Diffuse intrinsic pontine glioma (DIPG) is an aggressive type of brainstem cancer that targets young children. Novel approaches are urgently needed to treat this disease. We hypothesize that reversing signature gene expression derived from DIPG RNA-Seq samples will facilitate novel therapeutic discovery for DIPG. DIPG RNA-Seq reads obtained from EGA were processed through the TOIL pipeline developed by the UCSC treehouse project (n=22). RNASeq reads of normal tissue samples obtained through GTEx were processed through the same pipeline to mitigate batch effect (n=7,412). The best 50 normal tissue samples for the DIPG samples were selected using a machine learning method where deep-learning autoencoder was employed to embed both tumor samples and control samples. EdgeR was used to perform differential expression analysis and establish DIPG disease signature. A previously-published drug repurposing pipeline was used to compute summarized reverse gene expression score (sRGES) for each of the approximately 12,000 LINCS L1000 drugs and DIPG disease signature. Subsequently, FDA-approved drugs were selected, which were not previously studied in DIPG, tested more than once in LINCS L1000, and showing sRGES < -0.2. The candidates were tested against multiple DIPG cell lines in viability assays. RNA-Seq before and after drug treatment in DIPG cells was further profiled.

A total of 1,131 disease signature genes were identified for DIPG. Among these, 49 genes were included for drug prediction through sRGES pipeline. The sRGES is significantly correlated with drug efficacy for 35 compounds which have both gene expression profiles and drug efficacy data (Spearman correlation: 0.53). Four novel drugs are consistently predicted among multiple runs where parameters in the pipeline were changed. These drugs were tested against three DIPG cell lines (SF8628, DIPG4, and DIPG-NYU) and normal human astrocytes. All three drugs reduced cell viability in three cell lines with average IC50 < 10um for each. Two drugs are at least 8 fold more cytotoxic to DIPG cells than normal astrocytes. Further RNA-Seq analysis of the treatment samples in DIPG cells confirmed that expression of the 49 genes was significantly reversed after the drug treatment, suggesting the feasibility of the computational approach (Spearman correlation < -0.3, p value < 0.001).

This study helped to identify clinically available drugs with the ability to reverse DIPG gene expression signatures and significantly decrease the growth rate of primary DIPG cells in vitro. The pipeline developed in this study has the potential to identify novel therapeutic candidates for other cancers, especially for those where adjacent normal tissue samples were not readily accessible.