Amie Davé
Irving Center for Clinical Research Rotation

Everolimus and Temozolimide in pediatric recurrent or progressive high grade-gliomas

A. Study Purpose and Rationale

The therapeutic success for pediatric brain tumors has not been as good as it has been for other pediatric cancers. Less than 20 percent of children with high-grade gliomas are long-term survivors.[1] Despite surgical intervention, radiation therapy, and chemotherapy, most children with high-grade gliomas will die from recurrence or progression of their tumor. The predominant types of high-grade gliomas in children are anaplastic astrocytoma and glioblastoma multiforme, which account for approximately 14 percent of primary central nervous system tumors in children.[1, 2] These two histologic types of tumor are of pure astrocytic origin. Other histological variants of high-grade gliomas in children with oligodendroglial or neuronal components are rare.[1]

Chemotherapy is usually the only therapeutic option available for the treatment of recurrent high-grade gliomas. There are several new agents that are currently in Phase I and II studies for recurrent high-grade gliomas. These include EGFR (epidermal growth factor receptor) inhibitors, farnesyltransferase inhibitors, antiangiogenic agents, inhibitors of MGMT (methylguanine methyltransferase), and mTOR (mammalian target of rapamycin) inhibitors.[1] In addition to these newer agents which are small-molecule inhibitors, anti-angiogenic agents, biologic modulators, several conventional cytotoxic chemotherapeutic agents are being used as well. The dosing and scheduling of some of these cytotoxic agents is being changed to improve efficacy. Based on the experiences of adult trials, some of these changes are being employed in the treatment of pediatric patients.

The metronomic dosing of temozolomide, an oral imidazotetrazine second-generation alkylating agent, for the treatment of brain tumors looks promising. Low-dose metronomic chemotherapy, also known as antiangiogenic chemotherapy, uses conventional cytotoxic chemotherapy administered at lower doses more frequently, without a long interruption in therapy. The goal of such a dosing schedule is to allow for more sustained apoptosis of endothelial cells in the tumor vasculature. This antiangiogenic effect was demonstrated by Browder et al. and Klement et al.[3, 4] It has been shown during metronomic chemotherapy that endothelial cell apoptosis and capillary dropout occur before the death of tumor cells surrounding each capillary.[5] Studies have shown that drug-resistant tumors can be converted to drug-sensitive tumors by metronomic dosing of conventional cytotoxic chemotherapy.[5] Thus, this mode of treatment may be beneficial in a heavily pretreated patient population with relapsed disease.

In addition to metronomic dosing having a potential antiangiogenic effect, protracted administration of temozolomide even at low daily doses led to a significant and prolonged depletion of O6-alkylguanine-DNA alkyltransferase, AGAT, activity. This may enhance the anti-tumor effect of the agent, even at low daily doses.[6] Pre-clinical studies of TMZ in pediatric solid tumors, including brain tumor lines, have shown promising results especially in cell lines with low AGAT.[7] A phase I study of metronomic scheduling of temozolomide in pediatric patients with recurrent brain tumors recommended a dose for future studies of 85 mg/m^2 for a 42 day cycle.[8]

The phosphatidylinositol 3'kinase (PI3K)/Akt/mTOR signaling pathway plays a central role in modulating cell proliferation and angiogenesis. The abnormal signaling through this pathway demonstrated by cancer cells has made various components of the pathway potential targets for cancer therapy.[9, 10]

There is a good understanding of the role the PI3K/Akt pathway plays in brain tumors and provides the rationale for targeting this pathway in the treatment of brain tumors. Activity of pathway is upregulated in brain tumors as a result of excess stimulation by growth factors and Ras.[11] Activation of the PI3K pathway in humam gliomas has been found to be significantly associated with increasing tumor grade, lower levels of apoptosis, and adverse clinical outcome.[12] EGFR is a ligand that can activate the PI3/Akt pathway and is amplified or mutated in 40 to 60 percent of GBM.[13] In GBM, aberrant EGFR expression and abnormal PI3/Akt signaling modulate the migration levels of tumor astrocytes[14]. In addition, deletions or mutations of the PTEN tumor suppressor gene, which are found in 30 to 40 percent of GBM, also cause activation of the PI3/Akt pathway. PTEN is an important component of the pathway since it causes inactivation of the products of PI3K activity.[13]

Migrating cells of maligant gliomas that diffusley invade brain parenchyma have been shown to demonstrate decreased levels of apoptosis, Type I programmed cell death.[11] This decrease in apoptosis causes the cells to be resistant to cytotoxic insult since most chemotherapeutic agents are associated with proapoptotic effects.[14] Several intracellular signalling pathways can become constitutively activated in migrating glioma cells allowing them to become resistant to apoptosis, the most important of which appears to be the PI3K/Akt pathway.[14] The clinical radioresistance exhibited by GBMs may in part be due to rapid tumor proliferation secondary to low apoptotic levels. Disrupting mTOR signaling with rapamycin can cause radioresistant GBM cells to return to levels of radiosensitivity.[7]

Since resistance to apoptosis is linked to tumorigenesis, another mechanism of cell death would be valuable to bypass these cells' resistance to apoptotic death.[11] This may help to kill the migrating glioma cells that are resistant to apoptosis. Autophagy, Type II programmed cell death, is a potential mechanism of cell death since migrating GBM cells are less prone to resist it. Compounds that induce autophagy have been found to be active against multidrug-resistant cells, as most glioma cells are.[11, 14]

Aberrant signaling through the PI3K/Akt pathway results in abnormal cell proliferation as well as cell migration in glioma cells.[11] This aberrantly activated pathway contributes to the resistance that gliomas have to chemotherapy and radiotherapy.[11, 14] Inhibition of the pathway has shown to increase or restore the effectiveness of chemotherapy on glioma cells.[15] mTOR is a direct target of the PI3K/Akt pathway and plays a central role in modulating cell proliferation and angiogenesis. mTOR also has a role in controlling cell migration in GBM.[11] mTOR appears to be involved in the process of autophagy. Inactivation of mTOR can induce autophagy.[14] Rapamycin, an inhibitor of mTOR, induced autophagy in rapamycin-sensitive malignant glioma U87-MG and T98G cells and found that this was the mechanism by which rapamycin exerted its antitumor effect on GBM cells.[16]

Temozolomide is a cytotoxic drug that has been shown to induce autophagy, not apoptosis, in human glioblastoma cells.[14] The inhibition of mTOR by rapamycin would reinforce the therapeutic benefits of temozolomide. Therefore, a combination therapy with temozolomide with metronomic dosing and an mTOR inhibitor would be a novel and promising approach to the treatment of progressive or recurrent high-grade gliomas in the pediatric population. Temozolomide is an oral agent and there is a rapamycin analog, Everolimus (RAD-001) which is also an oral agent. A combination of Temozolomide and Everolimus, two oral agents, would be especially beneficial in the pediatric population and for quality of life purposes.

B. Study Design and Analyses

This study is a Phase II prospective randomized control trial to determine the efficacy of a combination regimen of temozolomide and everolimus in patients with recurrent or progressive high-grade gliomas. In addition, toxicity data will be elaborated.

The overall survival and time to progression data will be analyzed using the Wilcoxon rank-sum test and its variation of the t-test. It can not be assumed the results will have a normal distribution (eg. a group of patients may have no progression of disease), therefore a t-test can not be used. By using the Wilcoxon test to analyze the data, the disadvantage will be that the magnitude of the difference between the two groups being studied will be lost. However, given that that data may not be normally distributed, the Wilcoxon test provides a way to analyze such data. The Wilcoxon test will rank the observations of both groups of patients combined. Each observation will then be assigned a rank (eg. lowest time to progression will receive rank of 1, next lowest will receive rank of 2, etc.). A t-test can then be done using the ranks values assigned.

Given that nearly all patients with recurrent or progressive disease will be enrolled in various Phase I/II trials and that these patients vary with respect to aggressiveness of disease and number of relapses, there is a wide range of overall survival times for these patients in the various studies they are enrolled in. The data from one study of pediatric patients with high-grade gliomas will be used to determine the sample size needed for this study. An unpaired t-test is used:

$N = 1+16 (SD / effect)^2$

$N = 1+16 (¼*range / effect)^2$

$N = 1+16 (5.6 / 4)^2$

$N = 32.4$

Since Wilcoxon test will be used to analyze data, will need 10 percent more subjects.

$N*10\% = 3.2$

$N = 32.4 + 3.2$

$N = 35.6$

36 patients will be needed in each group of this study.

C. Study Procedures

Eligible patients will be assigned randomly to enroll in this study protocol or another Phase I/II protocol for which they are eligible. Informed consent will be obtained from patients, parents, or guardians at the time of protocol enrollment. The dosages for the chemotherapy regimen used in this study are based on Phase I/II data. Each patient will receive the

following: 1) temozolomide 85mg/m^2 X 42 days repeated every 56 days and 2) everolimus 5 mg daily. Pneumocystis carinii prophylaxis with sulfamethoxazole/trimethoprim will be administered to all patients. Treatment will be discontinued in patients who experience tumor progression. It will be recommended that patients who have at least stable disease continue therapy until 6 cycles of temozolomide are completed.

Primary efficacy analysis of temozolomide and everolimus requires assessment of response rates. Radiologic tumor assessment will be performed by MRI or CT scan on the last scheduled day of each cycle of temozolomide or at the time of clinical disease progression. Patients who die before Day 56 will be considered non-responders. Patients who die of complications related to temozolomide and/or everolimus before day 56 will be considered nonresponders. Disease assessment will be classified as 1) complete response, CR, if there is complete disappearance of all evidence of disease and disease-related symptoms; 2) partial response, PR, is there is a $\geq$ 50 percent decrease in the sum of the products of the perpendicular dimensions of all measurable lesions compared to baseline and no evidence of new disease; 3) progressive disease, PD, if there is a 25 percent increase in the sum of the products of the greatest perpendicular dimensions of any measurable lesion compared with baseline or the appearance of any new disease; and 4) stable disease, SD, if does not qualify for CR, PR, or PD. Overall survival, OS, will be assessed from the first day of initiation of therapy. Time to progression, TTP, will be defined from the date of therapy initiation to radiographic progression.

Grade III and IV toxicities will be recorded. DLT for outpatient txt (oral topotecan)

D. Study Drugs

Temozolmide is FDA approved for the treatment of adult patients with refractory anaplastic astrocytoma and newly diagnosed glioblastoma multiforme. Temozolmide is not directly active but undergoes rapid non-enzymatic conversion at physiologic pH to the compound MTIC. The cytotoxicity of MTIC is primarily due to alkylation of DNA. Temozolomide is rapidly and completely absorbed after oral administration. This agent can cross the blood-brain barrier and has good CNS penetration. Oral administration is the standard route of administration. The dosage that will be used in this study is 85mg/m^2 X 42 days repeated every 56 days. This dose was recommended based on a Phase 1 study of study of metronomic scheduling of temozolomide in pediatric patients with recurrent brain tumors.[8]

In a review by Kuo et al. of temozolomide given to pediatric patients with progressive, unresectable, low-grade gliomas, nine of the 13 patients had partial responses, minor responses, or disease stabilization. The patients received either five-day regimen of temozolomide (150 mg/ m^2 per day) repeated every 28 days or a metronomic dosing schedule, a 42-day regimen (75 mg/m^2 per day) for a 56 day cycle. Of the nine patients who received metronomic dosing, there was one patient with minor response, two with partial response, two with stable disease, and four with progressive disease. The four patients with progressive disease were subsequently succuessfully treated with other therapies. The 42-day regimen appeared less toxic than the five-day regimen. Four of the five patients who experienced thrombocypoenia were on the five-day regimen and both of the patients with episodes of neutropenia were on the five-day regimen. In the Phase I study of metronomic

temozolomide by Baruchel et al. in pediatric patients with recurrent brain tumors, of the three patients with high-grade gliomas enrolled, three showed a partial response.

The Phase 1 study of study of metronomic scheduling of temozolomide in pediatric patients with recurrent brain tumors showed the following toxicities[8]:
Grade 3/4 toxicities: Hemoglobin 5%, Leukocytes 20%, Lymphocytes 45%, Neutrophils 10%, Thrombocytes 15%, HSV infections 20%, Abdominal pain O, Anorexia 10%, Vomiting 15%.
Other milder toxicities: fatigue, fever, flu-like symptoms, gastrointestinal symptoms, lethargy, decreased motor function, and restlessness. A non-specific grade 2 rash was present in 6 patients. The increased drug exposure in the regimen was not associated with increased drug toxicity.

Everolimus (RAD001) is not presently FDA approved in the United States, it is approved in Europe to prevent rejection in recipients of kidney or heart transplants. Everolimus is an orally available ester derivative of rapamycin. It is a macrolide immunosuppressant that binds to the FK-binding protein and the RAD-FKBP complex then binds to and inhibits the activity of mTOR. This subsequently inhibits progression from G1 to the S phase of the cell cycle. The drug will be orally administered, which is its standard route of administration at a dose of 5mg daily.

There have been several trials showing that rapamycin (sirolimus) has some efficacy in halting tumor growth or tumor regression in various tumor types.

The Phase I study by Yee et al. of everolimus in patients with recurrent or refractory hematologic malignancies showed the following toxicities[17]:
Grade 3/4 toxicities: Hyperglycemia 22%, Anorexia 4%, Diarrhea 4%, Hypophosphatemia 7%, Fatigue 7%, cutaneous leukocytoclastic vasculitis 4%.
Grade 1/2 toxicities: Hyperglycemia 26%, Hyperlipidemia 44%, Anorexia 37%, Hepatic 41%, Oral aphthous ulcers 37%, Diarrhea 29%, Hypophosphatemia 18%, Fatigue 15%, Hypomagnesemia 22%, Dermatologic 18%, Hypocalcemia 18%, Constipation 18%, Cramps 18%, Dysguesia 18%, Nausea/vomiting 15%, Weight loss 15%, Xerostomia 15%, Headache 11%, Hypokalemia 8%.

E. Medical Devices
This study will not utilize any medical devices.

F. Study Questionnaires
This study will not utilize questionnaires.

G. Study Subjects
72 male and female patients aged 18 and younger with a recurrent or progressive high grade glioma that is refractory to standard therapy or for which there is no standard salvage treatment will be recruited.

Inclusion criteria:
1. Patients ≤18 years of age at original diagnosis

2. Histopathologic confirmation of a high-grade glioma arising primarily outside the brainstem but intracranial in location. High grade gliomas include anaplastic astrocytoma, glioblastoma multiforme, or other WHO grade III/IV astrocytomas.
3. Disease measureable by MRI or CT scan
4. Life expectancy of greater than 12 weeks
5. Full recovery from any toxic effects of previous chemotherapy
6. Adequate bone marrow function:
    - Absolute neutrophil count ≥1000/μL
    - Platelet count ≥100,000/μL
    - Hemoglobin ≥ 8 g/μL
7. Adequate kidney function: Normal creatinine for age
8. Adequate liver function:
    - Bilirubin < 1.5x upper limit of normal for age
    - AST/ALT < 2.5x upper limit of normal for age
    - Albumin ≥ 2g/dL
9. Females of reproductive potential have to have negative pregnancy test
10. Written informed consent signed by patient, parent, or guardian

Exclusion criteria:

1. Bone marrow transplantation within last six months
2. Pregnancy or nursing
3. Patients with frequent vomiting or other condition that would interfere with the oral intake of medications

H. Recruitment of Subjects

Subjects will be recruited from the Pediatric Oncology Clinic at Columbia University. Upon diagnosis of recurrent or progressive disease, the patients will be provided the option of enrolling in this study.

I. Confidentiality of Study Data

A unique code number will be used for all subjects and no identifiers will be used. Patient data will be stored such that only study investigators will have access to the information.

J. Potential Conflict of Interest

There are no potential conflicts of interest related to this protocol.

K. Location of Study

Patients for this study will be recruited at Columbia University. Data analysis, interpretation, and manuscript preparation will be completed at Columbia University.

L. Potential Risks

The efficacy of the regimen being used in this protocol has not been proven in the setting of recurrent or progressive high grade gliomas or in any other cancers. The combination of the two agents as therapy in this study is novel and all potential interactions are not known.

M. Potential Benefits

The efficacy of this combined regimen is unknown, the purpose of this study is to assess efficacy. There may be a potential benefit to subjects in terms of prolonging time to progression of disease and overall survival, but this can not be guaranteed.

N. Alternatives
The alternative to participating in this protocol would be for a patient to enroll in another Phase I/II study given their recurrent or progressive disease status. The patient may also undergo no further therapy and receive supportive care.

O. Compensation to Subjects
There will be no compensation for subjects for participating in this protocol.

P. Costs to subjects
There will be no additional cost for a subject to participate in this protocol.

Q. Minors as Research Subjects
This study will involve minors as research subjects, the appropriate written informed consent will be obtained from parents or guardians if necessary and assent from the minors as appropriate.

R. Radiation or Radioactive Substances
This study will not involve the use of radiation or radioactive substances.

References:

1. Broniscer, A., *Past, present, and future strategies in the treatment of high-grade glioma in children.* Cancer Invest, 2006. **24**(1): p. 77-81.
2. Ullrich, N.J. and S.L. Pomeroy, *Pediatric brain tumors.* Neurol Clin, 2003. **21**(4): p. 897-913.
3. Klement, G., et al., *Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity.* J Clin Invest, 2000. **105**(8): p. R15-24.
4. Browder, T., et al., *Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer.* Cancer Res, 2000. **60**(7): p. 1878-86.
5. Folkman, J., *Angiogenesis.* Annu Rev Med, 2006. **57**: p. 1-18.
6. Tolcher, A.W., et al., *Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules.* Br J Cancer, 2003. **88**(7): p. 1004-11.
7. Middlemas, D.S., et al., *Biochemical correlates of temozolomide sensitivity in pediatric solid tumor xenograft models.* Clin Cancer Res, 2000. **6**(3): p. 998-1007.
8. Baruchel, S., et al., *Safety and pharmacokinetics of temozolomide using a dose-escalation, metronomic schedule in recurrent paediatric brain tumours.* Eur J Cancer, 2006. **42**(14): p. 2335-42.
9. Chan, S., *Targeting the mammalian target of rapamycin (mTOR): a new approach to treating cancer.* Br J Cancer, 2004. **91**(8): p. 1420-4.
10. Law, B.K., *Rapamycin: an anti-cancer immunosuppressant?* Crit Rev Oncol Hematol, 2005. **56**(1): p. 47-60.
11. Lefranc, F., J. Brotchi, and R. Kiss, *Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis.* J Clin Oncol, 2005. **23**(10): p. 2411-22.
12. Chakravarti, A., et al., *The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas.* J Clin Oncol, 2004. **22**(10): p. 1926-33.
13. Galanis, E., et al., *Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study.* J Clin Oncol, 2005. **23**(23): p. 5294-304.
14. Lefranc, F. and R. Kiss, *Autophagy, the Trojan horse to combat glioblastomas.* Neurosurg Focus, 2006. **20**(4): p. E7.
15. Shingu, T., et al., *Growth inhibition of human malignant glioma cells induced by the PI3-K-specific inhibitor.* J Neurosurg, 2003. **98**(1): p. 154-61.
16. Takeuchi, H., et al., *Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors.* Cancer Res, 2005. **65**(8): p. 3336-46.
17. Yee, K.W., et al., *Phase I/II Study of the Mammalian Target of Rapamycin Inhibitor Everolimus (RAD001) in Patients with Relapsed or Refractory Hematologic Malignancies.* Clin Cancer Res, 2006. **12**(17): p. 5165-73.