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Phase I trial of p28 (NSC745104), a non-HDM2-mediated peptide inhibitor of p53 ubiquitination in pediatric patients with recurrent or progressive central nervous system tumors: A Pediatric Brain Tumor Consortium Study

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Background. p53 is a promising target in human cancer. p28 is a cell-penetrating peptide that preferentially enters cancer cells and binds to both wild-type and mutant p53 protein, inhibiting COP1-mediated ubiquitination and proteasomal degradation. This results in increased levels of p53, which induces cell cycle arrest at G_2/M. We conducted a phase I study to determine the maximum-tolerated dose (MTD) and describe the dose-limiting toxicities (DLTs) and pharmacokinetics (PKs) of p28 in children.

Methods. Children aged 3–21 years with recurrent or progressive central nervous system tumors were eligible. Intravenous p28 was administered 3 times weekly for 4 consecutive weeks of a 6-week cycle at 4.16 mg/kg/dose (the adult recommended phase II dose) using a rolling-6 study design. Expression status of p53 was characterized by immunohistochemistry, and serum PK parameters were established on the second dose.

Results. Of the 18 eligible patients enrolled in the study, 12 completed the DLT monitoring period and were evaluable for toxicity. p28 was well-tolerated; 7 participants received $\geq$2 courses, and the most common adverse event attributed to the drug was transient grade 1 infusion-related reaction. PK analysis revealed a profile similar to adults; however, an increased area under the curve was observed in pediatric patients. High p53 expression in tumor cell nuclei was observed in 6 of 12 available tissue samples. There were no objective responses; 2 participants remained stable on the study for >4 cycles.

Conclusions. This phase I study demonstrated that p28 is well-tolerated in children with recurrent CNS malignancies at the adult recommended phase II dose.

Keywords: azurin, central nervous system tumors, p28, pediatric, phase I.

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Survival rates for many types of pediatric central nevous system (CNS) tumors continue to improve. In contrast, patients with recurrent or progressive high-grade tumors generally have a poor prognosis despite current treatment regimens.[1] The lack of long-term response to therapy has prompted detailed analyses of the molecular origins of adult and pediatric CNS tumors.[2–6] Structural alterations in the tumor suppressor protein p53 are of fundamental importance to the pathogenesis and progression of both adult and pediatric CNS tumors.[7,8] p53 is central to the regulation of the cell cycle, DNA repair, development, and programmed cell death (apoptosis) through a myriad of signaling pathways.[9] The *TP53* gene is mutated in ~50% of all human solid tumors. These tumors can express constitutively high levels of mutant p53 due to a lack of feedback control of p53 protein levels.[10–12] In malignant glioma, p53 mediates an initial response to conventional chemotherapy agents, and p53 regulation is also intimately involved in resistance to these agents.[13–15] Overexpression of p53 in malignant gliomas during childhood is strongly associated with an adverse outcome, independent of clinical prognostic factors and histologic findings.[16]

To date, strategies for restoration of p53 functions in tumors have focused on targeting wild-type p53 with the aim of protecting p53 from degradation by a major endogenous regulator, HDM2.[17,18] p28 is a novel anticancer agent derived from azurin, a 128 amino acid cupredoxin, which is secreted by the opportunistic pathogen *Pseudomonas aeruginosa* and contains an amphipathic α-helical motif that is responsible for the preferential penetration of azurin and p28 into human cancer cells.[15,19,20] As such, p28 acts as a cell-penetrating peptide that is processed into the nucleus and blocks the binding of constitutional morphogenic protein 1 (Cop1) to p53. The decrease in Cop1 through autodegradation results in an increase in intracellular levels of wild-type and mutant p53 and induces cell cycle arrest at G2/M.[20–22] p28 is the lead agent in a series of cell-penetrating peptides that enhance the stability of p53 (Fig. 1).

p28 also enters endothelial cells, where it exerts a direct antiangiogenic effect halting tumor neoangiogenesis.[23] p28 exerts this activity through a non-p53-mediated mechanism: a noncompetitive inhibition of the VEGFR2 and FGFR1 kinases, which in turn significantly reduces the phosphorylation of their downstream targets FAK and Akt, inhibiting endothelial cell motility and migration.[23] Even more importantly, p28 transcends the endothelial cell, crossing the blood-brain barrier and saturating the brain parenchyma in a dose-related manner.[24]

In preclinical testing, the antitumor efficacy of p28 was assessed on human breast cancer, prostate cancer, and melanoma cells in vitro and resulted in dose-dependent reduced proteasomal degradation of p53 and induction of G2-M cell cycle arrest.[19,20] Subsequently, a phase I trial in adults with metastatic solid tumors excluding CNS tumors with >10% p53 expression by immunohistochemistry (IHC) did not report any dose-limiting toxicities (DLTs) or significant adverse events in the 15 participants enrolled. The highest dose level (4.16 mg/kg/dose) was selected as the recommended phase II dose (RP2D). Best responses included 1 complete response (CR), 3 partial responses (PRs), and 7 patients with stable disease (SD). Three participants with melanoma or colon cancer were alive at 25, 32, and 36 months after therapy completion

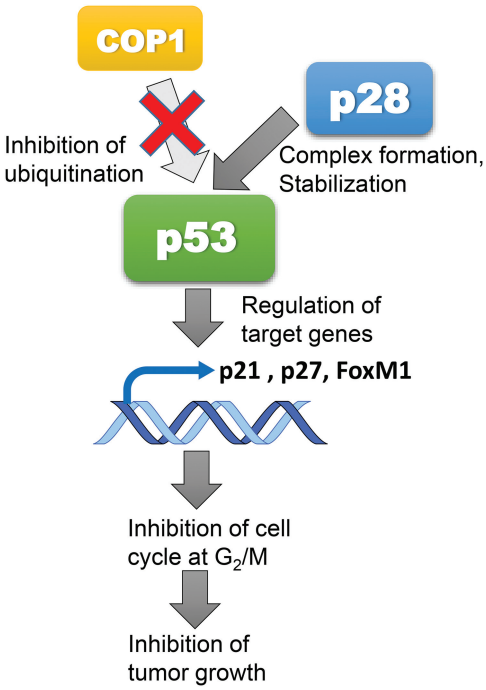

Fig. 1. p28 Mechanism of antitumor action. p28 binds with high affinity to the p53 DNA binding domain blocking COP1-mediated proteasomal degradation of p53. The posttranslational increase in the level and activity of p53 regulates the activity of the downstream genes, *p21*, *p27* and *FoxM1*, leading to inhibition of the cancer cell cycle at G_2/M and subsequent apoptosis.

at the time of publication. Consistent with animal models, no immune response to the peptide was observed in any participant at any dose level.[25]

These promising data led to the development of this phase I trial within the Pediatric Brain Tumor Consortium (PBTC). The primary objectives of this study were to establish whether the adult R2PD of p28 was safe for children with recurrent or refractory CNS tumors and to characterize the serum PKs of p28 in children. Secondary objectives were to describe the antitumor activity of p28 in this patient population and characterize the level of p53 expression in available tumors.

Materials and Methods

Children aged 3–21 years with histologically confirmed progressive, recurrent, or refractory high-grade glioma, medulloblastoma, primitive neuroectodermal tumors, atypical teratoid rhabdoid tumor (AT/RT), diffuse intrinsic pontine glioma (DIPG), or choroid plexus carcinoma for whom no curative therapy existed were eligible. A histopathologic diagnosis was not required for participants with DIPG.

Other eligibility criteria included Karnofsky (for patients aged >16 y) or Lansky (for patients ≤16 y) performance status of ≥50, adequate renal, hepatic, and hematologic function, and recovery from prior therapy including myelosuppressive chemotherapy (3 weeks from the last dose and 6 weeks for nitrosureas), immunotherapy (3 weeks from the last dose), biological agents (≥7 days from the last dose), monoclonal antibody

treatments (30 days or 3 half-lives from the last dose). Participants were required to be neurologically stable and on stable or decreasing doses of corticosteroids for at least one week before enrollment. For participants who had recently received radiation therapy, an interval of ≥3 months from craniospinal radiation, ≥8 weeks from local irradiation to the primary tumor, and ≥2 weeks from focal irradiation to symptomatic sites was required.

Patients were excluded if they were receiving other anticancer or experimental agents, required growth factors, or had uncontrolled infections, seizures, or other systemic illness. Women who were pregnant or lactating were also excluded. The institutional review board of each PBTC participating site approved the trial. Written informed consent was obtained from all participants or legal guardians, and assent was obtained from minor subjects according to institutional guidelines.

Drug Administration

p28 (NSC #745104) was supplied by CDG Therapeutics, Inc. as a sterile, preservative-free, lyophilized powder. The reconstituted solution was administered intravenously, followed by a 15–30 minute infusion of 50 mL normal saline or dextrose. Alternatively, p28 could be administered in 50 mL normal saline and infused over 15–30 minutes. p28 was given 3 times a week for 4 consecutive weeks followed by a 2-week rest (1 course = 6 weeks). It was generally administered in the outpatient setting. Participants were to receive up to 10 courses of therapy unless they experienced unacceptable toxicity or disease progression. The starting dose of p28 was 4.16 mg/kilogram/dose, which is the adult R2PD, considering that the drug was extremely well tolerated in the adult phase I study. Dose de-escalation was planned and governed by the rolling-6 design (to be implemented in the event that dose level 1 was found to be too toxic). All subsequent courses required a minimum of stable disease and organ function that met eligibility criteria prior to drug administration.

Table 1. Patient characteristics

Characteristic	No. of Patients
Total patients enrolled	18
Evaluable	12
Inevaluable	6
Diagnosis	
Atypical teratoid/rhabdoid tumor	1
Choroid plexus carcinoma	2
Diffuse intrinsic pontine glioma	2
High-grade glioma	
Anaplastic astrocytoma	1
Giant cell glioblastoma	1
Glioblastoma multiforme	3
Glioma, other	4
Medulloblastoma	2
Pineoblastoma	2
Sex	
Male	11
Female	7
Age, y	
Median	11.8
Range	3–19
Ethnicity	
Hispanic or Latino	3
Non-Hispanic	15
Race	
Black	5
Unknown	1
White, non-Hispanic	12

Monitoring

Toxicity monitoring included weekly history and physical examination, complete blood counts, metabolic profile, and serum pregnancy test for females of child-bearing potential during the first course. For subsequent courses, an interval history and physical assessment as well as a complete blood count and metabolic profile were required prior to receiving p28. Assessment of tumor status was performed by MRI of the brain (and spine, if applicable) at the end of courses 2, 4, 6, 8, and at the time of disease progression or end of therapy.

Trial Design

The rolling-6 phase I design was used to assess safety of the adult recommended dose (4.16 mg/kg/dose). Higher dose levels were not pursued due to solubility concerns. If dose de-escalation was needed, the MTD was to be defined as the highest dose level in which no more than 1 of 6 participants experienced DLTs. Patients who received <75% of the total dose of drug in the first course of protocol therapy for reasons other than toxicity were considered inevaluable for MTD evaluation and were replaced. Once the RP2D was achieved, there was a planned expansion to 12 participants.

Toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0). DLT was defined as any of the following events occuring during the 6-week dose-finding period: grade 4 neutropenia or thrombocytopenia, any grade 4 nonhematologic

Table 2. Summary of grade 2 and higher adverse events possibly, probably, or definitely related to p28 for 17 participants and a total of 32 courses

Adverse Events [events (pts.)]	2	3	4
Platelet count decreased	10 (2)	10 (1)	4 (1)[a]
Lymphocyte count decreased	7 (4)	2 (2)	
White blood cell decreased	4 (4)		
Anemia	3 (2)	1 (1)	
Neutrophil count decreased		2 (2)	1 (1)[a]
Nausea	1 (1)		
Fatigue	1 (1)		
Hypoglycemia	2 (2)		
Constipation	1 (1)		
Hyperglycemia	1 (1)		
Abdominal pain		1 (1)	

[a]All grade 4 events were in a single participant.

toxicity at least possibly related to p28 persisting for ≥7 days, any grade 3 nonhematologic toxicity at least possibly related to p28 (except fever or infection <5 days duration, nausea and vomiting <3 days duration, electrolyte abnormalities responsive to supplementation, or elevation of transaminases that returned to baseline within 7 days of drug interruption and did not recur upon restarting therapy). A DLT was further defined as any drug-related toxicity resulting in the permanent cessation of therapy or that resulted in missing more than 3 consecutive doses of p28.

Standard 2-dimensional imaging criteria were used for response assessment. Response findings for stable disease must have been maintained for 24 weeks (4 courses).

Pharmacokinetics

During course 1, blood samples for pharmacokinetics (PK) were required preinfusion and 5, 10, 20, 30, and 60 minutes after the second dose of p28 in all participants. Blood samples were collected from the opposite limb or a site other than the site of administration. Samples were assayed for p28 and 2 major metabolites by fast liquid chromatography/tandem mass spectrometry as previously described.[26,27] Individual participant serum concentration–time data for each dose of p28 were analyzed by standard noncompartmental methods, and dose- and time-related increases in the amount of each metabolite in serum were quantified as percentage of the total peak area of p28.[26]

Immunohistochemistry

For consenting participants for whom previous tumor tissue was available, representative tissue sections were stained for p53 status as previously described[28] using a monoclonal antibody to mutant and wild-type p53 (DO-1, sk-126; Santa Cruz Biotechnology) and visualized by biotinylated secondary antibody and ABC kit (Vector). Sections were counterstained with hematoxylin to identify tumor morphology. Ten separate areas from each tumor slide were evaluated, and a minimum 1000 tumor cells were counted for statistical analysis. All slides were evaluated by 2 independent pathologists without prior knowledge of patient status. Only cells with nuclear staining for p53 were considered positive; a tumor was classified as p53-positive when ≥10% of cells analyzed were positive.

Results

A total of 18 eligible participants were enrolled from October 2013 to August 2014. Twelve of the 18 participants were fully evaluable for toxicity. Among the 6 participants who were determined to be inevaluable, 5 received <75% of study drug during the first course and came off treatment due to progressive disease, and 1 participant progressed prior to receiving any study medication. Patient characteristics are detailed in Table 1.

Adverse Events

p28 was well-tolerated. Among the 12 evaluable participants, the most common adverse events attributed to the drug were transient grade 1 or 2 infusion-related reactions manifested as flushing, hot flashes, dizziness, headache, or changes in blood pressure. During the dose-finding period, 1 participant with metastatic pineoblastoma had 2 DLTs of grade 4 neutropenia and thrombocytopenia. Table 2 summarizes all grade 2 or higher adverse events that were at least possibly related to p28 among eligible participants on the study.

Pharmacokinetics of p28

PK analysis was performed on 16 participants. The overall concentration of p28 with time after administration was similar among the 16 participants as shown in Fig. 2A. The time to reach maximum serum concentration (T_{max}) was 11.3 ± 0.8 minutes, and half-life was 0.12 ± 0.02 hours (Fig. 2B). An increased area under the curve (AUC) was observed in pediatric participants when compared with adult participants, which is likely a result of a higher C_{max} concentration (pediatric patients 22.6 μg/mL; adult patients 13.7 μg/mL), a prolonged half-life of β-phase (elimination), and shorter γ-phase. PK parameters are summarized in Table 3.

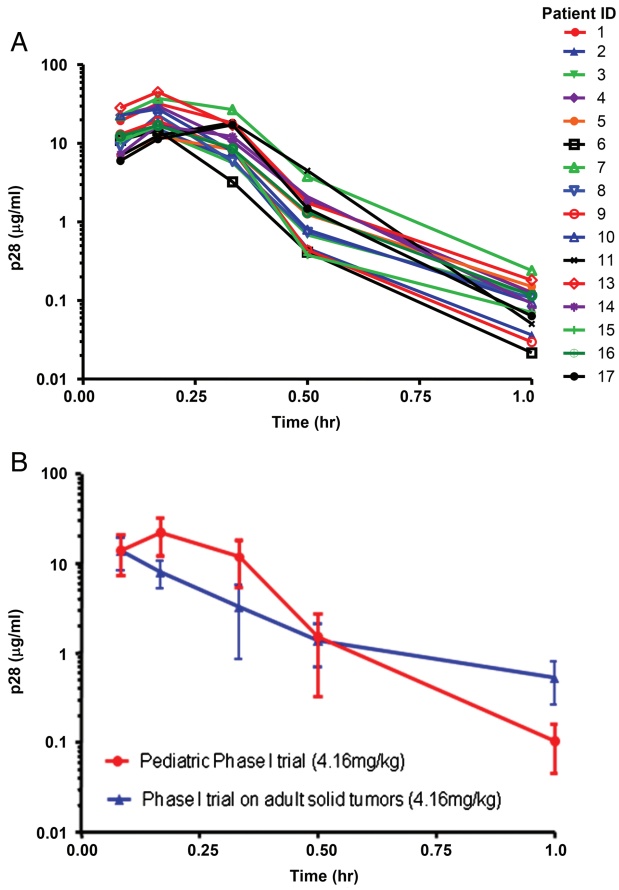

Fig. 2. (A) Individual participant p28 serum concentration. Plots of p28 concentration versus time in pediatric patients receiving 4.16 mg/kg of p28. Serum samples were applied to liquid chromatography-tandem mass spectrometry and p28 concentration was determined from the elution profiles. (B) Concentration profiles of p28. p28 concentration versus time profiles of 16 pediatric patients (red) and 7 adult patients (blue) receiving 4.16 mg/kg dose.

p53 Expression in Tumor Tissue

p53 expression in formalin-fixed, paraffin-embedded tumor slides of 12 eligible participants was evaluated by IHC. Six tumors tested positive for p53 (10% to 87%), representing 1 AT/RT and 5 malignant gliomas.

Clinical Outcomes

Seven participants received ≥2 courses of p28 (median 2; range, 2–7). No complete or partial responses were observed.

Table 3. Summary of pharmacokinetic parameters for 16 pediatric participants receiving p28

p28 dose (mg/kg)	4.16
C_{max} (μg/mL)	22.6 ± 2.2
T_{max} (min)	11.3 ± 0.8
$t_{1/2}$ (h)	0.12 ± 0.02
$t_{1/2\alpha}$ (h)	0.01 ± 0.002
$t_{1/2\beta}$ (h)	0.07 ± 0.01
$t_{1/2\gamma}$ (h)	0.43 ± 0.1
AUC_{last} (h-μg/mL)	6.4 ± 0.6
Cl (mL/kg/r)	743 ± 64
Vdss (mL/kg)	168 ± 13

Abbreviations: h, hour; min, minute.
Pharmacokinetics parameters (C_{max} = p28 maximum concentration in serum, T_{max} = time to C_{max}, $t_{1/2}$ = terminal half-life of p28, $t_{1/2\alpha}$ = rapid distribution half-life, $t_{1/2\beta}$ = slow distribution half-life, $t_{1/2\gamma}$ = elimination half-life, AUC_{last} = area-under curve, Cl = total clearance and Vdss = volume distribution at steady state) were calculated from the p28 concentrations in serum versus postinjection time. The concentration at 0 min is defined as 0 ng mL^{-1}.

Two participants (both with malignant glioma) with stable disease in course 2 and course 7 came off study without disease progression because of a lack of drug supply. Duration of therapy and best response for all participants who received at least 1 dose of p28 ($n = 17$) are detailed in Table 4. Within the limitation of this phase I study, there was no correlation between response to p28 and p53 expression evaluated by IHC.

Discussion

The search for novel treatments for recurrent and progressive pediatric CNS tumors has prompted investigation of agents targeting multiple oncogenic pathways. p53 is central to the regulation of the cell cycle, DNA repair, development, and a myriad of signaling pathways. Dysregulation of p53 has been found in virtually all malignancies including pediatric CNS tumors. In this study, we present results from a pediatric phase I trial of p28, a novel cell-penetrating peptide targeting the p53 pathway. Past studies have utilized compounds primarily focused on protecting p53 from degradation by the endogenous regulator HDM2 in hopes of suppressing the transcriptional activity of p53.[29] In contrast, p28 blocks the binding of constitutional morphogenic protein 1 (Cop1) to the DNA binding domain of p53.[22,30] As an E3 ubiquitin ligase, Cop1, like MDM2, is a known major negative regulator of p53 activity in many cancers. p28 exerts its antitumor affect by inducing cell-cycle arrest at G2/M. Furthermore, p28 also has an antiangiogenic effect that is independent of p53 status. Thus, p28 represents a novel and potentially promising agent for anticancer therapy.

p28 was well-tolerated in this population of heavily pretreated children. Given the favorable safety profile in adult patients, we evaluated and demonstrated the tolerance of the adult R2PD in the participants on our study. The most common adverse event related to p28 was grade 1 or 2 infusion-related

Table 4. Clinical outcomes and p53 expression by immunohistochemistry for participants on PBTC-041

Patient ID	Diagnosis	Days of Treatment	Total Courses	Best Response	IHC % p53
1	CPC	80	2	PD	7%
2	DIPG	84	2	PD	N.A.
3	AT/RT	83	2	PD	23%
4	AA	7	<1	PD	30%
5	Malignant glioma	36	1	PD	8%
6	GBM	4	<1	PD	>75%
7	Medulloblastoma	34	1	PD	5%
8	Pineoblastoma	44	1	PD	6%
9	DIPG	9	<1	PD	N.A.
10	Malignant glioma	7	<1	PD	5%
11	Pineoblastoma	209	5	SD	N.A.
12	GBM	14	<1	PD	87%
13	CPC	75	2	PD	< 1%
14	GBM	303	7	SD	10%
15	Medulloblastoma	37	1	PD	N.A.
16	GBM	36	1	PD	64%
17	Malignant Glioma	65	2	SD	N.A.

Abbreviations: AA, anaplastic astrocytoma; CPC, choroid plexus carcinoma; DIPG, diffuse intrinsic pontine glioma; GBM, glioblastoma; IHC, immunohistochemistry; SD, stable disease; PD, progressive disease.

reactions, which were short and rarely required intervention. A single participant with extraneural metastatic pineoblastoma with pre-existing bone marrow metastases experienced 2 DLTs of neutropenia and thrombocytopenia during course 1.

PK parameters of p28 in children closely correlated with the adult experience. PK analysis of 16 participants revealed an overall $t_{1/2}$ and $t_{1/2\alpha}$ similar to those in adults. An increased AUC was observed in pediatric participants as a result of a higher C_{max} and longer $t_{1/2\alpha\beta}$. The PK parameters identified here as well as the prolonged intranuclear half-life of p28 may suggest evaluating the drug under a less intensive dosing schedule.

As expected, approximately one-half of the tumor specimens available were positive for p53 by IHC. All positive samples were either AT/RT or malignant glioma. The expression of p53 as determined by IHC was not correlated with best response to p28 mirroring the adult experience, in which p28 demonstrated antitumor activity independent of p53 status.[32]

As a single cytostatic agent, p28 is not likely to be effective against pediatric CNS tumors. However, combinatorial strategies may prove more promising. Preliminary data have shown additive cell kill with agents such as dacarbazine and temozolomide in a number of high-grade glioma cell lines including U87 ($p53^{wt}$) and LN229 ($p53^{mut}$). (T. Yamada et al, unpublished). Further combination strategies are being explored preclinically.

In conclusion, the results of this trial have established that p28 is safe and well-tolerated in children with progressive CNS malignancies. The further development of this agent in combination with other agents is currently being explored.

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Conflict of interests statement. T.Y. and C.W.B. are Director of Drug Development and Chief Scientific Officer, respectively, of CDG Therapeutics Inc.

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