NCI Protocol #: PBTC-042

Local Protocol #: PBTC-042

TITLE: ***Phase I study of CDK 4-6 inhibitor PD-0332991 (palbociclib; IBRANCE) in children with recurrent, progressive or refractory central nervous system tumors***

Coordinating Center:

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

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SCHEMA

Description

This is a multicenter, Phase I trial for children with Retinoblastoma Protein 1 (Rb1) positive recurrent, progressive or refractory central nervous system tumors.
In the recent years there have been several clinical trials in adults with cancer utilizing small molecule inhibitors targeting cell cycle regulatory genes such as CDKs (cyclin-dependent kinase). In normal cells, these kinases are kept under control by a gene, however, this gene is frequently deleted in cancers and these two kinases are uncontrollably activated which drives the cell to divide and form cancer. This trial is using PD-0332991 (palbociclib; IBRANCE), a selective CDK inhibitor, to be evaluated in children with CNS tumors.

PD-0332991 (Pfizer Corporation, USA) is an orally active, water soluble, cell-permeable highly specific inhibitor of CDK4 and 6. It is designed to shut down the activity of molecules, CDK4 and 6 that drive cell division. PD-0332991 is expected to work only if the cancer cells demonstrate expression of the tumor suppressor retinoblastoma (Rb1) protein which is needed to control cell growth even if CDK4/6 is inhibited. A screening test for presence or absence of Rb1 will therefore be performed in all types of CNS tumors except diffuse pontine glioma (DIPG), medulloblastoma, or Atypical Teratoid Rhabdoid Tumor (ATRT) to confirm eligibility for this trial. .

Schema

This is a dose escalation trial of PD-0332991 to determine the maximum tolerated dose in children with recurrent brain tumors. PD-0332991 is taken orally once a day for 21 days followed by a week rest. One course is therefore equivalent to 28 days. Therapy may continue for up to two years (26 courses) in the absence of disease progression or unacceptable toxicity.

Dosing is based on BSA calculated at the beginning of each course. The starting dose is 50 mg/m^2 for stratum I and stratum II. PD-0332991 dose escalations will be performed according to the table below until the maximum tolerated or recommended Phase II dose is reached. Since myelosuppression is the main dose limiting toxicity (DLT) of this drug, patients who have been more heavily pretreated are likely to experience hematologic toxicity with this agent. Therefore, patients will be divided into two strata; stratum I- patients who have received either focal radiotherapy (RT) only or focal RT ± $\leq$ 4 prior myelosuppressive chemotherapy and/ or biologic therapy regimens or stratum II- those who have received > 4 prior regimens (either chemotherapy or biologic agent), ± craniospinal irradiation, and ± myeloablative chemotherapy plus bone marrow or peripheral blood stem cell rescue.

Enrollment to Stratum I is now complete and accrued 21 patients at dose level 1 (n=3), 2 (n=12), and 3 (n=6). Two patients enrolled at dose level 3 were inevaluable for DLT. Of the 10 evaluable patients, 2 of 4 patients at dose level 3 experienced a DLT of grade 4 neutropenia. Hence the MTD was exceeded at dose level 3. The maximum tolerated dose (MTD) for stratum I was dose level 2 (75 mg/m^2/day for 21 days). Since the possibility of myelosuppression is expected to be greater in patients enrolled in stratum II (heavily pretreated patients), we consider it more prudent to commence enrollment of patients at dose level 1 which is 50 mg/m^2/day. An MTD is currently being determined for this stratum.

Stratum I and II - PD-0332991 (IBRANCE) Dosing Regimen and BSA Restrictions

Dose Level	Dose (mg/m^2)	BSA (m^2)
1*	50	≥ 1.20
2**	75	≥ 0.93
3	95	≥ 0.70

*Starting dose for stratum II
** MTD for stratum I

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To determine the maximum tolerated dose (MTD)/Phase II recommended dose and describe toxicities related to PD-0332991 in children with Retinoblastoma Protein 1 (Rb1) positive recurrent, progressive, or refractory primary CNS tumors. MTD will be determined separately in less-heavily pre-treated vs. heavily pre-treated patients.

1.1.2 To determine plasma pharmacokinetics of PD-0332991 in children with Rb1positive recurrent, progressive or refractory primary CNS tumors

1.2 Secondary Objectives

1.2.1 To record preliminary evidence of efficacy of PD-0332991 in children with recurrent CNS tumors

1.2.2 To evaluate CDK4/6, cyclin D1-3, Ink4a-ARF copy-number variations in available tumor tissue by array comparative, genomic hybridization (aCGH)

1.2.3 To explore the potential relationships between the pharmacokinetics of PD-0332991 and pharmacodynamic response (e.g. percentage change in absolute neutrophil count (ANC), platelet counts)

1.2.4 To explore the pharmacogenetic polymorphisms in PD-0332991 metabolizing enzymes and transporters and relate these polymorphisms to PD-0332991 pharmacokinetics

2 BACKGROUND

2.1 Study Disease

Recurrent pediatric primary Central Nervous System (CNS) tumors

The incidence of pediatric central nervous system (CNS) tumors is approximately 4000 new cases per year in the United States. While excellent cure rates can be achieved with low-grade gliomas and medulloblastoma with either surgery alone or in combination with chemotherapy and radiotherapy, the outcome for other types of CNS tumors remains suboptimal. Furthermore, cure rate in children with recurrent CNS tumors following standard therapy is dismal. Recent studies at the genomic level have revealed specific molecular alterations that drive the relentless progression of these tumors and might serve as targets for specific inhibitors that can control tumor proliferation and spread and, in contrast to cytotoxic therapy, with less damage to normal tissues.[5,6]

Cell cycle phases in mammalian cells (accelerators, brakes, and check points)

Tumor cell proliferation requires repetitive cell divisions involving doubling of DNA content (during the synthetic or S phase) and halving of the genome during mitosis (M phase) that results in two identical cells. Between the S and M phase, a gap occurs referred to as G_1 during which the cell decides to commit to another round of cell division or exit the cell cycle and enter a state of quiescence called the G_0 phase.[7] During the G_1 phase, cells grow and prepare for chromosomal replication during the S phase. Following the S phase, the cell enters into another gap phase called G_2 during which time the cell prepares for the equal division of its DNA into two identical daughter cells (M phase). Checkpoints are transitions between various phases of the cell cycle when the integrity of replication and division are carefully monitored before allowing the cell to move to the next phase.[7] Any error in the process of replication will result in the normal cell arresting the cell cycle till the error can be rectified. Cancer cells however continue the replication process despite the breach in DNA integrity. The different phases of the cell cycle are orchestrated by two distinct groups of proteins, the cyclins and their closely associated cyclin-dependent kinases (CDK).[7] In mammalian cells, cyclins and the CDKs can be divided into G_1 Cyclins (cyclin D1-3, and E) and CDK 4, 6, and 2; S phase cyclins (cyclins A and E) and CDK2, and mitotic cyclins (Cyclin A and E) and CDK 1 and 2.[7] The "brakes" in the cell cycle are by regulation of CDK activity that can occur at multiple levels including phosphorylation and dephosphorylation of CDK, cyclin synthesis and degradation, and expression/degradation/ availability of naturally occurring protein inhibitors and subcellular localization of these regulatory components. The two main classes of inhibitors include inhibitor of CDK4 (INK4) family of inhibitors and the CDK inhibitor protein (CIP)/kinase inhibitor protein family of inhibitors.[7] Whereas the INK4 family of inhibitors ($p16^{INK4A}$ and $p14^{ARF}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$) specifically inhibits only CDK4/6 during G_1, CIP/KIP ($p21^{CIP1}$, $p27^{KIP1}$, $p57^{KIP2}$) are capable of inhibiting kinases and cyclins during all phases of the cell cycle.[7]

G_1-S transition in the mammalian cell cycle is divided into the early G_1(pre-restriction point (RP) during which activation CDK-4 /Cyclin D complex occurs following release of INK4 from CDK4/6.[7] This results in hypophosphorylation of the retinoblastoma protein (Rb) and partial release of the E2F-DP1 transcription complex. In late G_1, release of cyclin E/CDK2 from the inhibitory effect of $p27^{KIP1}$, results in hyperphosphorylation of Rb1 and complete release of E2F-DPI complex which then binds to promoter regions of genes whose transcription are necessary for S phase entry and progression (**Figure 1**).[7,8]

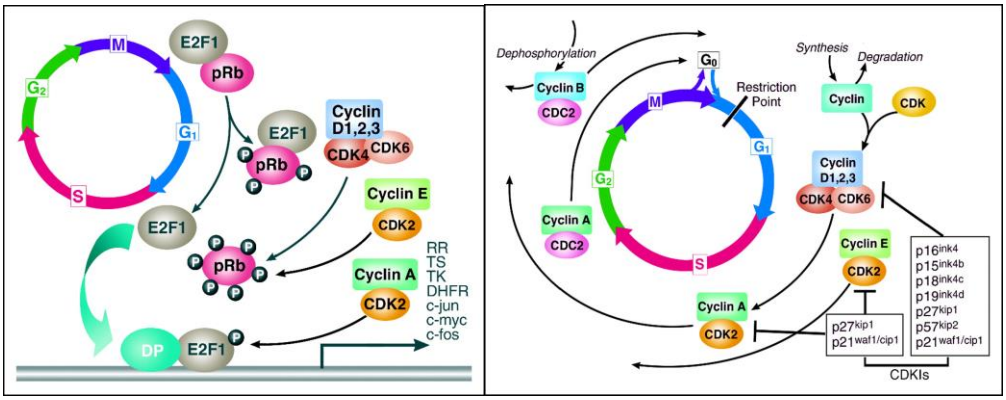


Figure 1: The CDK4,6/p16/Rb-E2F pathway

Alterations of cell cycle regulating genes in pediatric CNS tumors

Recent studies on the genomic aberrations of pediatric CNS tumors have demonstrated that a subset harbor focal gains in CDK4, CDK6, Cyclin D1-3, or homozygous Ink4a-ARF loss suggesting that patients with these tumors may benefit from therapies targeting CDK4/6. About 35% of pediatric brainstem gliomas harbor focal gains in CDK4, CDK6, or cyclin D1-3.[9]

About 20% of pediatric brainstem gliomas have been shown to have p16 loss.[10] Although one report did not identify any Rb1 mutations in 11 DIPG (Diffuse Intrinsic Pontine Glioma) tumor samples,[11] in another recent unpublished study homozygous Rb1 deletions have been observed in approximately 30% of tumor specimens (Oren Becher MD., Durham, NC 2013; unpublished observations). In a yet unpublished study of over 50 DIPG samples from St. Jude children's Research Hospital no Rb1 mutations were found (Suzanne Baker Ph.D, Memphis, TN; personal communication 2013). **On this basis, patients with DIPG will not be screened for Rb1 mutation prior to enrollment.**

Homozygous Ink4a-ARF loss is seen at the genomic level in 20% of non-brain stem gliomas.[12] In addition focal gain in CDK6, CDK4, and Cyclin D2 is observed in up to 6% in these tumors.[12,13] Rb1 deletions have been observed with this tumor subtype in only 5/49 (10%) of cases.[13] In pilocytic astrocytomas (PA), the most common subtype of pediatric CNS tumors, a subset of patients with poor prognosis harbor p16 loss (13.6% or 9/66), suggesting that some recurrent PAs may respond to CDK4/6 inhibitor.[14]

Homozygous CDKN2A (the gene for p16) deletions have been observed to be a poor prognosis marker in ependymoma.[15] Rb1 and p16 deletions were seen in 22 of 92 (24%) and 22 of 89 (25%) of ependymomas respectively.[16] Therefore recurrent ependymoma patients may benefit from a CDK 4/6 inhibitor but should be screened for the presence of Rb1 protein expression by immunohistochemistry (IHC) prior to enrollment on this trial.

CDK6 is overexpressed in 30% of medulloblastomas. Overexpression of CDK6 correlates significantly with poor prognosis and represents an independent prognostic marker of overall survival on multivariate analysis (P = 0.02).[17] In contrast, Rb1 homozygous loss or Rb1 mutations have only been rarely observed in this tumor.[17-19] In a genomic analysis of 22 medulloblastoma samples, Parsons et al., found no *Rb1* mutations.[18] Jones et al., in another

study observed only one non-synomyous single nucleotide variation (SNV) in 1/38 medulloblastoma tumors.[19] Similarly, atypical teratoid rhabdoid tumors (ATRT) have not been found to harbor Rb1 mutations.[20] Kieran et al., evalauted 25 Rhabdoid (n = 8) ATRT (n = 17) newly diagnosed and histologically verified tumor samples using an Oncomap-3 platform to interrogate 983 genes for mutations and amplifications. Other than *SMARCB1* mutations in all samples and *NRAS* mutation in one case of a brain ATRT, no other gene alterations were observed including *Rb1* mutations.[20] **Based on the data above, screening for *Rb1* mutations will not be required for enrollment in this study for patients with medulloblastoma and ATRT as well as DIPG**. About 30% of supratentorial PNETs have been noted to have p16 loss (7/21) and may therefore respond to a CDK 4/6 inhibitor.[1] In addition, about 25% have been reported to harbor CDK/Cyclin D amplification.[2]

There is suggestion in the literature that germ-cell tumors may be sensitive to CDK inhibition including CDK4/6.[3,4] Mature teratomas express high levels of phospho Rb1 (pRb1) in their epithelial components.[4] A prolonged PR was observed in an adult patient with a recurrent germ cell tumor treated in the Phase I study with PD-0332991.[3] Three adult patients with growing teratomas (with strong nuclear expression of pRb1) had either sustained PR (one patient) or stable disease (2 patients) following treatment with the CDK 4/6 inhibitor PD-0332991.[4]

2.2 PD-0332991

Specificity for CDK4/6

PD-0332991 (Pfizer Corporation, USA) is a pyrido [2, 3-d] pyrimidin-7-one (Figure 2) and an orally active, water soluble, cell-permeable highly specific inhibitor of CDK4 and 6.[21] The IC_{50} value for both CDK4 and CDK6 is between 0.009-0.015μM and >10 μM for 274 other protein kinases including CDK2.[22] PD-0332991 works by competing for ATP-binding sites on the enzyme.[23] The mean plasma half- life of the drug is about 26 hours.[3,22,24] The drug is moderately protein bound with unbound fraction in human plasma of 12-19%.

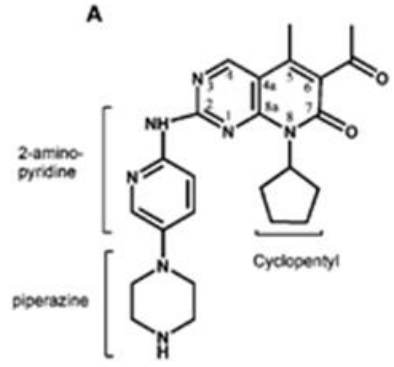


Figure 2: Structure of PD-0332991

2.2.1 Pre-clinical Studies of PD-0332991

PD-0332991 has undergone extensive pre-clinical testing for toxicity and efficacy. Pivotal 3-week toxicologic studies in rats identified 300 mg/m^2 as the dose that produced severe toxicity (mostly myelosuppression and testicular degeneration) in 10% of rats.[22] The maximum tolerated

dose of the drug in rats was 150 mg/day for 14 days. Dogs appeared to suffer more myelosuppression and testicular degeneration than rats.[22] (See section 2.2.2.1)

Following an oral dose of [^{14}C] PD-0332991, radioactivity levels were consistently greater in peripheral tissue than in blood including brain, meninges, pituitary gland, choroid plexus, and CSF. *In vitro* studies using pooled human liver microsomes demonstrate a low potential of PD-0332991 to inhibit cytochrome P450 enzymes including CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, and 2D6, based on IC_{50} values >30 μM. No significant induction of either CYP3A4 or CYP1A2 in Fa2N-4 cells was observed at PD-0332991 concentrations of 0.78 and 6.25 μM (349 and 2790 ng/mL), suggesting low potential for PD-0332991 to induce CYP3A4 and CYP1A activities at clinically relevant concentrations (0.19 μM or 86 ng/mL).

The ability of cytochrome P450 enzyme to metabolize PD-0332991 was evaluated by incubation of PD-0332991 (6 μM) with the 5 major cytochrome P450 enzymes (CYP1A2, 2C9, 2C19, 2D6, and 3A4). CYP3A4 was the only enzyme that metabolized PD-0332991. Concordantly, metabolism of PD-0332991 (6 μM) in human liver microsomes was significantly reduced (>80% decrease) in the presence of the CYP3A4 enzyme-specific inhibitor ketoconazole (1 μM). These data indicate that *in vitro*, CYP3A enzyme mediated oxidative pathway is primarily responsible for PD-0332991 metabolism. After oral administration of PD-0332991 to human subjects, sulfonation of parent drug constituted another major clearance pathway. In vitro, SULT 2A1 was found to be the predominant enzyme involved in the sulfonation of PD-0332991, with potential for minor contributions from other SULT isoforms.

2.2.2 Pre-clinical evidence of anti-tumor efficacy of PD-0332991

Several *in vitro* and *in vivo* studies in solid tumor xenografts have demonstrated efficacy of PD-0332991 in tumors that express wild type Rb1. The latter, not surprisingly, is a pre-requisite for efficacy of this agent. Of the known 16 phosphorylation sites on Rb1 protein, Ser^{780} and Ser^{795} are the 2 sites phosphorylated by CDK4/6 and represent a biomarker for inhibition of CDK4/6 by PD-0332991.[21,24] A panel of solid tumor cell lines including breast cancer and colorectal cancer[21] showed significant reversible inhibition of Rb1 phosphorylation at these two sites that peaked at 16 hours and began to return to baseline 2 hours after drug removal (Figure 3) with an IC_{50} of 0.066 μmol/L for MDA-MB-435 breast carcinoma cells.[21] At an optimal dose of 150 mg/kg daily for 14 days given by gavage after flank tumor implantation had reached a size of 100-150 g, the drug caused significant suppression of Rb1 phosphorylation *in vivo*, decreased cell proliferation, G-1 arrest (as measured by flow cytometry), and tumor regression or disease stabilization in a variety of adult solid tumor xenografts.[21] In a colorectal carcinoma (Col-205) tumor, treatment initially resulted in significant tumor shrinkage (Figure 4). **When tumors regrew after drug withdrawal, they were re-harvested and implanted in naïve mice. Retreatment with PD-0332991 at the same dose caused tumor regression (Figure 5) suggesting that initial tumor growth after stopping drug was not due to acquired resistance. None of the animals died due to toxicity and the maximum tolerated dose of PD-0332991 was 150 mg/kg/day.**

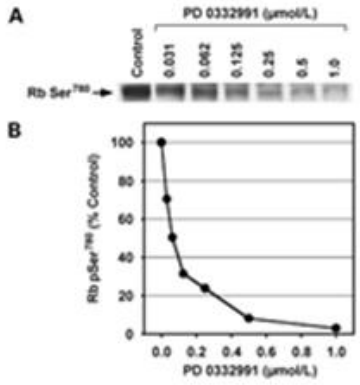


Figure 3

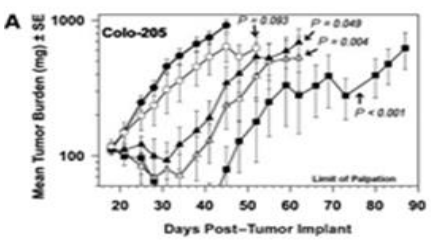


Figure 4

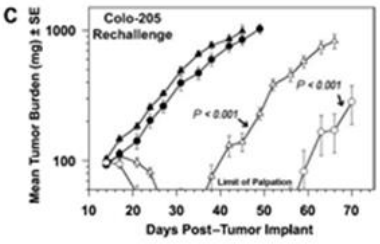


Figure 5

Michaud et al. investigated **the efficacy of PD-0332991 in 21 GBM cell lines and xenografts** with either intact or homozygously deleted Rb1 protein and at least one defect in the CDK4/6/cyclin D/ink4B signaling pathway.[25] Similar to what has been described with other solid tumor xenografts, treatment of cell lines and intracranial xenografts resulted in a predictable decrease in Rb1 phosphorylation, proliferative rate (Figure 6), G-1 arrest, and apoptosis that was dose dependent and occurred only in Rb1 proficient cell lines and xenografts and significant tumor regression (Figure 7).[25] In addition, Rb1 depletion using siRNA resulted in abrogation of PD-0332991 activity.[25] **Also, the study demonstrated that the drug penetrated the blood-tumor barrier efficiently (**Figure 8**). Synergistic effect could be demonstrated by combining radiotherapy and Temozolomide with PD-0332991**.[25]

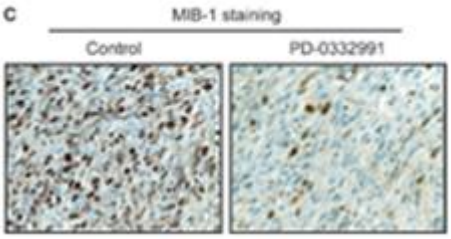


Figure 6: PD 0332991 decreases proliferative rate in GBM

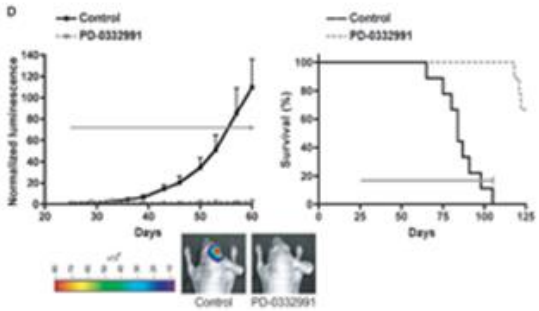


Figure 7: PD 0332991 causes significant tumor regression of IC GBM xenograft with improved survival

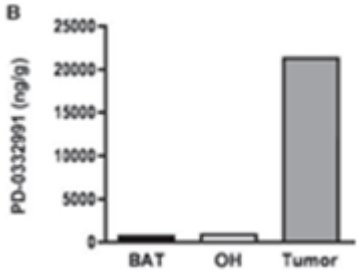


Figure 8: PD0332991 penetrates well into tumor as compared to brain around tumor (BAT) or opposite hemisphere

In another **study of intracranial malignant glioma xenografts** that were derived from DBTRG05-MG astrocytoma cell line harboring $BRAF^{V600E}$ mutation and INK4a-ARF deletion, treatment with PLX4720 (a $BRAF^{V600E}$ inhibitor) and PD-0332991 extended survival additively compared to either drug alone (Figure 9).[26]

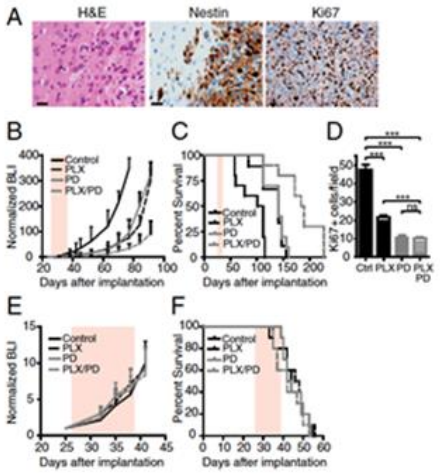


Figure 9: SCID mice with intracranial malignant glioma xenografts derived DBTRG05-MG astrocytoma cell line (harboring $BRAF^{V600E}$ and INK4a-ARF deletion) with histologic appearance, nestin expression, and high KI-67 (A). Treatment with PLX4020 or PD

0332991 results suppression of tumor growth but the combination significantly outperforms monotherapy (B) and better prolongation of survival (D) and decreased proliferation rate (D). In comparison, treatment of IC xenografts from GS2 malignant glioma cell line (wild type BRAF and Intact Ink4-ARF) shows no change in tumor growth or survival with either mono or combination therapy

PD-0332991 has also been tested **in brain stem glioma animal models**. Aoki et al., have shown that treatment of a brainstem glioma xenograft model with PD-0332991 provides significant survival benefit relative to vehicle-treated mice.[27] In addition, the Becher laboratory is carrying out preclinical studies with PD-0332991 in a genetically engineered brainstem glioma mouse model that is 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 as 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.

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 (Olson J, Seattle, WA; personal communication 2013). Significant tumor regressions (Figure 10) were observed associated with decrease in proliferation rate (Figure 10), Rb1 phosphorylation (Figure 12) and improvement in animal survival (Figure 11) 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 11).

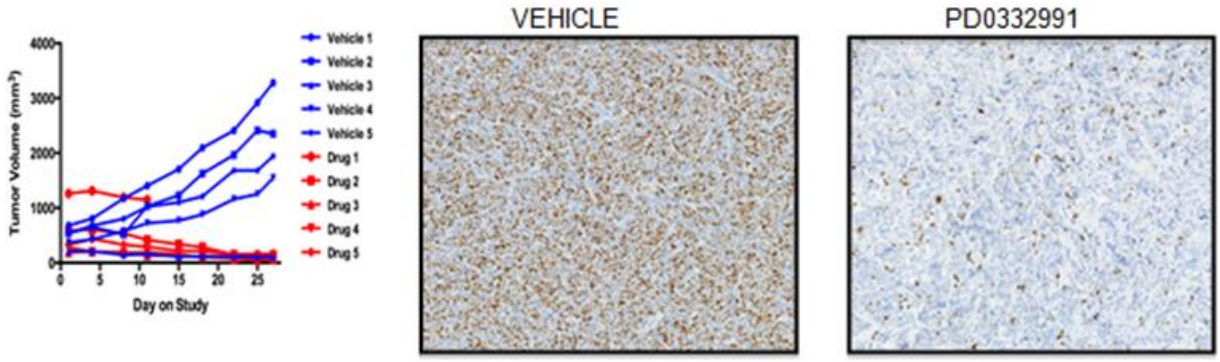


Figure 10: Medulloblastoma flank xenografts in mice treated with vehicle or PD0332991 (150 mg/kg/day for 14 days)

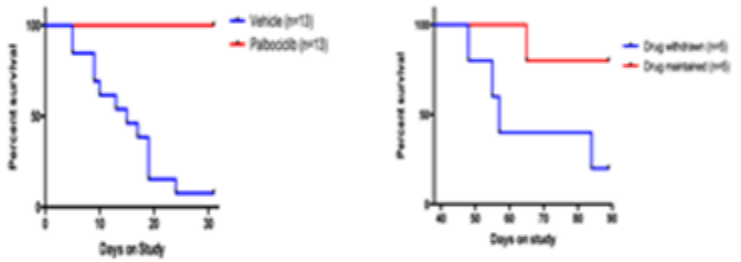


Figure 11: Survival of mice with Medulloblastoma flank tumors treated with vehicle or PD 0332991

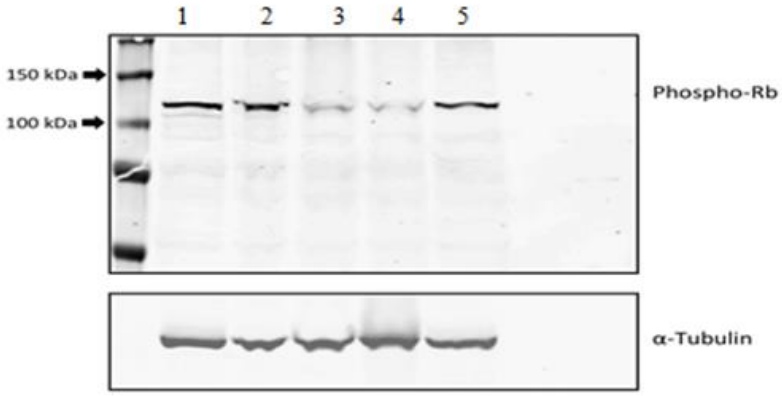


Figure 12: Western blot to demonstrate decrease in RB phosphorylation following treatment with PD-0332991 (Lanes 3, 4, and 5) compared to vehicle (lanes 1 and 2). Tumor from mouse in lane 5 was sacrificed at 24 hours as compared to 4 hours for those on lane 3 and 4.

Dr. Olson has similarly used an **Atypical Teratoid Rhabdoid Tumor (ATRT) flank xenograft** to demonstrate the efficacy of PD-0332991 in this tumor type. As in the medulloblastoma model, treatment with PD-0332991 at a dose of 150 mg/kg/day for 14 days resulted in excellent tumor regression 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.

2.2.2.1 Animal Toxicity

Results of animal toxicity studies in this section have been obtained from the Investigator's Brochure and have not been published yet. Pivotal toxicity studies were performed in rats and dogs. The latter animal was chosen due to the drug's better bio-availability in this species than in non-human primates. The predominant toxicities were related to bone marrow, lymphoid, and testicular tissues. In the pivotal 3-week toxicity study, deaths occurred at a dose of 1200 mg/m^2 and 2400 mg/m^2 given once daily for 3 weeks in male and female rats respectively. Death occurred in dogs at > 200 mg/m^2 daily for 2 weeks. Reversible bone marrow suppression (especially neutropenia) and lymphoid depletion (mesenteric lymph node, spleen, and thymus)

were observed in rats (males > females) at doses of > 300-1200 mg/m^2. Decrease in reticulocytes, neutrophils, and monocytes were observed within one week of treatment with variable nadir times and recovery within 2 weeks of cessation of therapy. In dogs, similar changes were observed at doses > 12mg/m^2 and nadir time was typically seen at the end of the dosing period (day 21).

Irreversible testicular degeneration occurred in rats at > 300 mg/m^2 and dogs at > 12 mg/m^2. There was decrease in spermatocytes in the spermatogenic epithelium and associated with decrease in testicular weight that was related to the administered dose. These findings are consistent with CDKs and cell cycle proteins being involved in the normal tissue of the bone marrow, lymphoid organs, and testis indicating the rapid turnover of cells in these compartments. In another study, PD-0332991 was administered by oral gavage to Sprague-Dawley rats at doses of 0, 10, 30, and 100 mg/kg/day in males and 0, 50, 100, and 300 mg/kg/day in females (20/sex/group; 15/sex/group in main and 5/sex/group in recovery) on an intermittent dosing schedule (7 cycles of treatment where each cycle consisted of 3 weeks of continuous daily dosing followed by a 1-week non-dosing period) for 27 weeks. Animals were evaluated by indirect ophthalmoscopy pre-dose and by indirect and slit lamp ophthalmoscopy prior to termination (within 7 days prior to scheduled necropsy). Slit lamp ophthalmoscopy was included for a more accurate assessment of ocular changes that were observed through indirect ophthalmoscopy. Indirect ophthalmoscopy results revealed a degraded view of the fundus and the presence of cataracts in high dose males (100 mg/kg/day) that had normal fundus examinations prior to the start of the study. Cataracts (anterior cortical, incomplete, and complete) were identified by slit lamp ophthalmoscopy in male rats at ≥ 30 mg/kg/day with dose-related severity (Table 1), but not in females at any dose tested (300 mg/kg/day).

Table 1: Slit Lamp Ophthalmoscopy

	Males				Females			
Dose (mg/kg/day)	0	10	30	100	0	50	100	300
N[a]	20	20	20	13[b]	20	20	19[b]	20
Cataract (total):	0	0	2	9	0	0	0	0
Anterior cortical	0	0	2	5[c]	0	0	0	0
Incomplete	0	0	0	3[d]	0	0	0	0
Complete	0	0	0	1	0	0	0	0

N = Total number of animals examined.
a. 20/sex/group includes 15 main and 5 recovery animals.
b. Unscheduled deaths prior to ophthalmic examination; 7 and 1 unscheduled deaths at 100 mg/kg/day in males and females, respectively.
c. Includes 3 main and 2 recovery animals.
d. Includes 2 main and 1 recovery animal.

Lens degeneration was observed microscopically with dose-related incidence and with minimal to moderate severity in males at ≥ 30 mg/kg/day and minimal severity in males at 10 mg/kg/day and females at ≤ 100 mg/kg/day (not identified in females at 300 mg/kg/day) (Table 2). Among the animals that have been evaluated microscopically, lens degeneration was identified in all animals that were noted to have cataracts on slit lamp examination.

Table 2: Microscopic examination of the eyes

	Males				Females			
Dose (mg/kg/day)	0	10	30	100	0	50	100	300
N (unscheduled deaths)[a]	15	15	15	15 (7)	15	15	15(1)	15
Lens degeneration:	0	1	3	9 (2)	0	1	2	0
Minimal	0	1	1	2 (1)	0	1	2	0
Mild	0	0	2	3	0	0	0	0
Moderate	0	0	0	4 (1)	0	0	0	0

N = Total number of animals examined.
a. Unscheduled deaths prior to ophthalmic examination in parentheses. The number of unscheduled deaths are included in the total number of animals (N).

The identification of cataracts in rats following 27-weeks of intermittent dosing represents new target organ toxicity. In the previous rat and dog repeat-dose toxicity studies up to 15 weeks duration, the primary target organ findings were observed in the hematolymphopoietic and male reproductive tissues. There were no PD-0332991-related ocular toxicities at doses up to 200 and 2 mg/kg/day in rats and dogs, respectively, in the 15-week toxicity studies (unbound systemic AUC exposures of 5200 and 684 ng•h/mL, respectively). In addition, no cataracts have been identified in PD-0332991-treated dogs at doses up to 3 mg/kg/day following 27-weeks of intermittent dosing (equivalent duration to that at which cataracts were observed in the rat study) using slit lamp ophthalmoscopy in an ongoing 39-week repeat-dose oral toxicity study (Study 8282225; Sponsor Reference Number 13LJ037; exposure data not available).

A no observed effect level has not been identified for cataract formation from the 27-week rat toxicity study, based on the histological presentation of lens degeneration. The available data suggests that the lens degeneration observed at the lowest doses in both male and female rats is PD-0332991-related. Day 189 systemic AUC (total) exposure was 6000 ng•h/mL (750 ng•h/mL, unbound, based on Fu of 0.125 in the rat) and 2650 ng•h/mL (331 ng•h/mL, unbound) in males at 10 mg/kg/day and females at 50 mg/kg/day, respectively. These exposures are comparable to clinical exposure at the recommended human dose of 125 mg QD (unbound AUC 301 ng•h/mL). The mechanism for cataract formation in PD-0332991-treated rats is unknown; however, its pathogenesis may be related to primary pharmacology. CDK4 expression (mRNA and protein) has been identified in the lens epithelial layer and in lens fibers of rats, suggesting its importance to lens differentiation. Altered cell growth of the lens epithelium is also recognized as a potential mechanism for cataract formation.

In addition to the above toxicities, pulmonary toxicities of rales and dyspnea were observed in rats given oral doses > 300 mg/m^2. Histopathologic examination revealed accumulation of foamy alveolar macrophages and tracheal mucosal atrophy. In dogs, apnea occurred following intravenous (i.v) administration of PD-0332991 at a dose of 5 mg/kg that was attributed to central respiratory depression compounded by the effects of propofol induced general anesthesia. The in vitro Purkinje fiber and human ether -a-go-go (hERG) assays along with cardiovascular study in dogs given doses over 10 mg/kg indicate a risk for QT-interval prolongation. The IC_{20} in the hERG assay was 1 µM (447.5 ng/mL) and QT prolongation occurring dogs was associated with a C_{max} > 211 ng/mL. No effects on ECG parameters were noted in dogs given 2 mg/kg in 3- or 15-week toxicity studies, with unbound C_{max} values (80 and 42 ng/mL respectively) that

exceeded those associated with human clinical dose of 125 mg QD (18 ng/mL).

2.2.2.2 Pre-clinical Pharmacology

PD-0332991 was given via oral and i.v routes to Sprague-Dawley rats, Beagle dogs, and Cyanomolgus monkeys. The PK parameters following single dose administration are given in Table 3. In general, mean plasma clearance values were low to moderate in all species and volume of distribution at steady state was about 10 fold greater than total body water indicating drug distribution into peripheral tissues.

Table 3

Species (Strain)	Dose (mg/kg)	Route (n)	C_{max} (mg/mL)	T_{max} (h)	$t_{½}$ (h)	CL (mL/min/kg)	V_{SS} (L/kg)	$AUC_{(0-\infty)}$ (µg·h/mL)	F (%)
Rat (Sprague-Dawley)	1	IV[a] (3)	NA	NA	2.2 (0.34)	38.0 (3.79)	5.65 (0.736)	0.442 (0.0467)	NA
	5	IV[a] (3)	NA	NA	2.6 (0.19)	37.4 (1.58)	7.07 (0.317)	2.23(0.103)	NA
	5	PO[b] (3)	0.178 (0.0474)	3.5 (1.9)	2.1 (0.12)	NA	NA	1.20 (0.393)	56.1[c]
	20	PO[b] (3)	1.11 (0.0618)	5.0 (1.2)	2.8 (0.36)	NA	NA	10.8 (0.651)	ND
	50	PO[b] (3)	1.66 (0.245)	4.5 (1.9)	4.9 (1.4)	NA	NA	23.0(6.74)	ND
	200	PO[b] (3)	2.24 (0.166)	30 (0)	NC	NA	NA	76.8[d] (8.9)	ND
Dog (Beagle)	1	IV[a] (3)	NA	NA	11 (0.29)	7.22 (0.853)	6.22 (0.789)	2.33 (0.258)	NA
	20	PO[b] (3)	0.664 (0.247)	8.7 (3.1)	21 (5.7)	NA	NA	17.4±6.9[e]	36.9 (12.4)[f]
Monkey (cynomolgus)	0.5	IV[a] (3)	NA	NA	4.7 (1.4)	13.4 (0.896)	5.05 (1.01)	0.624 (0.0428)	NA
	2.66	PO[b] (3)	0.0862 (0.0231)	2.7 (1.2)	5.3 (0.89)	NA	NA	0.768 (0.150)	23.1 (3.6)

Note: Values in parentheses indicate "± standard deviation."
$AUC_{(0-\infty)}$ = AUC from time zero extrapolated to infinity.
F (%) = Absolute (oral) bioavailability.
NA = Not applicable.
ND = Not determined.
NC = Not calculated—the concentration time-profile would not allow calculation of $t_{½}$.
[a] In solution (5% DMA/25% Propylene glycol/70% D5W).
[b] In suspension for rat and monkey (5%/5% PEG200/0.5% methylcellulose), and by capsule for dogs.
[c] F was calculated when IV and PO doses were equivalent, based on mean $AUC_{(0-\infty)}$ after IV and PO administration.
[d] Value represents $AUC_{(0-t)}$, because the concentration time-profiles would not allow calculation of $AUC_{(0-\infty)}$.
[e] Value represents $AUC_{(0-t)}$, because the $AUC_{(0-\infty)}$ was extrapolated >20% and thus not reported.
[f] Absolute oral bioavailability calculated using $AUC_{(0-t)}$ value following PO administration, because the $AUC_{(0-\infty)}$ was extrapolated >20%.

PK parameters following multiple dosing of PD-0332991 in the same species is given in Table 4.

The C_{max} and AUC values increased in a dose-related manner in all species. There was gender related differences in rats (higher rates of clearance in females compared to males). Dose accumulation (3-fold) was observed following multiple dosing both rats and dogs.

Table 4

Species	Study Day	Dose (mg/kg/day)	Cmax (ng/mL)		AUC(0-24) (ng·h/mL)	
			Male	Female	Male	Female
Rat[a]	1	30	ND	211	ND	887
		100	ND	291	ND	2960
		300	3250	2400	50500	37300
		600	3140	ND	64400	ND
	8	300	5620	ND	104000	ND
	14	30	ND	191	ND	1020
		100	ND	403	ND	3170
		300	ND	634	ND	9370
	1	50	1170	ND	18600	ND
		100	1740	306	30900	2050
		200	1710	435	35200	4090
		400	ND	319	ND	4850
	21	50	1800	ND	17300	ND
		100	2220	296	45300	2530
		200	2310	494	46600	5930
		400	ND	954	ND	11100
			Male and Female		Male and Female	
Dog	1[b]	1	26.0±3.45		392±79.7	
		3	119±17.1		1890±197	
		10	449±194		7720±3530	
		20	559±280		9630±4860	
		40	560±197		9430±2970	
	9[c]	10	1120		24500	
		20	849		18900	
		40	1250		25600	
	14[b]	1	59.5±14.1		992±186	
		3	313±28.6		5700±466	
	1[b]	0.2	6.96±1.68		106±29.3	
		0.6	18.0±3.52		279±59.2	
		2	80.6±15.2		1150±202	
	21[b]	0.2	12.2±3.18		206±63.7	
		0.6	35.3±8.72		581±143	
		2	193±35.3		3260±861	

ND = Not determined.
[a]Toxicokinetic parameters were derived from composite plasma concentration-time profiles in rats, standard deviation (SD) not calculated.
[b]Cmax and AUC values presented as mean±SD.
[c]N = 1 female, SD not calculated.

2.2.3 Adult Clinical Trials

2.2.3.1 Phase I trial of PD-0332991 in adults with recurrent solid tumors

PD-0332991 has been tested in a Phase I trial in adults with recurrent solid tumors and lymphomas (A5481001) that explored two different schedules of the drug. In the first schedule reported by Schwartz et al., adult patients with Rb1 positive recurrent solid tumors were enrolled

across four cohorts using doses of 100 mg, 150 mg, 200, and 225 mg given orally daily for 14 days every 3 weeks (Schedule 2/1).[3] No DLTs were observed at the 100 mg or 150 mg dose level. Dose limiting myelosuppression occurred in 2/3 patients at the 225 mg dose level [Grade IV neutropenia and thrombocytopenia (n=1) and Grade III neutropenia with delayed recovery (n=1)]. An intermediate dose of 200 mg/day was chosen. After only one DLT was observed (Grade III neutropenia and thrombocytopenia) in 6 enrolled patients at this dose, the cohort was expanded to a total of 20 patients. Three additional patients suffered DLT (Grade III myelosuppression with delayed recovery) with an overall 20% rate of DLT at this dose level. Since this did not exceed the preset limit of 33%, 200 mg/day for 14 days every 4 weeks was declared the MTD. Additional toxicities were mild and included Grade I fatigue and Grade I-II diarrhea. No patient had Grade 3 prolongation of QTc interval on EKG during therapy. Plasma PK studies revealed a dose proportional increase in C_{max} and AUC_{0-10} on days 1 and 8. In samples obtained from patients receiving 200 mg q daily, repeated dosing led to steady state levels. The median T_{max} was 4.2 hours and volume of distribution was 3241 liters (more than the adult total body water of 42 liters) suggesting extensive tissue penetration. PD-0332991 was eliminated slowly with a mean $T_{1/2}$ of 26.7 hours.[21] One patient with a non-seminomatous germ cell tumor of the testis achieved a prolonged partial response and 9 had SD. Six patients with SD had treatment for ≥ 4 cycles and 2 for ≥ 10 cycles.

In the second schedule of the same Phase I study, daily treatment for 21 days every 4 weeks (Schedule 3/1) was evaluated in 41 adult patients with recurrent Rb1 positive solid tumors.[22] Dose levels studied included 25, 50, 75, 100, 125 and 150 mg orally once daily. Five DLTs (either Grade III or IV neutropenia) were observed; 2 at 75 mg, 1 at 125 mg, and 2 at 150 mg. Thus 125 mg was declared the MTD. In an expanded cohort of 22 patients at this dose level, only 2 additional patients suffered Grade III neutropenia, making this the recommended Phase II dose. Additional toxicities were mild and included nausea, fatigue, and diarrhea. There was only one instance of a mild QTc prolongation in one patient that was felt not related to study drug. Plasma PK study results were similar to the previous Phase I trial.

2.2.3.2 Phase II trial of PD-0332991 in adults with recurrent mantle cell lymphoma and breast cancer

A Phase II trial of PD-0332991 was recently reported in adults with recurrent mantle cell lymphoma (a tumor that has aberrant cyclin D1 expression and homozygous loss of CDKN2A and hence dysregulation of the cell cycle signaling pathway).[24] Seventeen patients (median age, 66 years) were treated with PD-0332991 at 125 mg orally once daily for 21 days every 4 weeks until disease progression or unacceptable toxicity. The treatment was well tolerated and most Grade III or IV events were neutropenia or thrombocytopenia. No patient came off study for toxicity. The objective response rate was 18% (PR 2, CR 1) in 16 evaluable patients. An additional 7 patients experienced stable disease. Objective responses were observed between 4-8 cycles. Three patients have had prolonged stable disease on treatment between 565 – 903+ days. Five patients received study drug for more than one year. Six patients suffered PD. Evaluable lymph node biopsies in 10 paired tumor samples (done before and after 21 days of treatment) showed significant reduction of phosphor-RB (pS807/811) [mean, 89% (range, 51.6-100)] although total Rb1 staining was preserved. There was also similar reduction of Ki-67 staining (mean, 78%, range, 5-98.2%). Degree of reduction of phosphor-RB correlated with that of Ki-67.

All patients had Fluoro-deoxy glucose (FDG) PET and Fluoro-thymidine (FLT) scans at baseline and demonstrated avid tumor uptake of both FDG and FLT. Sixteen patients had both a baseline and post-treatment scans (day 21) for evaluation. The 3 patients who had objective responses demonstrated significant reduction (> 80%) of FLT uptake and a 24% decrease in FDG. Even patients who had SD or PD by routine imaging demonstrated > 25% reduction in both PET studies.[24]

In a randomized Phase I/II trial of PD-0332991 in combination with letrozole vs. letrozole alone as first-line treatment in 165 post-menopausal women with ER+/HER2- advanced breast cancer, preliminary analysis of median progression-free survival (PFS) was significantly higher in patients receiving PD-0332991 plus letrozole vs. letrozole alone (26.2 vs. 7.5 months respectively) with a hazard ratio (HR) = 0.32 (95% CI 0.19-0.56).[28] The most commonly reported treatment-related adverse events included neutropenia, leucopenia, anemia, and fatigue.

2.2.3.3 Pharmacology/Pharmacokinetics

Pharmacokinetic data of PD-0332991 is available from Phase I and II studies done in adults (n=137) with recurrent solid tumors. On Day 1, all patients had detectable plasma concentrations of PD-0332991 at the first measured time point (1 hour) following oral administration. The exposure ($AUC_{(0-10)}$ and C_{max}) increased in a dose-proportional manner over the dose range of 25-225 mg QD following PD-0332991 administration on Days 1 and 8 of Cycle 1, although some variability (low to moderate) around these doses was observed particularly at the 150 mg QD dose level. Following repeated daily dosing to Day 14 and Day 21 (assumed to be steady-state), PD-0332991 was absorbed with a median T_{max} of ~4 hours (Table 5). The mean PD-0332991 V_z/F was 3103 L, which is significantly greater than total body water (42 L), indicating that PD-0332991 extensively penetrates into peripheral tissues. PD-0332991 was eliminated slowly; the mean t½ was 26.5 hours and the mean CL/F was 86.1 L/hour. PD-0332991 accumulated following repeated dosing with a median R_{ac} of 2.4, which is consistent with a half-life of ~27 hours. Renal excretion of PD-0332991 was a minor route of elimination with ~1.7% of the drug excreted unchanged in urine over the 10-hour collection period in the 125 mg and 200 mg dose group, combined. No dose adjustment is required for patients with mild, moderate or severe renal impairment (creatinine clearance [CLCR] ≥15 mL/min). Insufficient data are available in patients requiring hemodialysis to provide any dosing recommendation in this patient population. PD-0332991 is primarily metabolized by the liver and eliminated via feces.. Studies suggest that no dose adjustment is required for patients with mild or moderate hepatic impairment (Child-Pugh classes A and B). For patients with severe hepatic impairment (Child-Pugh class C), dose reduction is warranted.

Table 5

Treatment Description	C_{max} [1] (ng/mL)	T_{max} [2] (hour)	$AUC_{(0-24)}$ [1] (ng•hr/mL)	$AUC_{(0-72)}$ [1] (ng•hr/mL)	$t_{1/2}$ [1] (hour)	CL/F [1] (L/hr)	V_z/F [1] (L)	R_{ac} [2,3]
Dose corrected 125 mg QD (n = 13)	104 (48)	4.2 (2-9.8)	1863 (59)	3549 (71)	26.5 (26)	86.1 (50)	3103 (40)	2.4 (1.5-4.2)

[1] mean (%CV)
[2] Median (Range)
[3] For Rac, n = 12 ($AUC_{(0-24)}$ was not estimable for Patient 10021099 on Cycle 1, Day 1 in the 200 mg group)
Note: Combined PK parameter data from Day 14 (200 mg) and Day 21 (125 mg) dose corrected to the 125 mg dose level.

Final pharmacokinetic data are available from 24 healthy male volunteers from Protocol A5481009. This was an open-label, randomized, 4-period, 4-treatment, 4-sequence, crossover, single-dose study employing administration of 4 PD-0332991 formulations in the fasted state to healthy adult volunteers. The primary objectives of the study were to identify a formulation that is bio-equivalent with the isethionate formulation used in the Phase 1-2 studies for use in phase 3 studies, and to explore the pharmacokinetics of the PD-0332991 active lactam metabolite, PF-05089326, following single oral doses of PD-0332991. Each subject received 4 single oral doses of PD-0332991 as 4 distinct formulations (Treatments A, B, C and D) separated by a washout period of at least 10 days. Treatments A (Reference), B, C, and D were single oral doses of PD-0332991 125 mg isethionate capsule; freebase small particle capsule; freebase large particle capsule and 50 mg oral solution, respectively.

Plasma PD-0332991 PK parameters are summarized descriptively in Table 6. The mean apparent t½ of PD-0332991 was similar for each of the 4 treatments with mean values ranging from 22.4 to 22.7 hours. Variability for PD-0332991 AUC_{inf}, AUC_{last} and C_{max} was somewhat higher for the freebase small and large particle capsule formulations (Treatments B and C) compared to the isethionate capsule and oral solution formulations (Treatments A and D), with CV% values in the range of 22% to 48% for AUC_{inf} and AUC_{last} combined and 24% to 58% for C_{max}, respectively.

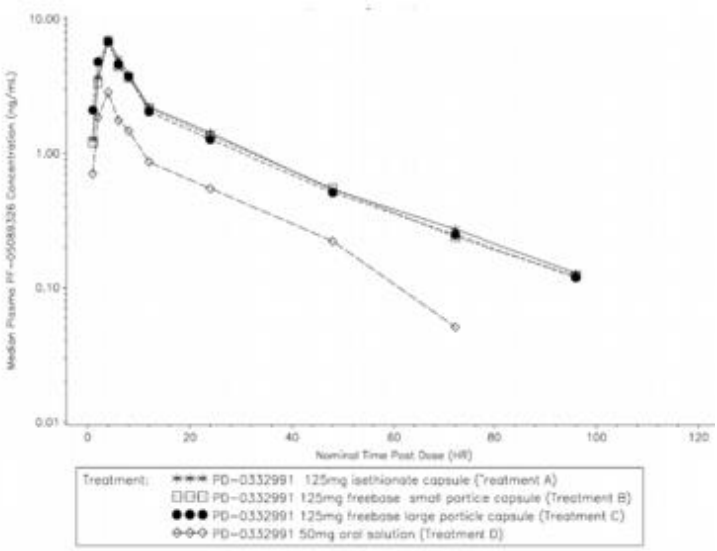


Figure 13: Median Plasma PD-0332991 concentration-time profiles following single oral doses of PD-0332991 Study A5481009

	Parameter Summary Statistics[a] by Treatment			
Parameter (units)	**125 mg Isethionate Capsule (Treatment A)**	**125 mg Freebase Small Particle Capsule (Treatment B)**	**125 mg Freebase Large Particle Capsule (Treatment C)**	**50 mg Oral Solution (Treatment D)**
N, n	24, 24	24, 23	24, 24	24, 23
AUC_{inf}DN125 (ng•hr/mL)	1388 (24)	1322 (22)	1267 (38)	1302 (22)
AUC_{last}DN125 (ng•hr/mL)	1337 (25)	1168 (48)	1213 (41)	1156 (26)
AUC_{inf} (ng•hr/mL)	1388 (24)	1322 (22)	1267 (38)	520.8 (22)
AUC_{last} (ng•hr/mL)	1337 (25)	1168 (48)	1213 (41)	462.0 (26)
C_{max}DN125 (ng/mL)	43.29 (24)	36.35 (58)	39.44 (52)	37.09 (27)
C_{max} (ng/mL)	43.29 (24)	36.35 (58)	39.44 (52)	14.82 (27)
T_{max} (hr)	6.01 (5.98-12.0)	8.00 (5.98-12.0)	8.00 (4.00-12.0)	8.00 (6.00-12.0)
CL/F (L/hr)	90.07 (24)	94.56 (22)	98.76 (38)	96.00 (21)
Vz/F (L)	2880 (19)	3049 (25)	3175 (46)	3123 (23)
$t_{½}$ (hr)	22.40 (±3.329)	22.60 (±3.457)	22.57 (±3.688)	22.72 (±2.812)

Abbreviations: %CV = percent coefficients of variation; hr = hour(s); N = number of subjects in the treatment group; n = number of subjects with reportable AUC_{inf}, CL/F, Vz/F and $t_{½}$ parameters; SD = standard deviation.
[a]Geometric mean (geometric %CV) for all except: median (range) for T_{max}, arithmetic mean (±SD) for $t_{½}$.

Table 6: Summary of Plasma PD-0332991 PK parameters following single doses of Treatment A, B, C and D (Study A5481009)

The results for statistical comparisons of the Test treatments relative to the Reference treatment (Treatment A) are summarized in Table 6. PD-0332991 freebase large particle capsule (Treatment C) prepared with an API lot with large particle size was shown to be bio-equivalent to the PD-0332991 isethionate hard capsule IR formulation (Treatment A) with respect to adjusted geometric mean ratios (90% CIs) of AUC_{inf}, AUC_{last} and C_{max} using pre-determined

equivalence limits of 80% to 125%. Therefore, the freebase large particle size (API) capsule will be used in this trial. The preliminary results from the recently performed food-effect study ("A5481021, a Phase 1, open-label 4 sequence 4 period crossover study of PD-0332991 in healthy volunteers to estimate the effect of food on the bioavailability of PD-0332991") has provided evidence that when a single 125 mg dose of PD-0332991 as administered under fed conditions (including high fat or low fat meal given together with PD-0332991, or moderate fat meal given 1 hour before and 2 hours after PD-0332991) as a freebase formulation the PD-0332991 exposure levels were more uniform across the population than when taken in the fasting condition. The preliminary data from the A5481021 food-effect study indicated that there was no clinically significant increase in PD-0332991 drug exposures when a freebase formulation of PD-0332991 was administered with food when compared to administration in the fasted state. The safety profile of the drug should remain unchanged in the presence of food. A small percentage of subjects who take PD-0332991 in the fasted state may experience variable drug exposures, and administration of PD-0332991 with food can make drug exposure in these subjects more uniform with the population.

2.3 Rationale

2.3.1 Rationale for version 2.0

Survival for children with recurrent CNS tumors is dismal and novel therapeutic options need to be developed in order to improve outcome. Cancer initiation and progression is dependent on deregulation of the components and phases of the cell cycle. Ample evidence exists (see background section) on alterations of CDKs, Cyclins, cell cycle inhibitors, and checkpoint proteins in pediatric CNS tumors. The presence of specific mutations in the proteins involved in G1-S phase transition of the cell cycle in these tumors is particularly relevant for the use of a specific CDK4/6 inhibitor like PD-0332991. Furthermore, it is likely that over 60% of pediatric CNS tumors are likely to have intact Rb-protein, a pre-requisite for the efficacy of this cell cycle inhibitor. PD-0332991 has not been tested in children with cancer thus far. A Phase I study is therefore needed to assess the toxicity, MTD, pharmacokinetics, and preliminary evidence of efficacy of this agent in this patient population. Since myelosuppression is the predominant side effect of this agent, the Phase I study will first evaluate the toxicities in children who have not been heavily pre-treated previously. Once MTD has been established in this cohort of patients, consideration will be given to expanding the trial to include those who have received intensive cytotoxic therapy including myeloablative chemotherapy +/- craniospinal irradiation. In addition, only patients who have no evidence of cataract formation on ophthalmologic examination will be eligible for this study based on pre-clinical evidence of development of cataracts and lens degeneration in rats treated with PD-0332991 on a 21-day cycle at doses > 30 mg/kg/day for 27 weeks.

2.3.2 Rationale for Amendments

Version 3.0

The purpose of Amendment Version 3.0 was to update CAEPR based on the February 2015 IB.

Version 4.0

Since myelosuppression was the DLT in adult patients with recurrent solid tumors treated with PD-0332991, the current PBTC-042 study was open only to patients who had either focal RT

and/or ≤ 2 prior myelosuppressive chemo or biologic therapy regimens (stratum I) and three dose levels tested; dose level 1 (50 mg/m^2/day), dose level 2 (75 mg/m^2/day), and dose level 3 (90 mg/m^2/day). Treatment was given daily for 21 days with a week of rest (one course = 28 days). Dosing was based on BSA calculated at the beginning of each course. Enrollment to Stratum I is currently ongoing and has accrued 12 patients at dose levels 1 (n=3), 2 (n=3), and 3 (n=6). Two patients enrolled at dose level 3 were not evaluable for DLT. Of the 10 patients evaluable for DLT, 2 of 4 at dose level 3 experienced grade 4 neutropenia as their DLT. Hence the MTD was exceeded at dose level 3, and 3 additional patients have been enrolled at dose level 2. The MTD for stratum I is expected to be 75 mg/m^2/day for 21 days.

With the MTD identified in less-heavily pre-treated patients (stratum I), this phase I study will be expanded to include heavily pre-treated patients (defined as those who have received more than 4 prior regimens (either myelosuppressive chemotherapy or biologic) ± craniospinal irradiation ± myeloablative chemotherapy plus stem cell rescue) in a separate cohort (stratum II). Treatment of these patients will be initiated at 50 mg/m^2/day (one dose level below the potential MTD in stratum I) as the starting dose level. Stratum II is likely to mostly include patients with recurrent medulloblastoma, other central PNETs, ATRT, and germ cell tumors. Recent genomic studies of medulloblastoma and ATRT have demonstrated that Rb1 protein is intact in these tumors.[18-20] Therefore, patients with medulloblastoma or ATRT will not be required to have their tumors screened for Rb1 expression.

2.3.3 Rationale for version 5.0

Per the recommendation from Pfizer, Cerebrospinal Fluid Pharmacokinetic Studies have been removed in Version 5.0 due to the high rates of non-specific binding of PD-0332991 to collection syringes/tube/vials/etc. when collected in media with low protein content (i.e. CSF).

2.3.4 Rationale for version 6.0 and 7.0

The purpose of Amendment Version 6.0 was to update CAEPR based on the December 2015 IB. The amendment version 7.0 was mainly due to the change of the protocol title because of a change in a drug supply of PD-0332991 from Pfizer to a commercial agent IBRANCE. The Principal Investigator and the IND Sponsor information were updated to Dr. David Van Mater.

2.3.5 Rationale for version 8.0

The protocol has been amended to reflect new and/or modified risk information for palbociclib (PD-0332991) based on the March 2017 IB. Additionally, language was added to clarify results of PK studies conducted in patients with renal and hepatic impairment as reflected in the July 2017 Investigator's Brochure.

2.4 Correlative Studies Background

2.4.1 Rationale for Biology Studies

2.4.1.1 Hypothesis:

To evaluate CDK4/6, cyclin D1-3, Ink4a-ARF copy-number variations in available tumor tissue by array comparative, genomic hybridization (aCGH).

2.4.1.2 Clinical Data

Recent studies on the genomic aberrations of pediatric CNS tumors have demonstrated that a subset harbor focal gains in CDK4, CDK6, Cyclin D1-3, or homozygous Ink4a-ARF loss

suggesting that patients with these tumors may benefit from therapies targeting CDK4/6. About 35% of pediatric brainstem gliomas harbor focal gains in CDK4, CDK6, or Cyclin D1-3.[9] About 20% of pediatric brainstem gliomas have been shown to have p16 loss.[10] Homozygous Ink4a-ARF loss is seen at the genomic level in 20% of non-brain stem gliomas.[12] In addition focal gain in CDK6, CDK4, and Cyclin D2 is observed in up to 6% in these tumors.[12,13] In pilocytic astrocytomas (PA), the most common subtype of pediatric CNS tumors, a subset of patients with poor prognosis harbor p16 loss (13.6% or 9/66), suggesting that some recurrent PAs may respond to CDK4/6 inhibitor.[14] Homozygous CDKN2A (the gene for p16) deletions have been observed to be a poor prognosis marker in ependymoma.[15] CDK6 is overexpressed in 30% of medulloblastomas. Overexpression of CDK6 correlates significantly with poor prognosis and represent an independent prognostic marker of overall survival on multivariate analysis ($P = 0.02$).[17] About 30% of supratentorial PNETs have been noted to have p16 loss (7/21) and may therefore respond to a CDK 4/6 inhibitor.[1] In addition, about 25% have been reported to harbor CDK/Cyclin D amplification.[2]

Based on this existing data and notwithstanding the confines of a Phase I study, it might be worthwhile evaluating and describing the cell cycle protein dysregulation in the tumors of patients who consent to have previously stored formalin fixed paraffin tumor tissue analyzed by aCGH. Feasibility of this approach would help us design a Phase II study in which responses would be correlated with alterations of specific targets within the cell cycle in patients given the MTD of PD-0332991 derived from this study.

2.4.2 Rationale for Pharmacokinetic Studies

Because the pharmacokinetics of this agent are relatively unknown in the pediatric population, this information will be essential for evaluating toxicity and disease response and for refining dosing in future clinical trials of PD-0332991. The results of these studies will provide the basis for assessing the relationship between the disposition of PD-0332991 in adult and children. Mandatory pharmacokinetic studies are needed to characterize the disposition of PD-0332991in this patient population. Insights into the biologically active dosage will be gained by relating PD-0332991systemic exposure (e.g., AUC) to results of pharmacodynamic studies (e.g., percentage change in ANC and platelets compared to baseline).

In vitro studies indicate that PD-0332991 is a substrate of CYP3A4, and dexamethasone is a known inducer of CYP3A4. Thus, one might expect lower exposures of PD-0332991 when administered concurrently with dexamethasone. Indeed, following multiples doses of PD-0332991 in A5481004 Phase 2 (dosing Schedule B), the mean PD-0332991 24-hr trough drug concentrations seem to decrease with time. The mean PD-0332991 trough concentrations on Cycle 1 Day 8, Cycle 1 Day 11, and Cycle 2 Day 11 were 45.33, 44.09, and 32.00 ng/mL, respectively, which is suggestive of a decreased PD-0332991 exposure when administered after dexamethasone-mediated enzyme induction has occurred. It should be noted that the exposure of PD-0332991 observed in this study is considerably lower compared to those observed in Studies A5481001 and A5481003 at the dose levels likely due to the induction effect of dexamethasone. Hence, patients who are receiving dexamethasone will have optional plasma PK studies done following discontinuation of this drug.

2.4.3 Rationale for Pharmacogenetic Studies

PD-0332991 has been shown in-vitro and in-vivo to be a substrate for the efflux-transporter proteins P-glycoprotein (P-gp; ABCB1) and breast cancer resistance protein (BCRP; ABCG2).[29] PD-0332991 is also metabolized hepatically by CYP3A4. Therefore, polymorphisms in *ABCB1* and *ABCG2*, which encode for P-gp and BCRP, could potentially alter inter-individual variability in the PD-0332991 dose-exposure-response relationships. Hence, pharmacogenetic studies will be included as a secondary objective. In order to examine the role of genetic variants in these transporters/metabolizing enzymes, genomic DNA will be isolated from peripheral blood cells to determine the relevant polymorphisms. We will then examine associations between these polymorphisms and PD-0332991 pharmacokinetic parameters.

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 three weeks prior to enrollment. All other clinical evaluations to establish eligibility must be done within 14 days prior to enrollment.

3.1 Screening Criteria

3.1.1 Screening Consent

Patients who are candidates for enrollment are willing to sign a screening consent and provide pre-trial tumor material for Rb1 testing. Screening applies to patients with all types of CNS tumors except DIPG, medulloblastoma, or ATRT.

3.1.2 Tumor

Patients must have recurrent, progressive or refractory central nervous system (CNS) tumors. Patients with secondary malignant gliomas will be eligible for this study but should conform to the strict prior treatment exposure criteria irrespective of the number of individual tumors treated previously. **Patients with low grade gliomas are excluded.**

3.1.3 Prior Therapy

Stratum I: Less-Heavily Pretreated Stratum

- Patients who have received the following:

i. ≤ 4 prior treatment regimens* with either myelosuppressive chemotherapy or biologic agents and/or
ii. focal radiotherapy

Stratum II: Heavily Pretreated Stratum

- Patients who have received ANY one of the following 3 criteria:

i. > 4 prior treatment regimens* with either myelosuppressive chemotherapy or biologic agents and/or
ii. craniospinal irradiation and/or
iii. myeloablative chemotherapy with autologous stem cell rescue

*A treatment regimen is defined as a single agent (chemotherapeutic or biologic), or a sequential combination of therapies that can include radiotherapy (with or without concurrent radiosensitizer, chemotherapy, or biologic therapy) followed by maintenance therapy (either single or combination) given over a period of time at either diagnosis or relapse.

3.1.4 Age

Patient must be ≥ 4 years and ≤ 21 years of age.

3.1.5 Patient must be able to swallow capsules.

3.1.6 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. Only patients with recurrent diffuse intrinsic brain stem glioma (DIPG), medulloblastoma, or ATRT can be enrolled without the need for available tumor tissue for Rb1 protein status confirmation.

3.1.7 BSA:

- Patients enrolled on dose level 1 (50mg/m^2) must have BSA≥ 1.20m^2
- Patients enrolled on dose level 2 (75mg/m^2) must have BSA≥0.93m^2
- Patients enrolled on dose level 3 (95mg/m^2) must have BSA≥0.70m^2

3.1.8 Potential Eligibility for Study Enrollment

Patients screened for this trial should be expected to meet the criteria for treatment.

3.2 Eligibility for Treatment Criteria

3.2.1 Eligible Diagnosis

Tumor type:
Histologically confirmed retinoblastoma protein (Rb1) positive primary recurrent, progressive, or refractory central nervous system tumors. **Patients with low grade gliomas are excluded.**

Formalin fixed paraffin embedded tumor tissue (preferably from the most recent recurrence) must be available to assess Rb1 protein status prior to enrollment. Patients with recurrent diffuse intrinsic brain stem glioma (DIPG), medulloblastoma, or ATRT can be enrolled without the need for available tumor tissue for Rb1 protein status confirmation.

3.2.2 Measureable Disease

Patients must have measurable disease (in 2-dimensions) on MRI scan of brain and/or spine to assess preliminary evidence of response.

3.2.3 Age

Patient must be ≥ 4 years and ≤ 21 years of age at the time of enrollment.

3.2.4 BSA:

- Patients enrolled on dose level 1 (50mg/m^2) must have BSA≥1.20m^2
- Patients enrolled on dose level 2 (75mg/m^2) must have BSA≥0.93m^2
- Patients enrolled on dose level 3 (95mg/m^2) must have BSA≥0.70m^2

3.2.5 Prior Therapy

Stratum I: Less-Heavily Pretreated Stratum

- Patients who have received the following:
  i. ≤ 4 prior treatment regimens* with either myelosuppressive chemotherapy or biologic agents and/or
  ii. focal radiotherapy.

Stratum II: Heavily Pretreated Stratum

- Patients who have received ANY one of the following 3 criteria:
  i. > 4 prior treatment regimens* with either myelosuppressive chemotherapy or biologic agents and/or
  ii. craniospinal irradiation and/or
  iii. myeloablative chemotherapy with autologous stem cell rescue

*A treatment regimen is defined as a single agent (chemotherapeutic or biologic), or a sequential

combination of therapies that can include radiotherapy (with or without concurrent radiosensitizer, chemotherapy, or biologic therapy) followed by maintenance therapy (either single or combination) given over a period of time at either diagnosis or relapse.

Patients must have fully recovered from the acute treatment- related toxicities of all prior therapies prior to entering this study. For those acute baseline adverse events attributable to prior therapy, patients must meet organ function criteria (section 3.2.10) in the Inclusion and Exclusion Criteria.

3.2.5.1 Chemotherapy: Patients must have received their last dose of known myelosuppressive anticancer chemotherapy at least three (3) weeks prior to enrollment in the study or at least six (6) weeks for those receiving nitrosourea.

3.2.5.2 Bone marrow/Stem Cell Infusion:

≥ 3 months since autologous bone marrow/stem cell infusion prior to enrollment (stratum II only)

3.2.5.3 Biologic therapy: Patients should have received their last dose of biologic agent ≥ 7 days prior to enrollment. In the event the patient has received another biologic agent and has experienced ≥ Grade 2 myelosuppression, then at least three (3) weeks must have elapsed prior to enrollment. If the investigational or biologic agent has a prolonged half-life then at least three (3) weeks interval is required. For patients on monoclonal antibodies including Bevacizumab, please refer to list of half-lives available on the PBTC webpage under Generic Forms and Templates.

3.2.5.4 Radiotherapy:

Patients must have had their last fraction of:

- Focal irradiation > 2 weeks prior to enrollment
- Craniospinal irradiation > 3 months prior to enrollment (stratum II only)

3.2.5.5 Corticosteroids: Patients who are receiving dexamethasone or other corticosteroids must be on a stable or decreasing dose for at least 1 week prior to enrollment. It is recommended that patients be off all steroid therapy or receive the least dose that will control their neurologic symptoms

3.2.5.6 Growth factors: All colony forming growth factor(s) have been discontinued for at least one week prior to enrollment (filgrastim, sargramostim, and erythropoietin). For patients on long acting growth factors, the interval should be two weeks.

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 that are stable for a minimum of one week prior to enrollment.

3.2.8 Patients must be able to swallow capsules.

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

3.2.10 Organ Function
Patients must have normal organ and marrow function as defined below:

- Absolute neutrophil count ≥1,000/mm^3
- Platelets ≥100,000/ mm^3 transfusion independent (no platelet transfusion one week prior to enrollment)
- Hemoglobin ≥ 8g/dl
- Total bilirubin ≤ 1.5 times upper limit of institutional normal (ULN) for age
- AST (SGOT)/ALT(SGPT) ≤ 3 x institutional upper limit of normal for age
- Serum albumin ≥ 3g/dL
- Creatinine clearance or radioisotope GFR ≥ 70 mL/min/1.73 m^2 or a serum creatinine based on age/gender as follows:

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.		

3.2.11 Pregnancy Status
Female patients of childbearing potential must have a negative serum pregnancy test at the time of enrollment.

3.2.12 Pregnancy Prevention
Patients of childbearing or child fathering potential must agree to use adequate contraceptive methods while being treated on this study and for 97 days after completing therapy.

3.2.13 Informed Consent

Patient and/or guardian have the ability to understand and the willingness to sign a written informed consent document according to institutional guidelines.

3.3 Exclusion Criteria

3.3.1 Patients with any clinical significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction) that is likely to interfere with the study procedures or results

3.3.2 Patients with low grade gliomas and Rb1 negative tumors

3.3.3 Patients with QTc interval of > 450 msec or those on medications known to prolong QTc interval (see Appendix B)

3.3.4 Prior treatment on a CDK inhibitor (e.g. Flavopiridol)

3.3.5 Patients who are receiving drugs that are strong inducers or inhibitors of CYP3A4 (see

Appendix C)

3.3.6 Patients who are receiving any other investigational therapy

3.3.7 Patients who require enzyme inducing anti-convulsants to control seizures

3.3.8 Patients with cataracts on ophthalmologic examination. The ophthalmology report from the institution should clearly specify the presence or absence of cataracts per slit lamp examination.

3.4 Treatment at Primary Institution

All experimental protocol therapy should be dispensed and all on treatment imaging studies should be obtained at the primary 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.

3.5 Criteria to Start Treatment

3.5.1 Subjects must start therapy within seven (7) working days of enrollment.

3.5.2 Laboratory values used to assess eligibility must be no older than seven (7) days prior to the start of therapy. Laboratory tests used to determine eligibility need not be repeated if therapy starts within seven (7) days. If a test that is repeated after enrollment and prior to the start of therapy is outside the limits for eligibility, it must be rechecked 48 hours prior to the start of therapy. If the recheck is still outside the limits for eligibility, the patient may not receive protocol therapy and will be considered off study.

4 REGISTRATION and ENROLLMENT 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 treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/investigator_registration_packet.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).

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
- 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-042 Site Registration:

- IRB approval (local IRB documentation, a CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

Downloading Site Registration Documents:
Site registration forms may be downloaded directly from the CTSU 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, followed by the Home tab
- Scroll down to "printable CTSU Forms"
- Select and download 2 forms: CTSU IRB Transmittal and CTSU IRB Certification

Requirements for PBTC-042 Site Registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet (Optional)

Checking Your Site's Registration Status:
Check the status of your site's registration packets by querying the RSS site registration status page of the members' section of the CTSU website. (Note: 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 at the top of your screen
- 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.

4.3 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.

All site staff will use OPEN to 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 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.

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 ensure 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.

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.

Patients with DIPG, Medulloblastoma, or ATRT

For patients with DIPG, Medulloblastoma, or ATRT 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

All patients with recurrent, progressive or refractory CNS Tumors **except DIPG, medulloblastoma, or ATRT,** must be screened prior to enrollment. Patients must sign the

screening consent to send the pre-trial tumor tissue to **BTRC Tissue Bank** at the University of California, San Francisco for Rb1 testing (see Section 9.1.1 for requirement) and have their Rb1 status confirmed prior to enrolling in the PBTC-042 study. Only patients who are Rb1 positive will be eligible to enroll.

4.3.1 Screening

Pre-enrollment screening will be performed for patients who are consented for screening and willing to provide pre-trial tumor material for Rb1 testing. Sites are required to make a reservation using the CTSU OPEN Slot Reservation System before they ship the samples to UCSF 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 Enrollment tracking number which will be used for labeling the slides. OPEN enrollment will not proceed from the Prerequisite screen until the UCSF BTRC lab receives the tumor material, analyzes the tissue and captures the Rb1 result into the PBTC ProtoLab database.

Completing the Prerequisite screen sends an automatic email to the UCSF BTRC lab and site registrar with information on the screening process. Once the Rb1 result is entered in ProtoLab by UCSF BTRC 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 completes enrollment of a patient for treatment who passes the screening, or removes the reservation of the patient who fails 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-042 study must be enrolled via OPEN within 10 days of the site notification of a patient's Rb1 status.

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 will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6.2. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's tumor. Treatment must be initiated within seven (7) days of enrollment.

5.1 Agent Administration

PD-0332991 (IBRANCE) is currently available as 75, 100, and 125 mg capsules (No liquid formulation is currently available). PD-0332991 (IBRANCE) will be given oral daily for 21 days followed by a week rest (one course = 28 days) for 24 months (26 courses). The starting dose will be 50 mg/m^2/day for stratum I. The starting dose for stratum II will be one dose level below the MTD for stratum I (50 mg/m^2/day).

Dosing is 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 capsule sizes. Dosing Tables are available in Appendix D. Patients will be provided with a Medication Diary for PD-0332991 (IBRANCE), instructed in its use, and asked to bring the diary as well as the remaining capsule bottles with them to each appointment. The Patient Diary is available on the PBTC-042 webpage.

PD-0332991 (IBRANCE) should be taken with food. Patients are encouraged to take their dose at approximately the same time each day. Capsules should be swallowed whole (do not chew, crush or open them prior to swallowing). Capsules should not be ingested if they are broken, cracked, or otherwise not intact. Patients should be advised to drink plenty of water or take hydration fluids to avoid dehydration if diarrhea occurs. Patients who vomit a dose of PD-0332991 (IBRANCE) can be re-dosed if capsule is intact in the vomitus, and appropriate anti-emetic therapy should be implemented prior to the next scheduled dose (see Appendix B for contraindicated anti-emetic medications).

5.1.1 Dose-finding Period

The dose finding period begins with the initial dose of PD-0332991 (IBRANCE) 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.
Dose escalations will be based on toxicities observed during the dose finding period.

5.1.2 Dose Escalation Schedule

The pediatric equivalent of the adult MTD for the once daily administration of PD-0332991 (IBRANCE) based on a 3-week on/1 week off schedule is approximately 75mg/m^2. PD-0332991 (IBRANCE) dosing will begin at 50 mg/m^2. The PD-0332991 (IBRANCE) dose will be increased in subsequent cohorts until the MTD is reached (
Table 7) or the highest proposed dose is determined to be safe.

The
Table 7 below lists the proposed dose levels to be studied along with the BSA restrictions for each dose level for both Strata I and II. At the time of submission of version 8.0, a total of 21 patients had been enrolled in stratum I. No DLTs were observed in 3 patients enrolled in dose

level 1 and 6 patients enrolled in dose level 2. At dose level 3, a total of 6 patients were enrolled and 2 were inevaluable for toxicity assessment. Two of 4 evaluable patients at this dose level experienced DLTs (grade 4 neutropenia); hence the MTD was exceeded at dose level 3. The MTD for stratum I was determined as dose level 2 (75 mg/m^2/day) and this stratum has been closed to accrual.

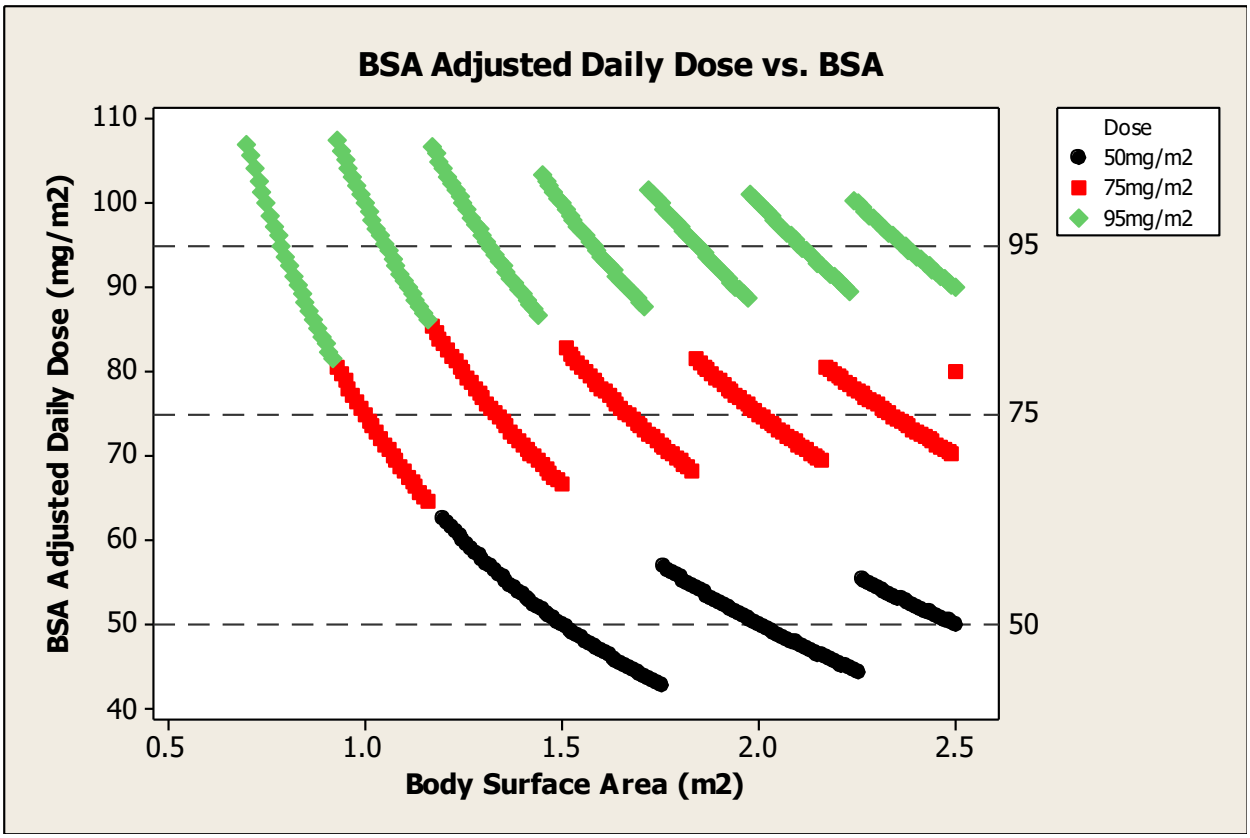
The starting dose level for stratum II is one dose level below the stratum I MTD (i.e. 50 mg/m^2/day). If this dose is well tolerated in the initial cohort of patients enrolled on Stratum II, the dose will be escalated to 75 mg/m^2/day for the next cohort of patients. No further escalation will be allowed in this stratum.

The plot below the table demonstrates that dosing using the available capsule sizes is feasible for the proposed dose levels using the BSA restrictions outlined in the table below. It is not however possible to include a level below 50mg/m^2/day due to the capsule size limitations. No intra-patient dose escalation will be permitted on the protocol. Only DLTs observed during the dose-finding period will be used to guide dose escalation. Dose escalation will be governed by the Rolling-6 statistical design separately in each of the two strata, as described in section 13.2 of the protocol.

Table 7
Stratum I and II: PD-0332991 (IBRANCE) Dosing Regimen and BSA Restrictions

Dose Level	Dose (mg/m^2)	BSA (m^2)
1*	50	≥ 1.20
2**	75	≥ 0.93
3	95	≥ 0.70

* Starting dose for Stratum II
** MTD for stratum I



5.2 Dose Limiting Toxicity

Toxicities will be graded according to CTCAE version 4.0 of the NCI Common Terminology Criteria for Adverse Events CTCAE version 4.0 is identified and located on the CTEP website at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

All appropriate treatment areas should have access to a copy of CTCAE version 4.0. 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.

Patients who come off therapy for reasons other than toxicity before completing the dose-finding period may be replaced for purposes of estimating the MTD, as discussed in section 13.1.

5.2.1 The following are Dose-Limiting Toxicities (DLT's)

- A PD-0332991(IBRANCE) related adverse event will be considered dose-limiting if it leads to a dose reduction or permanent cessation of treatment during the first course of therapy. All dose modifications in all courses are to follow the guidelines provided in section 6.2.

- Inability to receive the next cycle of PD-0332991(IBRANCE) within two weeks of the last dose because of lack of hematologic recovery or persisting non-hematologic toxicity.

5.2.1.1 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 of < 5 days
  - Grade 3 diarrhea and/or electrolyte disturbances which have not been maximally treated
  - Grade 3 AST/ALT elevation that returns to levels meeting eligibility criteria within 7 days of study drug interruption and does not recur upon restarting drug.
- Any Grade 2 non-hematologic toxicity that persists for > 7 days and is considered medically significant or sufficiently intolerable by patients requires treatment interruption.

5.2.1.2 Hematologic dose limiting toxicity is defined as:

- Grade 3 neutropenia with fever and sepsis
- Any Grade 4 hematologic toxicity with the exception of lymphopenia
- Grade 3 thrombocytopenia and/or requiring a platelet transfusion on 2 separate days within a 7-day period

5.3 Criteria for starting subsequent courses

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 3.2.10. The labs can be performed within 48 hours prior to the start of each course. If a patient does not meet these parameters at the end of the treatment course (at day 28), then PD-0332991 (IBRANCE) can be held for up to 7 days until parameters meet the eligibility criteria. 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.

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

Only non-enzyme inducing anticonvulsants drugs should be used, if indicated. Use of such anticonvulsants should be recorded in the Rave database.

5.4.3 Growth Factors

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

5.4.4 Anti-emetics

The use of anti-emetics will be at the investigator's discretion (see Appendix B for contraindicated medications). Use of anti-emetics should be recorded in the Rave database.

5.4.5 Proton Pump Inhibitors

The use of proton pump inhibitors (PPI) (e.g. rabeprazole, omeprazole, pantoprazole, lansoprazole or esomeprazole) is not permitted due to a significant decreased exposure to PD-0332991 (IBRANCE) when concurrently given. If a patient is already receiving a PPI, treatment should be discontinued and a wash-out period of at least 24 hours should have elapsed before beginning therapy with PD-0332991 (IBRANCE). However, alternative antacid therapies may be used including H2-receptor antagonists, and locally acting antacids. H2-receptor antagonists should be administered with twice daily dosing regimen. PD-0332991 (IBRANCE) should be given at least 10 hours after H2-receptor antagonist evening dose and 2 hours before the H2-receptor antagonist morning dose. Local antacid should be given at least 2 hours before or after PD-0332991 (IBRANCE) administration.

5.4.6 Febrile neutropenia

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

5.4.7 Pneumocystis jiroveci pneumonia (PJP) prophylaxis

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

5.4.8 Neurosurgical Procedures

If a neurosurgical 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". Due to the possible effect of myelosuppression on wound healing, PD-0332991 (IBRANCE) should be held for at least 7 days or until the patient has clinically recovered from the effects of surgery and has demonstrable wound healing before resuming treatment. Treatment can be held for up to 14 days.

5.4.9 Concomitant Therapy

Other anti-cancer or experimental agents are not permitted. Drugs that are known to interact with the investigational agent are not allowed during the trial and are listed in Appendix B,

Appendix C and Section 8.1.1.11.

5.5 Duration of Therapy

In the absence of treatment delays due to adverse event(s) or disease progression, treatment may continue for 26 courses (approximately two years) or until one of the Off Treatment Criteria applies as noted in 5.5.2.

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-042 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-042 Protocol Webpage.

5.5.2 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 5.2.1. See section 7 for specific reporting requirements.
- Progressive disease (PD) as described in section 11.1.4.
- 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.
- Interruption greater than 14 days of continued drug administration for reasons unrelated to toxicity
- The patient, parent or legal guardian refuses further treatment on this protocol.
- Completion of all protocol defined treatment
- Pregnancy
- Non Compliance that in the opinion of the investigator does not allow for ongoing participation.
- Termination of the study by the sponsor
- Investigational Agent/Device manufacturer can no longer provide the study agent.

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

5.5.3 Data Submission Schedule for Patients Off Treatment

Subjects will be monitored for the resolution (or return to baseline) of all toxicities considered at least possible related to PD-0332991 (IBRANCE) occurring while on treatment and/or within30 days after 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.

5.5.4 Criteria for Removal from Study (Off Study Criteria)

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, 12).

- Patient determined to be ineligible.
- Parent, patient, or guardian withdraws consent for continued participation.
- Patient death while on study. The IRB, Investigational Drug Branch (IDB), Study Chair and OBDMC must be notified as per section 7.
- Initiation of another anti-cancer therapy
- Patient 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.

5.5.5 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 the study drug. (see section 7).

6 DOSING DELAYS/ DOSE MODIFICATION

6.1 Notification of 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

Table 8

Event	AE Grade or Observation	Dose Modification
Diarrhea or other non-hematologic toxicity	Grade 1 or 2	Maintain dose; continue anti-diarrheal treatment.
	Grade 3 (non-dose limiting)	Hold PD-0332991 (IBRANCE) and maximally treat. If toxicity resolves to meet on study parameters within 14 days of drug discontinuation or (as outlined in Section 5.2.1.1), restart treatment at the same dose.
	Grade 3 or 4 (dose limiting)	Hold PD-0332991 (IBRANCE) and maximally treat. If toxicity resolves to meet on study parameters with 14 days of drug discontinuation, restart treatment at next lower dose level if patient at dose level 2 or 3 and meets BSA requirements for the next lower dose level. If patient at dose level 1 or with BSAs smaller than what is required should come off treatment permanently. If toxicity does not resolve to meet study eligibility parameters within 14 days of drug discontinuation, the patient must be removed from protocol therapy. If dose limiting toxicity recurs in a patient who has resumed treatment at the reduced dose level, the patient must be removed from protocol therapy.
Hematologic	Grade 1 or 2	Maintain dose.
	Grade 3 (non-dose limiting)	Maintain dose.
	Grade 3 or 4 (dose limiting)	Hold PD-0332991 (IBRANCE). Counts should be checked at least twice weekly (3-4 days apart) during this time. If toxicity resolves to meet on study parameters with 14 days of drug discontinuation, restart treatment at next lower dose level, if patient is at dose level 2 or 3 and meets BSA requirements for the next lower dose level. If patient at dose level 1 or with BSAs smaller than what is required should come off treatment permanently. If toxicity does not resolve to meet study eligibility parameters within 14 days of drug discontinuation, the patient must be removed from protocol therapy. If dose limiting toxicity recurs in a patient who has resumed treatment at the reduced dose level, the patient must be removed from protocol therapy.

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 the characteristics of an observed AE (Sections 7.2 and 7.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting".

- 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 Grades 1 and 2 adverse events if the attribution is at least possibly related to PD-0332991. Record all adverse events Grades 3 through 4 and deaths), regardless of attribution on the electronic case report forms.

7.1 Comprehensive Adverse Events and Potential Risks list (CAEPR)

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
Palbociclib (PD-0332991, NSC 772256)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1751 patients.* Below is the CAEPR for Palbociclib (PD-0332991).

Version 2.3, January 25, 2018[1]

Adverse Events with Possible Relationship to Palbociclib (PD-0332991) (CTCAE 5.0 Term) [n= 1751]		
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
Anemia		
		Febrile neutropenia
EYE DISORDERS		
	Blurred vision	
	Dry eye	
	Watering eyes	
GASTROINTESTINAL DISORDERS		
	Constipation	
	Diarrhea	
	Mucositis oral	
Nausea		
	Vomiting	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
Fatigue		
	Fever	
INFECTIONS AND INFESTATIONS		
Infection[2]		
INVESTIGATIONS		
	Alanine aminotransferase increased	
	Aspartate aminotransferase increased	
	Lymphocyte count decreased	
Neutrophil count decreased		
	Platelet count decreased	
White blood cell decreased		
METABOLISM AND NUTRITION DISORDERS		
	Anorexia	
NERVOUS SYSTEM DISORDERS		
	Dysgeusia	
	Headache[3]	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		

Adverse Events with Possible Relationship to Palbociclib (PD-0332991) (CTCAE 5.0 Term) [n= 1751]		
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**
	Epistaxis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
	Alopecia	
	Dry skin	
	Skin and subcutaneous tissue disorders - Other (rash)[4]	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Infection includes all 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

[3]Headache has been observed in trials using Palbociclib (PD-0332991) in combination with fulvestrant.

[4]Rash includes rash, rash maculo-papular, erythema, erythematous rash, erysipelas, rash pruritic, rash papular, generalized rash, exanthema, allergic dermatitis, dermatitis acneiform, and dermatitis.

[5]Peripheral neuropathy includes both peripheral motor neuropathy and peripheral sensory neuropathy under the NERVOUS SYSTEM DISORDERS SOC.

Adverse events reported on palbociclib (PD-0332991) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that palbociclib (PD-0332991) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Bone marrow hypocellular; Blood and lymphatic system disorders - Other (pancytopenia)
CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Cardiac disorders - Other (paroxysmal atrial fibrillation with rapid ventricular response); Palpitations; Pericarditis; Sinus bradycardia; Supraventricular tachycardia
EYE DISORDERS - Cataract; Eye disorders - Other (retinal hemorrhage)
GASTROINTESTINAL DISORDERS - Abdominal distension; Abdominal pain; Ascites; Colitis; Colonic perforation; Dry mouth; Dyspepsia; Dysphagia; Esophageal stenosis; Flatulence; Gastric hemorrhage; Gastrointestinal disorders - Other (gastrointestinal hemorrhage); Intra-abdominal hemorrhage; Lower gastrointestinal hemorrhage; Small intestinal obstruction; Small intestinal perforation; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; Edema limbs; Localized edema; Malaise; Non-cardiac chest pain; Pain; Sudden death NOS
HEPATOBILIARY DISORDERS - Hepatic failure; Hepatobiliary disorders - Other (bile duct obstruction); Hepatobiliary disorders - Other (jaundice)
IMMUNE SYSTEM DISORDERS - Allergic reaction
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall; Fracture
INVESTIGATIONS - Alkaline phosphatase increased; Blood bilirubin increased; CPK increased; Creatinine increased; Ejection fraction decreased; GGT increased; INR increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hypermagnesemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypokalemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (failure to thrive)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Flank pain;

Generalized muscle weakness; Muscle cramp; Musculoskeletal and connective tissue disorder - Other (osteomyelitis); Myalgia; Neck pain; Osteonecrosis; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL. CYSTS AND POLYPS) - Treatment related secondary malignancy
NERVOUS SYSTEM DISORDERS - Dizziness; Dysesthesia; Dysphasia; Intracranial hemorrhage; Nervous system disorders - Other (peripheral neuropathy)[5]; Syncope
PSYCHIATRIC DISORDERS - Confusion; Insomnia; Psychiatric disorders - Other (altered mental status)
RENAL AND URINARY DISORDERS - Acute kidney injury; Hematuria
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea; Hypoxia; Oropharyngeal pain; Pleural effusion; Pneumonitis; Postnasal drip; Pulmonary edema; Pulmonary hypertension
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis; Pruritus
VASCULAR DISORDERS - Hypertension; Hypotension

Note: Palbociclib (PD-0332991) 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 Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting in the RAVE database. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized until March 31, 2018 for expedited AE reporting. CTCAE version 5.0 will be utilized for expedited AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE versions 4.0 and 5.0. A copy of the CTCAE versions 4.0 and 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **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.

7.3 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 (http://ctep.cancer.gov). 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). These requirements are briefly outlined in the tables below (Section 7.3.2).

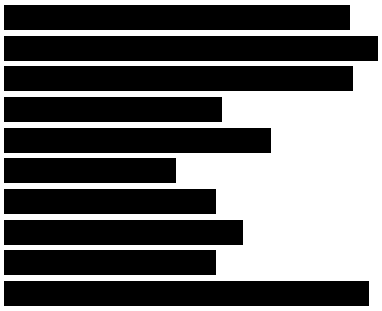
In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made by telephone [redacted] to the PBTC Operations Office for instances when the Internet fails. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.1 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients. For this protocol the following groups should be notified:

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

Study Chair & IND holder:
David Van Mater, MD, PhD



PBTC OBDMC:
Emily Carps, MBA, CCRA

The site principal investigator should report SAEs as soon as they are determined to meet the definition, even if complete information is not yet available. The institution will assist Pfizer in investigating any SAE and will provide any follow-up information requested by Pfizer.
The IND holder will fax the SAE / CTEP-AERS report, within 24 hours of their awareness of the event to Pfizer. (Fax 1-866-997-8322). The *Reportable Event Fax Cover Sheet* provided by Pfizer must also be included with each SAE submitted.

The IND holder, Duke University Medical Center 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 must also be submitted, as requested.

- If a suspected adverse reaction is determined by the Sponsor to be unexpected and is either fatal or considered life threatening, the FDA must be notified as soon as possible but no later than 7 calendar days after the initial report was received by the sponsor. A further 8 days is allowed for any additional information to be sent.
- Serious, suspected adverse reactions that are both unexpected and associated with the use of the investigational product, but are not fatal or life-threatening, will be reported to the FDA no later than 15 calendar days after the initial report was received by the Sponsor.
- All other serious adverse events will be reported at the time of submission of the annual report.

The PBTC OBDMC will post all IND Safety Letters on the PBTC-042 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, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local

regulators by the sponsor-investigator. This submission will be cross-referenced according to local regulations to the Pfizer product number at the time of submission. Additionally, a copy of these reports will be submitted to Pfizer [redacted] at the time of submission to the appropriate regulatory agency.

7.3.2 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 treatment 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 CTEP-AERS 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	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in protocol section 7.3.3.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS 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 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

7.3.3 Expedited Reporting Exceptions

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 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine (CTMS or CDUS) study data submissions. **AEs reported through** CTEP-AERS **must also be reported in routine study data submissions.**

7.5 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 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.

7.6 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 reporting via CDUS unless otherwise specified.

8 AGENT INFORMATION

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

8.1 PD-0332991 (palbociclib; IBRANCE®)

8.1.1 Agent Information

8.1.1.1 Chemical Name:
6-acetyl-8-cyclopentyl-5-methyl-2-((5-(piperazin-1-yl) pyridin-2-yl) amino)pyrido[2,3-d]pyrimidin-7(8H)-one

8.1.1.2 Other Name: palbociclib, IBRANCE®

8.1.1.3 Classification: A Cyclin-Dependent Kinases Inhibitor

8.1.1.4 CAS Registry Number: 571190-30-2

8.1.1.5 Molecular Formula:
Freebase (PD 0332991-00) - $C_{24}H_{29}N_7O_2$

8.1.1.6 Molecular Weight:
Freebase (PD 0332991-00) - 447.54 Daltons

8.1.1.7 Mechanism of Action:
Oral PD-0332991 (IBRANCE) is a highly selective, reversible inhibitor of cyclin-dependent kinases 4 and 6. Inhibition of CDK 4/6 blocks DNA synthesis by prohibiting progression of the cell cycle from G1 to S phase.

8.1.1.8 Formulation:
PD-0332991 (IBRANCE) is an immediate release capsule. PD-0332991 (IBRANCE) is currently available as 75, 100, and 125 mg hard gelatin capsules (No liquid formulation is currently available). Dispense in the original container--do not repackage capsules.

8.1.1.9 Availability and Storage:
The study drug should be stored in a safe and securely locked area. Store at 20°C to 25°C (68°F to 77°F); brief excursions are permitted between 15° – 30° C (59°F to 86°F). It will be shipped to Investigator sites upon request.

8.1.1.10 Stability:
Stability is about 18 months and will increase with time as stability studies continue to accrue data.

8.1.1.11 Route of Administration:
PD-0332991 (IBRANCE) is an oral capsule. PD-0332991 (IBRANCE) should be taken with food. Capsules should be swallowed whole. Do not chew; crush or open capsules prior to swallowing. Do not take any capsule that is broken, cracked or otherwise not intact.

Potential Drug Interactions:
PD-0332991 is metabolized in vitro primarily via CYP3A4. Concomitant administration of agents known to inhibit CYP3A isoenzymes (e.g, ketoconazole, miconazole, itraconazole, posaconazole, clarithromycin, erythromycin, tilithromycin, nefazodone, diltiazem, verapamil, indinavir, saquinavir, ritonavir, nelfinavir, lopinavir, atazanavir, amprenavir, fosamprenavir, and grapefruit juice) may increase PD-0332991 exposure and thus are not recommended. Concomitant administration of agents that are strong CYP3A inducers (such as phenobarbital, rifampin, phenytoin, carbamazepine, rifabutin, rifapentin, clevidipine, and St. John's Wort) may reduce the exposure of PD-0332991 and thus are also not recommended. Proton pump inhibitors (e.g., rabeprazole, omeprazole, pantoprazole, lansoprazole, or esomeprazole) have also been

shown to produce a significant decrease in PD-0332991 exposure and should be avoided. Based on in vitro studies (human liver microsomes and human hepatocytes), PD-0332991 has a low potential to produce drug interactions through inhibition of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, (IC50 values all >30 μM) and by inducing CYP3A4 and CYP1A2 activity. In vitro, PD-0332991 demonstrated time-dependent inhibition of CYP3A in human liver microsomes.

In vivo, inactivation of this enzyme by PD-0332991 may result in drug interactions with compounds that are predominantly metabolized by CYP3A. Therefore, caution must be exercised in patients receiving PD-0332991 in combination with drugs that are predominantly metabolized by CYP3A. In particular, co-administration of PD-0332991 with CYP3A4 substrates with narrow therapeutic indices (e.g., astemizole, terfenadine, cisapride, pimozide, quinidine, tacrolimus, cyclosporine, sirolimus, alfentanil and fentanyl, excluding transdermal patch) or ergot alkaloids (ergotamine, dihydroergotamine) must be avoided. Similarly, PPIs (including rabeprazole, omeprazole, pantoprazole, lansoprazole or esomeprazole) should be avoided.

The potential for PD-0332991 to inhibit the efflux transporter P-glycoprotein (P-gp, also known as multidrug resistance protein 1 [MDR1 and ABCB1]) was investigated in vitro in a Madin-Darby Canine Kidney (MDCK) II-MDR1 cell line using the known P-gp substrate digoxin. In addition, the potential for PD-0332991 to inhibit the breast cancer resistance protein (BCRP) was evaluated using the probe substrate, prazosin, in MDCKII-BCRP cells. PD-0332991 was a weak inhibitor of both P-gp and BCRP, with an IC50 value of >32 μM. The potential of palbociclib to inhibit P-gp or BCRP in the GI tract was assessed according to the draft FDA guidance (2012) and the EMA guideline (2012). When the intestinal concentration was calculated to be dose/250 mL, the values obtained from assessments exceeded the regulatory threshold of a potential drug - drug interaction (DDI). However, given the solubility of PD-0332991 is 0.019 mg/mL at neutral pH, which is far below 0.5 mg/mL (125 mg/250 mL), the risk for GI interactions with P-gp and BCRP is considered to be low at the clinically relevant doses. At clinically relevant concentrations, no drug interaction was observed with hepatic uptake/efflux (organic anion transporting polypeptide [(OATP) 1B1 and 1B3] and renal transporters [organic anion transporters (OAT1 and 3) and organic cation transporter (OCT-2)].

8.1.2 Agent Supply and Distribution

Pfizer will supply IBRANCE for PBTC-042 study as Pfizer will not be replenishing the pool of PD-0332991. The description of IBRANCE capsule is identical to the capsule of PD-0332991 except for the presence of Pfizer logo on the IBRANCE capsule. Each bottle of IBRANCE contains 21 capsules.

IBRANCE is supplied in the following strengths and package configurations:

IBRANCE Capsules			
Package Configuration	**Capsule Strength (mg)**	**NDC**	**Capsule Description**
Bottles of 21 capsules	125	NDC 0069-0189-21	opaque, hard gelatin capsules, size 0, with caramel cap and body, printed with white ink “Pfizer” on the cap, “PBC 125” on the body

Bottles of 21 capsules	100	NDC 0069-0188-21	opaque, hard gelatin capsules, size 1, with caramel cap and light orange body, printed with white ink "Pfizer" on the cap, "PBC 100" on the body
Bottles of 21 capsules	75	NDC 0069-0187-21	opaque, hard gelatin capsules, size 2, with light orange cap and body, printed with white ink "Pfizer" on the cap, "PBC 75" on the body

8.1.3 Agent Ordering and Agent Accountability

PD-0332991 (IBRANCE) may be requested by the Principal Investigator or designees 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-042 protocol and resources webpage. The form can be emailed or faxed to Pfizer.

The investigator, or a responsible party designated by the investigator, must maintain accurate records regarding study drug receipt, dispensing, use and disposal using the NCI Drug Accountability Record Form (DARF). See the CTEP home page at http://ctep.cancer.gov or the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.

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.

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.

Test Name	Sample Type and Amount	Analyzing Laboratory	Required or Optional	Section ID
Assessment for tumor Rb1 protein	FFPE tumor tissue – 2 unstained slides + 1 H&E stained slide	UCSF BTRC	Required	9.1.1
Laboratory Correlative Studies				
Pharmacogenetic	Whole blood – 5 mL	Stewart Laboratory	Optional	9.2.1
Pharmacokinetics	Plasma – 2 mL per sample	Stewart Laboratory	Required	9.2.2
Pharmacokinetics after discontinuation of dexamethasone	Plasma – 2 mL per sample	Stewart Laboratory	Optional	9.2.2
Assessment of Cyclin D1,Cyclin D2, Cyclin D3, CDK4, CDK6 and Ink4a-ARF loss	FFPE tumor tissue 10 unstained slides or tissue block	Will Parsons, MD Texas Children's Hospital	Optional	9.3.3
Central Neuropathology Review	1 H&E stained slide	PBTC CRB at CHLA	Optional	9.3.2
PBTC CRB Repository Samples	20 unstained slides and blood for future research	PBTC CRB at CHLA	Optional	9.3.1

9.1 Integral Biomarker Studies

9.1.1 Assessment for Tumor Retinoblastoma (Rb1) protein

Formalin fixed paraffin embedded tumor tissue will be collected from all patients prior to enrollment (except for those with diffuse intrinsic pontine gliomas, medulloblastoma, or ATRT who do not require testing). Unstained slides and a corresponding H&E stained slide will be sent to UCSF BTRC for Rb1 immunohistochemistry and interpretation. The turn-around time for this test is approximately 2 weeks. Tumor Rb1 protein status will be denoted as "positive" if ≥20% of tumor cells have positive nuclear staining. Rb1-positive endothelial cells will serve as an internal positive control. All slides will be centrally reviewed by the Neuro-pathologist, Joanna J. Phillips, MD.

9.1.1.1 Assay Method

Immunohistochemistry for Rb1 will be performed as previously described[30] using a mouse monoclonal anti-Rb1 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, pH8.0 at 95C for 1 hour, followed by incubation in 3% H_2O_2 for 16 min, 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 in 20% to 50% of tumor cells; and 2, indicates 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 to perform high complexity testing. The immunohistochemistry assay for Rb1 is not FDA approved.

9.1.1.2 Collection and shipping of Specimen(s)

Two unstained slides from formalin-fixed paraffin-embedded tumor material will be collected along with one H&E stained slide prepared from the same block.
Slides must be shipped from the site to UCSF BTRC within 2 business days of reservation. Slides must be labeled with the PBTC Enrollment tracking number, patient's date of birth, protocol number, and date of collection. Samples should be shipped Monday through Thursday using the PBTC-042 FedEx account. Weekend deliveries are not permitted. The sample collection and shipping dates must be documented in the eCRF by the sites.

Send the following to UCSF BTRC Biomarkers Laboratory:
1-H&E stained slide from best block
2-Unstained slides from best block
Completed Specimen Transmittal Form

All specimens should be shipped at room temperature with a completed Specimen Transmittal Form (found on the PBTC-042 website) to:

Attn: Anny Shai, Ph.D.
[redacted]

9.2 Laboratory Correlative Studies

9.2.1 Pharmacogenetic Studies

Pharmacogenetic studies will be obtained from consenting patients enrolled on this protocol prior to starting treatment. Prior to the first PD-0332991 (IBRANCE) dose, collect 5 mL of whole blood for isolation of peripheral mononuclear cells, which will be used for DNA extraction by standard techniques. Samples may be collected after PD-0332991 (IBRANCE) treatment has begun with the prior approval of Dr. Clinton Stewart or his designee (901-595-2400). Pharmacogenetic samples should be shipped using PBTC-042 PK/PG FedEx account details, as per PBTC member website.

9.2.1.1 Sampling Collection and Processing Instructions

Upon request, each participating institution will receive a Pharmacogenetics (PG) Kit from Dr. Clinton Stewart for the collection of samples from consenting patients. Collect 5 mL of blood in a purple-top tube for PG studies before starting PD-0332991 (IBRANCE) treatment. **The sample should not be batched** and **should be shipped as soon as possible**, **within 48 hours of**

sample collection, to St. Jude Children's Research Hospital with the cold ice pack included in kit. The blood sample should be kept on ice or at 4°C until shipped. Shipments should be made Monday through Thursday; weekend and holiday deliveries should be avoided. Please contact the Stewart Laboratory [redacted] to request a Pharmacogenetics Kit.

DNA extraction by standard techniques will be performed [redacted] Genotyping for pharmacogenetic polymorphisms will be assessed using standard molecular techniques. No patient identifiers will be available to the laboratory personnel conducting the genotyping analyses, as they will be conducted using designated PBTC accession numbers alone.

Patient and institution information should be recorded on the Pharmacogenetics Sample Transmittal Form (can be found on the PBTC – 042 'Protocol and Resources' web page as 'PG sample transmittal form') on the day the sample is collected. The PG Sample Transmittal Form should be sent with the sample to the address below to maintain confidentiality of the pharmacogenetic specimens. Samples should be shipped using PBTC-042 PK/PG FedEx account details, as per PBTC member website **within 48 hours of sample collection**.

Stewart Laboratory
[redacted]

9.2.2 Plasma Pharmacokinetics Studies

Plasma pharmacokinetic studies will be obtained from all patients enrolled on this study on days 1, 2, 3, 21 and 22 of Course 1 to evaluate the pharmacokinetics of PD-0332991 (IBRANCE). ***On Day 2 of Course 1, the dose of PD-0332991 (IBRANCE) will be held to obtain pharmacokinetic studies.*** No other doses should be held for pharmacokinetic purposes. Serial pharmacokinetic studies will be repeated approximately 2 weeks after discontinuation of dexamethasone in patients who were previously receiving this medication.

9.2.2.1 Sampling Strategy

On Day 1 of Course 1, serial blood samples for PD-0332991 (IBRANCE) pharmacokinetic studies will be collected at the following times: pre-dose and at 0.5, 1, 2, 4, 8 (±1), 10 (±0.5) [optional], 24 (±4), and 48 (±4) hours after the oral dose. **In order to obtain the 24 and 48-hour time points, the dose on Day 2 will be held. It is crucial that the 48-hour time point be obtained prior to dose administration on Day 3.**

On Day 21 of Course 1, serial blood samples for PD-0332991 (IBRANCE) pharmacokinetic studies will be collected at the following times: pre-dose and at 1, 2, 4, 8 (±1), 10 (±0.5) [optional], and 24 (±4) hours after the dose (prior to the next administered dose).

For children less than 13 kg, contact Dr. Clinton Stewart or his designee [redacted] for a sampling strategy with reduced blood requirements.

Pharmacokinetic Studies after Discontinuation of Dexamethasone (if consented)
Any patient who received dexamethasone during the mandatory PD-0332991 (IBRANCE) pharmacokinetic studies should have serial blood samples obtained at least 14 days after discontinuation of dexamethasone, and after 14 days of continuous dosing with PD-0332991 (IBRANCE). At a routine clinic visit samples for pharmacokinetic studies should be obtained at the following times: pre-dose and at hours 1, 2, 4, 8 (±1), 10 (±0.5) [optional], and 24 (±4) hours after the dose (prior to the next administered dose).

9.2.2.2 Sampling Collection and Processing instructions

At each time point, 2 mL of blood will be collected into appropriately labeled tubes containing K_2EDTA. Two mL of blood is needed to provide approximately 1 mL plasma for pharmacokinetic analysis. 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 that is available on the PBTC-042 webpage.

During the sample collection and processing steps, the blood and plasma should be kept out of direct sunlight and unfiltered lab light. Upon collection of the blood PK samples in K_2EDTA blood collection tubes, keep the blood samples on wet ice at all times prior to processing to plasma. Samples will be centrifuged at approximately 1700 x g for about 10 minutes at 4°C (Note: If a refrigerated centrifuge is not available, please refer to the Shipping Manual located on the PBTC-042 webpage for further instructions). The plasma will be stored in an appropriately labeled amber screw-capped polypropylene tube at approximately -20°C within 1-hour of collection.

9.2.2.3 Handling of Specimens(s)

The blood to plasma sample processing requires using refrigerated centrifuges. The samples should be stored at -20ºC. The established long-term stability (LTS) of PD-0332991 (IBRANCE) in frozen plasma stored at -20 ºC or -70 ºC is 439 days. Additional testing is to be conducted to extend the LTS.

9.2.2.4 Shipping of Specimen(s)

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 at St. Jude Children's Research Hospital, at [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-042 PK/PG 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 042 webpage in the "Protocol Specific Instructions/forms" section) to:

Stewart Laboratory
[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 Description of PD-0332991 Assay

PD-0332991 PK is measured in K2EDTA plasma samples. The LC-MS/MS-based method identifies both PD-0332991 and its lactam metabolite ((PF-05089326) but will be used for measuring PD-0332991 levels only. The assay is fully validated and meets the criteria for the validation method defined in the FDA and EMA Guidance documents and executed in a Pfizer approved US-based laboratory (not CLIA certified).
The method has been successfully used for the analysis of PD-0332991 PK samples from multiple Pfizer sponsored studies. The assay dynamic range for PD-0332991 is from 1 to 250ng/mL.

NOTE: The validated dilution may change depending upon the concentrations of the study samples outside the curve and are validated during the sample analysis phase if required more than a 10-fold dilution.

9.2.2.6 Description of Pharmacokinetic analysis

The PD-0332991 plasma concentration-time data will be analyzed with both compartmental and non-compartmental approaches. The noncompartmental analysis will provide an estimate of the maximum concentration (C_{max}), minimum concentration (C_{min}), area under the concentration-time curve, and apparent oral clearance.

Pharmacokinetic data will also be analyzed using nonlinear mixed effects modeling (NONMEM v.7). For PD-0332991, an appropriate compartmental pharmacokinetic model will be fit to the data. Estimated pharmacokinetic parameters may include the absorption rate constant (ka), apparent oral clearance (CL/F), apparent volume of distribution (V1/F), peripheral volume of distribution (V2/F), and distributional clearance (Q/F). The typical population value and inter-individual variability of each parameter will be determined using NONMEM. Demographic and clinical chemistry covariates will be examined graphically (via individual Bayesian parameter estimates) and by using generalized additive modeling. Potentially significant covariates will be tested in the NONMEM population model.

9.3 Pathology and Exploratory Correlative Studies

The Pathology Central Review and Biorepository (CRB)'s function is to collect, distribute and store specimens for central pathology review and 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 (9.3.3). If the patient does not consent to long term storage, remaining correlative study samples will be destroyed once the PBTC-042 analysis is complete.**

9.3.1 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) on this protocol that require slides, these submitted slides will be used for the conduct of studies outlined in the corresponding section below (9.3.3). 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

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

Specimen Collection and Processing by Ficoll tube

- Collect 2 – 5 mL 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 dispense into cryovials. Freeze the cryovials within 1 hour of collection and store immediately at -20°C or colder.
- 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. 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 (20° C to 25° 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 stripette, remove as much of the plasma component (upper half of the CPT tube) without disturbing the mononuclear layer if possible and aliquot it into cryovials. Freeze the cryovials within 1 hour of collection and store immediately at -20°C or colder.
- 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 (20° C to 25° C). Use acceleration 9, brake (“fast mode”).
- Remove supernatant, being careful not to aspirate the cellular pellet at the bottom of the tube.

- Add 1mL 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 section 9.3.3, the following specimens may also be submitted to the CRB for distribution and storage.

- Formalin-fixed, paraffin-embedded (FFPE) slides or an FFPE tissue: quantity and schedule of collection as outlined in section 9.3.3.

9.3.1.1 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.

- 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. FFPE tumor material should be shipped at room temperature.

- PBMCs are to be labeled with the PBTC Accession # and the PBTC study for which the sample is provided. Samples should be shipped overnight in a separate box with a 2-day supply of dry ice unless otherwise specified.

9.3.1.2 Shipment of Specimens

Samples collected for the repository should be sent to the PBTC CRB overnight via FedEx Monday through Thursday by completing the Internet form at http://www.fedex.com/us/ and requesting FedEx to e-mail researchsupportbiorepository@chla.usc.edu. FedEx user ID and password for pathology shipping can be found at PBTC-042 protocol webpage. Samples should be shipped in the appropriate environment as described in Section 9.3.1.1. Samples are to be shipped to:

PBTC CRB

[redacted]

9.3.2 Pathology Central Review

Central pathology review is optional for this study. In consenting patients, samples for central pathology review will be completed from the slides submitted to the PBTC CRB.

Pathologist review for this study will include the following elements:

- Examination of H&E stained slides and, if the tumor being reviewed is residual, recurrent or metastatic, examination of H&E slides from the original primary tumor. 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.

9.3.3 Assessment of Cyclin D1, Cyclin D2, Cyclin D3, CDK4, CDK6, and Ink4a-ARF loss

Description of Assay

Array CGH is a superior approach to detect amplifications and deletions of genes such as cyclin D1, cyclin D2, cyclin D3, CDK4, CDK6, and Ink4a-ARF loss. The assay will be array CGH using the Agilent 1M microarray. Minimum required genomic DNA from FFPE tissue is 2,000 ng. Ten unstained FFPE slides would be required (although it depends on the genomic DNA yield per slide). It is of particular interest to look for the following copy number variations: Cyclin D1, Cyclin D2, Cyclin D3, CDK4, CDK6, Ink4a-ARF loss, and possibly others. The median probe spacing is 2.1-kb which allows for the detection of smaller more focal amplifications and deletions.

9.3.3.1 Collection of Specimen(s)

10 unstained slides from formalin fixed, paraffin wax embedded (FFPE) tumor material will be collected at the time of enrollment from all patients who have agreed to participate in this correlative study.

Either 10 unstained 5 um formalin-fixed, paraffin-embedded (FFPE) slides or an FFPE tissue block from tumor material will be collected. Use air-dried non-charged, non-coated slides. Oven drying of slides should be avoided. Samples must be labeled with the PBTC accession number, protocol number and date of collection. Specimens should be shipped at room temperature along with a completed Specimen Transmittal Form found on the PBTC-042 website.

9.3.3.2 Handling of Specimens(s)

FFPE slides or tissue blocks should be stored at room temperature.

9.3.3.3 Shipping of Specimen(s)

The slides should be shipped at room temperature to the PBTC CRB Monday through Thursday via Federal Express Priority Overnight using the FedEx account information and transmittal form, found on the PBTC-042 Protocol and Resources web page. 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. Specimens should be sent to the following address:

PBTC CRB

[redacted]

9.3.3.4 Site(s) Performing Correlative Study

Will Parsons, MD PhD

[redacted]

9.4 Neuroimaging Studies

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

9.4.1 Image Transfer

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.

All patient specific data are stripped from the images and replaced with PBTC Subject Accession number prior to transmitting the images to the NIC. All image data transfer is accomplished using pretty-good-privacy (PGP) 128-bit encryption, which meets industry standard for secure communication.

9.4.2 Discipline Review: Neuro-imaging

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). 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.

NIC review will include assessment of response to therapy (as feasible). All patients with a documented PR/CR at any time point will have central review of the scan showing response, the confirmation scan obtained approximately 8 weeks later, as well as the corresponding baseline scan conducted for confirmation.

10 STUDY CALENDAR

	Pre-therapy	Course 1	Course 2-Course 26	Completion/ Discontinuation Of Treatment
PHYSICAL ASSESSMENTS				
Medical history	**X**	**Weekly**	**X**	**X**
Physical exam /height/weight	**X**	**Weekly**	**X**	**X**
Vital signs	**X**	**Weekly**	**X**	**X**
Performance status	**X**	**Weekly**	**X**	**X**
Neurologic exam	**X**	**Weekly**	**X**	**X**
LABORATORY EVALUATIONS				
CBC - WBC, HgB, Hct, Platelets, ANC, ALC	**X**	**Weekly[A]**	**Weekly[A, B]**	**X**
Serum Chemistry - Sodium, Potassium, Bicarbonate, Chloride, Calcium, BUN, Creatinine, Glucose, Phosphorous, Magnesium, Albumin, Total Protein	**X**	**Weekly**	**X[B]**	**X**
SGPT (ALT), SGOT (AST), Alkaline phosphatase, Total Bilirubin	**X**	**Weekly**	**X[B]**	**X**
Serum pregnancy test (for females of childbearing potential)	**X**	**X[B]**	**X[B]**	
CSF cytology (if clinically indicated)	**X**			
OTHER ASSESSMENT				
12-lead EKG	**X**	**X[C]**	**X[C]**	**X[C]**
Ophthalmology Examination (including slit lamp)	**X**		**X[D]**	
IMAGING ASSESSMENTS				
Brain MRI (standard)	**X**		**X[E]**	**X**
Spine MRI (if clinically indicated)	**X**		**X[F]**	**X**
CORRELATIVE STUDIES				
Screening Pre-trial tumor materials for Rb1 testing (required for patients with all types of CNS tumors except DIPG, medulloblastoma, ATRT)	**X[G]**			
Pharmacogenetic Studies (if consented)	**X[H]**			
Plasma Pharmacokinetic Studies (required)	**X[I]**	**X[I]**		
Plasma PK after discontinuation of dexamethasone (if consented)		**Any time after stopping dexamethasone [I]**		
Pre-trial tumor materials for Genomics (if consented)	**X[J]**			
Tumor Tissue and blood for Biorepository (if consented)	**X[K]**			

	Pre-therapy	Course 1	Course 2-Course 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 has no 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 48 hours of beginning protocol therapy and each course (see 5.3).				
C. 12-lead EKG at baseline, and 3 hours post dose on days 1, 8, and 15 of cycle 1 and on day 1 of 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).				
D. Eye exam is to be done at baseline, and every 3 months (after courses 3, 6, 9, 12, 15, 18, 21, 24). The ophthalmology exam report should clearly specify the presence or absence of cataracts and data must be recorded in Rave database.				
E. Standard imaging is to be done every 8 weeks (after courses 2, 4, 6) and then every 12 weeks thereafter through the 26 courses and at the completion of treatment.				
F. MRI Spine should be done at the same time points as the standard MRI brain, if clinically indicated.				
G. 2 unstained slides from FFPE block and 1 H&E slide required to be submitted for Rb testing (see 9.1.1)				
H. Obtain 5 mL of whole blood prior to 1st dose if consented (See section 9.2.1 for details)				
I. Plasma PK (required) – D1 Course 1: pre-dose, 0.5, 1, 2, 4, 8, 10, 24, 48 hrs after dose D21 Course 1: pre-dose, 1, 2, 4, 8, 10, 24 after dose (See section 9.2.2 for details of Plasma PK) Plasma PK after discontinuation of dexamethasone (if consented) – see section 9.2.2				
J. 10 unstained slides from FFPE block or FFPE tissue block [See section 9.3.3 for details of Pre-trial tumor materials for Genomics (if consented)]				
K. Biorepository samples (if consented) – 20 unstained FFPE slides and blood. Obtain 2-5 mL of whole blood at baseline. If unable to collect at baseline, it can be collected any time during protocol therapy. See section 9.3.1 for details.				

11 MEASUREMENT OF EFFECT

11.1 Tumor Response Criteria

11.1.1 Complete Response (CR)

Complete disappearance on MR of all enhancing 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 for malignant cells, it must be negative.

11.1.2 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. If CSF was positive for malignant cells, it must be negative.

11.1.3 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 PD. CSF can be positive or negative for malignant cells. If this category is to be reported as of possible clinical benefit, Stable Disease status must be maintained for at least 4 courses.

11.1.4 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, 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 lesion (including new appearance of malignant cells in the CSF), OR increasing doses of corticosteroids required to maintain stable neurological status or imaging.

12 DATA REPORTING/ REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.

12.1 Data Reporting

12.1.1 Responsibility for Data Submission

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.

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/).

12.1.2 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 Collaborative Agreements Language

Not applicable.

12.3 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 Evaluability

13.1.1 Evaluability for DLT Assessment

Patients who receive at least one dose of the study drug and are taken off treatment for toxicity during the first course (dose-finding period) are evaluable for estimating the MTD. Similarly patients who have to go off treatment due to inability to de-escalate following toxicity because of BSA restrictions are also evaluable for DLT assessment and for estimating the MTD.

Patients who receive approximately 85% (17 or more doses) 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 will be considered inevaluable for estimating the MTD and replaced.

Patients who receive less than 17 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. However as noted above, patients who go off treatment due to inability to de-escalate following toxicity as a result of BSA restrictions are evaluable.

13.1.2 Evaluability for preliminary evidence of response

All eligible patients who received at least one dose of the study drug will be evaluable for response assessment.

13.2 Study Design/Endpoints

This is a phase I study and we will use the Rolling-6 design for dose finding.
Since myelosuppression is the predominant side effect of this agent, the phase I study will first evaluate the toxicities in children who have not been heavily pre-treated previously. Once MTD has been established in this cohort of patients, consideration will be given to expanding the trial to include those who have received intensive cytotoxic therapy including myeloablative chemotherapy +/- craniospinal irradiation.

As of version 4 and following the declaration of MTD in Stratum I, we will initiate enrollment in Stratum II for heavily pre-treated patients. The initial treatment dose for these patients will be one dose level below the MTD in stratum I and escalation to the MTD of stratum I will be pursued, if the initial dose level is well tolerated.

Rolling-6 Design

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. The same algorithm will be used in both strata I and II but will proceed independently. The maximum dose to be studied in Stratum II will be the MTD for Stratum I.

Skolnik et al recently introduced the Rolling-6 design,[31] 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, 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 PBTC's own simulations indicate that this approach decreases the duration of a Phase I trial compared to the traditional method without increasing the overall incidence of toxicity.

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 a rolling 6 design.

Dose Escalation/De-Escalation Rules for the Rolling-6 Design					
				Decision when Next Patient is Enrolled	
# Pts. Enrolled	**# Pts. with DLTs**	**# Pts. w/o DLT**	**# Pts. 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

Based on the above-outlined escalation rules, if dose level 1 is found to be too toxic in either stratum, then accrual to that stratum will be suspended and the merits of amending or closing the stratum 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 not have been determined 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.

13.3 Sample Size/Accrual Rate

Projected Accrual Rages and Study Duration

Though it is difficult to come up with a precise estimate for recurrent pediatric brain tumor patients, we are estimating that up to 30% of patients screened will be Rb1 negative as measured by IHC and thus will not be eligible for this trial. The maximum sample size for stratum I is 24 eligible and evaluable patients if all 3 dose levels are studied and taking into account the expansion cohort at the MTD (barring excessive toxicities at the expansion cohort). Hence we may need to screen up to 35 patients in order to enroll 24 eligible patients in stratum I.

Based on the available information as of version 4.0, dose level 3 will not be studied in Stratum II and thus with two possible dose levels to be studied, the maximum number of patients to be enrolled on Stratum II is 18 (barring excessive toxicities at the expansion cohort). Though less information is available about the potential patient cohort for stratum II, if we assume that 30% of patients screened will be Rb1 negative as measured by IHC, then we may have to screen 25 patients in order to be able to enroll 18 eligible and evaluable patients.

As of version 8.0, 25 patients had been enrolled on this study; 21 in stratum I and 4 in stratum II.

For stratum I, the MTD has been determined as dose level 2 (75 mg/m^2/day) and this stratum has been closed to accrual. Accrual for stratum II was recently initiated at 50 mg/m^2/day, which is one dose level below the stratum I MTD. Four patients have been enrolled at this dose level to date. The intent is to either determine a new MTD for this stratum which is lower than 75 mg/m^2/day or establish that the stratum I MTD is also safe in this patient cohort. The accrual to the trial is ongoing.

Considering that there are always patients who enroll on Phase I trials but are not evaluable for dose finding we may need to screen as many as 55 patients in total for both strata.

The accrual rate for most of our Phase I trials open to all recurrent patients is typically 2-3 patients/month. Considering that eligibility to this study will be limited to patients with available tissue for IHC analysis and among those only to patients with Rb1 + tumors, accrual rate is estimated to be 1-2 eligible patients per month per stratum. Hence we expect to complete accrual to each stratum within 1-2 years of initiating enrollment.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	5	+	6	=	11
Not Hispanic or Latino	22	+	22	=	44
Ethnic Category: Total of all subjects	27	+	28	=	55
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	3	+	3	=	6
Black or African American	6	+	7	=	13
Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	18	+	18	=	36
Racial Category: Total of all subjects	27	+	28	=	55

13.4 Analysis of the Adverse Event Data:

All patients who receive at least 1 dose of protocol therapy will be included in the safety analyses. Adverse event data will be summarized in stratum specific tables which will incorporate dose, attribution as well as grade information.

13.5 Data analysis plan for the secondary objectives:

Any objective responses (PR+CR) which may be observed in this trial will be described by dose and by histology. We will also report prolonged disease stabilizations in a descriptive fashion.

The biology and PD/PG studies are optional in this trial and thus data will be available only from a subset of patients who are likely to represent a variety of histologies. The data generated as part of these correlative studies will be described and summarized in an exploratory fashion via summary statistics and frequency tables as appropriate.

13.6 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.

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.

Because this is a Phase I study, most patients will be treated at very low doses of the agent and will have various diseases; thus, observations of objective responses are expected to be rare. In this setting, correlations of copy number variations of various cell cycle proteins and clinical outcome will be reported descriptively.

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Appendix A Performance Scales

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

Appendix B Medications that may cause QTc Prolongation

The following table presents a list of drugs that may prolong the QTc. Patients on these medications are excluded from the study.

Compound	Compound Half Life	Possible Washout Period - Hours	Possible Washout Period - Days
Alfuzocin	~10 hours		7
Amantadine	17 +/- 4 hours (10-25)		4
Amiodarone (cordarone)	58 days (15-142) 36 days (active metabolite)		180
Amitriptyline*	> 24 hours, wide interpatient variability		
Arsenic trioxide	Not characterized		
Azithromycin	40 hours		
Bepridil	42 hr (26-64)		10
Chloral hydrate	Readily converted to Trichloroethanol (active metabolite $T_{1/2}$=7-10 hour)	48	
Chloroquine	Prolonged (days to weeks)		
Chlorpromazine	30 +/- 7 hours		7
Cisapride	6 – 12 hour, up to 20 hour	60	
Clarithromycin	• Non linear PK3-4 hr (250mg Q12) 5-7 hr (500mg Q12)	36	
Cloroquine	6 to 60 days; mean 20 days		
Desipramine*	> 24 hours, wide interpatient variability		
Disopyramide	6.7 hr (4-10)	36	
Dofetilide	10 hr	48	
Dolesetron	8.1 hr		
Domperidone	7-8 hr	48	
Doxepin*	> 24 hours, wide interpatient variability		
Droperidol	2.2 hours	10	
Erythromycin	* Each salt form has different Half life*		
Felbamate	20-23 hr		5
Flecainide	20 hr (12-27)		5
Foscarnet	87.5+/-41.8 hours *distribution and release from bone*		20
Fosphenytoin	12-29 hr		6
Gatifloxacin	7-14 hr	48	
Gemifloxacin	7 hours	48	
Grepafloxacin	16 hr		3
Halofantrine	6-10 days (variable among individual)		45
Haloperidol	18 +/-5 hr		5
Ibutilide	6 hours (2-12) * variable among subject*	36	
Imipramine*	> 24 hours, wide interpatient variability		
Indapamide	14 hours (biphasic elimination)		3
Isradipine	8 hours (multiple metabolites)	48	
Levofloxacin	6-8 hours	48	
Levomethadyl	Multiple compartment PK with active metabolite 2.6 day for LAAM, 2 day for nor-LAAM, 4 day for dinor-LAAM		

Compound	**Compound Half Life**	**Possible Washout Period - Hours**	**Possible Washout Period - Days**
Lithium	24 hour (10-50)		7
Mesoridazine	24-48 hours (animal study)		10
Methadone	15-30 hours		7
Moexipril/HCTZ	2-9 hour (include active metabolite) for moexipril; 5.6-14.8 hours for HCTZ	48	
Moxifloxacin	12 +/-1.3 hours		
Naratriptan	6 hours	36	
Nicardipine	~ 2 hour post IV infusion	12	
Nortriptyline*	> 24 hours, wide interpatient variability		
Octreotide	1.7 hours	12	
Ofloxacin	5 to 7.5 hours		2
Ondansetron	4 hours (IV/IM); 3 hours (PO)		1 to 3
Pentamidine	6.4+/-1.3 hours	36	
Pimozide	55 hours		10
Procainamide	3-4 hour for PA and NAPA (active metabolite)	24	
Protiptyline*	> 24 hours, wide interpatient variability		
Quetiapine	6 hours	36	
Quinidine	6-8 hours in adult; 3-4 hours in children	36	
Quinine	4-5 hours		
Risperidone	3-20 hours (extensive to poor metabolizer) 9-hydroxyrisperidone (active metabolite) T ½ =21-30 hours (extensive to poor metabolizer)		4
Salmeterol	5.5 hours (only one datum)	36	
Sotalol	12 hours	72	
Sparfloxacin	20 hours (16-30)		4
Sumatriptan	2.5 hours	12	
Tacrolimus	~34 hours in healthy; ~19 hours in Kidney transplant		7
Tamoxifen	5-7 days (biphasic)		30
Telithromycin	2-3 hr	24	
Thioridazine	20-40 hours (Phenothiazines)		7
Tizanidine	2.5 hours	12	
Vardenifil	4 to 5 hours		
Venlaflaxine	5 +/-2 hours for parent comp. 11+-2 hours for OVD (active metabolite)	60	
Voriconizole	6 hours; dose dependent		
Ziprasidone	7 hr	36	
Zolmitriptan	2.8-3.7 hours (higher in female)	18	

Appendix C Potential Drug Interactions with PD-0332991 (palbociclib; IBRANCE)

This appendix is a partial list of concomitant medications that should be avoided, if possible. Drugs that are known to be moderate to strong inhibitors and/or inducers of CYP3A and PPIs are listed below. Strong CYP3A inducers/inhibitors are prohibited and moderate inducers/inhibitors are not recommended. PPIs are also prohibited.

Inhibitors of CYP3A			
Ketoconazole	Miconazole	Itraconazole	Posaconazole
Clarithromycin	Erythromycin	Tilithromycin	Nefazodone
Diltiazem	Verapamil	Indinavir	Saquinavir
Ritonavir	Nelfinavir	Lopinavir	Atazanavir
Amprenavir	Fosamprenavir	Grapefruit Juice	

Inducers of CYP3A			
Phenytoin	Carbamazepine	Rifabutin	Rifapentin
Clevidipine	Phenobarbital	Rifampin	St. John's Wort

Proton Pump Inhibitors (PPI)			
Rabeprazole	Omeprazole	Pantoprazole	Lansoprazole
Esomeprazole			

Appendix D Dosing Tables for PD-0332991 (palbociclib; IBRANCE)

Dose Level 50 mg/m^2/day	Total Daily Dose (mg)	BSA Range		Capsules Required		
		low	high	75 mg	100 mg	125 mg
	75	1.20	1.75	1	0	0
	100	1.76	2.25	0	1	0
	125	2.26	2.50	0	0	1

Dose Level 75 mg/m^2/day	Total Daily Dose (mg)	BSA Range		Capsules Required		
		low	high	75 mg	100 mg	125 mg
	75	0.93	1.16	1	0	0
	100	1.17	1.50	0	1	0
	125	1.51	1.83	0	0	1
	150	1.84	2.16	2	0	0
	175	2.17	2.49	1	1	0
	200	2.50	2.50	0	2	0

Dose Level 95 mg/m^2/day	Total Daily Dose (mg)	BSA Range		Capsules Required		
		low	high	75 mg	100 mg	125 mg
	75	0.70	0.92	1	0	0
	100	0.93	1.16	0	1	0
	125	1.17	1.44	0	0	1
	150	1.45	1.71	2	0	0
	175	1.72	1.97	1	1	0
	200	1.98	2.23	0	2	0
	225	2.24	2.50	0	1	1

Appendix E Patient's Diary

CTEP-assigned Protocol #_PBTC-042___
Local Protocol # PBTC-042

PATIENT'S MEDICATION DIARY

Today's date _____ **Agent:** PD-0332991 (palbociclib; IBRANCE)

Patient Name_____ (*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment.
2. You will take PD-0332991 (IBRANCE) capsules by mouth with food and use water to swallow the whole capsule (s).
   Dose: Take _____# of capsules _____ daily.
3. Record the date, the number of capsules that you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring this form and your bottles of PD-0332991 (IBRANCE) when you return for each appointment.

Day	Date	Time of dose	# of capsules taken			Comments
			75 mg	100 mg	125 mg	
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						

Patient's signature_____

Physician's Office will complete this section:

1. Date patient started protocol treatment _____
2. Date patient was removed from study _____
3. Patient's planned total daily dose _____
4. Total number of capsules taken this month _____
5. Physician/Nurse/Data Manager's Signature _____

Appendix F Patient Drug Information Handout and Wallet Card

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

[Note to investigators: This appendix consists of an "information sheet" to be handed to the patient at the time of enrollment. Use or modify the text as appropriate for the study agent, so that the patient is aware of the risks and can communicate with their regular prescriber(s) and pharmacist. A convenient wallet-sized information card is also included for the patient to clip out and retain at all times.]

The patient _____ is enrolled on a clinical trial PBTC-042 using **PD-0332991 (IBRANCE)**. *This form is addressed to the patient, but includes important information for others who care for this patient.*

PD-0332991 (IBRANCE) interacts with many drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements such as St. John's wort.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**. These are the things that you and they need to know:

PD-0332991 (IBRANCE) interacts with a certain specific enzyme in your liver.

- The enzyme in question is **CYP3A4/5**. **PD-0332991 (IBRANCE)** is broken down by this enzyme in order to be cleared from your system. The dose of this drug that you are taking assumes that these enzymes are working normally.
- Certain drugs may reduce the activity of **CYP3A4/5**, which can increase the amount of active drug in your system. This increases your chances of experiencing harmful side effects. Other drugs might increase the activity of **CYP3A4/5**, reducing the level of active drug in your system and making it less effective.
- **PD-0332991 (IBRANCE)** must be used very carefully with such drugs; it is therefore vitally important that you provide your study doctor with a complete list of your medications. Before you begin the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "**strong inhibitors or inducers of CYP3A4/5**."
- Once the study begins, you and your healthcare providers must be very careful about adding or removing any drugs in this category. Your prescribers should look at the web site http://medicine.iupui.edu/clinpharm/ddis/main-table/, consult a medical reference, or contact your study doctor to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and

located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.

Be careful:

- If you take acetaminophen regularly: You should not take more than 4 grams a day if you are an adult or 2.4 grams a day if you are older than 65 years of age. Read labels carefully! Acetaminophen is an ingredient in many medicines for pain, flu, and cold.
- If you drink grapefruit juice or eat grapefruit: Avoid these until the study is over.
- If you take herbal medicine regularly: You should not take St. John's wort while you are taking **PD-0332991 (IBRANCE)**.

Other medicines can be a problem with your study drug.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is *Physician's name* and he or she can be contacted at *Physician's telephone number*.

INFORMATION ON POSSIBLE DRUG INTERACTIONS You are enrolled on a clinical trial PBTC-042 using **PD-0332991 (IBRANCE).** **PD-0332991 (IBRANCE)** interacts with drugs that are processed by your liver. Because of this, it is very important to: ➢ Tell your doctors if you stop taking regular medicine or if you start taking a new medicine. ➢ Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) 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.	**PD- 0332991 (IBRANCE)** interacts with a specific liver enzyme called **CYP3A4/5**, and must be used very carefully with other medicines that interact with this enzyme. ➢ Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers or inhibitors of **CYP3A4/5"**. ➢ Before prescribing new medicines, your regular prescribers should go to **http://medicine.iupui.edu/clinpharm/ddis/main-table/** for a list of drugs to avoid, or contact your study doctor. ➢ Your study doctor's name is _____ and can be contacted at _____