An enolase inhibitor for the targeted treatment of ENO1-deleted cancers

Awurama Akyianu[1], Brian Grindel[1], Sunada Khadka[1] Florian Muller[1], Steven Millward[1]

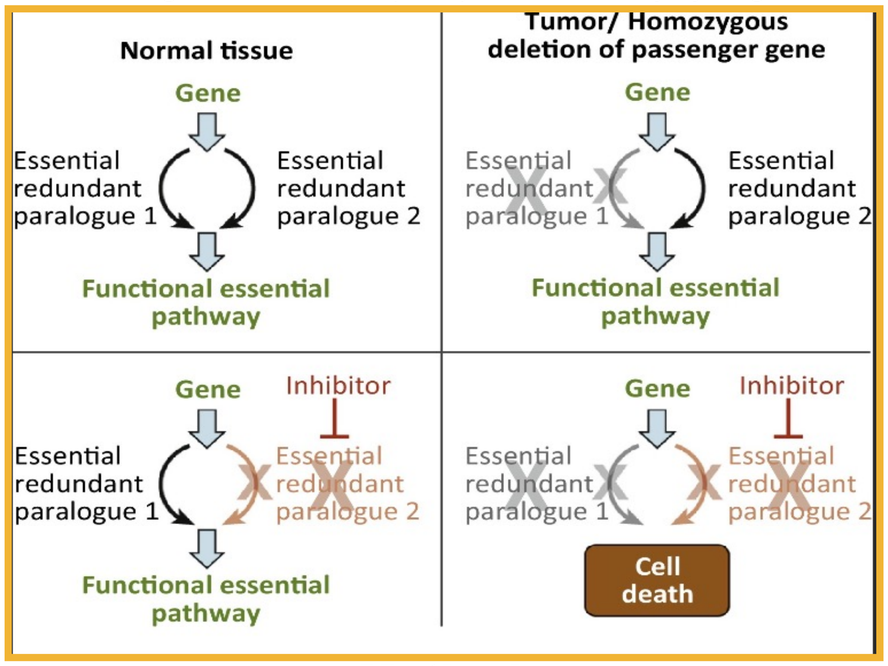
[1]Department of Systems Imaging, MD Anderson Cancer Center, Houston, Texas

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Background

- In cancers like **glioblastoma**, a grade IV astrocytoma with 22% five-year survival rate, there is **homozygous deletions of the 1p36 tumor suppressor locus (Figure 1)** resulting in the concomitant deletion of the glycolytic enzyme gene, ENO1 (encoding enolase 1)
- **Collateral lethality therapies** capitalize on the "cancer-specific metabolic vulnerabilities"(Muller et al), 2020) arising from the deletion of genes neighboring tumor suppressors. The two enolase paralogs, ENO1 and ENO2, are essential in the glycolytic pathway given their role in the **catalytic conversion of 2-phosphoglycerate (2 PG) to phosphoenolpyruvate** and this is what HEX targets.
- In this work, the inhibitory concentration (IC_{50}) of HEX was re-determined on glioblastoma cells with **different ENO1 deletions** using different viability assays and combined with a standard-of-care glioblastoma drug, temozolomide (TMZ), to assess any synergistic effects.



Method

- Cell culture experiments to test both **HEX and TMZ sensitivity** were performed in multiple glioma cell lines with two different cell viability assays.
- Glioma cell lines with **different ENO1 status** (D423: $ENO^{-/-}$; D423 ENO1 rescue; U343: $ENO1^{+/-}$; LN319: $ENO1^{+/+}$; D502: $ENO1^{+/-}$) were treated for 5 days and subjected to **a sulfrhodamine B (SRB) assay and MTT assay**: Cell viability via the SRB and MTT assay uses total protein content and cell metabolic activity, respectively.
- The cell binding substrate **Matrigel** ® was also tested for its ability to better adhere cells that detach during the assays.

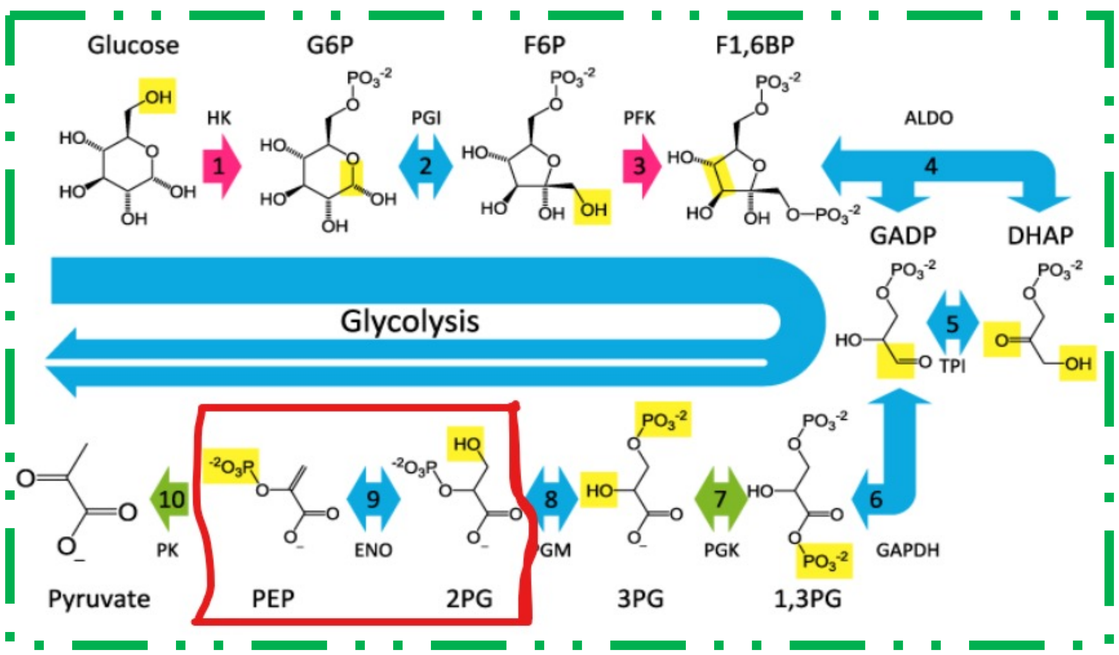


Figure 1 (A) Collateral lethality: a therapeutic strategy that capitalizes on the "cancer-specific metabolic vulnerabilities" arising from the deletion of passenger genes (B) A schematic of the glycolytic pathway, with the reaction of interest emboldened. This is the reaction HEX targets and inhibits.

Results

- With both assay types, HEX showed an (IC_{50}) of ~1100 nM-1300 nM against **$ENO^{-/-}$ D423 cells** while the ENO1 rescue cells showed essentially no sensitivity to HEX.
- These values are in line with previous observations using **crystal violet assays**.
- Matrigel coating increased initial adherence of cells but failed to **mitigate assay detachment**. Additionally, TMZ showed an IC_{50} of 628.2 uM **against $ENO^{-/-}$ D423 cells** and 1940 **uM against $ENO1^{+/-}$ D502 cells**

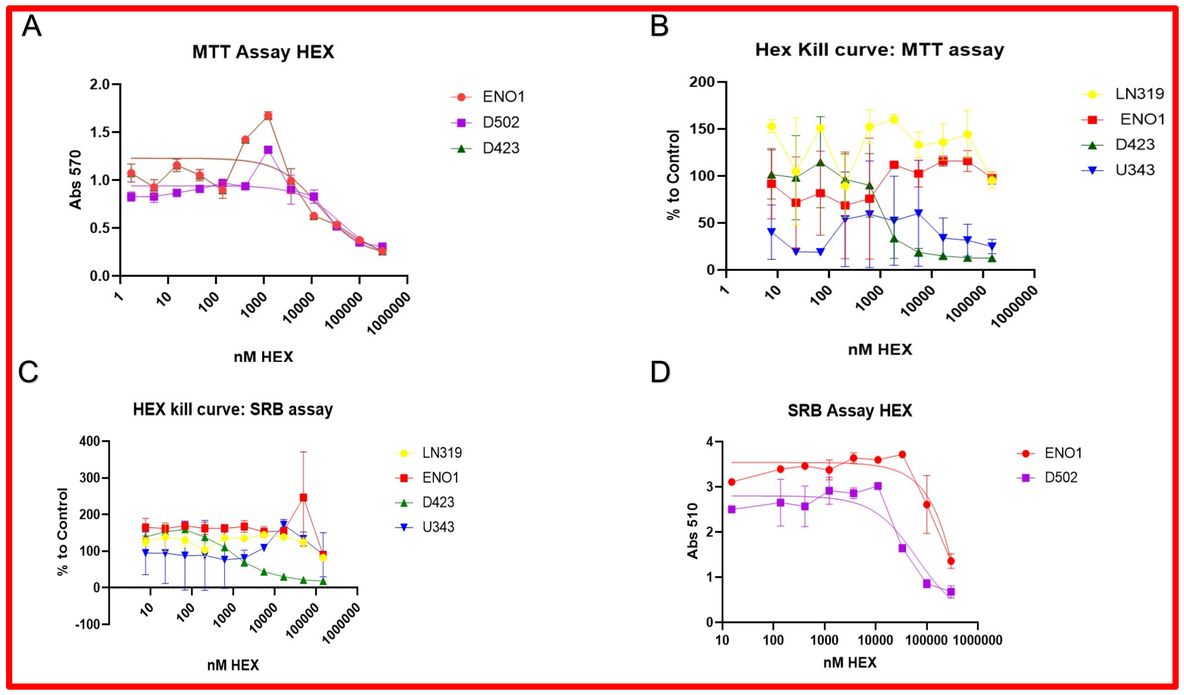


Figure 2: (A) HEX Kill curves generated from an MTT assay after incubation with HEX on ENO1, D502 and D423 cell lines; (B) HEX Kill curves generated from an SRB Assay after incubation with HEX on ENO1, LN 319, U343 and D423 cell lines; (C) HEX kill curves generated from an SRB assay after incubation with HEX on LN 319, ENO1, D423 and U343 cell lines. (D) HEX Kill curves generated from an SRB assay after incubation with HEX on ENO1 and D502 cell lines

Conclusions

- **HEX is effective** against ENO1-deleted cells leaving wild type cells unaffected.
- **TMZ, on the other hand had little effect,** in cell culture.
- HEX needs to be further assessed as **a combo treatment** with TMZ for glioma treatment.

References

(1) https://cdn1.byjus.com/wp-content/uploads/2019/02/Glycolysis-An-Introduction-To-Glycolytic-Pathway1.png

(2) https://www.cell.com/cms/attachment/2040746430/2054491688/gr3.sml

(3) Lin, Yu-Hsi, et al. "An Enolase Inhibitor for the Targeted Treatment of eno1-Deleted Cancers." *Nature Metabolism*, vol. 3, no. 1, 2020, pp. 122–122., doi:10.1038/s42255-020-00335-x.

Acknowledgements

I would like to thank the CPRIT-CURE Program for the opportunity to participate in this project