Executive Summary

DIPG is characteristically infiltrative (i.e. diffuse and intrinsic), and this infiltrative/invasive behavior is destructive both in the brainstem and in other areas of the central nervous system to which DIPG spreads during the course of the disease. In work previously funded by the Cure Starts Now and the DIPG Collaborative, we discovered that neuronal activity promotes DIPG cell invasion through activity-regulated secreted factors that includes an endogenous antagonist to the Nogo receptor (NgR). NgR signaling is a key mechanism that restricts the plasticity and regeneration of normal brain cells, and blocking the Nogo pathway results in increased motility of cells and cellular processes such as axonal outgrowth. In the normal brain, this signaling pathway plays a role during early brain development and may continue to play a role in ongoing brain plasticity. DIPG cells express NgR and in preliminary studies we have found that blocking NgR signaling with recombinant antagonist or by deleting the NgR gene via CRISPR gene editing from patient-derived DIPG cells dramatically increases DIPG invasion *in vitro*. In the proposed studies, we will expand these observations to a larger number of patient-derived DIPG cell cultures to determine how universal this mechanism may be, and will assess the importance of NgR signaling to DIPG invasion *in vivo* using genetic models. If NgR signaling proves to be an important mechanism controlling DIPG invasion, then stimulating the NgR receptor may be an innovative therapeutic strategy to control infiltration of DIPG cells throughout the brainstem and prevent spread more diffusely to the cerebrum and spinal cord.

The regulation of the NgR signaling by neuronal activity represents one of the many ways that experience shapes brain development and plasticity. The effects on DIPG invasion represents yet another way that DIPG cells hijack normal mechanisms of brain development to promote disease progression. Ultimately, by understanding the ways DIPG subverts mechanisms of childhood brain development and adaptability, we hope to develop effective and tumor-specific strategies to disrupt the ability of the tumor cells to use these crucial signals in the tumor microenvironment.