EXECUTIVE SUMMARY

Therapeutic reversal of pre-natal pontine ID1 signaling in DIPG

Background: Diffuse intrinsic pontine glioma (DIPG) is a lethal pediatric brain tumor with a median survival of just 10-11 months. Even with the advent of precision-based medicine, experimental therapies have yet to show benet beyond standard radiation, highlighting the dire need to identify and investigate novel genetic therapeutic targets in DIPG.

Inhibitor of DNA binding (ID) proteins are key regulators of tissue and lineagespecic gene differentiation during embryogenesis, acting through negative regulation of basic helix-loop-helix (bHLH) transcription factors. A preliminary study of cultured human astrocytes implicated a role for ID1 in DIPG, with ID1 up-regulation with introduction of H3F3A K27M mutation and/or ACVR1 mutation, but this has not been validated or targeted in human DIPG. Thus, there is a critical need to better understand the impact of H3K27M mutation on ID1 expression and targetability with CBD.

Hypothesis and goals: Our central hypothesis is that ID1 expression can be epigenetically up-regulated by H3K27M-mediated re-activation of prenatal pontine signaling and that ID1 expression and DIPG cell viability can be reduced with CBD treatment. (Fig. 1). This hypothesis was formulated based on our preliminary data showing: (1) increased ID1 expression in H3K27M-DIPG autopsy tissue, (2) reduced invasion and migration of cultured DIPG cells with ID1 knockdown, and (3) reduced ID1 levels and cultured DIPG tumor cell viability with CBD treatment. We propose to determine the epigenetic mechanisms of ID1 expression in DIPG and its targetability with CBD through two specic aims:

Aim 1: Identify the impact of H3 status on the epigenetic control and invasive phenotype of ID1. Our hypothesis is that H3K27M reduces H3K27me3 and increases H3K27ac at ID1 promoter and enhancer sites, thus contributing to increased ID1 expression and invasion/migration in H3K27M-DIPG cells. Aim 2: Determine the impact of H3 status on cannabidiol (CBD) reduction in invasion and motility. Our hypothesis is that H3K27M-mutant DIPG cells will display most prominent reduction in ID1 expression and invasion and improvement in survival in response to CBD treatment. Design and methods: In order to study the impact of regional and H3mutational differences on ID1 expression and targetability, in collaboration with Dr. Tim Phoenix's lab (CCHMC, collaborator on this grant), the Koschmann lab has employed an in utero electroporation (IUE) to transfect neural progenitor cells (NPCs) of pre-natal wildtype (Wt) CD1 mice on embryonic day E14 with plasmids bearing characteristics of pediatric high-grade glioma (pHGG) mutations. In our lab, we have successfully generated in vivo tumors in both the forebrain and hindbrain for: (i) PPW, mutant TP53, constitutively active PDGFRA-D842V, Wt H3; (ii) PPK, mutant TP53, PDGFRA-D842V, mutant H3K27M; (iii) PPG mutant TP53, PDGFRA-D842V, mutant H3G34V. Our lab has also generated primary cell lines de novo from the aforementioned IUEinduced pediatric high grade glioma model for in vitro studies. Multiple (n=2) mouse primary cell cultures were generated from dissociated primary tumor tissue from each condition (forebrain: PPW/PPK/PPG; hindbrain: PPW/PPK/PPG).

Aim 1.1: Determine the impact of ID1 knockdown on cell invasion and motility by H3 mutational status using mouse DIPGs generated with hindbrain intra-uterine electroporation (IUE) of plasmids expressing a dominant negative TP53 and over-expression of PDGFRA and various H3 mutations (H3.3K27M, H3.3G34V, H3WT). Aim 1.2: Examine differences in H3K27ac and H3K27me3 at enhancer and promoter of the ID1 locus by H3 mutational status and location (forebrain, hindbrain) using IUE-generated tumors. Aim 1.3: Examine differences in H3K27ac and H3K27me3 levels at ID1 loci by H3 mutational status in multi-focal human DIPG autopsy specimens.

Aim 2.1: Determine the impact of CBD on cell viability by H3 mutational status using isogenic primary murine and human pediatric HGG/DIPG cell lines. Aim 2.2: Determine the impact of CBD on cell invasion and migration by H3 mutational status using mouse isogenic DIPG cells. Aim 2.3: Determine the impact of CBD treatment on tumor proliferation and differentiation in IUE-generated murine DIPG, and differences by H3 mutational status and location (hindbrain vs forebrain). Clinical signicance: DIPG is currently the most aggressive primary brain tumor in children, and there is a dire need to identify novel therapeutic targets and therapies. Therapies are now tailored to the H3 K27M status of each tumor. A better understanding of the impact and epigenetic regulation of ID1 by H3mutational subgroups will help us to identify patients in which ID1 signaling is most important, as well as mechanisms underlying the role and regulation of ID1 in DIPG. CBD has been shown to target ID1 in pre-clinical models of other human cancers and is being used off-trial in human DIPG, but robust preclinical data is sorely lacking. By expanding our understanding of the impact of CBD on DIPG phenotypes in vitro and in vivo by H3-mutational status, we hope lay the groundwork for potential future clinical trials assessing the safety and efcacy of CBD in DIPG in a rationale manner.