Published in final edited form as:

Pediatr Blood Cancer. 2014 May ; 61(5): 833–839. doi:10.1002/pbc.24874.

Phase 1 Trial of Temozolomide in Combination with Irinotecan and Temozolomide in Children, Adolescents and Young Adults with Relapsed or Refractory Solid Tumors: A Children's Oncology Group Study

R Bagatell¹, RE Norris², AM Ingle³, CH Ahern⁴, S Voss⁵, E Fox¹, A Little¹, B Weigel⁶, PC Adamson¹, and SM Blaney⁴

¹The Children's Hospital of Philadelphia, Philadelphia PA

²Rainbow Babies and Children's Hospital, Cleveland OH

³Children's Oncology Group Statistics and Data Center, Arcadia CA

⁴Texas Children's Cancer Center/Baylor College of Medicine, Houston TX

⁵Dana-Farber Cancer Institute/Children's Hospital Boston, Boston MA

⁶University of Minnesota, Minneapolis MN

Abstract

Background—mTOR inhibitors have activity in pediatric tumor models. A phase I trial of the mTOR inhibitor temsirolimus (TEM) with irinotecan (IRN) and temozolomide (TMZ) was conducted in children with recurrent/refractory solid tumors, including central nervous system (CNS) tumors.

Methods—Escalating doses of intravenous TEM were administered on days 1 and 8 of 21-day cycles. IRN (50 mg/m²/dose escalated to a maximum of 90 mg/m²/dose) and TMZ (100 mg/m²/dose escalated to a maximum of 150 mg/m²/dose) were administered orally on days 1–5. When maximum tolerated doses (MTD) were identified, TEM frequency was increased to weekly.

Results—Seventy-one eligible pts (median age 10.9 years, range 1.0–21.5) with neuroblastoma (16), osteosarcoma (7), Ewing sarcoma (7), rhabdomyosarcoma (4), CNS (22) or other (15) tumors were enrolled. Dose-limiting hyperlipidemia occurred in 2 patients receiving oral corticosteroids. The protocol was subsequently amended to preclude chronic steroid use. The MTD was identified as TEM 35 mg/m² weekly, with IRN 90 mg/m² and TMZ 125 mg/m² on days 1–5. At higher dose levels, elevated serum alanine aminotransferase and triglycerides, anorexia, and thrombocytopenia were dose limiting. Additional grade 3 regimen-related toxicities included leukopenia, neutropenia, lymphopenia, anemia, and nausea/vomiting. Six patients had objective responses confirmed by central review; 3 of these had sustained responses through 14 cycles of therapy.

Conclusion—The combination of TEM (35 mg/m²/dose weekly), IRN (90 mg/m²/dose days 1–5) and TMZ (125 mg/m²/dose days 1–5) administered every 21 days is well tolerated in children. Phase 2 trials of this combination are ongoing.

Keywords

Phase 1; solid tumors; temsirolimus

Introduction

The serine/threonine kinase known as the mammalian target of rapamycin (mTOR) plays an important role in regulation of protein synthesis, cell growth, and proliferation when activated and associated with appropriate members of a functional complex. [1] Rapamycin and rapamycin analogs, including temsirolimus (TEM), form complexes with mTOR and FK506-binding protein, leading to inhibition of mTOR signaling. [2] These agents associate with binding protein FKBP12 to inhibit mTOR in the TORC1 multi-protein complex. Targets of activated mTORC1 include ribosomal S6kinase (p70S6K) and the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). [3] Phosphorylation of 4E-BP1 through mTORC1 results in the release of 4EBP1 from the eukaryotic translation initiation factor (eIF4E), permitting initiation of translation. Inhibition of mTORC1 therefore alters protein synthesis and results in changes in levels of regulatory proteins including HIF1 α , fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), MYC, cyclin D1, and ornithine decarboxylase (ODC). [3,4] Effects on S6K activity also alter translation and ribosomal biosynthesis and may affect apoptotic signaling. Upstream influences on mTOR activation are numerous, and include growth factor signaling, Ras-dependent mitogen-activated protein kinase (MAPK) pathway signaling, and AKT activation. TEM, through its effects on mTOR, can therefore disrupt the integration of signals from multiple pathways and affect cell survival, proliferation, metabolism and angiogenesis. [5,6]

Rhabdomyosarcoma, neuroblastoma, and Ewing sarcoma cells have been shown to be sensitive to mTOR inhibitors *in vitro* and *in vivo*, [7–11] and mTOR inhibition decreases metastasis in osteosarcoma models. [12] In early phase clinical trials, TEM has been well-tolerated and as a single agent has demonstrated preliminary evidence of benefit, primarily disease stabilization, in children with solid tumors. [13,14] This benefit, together with the results of preclinical studies demonstrating that mTOR inhibitors have synergistic or additive anti-tumor effects when combined with chemotherapeutic drugs used in treatment of pediatric malignancies [15,16], [17], [18], [19] provided the rationale for combining TEM with irinotecan (IRN) and temozolomide (TMZ). Additionally, the limited overlap in the toxicity profile of TEM and the combination of IRN and TMZ, two agents commonly used in the clinical care of children with relapsed solid tumors, further supported the use of this multi-agent combination regimen. The primary objective of this trial was to assess the tolerability of TEM in combination with a chemotherapy backbone of IRN and TMZ in patients with intra- and extracranial solid tumors.

Patient Eligibility

Patients >12 months and <22 years of age with measurable or evaluable recurrent or refractory solid tumors, including CNS tumors or lymphomas, were eligible. Histologic verification of malignancy was required with the exception of patients with an intrinsic brain stem glioma, optic pathway glioma, or pineal tumor associated with elevated serum or cerebrospinal fluid tumor markers. Other eligibility criteria included a Karnofsky/Lansky performance score

50; interval from prior therapy >21 days for myelosuppressive chemotherapy, >7 days for biologic agents, >6 weeks for immunotherapy, >3 half lives for monoclonal antibodies, >7 days for short-acting and >14 days for long-acting hematopoietic growth factors; 2 weeks for local palliative radiation; >24 weeks from total body, craniospinal, or radiation to 50% of the pelvis; >6 weeks from other substantial bone marrow radiation; 12 weeks from a stem cell transplant or rescue and no evidence of active graft versus host disease; and no prior treatment with the combination of the 3 anticancer agents comprising this regimen. Adequate renal function (age-adjusted normal serum creatinine, or GFR 70 mL/min/1.73 m²); adequate liver function [total bilirubin 1.5 x institutional upper limit of normal (ULN), albumin >2 g/dL, and alanine aminotransferase (ALT) 110 U/L]; a prothrombin time <1.2 x ULN, blood glucose ULN, serum triglyceride level 300 mg/dL, serum cholesterol 300 mg/dL, and adequate pulmonary function were required. Adequate bone marrow function, defined as an absolute neutrophil count (ANC) 1,000/mm³ and transfusion-independent platelet count 100,000/mm³ were also required. Exclusion criteria included pregnancy or lactation; uncontrolled infection; concurrent use of other investigational agents, anticancer agents, cytochrome P450 enzyme-inducing antiepileptic drugs, potent CYP3A4 inducers or inhibitors, therapeutic anticoagulants, angiotensin-converting enzyme inhibitors, agents to prevent organ rejection post-transplant, and chronic systemic corticosteroids. This trial was approved by the Institutional Review Boards of participating sites. All patients or their legal guardians signed a document of informed consent and assent was obtained according to institutional guidelines.

Drug administration

Temsirolimus was supplied by the Cancer Therapy Evaluation Program (NCI, Bethesda, MD) and administered intravenously over 30 minutes following pretreatment with diphenhydramine. The starting dose was 15 mg/m²/dose given on days 1 and 8 of a 21 day cycle with planned escalations to 20, 25, and 35 mg/m²/dose. The final cohort of patients was to receive temsirolimus on days 1, 8, and 15 of a 21 day cycle (35 mg/m²/dose). Irinotecan was administered orally (commercial supply) on days 1–5 of each cycle using a 20 mg/mL solution further diluted in juice just prior to administration. The initial dose of irinotecan was 50 mg/m²/dose; the dose was escalated stepwise to a maximum of 90 mg/m²/dose during the study. Temozolomide was administered orally (commercial supply) on days 1–5 of each cycle in capsule form. A dosing nomogram was used to standardize temozolomide dosing. The initial dose of temozolomide was 100 mg/m²/dose; the dose was escalated stepwise to a maximum of 150 mg/m²/dose. Patients maintained a diary to document intake of oral agents. Anti-diarrheal prophylaxis was required (cefixime or equivalent) as was pneumocystis prophylaxis.

Study Design

The primary objectives of the study were 1) to estimate the maximum tolerated dose (MTD) or recommended phase 2 dose and schedule of temsirolimus administered in combination with irinotecan and temozolomide every three weeks to children with recurrent or refractory solid tumors and 2) to define and describe the toxicities of the combination of temsirolimus, irinotecan and temozolomide administered on this schedule. To achieve these objectives, a rolling six dose-escalation scheme was used. [20] Monitoring for regimen-related toxicity included physical examinations (weekly during cycle 1, then prior to subsequent cycles), weekly serum chemistries, triglycerides and cholesterol; serum albumin before each cycle, and complete blood counts (twice weekly during cycle 1, then weekly).

Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 4.0 (<http://ctep.cancer.gov>). Hematologic DLT was defined as grade 4 neutropenia for >7days; grade 4 thrombocytopenia on 2 separate days, or requiring a platelet transfusion 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 attributable to the investigational regimen with the exclusion of Grade 3 nausea, vomiting or diarrhea of <3 days duration; grade 3 mucositis/stomatitis of <3 days duration; transaminase elevation that returned to grade 1 or baseline prior to the time for the next treatment cycle; grade 3 fever or infection; or grade 3 electrolyte abnormalities responsive to supplementation within 7 days; grade 3 hypertriglyceridemia (fasting) that returns to grade 2 prior to start of next treatment cycle; grade 3 hyperglycemia that returned to grade 2 or baseline prior to start of next treatment cycle; or grade 3 hypercholesterolemia that returned to grade 2 within 35 days of initiation of lipid lowering medication. The maximum-tolerated dose (MTD) was the highest dose at which fewer than one-third of patients experienced a DLT during cycle 1 of therapy. Disease evaluations were performed after every second cycle until the sixth cycle and every third cycle thereafter. Tumor response was reported using the Response Evaluation Criteria in Solid Tumors (RECIST)[21] or using the World Health Organization based bidirectional target lesion response assessment (Macdonald Criteria)[22,23], serum markers, and evidence of new lesions for CNS tumors.

Results

Of the 72 patients (median age 10.9 years; range 1.0–21.5 years) enrolled between July 2010 and February 2013, 71 were eligible and 62 were fully evaluable for toxicity. Inevaluable patients never received protocol therapy (n=1), did not complete cycle 1 therapy or received fewer than the required number of doses of medications during cycle 1 (n=6), were found to have had insufficient time elapsed since prior therapy (n=1), or did not complete laboratory monitoring required for accurate toxicity assessment (n=1). Characteristics of eligible and evaluable patients are shown (Table 1).

Maximum Tolerated Dose Determination

Cycle 1 DLTs and additional toxicities are shown (Tables 2 and 3). No DLTs were observed among evaluable patients treated on the first two dose levels, however following treatment

with irinotecan (50 mg/m²/dose), temozolomide (100 mg/m²/dose) and temsirolimus at a dose of 25 mg/m² on Days 1 and 8, two of four evaluable patients developed asymptomatic

Grade 3 hypercholesterolemia that met protocol-defined criteria for DLT. Both patients had CNS tumors and were receiving systemic dexamethasone on a chronic basis.

Cholesterol levels peaked during the third week of therapy. In both patients, cholesterol levels dropped below 315 mg/dL within 2 weeks after study treatment was held and lipid-lowering medication was initiated. Based on this experience, the protocol was amended to exclude patients receiving chronic systemic steroids. Recommendations regarding temsirolimus dose modifications and use of lipid-lowering agents were also revised.

None of the additional 6 patients treated with irinotecan (50 mg/m²/dose), temozolomide (100 mg/m²/dose) and temsirolimus at a dose of 35 mg/m² on Days 1 and 8 experienced Cycle 1 DLT. When the irinotecan dose was escalated to 65 mg/m²/dose combined with temozolomide (100 mg/m²/dose) and temsirolimus (35 mg/m²/dose), one patient experienced dose-limiting diarrhea and a Grade 3 elevation of GGT during Cycle 1. The patient had concurrent *C. difficile* colitis but a relationship between the toxicities observed and protocol-prescribed therapy could not be excluded. None of the other evaluable patients (n=4) treated at this dose level experienced DLT, nor did any of the patients treated with irinotecan at 90 mg/m²/dose together with temozolomide (100 mg/m²/dose) and temsirolimus (35 mg/m²/dose). The dose of temozolomide was then increased to 125 mg/m²/dose. One DLT was observed in this cohort (n=6). A 19-year-old with an anaplastic ependymoma developed headache, vomiting and weakness on day 2 of cycle 1; imaging on day 7 revealed intratumoral hemorrhage with hydrocephalus in the absence of thrombocytopenia. The event was deemed probably related to tumor but possibly related to protocol therapy. No other patient experienced DLT at this dose level, and the dose of temozolomide was increased to 150 mg/m²/dose. Dose-limiting but reversible thrombocytopenia and elevated ALT were observed in two of the first 6 patients treated at this dose level. Because these toxicities represented different classes of adverse effects, per protocol the dose level was expanded. Dose-limiting anorexia, thrombocytopenia and elevated triglycerides were subsequently observed, and the MTD was exceeded at this dose level.

The final cohort of patients (n=6) was treated with irinotecan 90 mg/m²/dose on days 1–5, temozolomide 125 mg/m²/dose on days 1–5, and temsirolimus 35 mg/m²/dose weekly. No DLTs were observed, and therefore these are the doses recommended for further study in the Phase 2 setting.

Additional toxicities

DLTs in subsequent 273 patient-cycles (218 therapeutic cycles and 55 follow-up periods) included grade 4 neutropenia (n=1), grade 3 anorexia and nausea (n=1), grade 4 vomiting (n=1), grade 3 weight loss (n=1), grade 3 elevation in GGT (n=1), and grade 3 abdominal pain in the setting of ascites, hyperbilirubinemia, elevated alkaline phosphatase and elevated transaminases (n=1). One patient was found to have diminished pulmonary function (formal testing performed because the patient had an objective response to therapy and was proceeding to stem cell transplantation) that met DLT criteria; this patient did not have

respiratory symptoms. One patient experienced a grade 2 infusion reaction during administration of temsirolimus. During the first infusion, grade 1 allergic symptoms were ameliorated by stopping the infusion, administering ranitidine, and restarting the infusion at a slower rate. During cycle 2 the patient developed lip swelling during the temsirolimus infusion, and no additional temsirolimus was given. One other patient had a grade 1 allergic reaction attributed to protocol therapy, however the patient went on to receive treatment on study for a total of one year. An additional patient experienced grade 1 pruritus deemed related to protocol therapy; dosing modifications were not required.

Two patients with primary CNS tumors experienced intracranial hemorrhage. One is the patient with an anaplastic ependymoma described in the preceding section. The second was a 5 year old male also with a recurrent anaplastic ependymoma treated with temozolomide 125 mg/m²/dose daily x 5 days, irinotecan 90 mg/m²/dose daily x 5 days, and temsirolimus 35 mg/m²/dose weekly. On Day 16 of Cycle 1 he developed headache and emesis accompanied by change in mental status. Imaging revealed a tumor-associated hemorrhage that was deemed related to tumor rather than protocol therapy by the treating team. Platelet count was normal. One additional patient with a primary CNS tumor did not have a documented intracranial hemorrhage but died while on study without clear cause. The subject was a 10-year-old female with metastatic medulloblastoma who received temozolomide 150 mg/m²/dose daily x 5 days, irinotecan 90 mg/m²/dose daily x 5 days and temsirolimus 35 mg/m²/dose on days 1 and 8. The patient experienced therapy-related nausea and vomiting following the first 4 days of cycle 4 therapy. She was found in bed deceased on the morning of day 5 and aspiration was suspected; an autopsy was not performed. The event was designated as probably related to the underlying malignancy but possibly related to study drugs. There were no bleeding events or unexplained deaths in patients who did not have CNS tumors.

Regimen-related diarrhea grade 3 was uncommon (n=4). Grade 3 nausea and vomiting were observed (n=4 and n=6, respectively), but only 2 patients had grade 3 regimen-related anorexia and only one patient had grade 3 weight loss possibly attributed to protocol therapy. One patient had grade 3 oral mucositis, however most of the mucositis observed was less severe (Grade 2; n=11), and was observed in cycles 1 and 2 only. Eight patients experienced Grade 4 neutropenia that was deemed regimen-related. One patient experienced febrile neutropenia, but this was not attributed to protocol therapy. Treatment-related lymphopenia was observed (Grade 4 n=2, Grade 3 n=23 patients), however no Grade 3 viral or fungal infections related to protocol therapy were reported.

Response

Among the 61 eligible patients in whom response could be evaluated by the cutoff date of 6/1/13, 48 had measurable disease. Objective responses confirmed by central review and follow-up imaging were observed in 5 patients with measurable disease. These included children with neuroblastoma, Ewing sarcoma, ependymoma, hepatoblastoma, and non-Hodgkin lymphoma. These objective responses were sustained for >14 cycles in the patients with neuroblastoma, Ewing sarcoma and ependymoma. The patient with lymphoma elected to pursue stem cell transplantation after sustaining a PR through cycle 5. The patient with

hepatoblastoma experienced disease progression after 5 cycles of protocol therapy. Stable disease of at least 9 cycles' duration was confirmed by central review in 5 patients with measurable disease (3 neuroblastoma, 1 rhabdomyosarcoma, one choroid plexus tumor). One patient with an undifferentiated sarcoma had a CR after cycle 2 (confirmed by central review) but experienced disease progression after cycle 4.

Among those with disease considered evaluable only, one patient with neuroblastoma (MIBG avid bony lesions in both femora and right humerus) had a confirmed CR but declined further therapy after 4 cycles. A second patient with neuroblastoma had MIBG avid disease in the spine that remained stable through 6 cycles of therapy, after which the family elected to pursue other treatment. One patient with a brainstem glioma had stable disease for the maximum duration of protocol therapy (17 cycles). One patient with Ewing sarcoma (multiple lung nodules <1 cm diameter) has had stable disease through 8 cycles and remains on study therapy.

Discussion

mTOR inhibitors have been approved for use in patients with renal cell carcinoma, hormone refractory breast cancer, pancreatic neuroendocrine cancer, and subependymal giant cell astrocytoma [24–28] but modest activity was demonstrated in a phase II study of temsirolimus as monotherapy in children with solid tumors. [14] Through effects on survival signaling and apoptosis, however, mTOR inhibitors may play a role in re-sensitizing tumor cells to chemotherapy. Because temsirolimus and other mTOR inhibitors may be most effective when used in combination with cytotoxic agents, this trial was conducted to determine the appropriate dose and schedule of temsirolimus when combined with irinotecan and temozolomide.

This trial established the maximum tolerated doses of these agents in combination to be irinotecan (90 mg/m²/dose daily for 5 days), temozolomide (125 mg/m²/dose daily for 5 days) and temsirolimus (35 mg/m²/dose weekly) during a 21-day cycle. Because a high rate of infectious complications was observed in adults with glioblastoma multiforme treated with temsirolimus in combination with radiation and temozolomide [29], the starting doses of all three agents in the current study were low. Although lymphopenia was common, infectious complications were rare. Specifically, none of the patients developed significant viral or fungal infections. Bacterial infections were also uncommon, and none of the patients developed febrile neutropenia while on study. Grade 3 neutropenia was seen with moderate frequency in this heavily pretreated cohort of patients, however Grade 4 neutropenia was relatively rare.

Thrombocytopenia was dose-limiting at higher dose levels, but there were no clinically significant bleeding events in the setting of thrombocytopenia. Two patients with CNS malignancies did have tumor-associated hemorrhages while on study, and one patient with a CNS tumor died without clear cause. None of the patients with other tumor types had bleeding events or unexplained deaths. The documented intracranial hemorrhages that occurred during this trial were designated as unrelated or possibly related to protocol therapy. To date, there are no published cases of intracranial hemorrhage attributed to

temsirolimus, however patients with CNS tumors who receive temsirolimus in any future study should be monitored closely for symptoms suggestive of intracranial bleeding.

Hyperlipidemia is common in adults treated with temsirolimus, occurring in >20% of patients in one trial. [24] While none of the patients enrolled on the pediatric phase I trial of temsirolimus experienced grade 3 hyperlipidemia or hypercholesterolemia, 42% and 37% had grade 1 and 2 elevations in lipids and cholesterol, respectively. [30] In a pediatric phase II trial of temsirolimus monotherapy, 4% of patients had grade 3 hyperlipidemia while 29% of patients experienced hyperlipidemia of any grade. [14] Fourteen percent of patients experienced hypercholesterolemia of any grade; none had grade 3 elevations in cholesterol. [14] Although there are data to suggest that mTORC1 regulates cholesterol synthesis through 4E-BP1, the mechanisms by which mTOR inhibitors cause elevations in serum cholesterol have not been entirely elucidated. [31,32] Two patients on this combination phase I trial experienced therapy-related grade 3 elevations in cholesterol; both were treated with a low dose of temsirolimus (25 mg/m²/dose days 1 and 8). Both were receiving chronic systemic steroids. Early initiation of lipid lowering drugs, as recommended in a protocol amendment, led to rapid decreases in lipid levels. A recent retrospective report suggests that temsirolimus-induced elevations in cholesterol (but not glucose or triglycerides) were associated with longer survival among adults with renal cell carcinoma. [32] Prospective evaluation of a larger number of children treated with a uniform dose of temsirolimus would be required to determine if such an association exists in young patients.

Overall, the combination of irinotecan, temozolomide, and temsirolimus was well tolerated in children. Both temsirolimus and irinotecan have been associated with diarrhea; cefixime prophylaxis likely prevented significant diarrhea during this study. Mucositis is an expected side effect of temsirolimus, but it was largely low grade in this cohort, and tended to improve during subsequent cycles of therapy. Additional data regarding the efficacy of an mTOR inhibitor combined with irinotecan and temozolomide will become available upon completion of two ongoing phase II trials for children with neuroblastoma, including a study of irinotecan, temozolomide and temsirolimus being conducted by the Children's Oncology Group, and a study of rapamycin combined with irinotecan, temozolomide and dasatinib in progress in Europe. A study of temsirolimus and irinotecan for children with liver tumors is expected to open in the near future.

References

1. Sarbassov DD, Ali SM, Sabatini DM. Growing roles for the mTOR pathway. *Curr Opin Cell Biol.* 2005; 17(6):596–603. [PubMed: 16226444]
2. Dancey J. mTOR signaling and drug development in cancer. *Nat Rev Clin Oncol.* 2010; 7(4):209–219. [PubMed: 20234352]
3. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol.* 2009; 10(5):307–318. [PubMed: 19339977]
4. Richter JD, Sonenberg N. Regulation of cap-dependent translation by eIF4E inhibitory proteins. *Nature.* 2005; 433(7025):477–480. [PubMed: 15690031]
5. Barrett D, Brown VI, Grupp SA, et al. Targeting the PI3K/AKT/mTOR signaling axis in children with hematologic malignancies. *Paediatr Drugs.* 2012; 14(5):299–316. [PubMed: 22845486]

6. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell*. 2007; 12(1):9–22. [PubMed: 17613433]
7. Mateo-Lozano S, Tirado OM, Notario V. Rapamycin induces the fusion-type independent downregulation of the EWS/FLI-1 proteins and inhibits Ewing's sarcoma cell proliferation. *Oncogene*. 2003; 22(58):9282–9287. [PubMed: 14681687]
8. Gallicchio MA, van Sinderen M, Bach LA. Insulin-like growth factor binding protein-6 and CCI-779, an ester analogue of rapamycin, additively inhibit rhabdomyosarcoma growth. *Horm Metab Res*. 2003; 35(11–12):822–827. [PubMed: 14710364]
9. Hosoi H, Dilling MB, Shikata T, et al. Rapamycin causes poorly reversible inhibition of mTOR and induces p53-independent apoptosis in human rhabdomyosarcoma cells. *Cancer Res*. 1999; 59(4):886–894. [PubMed: 10029080]
10. Wan X, Shen N, Mendoza A, et al. CCI-779 inhibits rhabdomyosarcoma xenograft growth by an antiangiogenic mechanism linked to the targeting of mTOR/Hif-1alpha/VEGF signaling. *Neoplasia*. 2006; 8(5):394–401. [PubMed: 16790088]
11. 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(20):2910–2922. [PubMed: 18026138]
12. Wan X, Mendoza A, Khanna C, et al. Rapamycin inhibits ezrin-mediated metastatic behavior in a murine model of osteosarcoma. *Cancer Res*. 2005; 65(6):2406–2411. [PubMed: 15781656]
13. Spunt SL, Grupp SA, Vik TA, et al. Phase I study of temsirolimus in pediatric patients with recurrent/refractory solid tumors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011; 29(21):2933–2940. [PubMed: 21690471]
14. Georger B, Kieran MW, Grupp S, et al. Phase II trial of temsirolimus in children with high-grade glioma, neuroblastoma and rhabdomyosarcoma. *Eur J Cancer*. 2012; 48(2):253–262. [PubMed: 22033322]
15. Mondesire WH, Jian W, Zhang H, et al. Targeting mammalian target of rapamycin synergistically enhances chemotherapy-induced cytotoxicity in breast cancer cells. *Clin Cancer Res*. 2004; 10(20):7031–7042. [PubMed: 15501983]
16. Piguat AC, Semela D, Keogh A, et al. Inhibition of mTOR in combination with doxorubicin in an experimental model of hepatocellular carcinoma. *J Hepatol*. 2008; 49(1):78–87. [PubMed: 18486258]
17. Gupta A, Dai Y, Vethanayagam RR, et al. Cyclosporin A, tacrolimus and sirolimus are potent inhibitors of the human breast cancer resistance protein (ABCG2) and reverse resistance to mitoxantrone and topotecan. *Cancer Chemotherapy & Pharmacology*. 2006; 58(3):374–383. [PubMed: 16404634]
18. Pencreach E, Guerin E, Nicolet C, et al. Marked activity of irinotecan and rapamycin combination toward colon cancer cells in vivo and in vitro is mediated through cooperative modulation of the mammalian target of rapamycin/hypoxia-inducible factor-1alpha axis. *Clin Cancer Res*. 2009; 15(4):1297–1307. [PubMed: 19190131]
19. Houghton PJ, Morton CL, Kolb EA, et al. Initial testing (stage 1) of the mTOR inhibitor rapamycin by the pediatric preclinical testing program. *Pediatr Blood Cancer*. 2008; 50(4):799–805. [PubMed: 17635004]
20. Skolnik JM, Barrett JS, Jayaraman B, et al. Shortening the timeline of pediatric phase I trials: the rolling six design [see comment]. *Journal of Clinical Oncology*. 2008; 26(2):190–195. [PubMed: 18182661]
21. 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(3):205–216. [PubMed: 10655437]
22. Macdonald DR, Cascino TL, Schold SC Jr, et al. Response criteria for phase II studies of supratentorial malignant glioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1990; 8(7):1277–1280. [PubMed: 2358840]
23. Henson JW, Ulmer S, Harris GJ. Brain tumor imaging in clinical trials. *AJNR Am J Neuroradiol*. 2008; 29(3):419–424. [PubMed: 18272557]

24. Hudes G, Carducci M, Tomczak P, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *New England Journal of Medicine*. 2007; 356(22):2271–2281. [PubMed: 17538086]
25. Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet*. 2008; 372(9637):449–456. [PubMed: 18653228]
26. Baselga J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *The New England Journal of Medicine*. 2012; 366(6):520–529. [PubMed: 22149876]
27. Yao JC, Shah MH, Ito T, et al. Everolimus for advanced pancreatic neuroendocrine tumors. *The New England Journal of Medicine*. 2011; 364(6):514–523. [PubMed: 21306238]
28. Franz DN, Belousova E, Sparagana S, et al. Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet*. 2013; 381(9861):125–132. [PubMed: 23158522]
29. Sarkaria JN, Galanis E, Wu W, et al. Combination of temsirolimus (CCI-779) with chemoradiation in newly diagnosed glioblastoma multiforme (GBM) (NCCTG trial N027D) is associated with increased infectious risks. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2010; 16(22):5573–5580. [PubMed: 20921209]
30. Spunt, SL.; Grupp, S.; Vik, T., et al. Phase 1 Safety, Pharmacokinetic, and Exploratory Biomarker Study of IV Temsirolimus in Children with Advanced Solid Tumors. Toronto: 2007.
31. Wang BT, Ducker GS, Barczak AJ, et al. The mammalian target of rapamycin regulates cholesterol biosynthetic gene expression and exhibits a rapamycin-resistant transcriptional profile. *Proc Natl Acad Sci U S A*. 2011; 108(37):15201–15206. [PubMed: 21876130]
32. Lee CK, Marschner IC, Simes RJ, et al. Increase in cholesterol predicts survival advantage in renal cell carcinoma patients treated with temsirolimus. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2012; 18(11):3188–3196. [PubMed: 22472176]

Figure 1.
Response in a 7 year old female with neuroblastoma who received 15 cycles of protocol therapy

Figure 2.
Response in an 8 year old male with ependymoma who received 17 cycles of protocol therapy

Table I

Patient Characteristics

Characteristic	Eligible Patients Number (percent) n=71	Evaluable Patients Number (percent) n=62
Age (years)		
Median (Range)	10.9 (1.0–21.5)	10.8 (1.0–21.5)
Sex		
Male	45 (63)	39 (63)
Female	26(37)	23 (37)
Diagnosis		
<i>Extracranial Solid Tumors</i>		
Neuroblastoma	16 (22)	15 (24)
Osteosarcoma	7 (10)	5(8)
Ewing Family Tumor	7 (10)	7 (11)
Rhabdomyosarcoma	4 (5)	3 (5)
Other Sarcoma	4 (5)	4 (6)
Hepatoblastoma	2 (3)	2 (3)
Hepatocellular Carcinoma	2 (3)	2 (3)
Other Solid Tumors	6 (8)	6 (10)
<i>Central Nervous System Tumors</i>		
Glioma	14 (20)	11 (18)
Ependymoma	3 (4)	2 (3)
Medulloblastoma	2 (3)	1 (2)
Primitive Neuroectodermal Tumor	2 (3)	2 (3)
Other CNS	2 (3)	2 (3)
Prior Chemotherapeutic Regimens		
Median (Range)	2 (0–8)	2 (0–7)
SCT		
Yes	19 (27)	15 (24)
No	52 (73)	47 (76)
Prior Irinotecan and/or Temozolomide Containing Regimen		
Yes	30 (42)	25 (40)
No	41 (53)	37 (60)
Prior Radiation Therapy	49 (69)	42 (68)

Table II

Cycle 1 DLT by Dose Level

Dose Level	Temozolomide mg/m ² /dose days 1 & 8	Irinotecan mg/m ² /dose days 1-5	Temozolomide mg/m ² /dose days 1-5	No. Patients Entered	No. Patients Evaluable	No. Patients with DLT	Dose Limiting Toxicities (n)
1	15	50	100	6	5	0	
2	20	50	100	8	6	0	
3	25	50	100	5	4	2 [#]	Elevated cholesterol (2)
4	25	50	100	8	7 ^Ω	0	
5	35	50	100	6	6	0	
6	35	65	100	7	5 [*]	1	Diarrhea (1) GGT increased (1)
7	35	90	100	6	6	0	
8	35	90	125	6	6	1	Headache (1) Hydrocephalus (1) Intracranial hemorrhage (1) Nausea (1)
9	35	90	150	13	11 ^{&}	4 ^{&}	ALT increased (1) Anorexia (1) Hypertriglyceridemia (1) Platelet count decreased (2)
10	35	90	125	7	6	0	

[#] Protocol amended to exclude patients receiving chronic systemic steroids and revise temsirolimus dose modifications; dose escalation subsequently continued;

^Ω One patient was deemed inevaluable for non-hematologic toxicities due to lack of required serum glucose monitoring during Cycle 1;

^{*} Six patients were considered evaluable at the time of dose escalation as per Rolling Six; one was later found to be ineligible;

[&] Per protocol, this dose level was expanded as first two DLTs represented different classes of adverse effects

Table III

Adverse Events Possibly, Probably or Definitely Attributed to Protocol Therapy

	Maximum Grade During Cycle 1 (n= 62 cycles)				Maximum Grade During Cycles 2–17 and follow up (n=218 cycles + 63 follow up periods)		
	Grade 2	Grade 3	Grade 4	Grade 4	Grade 2	Grade 3	Grade 4
Hematologic Toxicities							
Anemia	12	2			7	3	
White blood cell decreased	19	11	1		10	12	1
Lymphocyte count decreased	9	14	1		7	13	1
Neutrophil count decreased	22	11	4		8	13	5
Platelet count decreased	5	5	2		8	3	2
Gastrointestinal Toxicities							
Abdominal pain	1					1	
Anorexia	10	1			1	1	
Diarrhea	8	2			4	2	
Mucositis (oral)	9	1			3		
Nausea	5	3			6	2	
Vomiting	6	5			5	1	
Weight loss	1				2	1	
Metabolic/Laboratory Toxicities							
Elevated cholesterol		1	1		1		
Elevated triglycerides	7				1	3	
Hyperglycemia	1						
Hypokalemia		3				5	
Hypophosphatemia	3	2			2	2	
Increased ALT	7	1			2	3	
Increased AST	3				1	1	1
Other Toxicities							
Fatigue	4				2		
Headache	3	1			1		

* Non-hematologic toxicities are those that occurred in > 10% of patients