Harnessing Cellular Stress for Immune Targeting of DIPGs

Donghang Cheng[1], Ajay Sharma[1], Sreepradha Sridharan[1], Arif Harmanci[2], Robert Siddaway[3], Tara Dobson[1], Jyothishmathi Swaminathan[1], Rohinton S. Tarapore[4], Joshua E. Allen[4], Cynthia Hawkins[3], Soumen Khatua[1], Wafik Zaky[1] and Vidya Gopalakrishnan[1]

[1]Division of Pediatrics, University of Texas MD Anderson Cancer Center, Houston, TX 77030; [2]School of Biomedical Informatics, University of Texas Health Science Center, Houston, TX 77030; [3]The Hospital for Sick Children, The University of Toronto, ON, Canada, [4]Chimerix, Durham, NC.

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Children's Cancer Hospital®

Introduction

- Diffuse Intrinsic Pontine Glioma (DIPG) is a pediatric brain tumor with a peak age of incidence at 6-8 years.

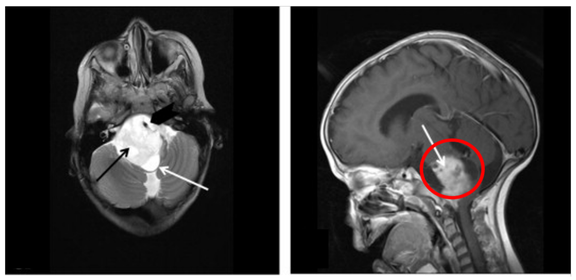


Figure 1. DIPGs are brainstem tumors. The median survival of patients is about 10 months with less than 10% of patients surviving past 2 years. Due to its location, surgical resection is not possible, radiation is palliative at best, and chemotherapy has been largely ineffective (Aly *et al*, the Egyptian Journal of Radiology and Nuclear Medicine, 2013).

- Epigenetic disease: about 80% of tumors are characterized by a hallmark mutation of a lysine to a methionine on histone H3.

H3F3A mutation-K27M.	60-71%
H3F3A mutation-G34R/V.	0
HIST1H3B mutation-K27M	18%

Figure 2. *H3K27M* mutation is a hallmark of DIPGs. Recurrent somatic mutation in histone *H3F3A* or *HIST1H3A* are found in a majority of DIPG tumors. A lysine (K) to methionine (M) mutation in DIPGs promotes aberrant activation of gene expression. In the context of other genetic events such as inactivation or p53 loss, the *H3K27M* mutation drives tumorigenesis.

- ONC201 was discovered in a screen as a p53-independent inducer of the pro-apoptotic cytokine TRAIL.
- It is known to inhibit dopamine receptor D2 (DRD2), a member of the G protein-coupled receptor (GPCR) family. It is also a ClpP agonist.
- It was shown to be effective in patients with midline gliomas that had the H3K27M mutation, suggesting that this drug was a potential therapeutic against DIPG.

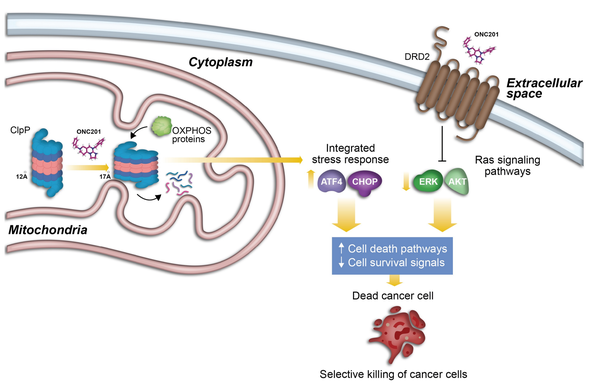
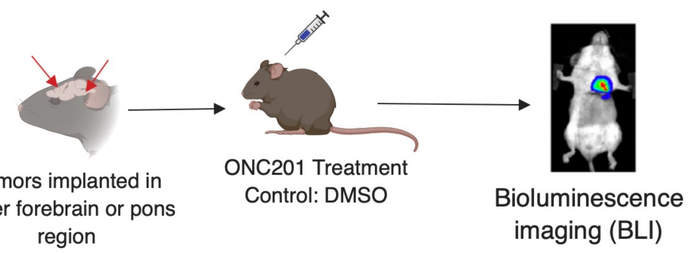


Figure 3. *ONC201 has been shown to engage many different pathways*. Works by inhibiting pro-survival signaling such as ERK and AKT and upregulating pro-apoptotic signaling, such as TRAIL and DR5, and stress signaling pathways, such as the EIF2 signaling pathway, to drive tumor cell death

- However, pre-clinical studies are needed to better understand its mode of action and efficacy in DIPGs.

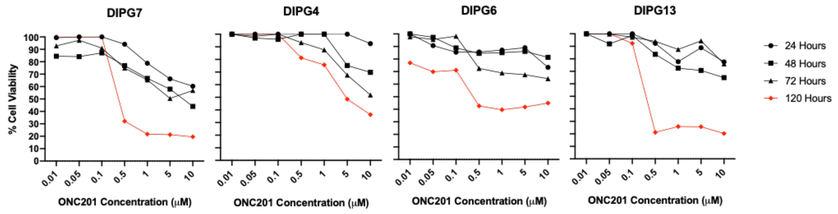
Methods

1. MTT assays were used to measure the sensitivity of four DIPG cell lines to ONC201.
2. Animal studies were performed to confirm the efficacy of ONC201 *in vivo*.



3. RNA-Seq analysis was conducted to determine molecular changes in DIPG cells in response to ONC201 treatment.
4. The synergistic effects of OC201 and NK cells in DIPG were determined by cytolytic assays.

Results



	72 Hours	120 Hours
DIPG7 (H3K27^{WT})	5μM	450nM
DIPG4 (H3.1K27M^{Mut})	10μM	5μM
DIPG6 (H3.3K27M^{Mut})	-	450nM
DIPG13 (H3.3K27M^{Mut})	-	350nM

Figure 4. DIPG cells are sensitive to ONC201 treatment. MTT assays were performed to determine sensitivity of DIPG4, DIPG6, DIPG7, and DIPG13 cells after 72 and 120 hours of treatment with 10nM, 50nM, 100nM, 500nM, 1μM, 5μM or 10μM ONC201. The IC50 value estimates from top panel figures are shown in the table.

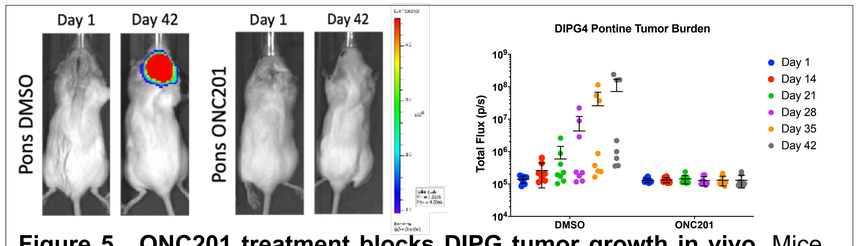


Figure 5. ONC201 treatment blocks DIPG tumor growth in vivo. Mice were injected IP with 50mg/kg of ONC201, or DMSO, once every seven days for 42 days. Bioluminescence imaging (BLI) imaging showed reduction in tumor burden in mice treated with ONC201 relative vehicle treated animals in pontine models. Longitudinal changes in flux is shown.

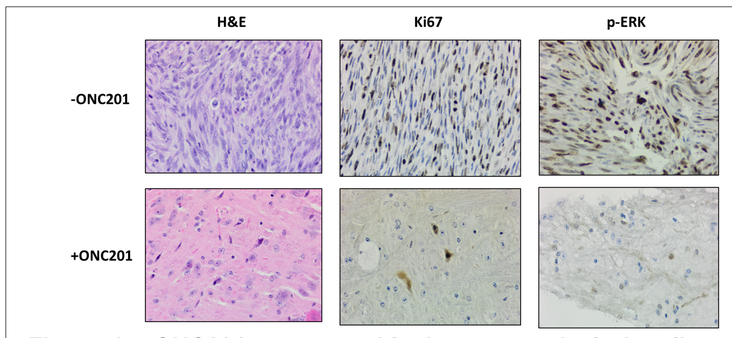


Figure 6. ONC201 treatment blocks pro-survival signaling. H&E and immunohistochemical analyses of Ki67 and p-ERK show confirmed that ONC201 causes a reduction in survival pathways in DIPG cells. These brain samples were collected after 6 weeks of ONC201 treatment.

Results

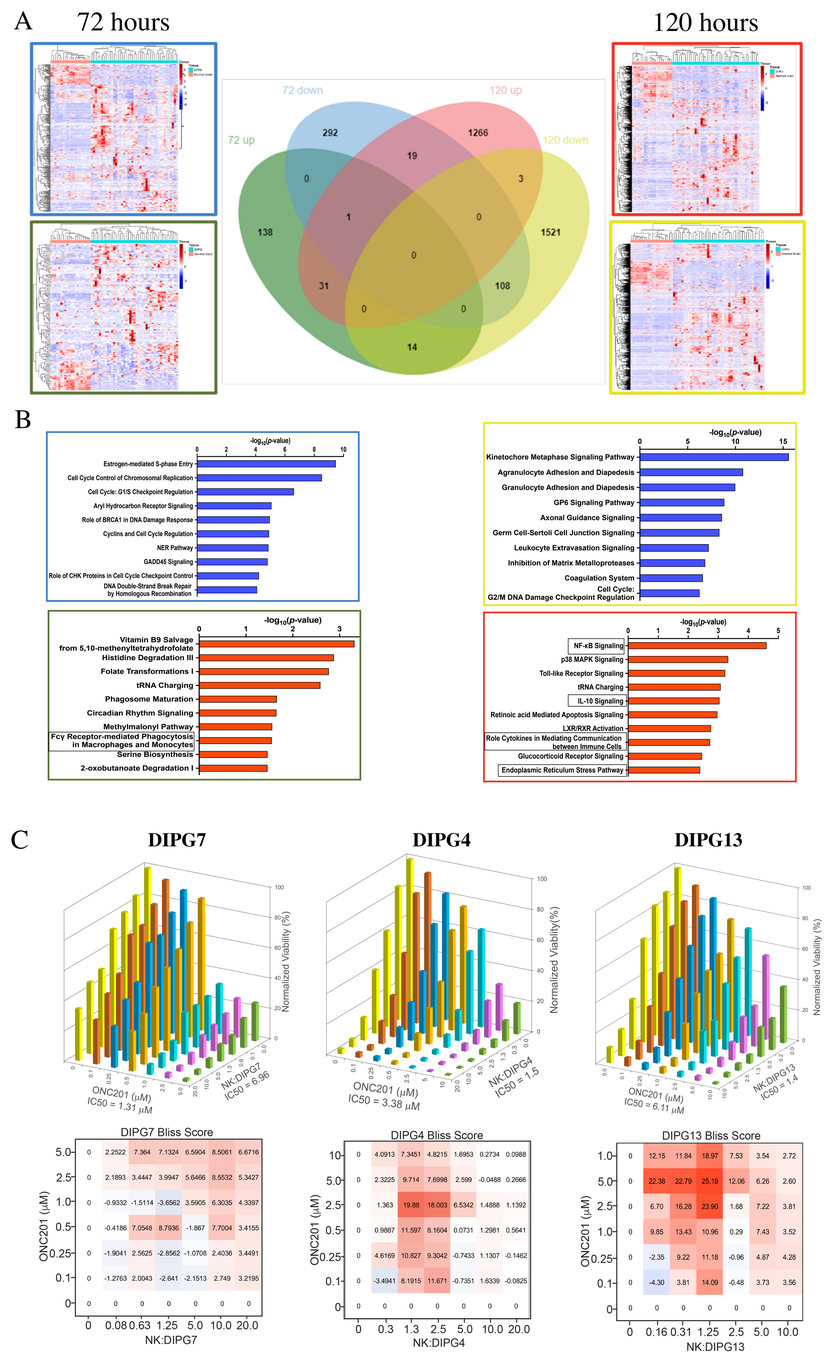


Figure 7. RNA Seq analyses reveals upregulation of immune related genes in DIPG cells in response to ONC201. A) Heatmap of gene expression across patient samples based on differentially expressed gene sets identified in cell lines. B) Top upregulated (red) and downregulated (blue) pathways in response to ONC201 treatment in DIPG4, DIPG7, and DIPG13 at 72 hours and 120 hours post-treatment. C) ONC201 sensitizes DIPG cells to NK-mediated cytolysis. DIPGs were treated with ONC201 for 48 hours and then NK cells were added for 5 hrs.

Conclusions

1. ONC201 induces cell death in DIPG cells *in vitro* and reduces tumor burden in mouse orthotopic models of DIPG4.
2. Bulk RNA seq analyses showed ONC201 upregulates immune-related pathways indicating potential to sensitize DIPG cell lines cytolysis by immune cells.
3. ONC201sensitizes DIPG cells to NK-mediated cytolytic activity *in vitro*.

Acknowledgements

Addi's Faith Foundation, Chance for Hope Foundation