Title: A phase I study of perifosine with temsirolimus for recurrent pediatric solid tumors

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Abbreviation Key table:

AKT	protein kinase B
AST	aspartate transaminase
AUC	area under the curve
CL	clearance
C_{max}	maximum concentration
DIPG	diffuse intrinsic pontine glioma
DLT	dose-limiting toxicity
FDA	Food and Drug Administration

IRB	Institutional Review Board
MSKCC	Memorial Sloan Kettering Cancer Center
MTD	maximum tolerated dose
mTOR	mammalian Target of rapamycin
mTORC	mammalian target of rapamycin complex
PI3K	phosphoinositide 3-kinase
PK	pharmacokinetic
PTEN	phosphatase and tensin homolog
$t_{1/2}$	half-life
V_{ss}	volume of distribution at steady state

Abstract

Background: The PI3K/AKT/mTOR pathway is aberrantly activated in many pediatric solid tumors including gliomas and medulloblastomas. Preclinical data in a pediatric glioma model demonstrated that the combination of perifosine (AKT inhibitor) and temsirolimus (mTOR inhibitor) is more potent at inhibiting the axis than either agent alone. We conducted this study to assess pharmacokinetics and to identify the maximally tolerated dose for the combination.

Procedure: We performed a standard 3+3 phase I, open-label, dose escalation study in patients with recurrent/refractory pediatric solid tumors. Four dose levels of perifosine (25 to 75 mg/m2/day) and temsirolimus (25 to 75 mg/m2 IV weekly) were investigated.

Results: 23 patients (median age 8.5 years) with brain tumors (diffuse intrinsic pontine glioma [DIPG] n=8, high-grade glioma n=6, medulloblastoma n=2, ependymoma n=1), neuroblastoma (n=4), or rhabdomyosarcoma (n=2) were treated. The combination was generally well tolerated and no dose limiting toxicity was encountered. The most common grade 3 or 4 toxicities (at least possibly related) were thrombocytopenia (38.1%), neutropenia (23.8%), lymphopenia (23.8%) and hypercholesterolemia (19.0%). Pharmacokinetic findings for temsirolimus were similar to those observed in the temsirolimus single agent phase II pediatric study and pharmacokinetic findings for perifosine were similar to those in adults. Stable disease was seen in 9 of 11 subjects with DIPG or high-grade glioma; no partial or complete responses were achieved.

Conclusions: The combination of these AKT and mTOR inhibitors was safe and feasible in patients with recurrent/refractory pediatric solid tumors.

Introduction

New agents are desperately needed for relapsed pediatric solid tumors because of their very poor outcome and the lack of effective salvage strategies. The phosphoinositide 3-kinases (PI3Ks) are a family of lipid enzymes which phosphorylate the phosphatidylinositols on the plasma membrane. They transmit signals received from activated tyrosine kinase receptors, G protein-coupled receptors, and activated Ras to molecules such as protein kinase B (AKT) and mammalian Target of rapamycin

(mTOR) that control cell metabolism, proliferation, size, and survival.[1] Activated PI3K recruits AKT to the cell membrane and activates it, which can indirectly activate mammalian target of rapamycin complex (mTORC) 1 which consists of mTOR, the catalytic subunit of this complex, and several other proteins. Activation of mTORC1 results in increased protein synthesis, cell growth, survival and proliferation.[2] An important negative regulator of PI3K/AKT/mTOR signaling is phosphatase and tensin homolog (PTEN), which is a lipid phosphatase that antagonizes the kinase activity of PI3K.

The PI3K/AKT/mTOR axis is aberrantly activated in a variety of cancers including pediatric solid tumors through gene amplification or mutation upstream at the level of the receptor (e.g., activating mutations in platelet-derived growth factor receptor-alpha, anaplastic lymphoma kinase, and fibroblast growth factor receptor 1, activating mutations in one of the subunits of PI3K, or inactivating mutations of the negative regulator PTEN.[3-8] AKT activation has been associated with a poorer outcome in neuroblastoma and with other markers of aggressive disease.[9] Similarly, PTEN loss has been noted to be a poor prognostic factor in pediatric high-grade gliomas and medulloblastomas.[10,11] In addition, there is evidence that the PI3K/AKT/mTOR pathway may also play an important role in resistance to radiation and/or chemotherapy.[12,13]

Perifosine, a synthetic alkylphospholipid, represents a new class of antitumor agents that act on cell membranes rather than on DNA. Perifosine's primary mechanism of action is thought to be through interference with the recruitment of AKT to the plasma membrane resulting in inhibition of AKT phosphorylation and activation.[14,15] Perifosine also has other mechanisms of action such as inhibition of de novo synthesis of cell membrane components.[15]

Temsirolimus is an ester derivative of sirolimus or rapamycin, a naturally occurring drug produced by a soil bacterium.[16] Both rapamycin and temsirolimus are inhibitors of mTORC1. However, mTOR can also form a second complex called mTORC2, which is insensitive to temsirolimus and sirolimus and is known to activate AKT.[16] Temsirolimus is FDA approved for the treatment of renal cell carcinoma. Recently, temsirolimus has been evaluated in children with solid tumors as a single agent (high-grade glioma, neuroblastoma, and rhabdomyosarcomas) with prolonged stable disease in some patients.[17] This suggests that inhibiting the mTOR pathway is a promising approach but that targeting a single molecule may not be adequate to achieve significant anti-tumor activity.

Research over the past decade has unraveled multiple feedback loops in this pathway and the scientific rationale for this study is the observation that mTOR inhibition alone with rapalogs like rapamycin or temsirolimus results in AKT activation through upregulation of receptor tyrosine kinase signaling.[18-20] In addition, AKT inhibition induces the expression and activation of multiple activated tyrosine kinase receptors.[1] Preclinical evaluation of perifosine and temsirolimus in a pediatric glioma model demonstrated that significant inhibition of both AKT and mTOR occurred only when both drugs were given together.[21] Therefore this phase I clinical study of perifosine in combination with temsirolimus (two targeted agents that inhibit different points of the same pathway) was developed to evaluate the preclinical findings of synergy in targeting the PI3K/AKT/mTOR pathway between the two drugs as observed in this preclinical model of glioma.

Methods

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The primary aim of the study was to determine the maximum tolerated dose (MTD) of the combination of perifosine and temsirolimus in patients with recurrent or refractory pediatric solid tumors. Secondary aims were to (1) determine whether pharmacokinetic (PK) serum levels of perifosine and temsirolimus correlate with toxicity, (2) assess preliminary data on the efficacy of the perifosine and temsirolimus combination, and (3) determine whether molecular features of the tumor were associated with likelihood of response.

Patients

Between February 10, 2010 and August 21, 2012, 23 patients with recurrent or refractory pediatric solid tumors were enrolled onto the study. One enrolled subject never received study prescribed therapy and is not included in this analysis (Figure 1). Twenty of the 22 treated subjects were less than 18 years old. The other two were young adults (21 and 24 years old).

Eligibility criteria included: (1) presence of any solid tumor that had failed standard therapy, (2) evidence of tumor by computed tomography, magnetic resonance imaging, or metaiodobenzylguanidine scan, serum markers, or tissue sampling, (3) age $\leq$ 21 years (age $\leq$ 35 years for biopsy proven medulloblastoma or neuroblastoma), (4) Karnofsky or Lansky performance status $\geq$ 50%, (5) adequate organ function [absolute neutrophil count $\geq$ 1000 at least 24 hours off filgrastim, platelet count $\geq$ 100,000/mcL at least 1 week post-platelet transfusion, hemoglobin $\geq$ 8g/dL at least 1 week post- packed red blood cell transfusion, aspartate transaminase (AST) and alanine transaminase $\leq$ 2 x the upper limits of normal, total bilirubin $\leq$ 2 mg/dL, serum creatinine $\leq$ 1.5 x the upper limit of normal for age, or calculated creatinine clearance or nuclear glomerular filtration rate $\geq$ 70

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ml/min/1.73 m2], (6) adequate lipid profile [cholesterol level < 350 mg/dL and triglycerides level <

400 mg/dL], (7) mandated interval since prior therapy [≥ 3 weeks since last non-nitrosourea

chemotherapy, ≥ 6 weeks since last nitrosoureas, ≥ 4 weeks since last radiation therapy], (8) ability to

swallow tablets whole, and (9) agreement to practice adequate contraception and not breast feed. Prior

exposure to single-agent perifosine and/or an mTOR inhibitor was permitted as long as the agent had

not been associated with a dose-limiting toxicity (DLT). Exclusion criteria included (1) pregnancy, (2)

uncontrolled active infection, (3) patients with human immunodeficiency virus receiving combination

anti-retroviral therapy, (4) enzyme-inducing anti-convulsant usage, and (5) history of pulmonary

hypertension or pneumonitis. The subjects (if adults or emancipated minors) or parents or legal

guardians of all patients gave informed consent. The Memorial Sloan Kettering Cancer Center

(MSKCC) Institutional Review Board (IRB) and the Food and Drug Administration (FDA) approved

the protocol.

Treatment protocol

This was a standard 3+3 phase I dose escalation study in which doses of both drugs were escalated.

Four dose levels were investigated (Table 1). Temsirolimus was administered weekly, intravenously

over 30 minutes following antihistamine pre-medication, at either 25 or 75 mg/m2/dose. Perifosine

was only available as 50 mg tablets. A loading dose was administered on day 1 and then the

maintenance dose was administered every 1 to 4 days, depending on the dose level and body surface

area of the subject. The complete dosing scheme is given in Table 1. Treatment was continued until

disease progression, intolerable toxicity, DLT or death was encountered. Subjects experiencing DLT

after 2 cycles of treatment, but with evidence of clinical benefit, were eligible to remain on study with

a dose level reduction. Some treatable laboratory abnormalities that were not associated with any

signs or symptoms originally met the DLT definition, but the MSKCC IRB and the FDA approved an amendment to exclude them.

Subjects were seen weekly for assessment and temsirolimus treatment. Laboratory assessments (complete blood count, coagulation studies, chemistries, lipid profiles) were performed weekly during cycle 1 and then every other week. Tumor assessments were performed about every 8 weeks.

Toxicity was assessed according to the Common Toxicity Criteria (version 3.0) of the National Cancer Institute, National Institutes of Health. Dose-limiting toxicity was defined in the final version of the protocol as (1) any non-hematological toxicity grade $\geq$ 3 [except for grade 3 nausea, vomiting, and diarrhea that could be controlled within 24 hours with supportive care measures, grade 3 or 4 electrolyte abnormalities that could be corrected by medical management or grade 3 or 4 cholesterol or triglyceride abnormality], (2) grade 4 neutropenia on 2 consecutive blood counts drawn at least 72 hours apart, (3) grade 4 febrile neutropenia or grade 4 documented infection with absolute neutrophil count < 1,000/mcL, (4) grade 3 thrombocytopenia with bleeding or a platelet count < 25,000/mcL.

Correlative studies

Samples for PK analyses were obtained at baseline and during cycle 1. Serum for perifosine levels was obtained on days 1, 8, 15 and 22 of cycle 1. One blood sample for temsirolimus and sirolimus levels was obtained pre-infusion on days 1, 8 and 22; on day 15 samples were obtained pre-infusion and at hours 1, 6 and 24.

Perifosine: At each time point, heparinized blood (7 to 10 ml) was collected into a plastic vacutainer to minimize adhesion of perifosine. Plasma was separated by centrifugation and stored in polypropylene cryovials at -80°C until assayed. Perifosine in plasma was measured by a validated reversed phase liquid chromatography/electrospray mass spectrometry method as previously described.[22]

Temsirolimus: At each time point, whole blood (2 ml) was collected into an ethylenediaminetetraacetic acid -treated tube and stored at -80°C until assayed. Concentrations of temsirolimus and sirolimus were measured by a validated liquid chromatography/tandem-mass spectrometry assay with internal standards at SFBC-Taylor (Princeton, NJ). Standard PK parameters such as maximum concentration (C_{max}), area under the curve (AUC), half-life ($t_{1/2}$), clearance (CL), and volume of distribution at steady state (V_{ss}) were calculated using non-compartmental approach using WinNonlin 6.3 software (Pharsight Corp).

Tumor tissue: Five micron formalin-fixed paraffin-embedded sections were used for all immunostaining procedures. Immunohistochemical staining was performed on a Discovery Ultra autostainer (Ventana Medical Systems, Tucson, AZ, USA) using the following antibodies and concentrations: PTEN (1:100) (cat# 9559, Cell Signaling Technology, Davers, MA, USA), p-AKT S473 (1:100) (cat#4060, Cell Signaling Technology), p-PRAS40 (1:40) (cat# 2997, Cell Signaling Technology). Stains were developed with standard DAB-based reagents with the exception of p-AKT, which employed multimeric chemistry (OmniMap anti-RB, Ventana Medical Systems). Following

staining, slides were dehydrated in graded alcohols and coverslipped manually with Permount

mounting media (Sigma-Aldrich, St. Louis, MO, USA).

Response criteria

For subjects with tumors other than neuroblastoma, responses were assessed via RECIST.[23] For

subjects with neuroblastoma, the International Neuroblastoma Response criteria were used.[24]

Statistics

The DLT assessment period was the first 28 day cycle. If therapy was discontinued during the

first cycle for reasons other than toxicity, an additional subject could be enrolled at that dose

level to ensure adequate evaluation of toxicity. No intra-patient dose escalation was

permitted.

Results

Patient Characteristics

The median age of the 22 subjects was 9 years (range 4 to 24 years). Eleven (50%) were male and 11

female. Sixteen subjects had central nervous system tumors (DIPG n=8, high-grade astrocytoma n=5,

medulloblastoma n=2, ependymoma n=1), 4 had neuroblastoma, and 2 had rhabdomyosarcoma. Table

2 contains additional details regarding patient characteristics.

Number of Cycles

The entire group of subjects initiated a total of 62 cycles of treatment. A median of 2 cycles was administered per patient, with a range from <1 to 6 cycles. Two subjects withdrew consent during the first cycle (not due to toxicity) and were replaced.

Toxicity

Table 3 contains a summary of toxicities considered at least possibly related to perifosine and/or temsirolimus including all hematologic toxicities and non-hematologic toxicities seen in > 10% of subjects or at least grade 3 (even if seen in < 10% of subjects). The perifosine and temsirolimus combination was generally well tolerated. The most common toxicities of any grade (at least possibly related) were hyperglycemia (95.2%), fatigue (90.5%), increased AST (81%), decreased hemoglobin (81%), and decreased platelets (81%), with the vast majority of these toxicities ≤ grade 2. The most common grade 3 or 4 toxicities (at least possibly related) were thrombocytopenia (38.1%), neutropenia (23.8%), lymphopenia (23.8%) and hypercholesterolemia (19.0%).

No subject suffered a DLT based on the criteria described in the Methods section above. The original version of the study included more stringent DLT definitions and 3 subjects suffered DLT. On dose level 2 one subject had grade 3 hypokalemia that spontaneously resolved 1 day later and on dose level 3 one subject each had grade 4 hypercholesterolemia and grade 3 hypophosphatemia.

Responses

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Nineteen subjects were evaluable for response and the results are detailed in Table 2. Three were considered inevaluable due to withdrawal of consent (n=2) and early removal from study (n=1) within the first month, prior to any response evaluation.

Five subjects with recurrent high-grade glioma were treated on study. One went off study after only 9 days due to electrolyte abnormalities (not considered to be DLT) and so was not evaluable for response. Four were evaluable for response and their best responses were stable disease (n=3) for 2, 2, and 4 months and progressive disease (n=1). None of them were treated on combined dose level 4, which is being proposed as the recommended phase 2 dose.

Eight subjects with recurrent DIPG were treated on study. One went off study due to withdrawal of consent following an infusion reaction (hives, cough, and hypoxia that responded promptly to supportive care) associated with the first dose of temsirolimus (not considered to be DLT) and so was not evaluable for response. Seven were evaluable for response and their best responses were stable disease (n=6) for 1.5, 2, 2, 4, 4 and 4 months and progressive disease (n=1). The 5 DIPG patients treated at the proposed phase 2 dose all had stable disease at first evaluation.

Four subjects with neuroblastoma were treated on study. All 4 had no response to treatment as evaluated after the first cycle of therapy. Three had progressive disease after 4 cycles and 1 received only 1 cycle of therapy before withdrawing consent.

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Pharmacokinetics

PK calculations for perifosine were possible from 18 patients. Perifosine plasma concentration was measured weekly (steady state levels) and presented in Figure 2. Average steady state levels of perifosine were calculated for each dose level. Linear dose response was found (Figure 2; average steady-state level vs. average actual daily dose given), albeit rather large inter-patient (especially in dose group 3) and in some cases intra-patient variability as illustrated in Figure 1. The average perifosine steady-state values were correlated with PK parameters obtained for temsirolimus and sirolimus; correlation coefficients are given in Supplemental Table S1. Despite some strong correlations found in case of dose group 1, the overall trend observed across dose groups and statistical power available is not enough to suggest that perifosine interferes with temsirolimus metabolism. The steady-state perifosine levels are similar to steady-state levels reported in clinical trials with perifosine for adults with cancer.[25]

PK calculations for temsirolimus and sirolimus were possible from 18 and 17 patients, respectively. PK parameters for temsirolimus and its major active metabolite, sirolimus, are listed in Supplemental Table S1. Large inter-patient variability in temsirolimus C_{max} measurements was observed, which was less pronounced with sirolimus and is in line with other studies.[17] There was no clear correlation pattern observed between perifosine steady-state levels and temsirolimus/sirolimus PK parameters.

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Tumor tissue biological assays

Eight subjects had DIPG tumors that had never been biopsied and tumor tissue was unavailable from 7 other subjects. The results of the biological assays performed on 7 subjects' tumor tissue are presented in Table 2.

Discussion

To the best of our knowledge, this is the first published experience regarding a clinical trial employing both AKT and mTOR inhibition. The most notable finding is that this is tolerable and joint AKT/mTOR inhibition potentially could be developed further with the addition of other agents.

There are few prior publications regarding the use of temsirolimus in pediatric oncology patients. Spunt et al treated 18 subjects at 10 to 150 mg/m2/dose weekly. One of 18 had DLT (grade 3 anorexia) at the 150 mg/m2/weekly dose level, but no MTD was identified. One subject with neuroblastoma achieved complete response; 5 other subjects achieved stable disease, 3 for more than 4 months (ependymoma, germ cell tumor, adrenocortical carcinoma).[26] The same group subsequently performed a phase II trial of temsirolimus in 52 children with high-grade glioma, neuroblastoma or rhabdomyosarcoma. They used a dose of 75 mg/m2/dose weekly and only 1 partial response (in a patient with neuroblastoma) was achieved. Stable disease at week 12 was seen in 7 of 17 patients with high-grade glioma, 6 of 19 with neuroblastoma, and 1 of 16 with rhabdomyosarcoma.[17]

Our team hypothesized that the failure of mTOR inhibition monotherapy might be due to compensatory AKT activation and that simultaneous inhibition of mTOR and AKT might be more effective. Prior to the initiation of this study a pediatric phase I study of single-agent perifosine opened at MSKCC and the preliminary safety data supported the development of this combination trial. The single-agent perifosine data will be published separately. The only other pediatric clinical experience with an AKT inhibitor that we are aware of was a phase I trial of MK-2206 conducted by the Children's Oncology Group.[27] Fifty children received MK-2206 orally on 2 schedules: every other day (n=23 evaluable) or weekly (n=17 evaluable). The recommended phase II dose was determined to be 45 mg/m2/dose every other day or 120 mg/m2/dose weekly. No objective response was observed; 7 subjects had stable disease for at least 3 courses (n=2 with ependymoma; n=1 each with malignant paraganglioma, gliomatosis, juvenile pilocytic astrocytoma, malignant peripheral nerve sheath tumor, and clear cell sarcoma).

The perifosine and temsirolimus combination has also been investigated in 34 adults with recurrent or refractory malignant gliomas, but thus far only reported in abstract form.[28] The MTD of perifosine was determined to be a 600 mg load, then 100 mg daily with temsirolimus 115 mg weekly. Two partial responses were achieved, but at a dose level that used a higher temsirolimus dose (170 mg) than the MTD.

The PK analysis of both perifosine and temsirolimus demonstrated large interpatient variability with perifosine steady state plasma levels in this pediatric cohort that are similar to those observed in adult studies and temsirolimus blood levels that are similar to those observed in the temsirolimus single agent phase II pediatric trial in a similar patient population suggesting that perifosine does not

interfere with the metabolism of temsirolimus. While the correlation between the perifosine dose administered and steady state plasma levels observed in this study suggest a linear dose response for perifosine at the dose range tested, this relationship should be interpreted with caution due to the large interpatient variability. By contrast, some perifosine studies in the adult population have observed an absence of a dose response at similar doses.[25, 29] In support of these latter observations are unpublished results from our phase I study of perifosine alone in children with recurrent solid tumors in which 25, 50, 75, 100, and 125 mg/m^2/day dose levels were investigated, and no dose response was observed beyond 50 mg/m^2/day (personal communication, Becher & Dunkel).

In conclusion, the combination of perifosine and temsirolimus is well tolerated in children with recurrent solid tumors. Although this was a phase I study, the lack of objective responses suggest that this combination may need to be combined with additional agent(s) in the future. We did not assess target inhibition in tumor tissue in response to the therapy so it is not clear whether we were successful in inhibiting the PI3K/AKT/mTOR pathway in our subjects' tumors. As most of the patients on the study had brain tumors, it is pertinent to know whether adequate levels of these drugs got to these tumors. Recently, the cerebrospinal fluid penetration of perifosine as a surrogate for blood-brain-barrier penetration was assessed in normal rhesus monkeys and was noted to be poor.[30] By contrast, another recent study identified the receptor for docosahexaenoic acid in endothelium of the blood-brain-barrier of mice and also noted that alkylphospholipids such as miltefosine may enter through this receptor.[31] Therefore, future studies with this combination particularly in brain tumor patients should investigate whether adequate concentrations of these drugs reach their targets.

Supplemental Table S1. Detailed pharmacokinetic results.

Acknowledgments

Conflict of Interest Statement

Dr. Ira Dunkel would like to disclose that after the completion of this clinical trial he began serving as a consultant for Pfizer, but not regarding temsirolimus, the Pfizer agent that is the subject of this investigation.

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Figure legends

Figure 1. Flow diagram regarding the enrolled subjects.
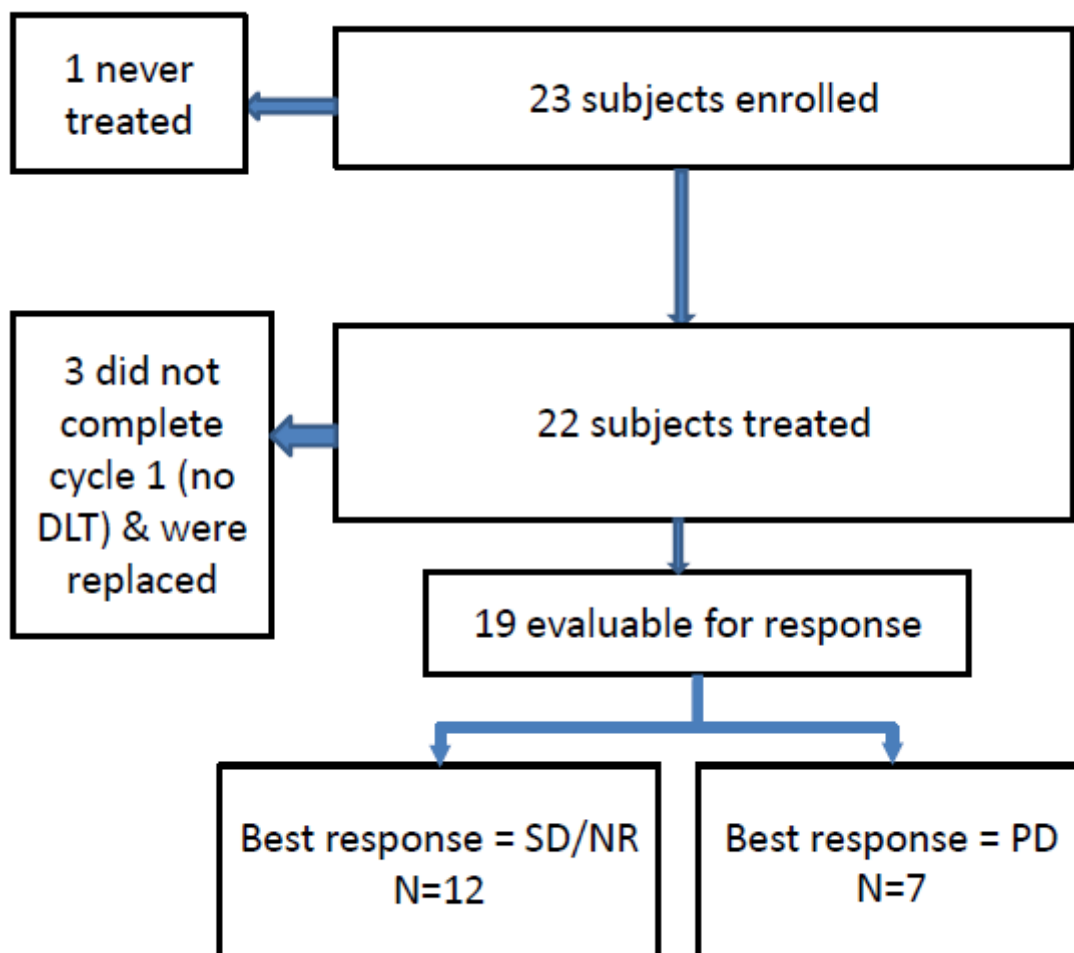
Figure 2. (A) Steady state plasma concentration of perifosine at weeks 2, 3, and 4 plotted for each study subject (1-24) across perifosine/combination dose groups; enumerated individual subject trace; grey semi-transparent thick line represents the average trace for the given dose group. Large inter-patient and occasionally intra-patient variability observed. (B) Correlation plot of perifosine steady state plasma concentration versus daily dose (actually administered); error bars represent single standard deviation; enumerated combination dose groups. The excellent correlation observed supports linear dose response (i.e. linear PK) in the dose range utilized but should be interpreted with caution due to the large inter-patient variability.
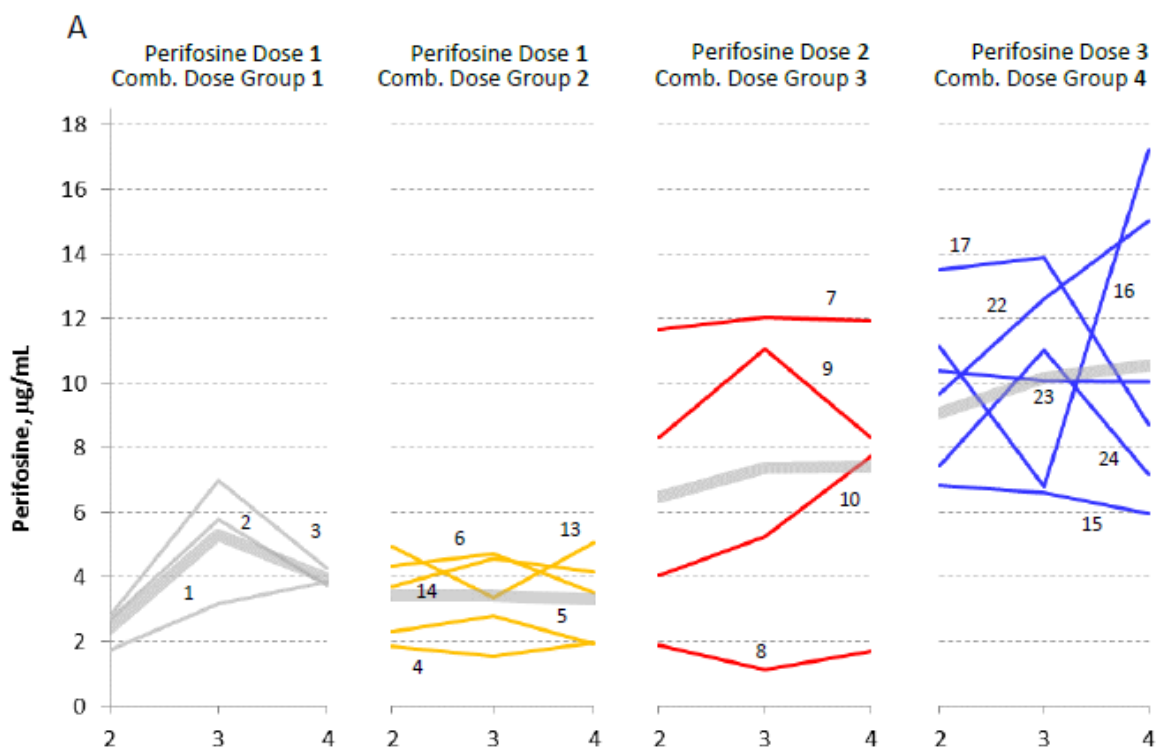
B

Perifosine, µg/mL

Average Daily Perifosine Dose , mg/m²/day

$R^2 = 1$
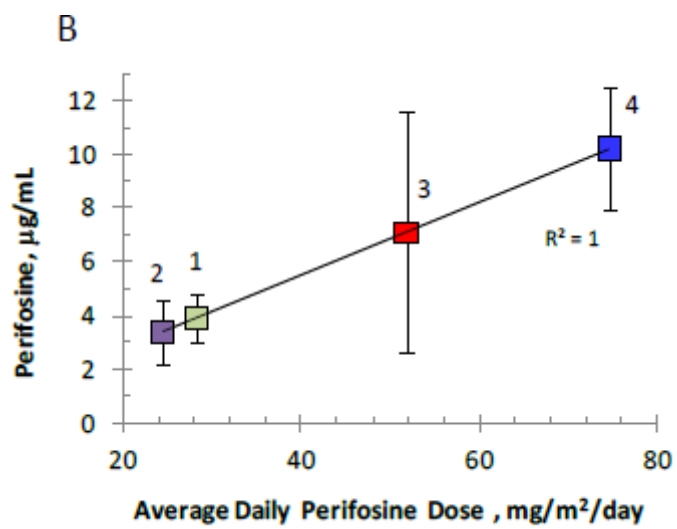
TABLE 1. Dose Escalation Scheme

Combination dose level	Perifosine dose level	Temsirolimus dose	BSA	Loading dose day 1	Maintenance dose
1	1	25 mg/m2	0.4 to 0.59	50 mg	50mg every 4 days
			0.6 to 0.79	50 mg	50mg every 3 days
			0.8 to 1.2	100 mg	50mg every 2 days
			1.21 to 1.6	150 mg	50mg 5 days per week
			> 1.6	150 mg	50mg daily
2	1	75 mg/m2	0.4 to 0.59	50 mg	50mg every 4 days
			0.6 to 0.79	50 mg	50mg every 3 days
			0.8 to 1.2	100 mg	50mg every 2 days
			1.21 to 1.6	150 mg	50mg 5 days per week
			> 1.6	150 mg	50mg daily
3	2	75 mg/m2	0.4 to 0.59	100 mg	50mg every 2 days
			0.6 to 0.79	100 mg	50 mg daily 5 days per week
			0.8 to 1.2	100 mg BID	50mg daily
			1.21 to 1.6	150 mg BID	100mg daily 5 days per week

			> 1.6	150 mg BID	100mg daily
4	3	75 mg/m2	0.4 to 0.59	100 mg	50 mg daily 5 days per week
			0.6 to 0.79	100 mg	50mg daily
			0.8 to 1.2	100 mg BID	50mg alternating with 100mg daily
			1.21 to 1.6	150 mg BID	100mg daily
			> 1.6	150 mg BID	100mg alternating with 150mg daily

Perifosine dose level 1 aim 25 mg/m2, mean dose (SD) achieved: 25.92 (3.61). Perifosine dose level 2 aim 50 mg/m2, mean dose (SD) achieved: 51.73 (8.36). Perifosine dose level 3 aim 75 mg/m2, mean dose (SD) achieved: 74.60 (11.59).

TABLE 2. Patient Characteristics and Responses

Patient	Dose level	Age	Sex	Dx	Prior RT	# prior Cx regimens	Best response (duration)	pAKT	PTEN	pPRAS40
1	1	7	F	HGG	Yes	3	PD	Negative	Negative	Positive
2	1	4	M	MB	Yes	4	PD			
3	1	11	M	NB	Yes	9	NR (1 mo)	Negative	Positive	Negative
4	2	24	M	NB	Yes	3	NR (4 mo)	Negative	Negative	Negative
5	2	9	F	NB	Yes	2	NR (4 mo)	Negative	Negative	Negative
6	2	4	F	DIPG	Yes	2	SD (4 mo)			
7	2	9	F	HGG	Yes	1	SD (4 mo)	AF	AF	AF
8	2	4	M	DIPG	Yes	3	PD			
9	3	10	M	RMS	Yes	2	PD	Positive	Negative	Positive
10	3	9	F	HGG	Yes	2	SD (2 mo)			
11	3	9	F	MB	Yes	5	PD			
12	3	17	M	HGG	Yes	2	SD (2 mo)	Positive	Positive	Positive
13	3	21	M	HGG	Yes	6	IE			
14	3	6	F	NB	Yes	3	NR (4 mo)			
15	4	8	M	DIPG	Yes	0	SD (4 mo)			
16	4	5	M	DIPG	Yes	1	PD			
17	4	5	F	DIPG	Yes	0	SD (1.5 mo)			
18	4	5	F	RMS	Yes	5	IE			
19	4	5	F	DIPG	Yes	0	IE			

20	4	15	M	DIPG	Yes	0	SD (4 mo)			
21	4	10	M	DIPG	Yes	0	SD (2 mo)			
22	4	9	F	Ep	Yes	2	PD			

Legend: AF=assay failure, Cx=chemotherapy, DIPG=diffuse intrinsic pontine glioma, Dx=diagnosis,

Ep=ependymoma, F=female, HGG=high-grade glioma, IE=inevaluable for response, M=male,

MB=medulloblastoma, mo=months, NB=neuroblastoma, NR=no response per INRC, PD=progressive

disease, RMS=rhabdomyosarcoma, RT=radiation therapy, Rx=treatment, SD=stable disease

TABLE 3. Toxicity Summary

	Any Grade		Grade 3 or 4	
Hematologic Adverse Events	No.	%	No.	%
Decreased hemoglobin	17	80.95%	1	4.76%
Decreased platelets	17	80.95%	8	38.09%
Decreased leukocytes	13	61.90%	2	9.52%
Increased PTT	11	52.38%	1	4.76%
Decreased neutrophils	9	42.85%	5	23.80%
Increased INR	9	42.85%	0	0
Lymphopenia	5	23.80%	5	23.80%
Nonhematologic Adverse Events				
Hyperglycemia	20	95.23%	2	9.52%
Fatigue (asthenia, lethargy, malaise)	19	90.47%	0	0
Increased AST	17	80.95%	2	9.52%
Increased ALT	15	71.42%	3	14.28%
Anorexia	13	61.90%	0	0
Hypercholesterolemia	13	61.90%	4	19.04%
Vomiting	13	61.90%	0	0
Hypertriglyceridemia	13	61.90%	1	4.76%
Hypokalemia	11	52.38%	2	9.52%

Constipation	10	47.61%	0	0
Nausea	9	42.85%	0	0
Hypernatremia	9	42.85%	1	4.76%
Pain - Head/headache	8	38.09%	0	0
Hypophosphatemia	8	38.09%	1	4.76%
Urinary frequency/urgency	7	33.33%	0	0
Hyponatremia	6	28.57%	3	14.28%
Fever (in the absence of neutropenia)	6	28.57%	0	0
Diarrhea	6	28.57%	0	0
Mood alteration - Agitation	6	28.57%	0	0
Muscle weakness - Whole body/general	6	28.57%	0	0
Hypoalbuminemia	6	28.57%	0	0
Pain - Joint	5	23.80%	0	0
Pain - Stomach	5	23.80%	0	0
Urinary retention	4	19.04%	0	0
Pain - Extremity-limb	4	19.04%	0	0
Hemorrhage, Nose	3	14.28%	0	0
Mood alteration - Anxiety	3	14.28%	0	0
Ocular/Visual - Other (eye discharge)	3	14.28%	0	0

Allergic rhinitis	3	14.28%	0	0
Hypoglycemia	3	14.28%	0	0
Infection w/ ≥ grade 3 neutropenia, Urinary tract NOS	1	4.76%	1	4.76%

Notes: Toxicities considered to be at least possibly related. Non-hematological toxicities seen in > 10

% of subjects