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Circulating Angiogenic Biomarkers in Pediatric Patients Receiving Bevacizumab and Irinotecan for High Grade Glioma

A. Study Purpose and Rationale

Angiogenesis is critical for tumor growth as well as metastatic spread and therapies targeting tumor angiogenesis continue to become more prevalent in the treatment of various cancers.[1] Agents that block the vascular endothelial growth factor (VEGF) pathway have been shown to effectively inhibit tumor angiogenesis and growth and are being incorporated in combination with traditional cytotoxic agents for the treatment of breast cancer, colorectal cancer, non-small cell lung cancer, renal cell carcinoma, and glioblastoma.[2-6]

Despite the clinical benefit and advances of antiangiogenic agents, their biologic activity is difficult to assess and there is a lack of correlates of biologic activity that can be reliably followed.[7] Direct evaluation of the tumor cells or vasculature could allow for the most direct assessment of biologic activity but tumor tissue is seldom routinely available and obtaining tissue would require an invasive approach.[8] Identification of serum biomarkers will be useful in assessing biologic activity as well as optimize dosing, identify patients most likely to benefit, monitor response to treatment, and explore mechanisms of resistance.[7]

Several studies have tried to identify and evaluate potential biomarkers in patients receiving antiangiogenic therapy for renal cell carcinoma, colorectal cancer, non-small cell lung cancer, and gastrointestinal stromal tumor. [7-10] Although some trends have emerged, findings are not necessarily consistent when different tumors and regimens are compared. For example, lower baseline serum VEGF levels are correlated with longer progression free survival in metastatic breast cancer and hepatocellular carcinoma but similar correlations were not seen with colon cancer, renal cell carcinoma or non-small cell lung cancer.[6]

Bevacizumab is a recombinant humanized monoclonal antibody that binds to human VEGF resulting in inhibition of angiogenesis and tumor growth.[11] Bevacizumab was approved by the U.S. Food and Drug Administration as a single agent for patients with glioblastoma multiforme with progressive disease following prior therapy. [3] Bevacizumab in combination with irinotecan has shown significant biologic and antiglioma activity in adult patients.[12-14] Retrospective series on the use of bevacizumab and irinotecan for the treatment for pediatric patients with gliomas have shown inferior results to that seen in the adult cohorts.[15, 16] Given the encouraging results in the adult population, however, a prospective clinical trial evaluating bevacizumab in combination with irinotecan in pediatric patients with recurrent or progressivehigh grade glioma is planned.

Given the paucity of information available on biomarkers in those receiving antiangiogenic therapies, particularly in those receiving therapy for gliomas, we propose a biomarker study that will prospectively assess the candidate systemic circulating biomarkers of interest in pediatric patients with recurrent or progressive high grade glioma receiving bevacizumab and irinotecan.

We propose following VEGF levels, soluble VEGF receptor 2 (sVEGFR-2) levels and monocytes in peripheral blood as potential biomarkers for antiangiogenic therapy in this cohort.

Although clinical correlations with VEGF levels during antiangiogenic therapy have been mixed among different tumor types, as discussed above, exploration of the potential relationship is warranted given the limited data currently available on VEGF levels during antiangiogenic therapy in glioma patients. Additionally, VEGF levels may be a potential pharmacodynamic biomarker of host therapy response as circulating VEGF levels increase after treatment with bevacizumab and subsequently decrease upon treatment discontinuation.[6] The VEGFR-2 mediates most functions of VEGF and is an endothelial-specific receptor controlling aspects of vascular growth and angiogenic responses.[17] A reciprocal relationship has been found between VEGF and sVEGFR-2 levels in patients being treated with VEGFR tyrosine kinase inhibitors with levels of sVEGFR-2 decreasing.[9, 10] In contrast, sVEGFR-2 level was shown to increase after treatment with bevacizumab. There is likely a difference in the modulation profiles based on the mode of VEGF pathway inhibiton which needs to be explored which is why we selected to study levels of sVEGFR-2 in this cohort.[10]

In a screen of peripheral blood to identify VEGF-binding cells, VEGF binding was predominantly observed in monocytes, which are positive for the monocyte marker CD14. In a group of patients receiving sunitnib, a multitargeted tyrosine kinase inhibitor, for gastrointestinal stromal tumor, monocyte levels were modulated by treatment. Patients with clinical benefit with sunitinib had a significantly smaller decrease in monocyte levels.[8] Monocytes can be followed with ease in this cohort and we would like to explore the impact and potential clinical correlation of monocyte levels in patients receiving bevacizumab.

B. Study Design and Statistical Analyses

This will be a prospective study assessing biomarkers (VEGF levels, sVEGFR-2 levels, and monocytes) and determining if there is a clinical correlation in pediatric patients with recurrent or progressive high grade gliomas receiving bevacizumab and irinotecan. Radiologic response, as defined by Macdonald's criteria, will be evaluated at eight-week intervals. Radiologic response will be classified as one of the following: complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Given the aggressive nature and poor prognosis in patients with high grade gliomas, patients with CR, PR, or SD will be grouped and called "stable/improved" and be compared to those with progressive disease. Serum biomarkers in the stable/improved group of patients will be compared to those with progressive disease. Biomarker levels from Days 1, 8, 15 will be compared between the two groups.

We plan to investigate:

1. If there are differences between baseline levels of biomarkers in the stable/improved group and the progressive disease group.
2. If changes in biomarker levels between day 1 to day 8 and between day 1 to day 15 of therapy are different between the stable/improved group and the progressive disease group.

3. If there is a correlation between baseline levels of the biomarkers and baseline tumor size.

We would like for biomarker levels or change in biomarker levels to have at least a one standard deviation difference between groups to be useful as a potential biomarker of antiangiogenic therapy and warrant further study. A unpaired t-test will be used to compare biomarker levels or change in biomarker levels between the two groups. 17 subjects will need to be needed in each the stable/improved group and the progressive disease group for 80% power, testing at p=0.05 level.

A correlation analysis will be used to describe the possible strength and direction of relationship between baseline biomarker levels and baseline tumor size, both continuous variables. A Pearson correlation coefficient will be determined. The Pearson product-moment correlation coefficient will have to be greater than 0.349, at p=0.05 for 34 subjects for findings to be significant.

C. Study Procedures

Pediatric patients who are receiving bevacizumab in combination with irinotecan for recurrent or progressive high grade gliomas will be eligible to participate in this biomarker study. The patients will be receiving bevacizumab at 10mg/kg concurrently with irinotecan at 125 mg/m^2 every two weeks. Magnetic resonance imaging (MRI) will be performed prior to initiation of therapy and at eight weeks. Radiologic response, as defined by Macdonald's criteria, will be evaluated at eight-week intervals. Progression-free survival, overall survival, and assessments of toxicities will also be analyzed.

Experimental Design Schema								
Week	1	2	3	4	5	6	7	8
Day	1	8	15	22	29	36	43	50
Bevacizumab&Irinotecan Administration	*		*		*		*	
MRI	*							*
Plasma Biomarker	*	*	*					

A treatment cycle will be a 28-day interval during which a patient receives bevacizumab and irinotecan treatments on day 1 and day 15 of each cycle. Plasma biomarkers will be measured Days 1, 8, and 15. Five to ten mL of blood will be drawn at each time point for the serum biomarker analysis.

D. Study Drugs

Patients will be receiving bevacizumab and irinotecan as per their treatment protocol. No additional drugs will be administered as part of this serum biomarker study.

E. Medical Devices

This study will not utilize any medical devices.

F. Study Questionnaires
This study will not utilize questionnaires.

G. Study Subjects
Inclusion criteria:

1. Patients must be > 12 months and ≤ 21 years of age at time of study entry.
2. Patients must have had histologic verification of the malignancy at original diagnosis.
3. Patients who have relapsed or have had progression with standard therapy and for whom there is no known curative chemotherapy.
4. Patients must have fully recovered from the acute toxic effects of all prior chemotherapy or radiotherapy.
5. Life expectancy of at least eight weeks
6. Disease measurable by MRI

Exclusion criteria:

1. Pregnancy
2. Patients with chronic, non-healing wound, ulcer, or bone fracture or history of a major surgical procedure or significant traumatic injury within 28 days prior to beginning therapy.
3. Known bleeding diathesis, coagulopathy, or thrombophilic condition
4. Proteinuria
5. History of cerebral hemorrhage

H. Recruitment of Subjects
Subjects that meet the eligibility criteria and plan to begin treatment with bevacizumab and irinotecan will be recruited from Columbia University and collaborating sites. They will be given the option to participate in this biomarker 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 and collaborating sites. Data analysis, interpretation, and manuscript preparation will be completed at Columbia University.

L. Potential Risks
There is minimal risk. An additional 5-10mL of blood will be drawn at the time points outlined above.

M. Potential Benefits
There is no direct potential benefit to the patients enrolled. The study of the biomarkers from the blood samples of enrolled patients will help further our understanding of antiangiogenic therapy and potentially help guide care of future patients.

N. Alternatives
The alternative is to decline participation in the biomarker component and not give additional blood samples. If subjects decline, they will continue to receive the treatment regimen as outlined.

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.

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