detectable increases in tumor size on imaging associated with the use of these treatments. Unfortunately, conventional imaging methods such as MRI do not distinguish whether that observed tumor growth reflects 'pseudoprogression' (swelling due to the activation of an immune response)
that indicates potential therapeutic success, or if it is due to true progression of resistant disease and represents treatment failure. This gap in knowledge poses a significant challenge for doctors to reliably ensure that they appropriately withdraw patients with refractory disease who will not benefit from the immunotherapy, while avoiding the removal of patients with inflammatory treatment responses from further participation in immunotherapeutic trials that may provide them benefit. Clinical trials using such immune-based strategies are therefore threatened by premature closure and misinterpretation of results due to inappropriate retainment or removal of enrolled patients. The accurate distinction between real tumor progression and immune reactivity to therapy is absolutely crucial to properly conduct immunotherapeutic trials and correctly interpret whether or not immune-based treatments are truly effective against brain tumors.

We seek to establish accurate, reproducible methods which distinguish whether increasing tumor size after administration of immune-based therapies is due to resistant disease progression or the expected infiltration of inflammatory immune cells. By leveraging PET- and MRI-based imaging approaches that are currently already used in human clinical trials and are readily available at our institution, we aim to address this critical need using CAR T cells and immune checkpoint blockade medications in mice with brain tumors, providing data that will directly inform the design of future immunotherapeutic clinical trials. Our studies are designed to identify which types of immune cells most contribute to tumor pseudoprogression, providing insight into potential ways to preemptively target and prevent immune therapy-induced inflammation. The clinical translation of this project is expected to re-define imaging-based immunotherapeutic response criteria and guide the appropriate and safe use of immune-based therapies for pediatric patients with brain tumors.

EXECUTIVE SUMMARY

Cancer is the leading cause of childhood death by disease in the United States, with central nervous system (CNS) tumors imparting the highest mortality contribution. Even when successful at tumor eradication, conventional treatments often confer devastating long-term sequelae, including risk for

secondary malignancies, without assured prevention of tumor recurrence. Therapies which potentiate T cell activity show promise, offering potential protection from relapse through immunologic memory while maximizing targeted anti-tumor efficacy. Previous studies using immune checkpoint blockade agents targeting CTLA-4 or PD-1 in immunocompetent murine models recapitulating human medulloblastomas (MBL) characterized the timing, quantity, and phenotypes of associated tumoral immune cellular infiltrates. Additionally, studies conducted by the Krenciute laboratory determined the time course of therapeutic response to stereotactically-injected T cells transduced with chimeric antigen receptors (CAR) in mice bearing orthotopic xenograft CNS tumors. Clinical trials using such approaches as immune checkpoint blockade or CAR T cells are currently underway for pediatric patients with CNS tumors. However, studies conducted in adult with CNS tumors utilizing checkpoint blockade and recent CAR T cell preclinical studies in orthotopic pediatric CNS tumor models revealed associated increases in tumor size. Conventional imaging modalities do not distinguish whether this radiographically-detected tumor growth observed after immunotherapeutic administration reflects a ‘pseudoprogression’ secondary to immunologic activation, or indicates disease progression. This knowledge gap poses a significant challenge for clinicians to reliably ascribe a definitive etiology ensuring justifiable withdrawal of patients with resistant disease, while avoiding inappropriate removal of patients from further participation in immunotherapeutic trials that provide them therapeutic benefit. The reliable discernment of tumor progression from immune reactivity is critical to accurately interpret immunotherapeutic anti-tumor efficacy. We seek to define accurate, reproducible methods which distinguish whether increasing tumor size is due to disease progression, representing treatment failure, or from immune cell infiltrates, indicating therapeutic success.

Positron emission topography (PET)-based imaging allows evaluation of dynamic molecular events and biochemical processes in vivo. Radionuclides such as 18F (110 minute half-life) and 11C (20 minute half-life) incorporated into biologically active radiotracers facilitate specific imaging of cells with high metabolic or proliferative activity, such as tumor cells or activated T cells, differentiating them from the surrounding less active environment. Though [18F]fluorodeoxyglucose (FDG), an indicator of glucose metabolism, is the most commonly used PET tracer for oncologic staging and response-monitoring, its intense uptake in normal brain renders it suboptimal for monitoring CNS tumors. Alternatively, PET imaging with other clinically-applicable radiotracers including 11C-labelled methionine (MET), an essential amino acid correlative to

protein synthesis, or [18F]fluorothymidine (FLT), a thymidine analog indicating DNA synthesis and cell proliferation, demonstrates minimal background uptake in the brain. Production of MET and FLT by our on-site cyclotron uniquely positions us to perform investigations using these modalities. Our institutional Center for In Vivo Imaging and Therapy (CIVIT) and Molecular Imaging core resources established a reliable method combining PET-, CT-, and MRI-based imaging modalities to detect orthotopic CNS tumors in small animals.

Our overarching hypothesis is that ***neuro-inflammatory responses elicited by T cell-dependent immunotherapeutic strategies are discernable from CNS tumor progression by clinically-applicable PET- and MRI-based imaging modalities.*** We propose correlating findings from combining MET- and FLT-based PET imaging with standard modalities like CT and MRI with tumor or T cell fluorescence and histopathologic evaluation of immune infiltrates to delineate biochemical processes of tumor growth from activated T cells. We will use immune checkpoint blockade agents in orthotopic murine MBL models and adoptively transferred human CAR T cells in pediatric MBL xenograft models with naïve tumor-bearing mice and mice without tumors who received immunotherapy as controls to test our hypothesis. We will confirm imaging findings by histopathologic analysis at defined time points to **(aim 1) determine whether clinically-applicable diagnostic imaging modalities distinguish tumor growth attributable to immunotherapeutic-responsive immune cellular infiltrates from non-responsive progressive disease.** We will correlate histopathologic evaluation of qualitative and quantitative changes in innate and adaptive immune cellular infiltrates and tumor-associated microglia in response to immunotherapeutic administration with time-matched imaging to **(aim 2) identify the innate versus adaptive and CNS-native versus systemic immune cellular contributions to tumor pseudoprogression.**

Therapeutic modalities which augment T cell responses have shown recent promise against brain tumors. Clinical trials utilizing immune checkpoint blockade or adoptive cellular immune strategies such as CAR T cells are threatened by the premature removal of patients demonstrating tumor pseudoprogression due to an inability to accurately differentiate them from patients with refractory tumor growth. By leveraging clinically-relevant PET- and MRI-based approaches readily available at our institution, we aim to address the critical need to discern inflammatory reactivity secondary to immunotherapeutic response from true disease progression. The proposed investigations of the mechanisms underlying this delineation of metabolically

active tumor from activated immune cells will provide data to directly inform the design of future immunotherapeutic clinical trials. These studies will additionally identify which immune cellular subtypes most contribute to immunotherapeutic-induced inflammatory tumor pseudoprogression, and provide mechanistic insight into investigations combining the rational preemptive use of targeted agents which may dampen or prevent immunotherapy-induced neuro-inflammation. The clinical translation of this project is thus expected to re-define relevant imaging-based immunotherapeutic response criteria and guide the appropriate, safe use of immune-based therapies for pediatric patients with brain tumors.

DESCRIPTION OF RESEARCH PROPOSAL

Specific Aims: Approximately 13,400 children are diagnosed with cancer each year in the United States, making it the leading cause of childhood and adolescent death by disease.[11] Central nervous system (CNS) tumors have recently emerged as the highest disease contributor to pediatric mortality.[1] Accounting for ~28% of the most common pediatric brain tumor diagnosis,[12,13] the Group 3 medulloblastoma (MBL) subtype is associated with a high rate of metastases and poor prognosis, with 5-year overall survival (OS) rates of 40-60%.[12-14] Pediatric high-grade gliomas (HGG), brain tumors which tend to metastasize early and are frequently refractory to currently available treatments, have extremely poor outcomes, with median survival times of merely 9 to 15 months.[15-17] Even when successful at tumor eradication, conventionally employed aggressive multi-modal treatment regimens often confer devastating long-term sequelae, including risk for secondary malignancies, without assured prevention of tumor recurrence.[2,3] Thus, new strategies using targeted therapies offering potential protection from relapse are critically needed. Clinical trials using such approaches as immune checkpoint blockade or chimeric antigen receptor (CAR) T cells are currently underway for pediatric patients with CNS tumors.[6] However, studies conducted in adult with CNS tumors utilizing checkpoint blockade and recent CAR T cell preclinical studies in pediatric CNS tumor models revealed associated increases in tumor size.[7,8] Conventional imaging modalities do not distinguish whether this radiographically-detected tumor growth observed after immunotherapeutic administration reflects ‘pseudoprogression’ secondary to immunologic activation, or indicates disease progression. We seek to define accurate, reproducible methods which distinguish whether increasing tumor size is due to disease progression, representing treatment failure, or from immune cell infiltrates, indicating therapeutic success.