Cancer Therapy: Clinical

Insulin Growth Factor-Receptor (IGF-1R) Antibody Cixutumumab Combined with the mTOR Inhibitor Temsirolimus in Patients with Refractory Ewing's Sarcoma Family Tumors

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Abstract

Purpose: Temsirolimus was combined with cixutumumab, a fully human IgG1 monoclonal antibody directed at the insulin growth factor-1 receptor (IGF-1R).

Experimental Design: Patients received cixutumumab, 6 mg/kg i.v. weekly, and temsirolimus, 25 to 37.5 mg i.v. weekly (4-week cycles), with restaging after 8 weeks. Median follow-up was 8.9 months.

Results: Twenty patients [17 with Ewing's sarcoma (EWS), 3 with desmoplastic small-round cell tumor (DSRCT)] were enrolled. Twelve patients (60%) were men with a median age of 24 years and six median prior systemic therapies in a metastatic setting. The most frequent toxicities were thrombocytopenia (85%), mucositis (80%), hypercholesterolemia (75%), hypertriglyceridemia (70%), and hyperglycemia (65%; mostly grade I–II). Seven of 20 patients (35%) achieved stable disease (SD) for more than 5 months or complete/partial (CR/PR) responses. Tumor regression of more than 20% (23%, 23%, 27%, 100%, 100%) occurred in five of 17 (29%) patients with EWS, and they remained on study for 8 to 27 months. One of six patients with EWS who previously developed resistance to a different IGF-1R inhibitor antibody achieved a CR. Four of the seven best responders developed grade III mucositis, myelosuppression, or hyperglycemia, which were controlled while maintaining drug dose.

Conclusion: Cixutumumab combined with temsirolimus was well-tolerated and showed preliminary evidence of durable antitumor activity in heavily pretreated EWS family tumors. Clin Cancer Res; 1–7. ©2012 AACR.

Introduction

Upstream tyrosine kinases, such as insulin and the insulin growth factor-1 receptor (IGF-1R), can regulate the PI3K/AKT/mTOR pathway (1). In vitro, in vivo, and tumor biopsy studies show that mTOR inhibitors activate a feedback loop resulting in upregulated AKT phosphorylation in tumor tissue, which occurs via an IGF-1R-dependent mechanism. This feedback can be abrogated, or at least limited, by IGF-1R pathway inhibition (2–4). These observations provide a rational basis for combining mTOR and IGF-1R inhibitors as a way to overcome resistance to the agents when given as monotherapy. This assumption is supported by preclinical evidence in vivo Ewing's sarcoma (EWS) and rhabdomyosarcoma models treated with the combination of an mTOR inhibitor and IGF-1R inhibitor, which showed enhanced antitumor activity compared with treatment with each agent alone (5, 6). Unfortunately, effective treatment for relapsed sarcoma has remained largely elusive, despite the fact that sarcomas are among the most common cancers of childhood and early adolescence (7, 8). EWS most frequently affects children and adolescents and is characterized by a translocation between the EWS protein and various fusion proteins, most commonly FLI1 (9). Desmoplastic small-round cell tumor (DSRCT) is a rare and aggressive soft tissue sarcoma, which primarily presents with abdominal masses, and is considered by some to be a part of the EWS family of tumors. Despite this controversy,
Patients and Methods

Eligibility criteria
Eligible patients had advanced or metastatic, histologically proven malignant EWS or DSRCT. Further requirements were age 14 years or older, ECOG performance status of 0 or 1, and life expectancy greater than 12 weeks. Patients were required to have an absolute neutrophil count (ANC) ≥ 1,500/mL, platelets ≥ 100,000/mL, creatinine < 2 × the upper limit of normal (ULN), bilirubin ≤ 1.5 × ULN; AST (aspartate aminotransferase) and/or ALT (alanine aminotransferase) ≤ 5 × ULN. There was no limit to number of prior treatment regimens permitted and patients could have been previously treated with an IGF-1R or an mTOR inhibitor. Treatment with radiotherapy (except palliative), endocrine therapy, or chemotherapy must have ceased at least 4 weeks before starting treatment. Patients with well-controlled diabetes and hyperlipidemia were allowed. Patient exclusions were treatment with concurrent strong CYP3A modifiers, major surgery within 4 weeks, significant comorbidities, brain metastases, and pregnant or breastfeeding females.

Study design
Patients were enrolled across 2 dose cohorts. Seventeen patients with EWS were enrolled in the first dose cohort of cixutumumab 6 mg/kg i.v. weekly and temsirolimus 25 mg i.v. weekly. Three patients with DSRCT were enrolled in the second dose cohort of cixutumumab 6 mg/kg i.v. weekly and temsirolimus 37.5 mg i.v. weekly because the previous dose level was well-tolerated. Treatment cycles were 4 weeks with restaging after approximately 8 weeks. This study was conducted according to the principles embodied in the Declaration of Helsinki and after approval by the Institutional Review Boards of both study centers [MD Anderson Cancer Center (Houston, TX) and Barbara Ann Karmanos Cancer Institute (Detroit, MI)]. Informed consent was obtained from all patients enrolled on the study.

Dose-limiting toxicity
Dose-limiting toxicity was defined as possibly/probably/definitely drug-related grade III to IV nonhematologic toxicity (excluding grade III nausea or grade III to IV vomiting or diarrhea in patients who had not received optimal prophylactic anti-emetic and anti-diarrheal treatment), grade III to IV thrombocytopenia lasting 7 days, or thrombocytopenia associated with active bleeding or requiring platelet transfusion, grade III anemia, grade IV neutropenia, and drug-related death.

Evaluation of safety
Adverse events were recorded for patients who received at least one dose of cixutumumab or temsirolimus. All patients were followed for a month after stopping treatment. Severity was assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 3.0. Temperature, blood pressure, and pulse were measured before each infusion. Hematology, blood chemistry and urinalysis, and physical examinations were also monitored regularly.

Evaluation of efficacy
Treatment efficacy was evaluated by computed tomography (CT) or MRI per Response Evaluation Criteria in Solid Tumors (RECIST 1.0; ref. 15) before treatment and approximately every 8 weeks thereafter. Briefly, a complete response (CR) was disappearance of all lesions, partial response (PR) was a 30% or higher reduction in the sum of the longest diameters of the lesions, SD was denoted in patients whose sum of longest diameters was not decreased more than 30% and not increased more than 20%, and progressive disease (PD) was a 20% or higher increase in the sum of the longest diameters of the lesions. A response had to last for at least 4 weeks to be considered a PR or CR. Patients with SD lasting 5 months

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**Translational Relevance**
This dose-expansion phase I study explored further the activities of the drug combination reported in our previously published manuscript entitled, "Phase I trial of cixutumumab combined with temsirolimus in patients with advanced cancer" (14). We now report the outcomes of 20 patients with refractory Ewing’s sarcoma (EWS) family tumors after being treated with cixutumumab combined with temsirolimus. This mechanism-based molecular targeted therapy approach showed the activity of the combination in some patients with advanced EWS. Our results further suggest that for responding patients, less stringent toxicity criteria and adequate supportive care should be applied to maintain the dose levels necessary to maintain response in this otherwise highly refractory disease.
or longer were considered to have durable SD. Patients who did not attain a PR but had a 20% tumor regression by RECIST and who felt well were also reported as having clinical benefit from the study treatment.

Results
Patient characteristics
A total of 20 patients (17 patients with EWS and 3 patients with DSRCT) were enrolled. All pathologic diagnoses were confirmed at MD Anderson Cancer Center. Patients’ demographic and clinical characteristics at study entry are summarized in Table 1. Ten patients with EWS had EWS–FLI1 fusion protein. Two of 3 patients with DSRCT were positive for EWS–WT1 fusion protein. The other patients were either not tested or were negative. Six patients received earlier IGF-1R treatment and 2 patients had been previously treated with temsirolimus. Most patients had been heavily pretreated, with the median number of prior therapies being 6 (range, 1–11).

Toxicities
The current study represents an expansion of a previous phase I dose escalation study (14). In the original study, 29 patients were treated with cixutumumab 6 mg/kg i.v. weekly and temsirolimus 25 mg i.v. weekly. One patient experienced a dose-limiting toxicity of grade III mucositis at this dose level. Six patients were treated with cixutumumab 6 mg/kg i.v. weekly and temsirolimus 37.5 mg i.v. weekly. One patient experienced grade IV thrombocytopenia and another patient had febrile neutropenia. As a result, cixutumumab 6 mg/kg i.v. weekly and temsirolimus 25 mg i.v. weekly was the recommended phase II dose for the combination (14).

The 20 patients reported in this study with EWS and DSRCT (only 3 of whom were part of the previous dose-escalation study) had the following toxicities that were at least possibly drug-related at both dose levels, although most instances of them were grade I or II (Table 2): thrombocytopenia (85%), mucositis (80%), hypercholesterolemia (75%), hypertriglyceridemia (70%), and hyperglycemia (65%; Table 2). Hyperglycemia was managed in collaboration with an endocrinologist and included the use of insulin together with metformin (n = 1). One of 20 patients developed diabetes mellitus on study. No patient was diabetic at baseline. Mucositis was managed with xylol

Table 1. Patient characteristics (N = 20)

<table>
<thead>
<tr>
<th></th>
<th>Median age, (range), y</th>
<th>Median no. of prior systemic therapies in the metastatic setting (range)</th>
<th>Men/women, n (%)</th>
<th>Prior IGF-1R inhibitor</th>
<th>Prior mTOR inhibitor</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 (14–41)</td>
<td>6 (1–11)</td>
<td>12/8 (60/40)</td>
<td>6</td>
<td>2</td>
<td>EWS 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DSRCT 3</td>
</tr>
</tbody>
</table>

Table 2. Treatment-related toxicities

<table>
<thead>
<tr>
<th>Dose</th>
<th>Cixutumumab 6 mg/kg 25 mg</th>
<th>Temsirolimus 6 mg/kg 37.5 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 17</td>
<td>N = 3</td>
</tr>
<tr>
<td>NCI CTCAE grade</td>
<td>I–II</td>
<td>III–IV</td>
</tr>
<tr>
<td>Endocrine</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>12 (71)</td>
<td></td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>10 (59)</td>
<td>2 (12)</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>10 (59)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Hematologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>8 (47)</td>
<td>6 (35)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>4 (24)</td>
<td>6 (35)</td>
</tr>
<tr>
<td>Anemia</td>
<td>2 (12)</td>
<td></td>
</tr>
<tr>
<td>Nonhematologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucositis</td>
<td>10 (59)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>9 (53%)</td>
<td></td>
</tr>
<tr>
<td>Rash/itching</td>
<td>9 (53)</td>
<td></td>
</tr>
<tr>
<td>Elevated AST/ALT</td>
<td>8 (47)</td>
<td></td>
</tr>
<tr>
<td>Elevated creatinine</td>
<td>6 (35)</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3 (18)</td>
<td>2 (67)</td>
</tr>
<tr>
<td>Anorexia/weight loss</td>
<td>3 (18)</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>2 (12)</td>
<td>1 (33)</td>
</tr>
</tbody>
</table>

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(1:1:1 ratio of diphenhydramine, antacid, lidocaine; 10 mL swish/swallow every 6 hours as needed). Caphosol solution (sodium phosphate; 15 mL swish/spit every 4 hours as needed), valacyclovir (500 mg per os 3 times daily), biotene mouth wash (every 4 hours as needed), and sulcrate, (1 g/10 mL; 10 mL swish/swallow or spit every day as needed).

In one patient, temsirolimus was reduced to 15 mg i.v. weekly (from 25 mg i.v. weekly). And in 2 patients, both drugs were reduced to 15 mg i.v. weekly of temsirolimus and 5 mg/kg i.v. weekly of cixutumumab. The reasons for dose reductions were mucositis, thrombocytopenia, and neutropenia. These patients were re-escalated to either full dose (for cixutumumab and/or temsirolimus) or, in one case, to temsirolimus 20 mg i.v. weekly, without recurrence of clinically limiting toxicities.

Antitumor activity

The best responses for the 20 study patients are shown in the waterfall plot in Fig. 1. Median follow-up was 8.9 months. Seven of 20 patients (35%) had SD lasting longer than 5 months or CR/PR. Median overall survival was 12.3 months [95% confidence interval (CI), 6.6–20+ months]. One of 17 patients with EWS (6%) who had previously developed resistance to a different IGF-1R inhibitor antibody achieved a CR, with time to treatment failure lasting 27 months. Tumor regression was seen in 5 of 17 (29%) patients with EWS (23%, 23%, 27%, 100%, 100%), and each of these patients has remained on study for 8 to 27 months. Two of 3 patients with DSRCT had SD lasting longer than 5 months. (The patient with EWS who had the best response of a 27% tumor regression remained on study for 15 months until financial/personal issues related to living in another country prevented him from continuing on study.)

Relationship between toxicity and response

There were no statistically significant differences in the degree of toxicity between patients with response versus those who progressed. However, 4 of the 7 patients with the best and most durable responses had grade III toxicities [hyperglycemia necessitating insulin and metformin initiation, mucositis, and neutropenia/thrombocytopenia (albeit without infection or bleeding)]. These patients were maintained on study, either without dose reduction or with transient dose reduction and re-escalation and with sponsor and Institutional Review Board notification. The patient who had a 27% regression lasting 15 months developed diabetes mellitus requiring insulin and metformin. He also had mucositis, which resolved with temsirolimus dose reduction and did not recur upon re-escalation. The patient with tumor regression of 23% lasting 18 months had a decrease in ANC to 0.8 × 10^3/mm^3. Growth factor support was given and ANC is maintained above 1.0 × 10^3/mm^3. One of the 2 patients who achieved a CR dropped his neutrophil count to 0.99 × 10^3/mm^3. The other patient who attained a CR had platelet counts between 29 × 10^3 and 50 × 10^3/mm^3 (grade III) with ANCs (grade III) that dropped as low as 0.79 × 10^3/mm^3. No infection or bleeding was noted and performance status remained at 0. Growth factor support maintained ANC above 1.0 × 10^3/mm^3 and platelet counts remained stable despite ongoing treatment. These results suggest that for responding patients, less stringent toxicity criteria and adequate supportive care should be applied to maintain the necessary dose levels to ensure an ongoing response.

Discussion

Weekly administration of cixutumumab, a fully human IgG1 monoclonal antibody directed against the IGF-1R, combined with temsirolimus, an mTOR inhibitor, was well-tolerated. The recommended phase II dose was established as cixutumumab 6 mg/kg i.v. weekly and temsirolimus 25 mg i.v. weekly, which is consistent with the results of the dose-escalation portion of the phase I study (14).

The most prevalent side effects were metabolic, hyperglycemia, hyperlipidemia, and thrombocytopenia. Most patients felt well on the study agents and their performance status was generally stable or improved on drug, unless progressive disease intervened. Patients with diabetes and hyperlipidemia were included in the study if they were well-controlled before enrollment. We worked closely with an endocrinologist to observe and carefully treat these patients when they developed associated adverse events. Of the 20 study patients, one (5%) developed grade III hyperglycemia (diabetes mellitus), which was rapidly controlled with metformin and insulin, even though the patient stayed on the same dose of drug. Twelve patients had grade I and II hyperglycemia, which was watched with home glucose monitoring, without further worsening. Only 3 of 20 patients (15%) developed grade III hypercholesterolemia or hypertriglyceridemia. These sequelae were abrogated.
with appropriate treatment, including statins, fibrates, and lifestyle changes. These observations suggest that the side effects of this regimen can be well-controlled with medication and the assistance of an endocrinologist, when necessary.

Responses in EWS have been reported with other IGF-1R antagonists, such as AMG 479 and R1507 (16–18), suggesting that a subset of patients with heavily pretreated EWS is particularly sensitive to IGF-1R antagonists. EWS–FLI can bind the IGFBP3 promoter and repress its activity, further supporting the role of IGF-1R signaling in this malignancy (19). However, many patients with EWS are resistant to IGF-1R antagonists, suggesting that signaling pathways in addition to IGF-1R are activated in these tumors (20).

The combination of an IGF-1R inhibitor, ganitumab (AMG 479), with the mTORC1 inhibitor, rapamycin, showed activity in EWS and osteogenic sarcoma models (21). When figitumumab, another IGF-1R inhibitor, was combined with the mTOR inhibitor everolimus, the combination was well-tolerated and 1 of 18 evaluable patients achieved a PR; 1 patient with EWS was treated and that patient had SD for 8 cycles (22).

Median follow-up in our study was 8.9 months. Best overall response rate (SD ≥ 5 months) was 35%. Median overall survival was 12.3 months (95% CI, 6.6–20+). In the present study, of the 17 patients with EWS, 2 (12%) achieved a CR; 1 patient with EWS was treated and that patient had SD for 8 cycles (22).

Importantly, 4 patients with significant durable responses had ≥ grade III toxicities by CTCAE criteria, including neutropenia, thrombocytopenia, mucositis, and hyperglycemic, requiring metformin and insulin, but these toxicities were not life-threatening. The patients with neutropenia and thrombocytopenia did not experience infection or bleeding. Furthermore, maintaining the dose did not result in worsening of these effects, which could be managed with supportive care and, in the case of hyperglycemia, the help of an endocrinologist. All responding patients maintained a near 100% performance status. Thus, it appears that the presence of metabolic or myelosuppressive toxicity may reflect a targeted agent effect and whether or not these side effects correlate with response merits further investigation. Future studies should therefore address this question and permit a greater range of such side effects in responding patients while on study.

Eight patients received prior treatment with an IGF-1R inhibitor (n = 6) or mTOR inhibitor (n = 2). The 2 patients who had previously not responded to treatment with an mTOR inhibitor did not respond to the combination of cixutumumab and temsirolimus. One patient who had previously received a different IGF-1R inhibitor (R1507) with response and then resistance achieved a CR on the current study (16). In biopsy samples taken at the time of emergence of resistance to the IGF-1R inhibitor, this patient’s tumor showed upregulation of mTOR pathway proteins, as determined by morphoproteomic analysis of the resistant tumor (23). This patient was treated durably on our study within 45 days of developing resistance to R1507. These results suggest that in selected patients with EWS...
family tumors, upregulation of the mTOR pathway serves as a resistance mechanism to IGF-1R inhibitors and shows that treatment with combined IGF-1R and mTOR inhibitors can reinduce response.

Interestingly, a second patient with EWS had a PR with prior IGR-1R treatment and then had a mixed, remarkable regression in 3 lung nodules after cixutumumab and temsirolimus were initiated but had disease progression in a fourth lesion. Morphoproteomic analysis of this patient’s resistant tumor showed that in addition to mTOR upregulation, extracellular signal-regulated kinase (ERK)/mitogen-activated protein (MAP)/ERK (MEK) signals were increased (23). The latter finding suggests the possibility that a combination of IGR/mTOR and MEK inhibitors might warrant investigation to reverse resistance.

In conclusion, this mechanism-based molecular approach shows preliminary evidence of activity in heavily pretreated patients with EWS family tumors. Further studies in larger numbers of patients with EWS and DSRCT as well as additional investigation into underlying resistance mechanisms in individual patients are needed.

Disclosure of Potential Conflicts of Interest
R.S. Benjamin has Ownership Interest (including patents in Pfizer and Merck). No potential conflicts of interests were disclosed by other authors.

References

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Naing, S. Fu, J. Ludwig, L.A. Doyle, R. Kurzrock
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