Section 2: Executive Summary

Background: Diffuse intrinsic pontine glioma (DIPG) is a lethal pediatric brain tumor that affects approximately 150 children in North America each year.[1] Though radiation therapy can prolong survival by 2-3 months, adjuvant chemotherapy has not improved outcome. The median survival is less than 12 months and has remained unchanged for the last 30 years.[3] Knowledge of the pathogenesis of DIPG has been historically limited by lack of tissue availability, as diagnostic biopsy has not been routinely performed due to safety concerns.[2] More recently, however, tissue procurement through autopsy programs[3-5] and slowly growing acceptance of the safety of DIPG biopsy[6-9] has provided the tissue necessary to gain critical knowledge of the genetic and epigenetic landscape of DIPG and has enabled development of *in vitro* and *in vivo* models through which novel therapies may be thoroughly tested.[10,11]

Since 2012, high-throughput sequencing studies have yielded unprecedented insight into the biologic basis of pediatric high-grade glioma (HGG) and DIPG.[12-18] Midline, non-brainstem HGGs harbor a genomic profile akin to DIPG (e.g. frequent H3K27M mutation) and carry a similarly dismal prognosis.[16,19] Mutations of chromatin remodeling genes *H3F3A*, *HIST1H3B*, and *HIST1H3C* are present in ~80% of thalamic HGGs[19] and 70-96% of DIPGs.[4-9] More recently discovered *ACVR1* mutations are found in ~25% of DIPGs. In addition, mutations in other canonical cancer pathways, including (RTK)-RAS-PI3K, TP53, and RB1, are commonly found in HGG and DIPG.[15,20] Unfortunately, our vastly improved understanding of tumor biology has not yet translated into a therapeutic breakthrough for children with HGG and DIPG. One critical factor that will significantly impact guiding appropriate therapy is histologic and molecular intra-tumoral heterogeneity. Although such heterogeneity has been well-documented in adult HGG[21-23], there is little understanding of intra-tumoral variation in pediatric HGG and DIPG. Studies of DIPG at autopsy, including our own investigations with correlative post-mortem MRI, have revealed distant tumor metastases to the periventricular and frontal brain regions.[5,24] Interestingly, we have observed some histologic variability between primary and distant tumor. Preliminary WES of primary, contiguous, and metastatic sites from 8 patients in our cohort also revealed relevant genomic heterogeneity (**Figure 2**). Although histone mutations, if present in the primary site, were detected at all disseminated sites, several interesting variations were seen among other clinically relevant mutations. Specifically, in one patient (patient 3) with six regions sequenced (all containing H3.3K27M), we detected regional variations in TP53 mutations. The primary tumour site (primary pons) contained a TP53 p.Arg248Gln alteration (allele frequency, AF = 0.46). Only 1 of 5 contiguous and metastatic sites (leptomeningeal spread) contained this alteration at an AF of 0.10. These 5 sites also harbored a TP53 p.Arg.275His alteration, which was not detected in the primary pons. In this same patient, a PDGFRA p.Glu229Lys alteration and ~8 copy amplification was detected in the right posterior pons, but not the primary pons or any other contiguous and metastatic sites. The leptomeningeal component did exhibit a *PDGFRA* copy number gain of ~4 copies.

We hypothesize that genomic heterogeneity exists within the primary tumor and between primary and distant disease and that better understanding of clonal evolution of DIPG and midline HGG will guide the rational design of molecularly targeted therapies for these lethal brain tumors.

- Specific Aim 1: To define spatial intra-tumoral heterogeneity using WES on contiguous, non-contiguous, and distant metastatic sites in DIPG and midline HGG obtained at autopsy
- Specific Aim 2: To demonstrate patterns of branched clonal evolution of DIPG and midline HGG using high-depth WES on contiguous, non-contiguous, and distant metastatic sites
- Specific Aim 3: To define temporal intra-tumoral heterogeneity using WES on matched biopsy and autopsy tumor specimens from patients with DIPG and midline HGG

Methods/Budget: We request $200,000 to perform WES on matched primary and metastatic (n=10) and matched biopsy and autopsy (n=3) specimens from patients with DIPG or midline HGG who underwent surgical biopsy and/or autopsy at Cincinnati Children's Hospital Medical Center (CCHMC) or The Hospital for Sick Children. A portion of funding will go toward salary support for the study PIs and for bioinformatics analyses. Complete clinical annotation is available for all patients.

Clinical Significance: Characterization of spatial and temporal heterogeneity and establishment of clonal evolutionary patterns in DIPG and midline HGG will have significant clinical impacts. First, this work will define the utility of primary tumor biopsy, which may misrepresent targetable genomic lesions across all disease locations, and a potential role for re-biopsy at progression. Additionally, exploration of early somatic events using high-depth sequencing and phylogenetic analyses has potential to define true disease "drivers" common across disease compartments that, if molecularly targeted, may hold more potent therapeutic potential.