2. Executive Summary:

Background: Recently, driver mutations in histone H3 have been found to correlate with particularly malignant forms of DIPG. These mutations change lysine 27 of H3 to methionine (K27M), abrogating methylation and acetylation at this site, both of which are epigenetic marks that influence chromatin architecture and are important for regulating the expression of numerous genes. Accordingly, molecules that inhibit histone deacetylases (HDACs) or the H3K27me3 demethylase inhibit DIPG growth *in vitro* and in tumor models *in vivo*. Despite this clear chromatin-dependent basis for DIPG pathology, a systematic survey of chromatin regulators important for the survival and proliferation of H3K27M mutant DIPGs has not been conducted. We **hypothesize** that additional chromatin regulators interact with the histone H3 mutations in DIPG to support proliferation and tumorigenesis, and that these regulators will be promising targets for developing novel therapeutics.

Research Design and Methods: We will apply multiple screening approaches to comprehensively query chromatin regulators for their effects on DIPG cell survival and proliferation. Recently, patient-derived cell lines expressing wild-type or mutant histone H3 have become available, making large-scale *in vitro* screens possible for the first time. We will first screen a specialized small-molecule collection that targets both conserved and specific domains found in chromatin regulators, focusing on molecules that preferentially inhibit the growth of H3-mutant DIPG cell lines, but not H3 wild-type glioblastoma cells. To obtain a more comprehensive view of epigenetic regulatory mechanisms important for DIPG growth, we will apply an shRNA-based screening platform that we developed and validated in ongoing work on chromatin regulators in acute myelogenous leukemia (AML). Cell lines harboring wild-type or mutant alleles of H3 will be transduced with a lentiviral library containing bar-coded shRNAs targeting 500 annotated chromatin regulators. After allowing these cells to propagate, we will sequence shRNAs in the population to to identify those that preferentially "drop out" of the H3-mutant population, which will theoretically identify genes that are essential for proliferation of H3-mutant cells. In parallel, CRIPSR technology will be used to target chromatin regulators in a similar screening platform, both to minimize identification of genes resulting from off-target effects of shRNAs, and to extend the coverage of the select chromatin regulators in the initial screening phase. High-priority targets will be selected based on their identification in multiple screens, and will be confirmed by targeted knock-down or knock-out strategies. The effects of these candidates on cell viability, proliferation, differentiation, and apoptosis will be determined in follow-up experiments to more completely characterize the functional roles of these chromatin regulators in H3-mutant DIPG. Future work will focus on understanding the epigenetic processes influenced by these genes in DIPG, and on assessing the importance of these target genes for DIPG tumor growth *in vivo*.

Innovation: This work harnesses the unique opportunities provided by the new DIPG cell-culture models to analyze chromatin sensitivities in a systematic and comprehensive manner. As epigenetic regulators commonly considered to be easily druggable, we hypothesize that focusing on these regulators will increase the likelihood of identifying strong candidates for small-molecule therapeutics. Our use of multiple, parallel screening approaches on both wild-type and mutant cell lines is expected to uncover high-confidence, genetically-focused targets in an efficient and high-throughput manner. By including a focused chemical screen, we hope to expedite the development of novel therapeutics, as future work will on identified hits will focus on compound optimization rather than *de novo* compound development. The genetic screens will use the technology we have recently developed and validated for AML screening, and will represent one of the first comprehensive assessments of chromatin-dependent mechanisms in pediatric glioma. The outcome of this work is expected to be highly **clinically significant**, as it will identify novel components to target for DIPG therapeutics, some of which may already have small-molecule inhibitors available, thus shortening the time to clinical applications.