A Multicenter, First-in-Pediatrics, Phase 1, Pharmacokinetic and Pharmacodynamic Study of Ridaforolimus in Patients with Refractory Solid Tumors

Lia Gore1,2, Tanya M. Trippett3, Howard M. Katzenstein4, Jessica Boklan5, Aru Narendran9, Amy Smith6, Margaret E. Macy1,2, Katherine Rolla3, for the Pediatric Oncology Experimental Therapeutics Investigators’ Consortium (POETIC), Narayana Narashimhan8, Rachel M. Squillace8, Christopher D. Turner8, Frank G. Haluska8, and Michael Nieder7

Abstract

Purpose: Ridaforolimus (MK-8669, AP23573) is a potent and selective mammalian target of rapamycin (mTOR) inhibitor. Preclinically, ridaforolimus displays antiproliferative activity against a variety of human tumors in vitro and tumor xenograft models in vivo, with additive or synergistic activity when combined with other anticancer agents. Antitumor activity has been confirmed in adults. This phase I study determined the safety, pharmacological, biological, and toxicology profiles of ridaforolimus in pediatric patients with refractory malignancies.

Experimental Design: Eligible children ages 1 to 18 years with advanced solid tumors were enrolled in a 3 þ 3 dose escalation design, to determine the safety, tolerability, and maximum tolerated dose (MTD)/dose-limiting toxicity (DLT) of ridaforolimus. Toxicities, pharmacokinetics, and pharmacodynamics were characterized.

Results: Fifteen patients were treated. No DLT was observed at any dose level tested; therefore, an MTD was not identified. Most adverse events were mild to moderate; the most common grades 3 and 4 adverse events were hematologic, including thrombocytopenia and anemia. Nonhematologic adverse events were mostly electrolyte disturbances. The observed pharmacokinetic profile of ridaforolimus in children was consistent with that previously showed in adults. Pharmacodynamic confirms that the dose range tested has pharmacological/pharmacodynamic activity. Forty percent of patients achieved stable disease including four of six with central nervous system tumors and two of eight with sarcomas.

Conclusions: This first-in-pediatrics study shows that the second-generation mTOR inhibitor ridaforolimus is well tolerated in heavily pretreated children with refractory solid tumors. No DLTs were observed over the dose range tested. Ridaforolimus may represent a therapeutic option for use in pediatric malignancies.

Introduction

The mammalian target of rapamycin (mTOR) is a ubiquitous serine–threonine kinase that regulates cell growth, proliferation, and apoptosis (1–3). Activation of mTOR is mediated by growth factor receptor tyrosine kinases that stimulate Ras-effector cascades; most importantly, the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway (4). Mutations in KRAS and PIK3CA and loss of tumor putative tumor suppressor genes such as p53, VHL, and PTEN are among the most common alterations in human malignancies, each of which may lead to activation of PI3K/AKT signaling and results in enhanced mTOR activity (5–8). Mutations or amplifications in upstream EGFR and BRAF may also lead to mTOR activation. mTOR is a component of a multiprotein complex, mTOR complex 1 (mTORC1) or mTOR-raptor, which regulates cell growth by phosphorylating downstream effector proteins including the 4E-binding protein 1 (4E-BP1) and p70 ribosomal S6 kinases. Phosphorylation of 4E-BP1 by mTORC1 promotes its dissociation from the translation-initiation factor eIF4E, thereby allowing cap-dependent translation of proteins critical to cell-cycle progression and cell growth (4, 9, 2). mTOR is also part of mTOR complex 2 (mTORC2) or mTOR-rictor, which

Authors' Affiliations: 1The Center for Cancer and Blood Disorders, Children's Hospital Colorado; 2The University of Colorado Cancer Center, Aurora, Colorado; 3Memorial Sloan-Kettering Cancer Center, New York, New York; 4Aflac Cancer Center, Children’s Healthcare of Atlanta, Emory University, Atlanta, Georgia; 5Phoenix Children’s Hospital, Phoenix, Arizona; 6University of Florida, Shands Cancer Center, Gainesville; 7All Children’s Hospital, Tampa, Florida; 8ARIAD Pharmaceuticals Inc., Cambridge, Massachusetts; and 9University of Calgary, Alberta Children’s Hospital, Calgary, Alberta, Canada

Corresponding Author: Lia Gore, Center for Cancer and Blood Disorders, Children’s Hospital Colorado, 13123 East 16th Avenue, Box B115, Aurora, CO 80045. Phone: 720-777-7772, Fax: 720-777-7289; E-mail: lia.gore@ucdenver.edu

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Translational Relevance

Ridaforolimus is a selective inhibitor of mammalian target of rapamycin (mTOR), which regulates cell proliferation and apoptosis. This study provides a detailed evaluation of the safety, toxicity, pharmacokinetics (PK), and pharmacodynamics of the mTOR inhibitor ridaforolimus when administered to pediatric patients with relapsed and refractory solid malignancies. No dose limiting toxicities were observed and therefore no maximum tolerated dose was defined for single agent use over the dose range and schedule tested. Because mTOR inhibitor use is increasing in oncology therapeutics, and future trials are likely to focus on combining mTOR inhibitors with other chemotherapeutic and/or biologic agents, the results described in this study including the toxicity profile, PK, and biological correlative data will help support future clinical trial design and use in combination studies with this agent and/or other mTOR inhibitors.

Patients, Materials, and Methods

Patient eligibility

Eligible patients were 1 to 18 years of age with histologically confirmed solid tumors, including primary central nervous system (CNS) tumors and non-Hodgkin’s lymphomas, with disease that had progressed despite standard therapy—or a disease for which no standard therapy was available. Other key inclusion criteria were Eastern Cooperative Oncology Group performance status 0 to 2 for patients aged 16 years and older; Karnofsky performance score ≥60 for children aged more than 10 years of age; life expectancy ≥12 weeks; full recovery to baseline from acute toxicities of all prior chemotherapy, immunotherapy, surgery, or radiotherapy; adequate bone marrow, renal, hepatic, cardiac, and pulmonary function; and serum cholesterol <350 mg/dl and triglycerides <400 mg/dl. Patients on steroids were required to be on a stable or decreasing dose for ≥7 days before study entry. Patients were excluded if they had leukemia, uncontrolled intercurrent illness, known HIV or AIDS, or had undergone any stem cell transplantation <3 months before study entry; had evidence of ongoing GVHD or required immunosuppressive therapy to control GVHD; had received prior therapy with an mTOR inhibitor; or were currently receiving any other investigational therapy or using an investigational device.

The study protocol was approved by the Institutional Review Board or Independent Ethics Committee at each participating site and was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and all local and federal regulatory guidelines. Each patient’s parent or legal guardian provided written informed consent, with patient assent as appropriate according to institutional requirements.

Study design

This multicenter, international, open-label, phase 1 study evaluated ridaforolimus administered via 30-minute i.v. infusion, once daily for 5 consecutive days every other week (ClinicalTrials.gov identifier NCT00704054; Protocol 028). All patients were expected to receive at least one 4-week cycle of study treatment in the absence of disease progression, unacceptable toxicity, or voluntary choice to discontinue participation. Patients were not premedicated before the first dose of study drug; however, hypersensitivity care guidelines were provided.

A traditional 3 + 3 dose escalation design was used, with dose levels of 8, 10, 13, and 16 mg/m² and maximum doses of 10 mg (for 8 mg/m²), 12.5 mg (for 10 mg/m²), and 18.75 mg (for both the 13 and 16 mg/m² dose levels), respectively. The 18.75 mg dose corresponds to the defined adult MTD, which had been established at the beginning of this study, and escalation beyond the adult MTD was not planned. Patients weighing less than 10 kg were dosed on a per-kilogram basis, calculated as the dose level in mg/m² × 0.033 = dose/kg. Escalation to the next dose level was allowed if DLT was not observed during cycle 1. If one patient...
developed DLT during cycle 1, then up to 3 additional patients were enrolled. Subsequent dose escalation was allowed only if fewer than 2 of the 3 to 6 patients enrolled experienced DLT. The MTD was defined as the highest dose at which no more than one of 6 patients had DLT in cycle 1.

Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0 (22). DLT was defined as any of the following: grade 3 nonhematologic toxicity lasting greater than 3 days despite optimal supportive care; any grade 4 nonhematologic toxicity (except alopecia); grade 4 neutropenia lasting greater than 10 days; grade 4 thrombocytopenia lasting greater than 10 days; any toxicity considered possibly related to study drug that prevented the patient from completing one treatment cycle; or treatment delay greater than 2 weeks due to any study drug-related toxicity.

Patients with disease control were permitted to continue treatment, providing toxicity remained acceptable and they continued to express interest in voluntary participation. Subsequent treatment cycles were initiated if the following criteria were met on day 1 of that cycle: absolute neutrophil count ≥1,000/μL; platelets ≥75,000/μL; grade ≤1 mucositis, nausea, or vomiting; and bilirubin ≤1.5 × institutional upper limit of normal. Each patient was allowed up to 2 dose reductions during the course of the study for specified drug-related adverse events. Treatment could be delayed at weekly intervals for up to 2 weeks to allow recovery from adverse events.

**On-study evaluation**

Physical examinations were conducted at baseline, day 15, and before the start of each subsequent treatment cycle. Hematology and clinical chemistry assessments, including serum cholesterol and triglyceride, were done on days 1 and 15 of each cycle (and on cycle 1, day 5). Patients were monitored for at least 4 hours after the first infusion, and for at least 1 hour after each subsequent infusion for evidence of any infusion or dose-related adverse events. Disease and response assessments were conducted at the end of each even-numbered cycle. The same imaging method used at baseline was used at each subsequent assessment, appropriate to the patient’s disease type, with computer tomography or MRI required for any pulmonary lesions. Responses in patients with measurable disease were determined based on the Response Criteria in Solid Tumors (RECIST) 1.0 guidelines (23).

**Pharmacokinetic and pharmacodynamic evaluations**

Blood samples for determination of plasma and blood ridaforolimus concentrations were collected at prespecified time points during cycle 1 (day 1: predose, mid-infusion, end of the infusion, and then 5, 15, and 30 minutes and 1, 2, 4, and 8 hours postinfusion; days 2–4 preinfusion; day 5 preinfusion and 5, 15, and 30 minutes and 1, 4, and 8 hours postinfusion; days 6–8 representing 24, 48, and 72 hours after the day 5 infusion; and day 15 preinfusion, 1 and 4 hours postinfusion) and before the first infusion in cycle 2. Whole blood and plasma sample pharmacokinetic analyses were done using a validated liquid chromatography/tandem mass spectrometry (LC/MS-MS) assay (Charles River Laboratories, Shrewsbury, MA). Pharmacokinetic parameters were determined by noncompartmental modeling using WinNonLin, professional version 5.2 (Pharsight).

Blood samples for pharmacodynamic evaluation of mTOR inhibition were collected at screening and on days 2, 5, 15, and 16 before ridaforolimus administration. Lysates of peripheral blood mononuclear cells (PBMC) were prepared (24) and probed by Western blot analysis for phosphorylated 4E-BP1 (p-4E-BP1) and total 4E-BP1, with percent phosphorylation normalized to predose samples (24).

**Results**

**Patients**

Nineteen patients were enrolled between July 2008 and January 2010. Four patients never received study drug due to

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
<td><strong>Number of patients</strong></td>
</tr>
<tr>
<td>Consulted/enrolled</td>
<td>19/18</td>
</tr>
<tr>
<td>Eligible/treated</td>
<td>16/15</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>12 (2–16)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>African-American</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Native American</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Ethnicity, Hispanic</td>
<td>7 (47%)</td>
</tr>
<tr>
<td>Performance status</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>90 (60–100%)</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
</tr>
<tr>
<td>Sarcoma*</td>
<td>8 (53%)</td>
</tr>
<tr>
<td>CNS*</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Other*</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Number of prior therapies</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>14 (93%)</td>
</tr>
<tr>
<td>Median number of prior regimens</td>
<td>6 (range 1–11)</td>
</tr>
<tr>
<td>Radiation</td>
<td>9 (60%)</td>
</tr>
<tr>
<td>Median number of prior regimens</td>
<td>1 (range 0–4)</td>
</tr>
<tr>
<td>Surgery</td>
<td>13 (87%)</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>Hormonal therapy</td>
<td>1 (7%)</td>
</tr>
</tbody>
</table>

*Osteosarcoma (2) and one patient each with clear cell sarcoma, chondrosarcoma, Ewing sarcoma, DSRCT, soft tissue nonhabdomyosarcoma, and undifferentiated sarcoma.

*Osteosarcoma (2) and one patient each with clear cell sarcoma, chondrosarcoma, Ewing sarcoma, DSRCT, soft tissue nonhabdomyosarcoma, and undifferentiated sarcoma.

*Osteosarcoma (2) and one patient each with clear cell sarcoma, chondrosarcoma, Ewing sarcoma, DSRCT, soft tissue nonhabdomyosarcoma, and undifferentiated sarcoma.

*Wilm’s tumor.
early progression (2), lack of confirmation of histology (1), or withdrawal of consent (1). Fifteen patients (median age: 12 years; range: 2–16 years) with sarcomas ($n = 8$), CNS tumors ($n = 6$), or Wilm’s tumor ($n = 1$) were treated with ridaforolimus at dose levels of 8, 10, 13, or 16 mg/m$^2$ as shown in Table 1. Thirteen patients were fully assessable for all study objectives. Two patients completed all toxicity assessments but did not complete all required imaging at the end of the DLT period.

**Toxicity**

Dose-limiting toxicity was not observed at any ridaforolimus dose-level tested; therefore, an MTD was not identified in this study (Table 2). Reversible, non-DLTs were observed in most patients (Table 3). The most common grades 3 and 4 adverse events were hematologic, including thrombocytopenia (27%; all grade 3), anemia (27%; one grade 4), neutropenia (13%; one grade 4), and lymphopenia (13%; one grade 4). Grades 3 and 4 nonhematologic

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### Table 2. Dose escalation and dose-limiting toxicity of 15 treated patients

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Dosage$^a$ (mg/m$^2$)</th>
<th>Number of patients enrolled/treated$^b$</th>
<th>Median (range) cycles delivered</th>
<th>Dose-limiting toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>7/5</td>
<td>1 (1–15)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>4/4</td>
<td>3 (2–6)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>4/3</td>
<td>2 (2–6)</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>3/3</td>
<td>4 (2–5)</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$The maximum dose administered was 10 mg in dose level 1, 12.5 mg in dose level 2, and 18.75 mg in doses levels 3 and 4.

$^b$Two patients in dose level 1 and one patient in dose level 2 had disease progression and did not complete all requirements for evaluable. These patients were replaced.

### Table 3. Adverse events across ALL treatment cycles reported in greater than 20% of 15 treated patients, as assessed by the CTCAE, version 3.0

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Any grade (n, %)</th>
<th>Grades 1 and 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematologic toxicities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>11 (73)</td>
<td>7 (47)</td>
<td>3 (20)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>10 (67)</td>
<td>9 (60)</td>
<td>1 (7)</td>
<td>–</td>
</tr>
<tr>
<td>Platelets</td>
<td>11 (73)</td>
<td>7 (47)</td>
<td>4 (27)</td>
<td>–</td>
</tr>
<tr>
<td>Neutropenia (ANC)</td>
<td>6 (40)</td>
<td>4 (27)</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>5 (33)</td>
<td>3 (20)</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
<tr>
<td><strong>Electrolyte disturbances</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate, low</td>
<td>8 (53)</td>
<td>8 (53)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Potassium, low</td>
<td>5 (33)</td>
<td>3 (20)</td>
<td>2 (13)</td>
<td>–</td>
</tr>
<tr>
<td>Sodium, low</td>
<td>6 (40)</td>
<td>4 (27)</td>
<td>2 (13)</td>
<td>–</td>
</tr>
<tr>
<td>Albumin, low</td>
<td>4 (27)</td>
<td>4 (27)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium, low</td>
<td>4 (27)</td>
<td>4 (27)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Other nonhematologic toxicities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride, high</td>
<td>10 (67)</td>
<td>9 (60)</td>
<td>1 (7)</td>
<td>–</td>
</tr>
<tr>
<td>Elevated AST, SGOT$^a$</td>
<td>8 (53)</td>
<td>8 (53)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Elevated ALT, SGPT$^a$</td>
<td>8 (53)</td>
<td>7 (47)</td>
<td>1 (7)</td>
<td>–</td>
</tr>
<tr>
<td>Anorexia</td>
<td>6 (40)</td>
<td>6 (40)</td>
<td>1 (7)</td>
<td>–</td>
</tr>
<tr>
<td>Fatigue (asthenia, malaise)</td>
<td>6 (40)</td>
<td>6 (40)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fever (no neutropenia)</td>
<td>4 (27)</td>
<td>4 (27)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glucose, high</td>
<td>7 (47)</td>
<td>6 (40)</td>
<td>1 (7)</td>
<td>–</td>
</tr>
<tr>
<td>Mucositis, oral</td>
<td>6 (40)</td>
<td>6 (40)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nausea</td>
<td>6 (40)</td>
<td>5 (33)</td>
<td>1 (7)</td>
<td>–</td>
</tr>
<tr>
<td>Cholesterol, high</td>
<td>5 (33)</td>
<td>5 (33)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (33)</td>
<td>4 (27)</td>
<td>1 (7)</td>
<td>–</td>
</tr>
<tr>
<td>Pain, head/headache</td>
<td>4 (27)</td>
<td>4 (27)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$Abbreviations: ANC, absolute neutrophil count; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.
adverse events were mostly electrolyte disturbances, such as hypokalemia and hyponatremia (13% each; all grade 3). Other individual grade 3 events occurring in one patient each were leukopenia, elevated alanine aminotransferase (ALT), anorexia, hyperglycemia, nausea, and vomiting. A total of 18 serious adverse events were reported in 7 unique patients in this trial. Excluding 2 patients that did not receive treatment, there were 14 SAEs in 5 unique patients; only 2 of these events (anemia and mucositis in one patient each) were reported as being "possibly" related to ridaforolimus treatment. No SAEs were reported as "definitely" related to ridaforolimus. All of the SAEs resolved without sequelae. Neither of the patients experiencing "possibly related" toxicities required dose reduction in subsequent cycles and neither recurred on reinstitution of ridaforolimus at the doses at which the toxicity was initially observed.

**Pharmacokinetics and pharmacodynamics**

Pharmacokinetic (380 samples) and pharmacodynamic data (88 samples) were available for all 15 patients (100% compliance). Following i.v. administration, ridaforolimus showed a rapid exponential decrease in blood concentrations followed by a slower terminal phase of elimination (Fig. 1). The mean terminal half-life was 41.7 to 63.7 hours measured after dosing on day 5 and was consistent across dose levels (Table 4). Exposure to ridaforolimus increased.

![Ridaforolimus blood concentration-time profile](image.jpg)

**Figure 1.** Mean ridaforolimus blood concentration-time profile measured in whole blood in cycle 1, on day 1, day 5, and day 15.

**Table 4.** Pharmacokinetic parameters of ridaforolimus in whole blood in cycle 1

<table>
<thead>
<tr>
<th>Dose level (No. of patients treated)</th>
<th>Dose (mg/m²)</th>
<th>Day of course</th>
<th>N (% cohort tested)</th>
<th>Cmax (ng/mL) Mean ± SD</th>
<th>Tmax (hours) Mean ± SD</th>
<th>AUC0-last (ng-h/mL) Mean ± SD</th>
<th>CL (mL/h/m²) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (5)</td>
<td>8</td>
<td>1</td>
<td>4 (80)</td>
<td>413 ± 97</td>
<td>18.0 ± 2.6e</td>
<td>2,004 ± 416</td>
<td>2,174 ± 1,240</td>
</tr>
<tr>
<td>5</td>
<td>2 (16)</td>
<td>1</td>
<td>3 (60)</td>
<td>255 ± 39</td>
<td>63.7 ± 20.9d</td>
<td>5,815 ± 2,035</td>
<td>1,113 ± 12e</td>
</tr>
<tr>
<td>4 (3)</td>
<td>10</td>
<td>1</td>
<td>4 (100)</td>
<td>446 ± 169</td>
<td>15.6 ± 2.6e</td>
<td>1,827 ± 225</td>
<td>3,778 ± 662</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>1</td>
<td>3 (100)</td>
<td>299 ± 41</td>
<td>41.7 ± 11.4d</td>
<td>5,663 ± 2,153</td>
<td>1,903 ± 799</td>
</tr>
<tr>
<td>4 (3)</td>
<td>16</td>
<td>1</td>
<td>3 (100)</td>
<td>680 ± 293</td>
<td>20.7 ± 10.9f</td>
<td>2,390 ± 728</td>
<td>3,613 ± 1690</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>1</td>
<td>5 (100)</td>
<td>355 ± 59</td>
<td>57 ± 5.4f</td>
<td>8,011 ± 4,493</td>
<td>1,875 ± 843</td>
</tr>
<tr>
<td>4 (3)</td>
<td>13</td>
<td>3</td>
<td>1 (100)</td>
<td>373 ± 186</td>
<td>17.2 ± 2.1f</td>
<td>2,423 ± 217</td>
<td>4,313 ± 700</td>
</tr>
</tbody>
</table>

Abbreviations: CL, clearance; F, oral bioavailability.

Data are mean ± SD; SD cannot be calculated where n = 2.

Day 1 and day 5 pharmacokinetic parameters are derived from blood levels during 0 to 24 hours and 0 to 240 hours after dose, respectively.

Based on 0- to 24-hour data only; there was insufficient data in the terminal elimination phase for accurate calculations and this resulted in short half-life values.

Based on 96- to 336-hour data following day 5 dose administration.
with increasing dose, but the increases in maximum concentration ($C_{max}$) and area under the curve (AUC$_{0–last}$) were not linearly proportional to doses of ridaforolimus. Following 5 once-daily 30-minute infusions, ridaforolimus trough concentrations appeared to approach steady state in both blood and plasma for the majority of patients. Mean accumulation when comparing concentrations 24 hours after dosing was ~1.4- to 1.7-fold and ~1.2- to 3-fold for blood and plasma, respectively. Mean apparent terminal $t_{1/2}$ were 38.1 to 66.2 and 22.7 to 31.3 hours in blood and plasma, respectively.

Decreased levels of p-4E-BP1 are a marker of mTOR inhibition. As such, we evaluated the pharmacodynamics of ridaforolimus by measuring p-4E-BP1 in PBMCs via Western blot analysis. Complete time course data were obtained for 13 patients, including all of those in the 3 highest dose levels. Levels of p-4E-BP1 were reduced by a median of 85% when measured at 24 hours after the first dose. Greater than or equal to 70% inhibition was seen in 13 of 15 patients analyzed at 24 hours across all dose levels as shown in Fig. 2. This pharmacodynamic effect was sustained when measured on day 5, after 4 days of dosing, with greater than or equal to 80% inhibition seen in 11 of 12 analyzable patient samples (92%). One patient at dose level 2 did not have any measurable 4EBP1, so no analysis could be completed on that patient. Prolonged inhibition was shown in 7 of 13 patients with greater than 50% inhibition observed on Day 15, 10 days out from dosing. Despite increases in AUC and $C_{max}$ with increasing dose, a similar robust inhibition of p-4EBP1 was obtained across all dose levels.

Figure 2. Representative data from one patient at each dose level showing ridaforolimus pharmacodynamics measured by p-4E-BP1 levels in PBMCs and its relationship to ridaforolimus blood concentrations. Western blots show p-4E-BP1 and total 4E-BP1 levels at screening and before dosing on days 2 and 5 (representing 24 hours after the preceding dose), on day 15 or 16 (representing 240-268 hours after the preceding dose on day 8), and on day 16 or 17 (representing 24 hours after the preceding dose). Note that at dose level 1, the samples taken at days 16 and 17 were both 1 day late. Asterisk (*) represents duplicate blots; there was insufficient sample for duplicate analysis of dose level 1 (8 mg/m$^2$).
levels tested by day 1 and 5 of treatment. This suggests that patients were exposed to sufficient drug levels to achieve mTOR inhibition at all doses tested at the given time points evaluated.

**Antitumor response**

Six of 15 patients (40%) had stable disease at the time of first tumor assessment. Four patients with CNS tumors and 4 with sarcomas had stable disease lasting for a median 4.5 cycles (range 1–14 cycles). No evidence for a dose–response relationship was observed over all doses tested. The patient with the most sustained and substantial response was treated at dose level 1 (8 mg/m²). This patient had recurrent, refractory, widely metastatic desmoplastic small round cell tumor (DSRCT), and achieved a greater than 15% reduction in tumor size with complete resolution of ascites and bilateral pleural effusions lasting for 15 months before eventual progression of disease. Median overall survival for the study cohort at the time of the last assessment was 8.6 months (range: 1–17.9 months).

**Discussion**

This first-in-pediatric trial shows that the second-generation mTOR inhibitor ridaforolimus is extremely well tolerated in very heavily pretreated children with refractory solid tumors. No DLT was identified over the dose range tested (8–16 mg/m² i.v. daily for 5 consecutive days every other week), and therefore, the MTD was not identified in this study. A similar dosing schedule was evaluated in a phase 1 trial involving 32 adult patients with advanced malignancies (25). These investigators defined the adult MTD at 18.75 mg/day the only DLT identified in cycle 1 was grade 3 mouth sores. Based on these results, a dose of 12.5 mg daily for 5 days each week was selected for further adult studies (16, 17).

In this trial, 18.75 mg/day was the maximum dose studied; therefore, the dose range tested encompasses and is in harmony with the clinically effective i.v. dose of ridaforolimus in adults. Although lack of an identified DLT/MTD suggests that further dose escalation could be pursued, and may in fact be informative, evidence of pharmacodynamic inhibition and clinical responses/benefit noted in all dose levels in patients treated on this study suggest that further dose escalation may be neither necessary nor beneficial. In fact, some investigators may argue that dose escalation beyond biomarker evidence of target inhibition in the presence of clinical benefit may only increase toxicity and not improve efficacy. Indeed not reaching MTD seems to be increasingly common in trials with targeted agents. In another study with ridaforolimus (PN016), modeling and simulation work showed better efficacy with increasing exposure but with a plateau at exposures above those associated with the clinical dose administered (internal data; Merck Sharp & Dohme Corp.).

Most adverse events reported in this study were mild to moderate in severity (grade 1 or 2), reversible, and easily managed. In general, the safety profile of ridaforolimus was consistent with that established in adults, and no new toxicities were noted which had not been previously reported. Mouth sores and rash were the most common adverse events reported in the adult phase 1 study (25) and fatigue, stomatitis, and hypertriglyceridemia were most prevalent in the recently reported adult phase II study (17). These toxicities were not highly prevalent in this pediatric study population, however hematologic toxicity and electrolyte disturbances were more common, a limited number of which were grade ≥3 events (one each of grade 4 neutropenia, anemia, lymphopenia). This may reflect the very heavily pretreated population enrolled to this study, including a greater number of prior myelosuppressive regimens and heavier use of ifosfamide in these patients compared to the adults studied. Ifosfamide is known to induce renal wasting of electrolytes in many patients (26), which may be exacerbated when such patients are treated further. In contrast to the adult profile, minimal alteration in cholesterol and triglyceride levels were observed in this pediatric population, and no patient required therapeutic intervention for hypertriglyceridemia or hypercholesterolemia. Moreover, only 2 serious adverse events (SAE) reported in the trial were considered “possibly related” to study drug (anemia, mucositis), both of which had been previously reported at higher prevalence in adults treated with ridaforolimus. No “definitely related” SAEs were reported.

The pharmacokinetic profile of ridaforolimus in children was consistent with that previously showed in adults. The terminal elimination half-life ranged from 42 to 64 hours in children compared with 45 to 74 hours in 2 phase 1 studies with i.v. ridaforolimus in adults treated on a similar dosing schedule (25, 27). Exposure to ridaforolimus did not increase linearly with increasing dose, as expected for the class due to saturable binding to FKBP, but the day 5 exposure to ridaforolimus (based on AUC0–last) achieved with dose levels 1 and 2 (8 and 10 mg/m²) was consistent with the exposure seen in adults receiving doses of 12.5 to 18.75 mg, which is at or above the adult recommended i.v. dose (25).

Decreased p-4E-BP1 in PBMCs confirms that the dose range tested has pharmacologic/pharmacodynamic activity. Sustained inhibition of p-4E-BP1 was evident at 24 hours after dosing and remained low for 4 days after the last dose. A similar reduction of p-4E-BP1 in PBMCs was reported in a phase 1 trial in adults treated with ridaforolimus (25). Of note, the reduction in p-4E-BP1 observed in this study was consistently inversely associated with ridaforolimus blood concentrations, thereby suggesting a strong pharmacokinetic/pharmacodynamic relationship.

The present results with ridaforolimus are generally similar to those seen for 2 other mTOR inhibitors that were examined in children with refractory solid tumors. In a phase 1 trial, temsirolimus was administered i.v. once a week to children with recurrent or refractory solid tumors; 1 of 7 patients who received the highest dose tested (150 mg/m²) had dose-limiting grade 3 anorexia (28). Fouladi and colleagues studied everolimus in a phase 1 trial, with oral dosing once daily to children with refractory...
solid tumors; the MTD was identified as 5 mg/m² and all 3 patients who received the highest dose (6.5 mg/m²) experienced DLTs consisting of grade 3 mucositis, diarrhea, and elevated aspartate aminotransferase (AST; ref. 29). Similar to this study, adverse events were primarily reversible grades 3 and 4 hematologic toxicity and electrolyte disturbances reported in a small number of patients.

Temsirolimus and everolimus also displayed pharmacokinetic profiles in pediatric patients that were consistent with those seen in adults, and both exhibited pharmacodynamic activity in PBMCs (28, 29). Both agents inhibit phosphorylation of AKT at Ser473, mediated by mTORC2 and is essential for maximal AKT activity. It had been postulated that this process was insensitive to inhibition by rapamycin analogs, but recent evidence suggests that prolonged mTOR inhibition impairs mTORC2 assembly and AKT activation in some cancer cell types (30–32). In contrast to our study where there was fairly consistent evidence of pharmacodynamic effect, marked interpatient variability in inhibition of phosphorylation of 4E-BP-1 and ribosomal S6 kinase was reported with temsirolimus treatment; consistent inhibition was not evident at all time points and dose levels, and the pharmacodynamic effects of temsirolimus appeared unrelated to serum concentrations (28).

Consistent with the other mTOR inhibitors studied in children, our study of ridaforolimus showed that stable disease was generally the predominant response, noted in 40% of evaluable patients (4 of 6 patients with CNS tumors and 2 of 8 patients with sarcoma). This included one patient with refractory DSRCT who had prolonged disease stabilization lasting greater than 15 months. This patient had an extended clinically significant response with complete resolution of abdominal ascites and bilateral pleural effusions and a prolonged improvement in symptoms including resolution of pain and fatigue. This is highly unusual for this particularly aggressive tumor, for which a typical life expectancy may be 5 to 8 months (33). A high proportion of patients (13 of 15, or 87%) in this study were evaluable for tumor response, which is somewhat unusual for a phase 1 study in a very heavily pretreated pediatric cohort with highly refractory metastatic solid tumors. In the phase 1 pediatric trial with everolimus, 69% of patients were evaluable, with stable disease lasting at least 4 cycles reported in 22% of patients (29). With temsirolimus, 68% of pediatric patients were evaluable in the phase 1 study; one patient with neuroblastoma had a complete response and 5 patients had stable disease for a clinical benefit rate of 46% among evaluable patients (28).

Along with clinical experience with these drugs in adult patients and recent experience with xenograft models (34), these studies suggest that the benefit of single-agent therapy with an mTOR inhibitor overall is typically disease stabilization. The low toxicity and favorable pharmacokinetics suggest that ridaforolimus could be easily combined with other anticancer agents in children over a broad range of doses. Preclinical studies have shown additive or synergistic activity when ridaforolimus is combined with a variety of other anticancer agents (14, 15). The feasibility of administering ridaforolimus in combination with cytotoxic agents has already been showed in adults with advanced malignancies (35, 36). An ongoing study of oral ridaforolimus in combination with dalotuzumab in children with refractory solid tumors will help inform dosing in combination and tolerance to different doses of the oral formulation of ridaforolimus. This is particularly relevant, as the i.v. formulation is being discontinued and any future studies would likely use the oral formulation.

Ridaforolimus was given i.v. in this study, but has also shown activity when administered orally. A phase 1 trial evaluating 7 different oral regimens in adults with advanced/refractory solid tumors found that a 40-mg dose administered daily for 5 consecutive days each week provided exposure comparable to that achieved with the 12.5-mg i.v. regimen (37). In the phase 3 SUCCEED trial (16), an oral regimen was administered as maintenance therapy to patients with advanced sarcomas who had previously attained a best clinical response of stable disease or better to other therapy, and was shown to prolong PFS compared with placebo in adults (16). The role of maintenance therapy has not been explored in pediatric sarcomas, although it is well accepted in children with neuroblastoma using cis-retinoic acid as a differentiation agent, and is standard of care in children with acute lymphoblastic leukemia (ALL) with pulses of steroids, vincristine and antimetabolites. In fact, the introduction of maintenance therapy in both of these tumor types has dramatically improved the outcome for these patients. The role of maintenance in pediatric sarcomas or CNS tumors could be examined, with particular attention to sub-set analyses to define the population(s) who may derive most benefit. In this instance, an oral agent would be particularly attractive.

Taken together with results from the current trial, similar pharmacokinetic profiles observed with i.v. ridaforolimus in children and adults, and with i.v. versus oral ridaforolimus in adults, suggest that oral ridaforolimus may represent an option for use in pediatric malignancies. An international study of oral ridaforolimus in pediatric patients with refractory solid tumors is underway, which when completed, will be combined with an antibody to the Insulin-like growth factor receptor in a combination regimen.

Disclosure of Potential Conflicts of Interest

C.D. Turner and F.G. Haluska have ownership interest (including patents) in ARIAD Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: L. Gore, T.M. Trippett, J. Boklan, C.D. Turner, F.G. Haluska, M. Nieder


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Gore, T.M. Trippett, H. Katzenstein, J. Boklan, A. Narendran, A. Smith, M.E. Mary, R.M. Squillace, C.D. Turner, F.G. Haluska, M. Nieder

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Gore, N.I. Narasimhan, R.M. Squillace, C.D. Turner, F.G. Haluska, M. Nieder


Other: L. Gore, T.M Trippett, J. Boklan, C.D. Turner, F.G. Haluska, M. Nieder


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