2. Executive Summary

Title: *Advanced RNAi therapeutics for DIPG*

Diffuse intrinsic pontine glioma (DIPG) is a form of brain tumor that arises in the brainstem of children and is nearly 100% fatal with a median survival of 9-12 months after diagnostic. The location of these highly infiltrating tumors in the ventral pons precludes surgical resection and clinical trials with different chemotherapeutic drugs have shown dismal results. Alterations in epigenetic regulation appear to be an important feature in DIPG as whole-genome/exome sequencing revealed recurring heterozygote mutations in H3.3 and H3.1 histones (K27M, G34R and G34V), respectively encoded by *H3F3A* or *HIST1H3B* genes. Mutations in *H3F3A* that result in the K27M substitution occur in 70-80% of DIPG while 11-31% of mutations are found in *HIST1H3B*. The evidence from mechanistic studies indicates that H3-K27M mutant histones exert their dominant effects through global reduction of methylase activity that may be critical for tumor maintenance as an inhibitor of JMJD3 demethylase has a considerable anti-tumor effect. Presently there are no drugs to target mutant histones specifically.

Development of effective therapies for DIPG is hampered by the inability to target the majority of cancer relevant proteins using traditional small molecules. In contrast gene specific silencing using RNA interference, or antisense oligonucleotides (ASO), has no theoretical limitation on genes that can be targeted. Moreover as the nucleotide backbone chemistry determines the PD/PK and the sequence determines target engagement these flexible platforms can be used to rapidly assess the therapeutic potential of new genes or modulation of multiple genes in a network. **Using RNA interference with chemically modified ultra-stable siRNAs for cancer therapy is an innovative approach with no apparent target limitations** that builds on the growing clinical experience using oligonucleotides to modulate gene expression in neurological diseases. Dr. Anastasia Khvorova in the RNA Therapeutics Institute at UMMS has developed new ultra-stable hydrophobically modified siRNAs (hsiRNA) capable of sustained gene silencing throughout the adult mouse brain after a single injection into the cerebral spinal fluid (CSF). Also our laboratories (**Sena-Esteves and Khvorova**) found that intracranial delivery of hsiRNA is effective in silencing gene expression in a highly migratory human glioblastoma multiforme (GBM) orthotopic tumor model. Therefore w*e hypothesize that CSF delivery of hsiRNAs is an effective approach to silence gene expression in orthotopic DIPG tumors and drive transformative therapeutic outcomes with translation potential*. Successful completion of this proposal will demonstrate that RNA interference is a potent platform to harness the wealth of information available on gene networks driving DIPG to develop new genetic therapies for this devastating disease that so desperately needs effective treatments. We propose the following aims:

Aim 1: *Target screening and validation in human DIPG lines.* The high frequency of H3-K27M mutations in DIPG suggests that it may have a fundamental role in tumor maintenance/progression despite not being sufficient for tumor initiation. Here we will develop hsiRNAs specific for the *H3F3A* gene where most H3.3K27M mutations are found through the use of SNPs that vary across the 15 histone genes as well as targeting the unique 3'untranslated region of this gene. Also we will investigate the therapeutic effect of targeting cell cycle proteins with no apparent genetic redundancy such as mitosis cyclins (CCNA2, CCNB1) and others. Initial screening will be conducted in human HEK293 cells using mRNA levels as outcome measure. The biological effect of the most efficient hsiRNAs for each target gene will be assessed in multiple DIPG lines with and without H3.3K27M.

Aim 2: *Efficacy studies in experimental models of DIPG*. The hsiRNAs shown to impact tumor growth/survival in vitro will be tested in orthotopic tumor models generated by stereotaxic injection of firefly luciferase expressing DIPG tumor cells (H3.3 WT or K27M) in the brainstem of athymic nude mice. hsiRNAs will be infused into the CSF via the cisterna magna to maximize brainstem delivery and we will assess impact on tumor growth over time via bioluminescence imaging and survival.