#7066 Inhibition of GSK3B Signaling in Pediatric Brain Tumors

Mohammad Haque[1], Elizabeth Wert[1,2], Divya Gandra[1], Jeremy Hengst[1], Muhammad Younis[1], Tarlan Arjmandi[1], Katherine McClain[1], Meenakshi Shukla[1], Jonathan Lerch[1], Thussenthan Walter-Angelo[1], Giselle Saulnier Sholler, MD[1]

1Penn State University College of Medicine, PA, USA.

2University of North Carolina, Chapel Hill, NC, USA

Background

Embryonal Tumor with Multilayered Rosettes (ETMR) and Atypical Teratoid Rhabdoid Tumor (ATRT) are rare pediatric brain tumors that confer a 5-year overall survival less than 30% [1,2].

Based on (epi) genomic profiles, ATRT has been defined to 3 subgroups including ATRT-TYR, ATRT-SHH, and ATRT-MYC.

The protein Glycogen Synthase Kinase 3B (GSK3B) has recently gained attention as a therapeutic target in multiple cancer types due to its role in numerous cellular processes and cancer pathways [3].

9-ING-41 is a potent GSK3β inhibitor that crosses the blood-brain barrier [4]

It shows good tolerability in Phase I human clinical trials [5] and demonstrates biological activity against patient-derived intracranial models of gliomas [6].

Objectives

Treat ETMR and ATRT cell lines with a range of concentrations of 9-ING-41 and assay response.

Perform transcriptomic and proteomic analysis of treated versus untreated ATRT and ETMR cell lines to assay 9-ING-41 specific changes.

Utilize mouse xenograft models of ATRT and ETMR and treat with 9-ING-41 to assess toxicity and response to therapy.

Methods

A total of 6 ATRT cell lines, 4 from CHOP, CHLA-02 from ATCC, BCCBIO-091-07 from BCC and 1 ETMR BT-183 cell lines were procured under IRB consent through the 55+ institution Beat Childhood Cancer Research Consortium led by this laboratory, and through our partnership with the Childhood Brain Tumor Tissue Consortium.

Transcriptomic profiles were established by RNA seq from the lysed sample. Proteomic profile were established by immunological staining using standard mass cytometry technique.

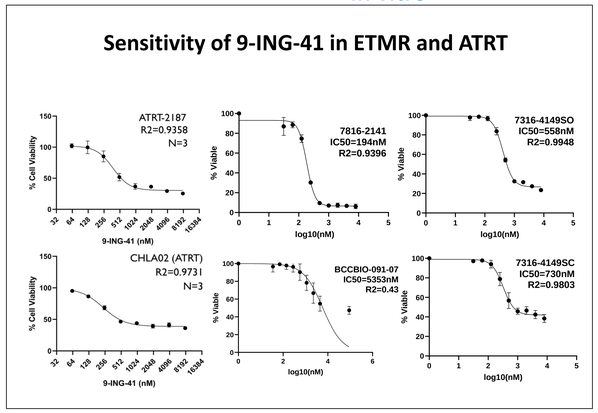
Neurosphere assays were done by seeding two cells per well in a 96 well plate followed by treatment with 9-ING-41. Neurosphere frequency and size were monitored weekly for up to 4 weeks using Incucyte S3 software.

Western Blot analysis and IHC were performed to investigate the expression of key proteins responsible for apoptosis.

In vivo mouse xenograft model were established by implanting ATRT cells lines into the mice through intra cranial injection. Bi-luminescent imaging were used to measure the tumor size and randomization prior to start treatment with 9-ING-41.

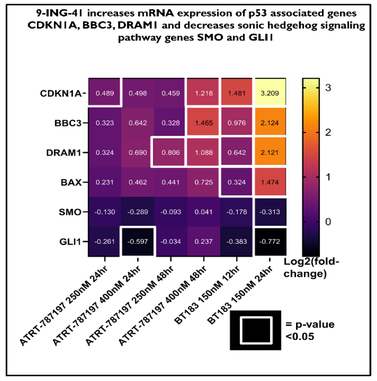
Results

FIG 1: Determine the efficacy of 9-ING-41 in treating ETMR and ATRT in vitro



ATRT Cell Line	IC50 (nM)
7316-2187	528.2
7316-2141	194.0
CHLA-02	481.2
7316-4149SO	558.0
7316-4149SC	730.0
BCCBIO-091-07	5353.0

FIG 2: Gene expression changes in 9-ING-41 treated ATRT/ETMR cell lines were determined with RNA-SEQ and Western Blot



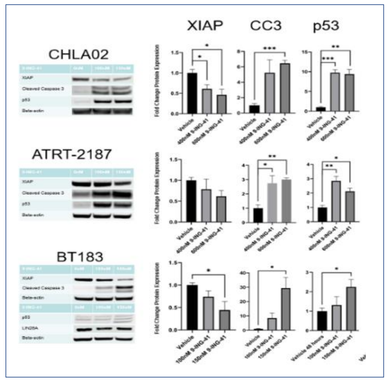
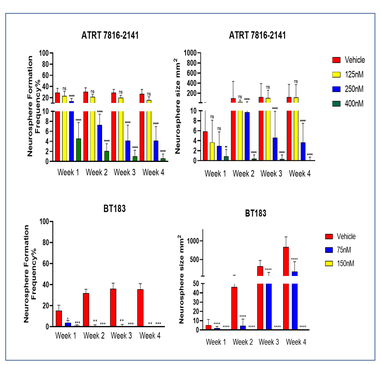


FIG 3: Inhibition of Neurosphere formation in 9-ING-41 treated ATRT/ ETMR Cell lines



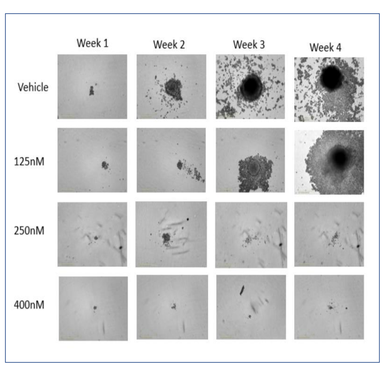


FIG 4: Reduced tumor size and enhanced survival are predominant in 9-ING-41 treated mice

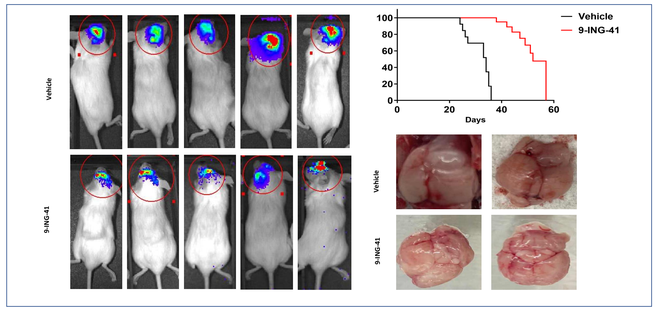
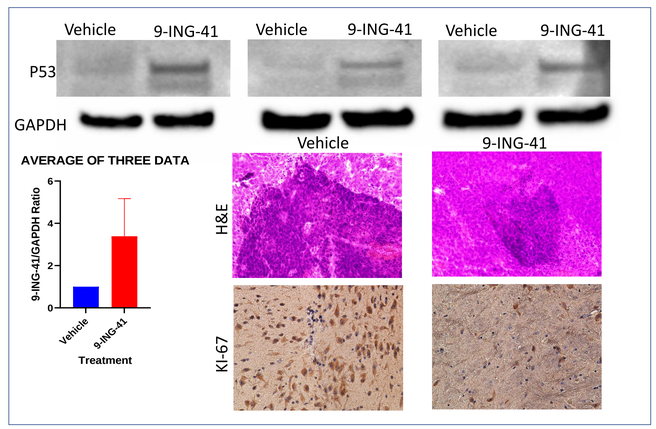


FIG 5: Enhanced tumor necrosis and reduced KI-67 are significant in 9-ING-41 treated mice



Conclusions

1. ATRT and ETMR cell lines revealed a significant therapeutic dosing response to GSK3B inhibitor.
2. P53 tumor suppressor genes are upregulated and Shh pathway genes are downregulated in GSK3B inhibitor treated cells.
3. Neurosphere formation are significantly reduced in 9-ING-41 treated cells.
4. Tumor size are reduced and mice survival are enhanced in treated mice.

References

1. Lambo, S., et al., ETMR: a tumor entity in its infancy. Acta Neuropathol, 2020. 140(3): p. 249- 266
2. Underiner, R.M., et al., Meta-Analysis of Treatment Modalities in Metastatic Atypical Teratoid/Rhabdoid Tumors in Children. Pediatr Neurol, 2020. 108: p. 106-112
3. Yang, L., et al., Targeting cancer stem cell pathways for cancer therapy. Signal Transduct Target Ther, 2020. 5(1): p. 8
4. Sin-Chan, P., et al., A C19MC-LIN28A-MYCN Oncogenic Circuit Driven by Hijacked Superenhancers Is a Distinct Therapeutic Vulnerability in ETMRs: A Lethal Brain Tumor. Cancer Cell, 2019. 36(1): p. 51-67 e7.
5. Ugolkov, A., et al., Combination Treatment with the GSK-3 Inhibitor 9-ING-41 and CCNU Cures Orthotopic Chemoresistant Glioblastoma in Patient-Derived Xenograft Models. Transl Oncol, 2017. 10(4): p. 669-678
6. Odia, Y., et al., Malignant glioma subset from actuate 1801: Phase I/II study of 9-ING-41, GSK3beta inhibitor, monotherapy or combined with chemotherapy for refractory malignancies. Neurooncol Adv, 2022. 4(1): p. vdac012

Contact

Giselle Saulnier Sholler - gsaulniersholler@pennstatehealth.psu.edu
Mohammad Haque - mhaque@pennstatehealth.psu.edu

Acknowledgments

BEAT CHILDHOOD CANCER FOUNDATION
HOPE 4 ATRT
FOUR DIAMONDS Conquering Childhood Cancer