Section 2: Executive Summary

Title: Defining the molecular mechanisms of DIPG development and progression to uncover novel therapeutic targets.

Diffuse Intrinsic Pontine Gliomas (DIPGs) are devastating pediatric brainstem tumors that lack effective treatment and are uniformly fatal. Patient studies have identified recurrent genetic lesions that drive the development of these tumors. Almost all DIPGs carry mutations in genes encoding either replication-dependent histone-H3 proteins (mostly *HIST1H3B*) or in a replication-independent histone (*H3F3A*). These mutations always substitute lysine with methionine at position 27 (K27M) of the H3 protein. The tumorigenicity of histone K27M mutations is thought to stem from epigenetic reprogramming of tumor-initiating glial cells in the brain. Moreover, recent genetic data strongly suggest that DIPGs can be clustered into distinct subtypes based on specific "partner" mutations that co-occur with the K27M H3 mutations. For example, most *H3F3A*^{K27M} mutant tumors carry lesions in the well-characterized tumor suppressor gene *TP53*. This is not the case for *HIST1H3B*^{K27M} mutant malignancies, which instead commonly harbor lesions that either cause a gain-of-function in ACVR1, a bone morphogenetic protein (BMP) type I receptor, or hyperactivate the PTEN/PI3K pathway. The oncogenic mechanisms of action of activating mutations in *ACVR1* are poorly characterized. Understanding the mechanisms that drive DIPG subtype development, and how these tumors might differ in therapeutic vulnerability, is crucial for the development of effective DIPG treatments.

In our proposal, we will test the hypothesis that oncogenic synergy between epigenomic reprogramming induced by *HIST1H3B*^{K27M} mutations and cellular hyperproliferation driven by *ACVR1* and PTEN/PI3K pathway mutations underlie unique therapeutic vulnerabilities in DIPG tumors. We will deploy a multidisciplinary approach that combines complementary areas of expertise and reagents, including the generation and analysis of the first pre-clinical mouse models harboring DIPG-causing mutations in the endogenous *Acvr1* and *Hist1h3b* genes. Using these models, we will characterize the molecular effects of *Acvr1* and *Hist1h3b* mutations and dissect their interaction and synergy. We will then harness an innovative direct *in vivo* CRISPR/Cas9 platform to combine multiple co-occurring mutations and describe their oncogenic mechanisms of action. We will pay particular attention to investigating how PTEN/PI3K pathway hyperactivation cooperates with *Acvr1* and *Hist1h3b* mutations. Finally, we will meld these analyses with human DIPG transcriptome data and perform functional experiments in patient-derived cell lines to uncover candidate therapeutic targets. We have already established a broad toolbox of reagents useful for our planned studies and have accumulated substantial preliminary data in support of our objectives.

We expect that our project will uncover the molecular mechanisms whereby *ACVR1*, *HIST1H3B* and PTEN/PI3K pathway mutations cooperate to drive DIPG development and progression. We further anticipate that our studies will reveal candidate therapeutic targets for tumors harboring this combination of lesions, and possibly for DIPGs in general.