Benjamin Kennedy
Doris Duke Fellow
IRB Proposal for CRC Rotation

GBM & ILT3

A. Study Purpose and Rationale

Glioblastoma Multiforme (GBM, or WHO Grade IV Astrocytoma) is the most common primary brain tumor and carries a dismal prognosis. Its median survival of less than a year from diagnosis has not changed appreciably over the past few decades, despite concurrent substantial efforts to research and implement new avenues of therapy. Chemotherapy, radiation, and surgery, even when providing modest survival and functional improvement, have not been adequate treatment modalities for this quickly fatal disease. Immunotherapy for neoplastic disease has shown promise for many malignancies, in some cases, impressively. However, immunotherapies for GBM have not yet proven efficacious. GBM has been shown to have significant immunosuppressive properties, both locally and systemically, and it is possible that these properties play a significant role not only in the natural history of the disease, but in its resistance to immunotherapy as well. For these reasons, characterizing the mechanisms of GBM-induced immunosuppression will be essential for developing effective immunotherapy and improving the survival for this disease.

Immunoglobulin-Like Transcript 3 (ILT3) is a cell-surface molecule found on many cells of hematopoietic origin, namely dendritic cells (DCs). Though the ligand of ILT3 is unknown, the molecule has an intracellular inhibitory motif (ITIM) which, when activated, causes the development of a tolerogenic phenotype in the DC. This tolerogenic phenotype is able to create $CD8^+$ T suppressor cells (T_S) and $CD4^+$ T regulatory cells (T_{reg}). The extracellular domain of ILT3 can induce T_S and T_{regs} on its own as well. These T_S and T_{regs} cause antigen-specific anergy and apoptosis in the $CD8^+$ cytotoxic T lymphocytes and $CD4^+$ T helper cells. Depletion of T_S and T_{reg} compartments prior to immune therapy has been shown to increase anti-tumor vaccine efficacy and survival in other neoplastic diseases. Therefore, T cell phenotype and the mechanisms by which these phenotypes are induced in cancer may lead to effective therapy innovations. It is as a result of these ideas that I propose to study the expression of ILT3 in antigen-presenting cells (APCs) in GBM.

B. Study Design and Statistical Analysis

This will be an observational study, and the choice of controls for the levels of GBM is important. I propose three control arms in addition to the GBM arm: low grade astrocytoma, meningioma, seizure focus. The rationale behind studying low grade glioma is that the natural history of glioma is still not fully characterized and controversial, and many believe that low grade astrocytoma is a precursor lesion to GBM. It is therefore of interest to determine if any overexpression of ILT3 is found in GBM with respect to low grade astrocytoma, suggesting that this aspect of immunosuppression is associated with disease progression. If there is no difference between these two groups, the overexpression could be specific to brain, specific to tumor, or nonspecific.

These possibilities will be addressed by the other two controls. If there is upregulated ILT3 expression in truly benign meningioma, this would suggest that neither the malignancy of the tumor (GBM) nor malignant potential nor astrocytic origin (low grade glioma) is necessary for this upregulation, but that some other aspect of tumor milieu is associated with these expression levels. Seizure focus, not to be confused with normal brain, is chosen as the third control arm as it is the only type of non-neoplastic surgical brain sample available. This is an essential piece as lower ILT3 levels in these samples could represent a tumor-specific upregulation of the molecule. Levels higher than meningioma would suggest a brain-specific expression of ILT3 unrelated to tumor formation. Of course, judgment of all findings from this arm must be tempered by the fact that seizure activity has an unknown effect on ILT3 expression and could upregulate or downregulate levels of this molecule.

Tumor samples will be received fresh during surgery and each will be split and used for immunohistochemistry (IHC) and flow cytometry. The IHC specimens will be frozen in liquid nitrogen while immersed in OCT gel. They will then be sectioned and co-stained with fluorophore-conjugated antibodies against CD11b, CD11c, and ILT3. CD11b and CD11c define the microglia and other CNS macrophages. The proportion of this defined population staining positive for ILT3 will be recorded. The tumors will be prepared into single cell suspensions, using mechanical and enzymatic digestion, followed by running the cells through a sucrose gradient to extract neurons and blood products. They will then be plated and glia will be removed, leaving APCs. They will be stained with fluorophore-conjugated antibodies against CD11b, CD11c, and ILT3. Using FACSCalibur, the proportion of cells positive for CD11b and CD11c that are also positive for ILT3 can be determined.

The proportions of APCs expressing high amounts of ILT3 by each of these methods will be compared between GBM and each of the controls using unpaired t-tests. I aim to detect differences on the order of one standard deviation or greater. Although all available subjects will have the opportunity to be consented and enrolled in this study, below is a simplified method of power analysis for unpaired t-test, with $\alpha=0.05$ and power of 80%, to estimate necessary sample size.

n (*in each group*) $= 1 + 16(\sigma/\delta)^2$
$n = 1 + 16(1)^2$
$n = 17$

17 subjects in each group will be needed to conduct these experiments, a number feasible in the year allotted for this study and with the operative load at this institution.

D. Study Procedure

The only procedure involved in this study is a partial or total intracranial tumor resection. Approach and extent of resection will vary based on type of tumor, location, and other patient-specific factors, but will be in accordance with neurosurgical standards of practice and the judgment of the attending surgeon. Involvement of subjects is strictly limited to this procedure, and the procedure will occur only once per subject. Secondary resections

for recurrence or other indications will not be studied. All decisions regarding whether to perform surgery, which surgeons will be involved in the case, how much tumor will be removed, surgical approach, and any other aspects of patient care will be made by the attending physician and the subject, to the extent that the subject is able, and will be based entirely on clinical judgment and subject's wishes, and without consideration of this study. Surgery will not be temporally extended as tumor is divided by staff and accepted by the researchers after removal as the surgery continues uninterrupted. No additional instruments will be required, and the subjects will not undergo any additional pain, discomfort, or inconvenience. Subjects will not be approached regarding this study until the decision for surgery has been made. Also, the amount of specimen available for study will consist only of superfluous tumor tissue, after the most appropriate tissue, designated by the attending surgeon, has been sent to pathology.

D. Study Drugs
No drugs will be used as part of this study.

E. Medical Device
No medical device will be used as part of this study.

F. Study Questionnaires
No study questionnaires will be used as part of this study.

G. Study Subjects
Inclusion Criteria: All patients undergoing intracranial tumorectomies through the Department of Neurosurgery at Columbia University. After pathology confirms a diagnosis of one of the relevant tumor types to be studied, the data will be included in the final analyses.

Exclusion Criteria: No patients will be excluded from this study. Nearly all patients will be using some form of immunosuppressive medications (i.e. dexamethasone) for tumor-related edema, and therefore excluding patients using immunosuppressive medications is not feasible. No age limits will be set; it is known that the tumor types being studied occur more commonly in different age groups, but this is an inherent flaw of the study which cannot be feasibly corrected.

H. Recruitment of Subjects
After the decision for surgery has been made with the subject, the attending neurosurgeon treating the subject will educate the subject on the study and consent the subject.

I. Confidentiality of Study Data
The conversion of MRNs to specimen numbers will be kept on an off-network computer in a locked office of the Bartoli Brain Tumor Research Laboratory in the P&S building on campus at Columbia University Medical Center.

J. Conflict of Interest
The investigators have no conflicts of interest to declare.

K. Location of the Study
All study procedures will take place in the neurosurgical operating rooms on the 4th floor of Milstein Hospital Building on campus at Columbia University Medical Center. Tissue analyses will take place in the P&S building.

L. Potential Risks
There is no deviation from standard of care and physician's best judgment in this study, and therefore, no additional risks will be encountered by the patients.

M. Potential Benefits
As there are no therapeutic options specific for patients with high or low levels of ILT3, it is not likely that there could be any potential for subjects to benefit from this study.

N. Alternative Therapies
There is no deviation from standard of care and physician's best judgment in this study, and therefore, all alternatives will be discussed prior to surgery to the extent that the physician and subject wish.

O. Compensation to Subjects
No compensation will be offered to subjects.

P. Costs to Subjects
No costs will be incurred by subjects.