

A Phase I Study of Cixutumumab (IMC-A12) in Combination with Temsirolimus (CCI-779) in Children with Recurrent Solid Tumors: A Children's Oncology Group Phase I Consortium Report

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Abstract

Purpose: To determine the MTD, dose-limiting toxicities (DLT), pharmacokinetics, and biologic effects of cixutumumab administered in combination with temsirolimus to children with refractory solid tumors.

Experimental Design: Cixutumumab and temsirolimus were administered intravenously once every 7 days in 28-day cycles. Pharmacokinetic and biology studies, including assessment of mTOR downstream targets in peripheral blood mononuclear cells, were performed during the first cycle.

Results: Thirty-nine patients, median age 11.8 years (range, 1–21.5), with recurrent solid or central nervous system tumors were enrolled, of whom 33 were fully assessable for toxicity. There were four dose levels, which included two dose reductions and a subsequent intermediated dose escalation: (i) IMC-A12 6 mg/kg,

temsirolimus 15 mg/m²; (ii) IMC-A12 6 mg/kg, temsirolimus 10 mg/m²; (iii) IMC-A12 4 mg/kg, temsirolimus 8 mg/m²; and (iv) IMC-A12 6 mg/kg, temsirolimus 8 mg/m². Mucositis was the predominant DLT. Other DLTs included hypercholesterolemia, fatigue, thrombocytopenia, and increased alanine aminotransferase. Target inhibition (decreased S6K1 and pAkt) in peripheral blood mononuclear cells was noted at all dose levels. Marked interpatient variability in temsirolimus pharmacokinetic parameters was noted. At 8 mg/m², the median temsirolimus AUC was 2,946 ng • h/mL (range, 937–5,536) with a median sirolimus AUC of 767 ng • h/mL (range, 245–3,675).

Conclusions: The recommended pediatric phase II doses for the combination of cixutumumab and temsirolimus are 6 mg/kg and 8 mg/m², respectively. *Clin Cancer Res*; 1–8. ©2014 AACR.

Introduction

Deregulated insulin-like growth factor signaling through the type 1 receptor is common to many childhood solid tumors (1), including Ewing sarcoma (2), rhabdomyosarcoma (3), osteosarcoma (4, 5), neuroblastoma (6–8), brain tumors (9–13), Wilms tumor (14), and hepatoblastoma (15). Similarly, the cell signaling pathways that activate the mTOR are altered in many pediatric cancers, making them susceptible to mTOR inhibition by siroli-

mus and its analogues (16–18). Although we have previously shown preclinical activity of mTOR inhibitors in pediatric cancers (17), monotherapy with these agents has had limited clinical activity (16, 19). This may, in part, be explained by a feedback loop in which inhibition of mTOR signaling leads to upregulation of IGFIR signaling through loss of S6K1 phosphorylation, leading to activation of PI3K and Akt (20–22). Indeed, Akt activation has been demonstrated in cancer cell lines and in patients treated with mTOR inhibitors (23). IGFIR inhibition should prevent rapamycin-induced Akt activation, hence abrogating at least one survival pathway and synergizing with mTOR inhibitors (20, 21, 24).

Cixutumumab (IMC-A12) is a fully recombinant IgG1 monoclonal antibody that specifically targets the human IGFIR1 with high affinity. It acts as an antagonist of IGF1 and IGFII ligand binding and signaling. It also blocks ligand binding to IGFIR and inhibits downstream signaling of the two major insulin-like growth factor pathways: MAPK and PI3K/AKT. Cixutumumab has potent dose-dependent *in vitro* and *in vivo* antitumor activity in a variety of cell lines and xenografts.

Temsirolimus is a small-molecule inhibitor of mTOR. Like sirolimus and everolimus, temsirolimus forms a gain-of-function complex with FK506-binding protein 12 (FKBP12) that binds and inhibits mTOR, leading to antiproliferative effects, including G₁-phase cell-cycle arrest (25), and apoptosis. The primary downstream targets of mTOR include eIF4E-binding protein (4E-BP1; refs. 26, 27) and p70S6 kinase, important in the translation regulation of mRNA encoding proteins involved in G₁ phase

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Translational Relevance

Deregulated insulin-like growth factor signaling through the type 1 receptor is common to many childhood solid tumors. Similarly, the cell signaling pathways that activate the mTOR are altered in many pediatric cancers. IGF1R inhibition should prevent rapamycin-induced Akt activation, hence abrogating at least one survival pathway and synergizing with mTOR inhibitors. This first pediatric phase I combination study established the recommended pediatric phase II doses for cixutumumab and temsirolimus at 6 mg/kg and 8 mg/m², respectively, with mucositis as the dose-limiting toxicity. Target inhibition (decreased p-S6K1 and p-Akt) in peripheral blood mononuclear cells was noted at all dose levels. One partial response was reported in a patient with medulloblastoma; prolonged stable disease was noted in 3 patients (median, 12 cycles). These results lay the groundwork for additional combination studies with IGF1R and mTOR inhibitors. A phase II study of this combination has recently been completed by the Children's Oncology Group (COG).

progression. mTOR inhibitors have potent *in vitro* activity against many human cancer cell lines and xenograft models. The Pediatric Preclinical Testing Program (17, 28) and others have reported preclinical single agent and synergistic combination activity of these agents in many solid tumors (24, 29, 30).

We report the results of a phase I trial of cixutumumab in combination with temsirolimus in children with recurrent or refractory solid tumors. The primary objectives were to estimate the MTD, determine dose-limiting toxicities (DLT), and characterize the pharmacokinetics of IMC-A12 and temsirolimus administered once weekly in combination, to children with refractory solid tumors. The secondary objectives were to assess the biologic activity of temsirolimus by measuring levels of phospho-S6^{Ser235/236}, phospho-AKT^{Ser473}, and phospho-4EBP1^{Ser65} in peripheral blood mononuclear cells (PBMC).

Patients and Methods

Patient eligibility

Patients >12 months and <22 years with measurable or evaluable solid tumors refractory to therapy were eligible. Histologic verification of malignancy was required except for patients with intrinsic brainstem glioma. Other eligibility criteria included Lansky or Karnofsky score ≥ 50 ; recovery from the acute toxic effects of prior therapy; ≥ 3 months since total body irradiation, craniospinal or hemi-pelvic radiation, and ≥ 2 months since a

stem cell transplant; adequate bone marrow function [peripheral absolute neutrophil count (ANC) $\geq 1,000/\mu\text{L}$, platelets $\geq 100,000/\mu\text{L}$ (transfusion independent), hemoglobin $\geq 8.0 \text{ g/dL}$]; adequate renal function (age-adjusted normal serum creatinine or a glomerular filtration rate $\geq 70 \text{ mL/min/1.73 m}^2$); adequate liver function [total bilirubin $\leq 1.5\times$ institutional upper limit of normal for age, SGPT (alanine aminotransferase, ALT) $\leq 5\times$ institutional upper limit of normal for age and albumin $\geq 2 \text{ g/dL}$]; prothrombin time and international normalized ratio $< 1.2\times$ upper limit of normal. Patients receiving corticosteroids had to be on a stable or decreasing dose for ≥ 7 days before study enrollment.

Patients were excluded if they had known bone marrow involvement; had received prior temsirolimus or monoclonal antibody therapy targeting IGF1R; were pregnant or lactating; had an uncontrolled infection; were receiving enzyme inducing anti-oncologics (EIAO), insulin, growth hormone therapy, or any of the following CYP3A4 inducers or inhibitors: erythromycin, clarithromycin, ketoconazole, azithromycin, itraconazole, grapefruit juice, or St. John's wort or other noncytotoxic anticancer agents. Also excluded were patients with a history of allergic reactions attributed to compounds of similar chemical or biologic composition to cixutumumab or temsirolimus, or patients who had undergone major surgery within 6 weeks before study enrollment. The Institutional Review Boards of participating institutions approved the protocol. Informed consent and assent, as appropriate, were obtained according to local institutional guidelines.

Drug administration

Cixutumumab [5 mg/mL (250 mg) or 10 mg/mL (500 mg vials)] and temsirolimus (25 mg/mL), supplied by the Cancer Therapy Evaluation Program (NCI, Bethesda, MD), were administered weekly as a 1 hour and 30 minute infusion, respectively, in 28-day cycles. In the absence of disease progression, patients could receive a total of 25 cycles of therapy.

The starting dose of cixutumumab was 6 mg/kg (one dose level below the recommended phase II dose of 9 mg/kg in the pediatric phase I study; ref. 31) and of temsirolimus was 15 mg/m², with planned dose escalations to a maximum of 9 mg/kg for cixutumumab and 35 mg/m² for temsirolimus. The starting dose of temsirolimus was based on published studies in adult combination trials (32). Because of toxicity, the study was amended to incorporate several dose de-escalations as shown in Table 1.

Trial design

Toxicities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v3.0. (<http://ctep.cancer.gov/forms/CTCAEv3.pdf>). Hematologic DLT was defined as grade 4 neutropenia for >7 days, or before drug administration on days 8,

Table 1. Dose-limiting toxicity

Dose level	IMC-A12 (mg/kg)	Temsirolimus (mg/m ²)	Number of patients entered	Number of patients evaluable	Number of patients with DLT	Type of DLT (n)
1	6	15	15	12	5	Platelets (1), fatigue (1), mucositis (1), ALT (1), hypercholesterolemia (1)
0	6	10	6	6	2	Mucositis/stomatitis (2)
-1	4	8	4	3	0	
Intermediate	6	8	8	6	1	Mucositis (1)
Expanded PK cohort	6	8	6	6	0	

Abbreviation: PK, pharmacokinetic.

15, or 21; platelet count $<20,000/\text{mm}^3$ on 2 separate days or requiring platelet transfusions on 2 separate days within a 7-day period, or myelosuppression that caused a delay of ≥ 14 days between treatment cycles. Nonhematologic DLT was defined as grade 3 or 4 nonhematologic toxicity with the specific exclusion of grade 3 nausea and vomiting of <3 days duration, grade 3 transaminase elevations that returned to grade ≤ 1 or baseline before the time for the next treatment cycle, grade 3 fever or infection, nonhematologic toxicity that caused a delay of ≥ 14 days between treatment cycles.

Any patient who experienced DLT at any time during protocol therapy was considered fully evaluable for toxicity. Patients without DLT who received at least 85% of the prescribed dose during the first 28-day cycle were considered evaluable for toxicity as well as response. Dose escalation proceeded using a modified 3 + 3 cohort design in which 3 patients were initially studied at each dose level. If none of these 3 patients experience DLT, the dose was escalated to the next higher level. If one of three patients experienced DLT, then up to three more patients were accrued at the same dose level. If none of these three additional patients experienced DLT, then the dose was escalated. If one or more of these three additional patients experience DLT, the MTD was exceeded, unless one of the DLTs did not appear to be related to dose or the DLTs were of different classes and the toxicities were readily reversible. In that circumstance, the cohort could be expanded to 12. The MTD was the maximum dose at which fewer than one third of patients experienced DLT. Once the MTD for the combination was defined, up to 6 patients with recurrent or refractory solid tumors were to be enrolled to acquire additional pharmacokinetic data in patients <12 years.

Response evaluation

Disease evaluations were obtained at baseline, at the end of cycle 1, and after every other cycle. Tumor response was reported using the RECIST (33).

Pharmacokinetics studies

Blood samples (5 mL) for pharmacokinetic analysis of cixutumumab were obtained before administration on days 1, 8, 15, 22, and 28 of cycle 1, and days 15 and 28 of cycle 2. In patients who agreed to have additional pharmacokinetic sampling, 3 mL of blood were collected at the end of the day 1 infusion, and 1, 3, 6, and 24 (± 2) hours, and 4 days (± 1 day) after the first dose. All blood samples were collected at a site distant from the infusion, in tubes without anticoagulant, allowed to clot at room temperature, centrifuged at 1,500 rpm for 15 minutes, and stored at less than -20°C . A validated ELISA was used to quantify levels of cixutumumab as previously described (31).

Blood samples (1 mL) for pharmacokinetic analysis of temsirolimus were obtained before temsirolimus administration on days 1 and 28 of cycle 1, and days 15 and 28 of cycle 2. In patients who agreed to have additional pharmacokinetic sampling, blood was collected prior at the end and at 15 minutes, 30 minutes, 1, 3, 6, and 24 (± 2) hours after the infusion on day 1. All blood samples were collected at a site distant from the infusion.

Temsirolimus and sirolimus concentrations were determined using validated isotope-dilution high-performance liquid chromatography-tandem mass spectrometry assays based on previously described principles (34).

The maximum plasma concentration (C_{max}) and time to maximum plasma concentration (T_{max}) were determined by visual

inspection of the plasma concentration-time profiles for subjects participating in the extended pharmacokinetic sampling. Temsirolimus clearance (CL), steady-state volume of distribution ($V_{d_{\text{ss}}}$), half-life ($t_{1/2}$), and area under the plasma concentration versus time curve (AUC_{0-24} and $\text{AUC}_{0-\infty}$) were analyzed using standard noncompartmental methods (NCA; Phoenix WinNonlin, Pharsight Corp). Sirolimus pharmacokinetic parameter estimates for $t_{1/2}$ and $\text{AUC}_{0-\infty}$ were generated using model-based Bayesian analysis (MW/Pharm; Version 3.60, Mediware; ref. 35).

Biologic assays

Whole blood (4 mL) was collected in BD Vacutainer CPT sodium citrate tubes before administration of protocol therapy on days 1, 8, and 28 of cycle 1 in consenting patients. Within 1 hour of acquisition, PBMC lysate was prepared as described (36), snap-frozen in liquid nitrogen, and stored at -80°C until phosphoprotein analysis. Phosphorylated and total S6K1, Akt, and 4E-BP1 detection and quantification were performed via Western blot analysis followed by densitometric analysis with ImageQuant 5.0 (Molecular Dynamics). The ratio of phosphorylated protein to total (phosphorylated and unphosphorylated) protein in each patient sample was then calculated.

Results

Of 39 patients enrolled, all were eligible, six were not fully assessable for toxicity: two never received protocol therapy because of clinical deterioration, two experienced progressive disease during cycle 1, and two withdrew consent for non-DLTs [grade 2 ALT/AST (aspartate aminotransferase; $n = 1$) and grade 2 mucositis ($n = 1$)]. Table 2 summarizes the characteristics of the eligible patients. Patients received a median of 2 cycles (range, 1–20) of therapy.

Toxicity

Table 1 summarizes the DLTs across all dose levels. At the starting dose [cixutumumab (6 mg/kg); temsirolimus (15 mg/m^2)], one patient in the initial cohort of three had dose-limiting hypercholesterolemia (grade 3). The cohort was expanded to include three more evaluable patients, one of whom had dose-limiting mucositis (grade 3). Because the observed DLTs observed were readily reversible, of different classes and unrelated to dose, the statistical design allowed enrollment of up to six additional patients at this dose level. Three different DLTs were observed among this expanded cohort of 6 (grade 3 ALT, fatigue, and prolonged thrombocytopenia >14 days), resulting in a dose de-escalation. At dose level 0 [cixutumumab (6 mg/kg); temsirolimus (10 mg/m^2)], 1 of 3 patients experienced grade 3 dose-limiting mucositis. Three additional patients were enrolled, one of whom experienced grade 3 dose-limiting mucositis. As a result, the protocol was amended to incorporate dose level -1 in which the doses of both agents were further decreased [cixutumumab (4 mg/kg) and temsirolimus (8 mg/m^2)]. None of the patients at dose level -1 experienced a DLT. An intermediate dose level [cixutumumab (6 mg/kg) and temsirolimus (8 mg/m^2)] was subsequently added and one of six patients experienced grade 3 mucositis; none of 6 patients experienced a DLT among an expanded cohort for children <12 years.

Table 3 summarizes all non-DLTs at least possibly attributable to cixutumumab or temsirolimus in the 33 patients fully assessable for toxicity.

Table 2. Characteristics of eligible patients ($n = 39$)

Characteristic	Number
Age (years)	
Median (range)	11.8 (1.0–21.5)
Male:female	20 (51%):19 (49%)
Diagnosis	
Rhabdomyosarcoma	9
Osteosarcoma	6
Ewing sarcoma	5
Malignant glioma (including GBM)	4
Medulloblastoma	2
Wilms tumor	2
Desmoplastic small round cell tumor	2
Astrocytoma NOS	1
Choroid plexus papilloma	1
Neuroblastoma	1
Neurilemmoma	1
Ganglioneuroblastoma	1
Gastrointestinal stromal tumor	1
Hepatocellular carcinoma	1
Atypical teratoid rhabdoid tumor	1
Epithelioid sarcoma	1
Prior therapy	
Chemotherapy regimens: median (range)	2 (0–7)
Prior radiotherapy	28

Abbreviations: GBM, glioblastoma multiforme; NOS, not otherwise specified.

Responses

One partial response, confirmed on central review, was observed following cycle 1 in a patient with medulloblastoma enrolled at dose level 1. Unfortunately, tumor progression was noted on surveillance imaging studies performed after cycle 4. Stable disease for ≥ 3 cycles was observed in 3 patients (median, 12 cycles; range, 11–20) with epithelioid sarcoma, neuroblastoma, and alveolar rhabdomyosarcoma, respectively.

Pharmacokinetics

Cixutumumab single-dose pharmacokinetics were studied in 19 patients, and steady-state pharmacokinetics were studied in 36 patients who received the combination of cixutumumab with temsirolimus. There were no changes in cixutumumab clearance as the temsirolimus dose was increased (Table 4). The median weight-adjusted clearance and half-life values for all patients were 0.17 mL/hr/kg (range, 0.07–0.43 mL/hr/kg) and 122 hours (range, 64–446 hours), respectively. Cixutumumab serum trough concentrations (C_{trough}) exhibited marked interpatient variability, increased throughout the first cycle of treatment (Supplementary Fig. S1), and a median C_{trough} of approximately 200 $\mu\text{g/mL}$ was achieved on day 28 following a 6 mg/kg dose (Supplementary Table S1). The median cixutumumab C_{trough} at the recommended phase II dose was 197 $\mu\text{g/mL}$ (range, 60–544).

Results of the pharmacokinetic data ($n = 19$) following the first dose of temsirolimus at 8, 10, and 15 $\text{mg/m}^2/\text{dose}$ are summarized in Table 5. There was marked interpatient and interdose group variability. Sirolimus, the metabolite of temsirolimus, exposure did not appreciably increase with dose. The steady-state volume of distribution (V_{ss}) was large and increased with dose, ranging from 46.3 L (after 8 mg/m^2 dose) to 74.9 L (after 15 mg/m^2 dose). Similarly, temsirolimus clearance from whole blood increased with increasing dose, from 2.3 L/h after an 8 mg/m^2 dose to 6.7 L/h after a 15 mg/m^2 dose.

mTOR pathway activity

At the recommended phase II dose, a total of 23 PBMC samples were obtained from 9 consenting patients. Four patients had suitable samples from the days 1, 8, and 28 timepoints, three had samples from days 1 and 8, and one had samples from days 1 and 28. Baseline variation in total S6K1 protein was observed

Table 3. Non-DLTs related to protocol therapy observed in evaluable patients ($n = 33$)

Toxicity type	Maximum grade of toxicity across cycle 1 (total, 33 cycles)				Maximum grade of toxicity across cycles 2 to 20 (total, 77 + 22 follow-up cycles)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic								
Anemia	11	2	3		5	7		
Lymphocyte count decreased	11	4	1	1	8	2		2
Neutrophil count decreased	3	7	3	1	3	4		1
Platelet count decreased	17	3	4		9	1		2
White blood cell decreased	8	6	2		6	3		2
Nonhematologic ^a								
ALT increased	9	2			3	3		1
AST increased	6	2			4	3		
Cholesterol high	13	2			5	1		
Constipation	3	1						
Creatinine increased	4				1			
Fatigue	7	1			4			
Headache	5	1			2			
Hyperglycemia	15	6			6	6		
Hypermagnesemia	4				2			
Hypertriglyceridemia	14	4			7	3		
Hypocalcemia	6	1			4			
Hypokalemia	4				3			
Hyponatremia	5				5			
Hypophosphatemia	7	2	3		1	2		2
Mucositis oral	5	5			3	1		2
Nausea	6	3			3	1		1
Proteinuria	1	3				1		
Rash acneiform	5	1						
Weight loss	5	1			1	2		

^aNonhematologic toxicities are those that occurred in $>10\%$ of patients as determined in the first cycle of therapy.

Table 4. Summary of cycle 1 IMC-A12 pharmacokinetic parameters

IMC-A12 dose Temsirolimus dose	6 mg/kg 8 mg/m ² (n = 8)		6 mg/kg 10 mg/m ² (n = 3)		6 mg/kg 15 mg/m ² (n = 4)	
	Median	Range	Median	Range	Median	Range
C _{max} (μg/mL)	281	239–355	277	219–390	230	161–464
T _{max} (h)	2.0	1.0–7.0	1.1	1.1–2.2	2.1	1.1–7.0
t _{1/2} (h)	98	64–161	122	88–133	130	98–446
AUC _{0–7d} (h • μg/L)	20.6	11.6–29.7	25.6	20.6–28.7	26.4	14.8–34.0
CL (mL/h)	8.3	1.1–15.2	9.9	2.9–14.5	7.0	3.1–14.3
CL (mL/h/kg)	0.19	0.12–0.43	0.17	0.12–0.18	0.13	0.07–0.24
Vd _{ss} (mL)	1050	150–2,390	1750	470–1,840	1860	590–2,400
Vd _{ss} (mL/kg)	25.8	20.8–40.3	22.7	20.7–29.4	31.1	18.6–42.3

among patients, but inpatient levels were consistent at each time point (days 1, 8, and 28). Phospho-S6K1 (T389) was reduced at day 8 in the seven evaluable patients when compared with baseline (day 1), and in the majority of cases was further reduced at day 28; in only one patient was S6K1 phosphorylation higher at day 28 when compared with baseline. Unlike S6K1, total and phospho-4EPB1 (S65) followed expression levels at each time point, with no observed change in phosphorylation as a function of treatment on days 8 or 28. Akt phosphorylation (S473) was more variable than S6K1, showing a reduction at day 8 in five patients, and slight or no increase in two others. By day 28, Akt phosphorylation in the five evaluable patients remained variable, with evidence of an increase in three patients, and decrease in the other two, when compared with baseline. Figure 1 depicts a Western blot of representative patient PBMC samples showing inhibition of target proteins p-S6K1 T389 and p-AKT S473.

Discussion

The recommended pediatric phase II doses of the combination of cixutumumab and temsirolimus in children with refractory solid tumor are 6 mg/kg and 8 mg/m², respectively. Similar to adults, observed DLTs included mucositis, fatigue, hypercholesterolemia, and transaminitis. However, the MTD of temsirolimus for this combination in children was significantly lower than observed in adults with advanced cancers (37), relapsed Ewing sarcoma [ref. 38; cixutumumab 6 mg/kg i.v. and temsirolimus 25-mg flat dose (14 mg/m²)], or metastatic prostate cancer [cixutumumab 6 mg/kg i.v. and temsirolimus 20-mg flat dose

(11 mg/m²); ref. 39]. It was noted in the prostate cancer trial that the weekly schedule was difficult to maintain long term due to chronic toxicities. Pharmacokinetic data were not available for any of the adult trials. Interestingly, in the adult trials, grade 3 mucositis was observed in 3% to 27% of patients (37–39). In our study, 4 of 27 patients or 15% experienced grade 3 mucositis, leading to repeated dose de-escalations of the temsirolimus and cixutumumab dose.

Although the pharmacokinetics of cixutumumab (31) and temsirolimus (40) have been previously reported in single-agent pediatric studies, this is the first description of the disposition of these drugs when used in combination in children. Cixutumumab clearance, C_{max}, and half-life at 6 mg/kg when combined with temsirolimus at the MTD of 8 mg/m² were similar to those reported earlier in the pediatric phase I single-agent study of cixutumumab (31). Furthermore, cixutumumab half-life and steady-state trough concentrations of 122 hours and 197 mg/mL, respectively, at 6 mg/kg were similar to the values of 209 hours and 145 mg/mL reported for adults at that same dose (41).

Although there was large interpatient and interdose variability in pharmacokinetic parameter estimates for temsirolimus and its active metabolite sirolimus, the temsirolimus and sirolimus AUCs did not increase significantly with dose, which has previously been reported (42, 43). The steady-state volume of distribution (V_{ss}) of temsirolimus was generally large, increased with dose, and ranged from 230 L (after a 25-mg dose) to as high as 900 L (after a 250-mg dose). The distribution of temsirolimus into red blood cells appeared to be saturable at higher doses. In addition, the clearance from whole blood increased with increasing dose, as

Table 5. Summary of cycle 1 pharmacokinetic data for temsirolimus and its metabolite

Temsirolimus dose	8 mg/m ² (n = 11)		10 mg/m ² (n = 3)		15 mg/m ² (n = 5)	
	Median	Range	Median	Range	Median	Range
Temsirolimus						
C _{max} (ng/mL)	509	187	2,010	606	283	675
T _{max} (h)	0.8	0.5	1.2	0.7	0.6	1.3
CL (L/h)	2.3	1.1	8.2	5.5	1.5	11.4
Vd _{ss} (L)	46.3	6.2	124.5	50.4	40.5	68.1
t _{1/2} (h)	10.3	5.3	45.9	10.5	3.8	18.4
AUC _{0–24h} (h • ng/mL)	2,022	698	5,194	2,654	1,650	2,975
AUC _{inf} (h • ng/mL)	2,946	937	5,536	3,646	1,666	4,820
Sirolimus						
C _{max} (ng/mL)	20.3	3.4	83.2	21.3	10.2	28.1
T _{max} (h)	1.6	1.0	6.5	1.7	1.3	1.7
t _{1/2} (h)	51.0	25.4	126.8	68.9	60.8	71.0
AUC _{0–24h} (h • ng/mL)	273	77	1,252	285	160	353
AUC _{inf} (h • ng/mL) ^a	767	245	3,675	1,268	551	3,210
Sum AUC _{T+S} (h • ng/mL)	5,750	1,182	8,932	4,914	4,876	5,371
Ratio Sir/Tem	0.3	0.1	1.7	0.3	0.1	1.9

^aInf = from time zero to infinity (0–∞).

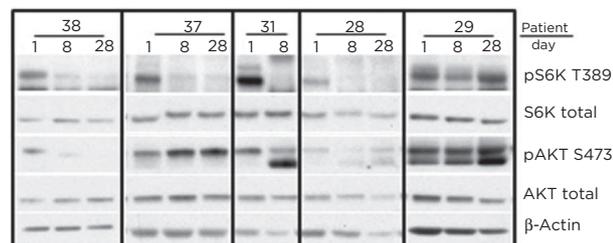


Figure 1.

Western blot of representative patient samples showing inhibition of target proteins pS6K1 T389 and pAKT S473 along with the respective total protein. Samples were taken before the first dose was administered (1) and on days 8 and 28 after treatment.

observed with V_{ssr} , with mean values ranging from 20 L/h after a 25-mg dose to 100 L/h after a 250-mg dose (coefficient of variation, approximately 16%–27%; ref. 43). We compared the pharmacokinetic parameters in the pediatric single-agent temsirolimus phase I trial with the current study. Spunt and colleagues (40) reported no MTD for temsirolimus at dose levels ranging from 10 to 150 mg/m². The median temsirolimus C_{max} and AUC values at the MTD of 8 mg/m² in our study were comparable with median values reported at the 25 and 75 mg/m² dose level reported by Spunt and colleagues. By contrast, the sirolimus AUC and C_{max} were substantially lower in our study at similar doses, with lower sirolimus to temsirolimus ratios at all dose levels tested. The AUCs and C_{max} of temsirolimus and AUCs and C_{min} of sirolimus and cixutumumab observed concentrations that did not show an association with toxicity or inhibition of target. The lower MTD observed in this study is possibly pharmacodynamic in nature and related to the combination of IGF1R and m-TOR inhibition, but requires further evaluation in a larger patient cohort. As these inhibitors affect signaling pathways that regulate glucose, lipid, and energy metabolism, it is possible that children may be biologically more sensitive to these drugs, particularly in combination; however, this determination would require further evaluation in a larger patient cohort.

We demonstrated that this combination significantly inhibited the phosphorylation of S6K1, but not 4E-BP1 in PBMCs at all dose levels. Phospho-S6K1 was reduced at day 8 in the 7 patients with evaluable samples, and was further reduced in 4 of 5 patients with samples at day 28. Although S6K1 and 4E-BP1 are both downstream effectors of mTORC1, these data are consistent with reports that in some cells, rapamycin and the rapalogs are incomplete inhibitors of mTORC1, affecting S6K1 phosphorylation more strongly than 4E-BP1 (44). Similar to previous pediatric and adult reports (40, 45), the phosphorylation status of S6K1, 4E-BP1, and Akt did not correlate with objective responses or prolonged stabilization of disease, limiting their use as biomarkers. There was considerable variation in Akt phosphorylation on days 8 and 28, with either an increase or decrease in Akt phosphorylation when compared with baseline. The pAkt variability is consistent with an earlier pediatric temsirolimus study that demonstrated a lack of correlation between temsirolimus dose and phosphorylation of Akt, S6, and 4E-BP1 (40). However, in the current study, phosphorylation trends of the mTORC1 targets S6K1 and 4E-BP1 were consistently decreased or unchanged, respectively. In 5 patients, Akt S473 phosphorylation was generally decreased in response to treatment, paralleling S6K1 phosphorylation and consistent with the inhibition of mTOR complex

2 (mTORC2) observed in cells exposed to long-term treatment with rapamycin (46). This decrease may also reflect the efficacy of cixutumumab, but the contribution of IGF1R signaling to PI3K and Akt may be minor in PBMCs. In two patients, there was an inverse correlation between pS6K1 (decreased) and pAkt (increased), which may be explained by temsirolimus-mediated inhibition of the mTORC1-S6K1-negative feedback loop (47–49), leading to increased PI3K signaling to Akt. The variability of pAkt among patients does not appear to correlate with the magnitude of S6K1 inhibition, but instead may be due to a multitude of factors that are patient-specific, including variability in the makeup of the lymphocytes and monocytes in the PBMC population, and unknown changes in the status of the immune system following prolonged treatment with temsirolimus and cixutumumab.

This study demonstrates that the combination of cixutumumab and temsirolimus can be safely given to children with recurrent solid tumors, albeit at lower doses than in adult combination studies. One patient with a medulloblastoma had a confirmed partial response and 3 patients experienced prolonged disease stabilization for ≥ 3 cycles. Target inhibition was noted in PBMCs at all dose levels as evidenced by a decrease in phospho-S6K1 and pAkt. A phase II study of this combination has recently been completed in children with recurrent tumors in the Children's Oncology Group.

Disclosure of Potential Conflicts of Interest

D.A. Krueger reports receiving a commercial research grant from, is a consultant/advisory board member for, and reports receiving speakers' bureau honoraria from Novartis. No potential conflicts of interest were disclosed by the other authors.

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