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Phase II trial of temsirolimus in children with high-grade glioma, neuroblastoma and rhabdomyosarcoma ☆

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ABSTRACT

Purpose: A phase II study of temsirolimus was conducted in children and adolescents with high-grade glioma, neuroblastoma or rhabdomyosarcoma.

Patients and methods: Temsirolimus 75 mg/m^2 was administered once weekly until disease progression or intolerance. Using the Simon 2-stage design, further enrolment in each disease cohort required $\geqslant$2 objective responses within the first 12 weeks for the first 12 evaluable patients (those who received $\geqslant$3 temsirolimus doses).

Results: Fifty-two heavily pretreated patients with relapsed (12%) or refractory (88%) disease, median age 8 years (range 1–21 years), were enroled and treated. One patient with neuroblastoma achieved confirmed partial response within the first 12 weeks; thus, none of the 3 cohorts met the criterion for continued enrolment. Disease stabilisation at week 12 was observed in 7 of 17 patients (41%) with high-grade glioma (5 diffuse pontine gliomas, 1 glioblastoma multiforme and 1 anaplastic astrocytoma), 6 of 19 (32%) with neuroblastoma and 1 of 16 (6%) with rhabdomyosarcoma (partial response confirmed at week 18). In the three cohorts, median duration of stable disease or better was 128, 663 and 75 d, respectively. The most common treatment-related adverse events were thrombocytopaenia, hyperlipidaemia and aesthenia. Pharmacokinetic findings were similar to those observed in adults.

Conclusions: Temsirolimus administered weekly at the dose of 75 mg/m^2 did not meet the primary objective efficacy threshold in children with high-grade glioma, neuroblastoma or rhabdomyosarcoma; however, meaningful prolonged stable disease merits further evaluation in combination therapy.

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1. Introduction

Despite 5-year survival rates in children with cancer approaching 80%, children with malignant brain tumours, metastatic neuroblastoma (over the age of 18 months) and soft-tissue sarcomas continue to experience poor outcomes.[1,2] Furthermore, substantial long-term morbidity and mortality are associated with treatment of these childhood cancers.[3–6] New therapeutic strategies are needed to improve survival rates and to reduce morbidity associated with current treatments.

Temsirolimus is an ester of sirolimus (rapamycin) and a specific inhibitor of mammalian target of rapamycin (mTOR), a signalling protein that regulates cell growth, proliferation, angiogenesis and cellular survival pathways.[7–9] Inhibition of mTOR leads to reduced synthesis of cell cycle regulatory proteins and, via inhibition of hypoxia-inducible factor alpha-1 (HIF-1α), reduces expression of vascular endothelial growth factor (VEGF), an important pro-angiogenic factor.[7,8,10,11] mTOR inhibitors have significant antitumour activity in paediatric cancer xenograft models, such as rhabdomyosarcoma, osteosarcoma, medulloblastoma and neuroblastoma, alone and in combination with chemotherapy.[12–16] In malignant glioma cells, silencing mTOR with small interfering ribonucleic acid increases autophagy, which mediates the growth inhibitory effect of rapamycin.[17] In neuroblastomas, the phosphatidylinositol 3-kinase (PI3-K)/Akt/mTOR signalling pathway is constitutively activated, and treatment with temsirolimus downregulates VEGF secretion and cyclin D1, as well as MYCN protein expression.[14] Temsirolimus inhibited tumour growth through an antiangiogenic mechanism and possibly through an insulin-like growth factor–mediated effect linked to the targeting of mTOR/HIF-1 α/VEGF signalling in subcutaneous rhabdomyosarcoma xenografts[15] and experimental lung metastasis in a metastatic murine osteosarcoma model (K7M2).[16] In a xenograft model of human primitive neuroectodermal tumour/medulloblastoma (DAOY), temsirolimus given alone delayed growth by 160% after 1 week and 240% after 2 weeks of treatment; additive antitumour activity was observed when given in combination with cisplatin or camptothecins.[12] Interestingly, temsirolimus also produced growth inhibition of xenografts derived from U251 malignant glioma cells, a human cell line resistant to rapamycin *in vitro*. These findings suggest that mTOR inhibition may be a targeted strategy for treatment of solid tumours in children.

A 2-part phase I/II study of temsirolimus was conducted in children with advanced solid tumours. In part 1, 75 mg/m^2 was selected as the temsirolimus dose for further study in paediatric populations.[18] In part 2, reported here, temsirolimus 75 mg/m^2 was explored in cohorts of children and adolescents with high-grade glioma, neuroblastoma or rhabdomyosarcoma.

2. Patients and methods

2.1. Study objectives

This study aimed to obtain preliminary information on the antitumour activity of temsirolimus in children with refractory or recurrent high-grade glioma, neuroblastoma and rhabdomyosarcoma. Antitumour activity was assessed by determining the proportion of patients with objective response (OR) (i.e. complete response [CR] or partial response [PR]) within 12 weeks. Secondary objectives included assessing safety, pharmacokinetics and proportion of patients exhibiting freedom from progression (i.e. disease stabilisation) at 12 weeks.

2.2. Eligibility criteria

Eligible patients were aged 1 through 21 years with refractory or relapsed high-grade glioma (glioblastoma multiforme, anaplastic astrocytoma and other high-grade gliomas, including diffuse pontine glioma), neuroblastoma or rhabdomyosarcoma. Eligibility and exclusion criteria are summarised in Table 1. Patients or their legal guardians provided written informed consent prior to enrolment.

This study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. The protocol was approved by the institutional review boards/ethics committees of each participating institution; continuing approval was maintained throughout the study.

2.3. Treatment

Temsirolimus 75 mg/m^2 was administered once weekly as an intravenous (IV) infusion over 60 min, with IV diphenhydramine (or comparable antihistamine) given 30 min before administration. One treatment cycle comprised three weekly infusions. Patients received temsirolimus until disease progression or unacceptable toxicity.

Treatment was held in the event of haematologic toxicity (ANC <1000/μL or platelets <75,000/μL) or grades 3–4 non-haematologic toxicity (National Cancer Institute Common Terminology Criteria for Adverse Events [CTCAE], v3.0). Dose reduction was allowed upon recovery to the required ANC and platelet levels or CTCAE grades 0–2 (or to within 1 grade of starting value for preexisting laboratory abnormalities). The next dose was reduced by 50% if the patient had experienced ANC <750/μL, platelets <50,000/μL, or grade 4 CTCAE toxicity, and by 25% if the patient had experienced ANC 750 to 999/μL, platelets 50,000 to <75,000/μL, or grade 3 CTCAE toxicity. If this dose was maintained for 4 weeks without further need for reduction, dose escalation to the prior level could be considered.

2.4. Evaluation of tumour response

Whereas the study was designed to conduct efficacy analyses only in those patients who received ⩾3 doses (i.e. 1 cycle) of temsirolimus, the presented results include data from all 52 enroled patients, all of whom had at least one dose of study drug. Excluding patients who had <3 doses of study drug from analysis could introduce a bias leading to an overestimation of benefit.

Glioma and rhabdomyosarcoma responses were categorised as CR, PR, stable disease (SD) or progressive disease (PD) using Response Evaluation Criteria in Solid Tumours (RECIST).[19] Neuroblastoma responses were categorised as

Table 1 – Eligibility criteria.

Inclusion criteria
Aged 1–21 years
Diagnosis of refractory or relapsed neuroblastoma, high-grade glioma (glioblastoma multiforme, anaplastic astrocytoma or other high-grade glioma), or rhabdomyosarcoma
Histologic confirmation at initial diagnosis (except for patients with diffuse pontine gliomas) and not at the time of relapse
Measurable disease according to RECIST for gliomas and rhabdomyosarcomas or the INRG criteria for neuroblastoma
$\geqslant$3 months since autologous or allogeneic bone marrow or stem cell transplant
$\geqslant$2 weeks since local radiotherapy
$\geqslant$3 months since craniospinal radiotherapy, radiotherapy to whole abdomen or pelvis, whole lungs, >25% of bone marrow reserve, or total body irradiation
$\geqslant$3 weeks since chemotherapy ($\geqslant$6 weeks for nitrosoureas), immunotherapy, or any investigational therapy (defined as treatment not approved for any indication)
Lansky (aged 1–10 years) or Karnofsky (aged 11–21 years) performance status $\geqslant$60%
Absolute neutrophil count $\geqslant$1000/mm^3
Platelet count $\geqslant$75,000/mm^3 ($\geqslant$50,000/mm^3 for patients with bone marrow involvement by tumour)
Haemoglobin $\geqslant$8 g/dL (red blood cell transfusion permitted if bone marrow involved by tumour)
Creatinine clearance (estimated by the Schwartz formula) $\geqslant$lower limit for age or serum creatinine $\leqslant 2\times$ normal for age
Bilirubin $\leqslant 1.5\times$ institutional ULN
SGOT and SGPT $\leqslant 3\times$ ULN.
Life expectancy $\geqslant$2 months
Among patients of childbearing potential or with partners of childbearing potential, willingness to use a reliable birth control method during the study and for 12 weeks after its completion
Exclusion criteria
Known hepatitis B, hepatitis C, or HIV infection
Active infection or serious intercurrent illness
Pulmonary hypertension or pneumonitis
Any other major illness which, in the investigator's judgment, would substantially increase the risk associated with the patient's participation in the study
Concomitant therapy with any other investigational agent
Receiving enzyme-inducing anticonvulsants
Major surgery within 6 weeks prior to study entry
Pregnancy or lactation
Known hypersensitivity to any components in the temsirolimus infusion
Medical reasons for being unable to receive protocol-required premedication
Unwillingness or inability to comply with protocol guidelines

INRG, International Neuroblastoma Risk Group; RECIST, Response Evaluation Criteria for Solid Tumours; ULN, upper limit of normal.

CR, very good partial response (VGPR), mixed response (MR), PR, SD or PD using International Neuroblastoma Staging System criteria. Patients with neuroblastoma who were followed by iodine meta-iodobenzylguanidine (MIBG) scan at the start of treatment were evaluable for MIBG response using International Neuroblastoma Risk Group criteria.[20] MIBG responses were reviewed by the Nuclear Medicine, Radiology Associates of Sacramento Medical Group and categorised as CR, PR or SD using the Curie scale. In all 3 cohorts, efficacy assessments were conducted every 6 weeks. Confirmed CR or PR required a confirmatory scan at least 4 weeks after initial response.

2.5. *Pharmacokinetic studies*

Whole blood samples were taken at the beginning of cycle 2 predose and at 1, 6, 24, 48, 72, 96 and 168 h after the start of the temsirolimus infusion. Concentrations of temsirolimus and its metabolite sirolimus were simultaneously measured using a validated liquid chromatography/tandem mass spectrometry with internal standard and were analysed using a non-compartmental method.[21,22] Parameters included peak concentration (C_{max}), average concentration (C_{ave}), time to C_{max} (t_{max}), half-life ($t_{½}$), area under the concentration–time curve (AUC) measured to last measured time (AUC_T) and through steady-state dosage interval (AUC_{ss}) and clearance (CL). For sirolimus, values of CL are reported as an apparent measure due to normalisation by the unknown fraction of dose metabolised (f_m).

2.6. *Study design and statistical analysis*

This open-label trial was conducted in 31 centres in the United States, Poland, Russia, France and Germany. The primary objective was to determine the proportion of patients with OR to temsirolimus within the first 12 weeks for children in each of 3 disease cohorts: high-grade glioma, neuroblastoma and rhabdomyosarcoma. Using a Simon 2-stage design[23] applied separately to each cohort, with $\leqslant$8% objective response rate (ORR) as unacceptable versus $\geqslant$30% ORR as acceptable and 10% types I and II error rates, a sample size of 25 evaluable patients were required for each of the 3 tumour types: 12 patients were to be enroled during the first stage and at least 2 ORs within 12 weeks were required to enrol an additional 13 patients in the second stage. Thus, it was expected that 25 subjects would be enroled in each of the 3 cohorts. Patients continued to be enroled in each disease cohort until confirmation that 12 evaluable patients had been treated for 12 weeks, at which time enrolment was halted. By this design,

treatment would be deemed ineffective if fewer than 4 responses were observed for a cohort of 25 patients.

3. Results

3.1. Patients

Between March 2006 and May 2008, 52 patients (17 with high-grade glioma, 19 with neuroblastoma and 16 with rhabdomyosarcoma) were enroled and treated. Baseline patient characteristics are listed in Table 2. The median age was 8 years (range 1–21 years). All patients had received chemotherapy, immunotherapy and/or hormonal therapy before entry. Eight (42%) patients with neuroblastoma received 6 or more prior chemotherapy regimens. Six (12%) patients (4 with neuroblastoma and 2 with rhabdomyosarcoma) had relapsed from CR on their last line of therapy prior to enrolment; the other 46 (88%) patients had refractory disease. Most patients (47 [90%]) had good performance status (⩾80%) at inclusion.

Other than the recommended premedication, concomitant medications were taken by all but one patient during the on-therapy phase. The most common therapies were antibiotics, antiemetics, antihistamines, corticosteroids, antacids, opioids, other analgesics and antipyretics.

3.2. Dose intensity and toxicities

The median number of temsirolimus doses received was 6 (i.e. 2 cycles; range, 1–>120 doses), and the median relative dose intensity was 0.95 (range, 0.35–1.08). Ten (19%) patients did not receive 3 doses because of early disease progression or symptomatic deterioration (7 patients), death due to disease progression (1 patient), or discontinuation for surgery or an adverse event (AE; 1 patient each). Nine (17%) patients received treatment for more than 6 months. At the date of analysis, 4 (8%) patients were still ongoing, of whom 2 with neuroblastoma currently remain on treatment.

A greater percentage of patients with neuroblastoma (58%) required a delay in temsirolimus administration versus those with high-grade glioma (41%) and rhabdomyosarcoma (31%). Also, ⩾1 dose reduction was required for 58% of patients with neuroblastoma, 12% with high-grade glioma and 25% with rhabdomyosarcoma. The most common drug-related AEs (Table 3) were thrombocytopaenia (28 patients, 54%), hyperlipidaemia and aesthenia (15 patients each, 29%), and anaemia, leucopenia and hypercholesterolaemia (14 patients each, 27%). Grades 3 or 4 drug-related AEs affected mainly the haematologic and metabolic systems and included thrombocytopaenia (9 patients, 17%), anaemia or increased SGOT or SGPT (4 patients each, 8%), neutropenia (3 patients,

Table 2 – Clinical characteristics of enroled patients.

Characteristic		Cohort			
		High-grade glioma (*n* = 17)	Neuroblastoma (*n* = 19)	Rhabdomyosarcoma (*n* = 16)	Total (*n* = 52)
Sex, *n* (%)	Male	11 (65)	13 (68)	11 (69)	35 (67)
	Female	6 (35)	6 (32)	5 (31)	17 (33)
Age, years	Median	12	7	11	8
	Range	1–21	3–14	1–21	1–21
Race, *n* (%)	White	12 (71)	12 (63)	10 (63)	34 (65)
	Black	3 (18)	4 (21)	3 (19)	10 (19)
	Other/unknown	2 (12)	3 (16)	3 (19)	8 (15)
Body surface area (m^2)	Median	1.5	0.9	1.3	1.1
	Range	0.5–2.2	0.6–2.0	0.6–2.0	0.5–2.2
Performance status,[a] *n* (%)	100	5 (29)	10 (53)	4 (25)	19 (37)
	90	8 (47)	3 (16)	5 (31)	16 (31)
	80	2 (12)	5 (26)	5 (31)	12 (23)
	⩽70%	2 (12)	1 (5)	2 (14)	5 (10)
No. prior chemotherapy regimens, *n* (%)	1	6 (35)	2 (11)	3 (19)	11 (21)
	2	6 (35)	1 (5)	3 (19)	10 (19)
	3	5 (29)	3 (16)	2 (13)	10 (19)
	4	0	3 (16)	2 (13)	5 (10)
	5	0	2 (11)	2 (13)	4 (8)
	⩾6	0	8 (42)	4 (25)	12 (23)
Best response to prior therapy, *n* (%)					
	CR	1 (6)	9 (47)	6 (38)	16 (31)
	PR	4 (24)	6 (32)	6 (38)	16 (31)
	SD	7 (41)	3 (16)	3 (19)	13 (25)
	PD	4 (24)	0	0	4 (8)
	Missing	1 (6)	1 (5)	1 (6)	3 (6)

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.
[a] Lansky Play Scale for children aged 1–10 years (*n* = 30) or Karnofsky Performance Status for adolescents aged 11–21 years (*n* = 21).

Table 3 – Number (%) of patients reporting drug-related adverse events (all grades; >10% of patients).

Adverse event	High-grade glioma ($n = 17$)		Neuroblastoma ($n = 19$)		Rhabdomyosarcoma ($n = 16$)		Total ($n = 52$)	
Grades	All	⩾3	All	⩾3	All	⩾3	All	⩾3
Any adverse event	17 (100)	5 (29)	18 (95)	11 (58)	13 (81)	7 (44)	48 (92)	23 (44)
Thrombocytopaenia	9 (53)	1 (6)	11 (58)	4 (21)	8 (50)	4 (25)	28 (54)	9 (17)
Hyperlipidaemia	4 (24)	2 (12)	7 (37)	0	4 (25)	0	15 (29)	2 (4)
Aesthenia	5 (29)	0	6 (32)	1 (5)	4 (25)	0	15 (29)	1 (2)
Anaemia	1 (6)	0	9 (47)	3 (16)	4 (25)	1 (6)	14 (27)	4 (8)
Leucopenia	2 (12)	0	7 (37)	1 (5)	5 (31)	0	14 (27)	1 (2)
Hypercholesterolaemia	6 (35)	0	7 (37)	0	1 (6)	0	14 (27)	0
SGPT increased	5 (29)	2 (12)	6 (32)	2 (11)	1 (6)	0	12 (23)	4 (8)
Fever	1 (6)	0	7 (37)	1 (5)	3 (19)	0	11 (21)	1 (2)
SGOT increased	3 (18)	2 (12)	5 (26)	1 (5)	2 (13)	1 (6)	10 (19)	4 (8)
Hyperglycaemia	2 (12)	0	5 (26)	0	2 (13)	0	9 (17)	0
Neutropenia	2 (12)	0	5 (26)	3 (16)	1 (6)	0	8 (15)	3 (6)
Anorexia	1 (6)	0	4 (21)	1 (5)	3 (19)	0	8 (15)	1 (2)
Rash	4 (24)	0	2 (11)	0	1 (6)	0	7 (13)	0
Mucositis	2 (12)	0	2 (11)	0	2 (13)	1 (6)	6 (12)	1 (2)
Pain	1 (6)	0	3 (16)	0	2 (13)	0	6 (12)	0

6%) and infection, dysponea, hypoxia, hyperlipidaemia and hypophosphatemia (2 patients each, 4%). Patterns of grades 3 or 4 AEs were comparable among the 3 disease cohorts, except that a greater percentage of patients with neuroblastoma experienced grade 3 or 4 anaemia and neutropenia (16% each). Overall, thrombocytopaenia was the predominant AE requiring dose delay (18 patients; 35%) or dose reduction (13 patients; 25%).

One patient with neuroblastoma discontinued treatment because of grade 4 pneumonitis (listed above as dysponea) considered possibly related to temsirolimus. All 6 deaths that occurred within 30 d of last dose were due to disease progression.

3.3. *Tumour response*

The ORR confirmed within the first 12 weeks of treatment was 1.92% (95% confidence interval (CI), 0.05–10.26). Best overall tumour responses within the first 12 weeks for all 52 individual patients, along with treatment duration, are shown in Fig. 1. One confirmed PR occurred in a patient with neuroblastoma; no ORs were observed in patients with high-grade glioma or rhabdomyosarcoma. The PR was achieved on day 38 in a 4-year-old girl with a primary diagnosis of stage 4 neuroblastoma who relapsed during first-line intensive chemotherapy. She received temsirolimus for 247 d (Fig. 1).

The best tumour response within the first 12 weeks was SD for 18 (35%) patients: 7 (41%) with high-grade glioma (5 with diffuse pontine glioma, 1 with glioblastoma multiforme and 1 with anaplastic astrocytoma), 7 (37%) with neuroblastoma and 4 (25%) with rhabdomyosarcoma (Fig. 1). A 19-year-old male with rhabdomyosarcoma who had SD as best response within 12 weeks went onto experience a confirmed PR, with a 40% reduction in tumour size around day 126 (week 18). He received temsirolimus for 500 d. This PR was not included in the ORR owing to the predefined cut-off for analysis at 12 weeks. Median duration of SD or better was 128 d (95% CI, 97, not applicable) for the high-grade glioma cohort, 663 d (95% CI, 145, not applicable) for neuroblastoma and 75 d (95% CI, 56, 256) for rhabdomyosarcoma. Stable disease or better for more than 6 months was achieved in 3 patients with high-grade glioma and in 5 patients with neuroblastoma, but in only 1 patient with rhabdomyosarcoma. One patient with neuroblastoma continues on treatment, with SD for more than 3 years.

Five patients with SD as best tumour response no longer had SD at 12 weeks (2 patients with neuroblastoma, 3 with rhabdomyosarcoma). At week 12, disease control (i.e. PR + SD) was observed in 7 (41%) patients with high-grade glioma, 6 (32%) with neuroblastoma and 1 (6%) with rhabdomyosarcoma. The PFS rates at 12 weeks (Kaplan–Meier estimates) were 35% (95% CI, 14.5–57.0) for patients with high-grade glioma, 38% (95% CI, 16.2–59.4) for those with neuroblastoma, and 7% (95% CI, 0.4–26.0) for those with rhabdomyosarcoma. Median PFS intervals for the 3 respective cohorts were 60 d (95% CI, 33–127), 63 d (95% CI, 42–663) and 39 d (95% CI, 23–48).

Despite prolonged disease stabilisation, none of the cohorts met the criterion for advancing to the second stage of the Simon 2-stage design (i.e. ⩾2 of 12 evaluable patients in a cohort have an OR within the first 12 weeks). Therefore, enrolment was halted for all 3 tumour types.

3.4. *Pharmacokinetics*

Data for PK assessment were available from 35 patients following the first dose of temsirolimus 75 mg/m^2 in cycle 2. Patients comprised 2 infants or toddlers (aged 28 d–23 months), 20 children (aged 2–11 years), 8 adolescents (aged 12–18 years) and 5 young adults (aged 19–21 years).

Pharmacokinetic parameters for temsirolimus and its major active metabolite, sirolimus, are listed in Table 4. Temsirolimus concentrations appeared to decline in a polyexponential fashion, whereas sirolimus concentrations appeared to decline in a monoexponential fashion. Two patients (1 neuroblastoma, 1 high-grade glioma), both of whom had SD, exhibited unusually high C_{max} and AUC_{ss} values. These higher exposures contributed substantially to interpatient variability in the summary statistics but were not associated with indi-

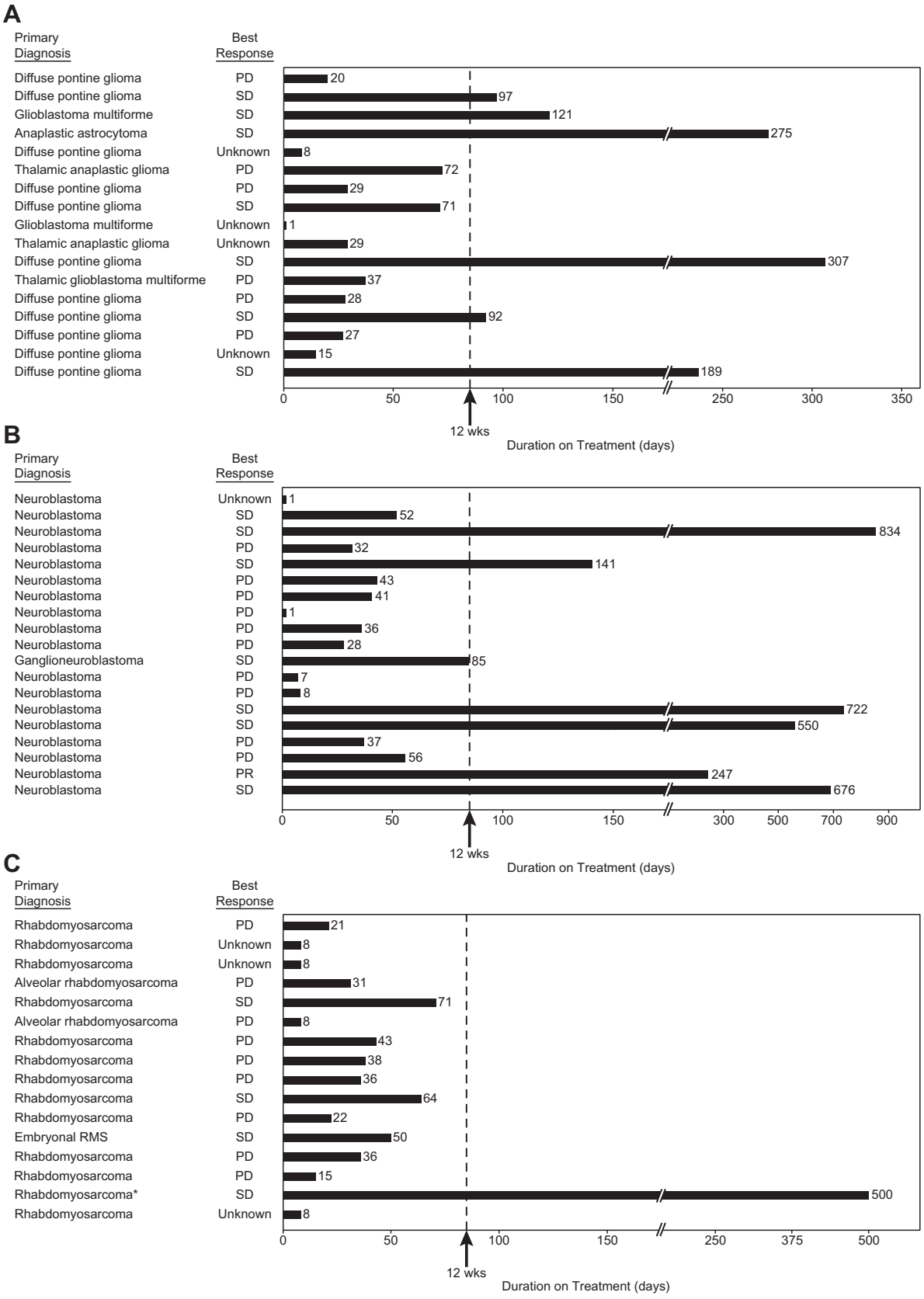

Fig. 1 – Best tumour response within 12 weeks of treatment and duration on treatment for individual patients with high-grade glioma (A), neuroblastoma (B), and rhabdomyosarcoma (C). Of the 8 patients with best tumour response as 'unknown,' 6 discontinued owing to early disease progression or symptomatic deterioration (includes 3 deaths due to disease progression), 1 stopped treatment for surgery, and 1 discontinued because of an adverse event. *Patient achieved confirmed partial response during week 18.

Table 4 – Pharmacokinetics summary for temsirolimus 75 mg/m^2, cycle 2, dose day 1.

Parameter	C_{max} (ng/mL)	C_{avg} (ng/mL)	t_{max} (h)	$t_{½}$ (h)	AUC_T (ng h/mL)	AUC_{ss} (ng h/mL)	CL (L/h)
Temsirolimus							
N	35	31	35	26	35	31	31
Mean	6280	82.8	1.29	30.7	13,100	13,900	14.3
SD	21,000	143	1.20	13.6	22,700	24,100	14.0
Min	117	11.9	0.00	12.0	1990	2000	0.378
Median	1180	32.4	1.00	28.4	5450	5450	11.1
Max	102,000	630	6.00	66.7	106,000	106,000	67.5
CV%	335	173	94	44	173	173	98
Sirolimus							
N	35	34	35	32	35	34	34
Mean	163	49.7	5.29	43.9	7920	8350	9.76
SD	70.7	28.9	8.94	18.2	3710	4860	3.40
Min	77.2	19.4	1.00	24.0	3260	3260	3.97
Median	143	38.8	1.00	39.0	6530	6510	9.46
Max	396	165	46.00	129.5	17,400	27,700	19.1
CV%	43	58	169	41	47	58	35

AUC_{ss}, area under the concentration–time curve (AUC) through steady-state dosing interval; AUC_T, AUC to last measured time; C_{avg}, average concentration; CL, clearance; C_{max}, peak concentration; CV, coefficient of variation; max, maximum; min, minimum; SD, standard deviation; $t_{½}$, half-life; t_{max}, time to C_{max}.

vidual differences in tolerance. For sirolimus, more consistent exposures were apparent for all patients. No trend in relationship could be seen when associating temsirolimus and sirolimus C_{max} or AUC_T with response.

Data from individual plots for temsirolimus C_{max} and AUC_{ss} did not exhibit any trends or relationship to age, weight or body surface area. In contrast, for sirolimus, dose-related trends in steady-state AUC were apparent with increases by age, body surface area and weight. An AUC_{ss} of approximately 6000 ng h/mL was observed for patients aged $\leqslant$12 years with a BSA $\leqslant$1.3 m^2 and body weight <50 kg. Patients aged >12 years with a BSA >1.3 m^2 or body weight >50 kg exhibited an exposure which increased to a plateau of AUC_{ss} of approximately 11,000 ng h/mL.

4. Discussion

The antitumour activity of once-weekly 75 mg/m^2 temsirolimus was evaluated in patients with high-grade glioma, neuroblastoma or rhabdomyosarcoma. The criterion for continuation to stage 2 of the Simon 2-stage design was not met (i.e. $\geqslant$2 ORs within 12 weeks for the first 12 evaluable patients [$\geqslant$17%] in each cohort) and enrolment was halted for all 3 disease cohorts. Disease stabilisation for 12 weeks or longer was observed in all 3 of the disease cohorts (27% of patients), including one confirmed PR within 12 weeks of treatment (neuroblastoma) and 1 PR that was confirmed at 18 weeks (rhabdomyosarcoma).

This was the first study of temsirolimus in children and adolescents with solid tumours, based on preclinical rationale. A possible reason for failure of the trial is that the study design required an OR of at least 17% in heavily pretreated children. However, in adults with cancer, randomised trials have demonstrated that, although objective responses were achieved in some patients, disease stabilisation (including prolonged SD) constitutes a major component of the clinical benefit of temsirolimus.[24,25] In the present study, children with solid tumours experienced prolonged disease stabilisation, suggesting that further study of temsirolimus, alone or in combination with other agents, should be explored.

Disease stabilisation was observed in a significant proportion of children with high-grade gliomas. Five of the 11 (45%) children with progressive/refractory diffuse pontine gliomas experienced durable disease stabilisation with temsirolimus. Diffuse pontine gliomas constitute 60–75% of tumours within the paediatric brainstem.[26] Resection is not a viable option and prognosis is dismal. Loss of phosphatase and tensin homologue (PTEN), a condition that has been suggested to predict for sensitivity to mTOR inhibitors, was recently reported in a high number of newly diagnosed diffuse pontine gliomas,[27] providing a basis for continued study of temsirolimus in this population. Only one patient with anaplastic astrocytoma was enroled, and this patient experienced prolonged SD (275 d) with temsirolimus. Also, 1 of 3 children with glioblastoma multiforme had SD for >3 months, which is consistent with disease stabilisation observed in adults with glioblastoma multiforme treated with 250 mg temsirolimus weekly in a phase II study.[28]

Neuroblastomas have been reported to have an activated PI3-K/Akt/mTOR pathway,[29] suggesting a biologic rationale for the observed activity of temsirolimus. In adults with advanced renal cell carcinoma, patients whose tumours have a highly activated mTOR pathway appear to benefit most from temsirolimus therapy.[30] In relapsed or refractory mantle cell lymphoma, the rationale for treatment with temsirolimus is based on the characteristic overexpression of cyclin D1, a G1 cyclin regulated by mTOR signalling.[31] In the present study, objective responses (1 PR in part 2 and 1 CR in part 1 of the study[18]) were achieved in children with advanced neuroblastoma, and 32% experienced prolonged disease stabilisation. Unfortunately, because *MYCN* amplification status was not collected and biomarkers of the PI3-K/Akt/mTOR pathway were not assessed, possible reasons for the observed antitumour activity in these patients are unknown. Molecular and

biomarker studies are needed to better define the activity of temsirolimus in neuroblastomas.

Although all patients met the eligibility requirement for performance status (60% or higher) and were considered appropriate for treatment by the study investigators, 10/52 patients did not complete at least 3 doses (1 cycle) of treatment. Seven of these patients discontinued owing to early disease progression or symptomatic deterioration, one discontinued due to death from disease progression and one discontinued for surgery. Additionally, one patient with rhabdomyosarcoma discontinued because of grade 3 somnolence and depressed level of consciousness (not related to treatment) and ultimately died of uncal herniation related to tumour progression.

The dose of temsirolimus (75 mg/m^2 once weekly) administered in this study is equivalent to 5 times the recommended dose for adults with renal cell carcinoma (i.e. flat dose of temsirolimus 25 mg once weekly). The safety profile was similar to that reported in adults with cancer, and treatment-associated AEs were primarily mild or moderate. However, a greater incidence of bone marrow involvement and intensity of prior treatment in neuroblastoma patients may have led to more delays and reductions for haematologic toxicity.

Temsirolimus exposures were comparable to those observed in part 1 of the study, although CL was higher in part 2 and AUC_{ss} was slightly lower (3500 ng h/mL; SD = 1140) in part 1.[18] In both parts of this trial, paediatric patients exhibited lower temsirolimus CL and higher AUC than did adults with cancer. Net exposure, as measured by AUC_{sum}, was comparable to that of adults owing to commensurately reduced sirolimus exposures. A parallel population analysis showed that temsirolimus CL (BSA-normalised) as a function of age is relatively constant.[32] These findings collectively suggest that the paediatric population is not at risk for excessive exposure to temsirolimus or its active metabolite, sirolimus. It should be noted that, despite sirolimus exposure in these patients, oral sirolimus has not been studied in this population. There is preclinical evidence that the high blood concentrations achievable with IV temsirolimus may result in mechanistic differences that can enhance antitumour activity.[33]

In conclusion, temsirolimus 75 mg/m^2 had insufficient antitumour activity in children with high-grade glioma, neuroblastoma or rhabdomyosarcoma to continue enrolment for the second stage of the Simon 2-stage design. Temsirolimus was well tolerated, with no new safety concerns identified in paediatric patients. Evidence of meaningful prolonged disease stabilisation in these heavily pretreated children, especially those with high-grade (including pontine) gliomas and neuroblastomas, suggests that investigators should continue to explore temsirolimus in combination with other therapies. Indeed, several ongoing studies are evaluating temsirolimus in combination with chemotherapy or targeted agents in children with refractory solid tumours.[34–42] Characterisation of molecular alterations or biomarkers that may be predictive of response to treatment are important components of these trials.

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This study was sponsored by Wyeth Research, which was acquired by Pfizer Inc. in October 2009. A. Berkenblit provided data acquisition and manuscript preparation and review. J. Boni provided study concepts and design (pharmacokinetics), data analysis and interpretation, and manuscript review. M. Krygowski provided statistical analysis, quality control analysis and interpretation of data, and manuscript preparation and review. R. Ananthakrishnan provided statistical analysis, quality control, analysis and interpretation of data and manuscript preparation, editing and review. J Clancy provided analysis and interpretation of data and manuscript preparation, editing, and review. Christine H. Blood, PhD, of Peloton Advantage provided assistance with manuscript preparation, which was funded by Pfizer Inc.

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Conflict of interest statement

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REFERENCES

1. Kaatsch P. Epidemiology of childhood cancer. *Cancer Treat Rev* 2010;**36**:277–85.
2. Smith MA, Seibel NL, Altekruse SF, et al. Outcomes for children and adolescents with cancer: challenges for the twenty-first century. *J Clin Oncol* 2010;**28**:2625–34.
3. Punyko JA, Mertens AC, Gurney JG, et al. Long-term medical effects of childhood and adolescent rhabdomyosarcoma: a report from the childhood cancer survivor study. *Pediatr Blood Cancer* 2005;**44**:643–53.
4. Armstrong GT, Liu Q, Yasui Y, et al. Long-term outcomes among adult survivors of childhood central nervous system malignancies in the Childhood Cancer Survivor Study. *J Natl Cancer Inst* 2009;**101**:946–58.
5. Armstrong GT, Liu Q, Yasui Y, et al. Late mortality among 5-year survivors of childhood cancer: a summary from the Childhood Cancer Survivor Study. *J Clin Oncol* 2009;**27**:2328–38.
6. Maris JM. Recent advances in neuroblastoma. *N Engl J Med* 2010;**362**:2202–11.
7. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. *Nat Rev Cancer* 2004;**4**:335–48.
8. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell* 2007;**12**:9–22.
9. Gibbons JJ, Abraham RT, Yu K. Mammalian target of rapamycin: discovery of rapamycin reveals a signaling pathway important for normal and cancer cell growth. *Semin Oncol* 2009;**36**:S3–S17.
10. Abraham RT, Gibbons JJ. The mammalian target of rapamycin signaling pathway: twists and turns in the road to cancer therapy. *Clin Cancer Res* 2007;**13**:3109–14.
11. Del Bufalo D, Ciuffreda L, Trisciuoglio D, et al. Antiangiogenic potential of the mammalian target of rapamycin inhibitor temsirolimus. *Cancer Res* 2006;**66**:5549–54.
12. Geoerger B, Kerr K, Tang CB, et al. Antitumor activity of the rapamycin analog CCI-779 in human primitive neuroectodermal tumor/medulloblastoma models as single agent and in combination chemotherapy. *Cancer Res* 2001;**61**:1527–32.
13. Dudkin L, Dilling MB, Cheshire PJ, et al. Biochemical correlates of mTOR inhibition by the rapamycin ester CCI-779 and tumor growth inhibition. *Clin Cancer Res* 2001;**7**:1758–64.
14. Johnsen JI, Segerstrom L, Orrego A, et al. Inhibitors of mammalian target of rapamycin downregulate MYCN protein expression and inhibit neuroblastoma growth in vitro and in vivo. *Oncogene* 2008;**27**:2910–22.
15. Wan X, Shen N, Mendoza A, Khanna C, Helman LJ. CCI-779 Inhibits rhabdomyosarcoma xenograft growth by an antiangiogenic mechanism linked to the targeting of mTOR/HIF-1alpha/VEGF signaling. *Neoplasia* 2006;**8**:394–401.
16. Wan X, Mendoza A, Khanna C, Helman LJ. Rapamycin inhibits ezrin-mediated metastatic behavior in a murine model of osteosarcoma. *Cancer Res* 2005;**65**:2406–11.
17. Iwamaru A, Kondo Y, Iwado E, et al. Silencing mammalian target of rapamycin signaling by small interfering RNA enhances rapamycin-induced autophagy in malignant glioma cells. *Oncogene* 2007;**26**:1840–51.
18. Spunt SL, Grupp SA, Vik TA, et al. Phase I study of temsirolimus in pediatric patients with recurrent/refractory solid tumors. *J Clin Oncol* 2011;**29**:2933–40.
19. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;**92**:205–16.
20. Brodeur GM, Pritchard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *J Clin Oncol* 1993;**11**:1466–77.
21. Punt CJ, Boni J, Bruntsch U, Peters M, Thielert C. Phase I and pharmacokinetic study of CCI-779, a novel cytostatic cell-cycle inhibitor, in combination with 5-fluorouracil and leucovorin in patients with advanced solid tumors. *Ann Oncol* 2003;**14**:931–7.
22. Boni JP, Hug B, Leister C, Sonichsen D. Intravenous temsirolimus in cancer patients: clinical pharmacology and dosing considerations. *Semin Oncol* 2009;**36**:S18–25.
23. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;**10**:1–10.
24. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med* 2007;**356**:2271–81.
25. Hess G, Herbrecht R, Romaguera J, et al. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. *J Clin Oncol* 2009;**27**:3822–9.
26. Frazier JL, Lee J, Thomale UW, et al. Treatment of diffuse intrinsic brainstem gliomas: failed approaches and future strategies. *J Neurosurg Pediatr* 2009;**3**:259–69.
27. Geoerger B, Hargrave D, Thomas F, et al. Innovative therapies for children with cancer: pediatric phase I study of erlotinib in brainstem glioma and relapsing/refractory brain tumors. *Neuro Oncol* 2010;**13**:109–18.
28. Galanis E, Buckner JC, Maurer MJ, et al. Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol* 2005;**23**:5294–304.
29. Brodeur GM. Getting into the AKT. *J Natl Cancer Inst* 2010;**102**:747–9.
30. Pantuck AJ, Seligson DB, Klatte T, et al. Prognostic relevance of the mTOR pathway in renal cell carcinoma. *Cancer* 2007;**109**:2257–67.
31. Coiffier B, Ribrag V. Exploring mammalian target of rapamycin (mTOR) inhibition for treatment of mantle cell lymphoma and other hematologic malignancies. *Leuk Lymphoma* 2009;**50**:1916–30.
32. Liao S, Spunt SL, Shapiro M, Boni JP. *Population pharmacokinetics of temsirolimus in pediatric patients with relapsed/refractory solid tumors*. Presented at: the 35th Annual European Society for Medical Oncology Conference; Milan, Italy, October 8–12; 2010 [abstract].
33. Shor B, Zhang WG, Toral-Barza L, et al. A new pharmacologic action of CCI-779 involves FKBP12-independent inhibition of mTOR kinase activity and profound repression of global protein synthesis. *Cancer Res* 2008;**68**:2934–43.
34. *Temsirolimus and valproic acid in treating young patients with relapsed neuroblastoma, bone sarcoma, or soft tissue sarcoma* [NCT01204450]. Available at: <www.clinicaltrials.gov>; 2011 [accessed April 25, 2011].
35. *Safety and efficacy study of Torisel and liposomal doxorubicin for patients with recurrent sarcoma* [NCT00949325]. Available from: <www.clinicaltrials.gov>; 2011 [accessed April 25, 2011].
36. *Doxil, bevacizumab and temsirolimus trial* [NCT00761644]. Available from: <www.clinicaltrials.gov>; 2011 [accessed April 26, 2011].

37. *Perifosine with temsirolimus for recurrent pediatric solid tumors* [NCT01049841, accessed April 26, 2011].
38. *IMC-A12 in combination with temsirolimus (CCI-779) in patients with advanced cancers* [NCT00678769; NCT00678769]. Available from: <www.clinicaltrials.gov>; 2011 [accessed April 26, 2011].
39. *A phase I Study of IMC-A12 in combination with temsirolimus in pediatric patients with recurrent or refractory solid tumors* [NCT01182883]. Available from: <www.clinicaltrials.gov>; 2011 [accessed April 26, 2011].
40. *Cixutumumab and temsirolimus in treating young patients with solid tumors that have recurred or not responded to treatment* [NCT00880282]. Available from: <www.clinicaltrials.gov>; 2011 [accessed April 25, 2011].
41. *Vinorelbine ditartrate and cyclophosphamide in combination with bevacizumab or temsirolimus in treating patients with recurrent or refractory rhabdomyosarcoma* [NCT01222715]. Available from: <www.clinicaltrials.gov>; 2011 [accessed April 26, 2011].
42. *Temsirolimus, irinotecan hydrochloride, and temozolomide in treating young patients with relapsed or refractory solid tumors* [NCT01141244]. Available from: <www.clinicaltrials.gov>; 2011 [accessed April 26, 2011].