Published in final edited form as:

Anticancer Drugs. 2013 April ; 24(4): 415–421. doi:10.1097/CAD.0b013e32835dc7c5.

Valproic Acid Reduces the Tolerability of Temsirolimus in Children with Solid Tumors

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Abstract

Objectives—A pediatric study has established a maximum tolerated dose (MTD) for temsirolimus (Tem) of more than 150 mg/m^2 IV/week. A phase I trial was conducted to establish the MTD for Tem in combination with valproic acid (VPA) in children and adolescents with refractory solid tumors. Secondary aims included expression of mTOR markers on archival tumor tissue; Tem pharmacokinetics (PK); assessment of histone acetylation (HA); and tumor response.

Methods—Patients were treated with VPA (5mg/kg PO TID) with a target serum level of 75–100 mcg/mL. Tem was started at an initial dose of 60 mg/m^2/week. PK and HA measurements were performed weeks 1 and 5.

Results—Two of the first 3 subjects experienced dose-limiting toxicity (DLT) (grade 3 mucositis). Tem at 35 mg/m^2/week was found to be tolerable. Peak Tem concentrations were higher in all subjects compared to those in previously published reports of single agent Tem. Increases in HA correlated with VPA levels. All tumor samples expressed mTORC1 and mTORC2. An objective response was seen in one patient (melanoma); transient stable disease was

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The authors have no conflict of interest.

seen in 4 other patients (spinal cord ependymoma, alveolar soft part sarcoma; medullary thyroid carcinoma; hepatocellular carcinoma).

Conclusions—The MTD of Tem when administered with VPA is considerably lower than when used as a single agent, with mucositis the major DLT. The combination merits further study and may have activity in melanoma. Attention to drug-drug interactions will be important in future multi-agent trials including Tem.

Keywords

Temsirolimus; Valproic; Pediatrics; Solid Tumors; Phase I

INTRODUCTION

Temsirolimus (Tem) is a selective inhibitor of the mammalian target of rapamycin (mTOR) which has demonstrated tolerability and efficacy against a wide range of adult cancers [1–6]. In adults, mucositis, rash, and asthenia have been the most common dose limiting toxicities (DLT) at doses up to 250 mg/m^2 [7,8]. However, doses of 15 mg/m^2 have been found to have biologic activity [8] and 25 mg/week is the dose recommended for the single agent treatment of advanced clear cell renal cell carcinoma, the US Food and Drug Administration approved indication for Tem [9].

Several mTOR inhibitors have demonstrated significant antitumor activity in both *in vitro* and *in vivo* pediatric solid tumor models, including rhabdomyosarcoma, gliomas, and neuroblastoma [10–12]. A recent phase I–II study of Tem as a single agent in children found the drug to be tolerable when given intravenously in doses as high as 150 mg/m^2/wk, with pharmacokinetics similar to those seen in adults [13].

Valproic Acid (VPA) is a histone deacetylase (HDAC) inhibitor that also has shown *in vitro* and *in vivo* anti-tumor activity against a range of cancers in children [14–16]. This is a drug which has a long history in pediatrics as an anticonvulsant at target serum levels of 50–100 mcg/mL. Both mTOR inhibitors and VPA are inducers of autophagy [17, 18]. Our rationale for combining Tem and VPA was based on the apparently minimal and largely non-overlapping toxicities of each of these drugs as single agents, the long track record of VPA in children, past demonstration of anticancer activity *in vitro* and *in vivo* of these drugs as single agents, and our results of additive effects of these drugs against neuroblastoma *in vitro* (D. Coulter, personal communication). A recent report using a similar combination *in vitro* in prostate cancer also suggests that it may have some additive effects [19]. The current report describes our phase I experience with escalating doses of Tem in combination with VPA.

METHODS

Patients

Eligibility criteria included male or female patients 2 to 18 years of age with radiographic evidence of persistent or progressive histologically verified solid tumor after standard therapy, normal renal, liver, and hematopoietic function using standard criteria (transfusion

support was permitted for patients with marrow involvement), and an age-appropriate performance status of at least 50%. Patients must have been off prior cancer-specific treatment for at least two weeks and must have recovered from prior toxicities. Current use of anticonvulsants including VPA, or use of CYP3A4 inducers or inhibitors and drugs that are CYP2D6 substrates were exclusions. Although the intent was to evaluate response based on the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 [20], this requirement was waived for one patient with measurable disease but with lesions smaller than 10 mm. The study was opened at the University of North Carolina at Chapel Hill in 2009, and at Levine Children's Hospital in 2010. Patients or their legal guardians provided written informed consent approved by the respective institutional review board before study participation.

Pretreatment evaluations included a medical history, physical examination, performance status assessment, a complete blood count (CBC) with differential, serum electrolytes, renal and liver function tests, fasting cholesterol and triglyceride levels, tumor measurements, and serum or urine pregnancy tests for female participants of childbearing age. Peripheral blood was collected for mononuclear cell isolation (below). Archival tumor tissue was obtained for correlative studies (below). Participation was not dependent on mTOR expression.

Study Design

This was an open label single arm dose escalation study of Tem in combination with VPA. Tem was provided by Pfizer (formerly Wyeth) Pharmaceuticals (Philadelphia, PA). VPA was commercially available and begun at a dose of 15 mg/kg/day divided TID orally 3–7 days before starting Tem with the intent to achieve trough plasma levels of 75–100 mcg/mL. Patients were asked to keep daily diaries of VPA use. Tem was administered intravenously over 30–60 minutes weekly following premedication with diphenhydramine (0.5 – 1 mg/kg to a maximum dose of 50 mg). After the first patient developed a moderate infusional reaction, Tem subsequently was infused over 60 minutes without problems. The starting Tem dose was 60 mg/m^2 based on ideal body weight, with the next dose level (dose level -1) of 35 mg/m^2. Achievement of target VPA levels was not required prior to the initiation of Tem, and VPA doses were titrated over time based on levels and toxicity (below).

A minimum of 3 patients assessable for toxicity was planned at each dose level. Patients were considered to have tolerated a given dose level if they had received at least 6 weeks of combination therapy without DLT (defined below). The MTD was to have been defined as the dose level immediately below that at which two of 6 patients experienced DLTs during the first 6 weeks of treatment, and the intent was to treat 6 evaluable patients at the MTD. There was no intra-patient dose escalation. Subjects who experienced a DLT could elect additional treatment on study at the next lower dose of Tem, or could continue combination treatment off study at every other week intervals and/or at lower Tem doses at the discretion of the family and treating clinician. Subjects without DLTs could continue therapy for up to a year or until disease progression.

Evaluation of Toxicity

Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.1. Grade 4 thrombocytopenia or neutropenia of more than 7 days in duration was classified as a hematologic DLT for subjects without marrow involvement by tumor. Nonhematologic DLTs included all grade 3 or 4 Tem-related toxicities resulting in delay in treatment for more than two weeks.

Evaluation of response

Tumor measurements were performed before every fourth month of treatment, in the event of DLT, or if the family elected to come off study after the first 6 weeks. Tumor response was evaluated using RECIST 1.1 [20]. Patients must have been on study for at least 6 weeks to be evaluable for efficacy.

Pharmacokinetic Studies

PK studies to measure Tem levels were performed during weeks 1 and 5. Whole-blood samples (2 mL) were collected in EDTA-treated tubes before Tem administration and at 0.5, 1, 2, 5 and 24 hours after administration. Samples were mixed, transferred into separate polypropylene tubes, and stored at − 20 °C within 60 minutes of the venipuncture. In all but subject #6, PK samples were obtained from a site different from that into which Tem had been administered. Tem concentrations were determined by Liquid Chromatography/Triple Quadrupole Mass Spectrometry (HPLC/MS-MS) using a previously validated method [21]. The HPLC-MS/MS system consisted of two Shimadzu Scientific (Columbia, MD) solvent delivery pumps, a Valco (Houston, TX) switching valve, a thermostated (6 °C) LEAP HTC autosampler (Carrboro, NC), and an Applied Biosystems (Foster City, CA) API3000 triple quadrupole mass spectrometer. Quality controls, also in whole blood, were prepared in triplicate. The lower limit of quantitation (LLQ) was 60 ng/mL with all standards and all controls achieving at least 85% accuracy and precision. A non-compartmental model was fit to the concentration-time data using Phoenix WinNonlin software, version 6.2 (Pharsight, Inc., Cary, NC). Individual subject pharmacokinetic profiles and the parameters of area-under-the-concentration time curve (AUC) 0 to 24 hours post dose, Tmax, Cmax, clearance, volume of distribution (Vd) and half-life were reported using descriptive statistics.

Immunohistochemistry

Slides from diagnostic paraffin-embedded tissue obtained prior to any chemoradiotherapy were stained with primary rabbit antibodies to Raptor (mTORC1), Rictor (mTORC2), and LC3 (Bethyl Laboratories, Montgomery, TX). Antibodies were used at a final dilution of 1:300 in PBS containing 2% horse serum and applied overnight at 4° C. Antibody-antigen complexes were visualized using a DAKO EnVision System HRP (DAB) kit (DAKO, Carpinteria, CA) according to the manufacturer’s protocol. Two slides from each sample were reviewed unblinded (BMMS, JB, SS) and subjectively scored as negative or positive (weak, intermediate, strong) compared to negative and positive control slides (human lung squamous cell carcinoma without and with primary antibody).

Histone acetylation measurements

Heparinized blood was obtained on 3 subjects prior to initiation of VPA, after 3–7 days on VPA but before initiation of Tem, and just before the 2nd or 5th Tem dose. Samples were obtained just prior to the morning dose of VPA. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation and stored at −80 C. Cell extracts, normalized for PBMC number, were separated by SDS-PAGE, transferred to nitrocellulose and blotted with rabbit anti-tetra-acetyl H4 (Active Motif, 1:2000) and rabbit anti-pan-H4 (Millipore, 1:1000). Western blots were scanned and quantified (Licor Odyssey v. 3.0).

RESULTS

Seven patients were enrolled on study over two years. One patient died within two weeks of starting treatment due to tumor progression and was considered to be not evaluable. Characteristics of the 6 evaluable patients are shown in Table 1.

Toxicity

Table 2 summarizes the toxicities observed in patients enrolled on this trial. Grade 3 mucositis was experienced by two of 3 subjects at dose level 1 (60 mg/m^2) and was considered to be dose limiting because it did not improve to grade 1 or 2 within 14 days. Both of these patients had received radiation therapy to the head and neck, though in each case the interval between radiation and Tem was more than a year. Subsequent patients received a decreased Tem dose of 35 mg/m^2. Even at this lower dose, all subjects experienced mucositis limited to grade 1 or 2, with the exception of one subject with grade 3 which did not meet DLT criteria (i.e., the subject recovered within 14 days of treatment and did not require dose or schedule modification of Tem). Other non-DLTs attributed to Tem included acne (n=1), a grade 3 allergic infusional reaction consisting of fever, chills and mild hypotension when Tem was given over 30 minutes (n=1), and chest pain (n=1). Toxicity seemed to correlate with VPA levels >75 mcg/mL.

The first 3 patients also experienced dose-limiting fatigue which was first noted prior to initiation of Tem. Because fatigue is a known side effect of VPA, and was identified as a dose limiting toxicity in a Phase I study of VPA in pediatric patients with solid tumors [16], the dose of VPA was reduced in an effort to preserve Tem dosing. Thus, serum levels below the targeted 75 – 100 mcg/mL were allowed, and grade 3 fatigue rather than serum level was used as the dosing end point. One patient developed grade 2 thrombocytopenia at a VPA level of 56 mcg/mL which resolved after holding VPA for 3 days. Tem was not held and VPA was resumed without a recurrent drop in platelets. One subject (#5) developed grade 3 poorly characterized bilateral plantar pain thought to be due to VPA (which is reported to be a cause of paresthesias), for which both VPA and Tem were suspended for 3 weeks. Because this toxicity was noted at the beginning of the 4th month of treatment and because it was not thought to be attributable to Tem, it was not considered to be a DLT. VPA level was 87 mcg/mL at the time of maximum symptoms. The subject was restarted on a reduced dose of VPA once symptoms had nearly resolved. VPA level after 24 hours at the reduced dose was 52 mcg/mL and Tem was restarted. The patient experienced no recurrence of DLT over her last 4 weeks of treatment. Patient #6 experienced transient grade 2 chest pain within 24

hours of courses 4 and 5 of Tem. Dosing of VPA, with confirmation by review of daily diaries, did not correlate reliably with serum levels in any of the patients. Whether diaries were true reflections of compliance is not clear.

Because of slow accrual, the study was discontinued after only 3 patients had received 35 mg/m^2. Although it is likely that this is the MTD of Tem when given in combination with VPA, the limited number of subjects treated at this dose raises the possibility that toxicity might have been observed eventually.

Immunohistochemistry

Expression of both mTORC1 and mTORC2 was intermediate or strong in pretreatment tumor samples in all patients. None of the pretreatment samples expressed LC3.

Tumor Response

As summarized in Table 3, an objective response (OR) not meeting criteria for a partial response was seen in one child (#3) with progressive metastatic melanoma. After dose limiting mucositis following 3 weeks at the 60 mg/m^2 dose level, Tem was continued at 35 mg/m^2. At his family's discretion, he came off study following 2 months and received treatment every 2 weeks. He had a 15% reduction in tumor size by RECIST criteria after 4 months on treatment. However, at his family's discretion treatment was extended to every 3–4 weeks with minimal increase in tumor size two months later and he came off treatment. He had clear progressive disease 12 months from the start of Tem/VPA. Two other patients treated at 60 mg/m^2 (alveolar soft part sarcoma [n=1] and spinal cord ependymoma [n=1]), and two patients treated at 35 mg/m^2 (MEN2b and metastatic medullary carcinoma of the thyroid, and one patient with metastatic hepatocellular carcinoma) had stable disease for 19, 9, 2, and 8 months, respectively. The last patient came off study at 8 months for quality of life considerations.

Pharmacokinetics

PK parameters were available from cycle 1 in five patients and from cycle 5 in three patients (Table 4). The median C_{max}, AUC, $T_{1/2}$, CL, and V_d increased with dose during the first cycle. Only the CL and V_d increased with dose in cycle 5; however, only one patient was evaluable at the 60 mg/m^2 dose level at cycle 5. Patient #2 had a C_{max} of 1,210 ng/mL at the 60 mg/m^2 dose level and did not receive cycle 5 PK as a result of stopping treatment secondary to a DLT. Of the two patients who received 60 mg/m^2 in cycle 1, the median C_{max} was 1,215 ng/mL, while the three patients who received 35 mg/m^2 in cycle 1 had a median C_{max} of 790 mg/m^2. The median C_{max} reported previously for patients on Tem 25 mg/m^2 and 75 mg/m^2 without VPA was 448 ng/mL and 442 ng/mL, respectively (13). The Cmax for our patients who had received 35 mg/m^2 in cycle 1 ranged from 649–1,260 ng/mL, averaging twice the C_{max} seen at even the 75 mg/m^2 dose level in patients without VPA.

HA measurements

As a marker of HDAC activity, we examined histone H4 acetylation in peripheral blood mononuclear cells isolated from three subjects for whom there were adequate samples that

represented pre-treatment as well as after initiation of VPA and after both VPA and Tem. For two of these subjects, we observed a significant increase in histone acetylation after treatment with both VPA and Tem but not VPA only (Fig 1AB). The one subject for whom histone acetylation was not increased had a low VPA level. As VPA doses were adjusted during the initiation of Tem, data from subject 6 suggest that VPA dose determines histone acetylation, and that plasma levels of >70 mcg/dL are required to inhibit HDAC activity in PBMC (Fig 1C).

DISCUSSION

In this phase I study in children and adolescents with refractory pediatric solid tumors, we demonstrated that a dose of Tem of 35 mg/m^2 appeared to be well tolerated when the drug was combined with VPA. The premature closure of the study due to slow accrual leaves open the possibility that further dose modification might be needed. Nonetheless, the MTD of Tem when given with VPA clearly is significantly lower than the MTD reported in multiple adult trials [1, 8, 9] and in a recent phase I pediatric study of Tem alone (150 mg/m^2) [13]. However, it is well above the 10 mg/m^2 dose identified by those researchers as inducing a complete response (CR) in a child with multiply relapsed neuroblastoma. Previous researchers have shown that doses of Tem as low as 25 mg inhibit mTOR activity and no relationship has been identified between the dose of Tem and the degree of mTOR inhibition [1]. The frequent occurrence of dose limiting mucositis in our study contrasts strikingly with the previously published phase I trial of Tem as a single agent [13], and suggests that VPA might increase the mucosal toxicity of Tem. Of note, several patients who continued for many months on study or off study at lower doses and every other week for prolonged periods after the initial 6 weeks experienced additional side effects, justifying prolonged monitoring of patients on Tem.

Most of our patients experienced some amount of fatigue. We attributed this effect to VPA since this often was noted prior to the initiation of Tem and is a known effect of VPA [16, 22], we attributed the fatigue to VPA. Interference with activities of daily living during the first few weeks of combination therapy required VPA dose reduction. Serum levels often fell well below the targeted levels of 75–100 mcg/mL, and somnolence became our dosing endpoint rather than serum levels. However, this seems to have resulted in reduced VPA levels that were not associated with HDAC activity.

Our PK results offer one explanation for the lower MTD for Tem when combined with VPA as compared with its MTD as a single agent. Tem C_{max} and AUC in children taking VPA was much higher than previously reported in children taking Tem without VPA [13]. Peak Tem levels in the 60 mg/m^2 dose level at both cycles ranged from 757–1,220 ng/mL compared to 369–630 ng/mL seen in children who had received 75 mg/m^2 Tem in the previous study. The higher C_{max} and greater exposure of Tem at the 60 mg/m^2 dose level may have resulted in DLTs. Interestingly, one patient who discontinued treatment due to dose-limiting mucositis had a C_{max} of 1,210 ng/mL during cycle 1, nearly three times the C_{max} reported in patients taking Tem 75 mg/m^2 without VPA. VPA is known to be a broad-spectrum metabolic inhibitor, primarily inhibiting CYP2C9, but having some competitive inhibitory effects on CYP3A4 [23]; Tem is primarily metabolized in the liver by CYP3A4

[21]. These data suggest a possible interaction between Tem and VPA resulting in increased concentrations of Tem.

In a phase I study of VPA alone [16], increased acetylated H4 was observed in half of the subjects associated with levels of 55–100 mcg/mL, although others have suggested that acetylation is not significantly inhibited until levels greater than 100 mcg/mL are achieved [24]. VPA levels in our subjects were quite variable and in all were below 100 mcg/mL. Nonetheless, with limited subjects there appeared to be an association between VPA level and histone acetylation suggesting that Tem might potentiate the affect of VPA on histone acetylation. Additional patients will need to be studied to clarify this interaction. If confirmed, a drug-drug interaction may explain the grade 1 or 2 fatigue which was seen even despite dose reductions and at relatively low plasma levels of VPA.

In the present study, we attempted to preserve Tem by reducing VPA doses. However, this strategy seems to have diminished HDAC activity which could have compromised the biological synergy predicted *in vitro* for combined VPA and Tem treatment. Our MTD of 35mg/m2/wk was met when VPA doses were dropped, using fatigue rather than serum levels as the eventual endpoint. Reduced Tem MTD has also been associated with combination treatment with metformin [25]. Together, these studies suggest that Tem, when given in combination with other agents, may exhibit a unique toxicity profile justifying study-based evaluation.

Because this was designed as a phase I and not a phase II trial, and because of the heterogeneous diagnoses of participating subjects, response data were inconclusive. However, an objective response--while not meeting RECIST criteria for partial response--was observed in one subject with disseminated melanoma. To what extent response was due to the combination rather than single agent activity is not clear from this single arm trial. Lesser responses were observed in children with other diagnoses for which there have been few therapeutic options. Response was not predicted in this small series by mTORC1 and mTORC2 staining, which were convincingly expressed in all pretreatment tumor samples.

The combination of Tem and VPA merits further study and may have activity in melanoma. Attention to drug-drug interactions will be important in future multi-agent trials including Tem. Additional attention should be paid to managing the side effects that might be specific to combination agent therapy in order to maintain the intended biologic effects without significantly compromising quality of life. Phase II trials for diseases such as melanoma for which there are limited single agent data may need to include two arms to compare the efficacy of combination therapy with single agent treatment. Because of concerns about compliance, we remain enthusiastic about intravenous administration of mTOR inhibitors for future studies.

Acknowledgments

This study was conducted with support from Pfizer Pharmaceuticals and UNC’s Clinical Translational Research Center.

The investigators would like to thank Patricia Robinson, R.N. and Teresa Nuttall, R.N. for help coordinating patients; the staff of the UNC Clinical Translational Research Center; and the staff of the Clinical Protocol Office, Lineberger Comprehensive Cancer Center, UNC Chapel Hill, NC.

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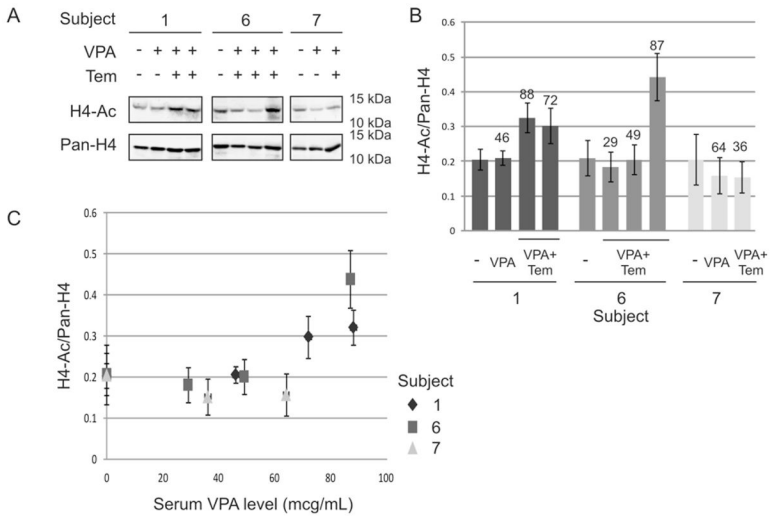

Figure 1. Increased histone acetylation was associated with Tem and VPA treatment

A. Extracts of purified peripheral blood mononuclear cells were immunoblotted for tetra-acetyl H4 and total H4. A representative blot is shown. B and C. Histone acetylation increased after both Tem and VPA treatment in two of three assayable subject. Signal from H4Ac relative to panH4 +/− SEM is shown. Relative acetylation corresponded with VPA levels (shown above bars), and levels of > 70 mcg/dL appear to be required to inhibit HDAC activity in PBMC.

Table 1

Characteristics of evaluable study patients*

Patient	Age (Years), Race, Gender	Diagnosis	Prior Treatments	Dose level of Tem, mg/m^2
1	16AAF	Alveolar Soft Part Sarcoma	ARO197; cyclophosphamide/celebrex; AEWS0031	60
2	13AsWM	Spinal cord ependymoma grade II	S; RT; cyclophosphamide/celebrex/etoposide	60
3	11WM	Melanoma	S; carboplatin/taxol	60
4	13AAWM	Undifferentiated Sarcoma, NOS	S; ifosfamide/doxorubicin, RT; temozolomide/irinotecan; cyclophosphamide/topotecan; cyclophosphamide/celebrex/ etoposide	35
5	18WF	Medullary carcinoma thyroid	S	35
6	17WF	Hepatocellular Carcinoma	Sorafenib, Doxorubicin	35

*Abbreviations: RT=radiation therapy; S=surgery; AA=African American; F=female; M=male; As=Asian; M=male; W=white

Table 2

Toxicity

Patient	Dose mg/m^2	Toxicity			VPA range, mcg/mL (level at time of grade 3 toxicity)
		Grade 1	**Grade 2**	**Grade 3**	
1	60	Elevated AST; rise in creatinine**	Anemia		26–72 (NA)
2	60			Mucositis (oral*, esophageal*, perianal); Anaphylactoid reaction/Fatigue	78–112 (85) (112)
3	60	Anemia, Thrombocytopenia	Pruritis**	Mucositis*/Fatigue	<10–91(91)
4	35	Mucositis/Fatigue			68–108 (108)
5	35		Mucositis; Acne;/Fatigue	Plantar pain**	13–87 (87)
6	35	Mucositis	Chest pain; thrombocytopenia		22–78 (78)

* DLT: dose limiting toxicity;

** after 4 months of therapy

Table 3

Response of Study Patients

Patient	Diagnosis	Dose Tem mg/m^2	Response	Duration of Treatment (on study)/Response (months)
1	Alveolar Soft Part Sarcoma	60	SD	19 (4.5)/19
2	Spinal cord ependymoma	60	SD	13 (1)/3
3	Melanoma	60	OR	6(2)/12
4	Undifferentiated Sarcoma, NOS	35	PD	2.5 (2.5)/na
5	Medullary Carcinoma Thyroid	35	SD	6 (4)/2
6	Hepatocellular Carcinoma	35	SD	7 (7)/7

Abbreviations: NOS: not otherwise specified; OR: objective response; PR: partial response; SD: stable disease; PD: progressive disease; n/a not applicable

Table 4

Summary of Temsirolimus Pharmacokinetic Parameters

Temsirolimus	**Dose level**			
Cycle 1	**35 mg/m^2**		**60 mg/m^2**	
	Median	**Range**	**Median**	**Range**
	N = 3		N = 2	
C_{max}, ng/mL	790	649–1,260	1,215	1,210–1,220
T_{max}, hr	0.66	0.5–1.0	1.0	1.0–1.0
$T_{1/2}$, hr	11.7	8.7–18.1	15.6	8.8–22.3
AUC_{last}, hr* ng/mL	7,554	6,983–9,816	8,330	7,892–8,768
CL, mL/hr	4.5	4.1–6.4	7.1	4.3–9.9
V_d, mL	107	56.5–107	132	126–138
Cycle 5	N = 2		N = 1	
C_{max}, ng/mL	1,094	968–1220	757	N/A
T_{max}, hr	0.5	0.5–0.5	0.5	N/A
$T_{1/2}$, hr	12.9	7.9–17.9	8.4	N/A
AUC_{last}, hr* ng/mL	9,089	7,638–10,539	6,200	N/A
CL, mL/hr	4.4	2.9–6.0	9.3	N/A
V_d, mL	71.0	67.8–74.1	112	N/A

Abbreviations: AUC, area under the concentration-time curve; CL, clearance; C_{max}, maximum concentration; T_{max}, time to C_{max}; $T_{1/2}$, half-life; V_d, steady-state volume of distribution

* Patient #2 did not receive cycle 5 secondary to toxicity

** Patient #3 was excluded from PK analysis secondary to an unexpected rise in temsirolimus concentration at the 24 hour PK sample resulting in inaccurate PK parameters

*** Patient #6, cycle 5 was excluded from PK analysis secondary to lack of 24 hour PK sample