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A phase I trial of the CDK 4/6 inhibitor palbociclibin pediatric patients with progressive braintumors: a Pediatric Brain Tumor Consortium study (PBTC-042)

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- OB: conducted pre-clinical work associated with the study, served as study PI during a transition process
- OC: Analyzed the palbociclib concentration-time data as well as PG data. She interpreted the PK and PG data, prepared the associated tables and figures, and wrote the results and discussion regarding PK and PG.
- SL: Co-chair of the trial and also conducted pre-clinical work that served as background of the study
- JJP: Reviewed immunohistochemistry for Rb for eligibility
- JH: Conducted real time data monitoring during the trial andconducted most of the data analysis for the manuscript.
- TL: Provided bioinformatic support for PG analyses.
- TYP: Study radiologist
- SG, PB, GD, GR,MDS and EH: served as participating site PI of the study and enrolled 10% or more of the subjects on the trial.
- CFS: Design, generation,and interpretation of pharmacokinetic and pharmacogeneticdata
- AOT: Statistical design and monitoring of study, operations oversight, data analysis and interpretation, manuscript writing
- MF:Study design and PBTC chair during the first half of the study
- ID: PBTC chair for the latter part of the study

CONFLICT OF INTEREST
I.D. declares advisory/consulting roles for Roche, Apexigen, AstraZeneca, Bristol Myers Squibb/Celgene, and Fennec. A.O-T. reports advisory roles for Roche and Lilly.

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Abstract

Background—Disruption of cell cycle regulators is a potential therapeutic target for brain tumors in children and adolescents. The aim of this study was to determine the maximum tolerated dose (MTD) and describe toxicities related to palbociclib, a selective cyclin dependent kinase 4/6 (CDK4/6) inhibitor in pediatric patients with progressive/refractory brain tumors with intact retinoblastoma protein.

Methods—Palbociclib was administered orally starting at 50mg/m^2 daily for the first 21 days of a 28 day course. Dose escalation was according to the Rolling-6 statistical design in less heavily (Stratum I) and heavily pretreated (Stratum II) patients, and MTD was determined separately for each group. Pharmacokinetic studies were performed during the first course, and pharmacodynamic studies were conducted to evaluate relationships between drug levels and toxicities.

Results—A total of 21 patients were enrolled on Stratum I and 14 patients on Stratum II. The MTD for both strata was 75mg/m^2. Palbociclibabsorption (mean T_{max}between 4.9 and 6.6 h) and elimination (mean half-life between 11.3 and 19.5 h) were assessed.The most common toxicity was myelosuppression. Higher palbociclib exposure was associated with grade 3/4neutropenia and leukopenia. Dose limiting toxicities included grade 4 neutropenia and grade 3 thrombocytopenia and dehydration. No patients had an objective response to palbociclib therapy.

Conclusions—Palbociclibwas safely administered to children and adolescents at a dosage of 75mg/m2 for 21 consecutive days followed by 7 days of rest in both strata. Future studies will establish its optimal utilization in pediatric patients with brain tumors.

Keywords

palbociclib; brain tumor; PBTC-042; pharmacokinetics; pharmacodynamics

INTRODUCTION

Children with relapsed/refractory primary central nervous system (CNS) tumors have a dismal prognosis, and there is a critical need for novel therapeutics.Cyclin-dependent kinases (CDKs) represent an appealing target.CDKs coordinate the transition between different stages of the cell cycle, and they are regulated by cyclins.Recent studies have shown that a significant subset of pediatric CNS tumors harbor mutations in the CDK4/6 signaling axis.[1–3]CDK4 and CDK6 drive the transition from the G1 growth phase to chromosomal replication in the S phase, and they are regulated by cyclins D1, D2, D3, and E.[4]A central node in the G1to S transition is the retinoblastoma (Rb) protein. In an unphosphorylated state, Rbsequesters E2F transcription factors that drive cells through the S phase.Phosphorylation of Rb by CDK4/6 releases the E2F transcription factors, which results in progression of the cell cycle through S phase.[4]This transition isvery carefully regulated to prevent mutated cells from progressing through mitosis in normal cells.However, this process is corrupted in pediatric CNS tumors by a variety of mechanisms including genetic loss of *RB1*, amplification of *CCND1*, and deletion of genes encoding CDK inhibitors such as *CDKN2a*.[5]

Palbociclib(IBRANCE®, Pfizer Inc, Kenilworth, NJ) is anorally bioavailable, highly specific inhibitor of CDK4/6.Palbociclib exhibits pH-dependent solubility and high permeability. Palbociclib is FDA-approved for the treatment of advanced or metastatic breast cancer in combination with either fulvestrant or letrozole.[6,7]Preclinical studies of palbociclib in a variety of CNS tumor cell lines and patient-derived xenograft mouse models demonstrated efficacy.[8–11]Additionally, preclinical studies confirmed that palbociclib could penetrate the blood-tumor barrier and that intact Rb is required for its therapeutic effect.[8]Here we report the results of a phase I trial assessing safety and efficacy of palbociclib in children withbrain tumors (PBTC-042).

PATIENTS AND METHODS

The primary objectives of this Phase I study (PBTC-042, NCT02255461) were to determine the maximum tolerated dose (MTD)/recommended Phase II dose (RP2D), describe toxicities, and characterize palbociclib pharmacokinetics. Secondary objectives were to record preliminary evidence of palbociclibantitumor effect, evaluate CDK4/6, cyclin D1–3, Ink4a-ARF copy-number variations, explore potential relationships between palbociclib pharmacokinetics and pharmacodynamics, and relate pharmacogenetic polymorphisms to palbociclib pharmacokinetics.

Eligibility

Subjects were ≥4 years and ≤21 years of age with progressive or refractory brain tumors (except low grade gliomas)with measurable disease and Lansky or Karnofsky score ≥ 60.All subjects were required to be able to swallow pills, and there were minimum body surface area restrictions at each dosing level based on available capsule strengths.Since Rb is required for palbociclib-mediated suppression of the CDK4/6 signaling pathway, a screening test for the presence or absence of Rb was performed in all types of brain tumors except for diffuse intrinsic pontine glioma (DIPG),[12] medulloblastoma,[13,14] and atypical teratoidrhabdoid tumor (ATRT),[15]all of which have been shown to have *RB1* mutation/loss or at a very low frequency or not at all.Since myelosuppression is the main dose limiting toxicity (DLT) in adults treated withpalbociclib[6,7]and more heavily pretreated patients are likely to experience more severe hematologic toxicity,subjectswere divided into two strata:Stratum I included patients that were not heavily pretreated, while

Stratum II included heavily pretreated patients, defined as havingreceived >4 prior regimens (either chemotherapy orbiologic agents with myelosuppressive effects), and/or craniospinal irradiation (CSI), and/or myeloablativechemotherapy plus bone marrow or peripheral blood stem cell rescue.Subjects not meeting these criteria were eligible for Stratum I.Subjects must have had at least 3 weeks from last myelosuppressive therapy, at least 6 weeks from nitrosourea, ≥7 days from a biologic agent (at least 3 weeks if prolonged half-life), >2 weeks from focal irradiation, and ≥3 months from a bone marrow/stem cell infusion or CSI.Subjects were required to be on a stable dose of corticosteroids and have a stable neurological examination for at least 1 week prior to study enrollment. Subjects had to have adequate bone marrow (absolute neutrophil count ≥1000/μL, platelet count ≥100,000/μL, hemoglobin ≥8.0 g/dL), renal (sex and age-adjusted normal serum creatinine or glomerular filtration rate ≥70 mL/min/1.73 m^2), and liver function (total bilirubin ≤1.5× and alanine aminotransferase ≤3× the institutional upper limit of normal for age and albumin ≥3 g/dL). Subjects were not eligibleif pregnant or lactating, cataracts noted on ophthalmologic examination, QTc>450 msec, or prior treatment with a CDK inhibitor. Subjects of childbearing/fathering potential had to consent to birth control.Informed consent and assent were obtained according to institutional guidelines. Institutional review boards of participating institutions maintained protocol approval throughout the study.

Treatment Regimen, Drug Administration, and Dose Escalation

Palbociclib was supplied by Pfizer as 75, 100, and 125 mg capsules, taken orally once a day for 21 days followed by a 7-dayrest. One course was equivalent to 28 days. Patients were encouraged to take palbociclib with food at the same time each day.The pediatric equivalent dosage of the adult MTD (125 mg daily on this same schedule)[6,7] was approximately 75 mg/m^2.The starting dosagefor Stratum I was 50 mg/m^2 with planned dose escalations to 75 mg/$m^2$and 95 mg/m^2.The planned starting dosage for Stratum II was one dosage level below the MTD for Stratum I.Dosage escalation was governed by the Rolling-6 statistical design separately in each of the two strata.[16]Therapy was allowed to continue for up to two years (26 courses) in the absence of disease progression or unacceptable toxicity.

Definition of MTD and DLT

Toxicities were graded according to version 4.0 of the NCI Common Terminology Criteria for Adverse Events.A non-hematologic DLT was defined as any grade 4 non-hematologic toxicity, any grade 3 non-hematologic toxicity (except for grade 3 nausea and vomiting <5 days, grade 3 diarrhea or electrolyte disturbance that has not been maximally treated, or grade 3 AST/ALT elevation that resolves within 7 days and does not recur), or any grade 2 non-hematologic toxicity that persists for >7 days and is considered medically significant or sufficiently intolerable by patients and requires treatment interruption.A hematologic DLT was defined as grade 3 neutropenia with fever and sepsis, any grade 4 hematologic toxicity with the exception of lymphopenia, grade 3 thrombocytopenia, or requiring a platelet transfusion on 2 separate days in a 7 day span.

MTD was defined based on the Rolling-6 design as the highest dose studied where no more than 1/6 patients experienced a DLT and the next higher dose level was determined to be intolerable. Patients were evaluable for MTD estimation if they received at least one dose of the study drug and were taken off treatment for toxicity during the first course (dose-finding period). In the absence of toxicity, patients needed to receive17 or more doses of prescribed therapy during the dose-finding period to be evaluable for MTD estimation.

Definition of Response

Tumor response was defined as follows:1) Complete response (CR), complete disappearance on MR of all enhancing tumor and mass effect.2) Partial response (PR), ≥50% reduction in tumor size by bi-dimensional measurement, as compared with the baseline measurements.Both CR and PR require a stable or decreasing dose of corticosteroids, accompanied by a stable or improving neurologic examination, and maintained for at least 8weeks.If cerebrospinal fluid (CSF) was positive for malignant cells at presentation, then it must be negative on repeat assessment. 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 progressive disease (PD).CSF can be positive or negative for malignant cells. 4) 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.All eligible patients who received at least one dose of the study drug were evaluable for response assessment.

Pharmacokinetics

Pharmacokinetic studies of palbociclib after an oral dosage were performed on days 1, 2, 3, 21, and 22 of course 1.On day 2 of course 1 the palbociclibdose was held. On day 1 of course 1, palbociclib single dose serial blood samples were drawn pre-dose and 0.5, 1, 2, 4, 8 (±1), 24 (±4), and 48 (±4) hours (immediately prior to the Day 3 dose) after the oral dose. On day 21 of course 1, palbociclib steady state serial blood samples were collected pre-dose and 1, 2, 4, 8 (±1), and 24 (±4) hours (immediately prior to the Day 22dose) after the dose. The blood samples were collected in K_2-EDTA tubes and spun to plasma within one hour of collection and stored at −20°C until analysis. Palbociclib plasma concentrations were measured by a validated liquid chromatography-mass spectrometric assay method with a lower limit of quantitation of 1 ng/ml.

Non-compartmental techniques were used to analyze the concentration-time data for palbociclib. The peak plasma concentration (C_{max}) and time to C_{max} (t_{max}) were determined from the plasma concentration-time profile. The last three measurable concentration-time data points in the serial sampling window were used to define the log-linear terminal slope (β), and the terminal half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2)/\beta$. The area under the plasma concentration versus time curve from time zero to the last measurable sampling time point ($AUC_{0\text{-}Tlast}$) was calculated using the linear-up/log-down trapezoidal ruleand the area under the curve from time zero to time infinity ($AUC_{0-\infty}$) was calculated by extrapolating $AUC_{0\text{-}Tlast}$ from the last measurable time point (C_{last}) using β: ($AUC_{0\text{-}Tlast} + C_{last} / \beta$). The

BSA-normalized apparent oral clearance (CL/F) was calculated as the BSA-normalized dose divided by $AUC_{0-\infty}$.

Exposure-toxicity associations

Associations were explored between palbociclibC_{max} and $AUC_{0\text{-}Tlast}$ after single and repeated doses and hematologic toxicities occurring during course 1 that were at least possibly attributable to the drug. Toxicities included neutropenia, thrombocytopenia, lymphopenia, and leukopenia. Patients were classified into three categories (0/1/2) based on their highest toxicity grade reported for course 1 for each toxicity: 0 = no toxicity reported, 1 = grade 1 or 2, and 2 = grade 3 or 4.Ordinal logistic regression models were built for each hematological toxicity outcome, and explanatory pharmacokinetic variables were transformed by dividing by 100. Statistical significance was defined by a p-value threshold of $P < 0.05$.

Pharmacogenetics

In consenting patients, prior to the first palbociclib dose, whole blood (5 mL) was collected for DNA extraction with a GentraPuregene BloodKit (#158389, Qiagen). DNA was quantified by using Nanodrop 2000 Spectrophotometer(Thermo Fisher Scientific).Genome-wide genotyping was performedin germline DNA with an Illumina Infinium Omni2.5Exome-8 Bead-Chip(Illumina Inc.) Selected genes included *CYP3A4*, *CYP3A5*, and *SULT2A1* involved in palbociclib metabolism, and *ABCB1*, *ABCG2*, *ABCC1*, and *ABCC4* which encode genes for known drug transporters. All available single nucleotide polymorphisms (SNPs) from these genes with a reported minor allelic frequency >5% (https://www.ncbi.nlm.nih.gov/snp/) were extracted.

Associations between palbociclibC_{max} and $AUC_{0\text{-}Tlast}$ after single and repeated doses and genotypes were evaluated using non-parametric Kruskal-Wallis tests, Mann-Whitney U-tests, and linear regression. For SNPs including data from wild-type (0), heterozygous (1), and homozygous mutant (2) patients, the distribution of the pharmacokinetic variables was compared between 0,1, and 2, and between 0+1 *vs* 2, and 0 *vs* 1+2. Statistical significance was defined by a p-value $P < 0.05$. Strong linkage disequilibrium (LD) between SNPs, defined as $r^2 > 0.80$, was verifiedusing the LD matrix tool (https://ldlink.nci.nih.gov/). Based on the population size,associations were tested using a univariate analysis only. All the tests were performed using R® software.

Immunohistochemistry for Rb

Formalin fixed paraffin embedded tumor tissue were collected from all patients prior to enrollment (except for those with DIPG, medulloblastoma, or ATRT). Immunohistochemistry for Rbwas performed as previously described[17] using a mouse monoclonal anti-Rb antibody (G3–245; BD Biosciences, San Jose, CA) and an automated IHC staining process (Benchmark XT; Ventana Medical Systems, Inc, Tucson, AZ). Briefly, antigen retrieval was 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. Tumor Rb1 protein status was denoted as “positive” if ≥20% of tumor cells had positive nuclear staining. Rb1-positive endothelial cells served as an internal positive control. All slides were centrally reviewed by a dedicated neuropathologist (JJP).

RESULTS:

Subject characteristics

In Stratum I, a total of 21 patients were enrolled. A total of 28 patients were pre-screened with 22 Rb-positive, 5 Rb-negative, and 1 with inadequate tissue. Of the 22 patients with Rb-positive tumors, 14 elected to enroll for treatment. The remaining 7 patients had DIPG and did not require Rb testing(Table 1). For Stratum II, 14 patients were enrolled. A total of 15 patients were pre-screened with 12 Rb-positive and 3 Rb-negative. Of the 12 patients with Rb-positive tumors, 9 elected to enroll for treatment. The remaining 5 patients had DIPG or medulloblastoma and did not require Rb testing (Table 1).The most common tumor typewas DIPG for stratum I (7/21 patients) and ependymoma for stratum II (7/14 patients).There was a male predominance in Stratum II (Table 1).

Toxicities

DLTs are summarized in Table 2.Three dosage levels were assessed in Stratum I. At dosage level 1 (50mg/m^2), 0/3 patients experienced a DLT. At dosage level 2 (75mg/m^2), there were no DLTs in the first 3 patients, prompting dose escalation. At dosage level 3 (95mg/m^2), there were 6 patients enrolled, 4 of which were evaluable (1 patient received less than the required amount of study agent due to progressive disease and 1 patient voluntarily withdrew after initiating protocol therapy).Two of the 4 patients developed grade 4 neutropenia. Hence the MTD was exceeded at dosage level 3, and a total of 9 additional patients were enrolled at dosage level 2. An MTD of 75 mg/m^2 was established for Stratum I, with only 2/12 patients experiencing a DLT (Table 2). Of note, the 2 DLTs occurred within the expansion cohort at this dose level (patient #8 and #9).

We proceeded to Stratum II after establishing an MTD for Stratum I. Given concern for myelosuppression in the heavily pretreated patients, enrollment in Stratum II was started at 50mg/m^2 (1 dose level below the MTD of Stratum I). Four patients were enrolled at 50mg/m^2, all were evaluablewith no DLTs. This prompted a dose increase to 75mg/m^{2}where a total of 10 subjects were enrolled and 7 were evaluable for DLT assessment (2 patients progressed before receiving sufficient study drug and 1 patient withdrew prior to treatment initiation). No DLT was observed among the first 6 evaluable subjects. There was no intent of escalating beyond the MTD of Stratum I, so the cohort was expanded with the aim to enroll a total of 12 patients. However,slow accrual prompted closure of the study prior to completing enrollment. One of 7 evaluable patients experienced a DLT, establishing 75mg/m^2 as the MTD for both Stratum I and II.

Adverse events associated with palbociclib in Stratum I and II are outlined in Table 3 and Table 4, respectively. Similar to the side effect profile in adults,[18] the most common adverse events were related to myelosuppression with decrease in white blood cells, neutrophils, lymphocytes, and platelets being the most common.

Treatment response

No patients on either stratum had an objective response to palbociclib. A patient with anaplastic ependymoma in Stratum I was treated on a dose of 75mg/m^2 for 18 courses before coming offtherapy. This patient was a 21 year-old male with neurofibromatosis type I and a multiply recurrent WHO grade III anaplastic ependymoma first diagnosed when he was 14 years-old. Prior treatment included multiple surgeries and 2 rounds of radiation therapy. He had 7 courses of 5-fluorouracil followed by a 1.5 year off-therapy period before starting palbociclib. Subsequent to his progression on palbociclib, he was maintained on additional chemotherapy regimens for 2.5 years before dying of his tumor. A mutation profile of his tumor confirmed a C11orf95-RELA fusion (pathognomonic of ependymoma), alterations in *NF1*, and homozygous deletion of *CDKN2A* and *CDKN2B*. He additionally had multiple segmental chromosomal gains and losses across the genome. Another patient with a GBM treated at 75mg/m^{2}stayed on treatment for 6 courses. In Stratum II, 3 subjects (2 at 50mg/m^2 and 1 at 75mg/m^2) remained on treatment for 4 courses. In Stratum I and II, 16/21 (76%) and 12/13 (92%) of patients, respectively, who initiated protocol therapy experienced disease progression while on treatment.The majority of patients received 2 or fewer cycles of therapy (Table 5)

Pharmacokinetics

Pharmacokinetic data after single dose palbociclib were collected and analyzed for a total of 34 patients (7 at 50 mg/m^2, 21 at 75 mg/m^2, and 6 at 95 mg/m^2). Data after repeated palbociclib doses were available for a total of 27 patients (6 at 50 mg/m^2, 18 at 75 mg/m^2, and 3 at 95 mg/m^2). The palbociclib plasma concentration-time profiles are depicted for each dosing group in Supplementary Figure S1, and the associated pharmacokinetic parameters are reported in Table 6.

Palbociclib was absorbed with a mean T_{max} between 4.9 and 6.6 h post-dose, and exhibited an elimination with a mean half-life between 11.3 and 19.5 h and a mean apparent oral clearance ranging from 26.8 and 52.6 L/h/m^2. Within 50 and 95 mg/m^2, following single and repeated doses, mean palbociclibC_{max} and $AUC_{0\text{-}Tlast}$ increased in proportional manner; however, large inter-individual variability was observed among the pharmacokinetic parameters.

Exposure-toxicity associations

Associations were found between palbociclib steady state pharmacokinetic variables and neutropenia. Higher values of palbociclib steady state C_{max} and $AUC_{0–24h}$were associated with severe (grade 3 or 4) neutropenia (p-values = 0.014 and 0.009, respectively). The odds ratio estimates (95% Wald confidence) were 7.49 (1.50–37.46) and 1.16 (1.04–1.30), respectively. In addition, associations were found between palbociclib pharmacokinetic variables and leukopenia. Higher values of single-dose palbociclib $AUC_{0–48h}$, and steady state C_{max} and $AUC_{0–24h}$were associated with severe leukopenia (p-values = 0.038, 0.044 and 0.036, respectively). The odds ratio estimates were 1.10 (1.01–1.20), 3.95 (1.04–15.0) and 1.10 (1.01–1.19), respectively. No association was found between palbociclib pharmacokinetic variables and thrombocytopenia orlymphopenia.

Pharmacogenetics

DNA was collected from 32 patients and a total of 795 SNPs (8 for *CYP3A4*, 7 for *CYP3A5*, 16 for *SULT2A1*, 110 for *ABCB1*, 85 for *ABCG2*, 179 for *ABCC1* and 390 for *ABCC4*) were extracted from the array. Significant associations between genotypes and palbociclibC_{max} and $AUC_{0\text{-}Tlast}$ following single (N=32) and repeated (N=24) doses are reported in Supplementary Tables S1–4 and Figures S2–5. The frequency of significant SNPs is reported in Supplementary Table S5. Multiples associations were found between palbociclib pharmacokinetic variables and SNPs from *SULT2A1*, *ABCB1*, *ABCG2*, *ABCC1*, and *ABCC4*; however, no associations were found with SNPs from *CYP3A4* and *CYP3A5* genes.

DISCUSSION

There is a critical need for novel agents to treat refractory/progressive brain tumors in children and adolescents. Palbociclib represents an intriguing candidate as it is a highly specific CDK4/6 inhibitor that isalready FDA approved for adult breast cancer patients. This was the first study to test the safety and tolerability of palbociclib in pediatric patients.Palbociclib has been associated with significant cytopenias in adults, so there was considerable concern that myelosuppression could impact tolerability inheavily pretreated patients with brain cancer. The study was therefore conducted in both less heavily pretreated and more heavily pretreated patient groups.The MTD was 75mg/m^2 for Stratum I and II patients. Myelosuppression was indeed the primary toxicity,among which grade 3/4 neutropenia and leukopenia were found to be significantly associated with higher palbociclib exposure.A similar exposure-toxicity association was also characterized in adult patients.[19]

No previous pharmacokinetic parameters have been reported for palbociclib in children with cancer. In this patient population, palbociclib displayed a similar pharmacokinetic profile as seen in healthy adults and adults with solid tumors.[20,21]PalbociclibT_{max}, C_{max}, and $AUC_{0\text{-}Tlast}$observed in this study were similar to the values previously reported in adults following 100 and 125 mg palbociclib doses.[20]The mean half-life (~15.6h) observed in this study was slightly lower that reported in adults (23–26 h); however this might be explained by the shorter sampling design used in this trial.[20]A population-based pharmacokinetic analysis will be performed to further characterize the disposition of palbociclib in this population, quantify the inter-individual variability, and determine the potential influence of patient covariates. Unfortunately, children <4 years of age were excluded from this study and no pharmacokinetic data is available.

Although palbociclib has been confirmed in vitro and in vivo to be a substrate for *CYP3A* genes,[22–24] no associations were found between palbociclib pharmacokinetics and variants from *CYP3A4* and *CYP3A5* genes in our pediatric population. Multiple associations were found with *SULT2A1* gene which is predominantly involved in palbociclib sulfonation,[23] and with genes coding for known drug transporters. Due to the small sample size, no multivariate analysis was performed, and p-values were not adjusted for multiplicity. Thus, these associations will need to be confirmed by further studies.

Only a few patients proceeded beyond 2 courses of therapy, and no patients had an objective response to palbociclib on this study. A similar lack of efficacy was reported in a recent phase 2 study of adult patients with recurrent, Rb-positive glioblastoma.[25] Notably, a subset of these patients were treated with palbociclib prior to surgery, and the tumor concentration of palbociclibwas greater than the 0.06 μM concentration felt to be biologically effective.[25]Thus palbociclib was able to cross the brain-tumor barrier in selected patients. The authors concluded that palbociclib is not likely to be effective as monotherapy in pretreated patients, but it may have a role earlier in therapy in patients with distinct mutational events or in combination with radiation or other biological agents.[25] There are ongoing clinical trials in adult breast cancer patients with CNS metastases to better elucidate the role of palbociclib and other CDK4/6 inhibitors in that clinical context; data to-date are sparse.[26]

CDK4/6is central to cell cycle control in cells with intact Rb, but enhanced vulnerability to CDK4/6 inhibition may be seen in tumors with genetic mutations that increase signaling though the cyclin D-CDK4/6-Rb pathway. Examples include amplification of *CCND1*, *CCND2*, *CCND3*, *CDK4*, or *CDK6*, or deletion of *CDKN2A*. One might expect enhanced efficacy of palbociclib in a patient population enriched with tumors harboring such mutations, though one must also be cognizant of the opposing possibility that such mutations could confer increased resistance to CDK4/6 inhibition. Indeed, amplification of CDK6 has been shown to be an acquired mechanism of resistance to CDK4/6 inhibitor in a breast cancer cell line.[27]The National Cancer Institute-Children's Oncology Group Pediatric MATCH Screening Trial (NCT NCT03155620) is conducting a study for refractory pediatric tumors in a tissue-agnostic fashion to address the question of whether cancers harboring mutations in cyclin D-CDK4/6-Rb have an enhanced vulnerability to palbociclib. The MTD of palbociclib in this study has informed the dose for the MATCH study and similar studies in the future.

Additionally, there is a growing recognition that CDK4/6 inhibitors such as palbociclib are maximally effective in combination with other agents.[28]Notably, palbociclib was FDA-approved in combination with anti-estrogen agents for the treatment of patients with breast cancer patients.[6,7] Preclinical studies are required to elucidate palbociclib resistance mechanisms that can exploited in individual cancer types. For example, mTOR[29] and c-Met/Trk[30] represent intriguing targets for intervention in glioblastoma.

In summary, we describe the MTD, toxicity, pharmacokinetic and pharmacogenomic data for palbociclib in children and adolescents. There was no notable antitumor activity efficacy seen for progressive/refractory brain tumors in this trial, but the cyclin D-CDK4/6-Rb pathway remains an intriguing target for future investigations. Future studies will strive to identify subsets of patients with enhanced vulnerability to CDK4/6 inhibition and agents that can be combined with palbociclib for synergy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Abbreviations:

ALT	alanine aminotrasnferase
AST	aspartate aminotransferase
ATRT	atypical teratoidrhabdoid tumor
AUC	area under the curve
BSA	body surface area
CDK4/6	cyclin dependent kinase 4/6
C_{max}	peak plasma concentration
CNS	central nervous system
CR	complete response
CSF	cerebrospinal fluid
CSI	craniospinal irradiation
DIPG	diffuse intrinsic pontine glioma
DLT	dose limiting toxicity
LD	linkage disequilibrium
MTD	maximum tolerated dose
PBTC	Pediatric Brain Tumor Consortium
PD	progressive disease
PR	partial response
Rb	Retinoblastoma

RP2D	recommended Phase II dose
SD	stable disease
SNP	single nucleotide polymorphism
T_{max}	time to peak plasma concentration
β	log-linear terminal slope

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TABLE 1.

Patient characteristics.

	Stratum I (N = 21)		*Stratum II (N = 14)*	
AGE (Years)	**At Initial Diagnosis**	**At Study Entry**	**At Initial Diagnosis**	**At Study Entry**
Median	9.1	11.9	7.9	12.8
Minimum	3.5	4.9	0.8	6.3
Maximum	16.4	21.1	15.1	21.6
	Number	*Percentage*	*Number*	*Percentage*
SEX				
Female	9	42.9	3	21.4
Male	12	57.1	11	78.6
ETHNICITY				
Hispanic or Latino	5	23.8	3	21.4
Not Hispanic or Latino	13	61.9	7	50.0
Unknown	3	14.3	4	28.6
RACE				
American Indian or Alaska Native	0	0.0	1	7.1
Asian	3	14.3	0	0.0
Black or African American	2	9.5	2	14.3
Multiracial	1	4.8	0	0.0
White	13	61.9	10	71.4
Unknown	2	9.5	1	7.1
CURRENT DIAGNOSIS				
Anaplastic astrocytoma	3	14.3	0	0.0
CNS primary tumor, NOS	1	4.8	1	7.1
Choroid plexus carcinoma	0	0.0	1	7.1
DIPG*	7	33.3	1	7.1
Ependymoma, NOS	3	14.3	7	50.0
Glioblastoma multiforme	6	28.6	0	0.0
High-grade astrocytoma, NOS	1	4.8	0	0.0
Medulloblastoma*	0	0.0	4	28.6

Race and ethnicity data are included to reflect our interest in recruiting a diverse patient population. Tumor types with an asterisk did not require immunohistochemistry for Rb; all other tumor types were screened and tested positive for Rb expression based on immunohistochemistry. NOS, not otherwise specified.

TABLE 2.

DLT summary.

Dose Level (mg/m2/dose)	Stratum	Number of Enrolled Patients	Number of Evaluable Patients	Number of Patients with DLTs	Description of DLTs
75	I	12	12	2	Grade 3 dehydration (n=1) Grade 4 neutrophil count decreased (n=1)
75	II	10	7	1	Grade 3 platelet count decreased (n=1)
95	I	6	4	2	Grade 4 neutrophil count decreased (n=2)

TABLE3.

Type and grade of adverse events experienced by ≥10% of Stratum I patients. Represents 21 patients and 56 courses.

	Grade				
Adverse Event	**1**	**2**	**3**	**4**	**Overall**
White blood cell decreased	14(9)	37(14)	19(3)		70(17)
Neutrophil count decreased	8(5)	26(7)	27(9)	3(3)	64(15)
Lymphocyte count decreased	22(8)	17(4)	12(7)		51(13)
Platelet count decreased	28(10)				28(10)
Fatigue	7(7)	3(3)			10(9)
Mucositis oral	5(2)	5(2)			10(3)
Anemia	7(5)	2(2)			9(7)
Constipation	8(6)				8(6)
Vomiting	5(4)	1(1)	1(1)		7(5)
Headache	4(3)	3(2)			7(4)
Hypokalemia	5(4)	1(1)			6(5)
Nausea	4(3)	1(1)	1(1)		6(3)
Ataxia	1(1)	3(3)	1(1)		5(5)
Alanine aminotransferase increased	4(4)				4(4)
Diarrhea	4(3)				4(3)
Dry skin	3(3)				3(3)
Gait disturbance		2(2)	1(1)		3(3)
Muscle weakness left-sided			3(3)		3(3)

The first number in each cell represents the number of episodes for each adverse event and the number in parentheses represents the number of patients for whom the adverse event was reported. All adverse events were reported regardless of attribution to palbociclib.

TABLE4.

Type and grade of adverse events experienced by ≥10% of Stratum II patients. Represents 13 patients and 28 courses.

	Grade				
Adverse Event	**1**	**2**	**3**	**4**	**Overall**
Neutrophil count decreased	6(4)	19(10)	13(9)	1(1)	39(11)
White blood cell decreased	13(5)	18(9)	7(6)		38(11)
Platelet count decreased	16(8)	5(3)	2(2)	1(1)	24(8)
Anemia	17(9)	3(3)			20(10)
Lymphocyte count decreased	10(5)	6(3)	3(3)		19(7)
Vomiting	5(3)		1(1)		6(4)
Fatigue	2(2)	2(2)			4(4)
Headache	1(1)	1(1)	2(2)		4(4)
Dizziness	3(2)	1(1)			4(3)
Anorexia	3(3)				3(3)
Hypokalemia	3(3)				3(3)
Seizure			3(3)		3(3)
Alanine aminotransferase increased	3(2)				3(2)
Constipation	1(1)	2(1)			3(2)
Electrocardiogram qt corrected interval prolonged	3(2)				3(2)
Aspartate aminotransferase increased	2(2)				2(2)
Hypercalcemia	2(2)				2(2)
Hyperkalemia	2(2)				2(2)
Mucositis oral	1(1)	1(1)			2(2)
Pain	1(1)		1(1)		2(2)

The first number in each cell represents the number of episodes for each adverse event and the number in parentheses represents the number of patients for whom the adverse event was reported. All adverse events were reported regardless of attribution to palbociclib.

TABLE 5.

Number of patients in each course receiving palbociclib.

Stratum I													
Dose	**Course**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**…**	**17**	**18**
	N	N	N	N	N	N	N	N	N	N	N	N	N
50 mg/m^2/day	3	2	1	1	0	0	0	0	0	0	0	0	0
75 mg/m^2/day	12	8	2	2	2	2	1	1	1	1	1	1	1
95 mg/m^2/day	6	3	0	0	0	0	0	0	0	0	0	0	0
Stratum II													
Dose	**Course**												
	1	**2**	**3**	**4**									
	N	N	N	N									
50 mg/m^2/day	4	3	2	2									
75 mg/m^2/day	9	6	1	1									

TABLE6.

Pharmacokinetic parameters of palbociclib after single dose (Day 1) and repeated oral doses (Day 21)

		Parameters[†]				
Dosage	**Day**	**C_{max} (ng/mL)**	**T_{max} (h)**	**$AUC_{0\text{-}Tlast}$ (h·ng/mL)**	**CL/F ($L/h/m^2$)**	**Half-life (h)**
50 mg/m^2	Day 1 (N= 7)	69.3 ± 43.7 54.9 (35.8–135)	6.6 ± 2.5 8.0 (2.0–8.0)	1327 ± 721 1156 (747–2834)	39.9 ± 15.1 36.3 (16.9–64)	14.4 ± 3.9 13.0 (9.9–22)
	Day 21 (N=6)	90.0 ± 62.7 77.2 (32.3–210)	5.3 ± 2.1 4.0 (4.0–8.0)	1517 ± 975 1227 (559–3301)	26.8 ± 20.0 19.5 (9.7–64)	19.5 ± 7.7 17.4 (10.5–30)
75 mg/m^2	Day 1 (N=21)	92.0 ± 34.9 94.0 (17.9–148)	5.2 ± 2.2 4.0 (2.0–10)	1872 ± 716 1831 (189–2951)	52.6 ± 71.3 35.2 (11.7–355)	16.6 ± 9.5 13.3 (7.1–49)
	Day 21 (N=17)[‡]	139.9 ± 67.1 137 (31.8–286)	4.9 ± 2.5 4.0 (2.0–8.0)	2219 ± 1086 2269 (521–4253)	30.5 ± 23.1 22.1 (12.1–100)	15.8 ± 7.9 14.4 (6.5–42)
95 mg/m^2	Day 1 (N=6)	132.5 ± 33.4 122 (98.1–180)	5.0 ± 2.5 4.0 (2.0–8.0)	2375 ± 820 2407 (1485–3252)	36.3 ± 16.6 33.7 (17.3–63)	15.9 ± 7.5 14.6 (8.4–30)
	Day 21 (N=2)[//]	190 ± 29.1 183 (165–222)	6.0 ± 3.5 8.0 (2.0–8.0)	2294 ± 828 2193 (1520–3168)	28.1 ± 10.3 28.1 (20.8–35)	11.3 ± 2.1 11.3 (9.8–13)_

C_{max}: maximum concentration, T_{max}: time to reach C_{max}, $AUC_{0\text{-}Tlast}$: area under the concentration curve from zero to the last measurable time-point, CL/F: apparent oral clearance.

[†]The parameters are reported as mean ± standard deviation (first row) and median (range) (second row).

[‡]N = 18 patients receiving 75 mg/m^2 palbociclib had samples collected and analyzed for Day 21 pharmacokinetics. However, one patient had 4 concentrations below the limit of quantification. Thus, this patient was excluded from the non-compartmental analysis.

[//]N = 3 patients receiving 95 mg/m^2 palbociclib had samples collected and analyzed for Day 21 pharmacokinetics. However, one patient had only 3 samples collected at times 2, 4, and 8 hours. Thus, this patient was excluded from the non-compartmental analysis.