Published in final edited form as:

Pediatr Blood Cancer. 2015 March ; 62(3): 440–444. doi:10.1002/pbc.25334.

Phase II Study of Cixutumumab in Combination with Temsirolimus in Pediatric Patients and Young Adults with Recurrent or Refractory Sarcoma: A Report from the Children's Oncology Group

Lars M. Wagner,

Cincinnati Children's Hospital Medical Center, Cincinnati, OH, Division of Oncology

Maryam Fouladi,

Cincinnati Children's Hospital Medical Center, Cincinnati, OH, Division of Oncology

Atif Ahmed,

Mercy Children's Hospital, Kansas City, MO, Department of Pathology

Mark D. Krailo,

University of Southern California, Los Angeles, CA, Keck School of Medicine, Department of Preventive Medicine

Brenda Weigel,

University of Minnesota, Minneapolis, MN, Division of Pediatric Hematology/Oncology

Steven G. DuBois,

University of California, San Francisco School of Medicine, San Francisco, CA, Division of Pediatric Hematology/Oncology

L. Austin Doyle,

Cancer Therapy Evaluation Program, National Cancer Institute

Helen Chen, and

Cancer Therapy Evaluation Program, National Cancer Institute

Susan M. Blaney

Texas Children's Hospital, Baylor College of Medicine, Houston, TX, Division of Hematology/Oncology

Abstract

Background—The combined inhibition of insulin-growth factor type 1 receptor (IGF-1R) and the mammalian target of rapamycin (mTOR) has shown activity in preclinical models of pediatric sarcoma and in adult sarcoma patients. We evaluated the activity of the anti-IGF-1R antibody cixutumumab with the mTOR inhibitor temsirolimus in patients with relapsed or refractory Ewing

Corresponding author: Lars Wagner, MD, University of Kentucky Pediatric Hematology/Oncology, 740 S. Limestone, Office J-457, Lexington, KY 40502, Phone: 859-218-0931, Fax: 859-323-8641, lars.wagner@uky.edu cc: pubs@childrensoncologygroup.org.

The authors have no conflict of interest to report.

sarcoma, osteosarcoma, rhabdomyosarcoma, and other soft tissue sarcoma, using the recommended dosages from a pediatric phase I trial.

Methods—Cixutumumab 6 mg/kg and temsirolimus 8 mg/m² were administered intravenously once weekly in 4-week cycles to patients ≥30 years of age. Temsirolimus was escalated to 10 mg/m² for subsequent cycles in patients who did not experience unacceptable first-cycle toxicity. A two-stage design was used to identify a response rate < 10% or > 35% for each tumor-specific cohort. Tumor tissue was analyzed by immunohistochemistry for potential biomarkers of response.

Results—Forty-three evaluable patients received a median of 2 cycles (range, 1–7). No objective responses were observed, and 16% of patients were progression-free at 12 weeks. Dose-limiting toxicity was observed in 15 (16%) of 92 cycles. The most common toxicities were mucositis, electrolyte disturbances, and myelosuppression. The majority of patients receiving a second cycle were not eligible for temsirolimus escalation due to first-cycle toxicity. The lack of objective responses precluded correlation with tissue biomarkers.

Conclusions—Despite encouraging preclinical data, the combination of cixutumumab and temsirolimus did not result in objective responses in this phase II trial of pediatric and young adults with recurrent or refractory sarcoma.

Keywords

cixutumumab; temsirolimus; Phase II; pediatric sarcoma

INTRODUCTION

The clinical benefit of molecularly targeted agents used as monotherapy is often limited by escape mechanisms that lead to tumor cell resistance. Rational targeting of multiple pathways implicated in both oncogenesis and resistance to therapy may improve efficacy. Signaling through the mammalian target of rapamycin (mTOR) pathway appears important for the growth and survival of many pediatric sarcomas [1]. However, single-agent activity of mTOR inhibitors may be limited by upstream activation of AKT through the release of feedback inhibition [2]. This upstream activation is mediated in part through the insulin-like growth factor-1 receptor (IGF-1R), and antibody blockade of IGF-1R can abrogate this escape pathway and synergize with mTOR inhibitors in preclinical models of pediatric sarcomas [3–7]. In fact, maintained complete responses have been observed in murine models of osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma when combining non-curative doses of an anti-IGF-1R antibody with an mTOR inhibitor [3]. In addition, clinical responses have been reported when these two classes of agents are used together in Ewing sarcoma patients who had prior progression after single-agent anti-IGF-1R antibody [8].

Cixutumumab (IMC-A12; ImClone Systems, a wholly-owned subsidiary of Eli Lilly and Company, Indianapolis, IN) is an investigational fully humanized monoclonal antibody against IGF-1R which reduces cell surface IGF-1R expression and blocks interactions with both IGF-1 and IGF-2 ligands. IGF-1R is an attractive therapeutic target due to its implication in the oncogenesis and maintenance of various sarcoma types [9]. Temsirolimus (CCI-779; Torisel®) is an mTOR inhibitor used for renal cancer and administered

intravenously on the same weekly schedule as IMC-A12. In addition to the therapeutic synergy seen preclinically with these classes of agents, patients with recurrent Ewing sarcoma and rhabdomyosarcoma have occasionally responded to single-agent cixutumumab and/or temsirolimus [10,11]. Further, two recent studies have shown encouraging activity with this combination in adult phase II trials of bone and soft tissue sarcoma [12,13].

This report describes the results of a COG phase II study designed to assess the objective response rate of temsirolimus in combination with cixutumumab in pediatric and young adult patients with recurrent or refractory sarcoma. We used the recommended phase II dosages obtained from a phase I trial of this combination in children with recurrent solid tumors [14]. In that study, frequent dose-limiting toxicities including mucositis led to a recommended temsirolimus dose of 8 mg/m² (approximate flat dose of 14 mg), which is lower than the flat temsirolimus dose of 25 mg used in some adult combination studies with cixutumumab [12,13].

PATIENTS AND METHODS

Patient Population

Eligible patients included those 1 and 30 years with relapsed or refractory bone or soft tissue sarcoma. Patients were divided into one of four cohorts, including: osteosarcoma; Ewing sarcoma; rhabdomyosarcoma; and non-rhabdomyosarcoma soft tissue sarcoma (NRSTS). Patients were required to have measurable disease on cross-sectional imaging 10 mm in one dimension, and have archived tumor tissue available for immunohistochemical evaluation. Other eligibility criteria such as standard organ function and performance status were similar to the previously reported pediatric phase I trial [15], with the exception that patients with metastatic bone marrow involvement were eligible for this study if they had an absolute neutrophil count $\geq 750/\mu\text{L}$, a supported platelet count of $\geq 50,000/\mu\text{L}$, and hemoglobin ≥ 8.0 g/dL. Patients were required to have normal blood glucose, and cholesterol and triglyceride levels that were $<$ grade 2 using the Common Terminology for Classifying Adverse Events (CTCAE) version 4. Patients were excluded if they had previously been treated with IGF-1R-targeted agents or mTOR inhibitors, or if they were receiving insulin, growth hormone, enzyme-inducing anticonvulsants, warfarin, or other anticancer agents. Patients with known diabetes, uncontrolled infection, or recent major surgery within three weeks of enrollment were also excluded.

This trial was approved by the National Cancer Institute Pediatric Central Institutional Review Board, as well as by local regulatory boards at all participating sites. A document of informed consent was signed by all patients or their parent/legal guardian, and assent was obtained as appropriate according to the local institutional guidelines prior to enrollment.

Drug Administration

Cixutumumab and temsirolimus were both supplied by the National Cancer Institute (Bethesda, MD). Cixutumumab was administered as a one-hour intravenous infusion, followed immediately by a 30-minute intravenous infusion of temsirolimus. Intravenous

premedication with an antihistamine such as diphenhydramine was required to minimize the potential for an infusion reaction.

During cycle 1, all patients received cixutumumab 6 mg/kg and temsirolimus 8 mg/m² (maximum 16 mg). Patients were eligible for a single inpatient dose escalation of temsirolimus to 10 mg/m² (maximum 20 mg) if during the first cycle they did not experience grade 2 mucositis or any grade 3 toxicity attributable to the study treatment. No further dose escalations were allowed beyond this dose.

Cixutumumab and temsirolimus were administered weekly in 28-day cycles, and treatment could continue for up to 25 cycles in the absence of disease progression or toxicity requiring discontinuation of treatment. Cycles were repeated provided initial eligibility criteria were met, including an absolute neutrophil count (ANC) $\geq 1,000/\mu\text{L}$ and platelet count $\geq 100,000/\mu\text{L}$. Hematologic dose-limiting toxicity (DLT) was defined as grade 4 neutropenia > 7 days or on day of scheduled drug administration, thrombocytopenia $< 20,000/\mu\text{L}$ on two separate days or requiring two or more platelet transfusions, or myelosuppression causing a delay of ≥ 14 days between treatment cycles. For patients with hematologic DLT or who did not meet blood count criteria to start a subsequent cycle, treatment was held until the ANC was $\geq 750/\mu\text{L}$ and platelets $\geq 75,000/\mu\text{L}$, and the temsirolimus was reduced by 2 mg/m² from the previous dose. Non-hematologic DLT was defined as any grade 4 non-hematologic toxicity attributable to study drugs, or any grade 3 non-hematologic toxicity excluding vomiting, reversible transaminase elevation, infection, or electrolyte disturbance responsive to oral supplementation. Patients with non-hematologic DLT that did not resolve to eligibility criteria within 21 days after the planned start of the next treatment cycle, or which recurred at a lower dosage, were removed from protocol therapy. Otherwise, patients received doses of cixutumumab and temsirolimus that were reduced by 2 mg/kg and 2 mg/m², respectively.

Study Design

A two-stage design was used to evaluate cixutumumab and temsirolimus in the four target disease strata. At the first stage for each stratum, 11 patients in each stratum were to be enrolled. If 1 patient experienced an objective response, defined as either partial response (PR) or complete response (CR), the combination was considered inactive in that stratum, and enrollment to that stratum was terminated. If 2 or more patients achieved an objective response, 8 additional patients were to be enrolled to that stratum. The combination would be considered active if 4 of 19 patients in an expanded stratum experienced a CR or PR. With this design, the combination would be identified as inactive if the true response rate was 10% with a probability of 0.90, and would be identified as active if the true response rate was 35% with a probability of 0.91. The point estimate of the response rate and 95% confidence intervals were calculated using the method of Jung and Kim [15]. Response was evaluated per the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria [16], with imaging performed at a minimum after every two cycles.

Any eligible patient who received at least one dose of each agent was considered evaluable for response provided: (1) the patient demonstrated progressive disease or died while on protocol therapy; or (2) the patient was observed on protocol therapy for at least one cycle

and the tumor was not removed surgically prior to the time a complete or partial response was confirmed; or (3) the patient demonstrated a complete or partial response as confirmed by central review of radiographic images. All evaluable patients who did not achieve CR or PR were considered non-responders. The evaluation period for determination of the overall best response was six treatment cycles.

Toxicity evaluation

Each cycle in which the combination was administered to an eligible patient was considered in the analysis of toxicity. The treating physician assigned an attribution for each CTCAE version 4 gradable adverse experience (AE) as unrelated, unlikely, possibly, probably, or definitely related to the combination. The relative frequency of each AE considered possibly, probably, or definitely related to the combination was estimated as the proportion of all toxicity-evaluable cycles in which such toxicity was observed.

Each cycle was evaluable for DLT if a patient either experienced DLT or received all four doses of the combination for that cycle without experiencing DLT. The per-cycle rate of DLT was monitored using a Bayesian rule. A beta prior distribution with $\alpha=0.6$ and $\beta=1.4$ was employed. The analytic unit for analysis was the patient cycle; all cycles were considered independent. If at any time the posterior probability that the per-cycle DLT probability exceeded 30% was greater than 0.80, the trial was to be identified for possible termination because of an excessive DLT rate.

Immunohistochemical Testing

Tumor tissue was assessed by immunohistochemistry on all patients for expression of putative biomarkers of response to these agents as suggested by previous studies [5,8,17–19]. Testing was carried out according to the manufacturer's specifications, and the following proteins were assessed: IGF-1R (Cell Signaling, Danvers, MA), insulin receptor (Abcam, Cambridge, MA), ERK (Cell Signaling, Danvers, MA), RON (Abcam, Cambridge, MA), p-AKT (Leica Biosystems, Buffalo Grove, IL), p-mTOR (Cell Signaling, Danvers, MA), and p-S6 ribosomal protein (Cell Signaling, Danvers, MA). Automated immunohistochemistry was performed with a Leica Bond Max Instrument, (Leica, Richmond, Illinois). A Bond Polymer Refine Detection system was utilized for single brown color staining. Deparaffinized slides were rinsed and then sequentially incubated with the primary antibody, a secondary antibody and a polymer conjugate. This procedure was performed for all tumor cases and positive controls. Tissues labeled as negative controls were treated similarly except for omission of the primary antibodies. The extent of expression was interpreted by an experienced pediatric pathologist (AA) and scored using the following system: 0 = no staining; 1+ = weak staining or < 20% of tumor cells stained; 2+ = moderate staining or between 20–80% of tumor cells stained; and 3+ = strong staining, or > 80% of tumor cells stained.

RESULTS

Patient Characteristics

This study (study code: ADVL1221; ClinicalTrials.gov identifier: NCT01614795) was opened in June 2012 and closed in June 2013. Data as of September 2013 were used in the analyses. Forty-six (46) patients were enrolled, including 11 with osteosarcoma, 12 with Ewing sarcoma, 11 with rhabdomyosarcoma, and 12 with NRSTS. One patient with Ewing sarcoma was ineligible because the individual was treated with myelosuppressive chemotherapy within 21 days of enrollment. One patient with NRSTS who did not receive protocol-mandated antihistamine premedication experienced an allergic reaction to the first treatment, and was removed from protocol therapy and considered inevaluable for both response assessment and toxicity. These two patients were replaced for evaluation of the primary statistical analysis. One patient with osteosarcoma underwent surgical removal of all measurable disease prior to the evaluation of response to chemotherapy, and was considered inevaluable for response assessment. This patient was not replaced, because at that point in the study, the osteosarcoma stratum would not have been expanded even if the replacement patient experienced a response. Therefore, 44 patients were evaluable for toxicity, and 43 patients for response. The characteristics of all eligible patients in the analytic cohort are described in Table I. At the time of the analysis all patients had completed protocol therapy.

Antitumor Activity

No objective responses were observed, and according to the protocol design, this combination was not considered of sufficient efficacy for further development in any of the strata considered.

The median number of treatment cycles for 44 eligible patients who received at least one complete cycle of therapy was 2 (range 1–7). Seven (16%) patients were progression-free through at least three cycles (12 weeks), and 5 (12%) were confirmed on repeat imaging to have stable disease, including two with Ewing sarcoma, two with rhabdomyosarcoma and one with NRSTS. These patients received 7, 6, 6, 4 and 7 cycles of protocol therapy, respectively.

Toxicity Evaluation

Grade 3 or higher CTC AE version 4 adverse experiences reported as at least possibly related to study therapy are displayed in Table II. DLT was reported in 15 (16%) of 92 cycles, and was confined to the first three cycles of therapy. A total of 19 CTC-gradable DLTs were reported, including: (1) ALT elevation, anaphylaxis, hyperglycemia, hypertriglyceridemia, hypoalbuminemia, hypokalemia, hyponatremia, hypophosphatemia – one incident each; (2) AST elevation and creatinine increase – 2 incidents each; (3) platelet count decrease – 3 incidents, and (4) oral mucositis – 4 incidents. No treatment-related deaths were reported. The posterior probability that DLT exceeds 30% is less than 0.01, and we concluded that this treatment was not associated with an excessive rate of DLT.

Of 44 patients evaluable for toxicity, 13 (30%) underwent dose escalation of temsirolimus during the second cycle, 12 (27%) had disease progression during or at the end of cycle 1, and 19 (43%) were not eligible for dose escalation based on first-cycle toxicity. Therefore, of the 32 patients receiving therapy for a second cycle, 13 (41%) were escalated, while 19 (59%) had first-cycle toxicity that precluded escalation. One patient with rhabdomyosarcoma was removed from protocol therapy due to persistent thrombocytopenia after 3 cycles despite reduction of the temsirolimus dose to 6 mg/m².

Immunohistochemistry Analysis

Archived tissue was available for testing on 44 patients, although for 3 patients, no tumor was identified in the submitted sample. Tissue samples were obtained at the time of previous recurrence or progression in all patients, and an additional 7 patients (16%) had paired samples from the time of diagnosis as well. The results of the immunohistochemistry tests for the various sarcoma types are reported in Table III. For patients with paired samples available for testing, only results from tissue obtained at the latest time point were included. Moderate or strong protein expression of IGF-1R, p-AKT, and p-mTOR was seen in 53%, 81%, and 23% of all tumors, respectively. Unfortunately, the absence of response precluded correlation with tissue biomarkers.

In the patients with samples both at diagnosis and recurrence, all four tumor strata were represented, and no consistent changes were observed. For example, there was loss of expression of IGF-1R in three patients, with gain of IGF-1R expression in three others. Loss of expression of IR, p-S6, and RON was observed in 4, 4, and 2 patients, respectively. No significant change was seen in expression of mTOR or p-AKT.

DISCUSSION

The rate of objective response and disease stability for the combination of cixutumumab and temsirolimus was disappointingly low in this phase II trial for children and young adults with recurrent sarcoma. In fact, we did not observe any complete or partial responses, and only 16% of patients remained on study progression-free at 12 weeks. Although direct comparison is difficult, these results appear inferior to a recently reported larger phase II trial of adults with bone or soft tissue sarcoma in which over one-third had disease stability for at least three months, and 4 (15%) of 27 Ewing sarcoma patients and 3 (13%) of 24 osteosarcoma patients experienced partial responses with this drug combination [12]. In another adult phase II trial, 2 (12%) of 17 Ewing sarcoma patients achieved complete responses, and three others had tumor reduction of > 20% [13].

The reasons for the poor activity seen on this study are unclear. The temsirolimus dose of 8 mg/m² (equivalent to an adult flat dose of 14 mg) in this trial was lower than the 25 mg flat dose used in adult sarcoma studies [12,13], although similar to the recommended phase II dose identified from a study in patients with metastatic breast cancer (cixutumumab 4 mg/kg, temsirolimus 15 mg flat dose) [20]. Our dose was based on a COG phase I trial in children with recurrent solid tumors, in which a variety of dose-limiting toxicities were encountered at temsirolimus doses higher than 8 mg/m² [14]. Supporting this decision was evidence of biologic effects of mTOR inhibition observed in peripheral blood mononuclear

cells at this dose [14], and the absence of any clear dose-response relationship in a previous adult single-agent study of temsirolimus comparing flat doses of 25, 75, and 250 mg [21]. Further, over half of patients treated in the large adult sarcoma phase II study required temsirolimus dose reduction due to toxicity, including 29% requiring two reductions [12]. Our finding that dose-limiting toxicity occurred in 16% of evaluable cycles is also consistent with results from the pediatric phase I trial, and the majority of patients receiving a second cycle of treatment were not eligible for escalation of temsirolimus from 8 mg/m² to 10 mg/m² due to first-cycle toxicity. In the 13 patients who did undergo cycle 2 escalation of temsirolimus, the increase appeared to be tolerable. However, these patients only received a median of three cycles of treatment, and the true importance of temsirolimus dosing in the context of combination therapy remains uncertain.

Among pediatric solid tumors, IGF-1R inhibition has been most thoroughly studied in Ewing sarcoma. Despite encouraging results from initial dose-finding trials, phase II studies of four different single-agent IGF-1R antibodies have produced relatively low response rates of <15% in adults and children with recurrent tumors, although some have had dramatic complete responses [22–26]. No studies have been done to directly compare the efficacy of one antibody against another in a controlled study. There has been great enthusiasm to identify predictive biomarkers which could allow selection of this small subset of patients most likely to benefit. Previous studies have not shown consistently meaningful results from serum markers such as IGF-1 and IGF-BP3 [25,27], and even the expression of IGF-1R by immunohistochemistry did not predict response in adult sarcoma patients receiving the drug combination used in our study [12]. We chose to study seven proteins that had been suggested as putative biomarkers in previous studies [5,8,17–19], hoping that a panel would better identify an expression signature correlating with response. We used immunohistochemistry to test archival tissues given the widespread availability of these assays and the benefit of not requiring re-biopsy for study purposes. Unfortunately, the lack of efficacy made clinical correlation with response impossible.

Given the low rate of responses following single-agent IGF-1R antibody therapy for sarcoma, and the continued absence of any predictive biomarker that would allow for enrichment of patient studies, the future of this strategy remains unclear. At the dosages used in our study, combination with temsirolimus likely increased toxicity but did not improve activity. It may be that the optimum use of these agents will be in combination with conventional cytotoxic drugs, since both cixutumumab and temsirolimus may affect chemotherapy-induced apoptosis and be synergistic with commonly used chemotherapy drugs such as cyclophosphamide and doxorubicin [28,29]. This approach is currently being studied by the COG in combination studies for treatment of rhabdomyosarcoma, and in a planned trial for patients with metastatic Ewing sarcoma.

Acknowledgments

This work was supported by study grants CA98543 (Chair's Grant), CA180886 (NCTN Operation Center Grant), CA98413 (Statistics and Data Center Grant), CA180899 (NCTN Statistics and Data Center Grant)

References

1. Wan X, Helman LJ. The biology behind mTOR inhibition in sarcoma. *Oncologist*. 2007; 12:1007–1018. [PubMed: 17766661]
2. Wan X, Harkavy B, Shen N, et al. Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. *Oncogene*. 2007; 26:1932–1940. [PubMed: 17001314]
3. Kurmasheva RT, Dudkin L, Billups C, et al. The insulin-like growth factor-1 receptor-targeting antibody, CP-751,871, suppresses tumor-derived VEGF and synergizes with rapamycin in models of childhood sarcoma. *Cancer Res*. 2009; 69:7662–7671. [PubMed: 19789339]
4. Kolb EA, Gorlick R, Maris JM, et al. Combination testing (Stage 2) of the Anti-IGF-1 receptor antibody IMC-A12 with rapamycin by the pediatric preclinical testing program. *Pediatr Blood Cancer*. 2012; 58:729–735. [PubMed: 21630428]
5. Beltran PJ, Chung YA, Moody G, et al. Efficacy of ganitumab (AMG 479), alone and in combination with rapamycin, in Ewing's and osteogenic sarcoma models. *J Pharmacol Exp Ther*. 2011; 337:644–654. [PubMed: 21385891]
6. Kolb EA, Kamara D, Zhang W, et al. R1507, a fully human monoclonal antibody targeting IGF-1R, is effective alone and in combination with rapamycin in inhibiting growth of osteosarcoma xenografts. *Pediatr Blood Cancer*. 2010; 55:67–75. [PubMed: 20486173]
7. Cao L, Yu Y, Darko I, et al. Addition to elevated insulin-like growth factor I receptor and initial modulation of the AKT pathway define the responsiveness of rhabdomyosarcoma to the targeting antibody. *Cancer Res*. 2008; 68:8039–8048. [PubMed: 18829562]
8. Subbiah V, Naing A, Brown RE, et al. Targeted morphometric profiling of Ewing's sarcoma treated with insulin-like growth factor 1 receptor (IGF1R) inhibitors: response/resistance signatures. *PLoS One*. 2011; 6:e18424. [PubMed: 21494688]
9. Kolb EA, Gorlick R. Development of IGF-IR Inhibitors in Pediatric Sarcomas. *Curr Oncol Rep*. 2009; 11:307–313. [PubMed: 19508836]
10. Malempati S, Weigel B, Ingle AM, et al. Phase I/II trial and pharmacokinetic study of cixutumumab in pediatric patients with refractory solid tumors and Ewing sarcoma: a report from the Children's Oncology Group. *J Clin Oncol*. 2012; 30:256–262. [PubMed: 22184397]
11. 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; 48:253–262. [PubMed: 22033322]
12. Schwartz GK, Tap WD, Qin LX, et al. Cixutumumab and temsirolimus for patients with bone and soft-tissue sarcoma: a multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2013; 14:371–382. [PubMed: 23477833]
13. Naing A, LoRusso P, Fu S, et al. Insulin growth factor-receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with refractory Ewing's sarcoma family tumors. *Clin Cancer Res*. 2012; 18:2625–2631. [PubMed: 22465830]
14. Fouladi M, Perentesis JP, Wagner LM, et al. A phase I trial of IMC-A12 and temsirolimus in children with refractory solid tumors: A Children's Oncology Group Study [abstract]. In: 2011 Annual Meeting of American Society of Clinical Oncology. *J Clin Oncol*. 2011; 29(suppl):abstract 9520.
15. Jung SH, Kim KM. On the estimation of the binomial probability in multistage clinical trials. *Statist Med*. 2004; 23:881–896.
16. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009; 45:228–245. [PubMed: 19097774]
17. Cho D, Signoretti S, Dabora S, et al. Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. *Clin Genitourin Cancer*. 2007; 5:379–385. [PubMed: 17956710]
18. Garofalo C, Manara MC, Nicoletti G, et al. Efficacy of and resistance to anti-IGF-1R therapies in Ewing's sarcoma is dependent on insulin receptor signaling. *Oncogene*. 2011; 30:2730–40. [PubMed: 21278796]

19. Potraz JC, Saunders DN, Wai DH, et al. Synthetic lethality screens reveal RPs6 and MST1R as modifiers of insulin-like growth factor-1 receptor inhibitor activity in childhood sarcomas. *Cancer Res.* 2010; 70:8770–8791. [PubMed: 20959493]
20. Ma CX, Suman VJ, Goetz M, et al. A Phase I trial of the IGF-1R antibody cixutumumab in combination with temsirolimus in patients with metastatic breast cancer. *Breast Cancer Res Treat.* 2013; 139:145–153. [PubMed: 23605083]
21. Atkins MB, Hidalgo M, Stadler WM, et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J Clin Oncol.* 2004; 22:909–918. [PubMed: 14990647]
22. Schöffski P, Adkins D, Blay JY, et al. An open-label, phase 2 study evaluating the efficacy and safety of the anti-IGF-1R antibody cixutumumab in patients with previously treated advanced or metastatic soft-tissue sarcoma or Ewing family of tumours. *Eur J Cancer.* 2013; 49:3219–3228. [PubMed: 23835252]
23. Juergens H, Daw NC, Geoerger B, et al. Preliminary efficacy of the anti-insulin-like growth factor type 1 receptor antibody figitumumab in patients with refractory Ewing sarcoma. *J Clin Oncol.* 2011; 29:4534–4540. [PubMed: 22025154]
24. Pappo AS, Patel SR, Crowley J, et al. R1507, a monoclonal antibody to the insulin-like growth factor 1 receptor, in patients with recurrent or refractory Ewing sarcoma family of tumors: results of a phase II Sarcoma Alliance for Research through Collaboration study. *J Clin Oncol.* 2011; 29:4541–4547. [PubMed: 22025149]
25. Tap WD, Demetri G, Barnette P, et al. Phase II study of ganitumab, a fully human anti-type-1 insulin-like growth factor receptor antibody, in patients with metastatic Ewing family tumors or desmoplastic small round cell tumors. *J Clin Oncol.* 2012; 30:1849–1856. [PubMed: 22508822]
26. Weigel B, Malempati S, Reid JM, et al. Phase 2 trial of cixutumumab in children, adolescents, and young adults with refractory solid tumors: A report from the Children's Oncology Group. *Pediatr Blood Cancer.* 2014; 61:452–456. [PubMed: 23956055]
27. Olmos D, Martins AS, Jones RL, et al. Targeting the Insulin-Like Growth Factor 1 Receptor in Ewing's Sarcoma: Reality and Expectations. *Sarcoma.* 2011:402508. [PubMed: 21647361]
28. Houghton PJ, Morton CL, Gorlick R, et al. Stage 2 combination testing of rapamycin with cytotoxic agents by the Pediatric Preclinical Testing Program. *Mol Cancer Ther.* 2010; 9:101–112. [PubMed: 20053767]
29. Benini S, Manara MC, Baldini N, et al. Inhibition of insulin-like growth factor I receptor increases the antitumor activity of doxorubicin and vincristine against Ewing's sarcoma cells. *Clin Cancer Res.* 2001; 7:1790–1797. [PubMed: 11410521]

Table 1

Characteristics of Eligible Patients

	OS (n=11)	ES (n=11)	RMS (n=11)	NRSTS (n=12)	All (n=45)
Age (years)					
Median	18	18	14	18	17
Range	7–25	9–27	1–23	13–26	1–27
Over 21	2	3	2	3	10
Gender					
Male:female	10:1	6:5	3:8	7:5	26:19
Race					
White	7	8	9	7	31
African American	2	0	0	1	3
Asian	1	1	0	2	4
Native American	0	0	0	1	1
Other	1	2	2	1	6

OS, osteosarcoma; ES, Ewing sarcoma; RMS, rhabdomyosarcoma; NRSTS, non-rhabdomyosarcoma soft tissue sarcoma

Table II

Grade 3–4 Toxicities Attributed as at Least Possibly Related to Protocol Therapy for All Cycles (Total 92 Cycles)

<u>Toxicity type</u>	<u>Grade 3</u>	<u>Grade 4</u>
<i>Hematologic</i>		
Neutropenia	6	
Leukopenia	3	
Anemia	3	
Thrombocytopenia	3	2
<i>Non-hematologic</i>		
Hypokalemia	5	
Oral mucositis	4	
Hypophosphatemia	4	
Aspartate aminotransferase	3	
Alanine aminotransferase	2	
Hypertriglyceridemia	2	
Pain	2	
Alkaline phosphatase	1	
Anaphylaxis	1	
Ascites	1	
Hyperbilirubinemia	1	
Elevated creatinine	1	
Epistaxis	1	
Intestinal obstruction	1	
Hyperglycemia	1	
Hypermagnesemia	1	
Hyperuricemia		1

Table III
Summary of Immunohistochemistry Expression Data from Archival Tumor Tissue

Extent of Expression	OS (n=10)	ES (n=11)	RMS (n=11)	NRSTS (n=11)	All (n=43)
IGF-IR					
0	2	4	1	3	10
1+	4	3	0	2	9
2+	2	2	2	5	11
3+	2	2	8	1	13
Insulin receptor					
0	4	4	4	3	15
1+	2	2	1	2	7
2+	2	3	4	2	11
3+	1	2	2	4	9
p-AKT					
0	1	0	0	0	1
1+	2	2	0	3	7
2+	3	3	2	3	11
3+	4	6	9	5	24
p-ERK					
0	2	5	6	3	16
1+	5	5	2	4	16
2+	1	0	3	3	7
3+	2	1	0	1	4
p-mTOR					
0	8	7	10	8	33
1+	2	3	1	3	9
2+	0	1	0	0	1
p-S6					
0	1	2	2	3	7
1+	2	4	6	2	15
2+	4	3	3	0	16
3+	3	2	0	0	5

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Extent of Expression	OS (n=10)	ES (n=11)	RMS (n=11)	NRSTS (n=11)	All (n=43)
RON					
0	7	10	10	6	33
1+	3	1	1	4	9
3+	0	0	0	1	1

OS, osteosarcoma; ES, Ewing sarcoma; RMS, rhabdomyosarcoma; NRSTS, non-rhabdomyosarcoma soft tissue sarcoma