Pediatric Patients with Refractory Central Nervous System Tumors: Experiences of a Clinical Trial Combining Bevacizumab and Temsirolimus

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Abstract. Background: Pre-clinical findings suggest that combination treatment with bevacizumab and temsirolimus could be effective against malignant pediatric central nervous system (CNS) tumors. Patients and Methods: Six pediatric patients were treated as part of a phase I trial with intravenous temsirolimus 25 mg on days 1, 8, 15, and bevacizumab at 5, 10, or 15 mg/kg on day 1 of each 21-day cycle until disease progression or patient withdrawal. Results: The median patient age was six years (range=3-14 years). The primary diagnoses were glioblastoma multiforme (n=2), medulloblastoma (n=2), pontine glioma (n=1) and ependymoma (n=1). All patients had disease refractory to standard-of-care (2-3 prior systemic therapies). Grade 3 toxicities possibly related to drugs used occurred in two patients: anorexia, nausea, and weight loss in one, and thrombocytopenia and alanine aminotransferase elevation in another. One patient with glioblastoma multiforme achieved a partial response (51% regression) and two patients (with medulloblastoma and pontine glioma) had stable disease for four months or more (20 and 47 weeks, respectively). One other patient (with glioblastoma multiforme) showed 18% tumor regression (duration=12 weeks). Conclusion: The combination of bevacizumab with temsirolimus was well-tolerated and resulted in stable disease of at least four months/partial response in three out of six pediatric patients with chemorefractory CNS tumors.

Primary central nervous system (CNS) malignancies are the second most frequent tumors in pediatric patients, with 3.4 cases per 100,000 person-years of the estimated incidence for children and adolescents at or below 19 years of age in the United States (1, 2). Advances in diagnostic and therapeutic modalities have improved the 5-year survival rates of children with primary CNS tumors (3). However, due to the emergence of drug-resistant clones, the outcome is poor in progressive disease. During the past decade, targeted therapies have been increasingly identified for the most common adult malignancies. In contrast, very few targeted therapies have been developed for children with solid tumors. CNS tumors, especially glioblastoma multiforme (GBM), are highly vascularized and characterized by abnormal blood vessels and the ability to invade normal brain structures (4). The binding of vascular endothelial growth factor (VEGF) to its various receptors plays an important role in angiogenesis and to pathogenesis of various cancers (5). Expression of pro-angiogenic factors and increased number of blood vessels has been associated with progressive disease and an unfavorable prognosis. Bevacizumab, a fully-humanized monoclonal antibody against VEGF, normalizes tumor vascularity through anti-angiogenic effects (6). Bevacizumab in combination with irinotecan demonstrated a response rate of 63% and a median progression-free survival (PFS) of 23 weeks in a study of adult patients with recurrent high-grade glioma (7). Therefore, the U.S. Food and Drug Administration (FDA) recently approved bevacizumab to treat GBM that has not responded to other chemotherapies. Despite such successes, treatment with anti-angiogenic agents has resulted in only modest durable responses.

Increased expression of hypoxia-inducible factor-1α (HIF-1α) leads to adaptive tumor responses under hypoxic conditions, which is a known mechanism of tumor resistance to anti-angiogenic therapy (8). In addition to angiogenesis, GBM is characterized by increased phosphotidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR)
pathway activity, with altered phosphatase and tensin homolog (PTEN) gene suppression (9). This subsequently activates mTOR, which increases the expression of key proteins associated with cell-cycle regulation.

Regarding the PI3K/AKT/mTOR pathway, oncogenic mutations in PIK3CA have recently been identified in diffuse intrinsic pontine glioma (2). Furthermore, medulloblastomas overexpress the insulin-like growth factor-1 receptor (IGF-1R) and ERBB4, which also activate PI3K/AKT signaling (10-12). Finally, a number of genetic syndromes, including neurofibromatosis type 2 (NF2), are associated with the development of ependymomas. Inactivation of the tumor suppressor gene NF2 (encoding merlin) induces the development of nervous system tumors, at least in part, through activation of PI3K/AKT/mTOR signaling (13).

Temsirolimus is an mTOR inhibitor, which abrogates the growth of numerous human tumor cell lines in xenograft models, including medulloblastoma and glioblastoma (14). Its highly lipophilic nature allows it to penetrate the blood–brain barrier, whose existence is often a major impediment to treating these tumor types (15). Importantly, mTOR inhibitors can also inhibit HIF-1α, down-regulating tumor and endothelial cell proliferative and survival functions, including compensatory responses to hypoxia (16). Because up-regulation of HIF-1α mediates chemoresistance to bevacizumab, we hypothesized that combination treatment with bevacizumab and temsirolimus might be effective against pediatric malignant gliomas. Here, we report our experience regarding toxicity and clinical response in pediatric patients with CNS tumors treated with the combination of these two targeted-agents.

**Patients and Methods**

**Study design and treatment plan.** The study reported is part of a single-Institution, phase I, open-label, dose-escalation clinical trial (NCT00610493). The primary objective of the trial was to determine the maximum tolerated dose and dose-limiting toxicities (DLTs) of combination treatment with bevacizumab and temsirolimus. Secondary objectives included preliminary descriptive assessment of antitumor efficacy and assessment of correlates of anti-angiogenesis. This trial successfully completed dose escalation to the highest specified dose level, that is dose level 13, which consisted of the highest FDA-approved doses of both drugs (bevacizumab 15 mg/kg i.v. every three weeks and temsirolimus 25 mg i.v. weekly) (17). This article focuses only on a subset of six pediatric patients with primary CNS tumors in order to provide information on the side-effects, tolerability and efficacy of this combination of targeted therapies in these patients.

Treatment was administered on an out-patient basis at The University of Texas MD Anderson Cancer Center. Treatment cycles were of 21 days’ duration. No commercial agents or therapies other than those described here were administered with the intent to treat the patient’s malignancy. For this subset of patients, temsirolimus was administered at a dose of 25 mg i.v. on days 1, 8 and 15, and bevacizumab was given at either 5, 10, or 15 mg/kg on day 1. The same schedules and doses of bevacizumab and temsirolimus were administered in subsequent cycles barring evidence of tumor progression or prohibitive toxicity.

For these pediatric patients, all biopsies except for one pontine glioma (Table I) were reviewed by an MD Anderson Cancer Center pathologist and classified using the World Health Organization classification (18). Consent was obtained and patients were treated in accordance to the MD Anderson Cancer Center Institutional Review Board guidelines.

**Eligibility criteria.** Patients were eligible for enrollment if they had histologically-documented, advanced or metastatic solid tumors refractory to standard therapy or for which no standard therapy was available that would induce a complete response (CR) rate of at least 10% or improve survival by at least three months. Other key inclusion criteria were: absolute neutrophil count ≥1,000/ml; platelets ≥50,000/ml; creatinine ≤3times the upper limit of normal (ULN); total bilirubin ≤3.0 mg/dl; alanine aminotransferase (ALT) ≤5times the ULN; fasting level of total cholesterol <350 mg/dl; and, triglyceride <400 mg/dl. Key exclusion criteria were: hemoptysis or clinically significant unexplained bleeding within 28 days of study entry; uncontrolled systemic hypertension; hypersensitivity to bevacizumab or temsirolimus and their metabolites. Prior exposure to mTOR and VEGF inhibitors were not exclusion criteria for study entry, nor were patients with a history of venous thromboembolism excluded. Because temsirolimus metabolism significantly increases with concurrent use of cytochrome P450 3A4-inducing medications, such as dexamethasone, or anti-epileptic drugs (e.g. phenytoin, carbamazepine, phenobarbital, oxcarbazepine and primidone), it was strongly recommended to treating physicians that these medications be discontinued and five elimination half-lives of such medication be allowed to pass before enrollment on this trial.

**Evaluation of safety and efficacy.** Adverse events were graded based on the Common Terminology Criteria for Adverse Events v3.0 (19). Side-effects were monitored in a prospective manner. History and physical examinations were done approximately weekly during the first cycle.

**Table I. Baseline demographics and clinical characteristics of study patients.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>6</td>
</tr>
<tr>
<td>Median age (range), years</td>
<td>6 (3-14)</td>
</tr>
<tr>
<td>Median (range) no. prior systemic therapies</td>
<td>2 (2-3)</td>
</tr>
<tr>
<td>Lansky performance status</td>
<td></td>
</tr>
<tr>
<td>80-100%</td>
<td>1 (17)</td>
</tr>
<tr>
<td>60-79%</td>
<td>2 (33)</td>
</tr>
<tr>
<td>40-59%</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Prior treatment</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>5 (83)</td>
</tr>
<tr>
<td>Radiation</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Primary diagnosis</td>
<td></td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>2</td>
</tr>
<tr>
<td>Pontine glioma</td>
<td>1</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>1</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>2</td>
</tr>
</tbody>
</table>
and then at the start of each subsequent cycle as per protocol. Laboratory analysis, including urinalysis, complete blood count with differential leukocyte count, renal function, hepatic enzymes and serum electrolytes, were performed weekly during the first cycle and then at the start of each subsequent cycle as per protocol.

Imaging evaluation. Magnetic resonance imaging (MRI) of the brain was performed within four weeks before initiation of treatment and at 6-week intervals thereafter, or earlier with clinical indications per the discretion of the treating physician. Tumor measurement for determining treatment response was according to the modified MacDonald criteria (20). For the purposes of this article, complete response (CR) is defined as resolution of all enhancing tumor, partial response (PR) as a decrease in tumor size of at least 50%, progressive disease (PD) as an increase in tumor size of 25% or more, stable disease defines all other patients. All responses were reviewed by our neuroradiologist (NGT).

Results

Patients’ characteristics. Between June 2009 and April 2010, six pediatric patients with refractory CNS tumors (median age=6 years; range=3-14 years) who met the inclusion and exclusion criteria were enrolled on this phase I clinical trial with combined temsirolimus and bevacizumab. The primary diagnoses included glioblastoma multiforme (n=2), medulloblastoma (n=2), pontine glioma (1) and ependymoma (n=1) (Table I). Most patients had a Lansky score ≤80% (21).

Patients’ characteristics are shown in Table I. Five patients had experienced relapse after surgery and subsequent radiation therapy. Patients had a median of two prior chemotherapy regimens (range=2-3). No patient had received a prior mTOR inhibitor. Four patients had previously been treated with a bevacizumab-containing regimen.

Toxicity. Therapy was generally well-tolerated with manageable toxicities. Two out of six patients experienced grade 3 toxicity possibly related to drugs used: (i) grade 3 anorexia, nausea and weight loss; and (ii) thrombocytopenia and ALT elevation, delaying treatment for two weeks in this patient. In both cases, treatment was stopped following disease progression. No patients experienced grade 4 toxicities. At a dose of 15 mg/kg of bevacizumab, one patient experienced grade 2 hypertension which was well-controlled with anti-hypertensive medication. Two patients had grade 2 skin toxicity, including complications of wound healing (superficial skin ulceration around skin folds and dehiscence of central venous catheter insertion site). There was no intra-cranial bleeding or other serious complications. All other side-effects were grade 2 or less, including neutropenia in two out of six patients (grade 1, n=1; grade 2, n=1), thrombocytopenia in three patients (grade 1, n=1; grade 2, n=2), grade 1 nausea in one patients, grade 2 diarrhea in one patient, hyponatremia in three patients (grade 1, n=1; grade 2, n=2), grade 1 hypokalemia in one patient, grade 2 hypophosphatemia in one patient, grade 1 hyperglycemia in two patients, and aspartate aminotransferase (AST) and ALT elevations in four patients (grade 1, n=1, grade 2, n=3). Five patients developed abnormal lipid profiles (grade 2 hypercholesterolemia/grade 2 hypertriglyceridemia). There were no dose reductions of either bevacizumab or temsirolimus due to toxicity.

Evaluation of antitumor activity. Median time-to-disease progression was 14 weeks (range=7-47 weeks; Table II). Although patients were heavily pre-treated, a PR was achieved in one patient. In this patient with GBM, restaging studies after four cycles of therapy show a 51% decrease in the size of the tumor (Figure 1). Stabilization of disease for a median of 16 weeks (range=8-47 weeks) was achieved in four other patients (one each with GBM, medulloblastoma, ependymoma, and pontine glioma). In fact, the patient with pontine glioma received 47 weeks of therapy before clinical deterioration. This patient was treated with therapy for 36
weeks before therapy was halted for radiation to the pontine lesion. Therapy was continued for 11 more weeks after radiation was completed for clinical benefit. One patient with medulloblastoma was taken off of treatment without progression after 20 weeks secondary to peritonitis associated with his ventriculoperitoneal shunt. Each patient’s best response by the modified MacDonald criteria is illustrated by a waterfall plot shown in Figure 2.

Discussion

Treatment planning for pediatric patients with chemorefractory CNS tumors is challenging because: i) definitive data regarding optimal therapy are lacking; ii) it is difficult to enroll patients in dose-defining pediatric studies; and iii) toxicities and tumor resistance are associated with prior conventional treatment. For these reasons, successful novel treatment regimens are required.
A bevacizumab-containing regimen resulted in a 6-month PFS of 38% in pediatric patients with recurrent high-grade glioma (22), which is similar to the outcomes of adult patients (23). The implication is that combining bevacizumab with a chemotherapeutic agent could also delay disease progression in pediatric patients with predominant contrast-enhancing disease on imaging. The common adverse effects of bevacizumab and other VEGF inhibitors are hypertension and proteinuria, and patients may be at risk for wound complications, hemorrhage, gastrointestinal perforation and thromboembolic events (24). However, the largest phase II study thus far was in 167 adult patients with recurrent malignant glioma treated with bevacizumab, with or without irinotecan. In that study, there were three cases of intracranial hemorrhage (1.8%), three arterial thromboembolic events (1.8%), three complications of wound healing (1.8%), and one patient had gastrointestinal perforation (0.6%) (25). In our study, 1 out of 4 patients receiving 15 mg/kg of bevacizumab had grade 2 hypertension and another had a grade 2 complication of wound healing associated with the central venous catheter insertion site. The remaining patients at this dose level had no bevacizumab-associated complications.

The FDA recently approved the mTOR inhibitors temsirolimus and everolimus for the treatment of patients with refractory renal cell cancer (26, 27). Inhibitors of mTOR have shown single-agent activity in other types of cancers and clinical trials are currently ongoing in several tumor types (28). In 18 pediatric patients with recurrent and refractory solid tumors, temsirolimus of 150 mg/m² weekly as the highest dose level was tolerable in a phase I study (29). One patient experienced dose-limiting anorexia at this dose level. Otherwise, toxicities were mild and tolerable. This was attributed to a shorter half-life and lower area under the curve (AUC) in pediatric compared to adult patients with solid tumors. Therefore, pediatric patients were administered more than 95% of the median dose intensity for all dose levels tested (29).

To better-characterize the relationship between dose and tolerability to temsirolimus, 111 patients with refractory renal cell carcinoma were randomly assigned to a phase II study of fixed doses of 25, 75, or 250 mg i.v. weekly (30). Overall, there was one CR, seven PRs, and 29 minor responses, but toxicity and efficacy were similar at each dose level. This observation, together with the greater number of dose reductions and treatment interruptions at the higher dose levels, led to 25 mg i.v. weekly as the optimum dose level being recommended for future temsirolimus studies. In our study, five out of six patients whose disease was refractory to prior chemotherapy, including bevacizumab in three patients, showed disease control with weekly i.v. temsirolimus at 25 mg. These results suggest the potential efficacy of this dose of temsirolimus in treating pediatric solid cancer. One important consideration is that patients with malignant glioma can require enzyme-inducing antiepileptic drugs or dexamethasone. Such patients exhibit increased drug metabolism by the CYP450 enzyme system, thereby being able to tolerate higher doses than patients not taking these agents. Accordingly, patients taking enzyme-inducing agents demonstrated a maximum tolerated dose of i.v. temsirolimus at 250 mg weekly in a phase I study (14). In a phase II study with glioma patients not on enzyme-inducing anti-epileptic drugs, 250 mg led to excessive toxicity, mainly stomatitis, so the dose was reduced to 170 mg (31). Therefore, one question to be answered is whether the dose of temsirolimus should be elevated to greater than 25 mg in pediatric patients taking enzyme-inducing antiepileptic drugs or steroids. Of note, none of the pediatric patients treated on this study were taking enzyme-inducing agents demonstrating a maximum tolerated dose of i.v. temsirolimus at 250 mg weekly in a phase I study (14).

Many mechanisms of resistance to VEGF inhibitors have been described, with recent emphasis on hypoxic responses. In cell culture models, rapamycins have also been shown to inhibit HIF-1α, a transcription factor that regulates the expression of VEGF, suggesting that combined VEGF and mTOR inhibition could have greater antiangiogenic and antitumor activity than either agent given as monotherapy (32-34). In our study, the fact that three out of four patients who had received previous bevacizumab treatment, and had stable disease for 8, 12 and 47 weeks, respectively, implies that temsirolimus may assist in overcoming resistance to VEGF inhibitors.

In conclusion, the combination of bevacizumab with temsirolimus was well-tolerated at the maximum FDA-approved doses and showed PR (51%) in one patient with...
glioblastoma multiforme and SD in four patients, with two of the latter patients having stable disease for over four months. Side-effects that were seen were expected and easily managed. There were no dose reductions of either bevacizumab or temsirolimus due to toxicity. Further study of this combination in larger populations of children with primary CNS tumors is warranted.

Conflicts of Interest

None of the Authors have any conflict of interest relevant to the subject of this article.

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References


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