patients exposure at the dose of 600 mg/day based on AUC) as evidenced by increased incidences of fetal abnormalities (malformations and external, visceral and skeletal variants). Fetal weights were decreased at 60 mg/kg. There was no evidence of embryo-fetal mortality. In view of these changes, the no-observed-effect level (NOEL) for maternal toxicity was considered to be at least 30 mg/kg and the NOEL for the embryo-fetal development, 10 mg/kg.

2.2.1.3 Ribociclib Preclinical Pharmacokinetic Studies

After oral ribociclib administration, bioavailability was moderate in mouse (65%) and dog (64%) and low to moderate in cynomolgus monkey (10 to 23%). After oral administration, maximum ribociclib plasma concentrations occurred between 2 and 4h across species.[48]

Initial studies of the tissue distribution of ^{3}H-ribociclib-derived radioactivity used quantitative whole body autoradiography (QWBA) in male albino rats after both intravenous and oral dosing. Total radioactivity was markedly distributed into the extravascular compartment, and was eliminated rapidly from most tissues. Radioactivity was not detected in the brain using QWBA. However, more recent studies using a cerebral microdialysis technique have demonstrated that at clinically relevant dosages the unbound brain or tumor to plasma partition coefficient (Kp,uu) for ribociclib was 0.12±0.09, 0.16±0.11, and 0.07±0.07 for normal brain, Group 3 medulloblastoma (Grp3 MB), and diffuse intrinsic pontine glioma (DIPGx7) tumors, respectively.[49] Although the Kp,uu for ribociclib varied among the groups studied, the median unbound ribociclib Cmax in all three groups was 100 nM. The median time to maximum ribociclib concentration (Tmax) was 2, 6, and 8 hours for normal brain, Grp3 MB, and DIPGx7, respectively.

The plasma protein binding of ribociclib was moderate. No major concentration dependency in the tested plasma concentration range (100-1000 ng/mL for mouse, rat, dog or 10-10000 for cynomolgus monkey and human) was observed. The unbound fractions in plasma (fu) ranged between 20 ± 1% to 32 ± 6 % (human: 30 ± 2%). The plasma volume of distribution at steady-state (Vss) was large across species (7.9 to 28 L/kg).[48]

Extensive in vitro drug metabolism studies have been conducted using mouse, rat, dog, monkey, and human hepatocytes. The primary metabolites formed included the M4 metabolite (LEQ803; N-demethylation at the amide moiety; all species), M8 (sulfation; rat and human), M6 (oxidation and glucuronidation; humans), and M13 (CCI284; N-hydroxylation; humans). Other metabolites are formed, but they are either in minor amounts (≤ 10% of ^{3}H-$AUC_{0\text{-}24h}$) or are not pharmacologically active. The LEQ803 metabolite is an active metabolite.[48]

In male rats, ribociclib was eliminated mainly by metabolism with minor contributions of direct biliary and renal clearance. The major metabolic route was sulfation of ribociclib to M8 and its excretion into the bile. The cumulative recovery of ribociclib in the urine, bile, and feces of bile duct-cannulated male rats accounted for 18.2% of the total plasma clearance.[50] Results from a rat ADME study showed that tritiated

ribociclib was primarily excreted with bile (~61% of dose). The proportion of the dose excreted in the urine of bile duct cannulated male rats ranged from ~5% after oral dosing to ~16% after i.v. dosing.

Based upon results of in vitro studies, ribociclib is primarily metabolized by CYP3A4 (hydroxylation and N-demethylation) and to a lesser extent (15 to 26%) the polymorphic enzyme FMO3 (hydroxylamine metabolite). The oxidative metabolic clearance of ribociclib is likely to be affected by co-administration of drugs that inhibit or induce CYP3A4. For example, the metabolism of ^{3}H-ribociclib in human liver microsomes was strongly inhibited by ketoconazole and azamulin (both CYP3A4 inhibitors), and partly by methimazole (FMO3 inhibitor).[48]

In vivo pharmacokinetics (PK)/pharmacodynamics (PD) studies demonstrated dose-related inhibition of pRb phosphorylation in tumors, with continuous dosing over at least 3-5 days being required to achieve optimal target inhibition. In male nude rats, a PK/PD/efficacy study indicated that plasma levels corresponding to approximately 0.5 - 4 µM over a 24 h dose interval are sufficient to obtain near complete inhibition of pRb phosphorylation and complete regression in the Jeko-1 MCL xenograft model.

2.2.1.4 Ribociclib Safety and Pharmacology Studies

Ribociclib was profiled in a safety pharmacology receptor/enzyme panel and showed activity against PDE4D (IC50 0.88 µM and 0.39 µM). Ribociclib did not induce vasculitis or cardiovascular toxicity in the toxicology studies performed so far (up to 15 weeks). Rat safety pharmacology studies did not reveal any effects on CNS or respiratory functions. In the in vitro manual patch clamp assay, ribociclib had an IC50 of 53 µM and LEQ803, a metabolite of ribociclib that represents 6 to 10% of the parent drug in dogs, had an IC50 of 4.5 µM. In the dog telemetry study, prolongation of the average QT and QTc was observed with the potential to induce incidences of PVCs at higher exposure levels. Ribociclib and LEQ803 likely contributed to the QT prolonging effects seen in vivo. Cardiac (ECG) monitoring in patients should be performed.

Oral single dose toxicity studies were performed in dogs only. Oral multiple dose toxicity studies were performed in rats and dogs (up to 15 weeks). In rats and dogs, effects on bone marrow, lymphoid tissue (thymus, spleen, lymph nodes, gut-associated lymphoid tissues), hepatobiliary system, testes and lungs were observed. In addition, in dogs, effects on intestinal mucosa, skin, bone/ribs and ovaries were also reported. In the 15-week rat study, the kidney was identified as an additional target organ of toxicity. The effects of ribociclib on the bone marrow (hypocellularity), lymphoid system (lymphoid depletion), intestinal mucosa (atrophy), skin (atrophy), bone (decreased bone formation) and testes (atrophy) can be regarded as related to the pharmacological inhibition of cell replication in these tissues due to CDK4/6 inhibition. An increased number of ovarian corpora lutea was observed in a single female dog at the highest dose tested (20 mg/kg/day) and this effect could also be related to the pharmacology (arrest of estrous cycle). The hepatobiliary system (proliferative changes, cholestasis, sand-like gallbladder calculi, and inspissated bile) and the kidney (concurrent degeneration and regeneration of tubular epithelial cells) were identified as additional target organs of toxicity, which are not likely related to the primary pharmacology of ribociclib. Inflammatory changes in the lung of dogs and the increase of alveolar macrophages in the rat studies were considered secondary to aspiration of test-article and are indicative of the irritant potential of the formulated test-article in the respiratory tract. Correlating hematological and/or biochemistry changes were seen for the effects described in the bone marrow, lymphoid system and liver. Generally, all changes demonstrated either reversibility or a clear tendency towards reversibility. It can be expected that after a prolonged recovery time incompletely recovered findings

would have totally reversed in both species.

It is advised to monitor carefully hematological, hepatobiliary and kidney parameters, as well as gastrointestinal effects in patients treated with ribociclib. In vitro and in vivo, ribociclib did not show an indication for a genotoxic potential. In vitro, ribociclib did not show a phototoxic potential. In conclusion, multiple biological effects were observed in toxicology studies up to 15 weeks duration in rats and dogs. The observed toxicities can be considered clinically manageable. The critical nature of many of these effects and the potentially severe consequences for patients, however, warrant careful monitoring.

2.2.2 Adult Clinical Trials

2.2.2.1 Adult Phase 1 Studies

As of April 2015, 132 patients have been treated with single agent ribociclib in the first-in-human phase I study; 85 patients have been treated in the dose escalation part and 47 patients in the dose expansion part of the study. Patients with advanced solid tumors or lymphomas were treated with increasing doses of ribociclib orally, once daily (qday) for 21 days followed by a 1-week rest (28-day cycle). Doses ranging from 50 mg to 1200 mg were evaluated on this schedule. In addition, continuous dosing of ribociclib at 600 mg was evaluated (qday for 28 days of a 28-day cycle). Doses tested include, 50 mg (n=4), 70 mg (n=2), 140 mg (n=4), 260 mg (n=4), 280 mg (n=4), 350 mg (n=5), 400 mg (n=5), 600 mg (n=67), 600 mg continuous (n=7), 750 mg (n=14), 900 mg (n=13), and 1200 mg (n=3). The median age of patients was 60 years (22 to 84), the male/female ratio was 1, and the distribution of Eastern Cooperative Oncology Group (ECOG) performance status of 0/1 at baseline was 47/84 patients, respectively. The most common cancer types were liposarcoma (33), ER+ breast cancer (20), head and neck (including salivary gland) cancer (16), colon cancer (14), lung cancer (8), and lymphoma (8). Treatment has been discontinued in 111 (84%) patients; the primary reasons for treatment discontinuation were: disease progression (96 [72%] patients); AEs (8 [6%] patients); withdrawal of consent (3 [2%] patient); and loss to follow up (1 [1%] patient).

A total of 10 events meeting DLT criteria were observed at the indicated doses in the 70 patients evaluable for dose determination and include Grade 3 mucositis/stomatitis (later determined to be due to herpes simplex virus infection) (n=1) at 50 mg, Grade 3 pulmonary embolism (n=1) at 280 mg, Grade 3 hyponatremia (asymptomatic and corrected with saline infusion) (n=1) and prolonged Grade 3/4 neutropenia (n=1) at 400 mg, prolonged Grade 2 elevated creatinine (n=1) at 600 mg, Grade 4 thrombocytopenia (n=1) at 750 mg, Grade 3 asymptomatic QTcF prolongation with Grade 3 neutropenia (n=1) at 900 mg and Grade 4 febrile neutropenia (n=1) and Grade 4 thrombocytopenia (n=1) at 1200 mg. There was also one DLT, prolonged Grade 3 neutropenia (n=1) at 600 mg on the continuous dosing schedule.

The most frequently reported AEs (>10%), regardless of grade, causality and ribociclib dose were: fatigue (53.8%); nausea (50.8%); neutropenia (47.7%); leukopenia (46.2%); anemia (37.1%); vomiting (34.8%); thrombocytopenia (34.1%); diarrhea (32.6%); lymphopenia (30.3%); decreased appetite, hyperglycemia (21.2% each); constipation (19.7%); hypoalbuminemia (18.9%); dyspnea (18.2%); cough (16.7%); fever, increased creatinine (15.9% each); abdominal pain, AST increase, edema, headache (15.2% each); back pain (14.4%); dizziness (13.6%); ECG QT prolonged (11.4%); blood alkaline phosphatase increased and hypocalcemia (10.6% each).

For either continuous or intermittent dosing, the onset of neutropenia (most frequently Grade 2) occurs by

Day 15, reaching a nadir in the third or fourth week with recovery during the week of drug holiday. Some patients require additional time for recovery (7 to 14 days). QT changes become evident in the first cycle by Day 8 and later (once steady state is reached), are associated with the maximum drug levels between 1 to 8 h post-dose, and remain stable or improve in subsequent cycles.

As of April 2015, asymptomatic Grade 2 QTcF prolongation was observed with increasing frequency when increasing the dose starting at 600 mg: twelve patients (18%) in the 600 mg cohort, three patients (21%) in the 750 mg cohort, four patients (31%) in the 900 mg cohort, and two patients (67%) in the 1200 mg cohort. Two patients (3%) at 600 mg and two patients (15%) at 900 mg had asymptomatic QTcF prolongation that resulted in a QTcF interval of 500 ms or more. As compared to baseline value, QTcF prolongation was at least 30 msec in 2 patients (50%) at 250mg, 2 (40%) at 350 mg and 400 mg, 46 (73%) at 600 mg, 10 (71%) at 750 mg, 11 (85%) at 900 mg and 2 (67%) at 1200 mg; and at least 60 msec as compared to baseline in respectively 16%, 0%, 39% and 67% of patients at 600 mg, 750 mg, 900 mg and 1200 mg. One grade 1 atrioventricular block of first degree was reported as related to ribociclib given at the dose of 140 mg. No other cardiac abnormalities were observed as related adverse events in any patient.

Paired skin biopsies from 55 patients treated with ribociclib at doses ranging from 50 to 900 mg and paired tumor biopsies from 20 patients (16 patients at 600 mg, 2 patients at 900 mg, and 1 patient each at 70 and 750 mg) were assessed for changes in Ki67 and pRb levels. Preliminary results indicate the following: in skin biopsies, reductions in Ki67 from baseline were observed across all dose levels with a more consistent trend from 400 mg onwards; in tumor biopsies, reductions in Ki67 from baseline were observed in 18/20 patients; however, limited samples and varied tumor types prevent conclusions about any dose-response relationship from being drawn. Changes in pRb were not significant or consistent in either skin or tumor samples, possibly due to varied tumor types.

Out of the 114 evaluable patients, 3 partial responses were observed at the 600 mg dose level; one each in BRAF/NRAS wild type with CCND1 amplified melanoma, and head and neck acinar carcinoma with CDKN2A loss (both on the 3 weeks on/1 week off regimen), and ER+/HER2-, PIK3CA mutant, CCND1 amplified breast cancer (on the continuous daily dosing regimen). This study is currently evaluating a dosing schedule with ribociclib 400 mg continuous dosing, as well as the safety and PK of the oral solution formulation. The MTD of ribociclib in adults is 900 mg qday with a 3 weeks on/1 week off schedule. The RP2D for future development is 600 mg qday with a 3 weeks on/1 week off schedule, which has an acceptable safety profile, lower risk for QT prolongation, adequate exposures, and preliminary evidence of clinical activity. There have been no deaths related to study drug reported on study CLEE011X2101.The following serious adverse events shown in Table 2 have been reported with a suspected causal relationship in study CLEE011X2101 as of August 2014.

A complete list of AEs, all grades and Grade 3/4 occurring in at least 10% of patients and suspected to be related to ribociclib are shown in Table 3.

Serious suspected adverse events which have occurred with LEE011 (single agent)	
System Organ Class Preferred Term	**Preferred Term**
Blood and lymphatic system disorders	Anaemia, Febrile neutropenia, Neutropenia,Thrombocytopenia
Gastrointestinal disorders	Diarrhoea, Nausea
General disorders and administration site conditions	Generalized oedema
Infections and infestations	Herpes simplex
Investigations	*Blood creatinine increased*

Events in *italic font* indicate those events which are newly included since the previous edition of the reference safety information.

Table 2: Serious adverse events with suspected causal relationship with Ribociclib

	LEE011 50 mg N=4		LEE011 70 mg N=2		LEE011 140 mg N=4		LEE011 260 mg N=4		LEE011 280 mg N=4		LEE011 350 mg N=5		LEE011 400 mg N=5	
Preferred term	**All grades n (%)**	**Grade 3/4 n (%)**	**All grades n (%)**	**Grade 3/4 n (%)**	**All grades n (%)**	**Grade 3/4 n (%)**	**All grades n (%)**	**Grade 3/4 n (%)**	**All grades n (%)**	**Grade 3/4 n (%)**	**All grades n (%)**	**Grade 3/4 n (%)**	**All grades n (%)**	**Grade 3/4 n (%)**
-Total	3 (75.0)	1 (25.0)	2 (100)	0	3 (75.0)	0	4 (100)	1 (25.0)	4 (100)	1 (25.0)	4 (80.0)	1 (20.0)	5 (100)	2 (40.0)
Neutropenia	0	0	0	0	0	0	0	0	1 (25.0)	0	1 (20.0)	0	2 (40.0)	1 (20.0)
Fatigue	2 (50.0)	0	2 (100)	0	3 (75.0)	0	4 (100)	0	1 (25.0)	0	1 (20.0)	0	1 (20.0)	0
Leukopenia	0	0	0	0	0	0	1 (25.0)	0	1 (25.0)	0	2 (40.0)	0	1 (20.0)	0
Nausea	0	0	1 (50.0)	0	0	0	0	0	2 (50.0)	0	2 (40.0)	0	1 (20.0)	0
Thrombocytopenia	0	0	0	0	0	0	0	0	1 (25.0)	0	0	0	1 (20.0)	0
Anaemia	0	0	0	0	0	0	0	0	0	0	2 (40.0)	0	2 (40.0)	0
Vomiting	0	0	1 (50.0)	0	0	0	0	0	0	0	2 (40.0)	0	2 (40.0)	0
Lymphopenia	0	0	0	0	0	0	2 (50.0)	0	1 (25.0)	1 (25.0)	1 (20.0)	1 (20.0)	0	0
Diarrhoea	0	0	0	0	0	0	0	0	1 (25.0)	0	1 (20.0)	0	0	0
Electrocardiogram Qt Prolonged	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Increased creatinine	0	0	0	0	0	0	0	0	0	0	3 (60.0)	0	1 (20.0)	0
Decreased Appetite	0	0	0	0	1 (25.0)	0	0	0	2 (50.0)	0	1 (20.0)	0	0	0
Hypoalbuminaemia	0	0	0	0	0	0	1 (25.0)	0	0	0	1 (20.0)	0	0	0
Aspartate Aminotransferase Increased	0	0	0	0	0	0	1 (25.0)	0	0	0	0	0	0	0
Dizziness	0	0	0	0	0	0	0	0	0	0	0	0	2 (40.0)	0
Stomatitis	1 (25.0)	1 (25.0)	1 (50.0)	0	0	0	1 (25.0)	0	1 (25.0)	0	0	0	0	0
Fever	1 (25.0)	0	0	0	0	0	0	0	0	0	0	0	0	0
Headache	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Preferred term	LEE011 50 mg N=4		LEE011 70 mg N=2		LEE011 140 mg N=4		LEE011 260 mg N=4		LEE011 280 mg N=4		LEE011 350 mg N=5		LEE011 400 mg N=5	
	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)
Alanine Aminotransferase Increased	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash	0	0	0	0	1 (25.0)	0	0	0	2 (50.0)	0	0	0	0	0

Preferred term	LEE011 600 mg N=67		LEE011 600 mg 28d cont. N=7		LEE011 750 mg N=14		LEE011 900 mg N=13		LEE011 1200 mg N=3		All subjects N=132	
	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)
-Total	63 (94.0)	33 (49.3)	7 (100)	6 (85.7)	14 (100)	7 (50.0)	12 (92.3)	8 (61.5)	3 (100)	3 (100)	124 (93.9)	63 (47.7)
Neutropenia	31 (46.3)	19 (28.4)	6 (85.7)	5 (71.4)	8 (57.1)	5 (35.7)	9 (69.2)	5 (38.5)	2 (66.7)	1 (33.3)	60 (45.5)	36 (27.3)
Fatigue	22 (32.8)	2 (3.0)	5 (71.4)	1 (14.3)	5 (35.7)	0	10 (76.9)	0	3 (100)	0	59 (44.7)	3 (2.3)
Leukopenia	31 (46.3)	13 (19.4)	6 (85.7)	5 (71.4)	6 (42.9)	0	9 (69.2)	4 (30.8)	0	0	57 (43.2)	22 (16.7)
Nausea	30 (44.8)	1 (1.5)	3 (42.9)	0	8 (57.1)	1 (7.1)	6 (46.2)	0	3 (100)	0	56 (42.4)	2 (1.5)
Thrombocytopenia	23 (34.3)	6 (9.0)	2 (28.6)	0	5 (35.7)	3 (21.4)	6 (46.2)	0	2 (66.7)	1 (33.3)	40 (30.3)	10 (7.6)
Anaemia	19 (28.4)	2 (3.0)	2 (28.6)	0	3 (21.4)	1 (7.1)	3 (23.1)	0	3 (100)	1 (33.3)	34 (25.8)	4 (3.0)
Vomiting	17 (25.4)	0	2 (28.6)	0	4 (28.6)	0	3 (23.1)	0	3 (100)	0	34 (25.8)	0
Lymphopenia	15 (22.4)	12 (17.9)	3 (42.9)	2 (28.6)	6 (42.9)	1 (7.1)	4 (30.8)	4 (30.8)	0	0	32 (24.2)	21 (15.9)
Diarrhoea	18 (26.9)	2 (3.0)	0	0	4 (28.6)	0	5 (38.5)	0	2 (66.7)	0	31 (23.5)	2 (1.5)
Electrocardiogram Qt Prolonged	6 (9.0)	0	0	0	2 (14.3)	0	4 (30.8)	2 (15.4)	2 (66.7)	0	14 (10.6)	2 (1.5)
Increased creatinine	4 (6.0)	0	1 (14.3)	0	3 (21.4)	0	2 (15.4)	0	0	0	14 (10.6)	0
Decreased Appetite	5 (7.5)	0	0	0	3 (21.4)	0	1 (7.7)	0	0	0	13 (9.8)	0

Preferred term	LEE011 600 mg N=67		LEE011 600 mg 28d cont. N=7		LEE011 750 mg N=14		LEE011 900 mg N=13		LEE011 1200 mg N=3		All subjects N=132	
	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)
Hypoalbuminaemia	7 (10.4)	0	1 (14.3)	0	1 (7.1)	0	1 (7.7)	0	0	0	12 (9.1)	0
Aspartate Aminotransferase Increased	8 (11.9)	0	1 (14.3)	0	1 (7.1)	0	0	0	0	0	11 (8.3)	0
Dizziness	6 (9.0)	0	0	0	2 (14.3)	0	1 (7.7)	0	0	0	11 (8.3)	0
Stomatitis	0	0	2 (28.6)	0	3 (21.4)	0	1 (7.7)	0	1 (33.3)	0	11 (8.3)	1 (0.8)
Fever	1 (1.5)	0	2 (28.6)	0	2 (14.3)	0	0	0	2 (66.7)	0	8 (6.1)	0
Headache	6 (9.0)	0	0	0	2 (14.3)	0	0	0	0	0	8 (6.1)	0
Alanine Aminotransferase Increased	5 (7.5)	1 (1.5)	1 (14.3)	0	1 (7.1)	0	0	0	0	0	7 (5.3)	1 (0.8)
Rash	1 (1.5)	0	0	0	0	0	2 (15.4)	0	1 (33.3)	0	7 (5.3)	0

Table 3: All grades and Grade 3 or 4 adverse events (occurring in at least 5% or more) suspected to be related to ribociclib by preferred term and treatment group. (data as of April 2014) (CLEE011X2101)

2.2.2.2 Phase II Studies

<u>Phase Ib/II trial of Ribociclib + Everolimus (RAD001) + Exemestane in postmenopausal women with ER+/HER2 negative locally advanced or metastatic breast cancer</u>

In the ongoing Phase Ib/II clinical trial (CLEE011X2106), as of August 2015, 77 patients treated with the triplet combination, ribociclib (200–350 mg 3 weeks on, 1 week off), everolimus (most at 2.5 mg), and exemestane (25 mg), the median number of prior regimens was 4, and 18 (23%) patients had received prior PI3K/AKT/mTOR or CDK4/6 inhibitors for metastatic disease.

(1) Ribociclib (600 mg) + exemestane (25 mg), n=3

(2) Ribociclib (200 mg) + everolimus (RAD001) (2.5 mg) + exemestane (25 mg)
(3) Ribociclib (250 mg) + everolimus (RAD001) (2.5 mg) + exemestane (25 mg)
(4) Ribociclib (300 mg) + everolimus (RAD001) (2.5 mg) + exemestane (25 mg)

All patients experienced at least one AE. The most frequent AEs (>10%), regardless of grade, causality and Ribociclib dose, were anemia, leukopenia and neutropenia (75.0% each); thrombocytopenia (68.8%); alanine aminotransferase increased, aspartate aminotransferase increased, fatigue and stomatitis (43.8% each); lymphopenia (37.5%); blood alkaline phosphatase increased, hypophosphatemia and nausea (31.3% each); diarrhea, edema, hyperglycemia and hypokalemia (25% each); constipation, dyspnea, headache, hypocalcaemia, insomnia and rash (18.8% each); decreased appetite, dermatitis acneiform, dysgeusia, dyspepsia, electrocardiogram QT prolonged, hypoalbuminaemia, hypomagnesaemia, hyponatraemia, pneumonia and sinusitis (12.5% each). The most common CTCAE Grade 3 or 4 AEs (>2%), regardless of causality and Ribociclib dose, were neutropenia (50.0%); leukopenia (31.3%); hypophosphatemia (18.8%); thrombocytopenia, alanine aminotransferase increased, aspartate aminotransferase increased, fatigue, stomatitis, lymphopenia, dyspnea, dermatitis acneiform, hyponatraemia and pneumonia (6.3% each). Grade 3/4 treatment-related AEs (≥5% patients) were neutropenia (45.7%), leukopenia (8.6%), and thrombocytopenia (5.7%). 17 patients (23%) experiences QTcF > 450 ms, 3 (4%) had QtcF > 480 ms and 2 (3%) had QtcF > 500 ms. Five (7%) patients discontinued due to AEs. Grade 3 AST/ALT AEs were appreciated in 4 patients, febrile neutropenia and hypophosphatemia (1 patient), oral mucositis (1 patient), rash and thrombocytopenia (1 patient), and thrombocytopenia with bleeding (1 patient). The RP2D was declared at ribociclib 300 mg 3 weeks on, 1 week off, everolimus 2.5 mg daily (continuous) and exemestane 25 mg daily (continuous). This combination dose appeared to be tolerable and safe. The following serious adverse events shown in Table 4 have been reported with a suspected causal relationship in study CLEE011X2106.

Serious suspected adverse events which occurred with LEE011 in combination with everolimus (RAD001) and exemestane	
System Organ Class Preferred Term	**Preferred Term**
Blood and lymphatic system disorders	Febrile neutropenia, Leukopenia, Lymphopenia, Thrombocytopenia
Hepatobiliary disorders	Drug-induced liver injury
Infections and infestations	*Pneumonia*
Investigations	Alanine aminotransferase increased, Aspartate aminotransferase increased
Respiratory, thoracic and mediastinal disorders	Acute respiratory failure, Pneumonitis
Skin and subcutaneous tissue disorders	Rash

Table 4: Serious adverse events with a suspected causal relationship with ribociclib in combination with everolimus (RAD001) and exemestane.

As of August 2015, when results of the combination of ribociclib + everolimus (RAD001) + Exemestane were presented at the San Antonio Breast Cancer Symposium (SABCS), 77 patients were evaluable for response. Seven patients (9%) had a confirmed partial response, 39 (51%) patients had stable disease, and 10 (13%) patients had neither complete response nor progressive disease. The median duration of stable disease was 115 days (range: 44-460) and 21 patients experienced stable disease for > 4 cycles. The median (range) duration of treatment exposure was 6 (1-15) months and 4 (1-17) months in patients with (n=11) and without (n=48) CCND1-amplification response resulting in a trend for longer duration of response in patients with CCND1 amplification. Six patients with partial responses had available genetic

data including the following alterations (amplifications/ mutations): PIK3CA (n=3), TP53 (n=2), amplification MDM2 (n=2), amplification MYC (n=2), ATM mutation (n=2) as well as alterations in KRAS, EGFR, FGFR, and ESR1 (n=1 each). One patient with a p16 (*CDKN2A*) deletion and cyclin D1 (*CCND1*) and insulin-like growth factor receptor 1 (*IGFR1*) amplification treated with ribociclib 200 mg + EVE 2.5 mg + EXE had SD >6 months.

2.2.2.3 Ribociclib Adult Pharmacokinetics

PK for Single Agent Ribociclib in Adults

As of June 2015, PK data were available from approximately 128 patients from the first-in-human study CLEE011X2101. Following oral dosing, Ribociclib was rapidly absorbed with median Tmax ranging from 1 to 5 hours. Ribociclib plasma exposure exhibited slightly over-proportional increases in exposure across the dose range tested (50 to 1200 mg), with no clear evidence of time-dependent auto-inhibition of its clearance. Steady-state was generally reached by Day 8, and the mean effective T1/2 based on accumulation ratio (i.e., T1/2,acc) ranged from 15.9 to 32.6 hours across the dose range tested. The accumulation ratio based on AUC obtained in a dosing interval (Racc) across the studied doses ranged from 1.55 to 2.52.

Ribociclib Food and Drug Interactions

A food effect study in 24 healthy subjects indicated that ribociclib administered as a drug-in-capsule formulation can be taken without regards to meals. The presence of food did delay the rate of absorption somewhat, but the extent was unaffected as predicted based upon the solubility characteristics of ribociclib.[51]

An additional study in healthy subjects (CLEE011A2103) demonstrated bioequivalence between the film-coated tablets formulation and capsule formulation. The study demonstrated no clinically important effect of high-fat, high-calorie meal on ribociclib exposure following oral administration of the film coated tablets formulation. Ribociclib exposure under fed conditions was similar to that under fasted conditions.[51]

Ribociclib is unlikely to be the object of a clinical drug interaction, with the exception of strong CYP3A4 inhibitors or inducers, which may markedly affect the exposure of ribociclib based on data from a clinical DDI study with ritonavir and rifampicin (CLEE011A2101).

A drug-drug interaction (DDI) study with ritonavir (a strong CYP3A4 inhibitor) and rifampicin (a strong CYP3A4 inducer) was conducted in 48 healthy subjects (CLEE011A2101). Compared to ribociclib alone, ritonavir (100 mg bid for 14 days) increased ribociclib Cmax and AUCinf by 1.7-fold and 3.2-fold, respectively, following a single oral dose of 400 mg ribociclib. Cmax and AUClast for LEQ803 decreased by 96% and 98%, respectively. In addition, compared to ribociclib alone, rifampicin (600 mg daily for 14 days) decreased LEE011 Cmax and AUCinf by 81% and 89%, respectively, following a single oral dose of 600 mg ribociclib. LEQ803 Cmax increased 1.7-fold and AUCinf decreased by 27%, respectively.

PK of Ribociclib, Everolimus and Exemestane in Adults

In an ongoing Phase Ib clinical trial CLEE011X2106, 2.5 mg of everolimus (RAD001) (qday) + 25 mg exemestane (qday) was administered with 200 or 300 mg ribociclib (qday, 3 weeks on/1 week off). As of March 2014, data for Cmax and AUC0-24h for ribociclib (300 mg, qday, 3 weeks on/1 week off) and everolimus (2.5 mg qday) were available on Day 1 (n=4) and after multiple doses on Day 15 (n=3). A dose-dependent drug interaction (DDI) was observed between ribociclib and everolimus based on clinical PK analyses in a separate dose escalation trial, where everolimus exposure increased 2- to 4-fold in the presence of ribociclib. Cmax and AUC0-24h for everolimus at steady-state suggested that in the presence

of 300 mg ribociclib, everolimus (2.5 mg qday) exposure (AUC0-24h) was increased and was approximately similar to exposure following 5 to 7.5 mg everolimus when compared to historical single agent data.[52,53]

2.2.3 Pediatric Clinical Trials

2.2.3.1 Phase I Trial of Ribociclib

Novartis has completed a phase I, multi-center, open-label study of ribociclib in children with recurrent malignant rhabdoid tumors (MRTs) and neuroblastoma (NB) or other tumors with cyclin D–CDK4/6 pathway aberrations (NCT01747876).[54] The initial treatment schema was as follows:

Dose Level	**Ribociclib (mg/m^2/day x 21 days)**
1 (starting dose)	280 mg/m^2/day
2	350 mg/m^2/day
3	470 mg/m^2/day

Fourteen patients had NB, 15 had MRT (13 primary CNS and two primary extra-CNS), 1 had CDK4-amplified rhabdomyosarcoma, and 1 had anaplastic meningioma with CDKN2A/B loss. Five patients received 280 mg/m^2, 14 received 350 mg/m2, and 12 received 470 mg/m^2 of ribociclib 1. Of 31 eligible patients, 24 (77%) have discontinued treatment for reasons that include disease progression (n=21), withdrawal of consent (n=2), or DLT. Four DLTs were reported, including 1 patient with grade 3 fatigue at 280 mg/m^2 and 3 patients with grade 4 thrombocytopenia at 470 mg/m^2. The most common (≥10%) grade 3/4 AEs suspected to be related to the study drug were neutropenia (61%), leukopenia (32%), and thrombocytopenia (29%). Adverse events were manageable and reversible upon discontinuation of ribociclib. Newly occurring post-baseline values of QTc ≥450 ms were observed in 6 patients (19%; all Grade 1), including 3 at 350 mg/m^2 and 3 at 470 mg/m^2. No treatment-related deaths were reported. Seven patients were still receiving treatment at the time of data cut-off on June 23, 2014. The MTD and RP2D were determined to be 470 mg/m^2 (adult equivalent dose ≈ 800 mg/day) and 350 mg/m^2 (adult equivalent dose ≈ 600 mg/day), respectively, on a 3-weeks-on/1-week-off dosing schedule, consistent with adult data. Three patients received ribociclib for ≥4 cycles, and stable disease was the best response observed.

2.2.3.2 Ribociclib Pediatric Pharmacokinetics

The pharmacokinetics of single agent ribociclib are being evaluated in an ongoing phase clinical trial in children (ages 1 to 21) with malignant rhabdoid tumors, neuroblastoma, and advanced solid tumors/ lymphoma. Patients received escalating ribociclib dosages (280, 350, and 470 mg/m^2) daily for 3 weeks and then 1 week off. Pharmacokinetic studies were conducted on days 1 and 15 of cycle 1 (pre-dose, 1, 2, 4, and 8 hr post-dose), and other samples collected in subsequent times/cycles. The preliminary noncompartmental pharmacokinetic results are presented in the table below.

Dosage (mg/m^2)	$AUC_{0\text{-}24h}$ (ng/mL*hr)	Cmax (ng/mL)
280	13800	1110
350	24500	2010
470 (MTD)	29100	2500

Other preliminary pharmacokinetic results included median time to maximum plasma concentration across cohorts ranged from 2 to 4 hours, and the median half-life ranged from 30 to 41 hours. The mean systemic exposure in children dosed with the 350 mg/m^2 was equivalent to the mean exposure observed

in adults at 600 mg. Based upon safety, tolerability, and pharmacokinetics, the MTD was determined as 470 mg/m^2 once a day for 3 weeks on and 1 week off.

2.3 Everolimus (RAD001)

RAD001 (everolimus) is a rapamycin analog and highly specific small-molecule mTOR inhibitor that functions by binding mTOR (when associated with raptor and mLST8 as part of the mTORC1 complex) and FK506-binding protein 12, leading to G1 phase cell cycle arrest and apoptosis.[55] The anti-cancer effects of everolimus are multifaceted and include transcriptional regulation of *cyclin D1* and *c-Myc*, as well as antiangiogenic effects mediated by down-regulation of hypoxia inducible factor 1 alpha (HIF1α), which decreases vascular endothelial growth factor (VEGF).[55,56] Rapamycin analogs have also been reported to inhibit mTORC2, preventing downstream signaling through Akt.[57]

2.3.1 Preclinical Studies

Everolimus has potent activity in many types of human cancer and has been intensely studied *in vitro* and *in vivo*.[58,59] Rapamycin analogs have been shown effective in rhabdomyosarcoma, neuroblastoma, medulloblastoma/PNET, and pediatric HGG cell lines with IC50s ranging from 0.37 to 4,680 ng/mL.[60,61] Rapamycin was extensively tested in the Pediatric Preclinical Testing Program, including in combination with cytotoxic agents.[56,62] It induced ≥50% cell death in 10 of 23 pediatric cell lines at nanomolar concentrations and showed 'intermediate or high activity' against 14 of 31 solid tumor xenograft models, including partial responses in rhabdoid tumor, rhabdomyosarcoma, and osteosarcoma.[56] Another rapamycin analog, CCI-779, was shown to inhibit growth *in vitro* and *in vivo* in medulloblastoma, PNET, and GBM with synergistic effect noted with cytotoxic agents, such as cisplatin and camptothecin.[63]

Rapamycin analogs are highly lipophilic and have been shown in preclinical studies to cross the blood-brain barrier.[63] This property of everolimus has also been demonstrated nicely in clinical practice, as it has been shown efficacious against certain brain tumors, such as subependymal giant cell astrocytoma (SEGA).[64,65] Preclinical studies with ribociclib showed enhanced antitumor activity in combination with everolimus and exemestane versus each agent alone.[51,66] Everolimus crosses the blood- brain barrier in a non-linear manner with the dose. Pre-clinically, the brain/blood concentration ratio at 2 hours post-dose was 0.2 after a 0.1 mg/kg intravenous dose but progressively increased to 3.1 after a 30 mg/kg intravenous dose where everolimus blood levels are far above the therapeutic concentration range.[66]

2.3.2 Everolimus (RAD001) Adult Clinical Studies

Everolimus has gone through multiple adult phase I, II, and III clinical trials and received its first FDA-approval for advanced renal cancer in 2009. It has since been FDA-approved for other indications, including SEGA associated with tuberous sclerosis in 2010 and in combination with exemestane for advanced hormone-positive, HER2-negative breast cancer in 2012. Everolimus is currently being studied in multiple adult combination studies with other targeted agents, such as dual PI3K/mTOR inhibitor BEZ235 (NCT01482156) and CDK4/6 inhibitor LEE011 + exemestane (CLEE011X2106).

2.3.3 Pediatric Studies

2.3.3.1 Phase I/II Studies

Fouladi *et al*. published a phase I study of everolimus in children with recurrent solid tumors and established the pediatric MTD as 5 mg/m^2 continuous daily administration.[67] At 3 mg/m^2, DLTs included reversible grade 4 hypokalemia (n=1) and grade 3 hypophosphatemia (n=2). No DLTs were observed at 5mg/m^2. At 6.5 mg/m^2, DLTs included grade 3 ALT elevation (n=1), mucositis (n=1), and diarrhea (n=1).

Pharmacokinetic findings of this phase I study are listed in Table 5. Population estimates for clearance (Cl)/F, V/F, and k_a were 12.4 L/h/m^2, 45.5 L, and 1.66 h^{-1}, respectively. Interindividual variability in Cl/F, V/F, and k_a was 47%, 46%, and 45% as determined by the percent coefficient of variation, respectively. Median apparent Cls for the 2.1, 3, 5, and 6.5 mg/m^2 dosage levels were 12.1 L/h/m^2 (range, 9.1 to 24.2 L/h/m^2), 9.3 L/h/m^2 (range, 4.3 to 28.5 L/h/m^2), 15.2 L/h/m^2 (range, 14.4 to 16.0 L/h/m^2), and 16.3 L/h/m^2 (range, 13.9 to 18.7 L/h/m^2), respectively.

	Dosage Level							
	2.1 mg/m^2 (n = 6)		3 mg/m^2 (n = 8)		5 mg/m^2 (n = 2)		6.5 mg/m^2 (n = 2)	
Parameter	Median	Range	Median	Range	Median	Range	Median	Range
k_a, h^{-1}	1.2	0.3-3.8	1.5	0.8-2.2	1.9	1.6-2.1	2.0	1.9-2.2
$t_{1/2}$, hours	18.0	15.2-19.9	15.9	13.9-22,5	17.7	15.8-19.6	15.2	14.8-15.5
$AUC_{0\to\infty}$, ng/mL·h	139.8	94.6-197.7	197.6	59.6-249.4	239.6	222.5-256.6	323.1	273.1-373.1
Noncompartmental pharmacokinetic parameters								
C_{max}, ng/mL	21.5	16.1-78.5	43.5	18-58	60.6	59.2-62	88.5	68.9-108
T_{max}, hours	1.0	0.5-1.2	1.1	0.6-2.3	1	1	0.6	0.5-0.7

Abbreviations: k_a, absorption rate constant; $t_{1/2}$, elimination half-life; $AUC_{0\to\infty}$, area under the concentration-time curve from zero to infinity; C_{max}, maximum everolimus concentration; T_{max}, time at which C_{max} occurred.

Table 5: Summary of everolimus pharmacokinetic parameters.

No objective responses were seen among 18 evaluable patients, but 6 patients exhibited prolonged stable disease, including one patient each with gliomatosis cerebri (low grade glioma; 14 cycles), osteosarcoma (8 cycles), brainstem glioma (5 cycles), peripheral PNET (5 cycles), anaplastic astrocytoma (4 cycles), and EPN (4 cycles). Inhibition of phosphorylated Akt^{S473} was observed at the MTD.

Krueger *et al*. published a phase I/II study of everolimus in patients > 3 years old with tuberous sclerosis and SEGA.[68] Everolimus was administered at 3 mg/m^2 and dose adjusted to achieve a trough concentration of 5 to 15 ng/mL. The median dose of everolimus at 3 and 6 months was 4.7 mg/m^2 and 5.6 mg/m^2, respectively. Stomatitis and upper respiratory infection were the most common adverse events. Four patients experienced severe adverse events, including upper respiratory infection (grade 3), pneumonia (grade 3), and convulsions (n=2; grades 2 and 4). Increases in total cholesterol, low-density lipoprotein cholesterol, and triglycerides were noted. Of 28 eligible patients (median age 11 years), 21 patients and 9 patients achieved at least 30% and 50% tumor reduction, respectively.

Kieran *et al*. reported a phase II study of everolimus in non-NF1 pediatric low-grade glioma (LGG), in which 23 patients (median age 9 years) were given everolimus 5 mg/m^2/day in 28-day cycles.[69] Four patients experienced partial response, and 13 had stable disease after a median of 10 cycles. Six patients experienced disease progression by 1 year. One patient discontinued therapy due to toxicity (mouth sores). PK was similar to that previously reported in children, and PD markers (phospho-S6 kinase, 4E-BP1 phosphorylation, and *cMyc* expression) were decreased by day 14 of therapy.

Geoerger *et al*. published a phase II study of single-agent temsirolimus (weekly oral administration at 75 mg/m^2), a rapamycin analog, in children with recurrent HGG, neuroblastoma, and rhabdomyosarcoma.[70] Only one patient with neuroblastoma had a PR, though prolonged disease stabilization (≥3 months) was observed in 7 of 17 (41%) of patients with HGG (including 5 DIPG, 1 GBM, and 1 anaplastic astrocytoma), suggesting potential utility if used in combination.

2.3.3.2 Phase III Studies

Franz *et al*. published an international phase III study confirming the efficacy of everolimus in patients with tuberous sclerosis and SEGA.[64] This study randomized 117 patients (median age 9.5 years in everolimus group and 7.1 years in placebo group) to receive everolimus (4.5 mg/m^2/day to trough concentration of 5 to 15 ng/mL) or placebo. The most common adverse events were mouth ulceration (32% everolimus, 5% placebo), stomatitis (31% everolimus, 21% placebo), convulsions (23% everolimus, 26% placebo), and pyrexia (22% everolimus, 15% placebo). Twenty-seven patients (35%) had a t least 50% reduction in the volume of SEGA versus none in the placebo group.

2.3.3.3 Pediatric Combination Studies including mTOR inhibitors

Wagner *et al*. published results of a phase II combination study with temsirolimus and cixutumumab, a monoclonal antibody against insulin-growth factor type 1 receptor (IGF-1R), in patients < 30 years old with recurrent solid tumors.[71] Temsirolimus (8 mg/m^2 with dose escalation to 10 mg/m^2 for subsequent cycles if tolerable in first cycle) and cixutumumab (6 mg/kg) were administered once weekly for 4 week cycles and were generally well tolerated. The most common toxicities were electrolyte abnormalities, mucositis, and myelosuppression. There were no objective responses.

Yalon *et al.* reported a feasibility and phase II study of rapamycin (0.8 mg/m^2/dose twice daily for trough concentration of 10 to 15 ng/mL) in combination with erlotinib (65 mg/m^2 daily continuous dosing), an EGFR inhibitor, in pediatric low-grade glioma.[72] Nineteen patients were evaluable. No DLTs were noted in the feasibility portion of the study. The most common toxicities were rash (n=11), oral apthous ulcers (n=9), and GI complaints (n=7). One patient (with NF1) had a PR and 6 patients remained on therapy for the planned 12 courses, while 10 patients progressed on therapy.

Becher *et al.* reported a feasibility Phase I study with combination of perifosine (AKT inhibitor) and temsirolimus (mTOR inhibitor) in patients with recurrent CNS/solid tumors. Twenty-three patients were treated. Diagnosis included DIPG (n=8), HGG (n=6), medulloblastoma (n=2), ependymoma (n=1), neuroblastoma (n=4) and rhabdomyosarcoma (n=2).[73] No DLTs were noted. The most common toxicities were thrombocytopenia (38.1%), neutropenia (23.8%), lymphopenia (23.8%), and hypercholesterolemia (19%). Stable disease was noted in 9 of the 11 patients diagnosed with DIPG or HGG. No partial or complete responses were achieved.[73]

2.4 Rationale for Proposed Pediatric Study

Aberrant cell cycle regulation and activation of the PI3K/Akt/mTOR pathway represent two highly important mechanisms of malignant potential in pediatric brain tumors. Joint inhibition of CDK4/6 and mTOR is promising due to strong biologic rationale, non-overlapping single-agent toxicities, and preliminary clinical experience in adults that suggests tolerability of this combination. Additionally, increased exposure of everolimus in the presence of ribociclib, as observed in pharmacokinetic profiling of samples from adults receiving the combination, suggest that lower doses of both drugs may also be used in the pediatric population.

We propose a phase I and surgical study of ribociclib and everolimus in children ≥ 1 and ≤ 21 years of age with recurrent, progressive or refractory HGG, DIPG, medulloblastoma, CNS embryonal tumor (NOS), Ependymoma, or ATRT with evidence of an intact Rb1 protein assessed on formalin fixed paraffin embedded tumor tissue (preferably from the most recent recurrence). DIPGs will not require molecular screening if tissue is not available since biopsy of DIPG is not currently standard of care and since they

frequently harbor cell cycle aberrations and activation of the PI3K/Akt/mTOR pathway, as detailed in the background section.

The proposed dose of ribociclib for the surgical study is the recommended single agent phase 2 dose 350 mg/m^2/dose which is a higher dose in comparison of ribociclib dose in combination with everolimus. Pre-clinical data reveals ribociclib crosses the blood brain barrier providing support for the surgical study. PK modeling and extrapolation will be completed. Administering the higher dose within the surgical cohort will provide information regarding exposure in the blood, tumor, and CSF generating knowledge of the RP2D. Initial evaluation at the surgical MTD (350 mg/m^2) will result PK of CSF, tumor, and plasma. For the proposed combination study with ribociclib and everolimus, we will evaluate plasma PK and extrapolate the PK in comparison to the surgical study PK analysis. The recommended time from dosing to surgery T max is 2-4 hours. Although ribociclib may cause myelosuppression, the timing of myelosuppression is usually after at least 3 weeks of therapy. However, we will have strict platelet requirements for this cohort pre-operatively. Ribociclib has not been reported to be associated with delayed wound healing. Following tumor resection and recovery from surgery (~2 weeks), patients enrolled on the Surgical study will be switched over to the phase I study and be treated with ribociclib + everolimus at the assigned dose level. Tumors resected following treatment with ribociclib will be assessed for Ki67 for ribociclib effect on archival tumor and recurrent tumor.

Primary Hypothesis: We hypothesize that ribociclib and everolimus will be safe and tolerable in children with recurrent malignant CNS tumors.

Secondary Hypothesis: We hypothesize that ribociclib will achieve adequate intra-tumoral drug concentration in children with recurrent malignant CNS tumors.

2.5 Correlative Studies Background

2.5.1 Rationale for Biology Studies

2.5.1.1 Hypothesis

Molecular markers and targeted genomics or RNA sequencing may predict sensitivity or mechanism of response to CDK4/6 inhibitors such as ribociclib and mTOR inhibitors such as everolimus.

2.5.1.2 Preclinical and Clinical Data

Mutations that dysregulate the cell cycle and the PI3-kinase/AKT/mTOR pathway are frequently encountered in adult glioblastoma[7] as well as in pediatric glioblastoma albeit at lower frequencies.[63] Dysregulation of these two critical pathways may also occur in the absence of genetic mutations, for example evidence for PI3-kinase/AKT/mTOR pathway activation in 80% of pediatric glioblastoma has been presented.[64] Furthermore, the PI3-kinase/AKT pathway can regulate cell cycle progression in an RB-dependent manner.[65] There are multiple potential mechanisms for crosstalk between these two core glioblastoma pathways including AKT-mediated phosphorylation and inhibition of GSK-3β leading to Cyclin D1 accumulation, direct phosphorylation and inhibition of the CDK2 inhibitors p21 and p27 by AKT, and AKT-mediated phosphorylation and inhibition of FOXO transcription factors which are known to promote p27 expression. Recent genomic studies also suggest the importance of cell cycle regulation in the oncogenesis of pediatric HGG, including TP53 mutations and Rb mutations, which were reported in 42-70% and 14-58% of pediatric HGG, respectively.[7-10] Wu *et al*. also reported amplification of G1 checkpoint regulators CCND1, CCND2, CCND3, CDK4, and CDK6 in 25% (14/57) of DIPGs and

collectively implicated mutations predicted to affect cell cycle regulation in 59% of pediatric HGG in their cohort.[8] Mutations of *PTEN*, a tumor suppressor gene involved in cell cycle regulation, were also recently reported by Buczkowicz *et al*. in 25% (9/36) of DIPGs.[7] Findings by Taylor *et al*. and Fontebasso *et al*. support the important role of PI3K/Akt/mTOR in pediatric HGG, as 46% (12/26) of DIPG and 26% (10/39) of DIPG or midline non-brainstem HGG in their respective studies exhibited mutations predicted to activate the (RTK)-PI3K-MAPK pathway.[9,10]

In this study, we will explore the relationships between the activation status of the cell cycle and PI3-kinase/AKT/mTOR pathways in patient samples. We will correlate our findings to the results from other correlative biological studies as well as to response to treatment and patient outcome.

2.5.2 Rationale for Pharmacokinetic Studies

2.5.2.1 Hypothesis

The pharmacokinetics and tolerability of ribociclib and everolimus in pediatric patients with recurrent/progressive brain tumors may differ from prior studies of ribociclib and everolimus in the adult population and may be affected by prior treatment, age, body surface area, steroid use or concomitant medications.

2.5.2.2 Ribociclib Preclinical Pharmacokinetic Studies

Because the pharmacokinetics of these agents in combination are relatively unknown in the pediatric population, this information will be essential for evaluating toxicity and disease response and for refining dosing in future combination clinical trials of ribociclib. The results of these studies will provide the basis for assessing the relationship between the disposition of ribociclib when administered in combination with everolimus in adult and children. Mandatory pharmacokinetic studies are needed to characterize the disposition of ribociclib and everolimus in this patient population.

In an ongoing Phase Ib clinical trial CLEE011X2106, 2.5 mg of everolimus (RAD001) (qday) + 25 mg exemestane (qday) was administered with 200 or 300 mg ribociclib (qday, 3 weeks on/1 week off). A dose-dependent drug interaction (DDI) was observed between ribociclib and everolimus based on clinical PK analyses in a separate dose escalation trial, where everolimus exposure increased 2- to 4-fold in the presence of ribociclib. Cmax and AUC0-24h for everolimus at steady-state suggested that in the presence of 300 mg Ribociclib, everolimus (2.5 mg qday) exposure (AUC0-24h) was increased and was approximately similar to exposure following 5 to 7.5 mg everolimus when compared to historical single agent data.[52,53]

Our pre-clinical work on Group 3 medulloblastoma (Grp3 MB), and diffuse intrinsic pontine glioma (DIPGx7) tumors reported the median unbound ribociclib Cmax in all three groups was 100 nM confirming distribution across the blood brain barrier.[49]

2.5.3 Rationale for Dexamethasone mouthwash

mTOR inhibitor associated stomatitis results in dose delays, reductions or discontinuation of the therapy resulting in compromise of duration and intensity of therapy. Anecdotal reports have suggested that topical steroids may improve healing of everolimus-associates apthous ulcers.[74,75] In a prior Phase 3 trial (BOLERO-2 trial) of everolimus and exemestane therapy for patients with hormone receptor-positive, HER2-negative metastatic breast cancer, all grade stomatitis occurred in 67% of patients including grade 2 or worse in 33% and grade 3 severity in 8%.[76] 89% of all grade stomatitis was most prominent within

8 weeks of starting the everolimus.[76] The SWISH trial was recently published in the Lancet Oncology. This is a multicenter, single-arm, phase 2 study of postmenopausal women who received everolimus and exemestane for hormone receptor-positive metastatic breast cancer and were evaluated for prevention of everolimus-related stomatitis with dexamethasone mouthwash compared to data from the BOLERO-2 trial as a historical control cohort for comparisons. 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 BOLERO-2 study. The most frequently reported grade 3 and 4 adverse events in the safety cohort included hyperglycemia (8%), rash (4%) and dyspnea (3%). The adverse events associated with the dexamethasone mouthwash were minimal (2 patients developed oral candidiasis).[77] Given the minimal toxicities related to dexamethasone mouthwash and significant decrease in everolimus associated stomatitis, we will incorporate using dexamethasone in our patient population for at least the first 2 courses of therapy and monitor closely for dexamethasone associated toxicities in addition to prevention of stomatitis.

3 PATIENT SELECTION

All subjects must meet the following inclusion and exclusion criteria. No exceptions will be given. Imaging studies to establish eligibility must be done within 3 (three) weeks prior to enrollment. All other clinical evaluations to establish eligibility must be done within 7 days prior to enrollment. Subjects enrolled on the surgical study, will be re-assessed following guidelines in Section 10.1 prior to starting the combination of ribociclib and everolimus.

3.1 Eligibility for Screening (Phase I and surgical study)

All subjects must meet the following screening criteria without exception.

3.1.1 Tumor

- Patients with a histologically confirmed diagnosis of HGG, medulloblastoma, CNS embryonal tumor (NOS), Ependymoma, or ATRT that is recurrent, progressive or refractory.
- Patients with recurrent DIPG with typical radiographic appearance who have undergone biopsy are eligible provided there is histologic confirmation of malignant glioma WHO II-IV. Rb1 screening for these patients is required only if adequate tissue is available.
- Patients with recurrent brainstem tumors with an atypical presentation who have undergone biopsy are eligible provided there is histologic confirmation of malignant glioma WHO II-IV. These patients must undergo Rb1 screening. These patients must have radiographic evidence of progression.
- Patients with secondary malignant gliomas will be eligible for this study but should conform to all other eligibility requirements. Patients with low-grade gliomas are excluded.

3.1.2 Pre-trial tumor tissue availability:

Formalin fixed paraffin embedded tumor tissue (preferably from the most recent recurrence) must be available to assess Rb1 protein status prior to enrollment on Phase I or surgical study. If the subject has results from prior Rb1 IHC testing in a CLIA-certified laboratory the requirement for screening to assess Rb1 protein status is waived. In these cases, patients will not be required to sign a screening consent.

Patients with recurrent diffuse intrinsic brain stem glioma (DIPG) that has an atypical presentation must also submit the tumor tissue for Rb1 protein status confirmation or provide previous testing results from a CLIA certified laboratory. Patients who have been biopsied for atypical DIPG but do not have sufficient tissue for Rb1 screening are not eligible.

3.1.3 Age

Patient must be ≥ 1 but ≤21 years of age at the time of enrollment.

3.1.4 BSA

- Patients enrolled on dose level -1 must have BSA≥0.55m^2
- Patients enrolled on dose level -0.5 must have BSA≥0.75m^2
- Patients enrolled on dose level 0 must have BSA≥0.55m^2
- Patients enrolled on dose level 1 must have BSA≥ 0.75m^2
- Patients enrolled on dose level 2 and 3 must have BSA≥ 0.45m^2

3.1.5 Screening Consent

Patients who are candidates for enrollment for the phase I or surgical studies must sign a screening consent and provide pre-trial tumor material for Rb1 testing unless testing is not needed due to diagnosis or the

availability of prior Rb1 IHC results. The screening consent is to be obtained according to institutional guidelines.

3.1.6 Potential Eligibility for Study Enrollment

Patients screened for this trial should be expected to meet the criteria for treatment as outlined in Section 3.2.

3.2 Eligibility Criteria: Prior to Study Enrollment

(applicable for Phase I and surgical cohort)

3.2.1 Rb1 Status

Patient has intact Rb1 protein confirmed either from previous results or screened tissue. All testing must be performed in a CLIA certified laboratory. DIPG patients with radiographically typical appearance will be waived from this requirement.

3.2.2 Diagnosis

3.2.2.1 Phase I (Stratum 1)

- Patients with Recurrent or Refractory CNS tumors

Patients with a histologically confirmed diagnosis of a primary CNS tumor that is recurrent, progressive, or refractory. All tumors must have histologic verification of HGG, medulloblastoma, CNS embryonal tumor (NOS), Ependymoma, or ATRT. Patients with low-grade gliomas are excluded.

- Patients with DIPG

Patients with progressive DIPG, as defined by progressive neurologic abnormalities or worsening neurologic status not explained by causes unrelated to tumor progression (e.g., anticonvulsant or corticosteroid toxicity wean, electrolyte disturbances, sepsis, hyperglycemia, etc.), OR an increase in the bi-dimensional measurement, taking as a reference the smallest disease measurement recorded since last treatment, OR the appearance of a new tumor lesion since diagnosis.

Please note:

- Patients with a radiographically typical DIPG, defined as a tumor with a pontine epicenter and diffuse involvement of more than 2/3 of the pons, are eligible without histologic confirmation.

- Patients with pontine lesions that do not meet these radiographic criteria will be eligible if there is histologic confirmation of malignant glioma WHO II-IV. These patients must have radiographic evidence of progression.

3.2.2.2 Surgical Study (Stratum 2)

- Patients must have recurrent or refractory disease with a histological diagnosis at either the time of diagnosis or at the time of recurrence of one of the following:
  - HGG
  - medulloblastoma,
  - CNS embryonal tumor (NOS),
  - Ependymoma, or
  - ATRT

- Patients for whom surgical intervention is clinically indicated (gross total resection or sub-total resection) at recurrence and are amenable to receiving ribociclib for 7 – 10 days prior to resection
    - Note: Patients with DIPG are excluded from the surgical study.

3.2.3 Age

Patient must be ≥ 1 and ≤ 21 years of age at the time of enrollment.

3.2.4 BSA

- Patients enrolled on dose level -1 must have BSA≥0.55m^2
- Patients enrolled on dose level -0.5 must have BSA≥0.75m^2
- Patients enrolled on dose level 0 must have BSA≥0.55m^2
- Patients enrolled on dose level 1 must have BSA≥ 0.75m^2
- Patients enrolled on dose level 2 and 3 must have BSA≥ 0.45m^2

3.2.5 Prior Therapy

Patients must have received prior therapy other than surgery and must have fully recovered from the acute toxic effects of all prior anti-cancer therapy (≤Grade 1 with the exception of alopecia) and must meet the following minimum duration from prior anti-cancer directed therapy prior to enrollment. If after the required timeframe, the defined eligibility criteria are met, e.g. blood count criteria, the patient is considered to have recovered adequately.

3.2.5.1 Myelosuppressive chemotherapy

Patients must have received their last dose of known myelosuppressive anticancer therapy at least 21 days prior to enrollment or at least 42 days if nitrosourea.

3.2.5.2 Investigational/Biologic Agent

- Biologic or investigational agent (anti-neoplastic):

Patient must have recovered from any acute toxicity potentially related to the agent and received their last dose of the investigational or biologic agent ≥ 7 days prior to study enrollment.

    - For agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time during which adverse events are known to occur.
- Monoclonal antibody treatment and agents with known prolonged half-lives: At least three half-lives must have elapsed prior to enrollment.

Note: A list of the half-lives of commonly used monoclonal antibodies is available on the PBTC webpage under Generic Forms and Templates.

3.2.5.3 Radiation

Patients must have had their last fraction of:

- Craniospinal irradiation (>24Gy) or total body irradiation > 12 weeks prior to enrollment.
- Focal irradiation > 2 weeks prior to enrollment

3.2.5.4 Stem Cell Transplant

Patient must be:

- ≥ 3 months since autologous bone marrow/stem cell transplant prior to enrollment

3.2.6 Inclusion of Women and Minorities

Both males and females of all races and ethnic groups are eligible for this study

3.2.7 Neurologic Status

- Patients with neurological deficits should have deficits that are stable for a minimum of 1 week prior to enrollment.
- Patients with seizure disorders may be enrolled if seizures are well controlled on an anti-epileptic drug that is not a strong inducer or inhibitor of CYP3A4/5 are eligible.

3.2.8 Performance Status

Phase I

Karnofsky Performance Scale (KPS for > 16 years of age) or Lansky Performance Score (LPS for ≤ 16 years of age) assessed within one week of enrollment must be ≥ 50.

Surgical Study

Karnofsky Performance Scale (KPS for > 16 years of age) or Lansky Performance Score (LPS for ≤ 16 years of age) assessed within one week of enrollment must be ≥ 60.

Patients who are unable to walk because of neurologic deficits, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

3.2.9 Organ Function

Patients must have adequate organ and marrow function as defined below:

- Absolute neutrophil count ≥ 1.0 x 10^9 cells/ L
- Platelets ≥ 100 x 10^9 cells/ L (unsupported, defined as no platelet transfusion within 7 days)
- Hemoglobin ≥ 8g/dl (unsupported)
- Total bilirubin ≤ 1.5 times institutional upper limit of normal (ULN)
- ALT(SGPT) ≤ 3 x institutional upper limit of normal (ULN)
- AST (SGOT) ≤ 3 x institutional upper limit of normal (ULN)
- Albumin ≥ 2 g/dl
- Serum creatinine based on age/gender as noted in Table 6. Patients that do not meet the criteria in but have a 24 hour Creatinine Clearance or GFR (radioisotope or iothalamate) ≥70mL/min/1.73m^2 are eligible.

Table 6

Table 6

Serum Creatinine for age/gender		
Age	**Maximum Serum Creatinine (mg/dL)**	
	Male	**Female**
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

3.2.10 Corticosteroids

Patients who are receiving dexamethasone must be on a stable or decreasing dose for at least 1 week prior to enrollment.

3.2.11 Growth Factors

Patients must be off all colony- forming growth factor(s) for at least 1 week prior to enrollment (i.e. filgrastim, sargramostim or erythropoietin). 2 weeks must have elapsed if patients received long-acting formulations.

3.2.12 Pregnancy Status

Female patients of childbearing potential must have a negative serum or urine pregnancy test.

3.2.13 Pregnancy Prevention

Patients of childbearing or child fathering potential must be willing to use a medically acceptable form of birth control while being treated on this study.
"Women of child-bearing potential" is defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception throughout the study and for 8 weeks after study drug discontinuation. Highly effective contraception methods include:

- Total abstinence when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception
- Combination of any of the two following (a+b or a+c or b+c)
  - Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception
  - Placement of an intrauterine device (IUD) or intrauterine system (IUS)
  - Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository

Note: Oral contraceptives are allowed but should be used in conjunction with a barrier method of contraception due to unknown effect of drug-drug interaction. In case of use of oral contraception, women should have been stable on the same pill for a minimum of 3 months before taking study treatment.

Female patients must agree not to breastfeed their infants while on study.

Patients of child fathering potential (defined as > Tanner stage 2) must use a condom during intercourse while taking the drug during treatment, for 8 weeks after stopping treatment and should not father a child in this period. A condom is required to be used also by vasectomized men during intercourse with a male or female partner in order to prevent delivery of the study drug via semen.

3.2.14 Informed Consent

The patient or parent/guardian is able to understand the consent and is willing to sign a written informed consent document according to institutional guidelines. Assent, when appropriate, will be obtained according to institutional guidelines.

3.3 Exclusion Criteria

3.3.1 Surgery

Patients who are otherwise deemed clinically unsuitable for surgical resection (applicable for surgical study only)

3.3.2 Breast feeding

Patients who are breast feeding.

3.3.3 Concurrent Illness

- Patients with any clinically significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction), that would compromise the patient's ability to tolerate protocol therapy, put them at additional risk for toxicity or would interfere with the study procedures or results.
- Patients with any other current malignancy, except patients with a secondary brain tumor if the patient's first malignancy has been in remission for at least 5 years from the end of treatment.

3.3.4 Concurrent Therapy

- Patients who are receiving any other anticancer or investigational or/and anti-neoplastic therapies, including chemotherapy, immunotherapy, target therapy, biological response modifiers.
- Previous treatment with CDK4/6 inhibitors (such as PD-0332991, abemaciclib) and/or mTOR inhibitors (such as sirolimus, temsirolimus or everolimus).
- Patients who are currently receiving treatment with agents that are known to cause QTc prolongation or induce Torsades de Pointes (see Appendix 15.2 and 15.4).
- Known need for major surgery within 14 days of the first dose of ribociclib and everolimus. Please note: Gastrostomy, insertion of a G tube, Ventriculo-peritoneal shunt, endoscopic ventriculostomy and central venous access are NOT considered major surgery.

3.3.5 Concomitant Medications

Patient is currently receiving any of the following medications and cannot be discontinued 7 days prior to enrollment (see Appendix 15.2 and 15.4 for details):

- Known strong and moderate inducers or inhibitors of CYP3A4/5, including enzyme inducing anti-convulsant drugs (EIACDs), grapefruit, grapefruit hybrids, pummelos, star-fruit, and Seville oranges
- Substrates of CYP3A4/5 with a narrow therapeutic index
- Herbal preparations/medications (except for vitamins) including, but not limited to: St. John's wort, Kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, black cohosh and ginseng. Patients should stop using all herbal medications and dietary supplements at least 7 days prior to enrollment.

3.3.6 Cardiac disease

Clinically significant active cardiac disease, uncontrolled heart disease and/or a history of cardiac dysfunction including any of the following:

- History of acute coronary syndromes (including myocardial infarction, unstable angina, coronary artery bypass grafting, coronary angioplasty, or stenting) or symptomatic pericarditis within 12

months prior to screening
- History of documented congestive heart failure (New York Heart Association functional classification III-IV)
- Documented cardiomyopathy
- Patient has a Left Ventricular Ejection Fraction (LVEF) <50% as determined by echocardiogram (ECHO)
- History of any cardiac arrhythmias, e.g., ventricular, supraventricular, nodal arrhythmias, or conduction abnormality within 12 months of screening
- Long QT syndrome or known family history of idiopathic sudden death or congenital long QT syndrome, or any of the following:
  - Risk factors for Torsades de Pointe (TdP) including uncorrected hypokalemia or hypomagnesemia, history of cardiac failure, or history of clinically significant/symptomatic bradycardia.
  - Concomitant use of medication(s) with a known risk to prolong the QT interval and/or days prior to starting study drug) or replaced by safe alternative medication.
- 12-lead Electrocardiogram (ECG)
  - QTc ≥450 msec
- Hypertension defined as:
  - Patients 1-12 years of age with blood pressure that is > 95th percentile for age, height and gender at the time of enrollment.
    - The normal blood pressure by height, age and gender tables can be accessed in the Generic Forms section of the PBTC members' webpage.
  - Patients who are ≥ 13 years of age with blood pressure > 130/80 mm of Hg at the time of enrollment.

* Note: If a BP reading prior to enrollment does not meet parameters, blood pressure should be rechecked and documented to be within eligibility range prior to patient enrollment.

3.3.7 Bleeding Disorder

Patient is currently receiving warfarin or other coumadin-derived anticoagulant for treatment, prophylaxis or otherwise. Therapy with heparin, low molecular weight heparin (LMWH) or fondaparinux is allowed as long as patient has adequate coagulation defined as: aPTT INR ≤ 1.5 x upper limit of normal.

3.3.8 Allergy

Patient has a known hypersensitivity to ribociclib or any of its excipients as described below:
- The capsules contain only the drug substance without any excipients.
- The film-coated tablets consist of drug substance and compendial quality colloidal silicon dioxide, crospovidone, hydroxypropylcellulose, magnesium stearate and microcrystalline cellulose. The film-coating is a mix of compendial quality iron oxides, lecithin, polyvinyl alcohol, talc, titanium dioxide, and xanthan gum. Patients allergic to peanut and soy are not permitted to take the film-coated tablet formulation.
- The oral solution consists of ribociclib succinate in water with an orange flavoring agent and common excipients such as preservatives, sweetener and pH modifier

3.3.9 Inability to Participate

Patients with inability to return for required follow-up visits or obtain follow-up studies required to assess toxicity to therapy or to adhere to drug administration plan, other study procedures, and study restrictions.

3.4 Criteria to Start Treatment

This section applies to both the Phase I study and the pre-surgical period of Surgical study. The following must be met prior to receiving ribociclib and everolimus combination for Phase I study or ribociclib alone for the Surgical study.

- Subjects must start therapy within seven (7) days of enrollment.
- Laboratory values must be no older than 7 days prior to the start of therapy. If a test that is repeated post enrollment and prior to the start of therapy is outside the limits for eligibility, it must be rechecked within 48 hours prior to the start of therapy. If rechecks are still outside the limits for eligibility, the patient may not receive protocol therapy and will be considered off study.

3.5 Criteria to Start Treatment with ribociclib and everolimus combination therapy

The following must be documented for Surgical patients during the post-surgical period when switching to Phase I treatment prior to receiving ribociclib and everolimus. If any of these criteria are not met, the patient is not eligible to start treatment. See section 10.1 for details of the assessments.

3.5.1 Imaging:

Subjects must have intra-operative or post-operative (<96 hours) MRI to document disease status.

3.5.2 Ribociclib and Everolimus Combination:

Patient amenable to receiving the drug combination therapy, ribociclib and everolimus, at least 2 weeks post- surgery and no later than 4 weeks post- surgery.

3.5.3 Organ functions

Laboratory parameters must meet the eligibility criteria as defined in Section 3.2.9. Laboratory values must be no older than 7 days prior to the start of combination therapy. If a test that is repeated post-surgery and prior to the start of therapy is outside the limits for eligibility, it must be rechecked within 48 hours prior to the start of therapy. If rechecks are still outside the limits for eligibility, the patient may not receive protocol therapy and will be considered off study.

3.5.4 Pregnancy status:

Female patients of childbearing potential must have a negative serum or urine pregnancy test.

3.5.5 Concurrent illness:

Patients must have recovered from surgical toxicities and cleared by Neurosurgery to start therapy.

3.5.6 Neurological status:

Patients must be neurologically stable and on stable or decreasing steroids for at least one week.

3.6 Treatment at the Primary Institution

All experimental protocol therapy should be dispensed and all on treatment imaging studies should be obtained at a PBTC institution. Laboratory studies, excluding pharmacokinetic and biologic assays, may

be performed at a CLIA certified laboratory of the investigator's choice. Imaging utilized to determine eligibility may be performed at an outside institution if all required imaging sequences are included and the study is deemed of adequate quality by the treating team. All required physical examinations, laboratory parameters need to be performed at the primary PBTC institution during the dose finding period of the protocol.

4 REGISTRATION PROCEDURES

4.1 CTEP Investigator Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN, RAVE, or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	**IVR**	**NPIVR**	**AP**	**A**
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the training, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR Help Desk by email at RCRHelpDesk@nih.gov.

4.2 CTSU Registration Procedures

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

4.2.1 IRB Approval

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federal Wide Assurance (FWA) number,
- An active roster affiliation with the Lead Network or a participating organization,
- A valid IRB approval, and
- Compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site.

Requirements for PBTC-050 Site Registration:

- IRB approval (For sites not participating via the NCI CIRB; local IRB approval documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

4.2.2 Downloading Site Registration Documents

Site registration forms may be downloaded directly from the CTSU members' website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab
- Click on the Home tab, then scroll down to the "printable CTSU Forms" section.
- Select and download this form:
    - CTSU IRB Certification

4.3 Requirements for Submitting Regulatory Documents

Submit completed forms along with a copy of your IRB Approval and approved Informed Consent to the PBTC via the PBTC document upload system. Once all documents are received, the responsible Protocol Coordinator listed on the study cover page will submit the regulatory documents to the CTSU Regulatory Office via the Regulatory Submission Portal, where they will be entered and tracked in the CTSU RSS.

4.3.1 Delegation of Task Log (DTL)

Each site must complete a protocol-specific DTL. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an Approved site registration status and enrolling patients to the study. The DTL application is located on the CTSU members' website at www.ctsu.org.

Any individual at the enrolling site on a participating roster may initiate the site DTL. Instructions on completing the DTL are embedded in the DTL application.

4.3.2 Checking Site Registration Status

You can verify your site's registration status on the members' section of the CTSU website. Sites will be notified by the PBTC when all required documents are uploaded to the CTSU Regulatory Office.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.4 General Guidelines

4.4.1 Prior to Consent

Prior to discussing protocol entry with the patient, all site staff must verify a slot is available via the CTSU OPEN Slot Reservation System which is accessible through https://open.ctsu.org/open/home.open.

4.5 Enrollment Procedures

Screening and patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. To access OPEN, the site user must have an active CTEP-IAM account (check at < https://ctepcore.nci.nih.gov/iam>) and a 'Registrar' role on either the LPO or participating organization roster. Registrars must hold a minimum of an AP registration type. If a DTL is required for the study, the registrar(s) must also be assigned the OPEN Registrar task on the DTL.

All site staff will use OPEN to screen and/or enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient in the PBTC RAVE database. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org. To assign an IVR or NPIVR as the treating, crediting, consenting, drug shipment (IVR only), or investigator receiving a transfer in OPEN, the IVR or NPIVR must list on their Form FDA 1572 in RCR the IRB number used on the site's IRB approval. If a DTL is required for the study, the IVR or NPIVR must also be assigned the appropriate OPEN-related tasks on the DTL.

Patients with DIPG (Rb1 screening is not required)

For patients with DIPG who are not being screened, reservations may also be made through the CTSU OPEN Slot Reservation system providing time to assess the patient's eligibility. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient on this study. Non screening reservations will be held for a maximum of 10 days. The patient's reservation should be canceled as soon as it is determined that the patient is not eligible or that the family/patient has decided not to consent to the trial.

Patients with all other CNS tumors (Rb1 screening is required)
All patients with recurrent, progressive or refractory CNS Tumors (HGG, medulloblastoma, CNS embryonal tumor (NOS), Ependymoma or ATRT) or with atypical presentation of DIPG must be screened prior to enrollment. Patients must sign the screening consent to send the pre-trial tumor tissue [redacted] for Rb1 testing (see Section 9.1.1 for requirement) and have their Rb1status confirmed prior to enrolling in the PBTC-050 study. Only patients who have intact Rb1 will be eligible to enroll.

For patients whose tumor material has previously undergone Rb1 IHC testing at another CLIA-certified lab, additional screening will not be required. If previous Rb1 IHC test confirms intact Rb1, then the patient may enroll using the same procedures as described for DIPG patients above.

4.5.1 Pre-enrollment Screening
Pre-enrollment screening will be performed for patients who are consented for screening and willing to provide pre-trial tumor material for Rb1 IHC testing. Sites are required to make a reservation using the CTSU OPEN Slot Reservation System before they ship the samples to CCHMC for screening. Screening is facilitated by having pre-enrollment screening questions completed by a site registrar in the Prerequisite screen of OPEN. The site must complete the steps up to Prerequisite to obtain the OPEN tracking number which will be used for labeling the slides. Patient enrollment will not proceed from the Prerequisite screen until the CCHMC pathology lab receives the tumor material, analyzes the tissue and enters the Rb1 result into the PBTC ProtoLab database.

Completing the Prerequisite screen sends an automatic email to the CCHMC pathology lab and site registrar with information on the screening process. Once the Rb1 result is entered in ProtoLab by CCHMC pathology lab, an automatic email will be sent to the site registrar and the responsible PBTC Protocol Coordinator indicating that enrollment may proceed. Based on the communicated Rb1 screening result, the site registrar must return to OPEN and complete the process – either to complete enrollment of a patient for treatment who has passed the screening, or to remove the reservation of the patient who failed the screening.

Sites will be notified of the results of the Rb1 testing within 2 weeks. All screened patients who are eligible for participating in the PBTC-050 study must be enrolled via OPEN **within 10 days of the site notification of a patient's Rb1 status as stated in the email notification.**

4.5.2 Patient Enrollment
Patient enrollment for this study will be facilitated using the Slot-Reservation System in conjunction with the Registration system in the Oncology Patient Enrollment Network (OPEN). Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System to insure that a slot on the protocol is available to the patient. Once a slot- reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

Patients must be enrolled prior to any protocol treatment. Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.

- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

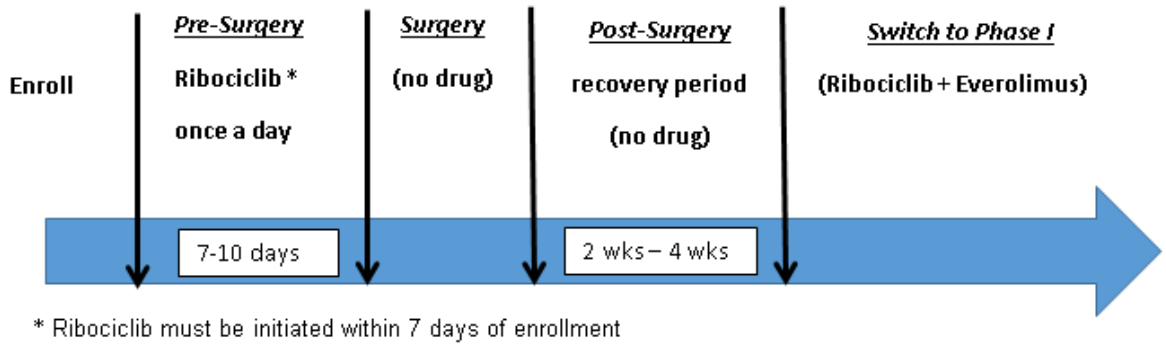
Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

5 TREATMENT PLAN

Treatment may be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's tumor. Timing of protocol therapy administration and response assessment studies are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable.

Phase I Study:
This study will enroll patients with recurrent or refractory malignant brain tumors to define the MTD/RP2D of ribociclib and everolimus.

Surgical study:
The surgical study will enable us in a small population of patients to measure the ribociclib concentration in brain tumor tissue and in plasma samples collected approximately at the same time. The surgical study will involve treatment of eligible patients with ribociclib alone at the pediatric MTD (350 mg/m2/day) for 7-10 days prior to tumor resection of their recurrent tumor, since the half- life of ribociclib in pediatric patients is approximately 30-41 hours. Following tumor resection and recovery from surgery (~2 weeks, maximum 4 weeks), patients enrolled on the surgical study will be switched over to the phase I study and be treated with ribociclib and everolimus at the assigned dose level.



Pre-surgery

For surgical patients, the period from enrollment until initiation of surgery will be considered 'pre-surgery'. Ribociclib should be initiated within 7 days of enrollment and surgery should be performed after 7-10 days of ribociclib treatment.

Post-surgery
"Post-surgery" is defined as the period after surgery until the initiation of combination treatment with ribociclib and everolimus on Phase I study (2 weeks – 4 weeks). During the recovery period, patients will be re-assessed per section 3.5 criteria prior to switching to Phase I study. The combination drug treatment must be commenced within 2-4 weeks of post- surgery period.

Switching to Phase I component
The dose level assigned for Phase I study will be determined based on the slot availability and the number of currently enrolled patients experiencing dose-limiting toxicities on the Phase I component. Each eligible surgical patient for Phase I will start treatment at the dose level that is recommended based on the available data and the Rolling 6 design. The potential dose for Phase I will be reviewed by the Study Chair, the protocol coordinator and the statistician and this dose will be made available as part of the patient's study record. This process may take about 1 – 2 days. Site should email the protocol coordinator to initiate the assignment of a dose level during the post-surgical recovery period (approximately week 2 – week 3) after re-confirmation of eligibility for Phase I study entry (see section 3.5). The treatment must start no later than 4 weeks post-surgery.

5.1 Starting Dose and Dose escalation Schedule

5.1.1 Surgical Study

Single agent ribociclib will be given at a dose of 350mg/m^2/day daily for 7-10 days prior to tumor resection. Formulation (capsule versus liquid) will be the same throughout the duration pre-operatively. If the starting dose of ribociclib is intolerable or if DLTs of the starting dose of ribociclib are clearly attributable to the study drug, the dose will be dose de-escalated to 280 mg/m^2/day.

Table 7: Dose De-escalation Schedule (Surgical Study)

Dose Level	**Ribociclib (mg/m^2/day) PO**
0	280 mg/m^2/day
1 (starting dose)	**350 mg/m^2/day**

5.1.2 Phase I

Subjects who enroll on the Phase I trial will be assigned a dose level at the time of enrollment. Subjects who enroll on the surgical study will be assigned a dose level once they meet the Phase I eligibility criteria during the post-surgery period. See Table 8 below for details of dose escalation schedule. See section 6 for dose modifications. Dosing should be adjusted based on BSA calculated at the beginning of each course of therapy. The dose prescribed should be rounded to the nearest deliverable dose based on the BSA adjustment and the available pill sizes. A Dosing Table for Ribociclib which reflects this approach is available in Appendix 15.5. Patients will be provided with a Medication Diary to record ribociclib and everolimus doses, instructed in their use, and asked to bring the diary as well as the remaining capsules/liquid (in supplied bottles) with them to each appointment. The Patient Diary is available on the PBTC-050 webpage. The diary and returned medication will serve as confirmation of adherence to the protocol-prescribed dosing.

The starting dose for everolimus will be 1.2 mg/m^2/day. The starting dose of ribociclib given on a 3-weeks-on/1-week-off dosing schedule will be 120 mg/m^2 per day which is approximately 70% of the adult RP2D (adult RP2D = 300 mg daily) adjusted for body surface area.

If the starting dosages are tolerable in combination, then dose levels 2 and 3 will be explored as tolerated. If the starting dosages of this combination are intolerable, then either dose level 0 or -0.5 will be explored. Since the toxicity profiles of ribociclib + everolimus do not generally overlap, if DLTs of the starting dose of the combination are clearly attributable to everolimus (e.g. mucositis, hyperlipidemia, etc.), the everolimus dose will be dose de-escalated (rather than the ribociclib dose) to 1 mg/m^2/day continuous dosing. However if the toxicities observed are more consistent with ribociclib side effects then the dose of ribociclib will be reduced (see Table 8 below, dose levels 0 and -0.5). If dose level 0 or -0.5 are not tolerable, doses will be reduced as outlined in dose level -1.

Patients are not allowed to take a combination of the liquid, capsules and tablets formulations for ribociclib within a course but may change to a different formulation when starting a new course. The dose levels in Table 8 have been determined in order to accommodate the smallest capsule sizes of 50 mg. While the increments between ribociclib dose levels are larger than 30% which is commonly used, this was necessary to avoid overlaps between the dosages.

No intra-patient dose escalation will be permitted on the protocol. Only DLTs observed during the dose-finding period of therapy will be used to guide dose escalation. Dose escalation will be governed by the statistical design as described in section 13.1 of the protocol.

Table 8 - Dose Escalation Schedule (Phase I)

Dose Level	**Ribociclib (mg/m^2/day PO days 1- 21)**	**Everolimus (mg/m^2/day PO days 1-28)**	**BSA Restriction**
-1	75 mg/m^2/day	1 mg/m^2/day	BSA≥0.55m^2
-0.5*	120 mg/m^2/day	1 mg/m^2/day	BSA≥0.75m^2
0	75 mg/m^2/day	1.2 mg/m^2/day	BSA≥0.55m^2
1(starting dose)	**120 mg/m^2/day**	**1.2 mg/m^2/day**	**BSA≥0.75m^2**
2	170 mg/m^2/day	1.2 mg/m^2/day	BSA≥0.45m^2
3	170 mg/m^2/day	1.5 mg/m^2/day	BSA≥0.45m^2

*Dose reduction if toxicities clearly attributable to Everolimus

5.2 Agent Administration

One course consists of a 28-day cycle. Ribociclib will be administered daily for 21 days on/7 days off on a 28-day cycle. Everolimus will be administered daily for 28 days. Subsequent courses will immediately follow with no break in administration of the agents in the absence of toxicity. Duration of therapy is defined in Section 5.5.

5.2.1 Ribociclib

Ribociclib is supplied as 50 mg capsules and 200 mg capsules or film coated tablets. The oral solution is available as 30 mg/mL. The liquid formulation may be administered via g-tube or nasogastric or nasojejunum tube. **Tablet and capsule dosing will be rounded to the nearest 50 mg. Liquid formulation (30mg/mL) will be rounded to the nearest 50 mg**. See Appendix 15.5 for the rounding

total daily dose (mg) tables. **Patients allergic to peanut and soy are not permitted to take the film-coated tablet formulation.**

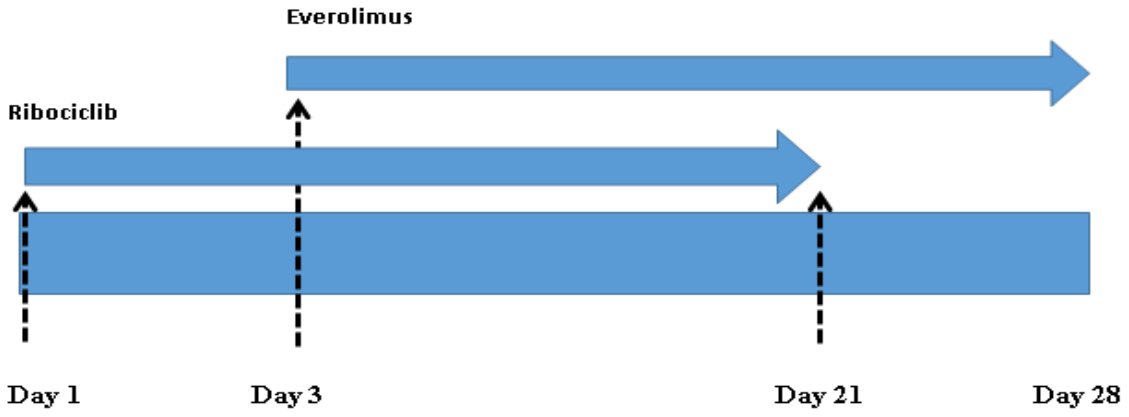
5.2.2 Everolimus

Everolimus dosing for this study will utilize the dispersible tablet (also referred to as 'tablets for oral suspension') formulation which is available as a 2 mg dosage form. **Dosing will be rounded to the nearest 0.1mg.**

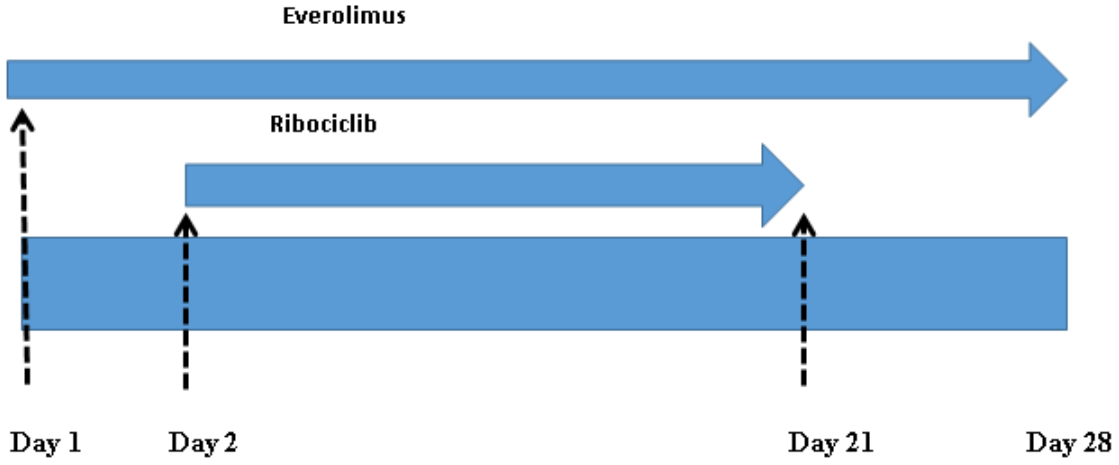
5.2.3 Treatment schedule

The PK studies will be done during courses 1 and 2. Please follow instructions for holding study medications until PK samples are collected noted in Section 9.2.2.

In Course 1, patients will start ribociclib on day 1 through day 21 followed by 7 days of rest and everolimus on day 3 through day 28 on a 28 day cycle.



In Course 2, patients will receive everolimus on day 1 through day 28 and Ribociclib on day 2 through day 21 followed by 7 days of rest.



For Course 3 and beyond:

For subsequent courses, patients will receive ribociclib once a day for 21 days followed by 7 days of rest and everolimus once a day for 28 days.

5.2.4 Instructions for taking the study drugs

Ribociclib and everolimus should be taken as follows:

- Patients should be instructed to take the Ribociclib dose with a large glass of water/clear liquid (~250 mL) at the same time each day preferably in the morning with the everolimus dose.
- On days when PK collection/ECG is scheduled at the clinic, patients will take ribociclib and everolimus in the clinic under the supervision of the investigator or designee. On all other days patients will take the ribociclib and everolimus combination at home.
- Ribociclib and everolimus can be taken without regard to meals.
- If emesis occurs within 10 minutes of administration of ribociclib and everolimus, the dose should be repeated.
- A missed dose (not taken within 6 hours of the intended time) should not be replaced or made up on a subsequent day, but the missed dose and reason for missing the dose should be recorded in the diary. The completed Patient dairy will be reviewed at each subsequent clinic visits throughout therapy.
- Patients must avoid consumption of grapefruit, Seville oranges or products containing the juice of each during the entire study and preferably 7 days before the first dose of study medication, due to potential CYP3A4 interaction with the study medications. Orange juice is allowed.
- No herbal or dietary supplements.
- Patients should be advised to drink plenty of water or take hydration fluids to avoid dehydration if diarrhea occurs.

5.2.5 Criteria to start subsequent courses

Patients will start each subsequent course of therapy with recovery of ANC $\geq 1.0 \times 10^9$ cells/ L and Platelet count $\geq 100 \times 10^9$ cells/ L (transfusion independent, no platelet transfusion for a $\geq$ 7 days). If a patient does not meet these parameters at the end of the treatment course, then ribociclib and everolimus must be held for 14 days until parameters are met. Drug can be held for up to 14 days in patients who undergo surgical procedures for reasons other than tumor progression to allow for proper wound healing.

A course may be repeated every 28 days if the patient has at least stable disease and has again met laboratory parameters as defined in Section 3.2.9. If a patient does not meet these parameters at the end of the treatment course then ribociclib/everolimus should be held until parameters meet the eligibility criteria.

5.3 Dose Limiting Toxicity

Toxicities will be graded based on CTCAE v5.0 unless otherwise specified in the protocol. Management and dose modifications associated with adverse events are outlined in Section 6. DLT will be defined as any of the events listed in this section that are at least possibly related to the investigational agent that occur during the dose-finding period regardless of expectedness.

Management and dose modifications for toxicities which occur outside of the dose-finding period should also follow section 6; however these will not be considered dose limiting for the purpose of dose escalation.

5.3.1 Phase I - Dose-Limiting Toxicities (DLT's):

- Any ribociclib/everolimus-related adverse event during the first course of therapy that leads to a dose reduction or results in the permanent cessation of therapy will be considered dose limiting. All dose modifications in all courses are to follow the guidelines provided in Section 6.2.
- Any ribociclib/everolimus -related adverse event during the first course of therapy that results in a delay of treatment > 14 days.

5.3.1.1 Non-hematologic dose limiting toxicity is defined as:

- Any grade 4 non-hematologic toxicity
- Any grade 3 non-hematologic toxicity with the exception of:
  - Grade 3 nausea and vomiting that respond to intervention within 5 days
  - Grade 3 fever or infection lasting fewer than 5 days in duration without the use of growth factors
  - Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia responsive to oral supplementation
  - Grade 3 anorexia
  - Grade 3 hyperlipidemia (hypercholesterolemia, hypertriglyceridemia)
- Any ≥ Grade 3 AST/ALT and/or bilirubin (see Table 9 for specific modifications)
  Any ≥ 2 Grade QTc (see Table 10 and
- Table 11 for specific modifications)
- Any ≥ Grade 2 mucositis (see Table 12 for specific modifications)
- Any ≥ Grade 2 pneumonitis
- Any grade 2 non-hematologic toxicity that persists for > 7 days and is considered medically significant or sufficiently intolerable by the patient as to warrant treatment interruption and/or dose reduction will be considered dose limiting.

5.3.1.2 Hematologic dose limiting toxicity is defined as:

- Grade 4 thrombocytopenia
- ≥ Grade 3 thrombocytopenia (platelet count < 50 $x10^9$ cells/L) with clinically significant bleeding, petechiae or grade 3 thrombocytopenia that persists for ≥ 7 days or that requires platelet transfusion on ≥ 2 separate days within a 7-day period
- Grade 4 neutropenia lasting >7 consecutive days
- Myelosuppression that causes a delay greater than 14 days between treatment cycles

5.3.2 Surgical study - Dose-Limiting Toxicities (DLT's):

- Any ribociclib-related adverse event defined below in sections 5.3.2.1 and 5.3.2.2 during the pre-operative (7-10 days prior to surgery), intra-operatively (during surgery) and post-operatively (2-4 weeks post- surgery).

5.3.2.1 Non-hematologic dose limiting toxicity is defined as:

- Any grade 4 non-hematologic toxicity
- Any grade 3 non-hematologic toxicity with the exception of:
  - Grade 3 nausea and vomiting that respond to intervention within 5 days
  - Grade 3 fever or infection lasting fewer than 5 days in duration

- Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia responsive to oral supplementation
- Grade 3 anorexia
- Grade 2 liver enzyme elevation, including ALT/AST OR total bilirubin that returns to Grade ≤ 1 or baseline within 14 days.

- Any ≥ Grade 3 AST/ALT and/or bilirubin (see Table 9 for specific modifications)
  Any ≥ 2 Grade QTc (see Table 10 and
- Table 11 for specific modifications)
- Any Grade 2 non-hematologic toxicity that persists for > 7 days and is considered medically significant or sufficiently intolerable by the patient as to warrant treatment interruption and/or dose reduction will be considered dose limiting.

5.3.2.2 Hematologic dose limiting toxicity is defined as:

- Grade 4 thrombocytopenia
- ≥ Grade 3 thrombocytopenia (platelet count < 50 x10^9 cells/L) with clinically significant bleeding, petechiae or grade 3 thrombocytopenia that persists for ≥ 7 days or that requires platelet transfusion on ≥ 2 separate days within a 7-day period
- Grade 4 neutropenia lasting >7 consecutive days
- Myelosuppression that causes a delay greater than 14 days between treatment cycles

5.3.3 Dose-finding period (Phase I)

The dose finding period of Phase I begins with the initial dose of ribociclib and ends on the last day of course 1. Should there be a delay starting the subsequent course, dose finding will complete on the start date of the subsequent course.

5.3.4 Dose Escalation/ De-escalation

Dose escalation will begin with dose level 1 and will be governed by the Rolling-6 design as described in section 13.1. No intra-patient dose escalation will be permitted on the protocol. Only DLTs observed during the dose-finding period of therapy will be used to guide dose de-escalation.

5.4 Concomitant Medications and Supportive Care Guidelines

5.4.1 Steroids

Corticosteroids should be used at the lowest dose to control symptoms of edema and mass effect, and discontinued, if possible. Use of corticosteroids should be recorded in the Rave database.

5.4.2 Anticonvulsants

Anticonvulsants drugs should be used, if indicated. Use of anticonvulsants should be recorded in the Rave database. Note: Use of enzyme inducing anti-convulsants is prohibited on this trial.

5.4.3 Growth Factors

Routine use of growth factors (e.g. G-CSF, GM-CSF and erythropoietin) is not permitted. However, therapeutic use of G-CSF or GM-CSF in patients with serious neutropenic conditions, such as sepsis, may be considered at the investigator's discretion. Use of growth factors should be recorded in the Rave database.

5.4.4 Anti-emetics

Patients may receive ondansetron or granisetron during therapy with CAUTION while taking ribociclib. The use of additional anti-emetics will be at the treating physician's discretion. Use of anti-emetics should be recorded in the Rave database.

5.4.5 Febrile neutropenia

Febrile neutropenia should be managed according to the local institutional guidelines. Measures include laboratory testing, blood cultures, and institution of broad spectrum antibiotics.

5.4.6 Pneumocystis jiroveci pneumonia (PJP) prophylaxis

The use of medication (i.e., Trimethoprim-sulfamethoxazole) for PJP prophylaxis in patients on chronic steroids is recommended, but is at the investigator's discretion.

5.4.7 Mucositis prophylaxis

Mouth care will be per standard institutional guidelines with the mandatory addition of dexamethasone and salt water rinses.

Overview of dexamethasone: Dexamethasone steroid-based oral solution comprised of 0.5 milligrams per 5mL of alcohol free dexamethasone.

Dexamethasone administration: All patients will be given 0.5 mg/5 mL dexamethasone steroid mouthwash. The patient will initiate the dexamethasone steroid mouthwash on the same day that they initiate the combination study treatment (Course 1 Day 3).
The alcohol-free, 0.5 mg/5 mL dexamethasone steroid mouthwash should be administered based on the following instructions:

- 5-10 mL of mouthwash swish and spit 3 times daily (TID). The mouthwash is to be held in mouth and swished around mouth to come in contact with entire buccal mucosa surface for a minimum of two minutes, and then spit out. If the patient is unable to hold mouthwash in mouth for 2 minutes, may spit out prior to 2 minutes or may use swab sponge.
- Patient should remain NPO (no food, no drinks, nothing in mouth) for at least an hour after administering mouthwash, with the exception of Nystatin (or another topical antifungal).

<u>Dexamethasone treatment regimen</u>
Course 1 Day 3 (C1D3) is the day the patient initiates the mouthwash regimen and study drugs.
Patients should use mouthwash regimen for two consecutive treatment cycles. Patients may continue for subsequent cycles, but this will not be mandated.

<u>Salt water mouth rinse</u>
The Salt water mouth rinse will only be administered once the onset of Grade 1 stomatitis has been noted. Salt water mouth rinse (0.9%): administered as 10mL swish and spit TID and must be done prior to the dexamethasone mouthwash. The patient should administer the salt water (0.9%) mouth rinse (swish and spit) and wait for 10-15 minutes before administering the dexamethasone mouthwash.

- If the patient is unable to swish and spit mouthwash, swab sponge may be used.

5.4.8 Neurosurgical or other surgical procedures

If a neurosurgical procedure or other surgical procedure is required for a reason other than tumor progression (i.e. the onset of hydrocephalus), these procedures should be documented, but will not constitute criteria for declaring the patient "off therapy". Ribociclib and everolimus should be held until the patient is clinically stable and has recovered from the acute effects of surgery.

5.4.9 Concomitant therapy

Because there is a potential for interaction of ribociclib and everolimus with other concomitantly administered drugs through the cytochrome P450 system, the local health care providers should be alerted if this patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes. Medications to be used with caution during in this study are listed in Appendix 15.3. This list is not comprehensive and is only meant to be used as a guide.

The heart's electrical activity may be affected by ribociclib. The study doctor should be concerned about QTc prolongation and any other medicine that is associated with greater risk for having QTc prolongation.

The case report form (CRF) must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize the use of while receiving ribociclib and everolimus.

Medications which should be excluded from patient use if possible are listed in Appendices 15.2 and 15.4. If they must be given based on the investigator's judgment, then use with caution and consider a ribociclib and everolimus interruption if the concomitant medication is only needed for a short time. The Study Chair should be contacted if there are additional questions regarding concomitant medications.

The Patient Drug Information Handout and Wallet Card (Appendix 15.6) should be provided to patients.

5.5 Duration of Therapy

In the absence of treatment delays due to adverse event(s) or disease progression, treatment will continue for 13 courses (approximately 1 year) or until one of the Off Treatment Criteria applies noted in Section 5.5.4.

5.5.1 On Study Data Submission Schedule

Pre-treatment, on-study and off-treatment data, as well as patient response data are to be recorded in the electronic data collection screens using the Rave database. See the Required Data and Timetable for Submission form located on the PBTC-050 Protocol webpage for the schedule. For assistance, contact the PBTC Protocol Coordinator listed on the cover page. An optional roadmap is located on the PBTC-050 Protocol Webpage.

5.5.2 Extended Therapy

Ribociclib and everolimus will be available beyond 13 courses of therapy for patients who have at least clinically and radiographically stable disease at the end of course 13 and the investigator and subject agree to continue treatment with ribociclib and everolimus for up to an additional 13 courses (total maximum duration of treatment is 26 courses).

5.5.3 Data Submission during Extended Therapy

For patients who are eligible for extended therapy (section 5.5.2), they will be followed during this additional period for dates of drug administration, adverse events that are possibly, probably or definitely related to study drug, laboratory results, date and result of disease evaluations by MRI, date of disease progression, and date of last follow up and date of death. At the completion of the additional 13 courses of therapy the patient is considered to have completed protocol therapy and should be taken off treatment (per protocol section 5.5.4). At the completion of the appropriate follow up period (per protocol section 5.5.6), the patient should be considered off study.

5.5.4 Off Treatment Criteria

At the discontinuation of treatment, the "Off Treatment Date" is to be recorded in the eCRF and is to be consistent with the reason given for going off treatment. The "Last Treatment Date" is defined as the last date that the patient received protocol based therapy. Date of "off treatment" must be the greatest of the date of last treatment, date of procedure, date of patient assessment, notification of patient/family decision, or decision made by the physician that resulted in the patient being taken off protocol treatment. The reason for discontinuation of treatment must be documented by the attending investigator in the medical record and recorded in the eCRF.

Patients will be considered Off Treatment for the following reasons:

- Development of unacceptable toxicity as outlined in section 5.3. See 7 for specific reporting requirements.
- Progressive disease (PD) as described in section 11.3.4.
- Development of a medical or psychiatric illness that in the investigator's judgment renders the patient incapable of further therapy on this protocol or the treating physician determines continuation on this study is not in the patient's best interest.
- The patient, parent or legal guardian refuses further treatment on this protocol. In this case the investigator should clarify if the family also wishes to withdraw consent for continued participation for data collection purposes.
- The patient was required to start a prohibited medication as noted in the exclusion criteria (Section 3.3.5).
- Completion of all protocol defined treatment
- Pregnancy
- Non Compliance that in the opinion of the investigator does not allow for ongoing participation.
- For surgical study participants, failure to meet criteria to start treatment within four (4) weeks of surgery

Patients who are off protocol therapy must be followed until an "Off Study Criterion" is met.

5.5.5 Data Submission Schedule for Patients Off Treatment

All patients will be followed until resolution (or return to baseline) of all adverse events considered at least possibly related to ribociclib and/or everolimus occurring while on treatment and/or within 30 days of the last administration of study drug. Toxicities that are ongoing at the end of day 30 of last treatment date will be followed until resolution or return to baseline or until the patient is off study.

Patients whose treatment is interrupted or permanently discontinued due to a ribociclib or everolimus related adverse event (AE), including abnormal laboratory value, should be followed at least once a week

for 4 weeks and subsequently at 4-week intervals until resolution to baseline or < Grade 2, whichever comes first.

5.5.6 Criteria for Removal from Study

The date and reason for the patient coming off study must be documented in the eCRF and the Operations, Biostatistics and Data Management Core must be notified according to standard reporting guidelines (see sections 7, 5.5.7, 12, and the Required Data and Timetable Submission from located on the PBTC-050 protocol webpage).

- Patient determined to be ineligible.
- Parent, patient, or guardian withdraws consent for continued participation.
- Patient death while on study. The IRB, Study Chair and OBDMC must be notified as per section 7.4.
- Initiation of another anti-cancer therapy
- Patients has been observed for the resolution of all toxicities occurring while on treatment and for 30 days following the last administration of study drug.
    - If the patient was found to be ineligible after starting treatment, the 30 day follow-up would still be necessary unless the patient commences a different anti-cancer therapy.
- Patients who have completed all protocol therapy or are removed from treatment for reasons other than withdrawal of consent, PD or Death will be followed until one of the following occurs:
    - Commencement of additional anticancer therapy
    - Progressive disease
        - If PD occurs within less than 30 days of off-treatment date, then follow-up should continue until 30 days from off-treatment date or until commencement of additional anticancer therapy, whichever occurs first.
        - If the off treatment reason is toxicity and the patient is subsequently found to have PD, then follow-up should continue for 30 days from the off-treatment date or until the toxicity resolves or returns to baseline, whichever is longer. If the patient commences new anticancer therapy within this specified follow-up period however, he/she will be taken off study on the date the new therapy is initiated.

5.5.7 Data Submission for Patients Off study

No data will be collected documenting treatment or reporting events or disease status that occur subsequent to the official "off study" date with the exception of adverse events with an attribution of possible, probable, or definite that occur after the "off study" date for agents being studied under an IND (see section 7).

6 DOSING DELAYS/DOSE MODIFICATION

6.1 Notification of the Study Chair

The study chair or co-chair must be notified of any dosage modifications, prior to the implementation of the dose modification.

6.2 Hematologic and Non-hematologic Adverse Events and Management

Each patient is allowed a maximum of two dose reductions as outlined in section 6.2.2, 6.2.3 and 6.2.4.

If toxicity does not resolve to meet eligibility parameters within 14 days of drug discontinuation, the patient must be removed from protocol therapy.

If a dose-limiting toxicity occurs in a patient who has resumed treatment at a reduced dose, the patients may have a second dose reduction to a lower dose level if available.

If a dose-limiting toxicity occurs in a patient who has had two dose reductions, or for whom there is no lower dose level available (i.e. At dose level -1), the patient must be removed from protocol therapy.

A dose-dependent drug-drug interaction (DDI) was observed between ribociclib and everolimus based on clinical PK analyses in a separate dose escalation trial among adults**,** where everolimus exposure increased 2- to 4-fold in the presence of ribociclib. If a dose adjustment of both ribociclib and everolimus are required, a dose reduction in ribociclib is considered as a method to reduce exposure to both drugs since everolimus exposure is increased with increased ribociclib doses through a DDI. However, if attribution is clearly due to everolimus, everolimus will be initially decreased.

First dose reduction will occur with ribociclib. However, if the attribution is clearly due to everolimus only, the everolimus dose will be reduced. The second dose reduction will occur for everolimus if the first dose reduction was ribociclib or will occur for ribociclib if the first dose reduction was everolimus. For patients enrolled in dose level 2, two dose level reductions are allowed.

6.2.1 Ribociclib specific dose reduction guidelines

For patients requiring a dose reduction enrolled on Dose Levels 2 and 3 (both at 170 mg/ m^2/ day ribociclib) with **BSA between 0.45 m^2 and 0.73m^2**, the ribociclib dose will be reduced to 120 mg/ m^2/ day (first reduction) then to 75 mg/ m^2/ day (second reduction) if needed. Ribociclib dose reduction will be based on the **actual calculated dose** and will not use the monograms in Appendix 15.5. The calculated dose will be rounded to the nearest 0.1 mL (or 3 mg).

For example: A patient with a BSA **0.5 m^2** enrolled at dose level 2 (170 mg/ m^2/ day). The first dose reduction will be 120 mg/ m^2/ day. The patient calculated dose reduction will be **60 mg** (120 x 0.5) or **2 mL** Ribociclib solution **(**30 mg/mL**).** If second dose reduction is needed, the second reduced dose will be **37.5 mg (or 1.2 mL**) Ribociclib solution (30 mg/mL).

Patients enrolled on dose levels 2 or 3 with a BSA of at least 0.74m^2 or higher, will follow the nomograms in appendix 15.5 for dose reductions.

6.2.2 Dose modifications for non-hematologic toxicities

Protocol therapy will be discontinued if a non-hematologic dose limiting toxicity occurs after two dose modifications.

If a patient experiences a dose-limiting non-hematological toxicity, as defined in Section 5.3, study drug should be withheld. Unless otherwise stated below, if the toxicity resolves to meet on study parameters within 14 days of drug discontinuation, the patient may resume treatment at the next lower dose level. For patients enrolled at Dose Level 1, the dose should be reduced to Dose Level 0 or -0.5 pending attributions. Patients already receiving Dose Level -1 at the time of dose limiting toxicity must be removed from study. Doses reduced for toxicity will not be re-escalated, even if there is minimal or no toxicity with the reduced dose.

6.2.3 Dose modifications for hematologic toxicities

≥ Grade 3 thrombocytopenia: Platelet transfusion is permissible and strongly encouraged for patients with dose-modifying thrombocytopenia to minimize risk of intra-tumoral hemorrhage. Ribociclib and everolimus should not be restarted until platelet count has recovered to ≥ 100 x 10^9 cells/ L (transfusion independent for 7 days).

Patients with grade 4 neutropenia (during any time throughout the course) may receive filgrastim (G-CSF) support (Investigator's discretion) only for documented Grade 3 or greater infection and/or sepsis. Ribociclib and everolimus should not be restarted until the ANC is ≥ 1.0 x 10^9 cells/ L and for at least 48 hours without G-CSF support. Patients should start the subsequent course at one lower dose level without G-CSF support.

In the event of hematological DLT, complete blood counts should be repeated twice weekly for any hematological toxicity described above until counts recover to meet criteria for starting subsequent course of maintenance therapy. Missed doses will not be made up if drug was held for a DLT. A patient who experiences any DLT at dose level -1 will be removed from study.

If a patient experiences myelosuppression (i.e. thrombocytopenia or neutropenia) that causes a delay of >14 days between treatment cycles, counts should be checked every 3-4 days for thrombocytopenia and for neutropenia during this period. Once neutropenia or thrombocytopenia has resolved to eligibility criteria, the patient may resume treatment at the next lower dose level for ribociclib and everolimus dose will remain the same. For patients on dose level -1, the patient must be removed from protocol therapy. Doses reduced for toxicity will not be re-escalated.

If a dose-limiting toxicity occurs in a patient who has had two dose reductions, or for whom there is no lower dose level available (i.e. if already at dose level -1), the patient must be removed from protocol therapy.

6.2.4 Dose modifications for specific toxicities

Table 9: Guidance for dose modifications for hepatic toxicities

Total bilirubin OR ALT/AST increased above baseline value	
Grade 3	Dose interruption of ribociclib and everolimus • If resolved to ≤ grade 1 in ≤14 days, lower 1 dose level of ribociclib and maintain everolimus dose Repeat liver enzymes and bilirubin tests twice weekly for 2 weeks after dose resumption
Grade 4 (>20.0 x ULN)	Discontinue ribociclib and everolimus
Confounding factors and/or alternative causes for increase of total bilirubin should be excluded before dose interruption/reduction.	
AST or ALT and concurrent Bilirubin	
For patients with normal ALT or AST or total bilirubin at baseline: AST or ALT ≥ grade 2 combined with total bilirubin >1.5-3 x ULN without evidence of cholestasis	Discontinue ribociclib and everolimus

Additional Follow-up for Hepatic Toxicities

Increase in transaminases combined with total bilirubin (TBIL) increase may be indicative of drug-induced liver injury (DILI), and should be considered as clinically important events.

The threshold for potential DILI may depend on the patient's baseline AST/ALT and TBIL value; patients meeting any of the following criteria will require further follow-up as outlined below:

- For patients with normal ALT or AST or TBIL value at baseline: AST or ALT Grade 3 combined with TBIL Grade 3.
- For patients with elevated AST or ALT or TBIL value at baseline: (AST or ALT > 2 x baseline AND > Grade 3) OR (AST or ALT Grade 3), whichever is lower, combined with (TBIL > 2 x baseline AND Grade 2).

In the absence of cholestasis, these patients should be immediately discontinued from study drug treatment, and repeat Liver Function Tests (LFTs) as soon as possible, preferably within 48 hours from the awareness of the abnormal results. The evaluation should include laboratory tests, detailed history, physical assessment and the possibility of obstructions/compressions, etc.
Hepatic toxicity monitoring includes the following LFTs: albumin, ALT, AST, total bilirubin, direct and indirect bilirubin, alkaline phosphatase (fractionated if alkaline phosphatase is grade 2 or higher), creatine kinase, prothrombin time (PT/INR) and GGT. For patients with Gilbert Syndrome: total and direct bilirubin must be monitored, intensified monitoring applies to changes in direct bilirubin only.

Close observation is recommended in case of AST, ALT, and/or bilirubin increase requiring dose interruption, which involves:

- Repeating liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or return to normal values.
- Obtaining a more detailed history of prior and/or concurrent diseases, including history of any pre-existing liver conditions or risk factors.
- Obtaining a history of concomitant drug use (including non-prescription medications, herbal and dietary supplements), alcohol use, recreational drug use, and special diets.
- Ruling out acute viral hepatitis types A, B, C, D, and E; hepatotropic virus infections (CMV, EBV or HSV); autoimmune or alcoholic hepatitis; Nonalcoholic steatohepatitis (NASH); hypoxic/ischemic hepatopathy; and biliary tract disease.
- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
- Considering gastroenterology or hepatology consultations.
- Assessing cardiovascular dysfunction or impaired liver oxygenation, including hypotension or right heart failure as possible etiologies for liver dysfunction.

Liver biopsy as clinically indicated to assess pathological change and degree of potential liver injury.

All cases confirmed on repeat testing meeting the laboratory criteria defined above, with no other alternative cause for LFT abnormalities identified, should be considered as "medically significant", thus met the definition of SAE (section 7.4.2) and reported as an SAE with a comment using the term "potential drug-induced liver injury". All events should be followed up with the outcome clearly documented.

Table 10: Guidance for Dose modifications for QT prolongation

Grade	Dose Modification
All Grades	1. Check the quality of the ECG and the QT value and repeat if needed. 2. Perform analysis of serum electrolytes (K+, Ca++, Phos, Mg++). If outside of the normal range, interrupt ribociclib administration, correct with supplements or appropriate therapy as soon as possible, and repeat electrolytes until documented as normal. 3. Review concomitant medication usage for the potential to inhibit CYP3A4 and/or to prolong the QT interval. 4. Check compliance with correct dose and administration of ribociclib.
1 (QTc 450- 480 ms)	Perform Steps 1-4 as directed in "For All Grades". No dose adjustment required.
2 (QTc 481-500 ms)	Interrupt ribociclib and everolimus. Perform steps 1-4 as directed in "For All Grades." Perform a repeat ECG within one hour after the first QTc of ≥481 ms • If QTc <481 ms, restart ribociclib by 1 dose level. Refer to Table 8 for dosing schedule.. • If QTc remains ≥481 ms, repeat ECG within 14 days until the QTc returns to <481 ms. Hold ribociclib and everolimus. Restart ribociclib reduced by 1 dose level and everolimus at the same dose level. • If QTc ≥481 ms recurs, ribociclib should be reduced again by 1 dose level and everolimus at same dose • Repeat ECGs 7 days and 14 days after dose resumption (then as clinically indicated) for any patient who has therapy interrupted due to QTc ≥481 ms
3 (QTc ≥ 501 ms on at least two separate ECGs)	Interrupt ribociclib and everolimus • Perform a repeat ECG within one hour of the first QTc of ≥501 ms. • If QTc remains ≥501 ms, consult with a cardiologist (or qualified specialist) and repeat cardiac monitoring as indicated until the QTc returns to <481 ms. • If QTc returns to <481 ms within 14 days, ribociclib should be reduced by 1 dose level and maintain everolimus dose. • If QTc remains ≥ 481ms after performing steps 1-2 as directed in "for all grade," discontinue Ribociclib and everolimus. • Repeat ECGs 7 days and 14 days after dose resumption for any patient who has therapy interrupted due to QTc ≥501 ms • If QTc of ≥501 ms recurs, discontinue ribociclib and everolimus
4 (QT/QTc ≥ 501 or > 60 ms change from baseline) and (Torsades de pointes or polymorphic ventricular tachycardia, or signs/symptoms of serious arrhythmia)	Discontinue Ribociclib and everolimus and removal from study

Table 11: Guidance for dose modification and management for cardiac dysfunction

Adverse drug reaction	Severity	Dose adjustment and management recommendations
Cardiac - Left Ventricular Systolic Dysfunction	Asymptomatic, resting ejection fraction 40-50%; or 10-20% drop from baseline	Maintain dose level, and continue ribociclib and everolimus with caution. Repeat LVEF within 4 weeks or as clinically appropriate.
	Symptomatic, responsive to intervention, ejection fraction 20-39% or >20% drop from baseline	Hold ribociclib and everolimus until resolved, then reduce ribociclib by one dose level; LVEF measurement to be repeated, if not resolved within 28 days permanently discontinue ribociclib and everolimus if applicable.

6.2.5 Dose Modifications for Everolimus related toxicities

Table 12: Dose modification and management for Mucositis

Adverse drug reaction	Severity	Dose adjustment and management recommendations
Mucositis characterized by ulceration or inflammation of the oral mucosa	**Grade 1** Asymptomatic or mild symptoms; intervention not indicated	No dose adjustment required. Manage with non-alcoholic steroid mouthwash and salt water (0.9%) mouthwash four times a day (section 5.4.7).
	Grade 2 Moderate pain or ulcer that does not interfere with oral intake; modified diet indicated	Interrupt everolimus and ribociclib until recovery to grade 1 within 14 days. • Re-initiate study treatment at the same dose. • If stomatitis recurs, interrupt ribociclib and everolimus until recovery to grade 1. • Re-initiate everolimus at a one dose lower. Manage with topical analgesic mouth treatments (e.g. benzocaine,butyl minobenzoate, tetracaine hydrochloride, menthol or phenol) with or without topical corticosteroids (i.e. triamcinolone oral paste).
	Grade 3 Severe pain; interfering with oral intake	Interrupt everolimus and ribociclib until recovery to grade ≤1. • Re-initiate everolimus at a reduced dose level and maintain ribociclib dosing • If reoccurs, interrupt ribociclib and everolimus until recovery to grade ≤1. • Re-initiate ribociclib at reduced dose level and maintain everolimus dosing.

Adverse drug reaction	Severity	Dose adjustment and management recommendations
		Manage with topical analgesic mouth treatments (i.e. benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol or phenol) with or without topical corticosteroids (i.e. triamcinolone oral paste).
	Grade 4 Life-threatening consequences; urgent intervention indicated	Hold ribociclib and everolimus and provide appropriate medical therapy. • If recovered to ≤grade 1 within 14 days, Re-initiate everolimus at a reduced dose. • If reoccurs, interrupt ribociclib and everolimus until recovery to grade ≤1. • Re-initiate ribociclib at reduced dose level and maintain everolimus reduced dosing

Table 13: Dose modification and management for Hypertension

Hypertension is to be reported as 'Vascular disorders-Other, Pediatric Hypertension" and graded as defined in Table 13.

Adverse drug reaction	Dose Adjustment and management recommendations
Grade 1 ***Children 1-13y:*** Elevated BP: ≥90th percentile to <95th percentile or 120/80 mm Hg to <95th percentile (whichever is lower)	No dose modifications
Children ≥13y Elevated BP: 120/<80 to 129/<80 mmHg	
Grade 2 ***Children 1-13y*** Stage 1 Hypertension (HTN): ≥95th percentile to <95th percentile + 12 mm Hg, or 130/80 to 139/89 mm Hg (whichever is lower)	No dose modifications
Children ≥13y Stage 1 HTN: 130/80 to 139/89 mm Hg	
Grade 3 ***Children 1-13y*** Stage 2 HTN ≥95th percentile + 12 mm Hg or ≥140/90 mm Hg (whichever is lower)	Hold ribociclib and everolimus and follow up blood pressure if the toxicity resolves to meet on study parameters within 14 days of drug discontinuation, the patient may resume treatment at the next lower dose level. If Grade 3 hypertension reoccurs, hold ribociclib and everolimus and follow up blood pressure if the toxicity resolves to meet on study parameters within 14 days of drug discontinuation, the patient may resume treatment at the next lower dose level (2 dose levels lower than starting dose).
Children ≥13y Stage 2 HTN: ≥140/90 mm Hg	

	If Grade 3 hypertension reoccurs after 2 dose level reductions, ribociclib and everolimus will be discontinued.
Grade 4 – All Ages Life-threatening consequences; urgent intervention indicated	Discontinue ribociclib and everolimus.

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1 and 7.2) and the characteristics of an observed AE (Section 7.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting. The coding of attribution in the Rave database pertains to adverse events related to ribociclib and everolimus.

- *Baseline Abnormalities*

Any baseline (pretreatment) abnormalities observed during the initial physical examination should be recorded in the Rave database.

- *Treatment or within 30 days of treatment*

Only record adverse events grades 1 and 2 if the attribution is at least possibly related to ribociclib and everolimus. Record all adverse events grades 3 through 4 and deaths), regardless of attribution on the electronic case report forms.

7.1 Adverse Event List(s) for ribociclib (LEE011, NSC 794613)

Adverse events suspected to be related to ribociclib –

Adverse events considered to be ***expected*** with ribociclib (3 weeks on 1 week off dosing regimen) for reporting purposes. May also refer to the most current version of the Ribociclib Investigator Brochure (available on the PBTC-050 webpage) for additional expected adverse events.

MedDRA system organ class	**Event**	**Frequency category* all grades**	**Frequency category* grade 3/4**
Blood and lymphatic system disorders	Anemia	Very common	common
	Febrile neutropenia	common	common
Eye Disorders	Dry eye	Common	Not reported
	Watering eyes	Common	Not reported
Gastrointestinal disorders	Abdominal pain	Very common	common
	Constipation	Very common	common
	Diarrhea	Very common	common
	Dyspepsia	Common	Not reported
	Mucositis oral	Very common	Uncommon
	Nausea	Very common	common
	Vomiting	Very common	common
General disorders and administration site conditions	Fatigue	Very common	Common
	Edema limbs	Very common	Not reported
	Fever	Very common	Uncommon
Hepatobiliary disorders	Hepatic Failure	Uncommon	Uncommon

Infections	Urinary tract infection	Very common	Uncommon
Investigations	Electrocardiogram QT corrected interval prolonged	common	Uncommon
	Alanine aminotransferase increased	Very common	Common
	Aspartate aminotransferase increased	Very common	Common
	Alkaline phosphatase increased	Very common	Common
	Blood creatinine increased	Common	Uncommon
	White blood cells decreased	Very Common	Very Common
	Neutrophil count decreased	Very common	Very Common
	Lymphocyte count decreased	Very Common	Very Common
	Platelet count decreased	Common	Uncommon
	Weight decreased	Common	Uncommon
Metabolism and nutrition disorders			
	Hypokalemia	Common	common
	Hypophosphatemia	Common	common
	Hypocalcemia	Common	Common
	Anorexia	Very common	Common
Musculoskeletal and connective tissue disorders	Back pain	Very common	common
Nervous System Disorders	Headache	Very common	Uncommon
	Syncope	Common	Common
	Dysgeusia	Common	Uncommon
Psychiatric disorder	Insomnia	Very Common	Uncommon
Respiratory, thoracic and mediastinal disorders			
	Dyspnea	Very common	common
	Epistaxis	Common	Not reported
Skin and subcutaneous tissue disorders	Rash maculo-papular	Very common	Uncommon
	Pruritus	Very common	uncommon
	Erythema	Common	Not reported
	Alopecia	Very common	Not reported
*Frequency category for each adverse drug reaction is based on the following: Very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1,000); very rare (<1/10,000). Unknown - insufficient data to determine the frequency of the ADR; none reported – for this event no grade 3-4 events have been reported,			

The adverse drug reactions frequencies are determined from the data from study CLEE011A2301 (a double-blinded randomized placebos controlled trial) and where reasonable evidence of a possible causal relationship with ribociclib was proven.
Note: Ribociclib in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event List(s) for everolimus (RAD-001, NSC 733504)

Adverse Events considered to be ***expected*** with everolimus for Reporting Purposes. Refer to most current version of **everolimus** Investigator Brochure located on the PBTC-050 webpage for additional expected adverse events.

MedDRA system organ class	Event	Frequency category* all grades

Blood and lymphatic system disorders	Anemia	Very common
Gastrointestinal disorders	Abdominal pain	Common
	Dry mouth	Common
	Oral pain	Common
	Dysphagia	Common
	Constipation	Common
	Diarrhea	Very common
	Mucositis oral	Very common
	Nausea	Very common
	Vomiting	Very common
	Dyspepsia	Common
General disorders and administration site conditions	Fatigue	Very common
	Edema limbs	Very common
	Fever	Common
	Non-cardiac chest pain	Uncommon
Immune System Disorders	Allergic reaction	Uncommon
	Anaphylaxis	Uncommon
Infections and Infestations	Infections[1]	Very common
Injury, poisoning and procedural complications	Wound complication[2]	Uncommon
Investigations	Weight loss	Very common
	Alanine aminotransferase increased	Common
	Aspartate aminotransferase increased	Common
	Alkaline phosphatase increased	Common
	Hypercholesterolemia	Very Common
	Blood creatinine increased	Common
	White blood cell decreased	Common
	Lymphocyte count decreased	Common
	Neutrophil count decreased	Common
	Platelet count decreased	Common
Metabolism and nutrition disorders	Hypertriglyceridemia	Common
	Anorexia	Very common
	Hyperglycemia	Very common
	Hypophosphatemia	Common
	Diabetes mellitus	Common
	Hyperlipidemia	Common
	Hypokalemia	Common
	Dehydration	Common
Musculoskeletal and connective tissue disorders	Arthralgia	Common
	Pain in extremity	Common
	Back pain	Common
Nervous System Disorders	Headache	Very common
	Dysgeusia	Very common
Psychiatric disorders	Insomnia	Common
Respiratory, thoracic and mediastinal disorders	Pneumonitis[3]	Very common
	Epistaxis	Very common
	Cough	Very common
	Dyspnea	common
Renal and Urinary disorders	Proteinuria	Common
	Renal Failure	Common
	Acute kidney injury	Uncommon
	Urinary frequency (daytime)	Uncommon
Reproductive system and breast disorders	Irregular menstruation	Common
	Amenorrhea	Uncommon
Skin and subcutaneous tissue disorders	Rash maculo-papular	Very common
	Pruritus	Very common
	Dry Skin	Common
	Nail Disorder	Common
	Acne	Common
	Angioedema	Rare

	Erythema	Common
	Hand-foot syndrome[4]	Common
Vascular disorders	Hypertension	Common
	Deep vein thrombosis	Uncommon
	Thromboembolic event – Pulmonary embolism	Uncommon
	Acute Respiratory Distress Syndrome	Rare
Bleeding	Hemorrhage[5]	Common

*Frequency category for each adverse drug reaction is based on the following:
Very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1,000); very rare (<1/10,000).

[1] Infection includes all reactions within the 'infections and infestations' system organ class including common: pneumonia, urinary tract infection; uncommon: bronchitis, herpes zoster, sepsis, abscess and isolated cases of opportunistic infections (e.g. aspergillosis, candidiasis, and hepatitis B) and rare: viral myocarditis.
[2] Everolimus delays wound healing and increases the occurrence of wound-related complications like wound dehiscence, wound infection, incisional hernia, lymphocele, and seroma.
[3] Includes common: pneumonitis, interstitial lung disease, lung infiltration, and rare: alveolitis, pulmonary alveolar hemorrhage and pulmonary toxicity.
[4] Reported as palmar-plantar erythrodysesthesia syndrome
[5] Includes different bleeding events from different sites not listed individually

7.3 Adverse Event Characteristics

7.3.1 CTCAE term (AE description) and grade:

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting with the exception of grading for Hypertension . Hypertension will be reported as "Vascular disorders – Other, pediatric hypertension", and is to be graded according to the definitions outlined in Table 14. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

In 2017, Flynn JT et al reported new clinical practice guidelines for high blood pressure in children and adolescents. [78] Definitions of blood pressure categories and stages have been redefined as noted in Table 14. These definitions are not aligned with CTCAE version 5.0 as the 99th percentile for pediatric pressure no longer exists in the definition of hypertension in children. Stage 2 hypertension is defined as the 95th percentile + 12mmHg. Per CTCAE version 5, the definition of Grade 3 hypertension in pediatrics is systolic and/or diastolic > 5mmHg above the 99th percentile.

Table 14: Updated Definitions of BP Categories and Stages

Grade	For Children Aged 1-13 years	For Children Aged ≥13 years
Normal	Normal BP: <90th percentile	Normal BP: <120/<80 mm Hg
1	Elevated BP: ≥90th percentile to <95th percentile or 120/80 mm Hg to <95th percentile (whichever is lower)	Elevated BP: 120/<80 to 129/<80 mm Hg
2	Stage 1 Hypertension (HTN): ≥95th percentile to <95th percentile + 12 mm Hg, or 130/80 to 139/89 mm Hg (whichever is lower)	Stage 1 HTN: 130/80 to 139/89 mm Hg
3	Stage 2 HTN ≥95th percentile + 12 mm Hg or ≥140/90 mm Hg (whichever is lower)	Stage 2 HTN: ≥140/90 mm Hg
4	Life threatening consequences (e.g. malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis); urgent intervention indicated	

7.3.2 Attribution of the AE:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

7.4 Expedited Adverse Event Reporting

Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm. These requirements are briefly outlined in the tables below (Section 7.4.2).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to the PBTC operation office by telephone at 901-595-7793. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.4.1 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and the responsible Protocol Coordinator at the PBTC, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

The following must be copied on expedited reports (24-hour notification and the complete report) submitted via CTEP-AERS:

Study Chair and IND Holder:
Mariko DeWire-Schottmiller, MD
[redacted]

PBTC OBDMC:
Stacye Richardson, MSHS
[redacted]

Regulatory Affairs:
Renee Doughman, PhD
[redacted]

The OBDMC will email and/or fax the SAE /CTEP-AERS report, within 24 hours of their awareness of the event to Novartis (fax#: 1- 877- 778- 9739).

The IND holder, [redacted] shall notify the FDA of any event that is both a serious suspected adverse reaction and unexpected in accordance with FDA rules and regulations (21 CFR 312.32). Follow-up information to a safety report will be submitted, as requested.

The IND Sponsor or designee will submit a report of the unexpected, suspected adverse event to the FDA using the FDA's reporting form (FDA 3500A MedWatch Form) and guidelines. The report should describe the event as fully as possible. Supporting documentation (lab reports, summary notes, and autopsy report) should accompany the report. A fatal or immediately life-threatening suspected adverse event will be reported to the FDA within 7 calendar days of the receipt of the initial report by the IND sponsor. A non-fatal, non-life threatening unexpected, suspected, serious adverse event will be reported to the FDA within 15 calendar days of receipt of the initial report by the IND Sponsor.

The PBTC OBDMC will post all IND Safety Letters on the PBTC-050 webpage. Sites will be notified via email of the receipt of the IND Safety Letter(s) and instructed to submit these to their local IRB in accordance with the institution's requirements.

A copy of the Annual Progress Reports is submitted by the IND holder as required by FDA, by the sponsor-investigator. This submission will be cross referenced according to local regulations to the Novartis product number at the time of submission.

Any SAEs experienced after this 30 days period should only be reported to Novartis if the investigator suspects a causal relationship to the study treatment. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval outside the 30-day period post last dose and related to therapy should be reported separately as a new event.

7.4.2 Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.
Note: A death on study requires both routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions". Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor /NCI **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.4.3 Additional Protocol Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting via CTEP-AERS. However, they still must be reported through the routine reporting mechanism (Section 7.4):

CTCAE SOC	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
Blood/Bone Marrow	Lymphopenia	≤4	Regardless	Any	Expedited reporting is not required.

7.4.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must also be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

7.4.5 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient's partner from the time of consent to 90 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

7.4.6 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy such as acute myelocytic leukemia (AML)
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.4.7 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

8 AGENT INFORMATION

A list of the adverse events and potential risks associated with the investigational and commercial agents administered in this study can be found in Section 7.1 and 7.2.

8.1 Everolimus (RAD001)

Chemical Name: (1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-[(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl]-19,30-

dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.04,9]hexatriaconta-16,24, 26,28-tetraene-2,3,10,14,20-pentaone

Other Names: RAD001, Afinitor

Classification: mTOR inhibitor

CAS Registry Number: 159351-69-6

Molecular Formula: $C_{53}H_{83}NO_{14}$ **M.W.:** 958.2

Mode of Action: Everolimus is an inhibitor of selective mammalian target of rapamycin (mTOR), the effects of which specifically target the mTOR-raptor signal transduction complex 1 (mTORC1). Inhibition of mTORC1, an essential regulator of global protein synthesis downstream on the PI3K/AKT/mTOR pathway, reduces tumor cell proliferation, glycolysis and angiogenesis. The PI3K/AKT/mTOR pathway is dysregulated in the majority of human cancers and is the object of many targeted agents.

Description: White to faintly yellow powder

How Supplied: Everolimus will be supplied by Novartis or its designee as dispersible tablets (also referred to as 'tablets for oral suspension') and is available as a 2 mg dosage forms. Each box of 28 tablets contains 4 blister cards of 7 tablets each.

Extent of absorption of everolimus through topical exposure is not known. Caregivers are advised to avoid contact with suspensions of everolimus. Wash hands thoroughly before and after preparation of the suspension. All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded.

Dispersible tablets contain butylhydroxytoluene/butylated hydroxytoluene (BHT), cellulose microcrystalline/microcrystalline cellulose, crospovidone, hypromellose/hydroxypropyl methylcellulose, lactose monohydrate, magnesium stearate, mannitol/D-mannitol, silica colloidal anhydrous/colloidal silicon dioxide.

Everolimus will be packaged in bottles. They will include storage conditions for the drug but no information about the patient. The study drug will be labeled and packaged under the responsibility of the Novartis drug supply management department. The medication will be supplied as open label supply to the site in a way that allows the patient to take medication at home.

Storage: Store at 25°C (77°F). Excursions permitted between 15 to 30°C (59 to 85°F).
If a storage temperature excursion is identified, promptly return everolimus to controlled room temperature and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to Novartis for determination of suitability.

Storage of the prepared oral suspension: The suspension should be discarded if not administered within 60 minutes of preparation.

Stability: Refer to the clinical labels for current shelf life, in-use, expiration date and proper storage condition. Store tablets in the original container and protect from light and moisture.

Stability or BUD (beyond-use date) of the prepared oral suspension: The oral suspension of everolimus prepared in an oral syringe or in a small drinking glass should be administered immediately after preparation. The oral syringe or glass should be rinsed with the same volume of water and the rinse should be completely swallowed to ensure that the entire dose is administered and there is no drug residue remaining in the oral syringe or glass.

Route and Method of Administration: Oral administration. The dispersible tablet is prepared as a suspension of undissolved medicine that is mixed with water, and then taken by mouth. The suspension can be prepared in an oral syringe or small drinking glass. Everolimus dispersible tablet is an *ad-hoc* drug compounding. Patient and/or guardian will be taught how to prepare the oral suspension by research nursing staff. A patient/caretaker educational template is available on the PBTC-050 webpage for step-by-step ad-hoc drug preparation and administration guidance for Everolimus dispersible tablet in the home settings and included in the protocol as Appendices 15.7 and 15.8.

They can be taken with or without food, but should be taken consistently the same time every day. If a dose is missed, it can still be taken up to 6 hours after the usual time of administration. Missed doses more than 6 hours late should be skipped for that day.

Potential Drug Interactions:
Everolimus is a substrate of CYP3A4 and P-glycoprotein (PgP). Concomitant treatment with strong inhibitors or inducers of CYP3A4 or PgP should be avoided. Use caution when everolimus is given concomitantly with moderate inhibitors or inducers of CYP3A4 and PgP. Consult the protocol document or study investigator prior to making any dose adjustments related to potential drug-drug interactions.

In vitro, everolimus is a competitive inhibitor of CYP3A4, a mixed inhibitor of CYP2D6, an inhibitor of transport proteins, OATP1B1 and OATP1B3 and a moderate inhibitor of P-gp. Exercise caution when everolimus is taken in combination with orally administered substrates of these enzymes and transporters, in particular, CYP3A4 and 2D6 substrates with a narrow therapeutic index. Everolimus does not appear to affect CYP 2C8 or 2C9 activity according to *in vitro* studies using reference substrates.

Co-administration with angiotensin-converting enzyme (ACE) inhibitors may increase the risk for angioedema.

Patient Care Implications: Patients taking everolimus should avoid the use of live vaccines and close contact with those who have received live vaccines. Examples of live vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid vaccines.

8.2 Ribociclib

Chemical Name: 7-Cyclopentyl-N, N-dimethyl-2-[[5-(piperazin-1-yl) pyridin-2-yl]amino]-7Hpyrrolo[2,3-d]pyrimidine-6-carboxamide succinate (1:1)

Other Names: LEE011, KISQALI

Classification: cyclin-dependent kinase (CDK) inhibitor

CAS Registry Number: 1211441-98-3 (free base)

Molecular Formula: C23H30N8O.C4H6O4 **M.W.:** 434.55

Mode of Action: An orally available cyclin-dependent kinase (CDK) inhibitor targeting cyclin D1/CDK4 and cyclin D3/CDK6 cell cycle pathway, with potential antineoplastic activity. Ribociclib specifically inhibits CDK4 and 6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation. Inhibition of Rb phosphorylation prevents CDK-mediated G1-S phase transition, thereby arresting the cell cycle in the G1 phase, suppressing DNA synthesis and inhibiting cancer cell growth. Overexpression of CDK4/6, as seen in certain types of cancer, causes cell cycle deregulation.

Description: Light tan to yellow powder

How supplied: Ribociclib will be supplied by Novartis or its designee. The available formulations are 50 mg (capsules) and 200 mg (capsules or film-coated tablets) and a 30mg/mL in 100 mL placed in a 180 ml glass bottle (oral solution) provided as an individual patient supply packaged in bottles. All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded.

Ribociclib capsules/tablets and the liquid form will be packaged in bottles. They will include storage conditions for the drug but no information about the patient. The study drug will be labeled and packaged under the responsibility of the Novartis drug supply management department. The medication will be supplied as open label supply to the site in a way that allows the patient to take medication at home.

Ribociclib may be administered orally in different ways based upon available formulations and capsule or tablet sizes:

- Capsule: swallow whole or open capsule and pour contents onto semi-solid food or dissolve contents of opened capsule in water or Ora-Sweet (or equivalent) and administer orally or via feeding tube (e.g. nasogastric or gastric)
- Liquid formulation: Since ribociclib did not show pH-dependent solubility in biorelevant media, the exposure/bioavailability is not expected to be limited by its dissolution rate due to different dosage form of administration. Liquid may be administered via feeding tube (e.g. nasogastric (NG)/nasojejunal (NJ)/gastric).

Storage: The shelf life of the drug product is established based on ongoing stability studies and may be extended during the clinical study. The capsules are stored in HDPE bottles. Storage for the capsules is "Do not store above 25 °C, protect from moisture". The oral solution is stored in brown glass bottles. Storage for the oral solution is "store at 2-8 degrees C, do not freeze". Refer to the clinical labels for current shelf-life, in-use and storage conditions for the capsules, film-coated tablets and oral solution.

Stability: Stable for at least 2 years after receipt when stored at -20°C

Route and Method of Administration: Ribociclib can be taken by mouth or NG/NJ/G-tube at the approximate same time daily for 3 weeks followed by one week off without regard to meals.

8.3 Agent Ordering and Agent Accountability

8.3.1 Agent Ordering

Ribociclib and Everolimus may be requested by the Principal Investigator (or their authorized designee) at each participating institution. All regulatory documents, as required by the PBTC, must be current and up to date prior to requesting the study drugs. Drug request form can be found on the PBTC-050 protocol and resources webpage. The form can be emailed to Novartis. In general, sites may order initial agent supplies when a subject is being screened for enrollment onto the study.

8.3.2 Agent Inventory Records

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing, use and disposal of ribociclib and everolimus received from Novartis using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate the Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

Any unused/used/expired study drug and containers may be destroyed according to the institutional standard operating procedure. The method and the record of destruction must be documented and maintained at the site.

8.3.3 Useful Links and Contacts

- CTEP Forms, Templates, Documents: https://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help email: ctepreghelp@ctep.nci.nih.gov.

9 BIOMARKER, CORRELATIVE AND SPECIAL STUDIES

This section contains the collection, shipping and handling information for all planned biomarker and exploratory correlative studies, neuropathology review and research imaging. The table below identifies the tests, sample type and amount, analyzing laboratory and whether it is required or optional. For additional details, please review the associated section below.

Tissue Requirements: Tissue for CLIA-certified Rb1 expression assessment for screening will be required for patients to enroll with the exception of DIPG patients. Prior testing for Rb1 IHC in a CLIA-certified laboratory will be accepted in lieu of tissue submission. For the Phase I study, tissue for exploratory correlative studies will be strongly recommended but not required.

Phase I

Test Name	Sample Type and Amount	Analyzing Laboratory	Required or Optional	Section ID
Screening: Assessment of Rb1 expression	(FFPE) tumor tissue - 2 unstained slides + 1 H&E stained slide	Christine Fuller, MD CCHMC	Required*	9.1.1

Pharmacokinetics	Plasma – 1 mL per sample	Stewart Laboratory	Required	9.2.2
Pharmacokinetics	Serial or single CSF – 2 mL	Stewart Laboratory	Optional	9.2.3
Genomics- Whole-exome sequencing and RNA sequencing	Frozen tumor tissue or FFPE and PBMCs**	Will Parsons, MD Texas Children's Hospital	Optional	9.2.5
PBTC CRB Repository Samples	20 unstained slides and blood for future research	PBTC CRB at CHLA	Optional	9.2.1

*Required for HGG, medulloblastoma, CNS embryonal tumor (NOS), Ependymoma, ATRT or with atypical presentation of DIPG. Patients with typical DIPG appearance who have biopsied may also submit tissue if available. If there is known Rb1 status, screening will not be required.
**In all cases, frozen tissue is preferable to FFPE, but either will be accepted.

Surgical Study

Rb1 testing will be completed on diagnostic tissue PRIOR to enrollment for the surgical study.
The PK sample collection requirements for patients enrolled on the surgical study are noted below. Surgical study patients should still be invited to participate in the optional genomics studies and submission of repository samples as noted in the Phase I table.

Test Name	Sample Type and Amount	Analyzing Laboratory	Required or Optional	Section ID
Screening: Assessment of Rb1 expression	(FFPE) tumor tissue - 2 unstained slides + 1 H&E stained slide	Christine Fuller, MD CCHMC	Required*	9.1.1
Intratumoral Pharmacokinetics	Tumor tissue (collected during surgery)***	Stewart Laboratory	Required	9.2.2
Pharmacokinetics	Plasma – 1 mL per sample	Stewart Laboratory	Required	9.2.2
Pharmacokinetics	CSF – 2 mL	Stewart Laboratory	Optional	9.2.3
Pharmacodynamics	FFPE – 2 unstained slides FFPE (1 FFPE each from pre-treatment tumor and post-treatment tumor***) + 1 H&E OR tumor block	Christine Fuller, MD CCHMC	Optional	9.2.4

*Required for HGG, medulloblastoma, CNS embryonal tumor (NOS), Ependymoma, ATRT. If there is known Rb1 status, screening will not be required.
*** For patients who consent to participate in the PD study, post-surgery tissue should be split between intra-tumoral PK and PD studies. Site should ensure sufficient tissue is sent per protocol requirement to each lab.

9.1 Integral Laboratory Studies

9.1.1 Assessment of Retinoblastoma (Rb1) expression

All diagnostic tumor samples will be evaluated for Rb protein by immunohistochemistry (IHC) in a CLIA certified lab – either at [redacted]) or local Lab. Tumor samples will be sent to CCHMC if the tissue has not been previously tested locally. Additionally, left over tumor samples will be stored after required studies are completed for future research with participant consent.

Formalin fixed paraffin embedded (FFPE) tumor tissue will be collected from all patients prior to enrollment (except for those with DIPG who have not undergone previous biopsy). Unstained slides and

a corresponding H & E stained slide will be prepared from the original and/or recurrent surgery by sites and sent to CCHMC for Rb immunohistochemistry and interpretation. Unstained sections should be cut to 5µm in thickness on (+) slides from a section containing at least 50% tumor cells. The turn-around time for this test is approximately 7-10 days. Tumor RB protein status will be denoted as "positive" if ≥ 20% of tumor cells have positive nuclear staining. Rb+ endothelial cells will serve as an internal positive control. All slides will be centrally reviewed by the Neuro-pathologist at CCHMC.

9.1.1.1 Assay Method

Immunohistochemistry for Rb will be performed as previously described[79] using a mouse monoclonal anti-RB antibody (G3-245; BD Biosciences, San Jose, CA) and an automated IHC staining process (Benchmark XT; Ventana Medical Systems, Inc, Tucson, AZ). Briefly, antigen retrieval will be performed in Tris, pH 8.0 at 95C for 1 hour, followed by incubation in 3% H2O2 for 16 minutes and primary antibody at 1: 100 at room temperature for 60 minutes. Immunohistochemistry will be scored as follows: 0, denotes expression in less than 20% of tumor cells; 1, denotes expression ins 20% to 50% of tumor cells; and 2, indicate positive expression in more than 50% of tumor cells. At least three 20x-fields will be evaluated per case. The assay is performed and interpreted in a laboratory that is CLIA certified (CCHMC) to perform high complexity testing.

9.1.1.2 Collection and shipping of Specimen(s)

Two unstained slides for FFPE material will be collected along with one H & E stained slide prepared from the same block will be submitted to CCHMC.

Slides must be shipped to CCHMC within 3 days of reservation. Sites will be notified of the results of the Rb1 testing within 2 weeks. All screened patients who are eligible for participating in the PBTC-050 study must be enrolled via OPEN within 10 days of the site notification of a patient's Rb1 status. Slides must be labeled with the PBTC enrollment tracking number (OPEN tracking #) and protocol number. Samples should be shipped Monday through Thursday using the PBTC-050 FedEx account. Weekend deliveries are not permitted. The sample shipping date must be documented in the eCRF by the sites. All specimens should be shipped at room temperature.

Send the following to CCHMC:

- 2 unstained slides
- 1 H&E stained slide
- Completed PBTC-050 specimen transmittal form

Attn: Christine Fuller, MD

[redacted]

9.1.1.3 Site(s) Performing Correlative Study

Christine Fuller, MD

[redacted]

9.2 Pathology and Exploratory Correlative Studies

The Biorepository (CRB)'s function is to collect, distribute and store specimens for planned correlative studies which support the laboratory objectives of this protocol.

The CRB will also serve as a central repository for specimens collected for future research and left over specimens (tumor tissue, blood, urine or CSF) returned to the repository following the planned analysis from patients who consent to long term storage of unused specimen. These samples will be stored in the repository for undefined future studies which support the mission of the PBTC. **If the patient does not consent to participation in the repository, correlative study samples should be submitted following the guidelines in the appropriate correlative study section below. If the patient does not consent to long term storage, remaining correlative study samples will be destroyed once the PBTC-050 analysis is complete.**

9.2.1 PBTC CRB Submission Guidelines

If the patient consents to provide slides for submission to the repository at the time of participation in a PBTC trial the following should be submitted:

- Tumor material

Slides from the original and/or recurrent surgery should be prepared for storage. The site should provide up to twenty (20) unstained sections cut at 4µm in thickness on (+) slides from the most representative section. For patients who have consented to other correlative studies (e.g. genomics, pharmacodynamics studies) on this protocol that require slides, these submitted slides will be used for the conduct of studies outlined in the corresponding sections below (9.2.4, 9.2.5). Fewer unstained sections may be submitted based on size and availability of tissue. Preference is for tissue that has not previously been frozen. The corresponding pathology report (s) including immunohistochemical, special stains, and molecular/genetic results is to be uploaded to the PBTC using the secure File Upload system. These reports will be made available to the pathologist via a link in the ProtoLab.

Suitability of sections would be established by preparing one (1) H&E to ensure that the sections meet the following criteria:

- histologically representative of the reported lesion
- contain at least 60% viable tumor
- no more than 40% necrosis

Slides submitted by patients who have consented to the PBTC CRB will undergo neuro-pathology review. Central pathology review is **not** required for this study. Pathologist review for this study will include the following elements:

- Examination of H&E stained slides. For each subject one (1) H&E stained slide per one representative block from the brain tumor, removed either at initial diagnosis or relapse, should be submitted for review.
- Review of the corresponding pathology report(s) of the immunohistochemical, special stains, and molecular/genetic results from current and/or original primary tumor
- If necessary, review of immunohistochemical or special stained slides.

Slides submitted to the PBTC CRB will be digitized to 40X. H&E stained sections will be retained and filed at the CRB. Original immunohistochemical or special stain slides will be returned to the submitting institution.

- Peripheral Blood Mononuclear Cells

PBMC may be collected by processing a 2-5mL whole blood specimen with Ficoll or collecting the specimen in a BD Vacutainer™ CPT™ Cell Preparation Tube with Sodium Citrate as noted below. Once separated, all pellets must be snap frozen and stored at least at -20ºC prior to dry ice shipment

Specimen Collection and Processing by Ficoll tube

- Collect 2 - 5mL of fresh blood into an EDTA tube.
- Transfer blood into a sterile 50-mL tube and add double the amount of PBS. MIX GENTLY.
- Set up another tube containing half of its total volume of Ficoll. For example, if there is 15 mL of blood + PBS, then use 7.5 mL of Ficoll (2:1 ratio).

At very slow pace (approx. 2 mL/minute), layer the blood + PBS mixture onto the Ficoll so that the solutions DO NOT MIX. Spin the blood/Ficoll at 750 g in slow mode for 30 minutes @ 25ºC. After spin you will see four distinct layers: plasma (top layer), white fluffy ring (2nd layer), Ficoll (3rd layer), and blood (bottom layer).

- Remove plasma layer down to about 1 mL above the white fluffy ring and discard.
- Collect the entire white fluffy ring. If ring is hard to see, also take extra liquid above. Then discard everything else.
- Place this fraction of white blood cells into a fresh 50 mL sterile tube with 20 mL of PBS. Spin down for 10 minutes @ 25ºC, 750 g in fast mode. Remove the supernatant. Add back to pellet 1 mL of PBS and spin for 5 min. at 4°C at 10000rpm. Remove supernatant.
- Freeze the pellet of WBCs and store at -80ºC until shipment.
- Ensure that all tubes are clearly labeled with the PBTC patient accession number. Please ensure that the labeling system used is designed to withstand temperatures down to -80ºC. Samples should be stored at -80ºC until shipment. For short term storage (2-3 weeks) -20ºC is acceptable. NOTE 4ºC IS NOT ACCEPTABLE STORAGE.

If it is not possible to collect the PBMC by Ficoll gradient then separation of PBMC can be conducted using CPT tube separation as an alternative. However the PBMC pellet MUST BE frozen immediately and stored at -80ºC.

Collection and Processing by CPT tube

- Peripheral blood should be collected in a BD Vacutainer CPT™ Cell Preparation Tube with Sodium Citrate or similar tube which is available locally. 8 mL and 4 mL CPT tubes can be obtained from Fisher Scientific (Cat# 02-685-125, 02-688-81) or Becton-Dickinson (BD No.362761, 362760). The 8 mL tubes have a 6 mL minimum draw and the 4 mL tubes have a 3 mL minimum draw.
- Centrifuge the CPT™ tube at 1500 x g for 30 minutes at room temperature (20º C to 25º C). DO NOT APPLY THE BREAK ON THE CENTRIFUGE. Use acceleration 5, brake 0 ("slow mode").
- It may be necessary to spin the tube longer to ensure that all of the red blood cell components have been separated from the plasma layer through the polyester gel barrier.

- The tube should be removed immediately from the centrifuge. The mononuclear layer and plasma lie above the polyester gel plug.
- Using a sterile pipette, remove as much of the plasma component (upper half of the CPT tube) without disturbing the mononuclear layer if possible and discard.
- Transfer mononuclear cell layer (and some residual plasma layer) to a labeled 15- mL conical centrifuge tube and add 5 mL sterile room temperature magnesium or calcium-free phosphate buffered saline (PBS) to fill the conical tube and recap.
- Centrifuge at 450 x g for 10 minutes at room temperature (20º C to 25º C). Use acceleration 9, brake ("fast mode").
- Remove supernatant, being careful not to aspirate the cellular pellet at the bottom of the tube.
- Add 1 mL of sterile PBS to the pellet and gently re-suspend by pipetting up and down. Transfer the entire suspended pellet to the labeled cryovial.
- Centrifuge the cryovial at 450 x g for 5 minutes (or spin down the microcentrifuge tube at 1300 x g for 5 minutes) at room temperature. Discard the supernatant. Store the cell pellet cryovial frozen at -80ºC. For short term storage (2-3 weeks) -20º C is acceptable.

If the patient consents to other secondary correlative studies as outlined in sections 9.2.4, 9.2.5), the following specimens may also be submitted to the CRB for distribution and storage.

- Fresh-frozen tissue: approximately 100 micrograms = pea-sized portion of tumor, frozen in foil in liquid nitrogen and stored at -70°C until time of shipping
- Peripheral blood mononuclear cells: quantity and schedule of collection as outlined in section 9.2.1.

9.2.1.1 Handling of Specimens

- Slides are to be labeled with the study ID and the patient PBTC Accession # and these slides should be designated as PBTCR # (where the # assigned from 1 to 20, or the highest number of unstained sections prepared, sequentially) or PBTCR H&E for the H&E stained section.

- Frozen tumor tissue or Formalin Fixed, Paraffin Embedded (FFPE) tumor materials are to be labeled with the PBTC Accession # and the PBTC study for which the sample is provided. When fresh frozen tumor (FFT) sample is available, we would additionally request a minimum amount of 20 mg. In the event that genomic DNA (gDNA) has already been extracted from tumor material, this can be submitted in lieu of FFT sample and we would request a minimum of 250 ng of gDNA.

9.2.1.2 Shipment of Specimens

Samples collected for the repository should be sent to the PBTC CRB overnight via FedEx by completing the Internet form at http://www.fedex.com/us/ and requesting FedEx to e-mail at researchsupportbiorepository@chla.usc.edu. FedEx user ID and password for pathology shipping can be found at PBTC-050 protocol webpage. Weekend deliveries are not permitted. Receipt and processing of biological materials will be recorded in ProtoLab, as appropriate, by staff at the PBTC CRB. The sites sending the materials must document sample collection and shipping dates in the eCRF.

Samples should be shipped in the appropriate environment as described below:

- Fresh frozen tumor sample should be shipped in a separate box on dry ice.
- FFPE tumor material should be shipped at room temperature.

- Blood samples should be shipped in a separate box with cold pack (1-10 degrees C), unless otherwise specified.

Samples are to be shipped to:
PBTC CRB
[redacted]

9.2.2 Plasma Pharmacokinetics

Phase I Study
Pharmacokinetic studies for ribociclib and everolimus will be performed on all patients enrolled on the Phase I component of the trial (including surgical patients who are eligible to initiate Phase I treatment). The pharmacokinetics of these agents alone and in combination are unknown in the setting of pediatric CNS neoplasia, and because various agents commonly used in patients with brain tumors (e.g., corticosteroids) can alter the disposition of these agents, this information will be useful for evaluating toxicity and disease response and for refining dosing in future clinical trials. Moreover, since both these agents are metabolized by CYP3A4 and studies in adults have suggested that the disposition of at least one of the agents is altered, it will be important to know if either of these agents alter the systemic disposition of the other in the setting of pediatric brain tumor therapy. Thus, patients will begin ribociclib therapy two days earlier than the everolimus so that we can perform ribociclib pharmacokinetic studies to gain a baseline of the ribociclib disposition, and then during Course 2 to hold the ribociclib dose on day 1 so that we can perform everolimus pharmacokinetic studies to gain a baseline of the everolimus disposition.

9.2.2.1 Sampling Strategy for Phase I Pharmacokinetic Study
Mandatory plasma pharmacokinetic studies will be obtained from all patients enrolled on this study on days 1, 2, 3, and 17 and 18 of Course 1, and days 1 and 2 of Course 2 to evaluate the pharmacokinetics of ribociclib and everolimus alone and the pharmacokinetics of the combination. A single dose of ribociclib will be administered on Day 1 of Course 1 to obtain a baseline ribociclib pharmacokinetic study with which to compare the effect of everolimus on ribociclib when they are administered in combination (i.e., Course 1 day 17/18). Moreover, the effect of ribociclib on everolimus will be evaluated by comparing the Course 2 day 1 (and 2) everolimus (without ribociclib) baseline when they are administered in combination (i.e., C1 day 17/18).

- ***Ribociclib Serial Sampling Strategy***

On Day 1 of Course 1:
Beginning on day 1 of Course 1, serial whole blood samples for ribociclib pharmacokinetic studies will be collected in a labeled lavender-top K_2-EDTA Vacutainer tube at the following times: pre-dose and at, 1, 2, 4, 8 (±1), 24 (±4), 32 (±4), and 48 (±4) hours after the oral dose (8 mL total). **The ribociclib dose on day 2 should be held during course 1 and resumed on day 3 after the 48 hour time point**

pharmacokinetic sample has been obtained. It is crucial that the 48-hour time point be obtained prior to administration of either drug (ribociclib or everolimus) on Day 3.

- ***Ribociclib and Everolimus Serial Sampling Strategy***

On day 17 (± 2 days) of Course 1:
On day 17 ± 2 days, serial whole blood samples for ribociclib and everolimus pharmacokinetic studies will be collected at the following times: pre-dose and at, 1, 2, 4, 8 (±1), and 24 (±4) (immediately prior to the next dose) hours after the dose (12 mL total). Because these samples will be used for both ribociclib and everolimus, 2 mL of whole blood will be collected in a labeled lavender-top $_{K2}$-EDTA Vacutainer tube at each time point. The sample will be split and 1 mL of blood will be processed to plasma for ribociclib, and 1 mL of whole blood will be used for the everolimus studies.

- ***Everolimus Serial Sampling Strategy***

On day 1 of Course 2:
Whole blood samples for everolimus pharmacokinetic studies will be collected into a labeled lavender-top K_2EDTA Vacutainer tube at the following times: pre-dose, 0.5, 1, 1.5, 2, 4, 6, 8 (±1), and 24 (±4) (immediately prior to the next dose) hours after the oral dose (9 mL total). **The ribociclib dose on day 1 should be held and day 2 should be delayed until the last everolimus sample is collected**.

Surgical Study

9.2.2.2 Sampling Strategy for Ribociclib Neurosurgical Study

Mandatory plasma and fresh frozen tumor tissue will be obtained from all patients enrolled on this aspect of the clinical trial after continuous ribociclib therapy of 7-10 days to determine the ribociclib plasma and brain tumor concentrations.

- ***Ribociclib Plasma and Brain Tumor Sampling Strategy***

After 7 – 10 days of continuous ribociclib dosing, a 1 mL whole blood sample will be collected into a labeled lavender-top $_{K2}$-EDTA vacutainer tube at the following times:.

- Prior to starting ribociclib (Day -7 or Day -10)
- Prior to dose on Day -5 (±1 day)
- Prior to dose on Day -2 (±1 day)
- On the day of surgery (Day 0)
- During surgery at time tumor tissue is collected

Record the exact time of day the sample is acquired and the time of the last dose of ribociclib on the Pharmacokinetic Data Collection Form.

During the neurosurgical procedure, brain tumor tissue will be acquired at the time of tumor resection. Record the time and day of sample acquisition, the anatomical location from which the tumor sample was acquired, and the condition of the sample at the time of acquisition (e.g., viable tumor material, necrotic tumor material). Each separate piece of tumor tissue should be placed into an appropriately labeled tube.

9.2.2.3 Collection and Handling of Specimens

- Ribociclib Plasma Sample Collection and Processing Instructions:

Collect the ribociclib blood samples according to institutional policies and procedures, ensuring that where applicable, sterile discard volume is returned to the patient after the blood sample is obtained. At times specified, 1 mL of whole blood should be drawn into an appropriately labeled lavender-top EDTA vacutainer tube. The samples should be inverted several times to mix, immediately aliquoted to microcentrifuge tubes, and centrifuged at 10,000 rpm for 2 minutes. For participating facilities equipped with lower speed centrifuges, samples may be centrifuged at 1,000 - 1,300 x g for 10 minutes (see vendor specific rotor instructions for rpm to rcf conversion). The plasma supernatant should then be transferred to an appropriately labeled, screw-capped polypropylene tube and stored at -80°. (Record the exact time that the sample is drawn along with the exact time that the drug is administered on the Pharmacokinetic Data Collection Form. Refer to the Pharmacokinetics Laboratory section with the Clinical Operations Manual for detailed instructions for the collection, handling, and processing of pharmacokinetic samples.)

- Everolimus Whole Blood Sample Collection and Processing Instructions:

At each time point, the designated amount of blood (see the amounts of blood collected in Section 9.2.2.1 and Correlative lab studies calendar 10.3) will be collected into appropriately labeled amber tubes containing K_2-EDTA. One (1) mL of blood is needed for bioanalysis. The Pharmacokinetic Data Collection Form should be completed with the exact time that the sample is drawn as well as the exact time that the drug is administered. The Pharmacokinetic Data Collection Form is located on the PBTC-050 webpage. The samples should be labeled with the PBTC Accession number and the Course, Day and Time information for each sample.

- Tumor Sample Collection and Processing Instructions for surgical study

Approximately 50 to 100 mg of fresh flash-frozen tumor material will be collected into an appropriately labeled 15-mL conical tube. All samples (i.e., plasma and tumor) should be shipped on dry ice.

9.2.2.4 Shipping of Specimens

At the start of the study, each participating institution will receive a Pharmacokinetics (PK) kit from Dr. Clinton Stewart for the collection of samples.

Samples should be shipped for delivery Monday through Thursday with a generous amount of dry ice enclosed to safeguard against shipping delays. Weekend and holiday deliveries should be avoided. Sites should contact the Stewart Laboratory [redacted] to request a Pharmacokinetics Kit for each patient enrolled on the study.

Samples should be shipped within 30 days of the last sample being taken. The site should use the PBTC-050 PK FedEx account details, located on the PBTC member website. Ship all pharmacokinetic samples on dry ice, along with a completed Pharmacokinetics Sample Transmittal Form located on the PBTC-050 webpage to:

Stewart Laboratory
[redacted]

[redacted]

Samples must be shipped from the site to the respective laboratory within 30 days of last sample collection in order to receive cost reimbursement. The sample collection and shipping dates must be documented in the eCRF.

9.2.2.5 Site Performing Correlative Study
Stewart Laboratory
[redacted]

9.2.3 Cerebrospinal Fluid (CSF) Pharmacokinetic Studies

Phase I
Pharmacokinetic studies for ribociclib, everolimus, or the combination of the two drugs will also be performed in all research participants with access to CSF who agree to participate.

9.2.3.1 Serial CSF Studies

- Ventricular CSF (0.5 mL) should be obtained in a screw-top tube pre-dose and at 1, 4, and 8 hours after drug administration on Course 1, Day 1 (C1D1); Course 1, Day 17 (C1D17) and Course 2, Day 1 (C2D1).
- The exact time that the CSF sample is obtained should be recorded along with the exact time that the drug is administered on the Pharmacokinetic Data Collection Form.

9.2.3.2 Single CSF Studies

- A single CSF sample may be available for collection where CSF is collected as part of the best clinical practice at any time during treatment (e.g., those requiring CSF diversion or VP shunt revision during therapy, or research participants requiring shunt externalization for infection), therapy should be preferentially continued without interruption, and all attempts should be made to obtain a sample of CSF for pharmacokinetic studies.
- A simultaneous plasma sample should be obtained at the time of all CSF sample collection(s).

Surgical study
If a CSF sample is obtained during a surgical procedure from a patient receiving ribociclib, the ribociclib concentration in that sample will be determined.

9.2.3.3 Collection and Handling of Specimen(s)

Phase I
Collect 0.5 milliliters of CSF in a screw-top tube along with concomitant plasma sample. Record the exact time that the CSF and plasma samples were obtained along with the exact time of drug administration on the CSF Pharmacokinetic Data Collection Form located on the PBTC-050 webpage in the "Protocol Specific Instructions/Forms" section. Samples should be labeled with the PBTC Accession number and the Course, Day information for each sample. Samples should be stored at -80°C until shipped.

Surgical Study

CSF will be obtained (2 mL in a screw-top tube) at the time of surgery after 7-10 days of daily administration of ribociclib (at steady state). Samples should be labeled with the PBTC Accession number and the Course, Day information for each sample. Samples should be stored at -80°C until shipped.

9.2.3.4 Shipping of Specimen(s)

The sample collection and shipping dates must be documented in the eCRF. All samples should be forwarded via FedEx, Monday through Thursday, by completing the internet form at http://www.fedex.com/us/ and requesting FedEx to email the laboratory. Fed Ex account information for the shipment of samples can be obtained on the PBTC -050 website. Weekend deliveries are not permitted.

In no instance should any CSF sample remain at a participating site for more than 30 days after its acquisition unless prior approval is obtained by Dr. Clinton Stewart or his designee. All CSF samples should be shipped on dry ice, along with a completed CSF PK Transmittal Form located on the PBTC-050 webpage to:

Stewart Laboratory
[redacted]

9.2.3.5 Site Performing Correlative Study

Stewart Laboratory
[redacted]

9.2.4 Pharmacodynamics (Only applicable for surgical study)

The rationale for this test is to look at change in Ki67 by comparing pre-treatment (prior to ribociclib) and post-treatment (after 7-10 days of ribociclib) tissue. Surrogate pharmacodynamics studies for a drug that targets the cell cycle can be performed on pre-treatment tumor and post-treatment tumor. Ki67 is a marker of cellular proliferation. In the adult clinical studies with Ribociclib, pharmacodynamics markers were tested in skin and tumor biopsies by IHC assessment of p-Rb and Ki67. A ≥ 50% reduction was observed a doses of 400 mg and higher. The study currently continues in the expansion phase for further assessment of toxicity and efficacy.[48]

For the Surgical Study, Ki67 will be evaluated in pre-treatment tumor (diagnostic) and post-treatment tumor (recurrent) tissue in consenting patients.

9.2.4.1 Collection of Specimen(s)

The following will be collected from representative (diagnostic and recurrent) tumor tissue:

- 2 (Two) unstained FFPE slides [1 each from pre-treatment tumor (diagnostic) and post-treatment tumor (recurrent) tissue] + 1 H&E
- *OR* tissue block from tumor material

9.2.4.2 Handling of Specimens(s)

The samples should be labeled with the PBTC Accession number and the Course, Day information for each sample.

9.2.4.3 Shipping of Specimen(s)

The sample collection and shipping dates must be documented in the eCRF. All samples should be forwarded via FedEx, Monday through Thursday, by completing the internet form at http://www.fedex.com/us/ and requesting FedEx to email the laboratory. Fed Ex account information for the shipment of samples can be obtained on the PBTC website. Weekend deliveries are not permitted. Specimens should be shipped in the room temperature, along with a completed PD Specimen Transmittal Form located on the PBTC-050 webpage to:

PBTC CRB

[redacted]

9.2.4.4 Site Performing Correlative Study

Christine Fuller, MD

[redacted]

9.2.5 Genomic Studies

As an ancillary/exploratory study in consenting patients, tumor tissue in the form of formalin fixed, paraffin embedded tumor material (FFPE) or frozen tumor, if available, from any previous surgical excision/biopsy of the primary tumor or recurrent tumor(s), and any additional resected tumor if surgery is performed while the subject is on study, will be analyzed as described above in 9.1.1, and 9.2.4.

For all tissue specimens submitted for genomics analysis, an additional 5 mL of blood should be collected prior to treatment, as a normal control for sequencing studies. This blood study is mandatory for those subjects who also consent to the tumor tissue genomics studies and have tissue available. The blood sample should be collected and processed as described in Section 9.2.1 Peripheral Blood Mononuclear cells.

After analysis, left over material will be stored at the PBTC specimen repository. Assays performed on FFPE and PBMCs will be undertaken in combination with the Biology and Pathology Committee and CRB on material that may include:

- Survey methylome analysis using Illumina 450k methylation array profiling of tumor tissue to confirm histological diagnosis, ascertain intra-diagnosis molecular subgroup, and evaluate copy

number gains/losses, including those in genes involved in cell cycle regulation, PI3K/mTOR signaling, or related cancer pathways (see Exploratory Objective in section 1.3)

- Targeted DNA/RNA panel analysis and/or whole exome sequencing of matched tumor and normal (blood) tissue to identify genetic alterations targeting cancer genes, including those in genes involved in cell cycle regulation, PI3K/mTOR signaling, or related cancer pathways that are known to be mutated in cancer and in pediatric brain tumors in particular.
- Other genomic tests including targeted deep sequencing, transcriptome (RNA) sequencing, or whole genome sequencing in order to identify cancer gene mutations, copy number alterations, structural rearrangements, and other types of genetic alterations.

9.2.5.1 Shipping of Specimen(s)

- Fresh frozen tissue tumor material should be rapidly transferred (without thawing) from long term storage to a Styrofoam box filled with dry ice.
- FFPE slides/blocks should be shipped at ambient temperature.
- PBMCs - Once separated, all pellets must be snap frozen and stored at least at -20°C until shipped. Samples should be shipped overnight in a separate box with a 2-day supply of dry ice.

The sample collection and shipping dates must be documented in the eCRF. All samples should be forwarded via FedEx, Monday through Thursday, by completing the internet form at http://www.fedex.com/us/ and requesting FedEx to email at researchsupportbiorepository@chla.usc.edu. Fed Ex account information for the shipment of samples can be obtained on the PBTC-050 website. Weekend deliveries are not permitted. Genomic specimens should be shipped as described in Section 9.2.5.1, along with a completed Genomics Specimen Transmittal Form located on the PBTC-050 webpage to:

PBTC CRB
[redacted]

9.2.5.2 Site(s) Performing Correlative Study

Will Parsons, MD, PhD
[redacted]

9.3 Neuroimaging Studies

Patients will have MRI Brain with and without contrast performed prior to therapy, after courses 2, 4, 6 then every 12 weeks thereafter until time of progression or completion of treatment. MRI Spine should be performed prior to therapy and at the same time points as standard MRI Brain, if clinically indicated.

Standard MR imaging will include Sagittal T1 MPRAGE, axial FLAIR, axial T2, post gadolinium sagittal T1 MPRAGE (with reconstructions) and axial T2 images. Standard MR Spine imaging protocol is Sagittal T1 images after gadolinium (slice thickness 3mm skip 0). Axial T1 images are after gadolinium (slice thickness 3mm skip 0). Axial T2 images are optional. Standard MR Spine imaging protocol is Sagittal T1 images after gadolinium (slice thickness 3mm skip 0). Axial T1 images are after gadolinium (slice thickness 3mm skip 0). Axial T2 images are optional. The standard MR parameters are listed on the PBTC NIC web page located at http://www.childrenshospital.org/research-and-innovation/research-labs/pediatric-brain-tumor-consortium-neuroimaging-center under Neuroimaging Studies/ Specific MR Imaging Sequences- Open PBTC Protocols. There is also a link to the same PBTC-NIC webpage from the PBTC-050 protocol study page.

Volumetric analyses will be done at the Neuroimaging Center (NIC, Children's Hospital Boston) via the Vitrea (Vitrea™) workstation, from the axial FLAIR and T1-weighted post-contrast brain images.

9.3.1 Neuroimaging Review

All patient specific data are stripped from the images and replaced with PBTC Accession numbers prior to transmitting the images to the NIC. All image data transfer is accomplished using PGP (pretty-good-privacy) 128-bit encryption which meets industry standard for secure communication.
Only scans showing a response, the confirmation scan obtained approximately 8 weeks later if available and the corresponding baseline scan will be electronically transferred to the PBTC Neuroimaging Center (NIC) for central review scan for confirmation.

Local review of MR imaging studies at each site and central review of the MR imaging studies will be conducted through the PBTC Neuroimaging Center (NIC). NIC review will include assessment of response to therapy (as feasible). The director and one neuroradiologist of NIC will review the imaging studies at study completion. If the local and central review are not in agreement the NIC neuroradiologist will confer with the participating site to determine why there is a discrepancy via conference call.

10 STUDY CALENDAR

Data is to be submitted according to the Data submission timelines located on the PBTC-050 webpage.

10.1 Surgical Study

	Screening	Pre- Enroll - Enrollment	Pre-surgery	Surgery	Post-surgery	Switch to Phase I (see 10.2)
Visit Window	**Week -5**	**Week -3**	**Week -2**	**Week 0**	**Week 2 - week 4**	**> Week 2-4**
OPEN registration system	X	X				
Screening consent	X					
Informed consent		X[A]				X[B]
Confirmation of intact Rb1 required for HGG, medulloblastoma, CNS embryonal tumor (NOS), Ependymoma, or ATRT	X					
Ribociclib administration [C]			X			

	Screening	Pre- Enroll - Enrollment	Pre-surgery	Surgery	Post-surgery	Switch to Phase I (see 10.2)
Physical Assessments						
Medical history		X			X	
Physical exam /height/weight		X			X	
Vital signs		X			X	
Performance status		X			X	
Neurologic exam		X			X	
Review of eligibility assessments, AE, concomitant meditations, prior therapy assessment		X			X	
Laboratory Evaluations						
CBC WBC, HgB, Hct, Platelets, ANC, ALC		X			X	
Serum Chemistry Sodium, Potassium, Bicarbonate, Chloride, Calcium, BUN, Creatinine, Glucose, Phosphorous, Magnesium, Albumin, Total Protein, SGPT(ALT), SGOT(AST), Total Bilirubin		X			X	
Lipid profile (fasting) Triglycerides, total cholesterol, HDL, LDL		X				
Serum or Urine pregnancy test (for females of childbearing potential)		X	X[D]		X[D]	
CSF cytology (if clinically indicated)		X				
Other assessments						
12-lead EKG		X				
ECHO		X				
Imaging Assessments						
Brain MRI (standard) with diffusion		X		X[E]	**X[E]**	
Spinal MRI (if clinically indicated)		X				
Correlatives						
PK Blood			X[F]	X[F]		
PK Tumor tissue				X[G]		
CSF PK (if consented)				X[H]		
PD (if consented)				X		

	Screening	Pre- Enroll - Enrollment	Pre-surgery	Surgery	Post-surgery	Switch to Phase I (see 10.2)
A. Informed consent for surgical study B. Informed consent for Phase I C. Ribociclib will be administered 7 – 10 days prior to surgery. D. Within 72 hours prior to starting study drug(s) E. Intraoperative OR post- operative (<96 hours) MRI F. PK blood – obtain 1 mL each prior to dose on day -7 or day -10, day -5, Day -2, on the day of surgery, and during surgery. See section 9.2.2.2. G. 50 to 100 mg of fresh flash-frozen tumor will be collected at the time of surgery. See section 9.2.2.3. H. CSF PK – obtain 2 mL at the time of surgery						

10.2 Phase I

	Pre-therapy	Course 1	Courses 2-Course 13	Courses 14-26	Completion/ Discontinuation Of Treatment
Study drugs administration (ribociclib + everolimus)		X	X	X	
Medical history	X	Weekly	X	X	X
Physical exam /height/weight	X	Weekly	X	X	X
Vital signs	X	Weekly	X	X	X
Performance status	X	Weekly	X	X	X
Neurologic exam	X	Weekly	X	X	X
CBC WBC, HgB, Hct, Platelets, ANC, ALC [A,B]	X	Weekly[A]	Weekly[A,B]	X[B]	X
Serum Chemistry Sodium, Potassium, Bicarbonate, Chloride, Calcium, BUN, Creatinine, Glucose, Phosphorous, Magnesium, Albumin, Total Protein, SGPT(ALT), SGOT(AST), Total Bilirubin	X	X[B]	X[B]	X[B]	X
Lipid profile (fasting) Triglycerides, total cholesterol, HDL, LDL [C]	X		X[C]	X[C]	X
Serum or Urine pregnancy test (for females of childbearing potential) [B]	X	X[B]	X[B]	X[B]	X
CSF cytology (if clinically indicated)	X				
12-lead EKG [D]	X	X[D]	X[D]	X[D]	X
ECHO	X		X[E]	X[E]	X[E]
Imaging Assessments					
Brain MRI (standard) with diffusion [F]	X		X[F]	X[F]	X[F]
Spinal MRI[F] (if clinically indicated) [G]	X		X[G]	X[G]	X

	Pre-therapy	Course 1	Courses 2-Course 13	Courses 14-26	Completion/ Discontinuation Of Treatment

A. CBC with differential at baseline and q weekly during each cycle for the first 3 cycles; consideration can be given to increase interval to q 2 weeks from course 4 onwards if the patient did not DLT in the previous cycle. If patient develops Grade 4 neutropenia or thrombocytopenia, then CBCs should be checked every 3-4 days until recovery to Grade 3.
B. Within 72 hours prior to starting the next course
C. Lipid profile is to be obtained every other course (prior to course 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26)
D. 12-lead EKG at baseline, days 15 of cycle 1 and prior to cycles 2 and 3. Thereafter, EKG should be done prior to the start of every 3rd cycle (prior to 6, 9, 12, 15, 18, 21, 24 and end of treatment). For patients with QTc ≥ 481 ms at any time, interrupt study treatment and follow the procedures described in Table 10**Error! Reference source not found.** EKG must be completed within 7 days prior to starting next course of ribociclib and everolimus.
E. ECHO must be obtained within 7 days prior to anticipated start of therapy of course 3. Additional studies may be done as clinically indicated at the investigator's discretion.
F. Standard imaging with diffusion is to be done every 8 weeks (after courses 2, 4, 6, and then every 12 weeks thereafter through the 26 courses of treatment or progression). All required imaging dates are to be entered in the database. However, only scans showing a partial response or complete response, and the corresponding baseline scan, will be uploaded.
G. MRI Spine should be done at the indicated time and when clinically indicated.

10.3 Correlative Lab Studies Calendar (Phase I)

Correlative Laboratory Studies	Screening	Pre-therapy	Course 1	Course 2
Rb1 expression (required)	X [A]			
Pharmacokinetics blood: Ribociclib serial samples (required)			**D1 of C1** • pre-dose, and 1, 2, 4, 8 (±1), 24 (±4), 32 (±4), and 48 (±4) hours after dose.[B] • 1 mL each (8 mL total)	
Pharmacokinetics blood: Everolimus serial samples (required)				**D1 of C2** • pre-dose, 0.5, 1, 1.5, 2, 4, 6, 8 (±1), and 24 (±4) hr after dose.[C] • 1 mL each (9 mL total)
Pharmacokinetics blood: Both ribociclib and everolimus serial samples (required)			**D17 of C1 (+/- 2 days)** • pre-dose, 1, 2, 4, 8 (±1), and 24 (±4) hrs after dose • 2 mL each (12 mL total)	
PK – serial CSF (if consented)			X[D]	
PK – single CSF (if consented)		X[E]		
Pre-trial tumor materials and PBMCs for Genomics (if consented)		X		
Tumor Tissue and blood for Biorepository (if consented)		X		

A. Required for patients with HGG, medulloblastoma, CNS embryonal tumor (NOS), Ependymoma, or ATRT or with atypical presentation of DIPG. Tissue may be submitted if available for patients with typical presentation of DIPG who have undergone previous biopsy but not mandated.
B. 48-hour time point will be obtained prior to administration of either drug (ribociclib or everolimus) on Day 3
C. The ribociclib dose on day 1 should be held and day 2 should be delayed until the last everolimus sample is collected on day 2.
D. See section 9.2.3 for details.
E. Anytime during treatment. See section 9.2.3 for details.

11 MEASUREMENT OF EFFECT

Although the clinical benefit of ribociclib/everolimus drug(s) has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every 8 weeks for the first 3 courses (Courses 2, 4, 6), then every 12 weeks (Courses 9, 12, and Course 15, 18, 21, 24, 26 during extended therapy period) thereafter until time of progression or completion of treatment. In addition to a baseline scan, confirmatory scans will also be obtained 8 weeks following initial documentation of an objective response.

11.1 Antitumor Effect

11.1.1 Definitions

- Evaluable for Toxicity

Patients who receive at least 1 dose of ribociclib or everolimus and are removed from treatment for toxicity during the dose-finding period (first 4 weeks of treatment) are evaluable for estimating the MTD.

Patients who receive approximately 85% (≥18/21 doses of ribociclib and ≥22/26 doses of everolimus) of prescribed therapy during the dose-finding period but who progress prior to completing the course may be considered evaluable for estimating the MTD, as long as no additional anti-cancer therapy or supportive care that would confound the interpretation of any observed toxicity or side effect is given. Patients must have completed all of the clinical and laboratory monitoring requirements specified by the protocol up to the time of disease progression for them to be considered evaluable for MTD.

Patients who have completed all therapy during the dose-finding period but who failed to comply with all the specified clinical and laboratory monitoring requirements for the first course may be considered inevaluable for estimating the MTD and replaced.

Patients who receive less than 85% (<18/21 doses of ribociclib and <22/26 doses of everolimus) doses of the protocol specified therapy and who go off treatment for reasons other than toxicity (e.g. progressive disease, withdrawal of consent etc.) during the dose finding period will be considered inevaluable for estimating the MTD and will be replaced.

- Evaluable for Objective Response

Only those patients who have measurable disease present at initiation of phase I treatment, have received at least one dose of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the next scheduled MRI will also be considered evaluable.)

- Evaluable for PK intra-tumoral PK on the surgical study

Patients with adequate tumor tissue (amount and quality) to be analyzable for the PK objective will be considered evaluable for the surgical study. Please note patients who may not be evaluable for the PK study may still be evaluable for the Phase I trial should they proceed to that component of the study.

11.2 Disease Parameters

Sites should report tumor measurements in 3 dimensions in the database. In order to completely document the assessment of response, the measurements of the longest tumor dimension, and its perpendicular, of all target lesions upon which the assessments of tumor response are based should be explicitly noted in the radiology report for the baseline and all subsequent follow-up exams. Reports for the follow-up exams should reiterate the measurements obtained at baseline for each target lesion. Non-target lesions or newly occurring lesions should also be enumerated in these reports, and changes in non-target lesions should be described. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Tumor response criteria are determined by changes in size using the longest tumor dimension, and its perpendicular. FLAIR, T2 or post contrast T1 weighted images may be used - whichever gives the best estimate of tumor size.

Since many tumors contain nonenhancing components (or, in some cases, the tumor may not enhance at all), both the enhancing and the non-enhancing components must be evaluated – on post contrast T1 weighted images and on FLAIR/T2 weighted images respectively. Increase in enhancement on T1 weighted images without accompanying increase in disease bulk on T2 or FLAIR images is not considered tumor progression. In return, enlarging areas of nonenhancing tumor (defined as mass effect/tissue thickening) are evidence of tumor progression. Conversely, decrease in enhancing tumor component without decrease in overall FLAIR/T2 extent may represent change in tumor permeability (commonly observed with antiangiogenic therapies) rather than represent tumor response.

11.2.1 Method

The following section describes the methodology. (See Figure 9 below for illustration.)

- For MRI imaging (preferred), the longest measurement of the tumor (or width, W) should be determined. It can be measured from the axial plane or the plane in which the tumor is best seen or measured, provided the same plane is used in follow ups. .

- The transverse measurement should be the longest length perpendicular to the selected W. These dimensions will serve as the comparator for subsequent disease response assessments.

  NOTE: A measurable lesion should have a minimal transverse measurement that is at least twice the combined thickness of the image slice and the interslice gap. For example, with a 4 mm slice and a 0.4 mm gap, minimal measurable lesion diameter is 8.8 mm. Smaller lesions would not be measurable for study purpose.

- Leptomeningeal tumor spread is usually not a target lesion, and usually cannot be measured accurately. Presence and location of leptomeningeal tumor spread should be noted, and change in extent/thickness assessed on follow up studies.
- Follow-up examinations should assess the same 2 largest dimensions assessed at baseline.

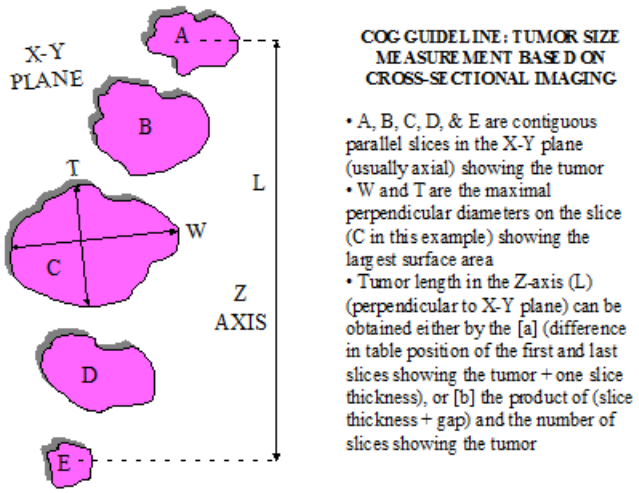


Figure 9: Tumor size measurement based on cross-sectional imaging

11.2.2 Overall Response Assessment

The overall response assessment takes into account response in both target and non-target lesion, and the appearance of new lesions, where applicable, according to the criteria described in the table below. The overall response assessment is shown in the last column, and depends on the assessments of target, non-target, and new lesions in the preceding columns.

Overall Response Assessment

Target Lesions	**New Lesions***	**Overall Response**
CR	No	**CR**
PR	No	**PR**
SD	No	**SD**
PD	Yes or No	**PD**
Any	Yes	**PD**
CR – Complete Response; PD – Progressive Disease; PR – Partial Response; IR – Incomplete Response; SD – Stable Disease *If CSF cytology becomes positive, it will be considered a new lesion and progressive disease.		

11.2.3 Selection of Target and Non-target Lesions

- For most CNS tumors, only one lesion/mass is present and therefore is considered a "target" for measurement/follow up to assess for tumor progression/response.
- If multiple measurable lesions are present, a minimum of the 2 largest lesions should be measured; a maximum of 5 should be selected as "target" lesions. Target lesions should be selected on the basis of size and suitability for accurate repeated measurements. All other lesions will be followed as non-target lesions.
- The lower size limit of the target lesion(s) should be at least twice the thickness of the slices showing the tumor to decrease the partial volume effect (e.g., 8 mm lesion for a 4 mm slice).

11.3 Tumor Response Criteria

11.3.1 Complete Response (CR)

Complete disappearance on MR of all evaluable tumor and mass effect, on a stable or decreasing dose of corticosteroids (or receiving only adrenal replacement doses), accompanied by a stable or improving neurologic examination, and maintained for at least 8 weeks. If CSF was positive, it must be negative.

11.3.2 Partial Response (PR)

Greater than or equal to 50% reduction in tumor size by bi-dimensional measurement, as compared with the baseline measurements, on a stable or decreasing dose of corticosteroids, accompanied by a stable or improving neurologic examination, and maintained for at least 8 weeks.

11.3.3 Stable Disease (SD)

Neurologic exam is at least stable and maintenance corticosteroid dose not increased, and MR/CT imaging meets neither the criteria for PR nor the criteria for Progressive Disease.

11.3.4 Progressive Disease (PD)

Progressive Disease (PD): Progressive neurologic abnormalities or worsening neurologic status not explained by causes unrelated to tumor progression (e.g., anticonvulsant or corticosteroid toxicity wean, electrolyte disturbances, sepsis, hyperglycemia, etc.), OR a greater than 25% increase in the bi-dimensional measurement, taking as a reference the smallest disease measurement recorded since the start of protocol therapy, OR the appearance of a new tumor lesion.

Increasing doses of corticosteroids required to maintain stable neurological status should be strongly considered as a sign of clinical progression unless in the context of recent wean or transient neurologic change due to treatment effect.

11.3.5 Progression-free Survival

Interval of time between date of initiation of protocol treatment and minimum date of documentation of PD, second malignancy, death due to any cause, or date of last follow-up.

11.3.5 Duration of Response

- Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

- Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

12 STUDY OVERSIGHT AND DATA REPORTING/ REGULATORY REQUIREMENTS/ CONFIDENTIALITY

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7 (Adverse Events: List and Reporting Requirements).

12.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through Medidata Rave.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the Pediatric Brain Tumor Consortium's (PBTC) data safety monitoring plan.

12.2 Data Reporting

Data collection for this study will be done exclusively through the Medidata Rave clinical data management system. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP-IAM account (check at https://ctepcore.nci.nih.gov/iam/) and the appropriate Rave role (Rave CRA, Read-Only, CRA (Lab Admin), SLA or Site Investigator) on either the LPO or participating organization roster at the enrolling site. To the hold Rave CRA role or CRA Lab Admin role, the user must hold a minimum of an AP registration type. To hold the Rave Site Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave. If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the “accept” link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave

resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

Users may also contact OBDMC to get help with study specific issues including clinical forms, data entry, data query, data sign-offs and/or uploading of regulatory and other required documents. For complete OBDMC contact details, click on the OBDMC Contact Information link that is available in the Members' Area of the PBTC website (http://www.pbtc.org/).

12.2.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. A protocol and subject-specific CDUS "Abbreviated" data set will be submitted electronically to CTEP on a quarterly basis via CDUS OPEN (Oncology Patient Enrollment Network). Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP website (http://ctep.cancer.gov/reporting/cdus.html).

12.2.2 Responsibility for Data Submission

The OBDMC for the PBTC is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.3 CTEP Multicenter Guidelines

N/A

12.4 Collaborative Agreements Language

N/A

12.5 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study and the data will be released to an unauthorized third party without the prior written approval of the Pediatric Brain Tumor Consortium (PBTC).

The PBTC protocol coordinators, other authorized representatives of the sponsor, regulatory representatives, PBTC auditors, representatives of the IRB or the pharmaceutical collaborator supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

Source documents which are the original records of clinical findings, observations or activities in a clinical trial are to be maintained at each participating site. Sites must upload all source documentation which supports the eligibility of the participant to the PBTC via the RAVE database. In the event the patient experiences unexpected events, additional source documentation may be requested to complete the event

review. These documents may include but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda and radiographic images.

Study participant study related data, which is for purposes of statistical analysis and scientific reporting will be transmitted to the Pediatric Brain Tumor Consortium electronically via the RAVE database. This will not include the participant's contact or identifying information. Rather, research participants and their research data will be identified by a unique study identification number assigned at the time of screening or registration. The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and institutional regulations.

The study data entry and study management systems used by clinical sites and by the PBTC will be secured and password protected. At the end of the study, all study data is maintained on a secure server.

After the study is completed, the data collected will be maintained on a server and may be used by other investigators, including those outside the study. With the participant's approval and as approved by local IRBs, biological samples labeled only with the participant's protocol specific identification number will be stored at the PBTC Central Review and Biorepository and could be made available to other investigators for future unspecified research. Investigators conducting future studies will not have access to the key for stored data collected while the participant is on study. Clinical data will be de-identified before it is shared with other investigators.

If the participant agrees to submit a repository sample, those samples contain genetic information that may be used for research related to brain tumors and their treatment. They may also be used to develop tests/assays to improve diagnosis and treatment of these diseases in the future. Genetic research may consist of the analysis of one or more genes or the analysis of genetic markers throughout the genome.

13 STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

Rolling-6 Design

Phase I Study
A ”Rolling-6” Phase I design will be used to estimate the maximum tolerated dose (MTD), where dose escalations are planned in cohorts of two-six patients. No intra-patient escalation will be allowed.

Skolnik et al[80] introduced the Rolling-6 design, motivated by the observation that most pediatric Phase I trials in oncology have not produced excessive toxicities. A possible explanation for this could be that pediatric studies are often preceded by adult trials and the knowledge gained in the latter is utilized towards ensuring safety in the former. The Rolling-6 design aims to shorten the duration of pediatric Phase I trials by minimizing the time the trial would be closed to accrual for toxicity monitoring. This is achieved by enrolling anywhere from 2 to 6 patients at a dose level without requiring that the DLT status of the patients already assigned to the same dose level are known; hence reducing the number of patients who would be turned away due to unavailability of open slots. The simulations in Skolnik et al. as well as Onar-Thomas and Xiong[81] indicate that this approach decreases the duration of a Phase I trial compared to the traditional

method and that the toxicity associated with the Rolling-6 design is not higher than the toxicity associated with the traditional method.

13.2 Dose Escalation/De-Escalation Rules

The Rolling-6 design allows for accrual of two to six patients concurrently onto a dose level. Decisions as to which dose level to enroll a patient are based on the number of patients currently enrolled and evaluable, the number of patients experiencing dose-limiting toxicities (DLT), and the number of patients still at risk of developing a DLT at the time of new patient entry. Dose escalation occurs if 0 of 3-6 or no more than 1 of 6 evaluable patients experience a DLT while being treated at a dose level; otherwise if 2 of 2-6 patients experience DLTs the dose is declared too toxic and thus above the MTD. Once a dose is determined to be too toxic, further dose escalation is not allowed. The MTD is empirically defined 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 too toxic.

The following table enumerates all possible scenarios and describes escalation/de-escalation rules for the rolling 6 design.

Dose Escalation/De-Escalation Rules for the Rolling-6 Design					
				Decision when Next Patient is Enrolled	
# Pats Enrolled	**# Pats with DLTs**	**# Pats w/o DLT**	**# Pats with Toxicity Data Pending**	**Not at the Highest Dose Level**	**At the Highest Dose Level**
2	0, 1	Any	Any	Stay	Stay
2	2	0	0	De-escalate	De-escalate
3	0	0, 1, 2	3, 2, 1	Stay	Stay
3	0	3	0	Escalate	Stay
3	1	0, 1, 2	2, 1, 0	Stay	Stay
3	≥ 2	Any	Any	De-escalate	De-escalate
4	0	0, 1, 2, 3	4, 3, 2, 1	Stay	Stay
4	0	4	0	Escalate	Stay
4	1	0, 1, 2, 3	3, 2, 1, 0	Stay	Stay
4	≥ 2	Any	Any	De-escalate	De-escalate
5	0	0, 1, 2, 3, 4	5, 4, 3, 2, 1	Stay	Stay
5	0	5	0	Escalate	Stay
5	1	0, 1, 2, 3, 4	4, 3, 2, 1, 0	Stay	Stay
5	≥ 2	Any	Any	De-escalate	De-escalate
6	0	0, 1, 2, 3, 4	6, 5, 4, 3, 2	Suspend	Suspend
6	0	5, 6	1, 0	Escalate	MTD not determined
6	1	0, 1, 2, 3, 4	5, 4, 3, 2, 1	Suspend	Suspend
6	1	5	0	Escalate	MTD not determined
6	≥ 2	Any	Any	De-escalate	De-escalate

As indicated in the above table, dose escalation occurs if 0 out of 3-6 or at most 1 out of 6 evaluable patients experience a DLT while being treated at a dose level; otherwise if 2 of 2-6 patients experience DLTs the dose is declared too toxic and thus above the MTD. Once a dose is determined to be too toxic, no escalation to higher dose levels is allowed.

Special Considerations for Surgical Patients to Contribute to the Phase I component:
Surgical patients will be counted towards the accrual of the Phase I trial when they initiate the combination treatment post-surgical recovery. Since we expect a delay of 4-6 weeks from enrollment of a surgical patient on the surgical study to his/her enrollment on the Phase I trial, it would be infeasible to reserve a slot on a given dose level for that long. Given the rarity of these patients we also do not want to miss enrolling a surgical patient due to unavailability of slots on the Phase 1 trial. Thus we propose the following contingencies for the surgical patients:

- As long as accrual to the Phase I trial remains open, accrual to the surgical study will continue.
- Surgical patients who meet treatment initiation criteria for the Phase 1 component post-surgical recovery will be enrolled on the current dose level where non-surgical patients are being enrolled (see section 5 for details).
    - If a slot is available at the time a surgical patient presents for Phase I enrollment, then the patient will count towards the maximum accrual of 6 patients at that dose level.
    - If a slot is not available at the current dose level i.e. 6 patients have been enrolled and accrual is temporarily on hold for DLT assessment, we will nonetheless enroll the surgical patient(s) at that dose level bringing the total to 7 (or more) patients. In this case, if the observed DLT rate is <0.33 (i.e. 2 or fewer DLTs in 7 or 8 patients) then we will allow escalation. Otherwise the dose will be declared too toxic and de-escalation will occur.
    - Given the rarity of these patients we do not anticipate that we will enroll any more than 8 patients at a dose level but in the unlikely event that we do we will use 0.33 as the threshold for escalation/de-escalation decisions.

Based on the above-outlined escalation rules, if the lowest proposed dose level is found to be too toxic, then the trial will be closed to accrual and the merits of amending or closing the trial permanently will be reconsidered. On the other hand, if the maximum dose level proposed for the study is deemed to be safe, then the MTD will be considered to be beyond the highest dose level and consideration may be given to investigate higher dose levels. Alternatively, the highest dose level may be recommended as the Phase II dose.

Once the MTD has been estimated or the recommended Phase II dose has been determined, 6 additional patients will be treated at that dose level to better describe the toxicity profile of the agent. If the number of toxicities observed in the expansion cohort suggests that the initial estimate of the MTD is too toxic then we will consider de-escalating to a lower dose level and treating additional patients at that level including an expansion cohort in an effort to estimate the phase II recommended dose.

Contingencies
If all the dose levels are investigated with acceptable toxicity, consideration may be given to investigating higher dose levels. A deliberate decision will be made by the study committee and the sponsor. If a decision is made to not study higher doses, then the highest dose level may be recommended for further study in Phase II trials.

In the event of a single fatal toxicity, the OBDMC will suspend enrollment, pending discussion with the study chair, IDB and others as necessary.

13.3 Sample Size/Accrual Rate

We believe that it is unlikely we will study all 6 dose levels proposed but rather 4-5 dose levels. If 6 patients are enrolled on each of the 5 proposed dose levels (including dose-de-escalation levels of either 0 or -0.5 based on toxicities observed), up to 30 patients will be enrolled on the Phase I Study. Once the MTD has been estimated or the recommended phase II dose determined, 6 additional patients will be treated at that dose level to better describe the toxicity profile of this combination, bringing the total expected sample size for the Phase 1 component to 36 evaluable patients.

Although it is difficult to estimate the number of patients who will be available for the surgical cohort, our past experience with PBTC studies suggests approximately 3-6 such patients may be enrolled. In order for patients to be considered evaluable, tumor samples must be analyzable for the PK objectives. There is limited information available regarding the variability associated with intratumoral PK parameters and thus the desired sample size of 3-6 was chosen on pragmatic grounds and based on the fact that for plasma PK dose cohorts of 3-6 patients are often used in Phase I trials. Enrollment to the surgical cohort will continue throughout the Phase I study and will be closed when accrual to the Phase 1 component is completed.

Considering that there are always patients who enroll but are not evaluable for dose finding, we may need to screen as many as 70 patients for both the Phase I and surgical cohorts.

13.3.1 Projected Accrual Rages and Study Duration

The projected accrual rate is 1-2 patients per month for the Phase I Study and 2-3 patients per year for Surgical Study, based on knowledge of the member institutions and their commitment to the Consortium. With 4-5 likely dose levels, the total sample size and the study duration are expected to be 35-45 and about 1.5-2.5 years, respectively, if four dose levels are investigated. We believe it is unlikely that both dose level -1 and dose level 3 will be evaluated.

Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	0	0	2	2	**4**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	3	3	2	2	**10**
White	10	11	3	3	**27**
More Than One Race	1	1	1	1	**4**
Total	**14**	**15**	**8**	**8**	**45**

13.4 Analysis of Primary endpoints

We will summarize toxicities observed on the study by dose level and by attribution in a table format. Similarly data from correlative studies will be reported descriptively. Tumor PK concentrations will be compared to plasma PK and described descriptively as there are no data on CNS distribution of Ribociclib in humans and the expected sample size for the surgical trial is small. Paired tests may also be used to capture any significant changes in these markers pre and post treatment.

13.4.1 Statistical Analysis of Pharmacokinetics

Plasma drug concentrations and pharmacokinetic parameters will be presented in tabular and graphical form. Pharmacokinetic parameters of interest, such as apparent volume of the central compartment (Vc/F), elimination rate constant (Ke), half-life (t1/2), apparent oral clearance (CL/F), and area under the plasma concentration time curve (AUC) will be estimated using compartmental methods. Dose proportionality in pharmacokinetic parameters will be investigated by performing one-way analysis of variance (ANOVA) on dose-normalized parameters.

13.5 Analysis of Secondary endpoints

Any objective responses (CR+PR) which may be observed in this trial will be described by dose and by histology. Prolonged stable diseases will also be reported in a descriptive fashion.

Target inhibition will be descriptively defined by changes in phospho-RB1 and ki-67 via plots and summary statistics. We will also descriptively summarize any associations between molecular subgroup designations and other biology correlates with observed response to ribociclib and everolimus if there are any.

Prevention of stomatitis with dexamethasone-based mouthwash during course 1 and 2 will be descriptively defined by reporting the number of events of stomatitis and grade.

In addition to estimating individual pharmacokinetic parameters, we will also estimate the population parameters using nonlinear mixed effects modeling methods (NONMEM). This method estimates the population parameters and both the inter- and intra-subject variability. Once the population parameters and corresponding covariance matrix are estimated, individual estimates can be obtained using post hoc analysis.

14 REFERENCES

1. 1. Ivanchuk SM, Rutka JT. The cell cycle: accelerators, brakes, and checkpoints. *Neurosurgery.* 2004;54(3):692-699; discussion 699-700.

2. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2006;24(11):1770-1783.

3. Paugh BS, Broniscer A, Qu C, et al. Genome-wide analyses identify recurrent amplifications of receptor tyrosine kinases and cell-cycle regulatory genes in diffuse intrinsic pontine glioma. *J Clin Oncol.* 2011;29(30):3999-4006.

4. Warren KE, Killian K, Suuriniemi M, Wang Y, Quezado M, Meltzer PS. Genomic aberrations in pediatric diffuse intrinsic pontine gliomas. *Neuro-oncology.* 2012;14(3):326-332.

5. Paugh BS, Qu C, Jones C, et al. Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. *J Clin Oncol.* 2010;28(18):3061-3068.

6. Schwartzentruber J, Korshunov A, Liu XY, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature.* 2012;482(7384):226-231.

7. Buczkowicz P, Hoeman C, Rakopoulos P, et al. Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. *Nature genetics.* 2014;46(5):451-456.

8. Wu G, Diaz AK, Paugh BS, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. *Nature genetics.* 2014;46(5):444-450.

9. Fontebasso AM, Papillon-Cavanagh S, Schwartzentruber J, et al. Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. *Nature genetics.* 2014;46(5):462-466.

10. Taylor KR, Mackay A, Truffaux N, et al. Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. *Nature genetics.* 2014;46(5):457-461.

11. Grill J, Puget S, Andreiuolo F, Philippe C, MacConaill L, Kieran MW. Critical oncogenic mutations in newly diagnosed pediatric diffuse intrinsic pontine glioma. *Pediatr Blood Cancer.* 2012;58(4):489-491.

12. Barton KL, Misuraca K, Cordero F, et al. PD-0332991, a CDK4/6 inhibitor, significantly prolongs survival in a genetically engineered mouse model of brainstem glioma. *PloS one.* 2013;8(10):e77639.

13. Aoki Y, Hashizume R, Ozawa T, et al. An experimental xenograft mouse model of diffuse pontine glioma designed for therapeutic testing. *J Neurooncol.* 2012;108(1):29-35.

14. Li M, Lockwood W, Zielenska M, et al. Multiple CDK/CYCLIND genes are amplified in medulloblastoma and supratentorial primitive neuroectodermal brain tumor. *Cancer genetics.* 2012;205(5):220-231.

15. Mendrzyk F, Radlwimmer B, Joos S, et al. Genomic and protein expression profiling identifies CDK6 as novel independent prognostic marker in medulloblastoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2005;23(34):8853-8862.

16. Pfister S, Remke M, Toedt G, et al. Supratentorial primitive neuroectodermal tumors of the central nervous system frequently harbor deletions of the CDKN2A locus and other genomic aberrations distinct from medulloblastomas. *Genes, Chromosomes and Cancer.* 2007;46(9):839-851.

17. Parsons DW, Li M, Zhang X, et al. The genetic landscape of the childhood cancer medulloblastoma. *Science.* 2011;331(6016):435-439.

18. Kleinman CL, Gerges N, Papillon-Cavanagh S, et al. Fusion of TTYH1 with the C19MC microRNA cluster drives expression of a brain-specific DNMT3B isoform in the embryonal brain tumor ETMR. *Nature genetics.* 2014;46(1):39-44.

19. Sangar MC, Pedro K, Ditzler SH, Girard E, Olson J. PD0332991, a cdk4/6 inhibitor, induces tumor regression and extends survival in Atypical Teratoid Rhabdoid Tumor (ATRT) and Medulloblastoma mouse models. Paper presented at: Neuro-oncology2013.
20. Korshunov A, Witt H, Hielscher T, et al. Molecular staging of intracranial ependymoma in children and adults. *J Clin Oncol.* 2010;28(19):3182-3190.
21. Rajaram V, Leuthardt EC, Singh PK, et al. 9p21 and 13q14 dosages in ependymomas. A clinicopathologic study of 101 cases. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc.* 2004;17(1):9-14.
22. Zhang ZK, Davies KP, Allen J, et al. Cell cycle arrest and repression of cyclin D1 transcription by INI1/hSNF5. *Molecular and cellular biology.* 2002;22(16):5975-5988.
23. Versteege I, Medjkane S, Rouillard D, Delattre O. A key role of the hSNF5/INI1 tumour suppressor in the control of the G1-S transition of the cell cycle. *Oncogene.* 2002;21(42):6403-6412.
24. Venneti S, Le P, Martinez D, et al. p16INK4A and p14ARF tumor suppressor pathways are deregulated in malignant rhabdoid tumors. *Journal of neuropathology and experimental neurology.* 2011;70(7):596-609.
25. Tsikitis M, Zhang Z, Edelman W, Zagzag D, Kalpana GV. Genetic ablation of Cyclin D1 abrogates genesis of rhabdoid tumors resulting from Ini1 loss. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(34):12129-12134.
26. Betz BL, Strobeck MW, Reisman DN, Knudsen ES, Weissman BE. Re-expression of hSNF5/INI1/BAF47 in pediatric tumor cells leads to G1 arrest associated with induction of p16ink4a and activation of RB. *Oncogene.* 2002;21(34):5193-5203.
27. Katsumi Y, Iehara T, Miyachi M, et al. Sensitivity of malignant rhabdoid tumor cell lines to PD 0332991 is inversely correlated with p16 expression. *Biochemical and biophysical research communications.* 2011;413(1):62-68.
28. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nature reviews. Drug discovery.* 2009;8(8):627-644.
29. Sartelet H, Oligny LL, Vassal G. AKT pathway in neuroblastoma and its therapeutic implication. *Expert review of anticancer therapy.* 2008;8(5):757-769.
30. Kawauchi K, Ogasawara T, Yasuyama M, Otsuka K, Yamada O. Regulation and importance of the PI3K/Akt/mTOR signaling pathway in hematologic malignancies. *Anti-cancer agents in medicinal chemistry.* 2009;9(9):1024-1038.
31. Shayesteh L, Lu Y, Kuo W-L, et al. PIK3CA is implicated as an oncogene in ovarian cancer. *Nature genetics.* 1999;21(1):99-102.
32. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res.* 2008;68(15):6084-6091.
33. Li SY, Rong M, Grieu F, Iacopetta B. PIK3CA mutations in breast cancer are associated with poor outcome. *Breast cancer research and treatment.* 2006;96(1):91-95.
34. Ruggeri BA, Huang L, Wood M, Cheng JQ, Testa JR. Amplification and overexpression of the AKT2 oncogene in a subset of human pancreatic ductal adenocarcinomas. *Molecular carcinogenesis.* 1998;21(2):81-86.
35. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. *Nat Rev Cancer.* 2004;4(5):335-348.
36. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell.* 2006;124(3):471-484.

37. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science.* 2005;307(5712):1098-1101.
38. Pollack IF, Hamilton RL, Burger PC, et al. Akt activation is a common event in pediatric malignant gliomas and a potential adverse prognostic marker: a report from the Children's Oncology Group. *J Neurooncol.* 2010;99(2):155-163.
39. Mueller S, Phillips J, Onar-Thomas A, et al. PTEN promoter methylation and activation of the PI3K/Akt/mTOR pathway in pediatric gliomas and influence on clinical outcome. *Neuro Oncol.* 2012;14(9):1146-1152.
40. Cho YJ, Tsherniak A, Tamayo P, et al. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *J Clin Oncol.* 2011;29(11):1424-1430.
41. Northcott PA, Shih DJ, Peacock J, et al. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. *Nature.* 2012;488(7409):49-56.
42. Castellino RC, Barwick BG, Schniederjan M, et al. Heterozygosity for Pten promotes tumorigenesis in a mouse model of medulloblastoma. *PloS one.* 2010;5(5):e10849.
43. Hartmann W, Digon-Sontgerath B, Koch A, et al. Phosphatidylinositol 3'-kinase/AKT signaling is activated in medulloblastoma cell proliferation and is associated with reduced expression of PTEN. *Clin Cancer Res.* 2006;12(10):3019-3027.
44. Johnson RA, Wright KD, Poppleton H, et al. Cross-species genomics matches driver mutations and cell compartments to model ependymoma. *Nature.* 2010;466(7306):632-636.
45. D'Cunja J, Shalaby T, Rivera P, et al. Antisense treatment of IGF-IR induces apoptosis and enhances chemosensitivity in central nervous system atypical teratoid/rhabdoid tumours cells. *Eur J Cancer.* 2007;43(10):1581-1589.
46. Darr J, Klochendler A, Isaac S, Eden A. Loss of IGFBP7 expression and persistent AKT activation contribute to SMARCB1/Snf5-mediated tumorigenesis. *Oncogene.* 2014;33(23):3024-3032.
47. Tamura K, Hashimoto K, Suzuki K, Yoshie M, Kutsukake M, Sakurai T. Insulin-like growth factor binding protein-7 (IGFBP7) blocks vascular endothelial cell growth factor (VEGF)-induced angiogenesis in human vascular endothelial cells. *European journal of pharmacology.* 2009;610(1-3):61-67.
48. LEE011 (Ribociclib) Investigator's Brochure. *Novartis*. 9 edMar 2016.
49. Patel Y. PDTB-12. CNS PENETRATION OF THE CDK4/6 INHIBITOR RIBOCICLIB (LEE011) IN NON-TUMOR BEARING MICE AND MICE BEARING ORTHOTOPIC PEDIATRIC BRAIN TUMORS. *Neuro-oncology.* 2016;18(suppl 6):vi152-vi152.
50. LEE011 (Ribociclib) Investigator's Brochure. *Novartis*. 7 ed2014.
51. LEE011 (Ribociclib) Investigator's Brochure. *Novartis* 10 edOct 2016.
52. Kovarik JM, Hartmann S, Figueiredo J, et al. Effect of food on everolimus absorption: quantification in healthy subjects and a confirmatory screening in patients with renal transplants. *Pharmacotherapy.* 2002;22(2):154-159.
53. Urva S, Bouillaud E, Delaney R, Jappe A, Cheung W. A phase I study evaluating the effect of everolimus on the pharmacokinetics of midazolam in healthy subjects. *Journal of clinical pharmacology.* 2013;53(4):444-450.
54. Geoerger B, Bourdeaut F, DuBois SG, et al. 455PPHASE I STUDY OF LEE011 (CDK4/6 INHIBITOR) IN PATIENTS WITH MALIGNANT RHABDOID TUMORS, NEUROBLASTOMA, AND CYCLIN D–CDK4/6 PATHWAY-ACTIVATED TUMORS. *Annals of Oncology.* 2014;25(suppl 4):iv151-iv152.
55. Houghton PJ. Everolimus. *Clinical Cancer Research.* 2010;16(5):1368-1372.

56. Houghton PJ, Morton CL, Kolb EA, et al. Initial testing (stage 1) of the mTOR inhibitor rapamycin by the pediatric preclinical testing program. *Pediatr Blood Cancer.* 2008;50(4):799-805.
57. Zeng Z, Sarbassov dos D, Samudio IJ, et al. Rapamycin derivatives reduce mTORC2 signaling and inhibit AKT activation in AML. *Blood.* 2007;109(8):3509-3512.
58. Treeck O, Wackwitz B, Haus U, Ortmann O. Effects of a combined treatment with mTOR inhibitor RAD001 and tamoxifen in vitro on growth and apoptosis of human cancer cells. *Gynecologic oncology.* 2006;102(2):292-299.
59. Haritunians T, Mori A, O'kelly J, Luong Q, Giles F, Koeffler H. Antiproliferative activity of RAD001 (everolimus) as a single agent and combined with other agents in mantle cell lymphoma. *Leukemia.* 2007;21(2):333-339.
60. Dilling MB, Dias P, Shapiro DN, Germain GS, Johnson RK, Houghton PJ. Rapamycin selectively inhibits the growth of childhood rhabdomyosarcoma cells through inhibition of signaling via the type I insulin-like growth factor receptor. *Cancer research.* 1994;54(4):903-907.
61. Hosoi H, Dilling MB, Liu LN, et al. Studies on the mechanism of resistance to rapamycin in human cancer cells. *Molecular pharmacology.* 1998;54(5):815-824.
62. Houghton PJ, Morton CL, Gorlick R, et al. Stage 2 combination testing of rapamycin with cytotoxic agents by the Pediatric Preclinical Testing Program. *Molecular cancer therapeutics.* 2010;9(1):101-112.
63. Geoerger B, Kerr K, Tang C-B, et al. Antitumor activity of the rapamycin analog CCI-779 in human primitive neuroectodermal tumor/medulloblastoma models as single agent and in combination chemotherapy. *Cancer research.* 2001;61(4):1527-1532.
64. Franz DN, Belousova E, Sparagana S, et al. Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. *The Lancet.* 2013;381(9861):125-132.
65. Franz DN, Belousova E, Sparagana S, et al. Everolimus for subependymal giant cell astrocytoma in patients with tuberous sclerosis complex: 2-year open-label extension of the randomised EXIST-1 study. *The lancet oncology.* 2014;15(13):1513-1520.
66. Everolimus Investigator's Brochure. *Novartis*. 15 edApr 2016.
67. Fouladi M, Laningham F, Wu J, et al. Phase I study of everolimus in pediatric patients with refractory solid tumors. *J Clin Oncol.* 2007;25(30):4806-4812.
68. Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *New England Journal of Medicine.* 2010;363(19):1801-1811.
69. Kieran MW, Yao X, Macy M, et al. Final results of a prospective multi-institutional phase II study of everolimus (RAD001), an mTOR inhibitor, in pediatric patients with recurrent or progressive low-grade glioma. A POETIC consortium trial. *Neuro-oncology.* 2014;16(suppl 3):iii27-iii27.
70. Geoerger B, Kieran MW, Grupp S, et al. Phase II trial of temsirolimus in children with high-grade glioma, neuroblastoma and rhabdomyosarcoma. *Eur J Cancer.* 2012;48(2):253-262.
71. Wagner LM, Fouladi M, Ahmed A, et al. Phase II study of cixutumumab in combination with temsirolimus in pediatric patients and young adults with recurrent or refractory sarcoma: a report from the Children's Oncology Group. *Pediatr Blood Cancer.* 2015;62(3):440-444.
72. Yalon M, Rood B, MacDonald TJ, et al. A feasibility and efficacy study of rapamycin and erlotinib for recurrent pediatric low-grade glioma (LGG). *Pediatric blood & cancer.* 2013;60(1):71-76.
73. Becher OJ, Gilheeney SW, Khakoo Y, et al. A phase I study of perifosine with temsirolimus for recurrent pediatric solid tumors. *Pediatr Blood Cancer.* 2016.
74. Divers J OSJ. Stomatitis associated with use of mTOR inhibitors: Implications for patients with invasive breast cancer. *Clinical Journal of Oncology Nursing.* 2015;19(4):468-474.

75. Pilotte AP HM, Polson, KMO, Huftalen TM, Treister N. Managing stomatitis in patients treated with mammalian target of rapamycin inhibitors. *Clinical Journal of Oncology Nursing.* 2011;15(5):E83-E89.
76. Rugo HS, Hortobagyi GN, Yao J, et al. Meta-analysis of stomatitis in clinical studies of everolimus: incidence and relationship with efficacy. *Annals of Oncology.* 2016;27(3):519-525.
77. Rugo HS, Seneviratne L, Beck JT, et al. Prevention of everolimus-related stomatitis in women with hormone receptor-positive, HER2-negative metastatic breast cancer using dexamethasone mouthwash (SWISH): a single-arm, phase 2 trial. *The lancet oncology.* 2017;18(5):654-662.
78. Flynn JT, Kaelber DC, Baker-Smith CM, et al. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. *Pediatrics.* 2017;140(3).
79. Goldhoff P, Clarke J, Smirnov I, et al. Clinical stratification of glioblastoma based on alterations in retinoblastoma tumor suppressor protein (RB1) and association with the proneural subtype. *Journal of Neuropathology & Experimental Neurology.* 2012;71(1):83-89.
80. Skolnik JM, Barrett JS, Jayaraman B, Patel D, Adamson PC. Shortening the timeline of pediatric phase I trials: the rolling six design. *J Clin Oncol.* 2008;26(2):190-195.
81. Onar-Thomas A, Xiong Z. A simulation-based comparison of the traditional method, Rolling-6 design and a frequentist version of the continual reassessment method with special attention to trial duration in pediatric Phase I oncology trials. *Contemp Clin Trials.* 2010;31(3):259-270.

15 APPENDICES

15.1 Performance Status Criteria

MODIFIED LANSKY SCORE (Score as 0 - 100)

A. Normal Range

100 = Fully active
90 = Minor restrictions in physically strenuous play
80 = Restricted in strenuous play, tires more easily, otherwise active

B. Mild to moderate restriction

70 = Both greater restrictions of and less time spent in active play
60 = Ambulatory up to 50% of time, limited active play with assistance/supervision
50 = Considerable assistance required for any active play; full able to engage in quiet play

C. Moderate to severe restriction

40 = Able to initiate quiet activities
30 = Needs considerable assistance for quiet activity
20 = Limited to very passive activity initiated by others e.g. TV)
10 = Completely disabled, not even passive play
0 = Unresponsive, coma

KARNOFSKY SCALE

100 = Normal; no complaints
90 = Able to carry on normal activities; minor signs or symptoms of disease
80 = Normal activity with effort
70 = Cares for self. Unable to carry on normal activity or to do active work
60 = Requires occasional assistance but able to care for most of his/her needs
50 = Requires considerable assistance and frequent medical care
40 = Disabled; requires special care and assistance
30 = Severely disabled; hospitalization indicated though death not imminent
20 = Very sick. Hospitalization necessary. Active support treatment necessary.
10 = Moribund
0 = Dead

15.2 Prohibited medications - Ribociclib alone or Ribociclib and Everolimus

Category	Drug Name
Strong CYP3A4/5 inhibitors	Atazanavir/ritonavir, boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir /ritonavir, darunavir/ritonavir, elvitegravir/ritonavir, grapefruit juice, idelalisib, indinavir, indinavir/ritonavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir,ombitasvir/paritaprevir/dasabuvir/ritonavir (VIEKIRA PAK), posaconazole, ritonavir, saquinavir/ritonavir, telaprevir, telithromycin, tipranavir/ritonavir, troleandomycinvoriconazole
Strong CYP3A4/5 inducers	carbamazepine[3], enzalutamide, lumacaftor, mitotane, phenobarbital, phenytoin[3], rifabutin, rifampin (rifampicin)[3], St. John's wort (hypericum perforatum)[2,3]
CYP3A4/5 substrates with NTI[1]	Alfentanil, astemizole, cisapride, cyclosporine, diergotamine (dihydroergotamine), ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus
Medications with a known risk for QT prolongation[4]	Amiodarone, anagrelide, arsenic trioxide, astemizole, azithromycin, chloroquine, chlorpromazine, cilostazol, ciprofloxacin, cisapride, citalopram, clarithromycin, disopyramide, dofetilide, domperidone, donepezil, dronedarone, droperidol, erythromycin, escitalopram, flecainide, fluconazole, gatifloxacine, grepafloxacin, halofantrine, haloperidol, ibutilide, levofloxacin, levomepromazine, levosulpiride, methadone, moxifloxacin, ondansetron, oxaliplatin, papaverine HCl (intra-coronary), pentamidine, pimozide, procainamide, propofol, quinidine, roxithromycin, sevoflurane, sotalol, sulpiride, sultopride, terlipressin, terodiline, thioridazine, vandetanib
Herbal preparations/ medications	Herbal preparations/medications known as strong inducers or inhibitors of CYP3A4/5 or those with a known risk of QT prolongation are prohibited throughout the study. These herbal medications include, but are not limited to: St. John's wort, Kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, and ginseng. Patients should stop using these herbal medications 7 days prior to first dose of study drug.
Other investigational and antineoplastic therapies	Other investigational therapies must not be used while the patient is on the study. Anticancer therapy (chemotherapy, all SERMS (including raloxifene), biologic or radiation therapy, and surgery) other than the study treatments must not be given to patients while the patient is on the study medication. If such agents are required for a patient then the patient must be discontinued study drug.

[1] NTI = narrow therapeutic index drugs whose exposure-response indicates that increases in their exposure levels by the concomitant use of potent inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes) or drugs which have <2-fold difference in the minimum toxic concentrations and minimum effective concentrations in the blood.

[2] Herbal product

[3] P-gp inducer

[4] The list provided is as of January 2018.Check https www crediblemeds.org/healthcare-providers/drug-list for the most updated list.

As far as possible, avoid co-administration of QT prolonging drugs or any other drugs with the potential to increase the risk of drug-related QT prolongation (e.g., via a potential DDI that increases the exposure of ribociclib or the exposure of the QT prolonging drug). A definitive list of drugs with a known risk, possible risk, or conditional risk of QT prolongation and/or Torsades de Pointes (TdP) is available online at qtdrugs.org.

Source: Novartis PK Sciences Memorandum: Drug-Drug Interactions (DDI) and Co-medication Considerations for Novartis Clinical Trials (January 2018), which is compiled from Indiana University "Clinically Relevant" Flockhart Table™, University of Washington Drug Interaction Database, and FDA Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers.

15.3 List of medications to be used with CAUTION during study drug treatment with Ribociclib alone or Ribociclib and Everolimus

Category	Drug Name
Moderate CYP3A4/5 inhibitors	Aprepitant, amprenavir, asafoetida resin (Ferula asafoetida) cimetidine, crizotinib, diltiazem, faldaprevir, imatinib, isavuconazole, netupitant, nilotinib, tofisopam, Schisandra sphenanthera (nan wu wei zi), verapamil
Moderate CYP3A4/5 inducers	Bosentan, dabrafenib, efavirenz, etravirine, genistein,lopinavir[5], modafinil, nafcillin, telotristat
Sensitive CYP3A4/5 substrates[1]	Alpha-dihydroergocryptine, aprepitant, atorvastatin, avanafil, bosutinib,brotizolam, budesonide, buspirone, cobimetinib, darifenacin, dasatininb, ebastine, eletriptan, eplerenone, everolimus, felodipine, fluticasone, grazoprevir, ibrutnib, isavuconazole, ivabradine, ivacaftor, , levomethadyl (LAAM), lomitapide, lovastatin, lumefantrine, lurasidone, maraviroc, midazolam, midostaurin, naloxegol, neratinib, nisoldipine, perospirone, quetiapine, ridaforolimus, sildenafil, simeprevir, simvastatin, ticagrelor, tilidine, tolvaptan, triazolam, ulipristal, vardenafil, venetoclax, vicriviroc, voclosporin
BSEP inhibitors	Alectinib, atorvastatin, bromocriptine, candesartan, clobetasol, clofaziminie, dabigatran, dipyridamole, glyburide, grazoprevir, ledipasvir, mifepristone,pioglitazone, reserpine, rifamycin, simeprevir, telmisartan,timcodar, troglitazone, valinomycin, velpatasvir
Medications that carry a possible risk for QT prolongation[2]	Alfuzosin, apomorphine, aripiprazole, artenimol+piperaquine , asenapine, , atomoxetine, bedaquiline, bendamustine, bortezomib, bosutinib, buprenorphine, cabozantinib, capecitabine, ceritinib, clomipramine, crizotinib,clozapine, cyamemazine (cyamepromazine), dabrafenib, dasatinib, degarilix, delamanid, desipramine, dexmedetomidine, dolasetron, efavirenz, eliglustat, epirubicin, eribulin mesylate, ezogabine(retigabine), famotidine, felbamate, fingolimod, flupentixol, gemifloxacin, granisetron, hydrocodone-ER, iloperidone, imipramine (melipramine), isradipine, ketanserin, lapatinib, lenvatinib, leuprolide, lithium, melperone, midostaurin, mifepristone, mirabegron, mirtazapine, moexipril/HCTZ, necitumumab, nicardipine, nilotinib, norfloxacin, nortriptyline, nusinersen, ofloxacin, osimertinib, oxytocin, paliperidone, palonosetron, panabinostat, pasireotide, pazopanib, perflutren lipid microspheres, perphenazine, pilsicainide, pimavanserin, pipamperone, promethazine, prothipendyl, rilpivirine, risperidone, romidepsin, sertindole, sorafenib, sunitinib, tamoxifen, tipiracil/trifluridine, tizanidine, tolterodine, toremifene,trimipramine, tropisetron, vardenafil, vemurafenib, venlafaxine, vorinostat, ziprasidone
MATE1/2 substrates[3]	Acyclovir, cephalexin, cimetidine, fexofenadine, ganciclovir, glycopyrronium, metformin, pindolol, plisicainide, ranitidine, topotecan, varenicline
OCT1/2 substrates[4]	Amantadine, 6-beta-hydroxycortisol, carboplatin, cisplatin, cephalexin, cephradine, , , , ipratropium, lamivudine, linagliptin, metformin, , oxyplatin, oxybutynin, phenformin, picoplatin, pilsicainide, pindolol, , ranitidine, sorafenib, tropisetron, trospium, umeclidinium,, , and zidovudine
BCRP substrates	Daunorubicin, dolutegravir, doxorubicin, hematoporphyrin, imatinib, methotrexate, mitoxantrone, pitavastatin, rosuvastatin, irinotecan, ethinyl estradiol, simvastatin, sulfasalazine, sofosbuvir, tenofovir, topotecan, venetoclax

[1] Sensitive substrates: Drugs whose plasma AUC values have been shown to increase 5-fold or higher when co-administered with a potent inhibitor.[2] The list provided is as of January 2018. Check https www crediblemeds.org/healthcare-providers/drug-list for the most updated list.

[3] MATE1 and MATE2 share considerable substrate specificity.

[4] OCT1 and OCT2 share considerable substrate specificity.

[5] Lopinavir is prohibited when combined with ritonavir (see Table 14-1)

Source: Novartis PK Sciences Memorandum: Drug-Drug Interactions (DDI) and Co-medication Considerations for Novartis Clinical Trials (January 2018), which is compiled from Indiana University "Clinically Relevant" Flockhart Table™, University of Washington Drug Interaction Database, and FDA Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers.

15.4 List of prohibited medications – Everolimus

Category	Drug Name
Strong PgP Inihibitors	alogliptin, canaglifozin, cremophor RH40, curcumin, ketoconazole, lapatinib, lopinavir/ritonavir, mirabegron, propafenone, simepravir, valspodar, vandetanib, voclosporin
Strong PgP/CYP3A4 dual inhibitors	amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, elacridar, erythromycin, felodipine, , fluvoxamine, ginkgo (Ginkgo biloba), indinavir, indinavir/ritonavir, itraconazole, mibefradil, milk thistle (Silybum marianum), nelfinavir, nifedipine, nitrendipine, paroxetine, quercetin, quinidine, ranolazine, rifampin, ritonavir, saquinavir/ritonavir, Schisandra chinensis, St John's wort (Hypericum perforatum), talinolol, telaprevir, telmisartan, ticagrelor, tipranavir/ritonavir, tolvaptan, verapamil
Herbal preparations/ medications	Herbal preparations/medications are prohibited throughout the study. These herbal medications include, but are not limited to: St. John's wort, Kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, and ginseng. Patients should stop using these herbal medications 7 days prior to first dose of study drug.
Other investigational and antineoplastic therapies	Other investigational therapies must not be used while the patient is on the study. Anticancer therapy (chemotherapy, biologic or radiation therapy, and surgery) other than the study treatments must not be given to patients while the patient is on the study medication. If such agents are required for a patient then the patient must be discontinued study drug.
Reference: Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated April-2015 which summarizes DDI data from three sources including the FDA's "Guidance for Industry, Drug Interaction Studies", the University of Washington's Drug Interaction Database, and Indiana University School of Medicine's Drug Interaction Table.	

15.5 Dosing Tables for Ribociclib

Surgical Study

- Based on 50mg and 200mg Capsules, round to the nearest 50mg. Patients allergic to peanut and soy are not permitted to take the film-coated tablet formulation.
- Ribociclib liquid formulation is 30 mg/ mL. **If a 1 mL syringe is used, each 0.1 mL is equivalent to 3 mg.**

	Total Daily Dose (mg)	BSA Range (m^2)		Capsules Required		Liquid Formulation	Liquid total dose
		low	high	50 mg	200 mg	ml	mg
Ribociclib Dose Level 280 mg/m^2/day	150	0.50	0.62	3	0	5	150
	200	0.63	0.80	0	1	6.6	198
	250	0.81	0.98	1	1	8.3	249
	300	0.99	1.16	2	1	10	300
	350	1.17	1.33	3	1	11.6	348
	400	1.34	1.51	0	2	13.3	399
	450	1.52	1.69	1	2	15	450
	500	1.70	1.87	2	2	16.6	498
	550	1.88	2.05	3	2	18.3	549
	600	2.06	2.23	0	3	20	600
	650	2.24	2.41	1	3	21.6	648
	700	2.42	2.50	2	3	23.3	699

	Total Daily Dose (mg)	BSA Range (m^2)		Capsules Required		Liquid Formulation	Liquid total dose
		low	high	50 mg	200 mg	ml	mg
Ribociclib Dose Level 350 mg/m^2/day	200	0.50	0.62	0	1	6.6	198
	250	0.63	0.78	1	1	8.3	249
	300	0.79	0.92	2	1	10	300
	350	0.93	1.07	3	1	11.6	348
	400	1.08	1.21	0	2	13.3	399
	450	1.22	1.35	1	2	15	450
	500	1.36	1.50	2	2	16.6	498
	550	1.51	1.64	3	2	18.3	549
	600	1.65	1.79	0	3	20	600
	650	1.80	1.92	1	3	21.6	648
	700	1.93	2.08	2	3	23.3	699
	750	2.09	2.21	3	3	25	750
	800	2.22	2.35	0	4	26.6	798
	850	2.36	2.49	1	4	28.3	849
	900	2.50	2.50	2	4	30	900

Phase I Study:

- Based on 50 mg and 200 mg Capsules, round to the nearest 50 mg. Patients allergic to peanut and soy are not permitted to take the film-coated tablet formulation.
- Ribociclib liquid formulation is 30 mg/ mL. **If a 1 mL syringe is used, each 0.1 mL is equivalent to 3 mg.**

Ribociclib Dose Level 75 mg/m²/day	Total Daily Dose (mg)	BSA Range (m²)		Capsules Required		Liquid Formulation	Liquid dose calculation
		low	high	50 mg	200 mg	30mg/ml	total
	50	0.55	1.02	1	0	1.6 ml	48 mg
	100	1.03	1.66	2	0	3.3 ml	99 mg
	150	1.67	2.33	3	0	5 ml	150 mg
	200	2.34	2.50	0	1	6.6 ml	198 mg

Ribociclib Dose Level 120 mg/m²/day	Total Daily Dose (mg)	BSA Range (m²)		Capsules Required		Liquid formulation	Liquid dose calculation
		low	high	50 mg	200 mg	30mg/ml	total
	100	0.75	1.02	2	0	3.3 ml	99 mg
	150	1.03	1.45	3	0	5 ml	150 mg
	200	1.46	1.87	0	1	6.6 ml	198 mg
	250	1.88	2.29	1	1	8.3 ml	249 mg
	300	2.30	2.50	2	1	10 ml	300 mg

Ribociclib Dose Level 170 mg/m²/day	Total Daily Dose (mg)	BSA Range (m²)		Capsules Required		Liquid formulation	Liquid dose calculation
		low	high	50 mg	200 mg	30mg/ml	total
	100	0.45	0.73	2	0	3.3 ml	99 mg
	150	0.74	1.02	3	0	5 ml	150 mg
	200	1.03	1.32	0	1	6.6 ml	198 mg
	250	1.33	1.61	1	1	8.3 ml	249 mg
	300	1.62	1.91	2	1	10 ml	300 mg
	350	1.92	2.20	3	1	11.6 ml	348 mg
	400	2.21	2.50	0	2	13.3 ml	399 mg

15.6 Patient Drug Information Handout

Information for Patients, Their Caregivers, and Non-Study Healthcare Team on Possible Interactions with Other Drugs and Herbal Supplements

The patient _____ is enrolled on a clinical trial using the experimental study drugs, ribociclib (LEE011) and everolimus (RAD001). This form is addressed to the patient, but includes important information for others who care for this patient.

These are the things that you as a healthcare provider need to know:
Ribociclib and everolimus interact with many drugs that are processed by your liver* and have an effect on the heart's electrical activity (QTc prolongation)**.

*The enzyme(s) in question are CYP3A4, FMO3, CYP2D6, and p-glycoprotein. *Ribociclib and everolimus are broken down by these enzymes and may be affected by other drugs that inhibit or induce these enzymes.*

**The heart's electrical activity may be affected by *ribociclib.* The study doctor may be concerned about QTc prolongation and any other medicine that is associated with greater risk for having QTc prolongation.

To the patient: Take this paper with you to your medical appointments and keep the attached information card in your wallet.
Ribociclib and everolimus may interact with other drugs which can cause side effects. For this reason, it is very important to tell your study doctors of any medicines you are taking before you enroll onto this clinical trial. It is also very important to tell your doctors if you stop taking any regular medicines, or if you start taking a new medicine while you take part in this study. When you talk about your current medications with your doctors, include medicine you buy without a prescription (over-the-counter remedy), or any herbal supplements such as St. John's Wort. It is helpful to bring your medication bottles or an updated medication list with you.

Many health care providers can write prescriptions. You must tell all of your health care providers (doctors, physician assistants, nurse practitioners, pharmacists) you are taking part in a clinical trial.

These are the things that you and they need to know:
Ribociclib and everolimus must be used very carefully with other medicines that use certain liver enzymes or transport proteins to be effective or to be cleared from your system or that may affect your heart's electrical activity. Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that are considered ***strong inducers/inhibitors or substrates of CYP3A4, FMO3, p-glycoprotein, or any medicine associated with greater risk for having QTc prolongation.***

- Please be very careful! Over-the-counter drugs (including herbal supplements) may contain ingredients that could interact with your study drug. Speak to your doctors or pharmacist to determine if there could be any side effects.

- Patients must avoid consumption of grapefruit, Seville oranges or products containing the juice of each during the entire study and preferably 7 days before the first dose of study medication, due to potential CYP3A4 interaction with the study medications. Orange juice is allowed.

Your regular health care provider should check a frequently updated medical reference or call your study doctor before prescribing any new medicine or discontinuing any medicine. Your study doctor's name is _____ and he or she can be contacted at _____.

STUDY DRUG INFORMATION WALLET CARD	
You are enrolled on a clinical trial using the experimental study drug **Ribociclib and Everolimus. Ribociclib and Everolimus** may interact with drugs that are processed by your liver, or use certain transport proteins in your body or affects the electrical activity of your heart. Because of this, it is very important to: ➢ Tell your doctors if you stop taking any medicines or if you start taking any new medicines. ➢ Tell all of your health care providers (doctors, physician assistants, nurse practitioners, or pharmacists) that you are taking part in a clinical trial. ➢ Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.	**Ribociclib and everolimus** interacts with a specific liver enzyme called CYP3A4, FMO3, CYP2D6, and p-glycoprotein,, transport protein, heart's electrical activity (QTc prolongation, and must be used very carefully with other medicines that interact with this enzyme, transporter, or agent. Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that are considered strong inducers/inhibitors or substrates of CYP3A4, FMO3, CYP2D6, and p-glycoprotein, or transporter; or affect the heart's electrical activity Before prescribing new medicines, your regular health care providers should go to a frequently-updated medical reference for a list of drugs to avoid, or contact your study doctor. ➢ Your study doctor's name is _____ and can be contacted at _____.

15.7 Caregiver and Patient instructions for administration of everolimus dispersible tablets using a Dosing Cup

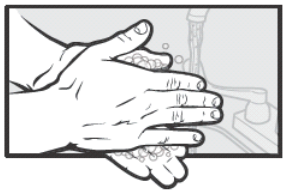
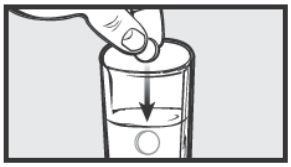
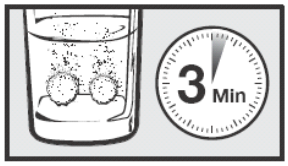
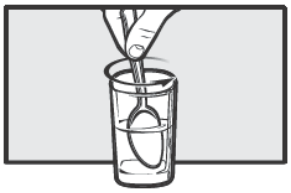
Please read this information before preparing and taking your study drug. **Please check with your study doctor or study team if you have any questions.**

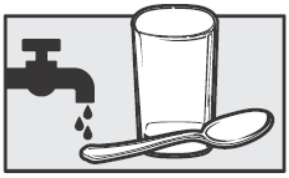
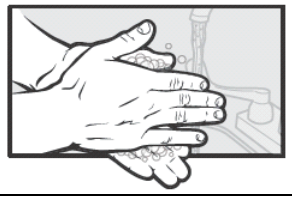
The everolimus dispersible tablets are made to be dissolved in water creating an oral suspension of the study drug. The oral suspension can be mixed in either a cup or syringe. If drug gets into eyes, flush with large amount of water while holding eyelids open for at least 15 minutes. Call your doctor or nurse immediately (insert phone #) and/or the Poison Control Center at (insert phone #). If you spilled the drug on your skin, remove contaminated clothing. Wash the area with soap and a large amount of water. Get medical attention if irritation develops or persists by calling the numbers listed above.

WHAT DO YOU NEED

- Everolimus dispersible tablets
- Dosing cup or syringe
- Drinking water or bottled water. Do not use seltzer (sparkling) water or juice.

HOW TO PREPARE THE EVEROLIMUS ORAL SUSPENSION - DOSING CUP

	Step 1: Wash and dry your hands before preparing the medication.
	Step 2: Add 5 mL of water into the dosing cup.
	Step 3: Remove the required number of dispersible tablets (_____) from tablet cards
	Step 4: Allow the dispersible tablets 3 minutes to disintegrate. Make sure to proceed further only when the 3 minutes are over and when the dispersible tablets have completely suspended.
	Step 5: Mix the content in the dosing cup by gentle swirling of the cup. The suspension is stable for up to one hour if cannot be given immediately.

	Step 6: Drink the full amount of liquid in the cup.
	Step 7: Refill the drinking glass with the same amount of water _____mL stir it again with the same spoon to suspend remaining particles and drink the full amount to ensure the entire dose is administered.
	Step 8: Rinse the dosing cup thoroughly with water. Wipe the dosing cup with a clean paper towel and store it in a dry and clean place until next dosing.
	Step 9: Wash your hands.

TABLET STORAGE
The everolimus tablets should be stored at room temperature. Store the drug away from food and out of reach of children and pets.

RETURN OF STUDY DRUG
All left over everolimus tablets need to be brought back to the study doctor at every clinic visit with the completed patient diary.

15.8 Caregiver and Patient instructions for administration of everolimus dispersible tablets using a Syringe

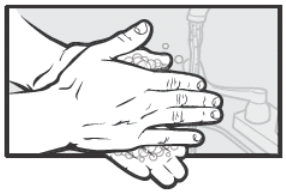
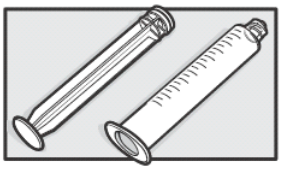
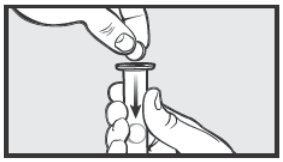
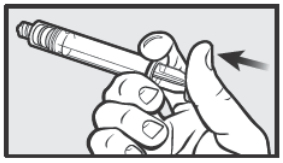
Please read this information before preparing and taking your study drug. **Please check with your study doctor or study team if you have any questions.**

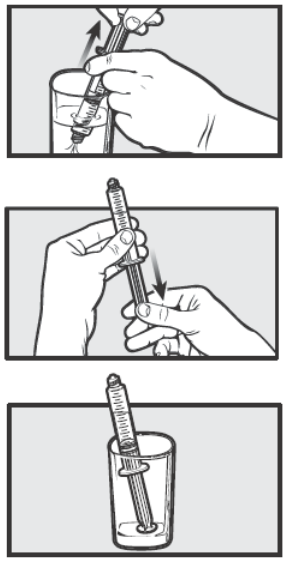
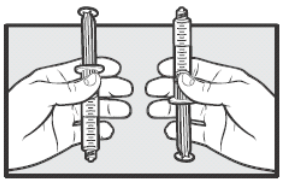
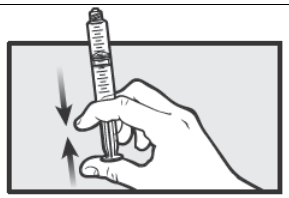
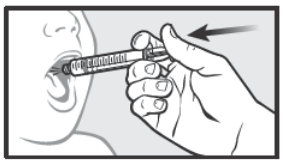
The everolimus dispersible tablets are made to be dissolved in water creating an oral suspension of the study drug. The oral suspension can be mixed in either a cup or syringe. If drug gets into your eyes, flush with large amount of water while holding eyelids open for at least 15 minutes. Call your doctor or nurse immediately (insert phone #) and/or the Poison Control Center at (insert phone #). If you spilled the drug on your skin, remove contaminated clothing. Wash the area with soap and a large amount of water. Get medical attention if irritation develops or persists by calling the numbers listed above.

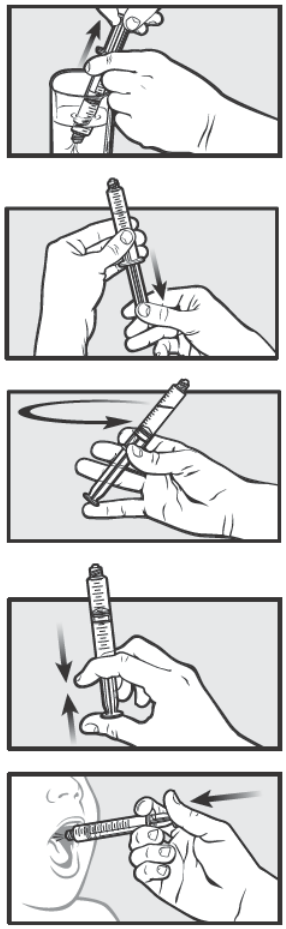
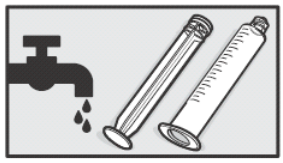
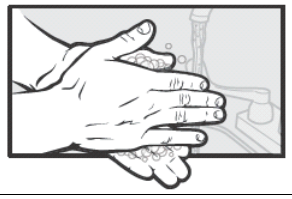
WHAT DO YOU NEED

- Everolimus dispersible tablets
- Dosing cup or syringe
- Drinking water or bottled water. Do not use seltzer (sparkling) water or juice.

HOW TO PREPARE THE EVEROLIMUS ORAL SUSPENSION - SYRINGE

	Step 1: Wash and dry your hands before preparing the medication.
	Step 2: Take a _____ml oral syringe and remove the plunger.
	Step 3: Remove the required number of dispersible tablets (_____) from tablet cards
	Step 4: Re-insert the plunger into the syringe and push it inward to make contact with the dispersible tablets.

	Step 5: Fill drinking glass with water and fill 5 mL of the water into the oral syringe by slowly pulling the plunger up. Turn the syringe upside-down so the tip points up. Then pull some additional _____ mL air. Place the filled syringe tip up in a glass.
	Step 6: Allow the dispersible tablets 3 minutes to disintegrate. Make sure to proceed further only when the 3 minutes are over and when the dispersible tablets have completely suspended.
	Step 7: Carefully turn the oral syringe upside down five times. Do not shake the oral syringe to avoid suspension leaking from the tip.
	Step 8: While holding the oral syringe in an upright position, carefully remove the excess air. Expel/dispose _____mLs of the solution in the oral syringe before giving to patient.
	Step 9: Dispense immediately the full content of the oral syringe slowly and gently into the mouth of the patient. Carefully remove the syringe from patient's mouth when empty The suspension is stable for up to one hour of cannot be given immediately.

	Step 10: Fill the oral syringe once more with the water by slowly pulling the plunger up. Turn the syringe so the tip is pointing up, and draw some additional mL air. Swirl the contents to suspend any remaining particles of the medication. While holding the syringe in an upright position, carefully remove the excess air. Then dispense the full contents of the oral syringe slowly and gently into the mouth of the patient.
	Step 11: Take apart the oral syringe and rinse all parts thoroughly with water. Store them in a dry and clean place until next dosing.
	Step 12: Wash your hands.

TABLET STORAGE
The everolimus tablets should be stored at room temperature. Store the drug away from food and out of reach of children and pets.

RETURN OF STUDY DRUG
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