BACKGROUND: Diffuse Intrinsic Pontine Glioma (DIPG) presents a significant clinical challenge due to its inoperable nature and poor response to current treatments. Sonodynamic Therapy (SDT) is a non-invasive therapy that combines low-intensity ultrasound stimulation with a sonosensitizing agent. 5-Aminolevulinic Acid (5-ALA) as a sonosensitizing agent has yielded positive outcomes in in-vivo glioblastoma models, and a clinical trial (NCT05123534) for DIPG patients is currently ongoing. 5-ALA is a precursor of Protoporphyrin IX (PpIX), which preferentially accumulates in cancer cells and generates cytotoxic reactive oxygen species upon activation by ultrasound or light. Our work is aimed at investigating the accumulation of PpIX in in-vitro DIPG patient-derived cell models to predict the efficacy of 5-ALA SDT treatment.

METHODS: Patient derived DIPG cell lines (H3K27M, TP53 mutant and wild type) were used to create three-dimensional spheroids. The spheroids were dosed with 5-ALA for 4 hours, and PpIX accumulation was quantified by measuring its fluorescence intensity within the cells. The spheroids were then exposed to controlled light stimulation as an in-vitro proxy for SDT. RESULTS: We confirmed the uptake of 5-ALA and subsequent PpIX accumulation in all patient-derived DIPG spheroids. Light exposure of 5-ALA treated DIPG spheroids resulted in reduced viability of the tumor models, while 5-ALA itself did not show cytotoxic effects. The extent of PpIX accumulation and cytotoxicity upon PpIX light activation varied among DIPG models with higher sensitivity of TP53 wild type cells. Further studies incorporating a larger number of DMG cells with p53 alterations are underway. CONCLUSIONS: These findings indicate that SDT using 5-ALA as sonosensitizer may offer clinical benefits for treatment of DIPG, however treatment efficacy could vary considerably between patients. In vitro evaluation of PpIX accumulation and sensitivity to activation could potentially provide a means to stratify patients and to identify those most likely to benefit from 5-ALA-mediated SDT.