GLIO-1 is a selective DHODH inhibitor that is effective in IDH-mutant gliomas and KDM6-mutated cancers

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Background

- Isocitrate dehydrogenase (IDH) mutations synthesize (*R*)-2-hydroxyglutarate (R2HG) and are common in glioma brain tumors.
- R2HG broadly inhibits 2-oxoglutarate (2OG)-dependent enzymes and renders gliomas sensitive to drugs that induce replication stress (e.g. Ataxia telangiectasia [ATR] inhibitors, dihydroorotate dehydrogenase [DHODH] inhibitors) in preclinical studies.
- Translation of these strategies to clinical testing has been limited in part by a lack of well-tolerated, effective, and on-target drugs that exploit this vulnerability.
- Mechanisms underlying sensitivity of IDH-mutant gliomas to replication stress remain incompletely understood.

Preclinical IDH-mutant glioma models are sensitive to DHODH inhibition

- Drug screen identified that inhibitors of de novo pyrimidine nucleotide synthesis, including dihydroorotate dehydrogenase inhibitors (DHODHi), preferentially kill IDH-mutant glioma cells.
- DHODHi are effective in multiple preclinical IDH-mutant glioma models, including isogenic IDH-mutant cell lines, IDH-mutant patient-derived xenografts, and surgically explanted organoids.
- Mechanistically, this reflects an underlying sensitivity of IDH-mutant gliomas to replication stress.

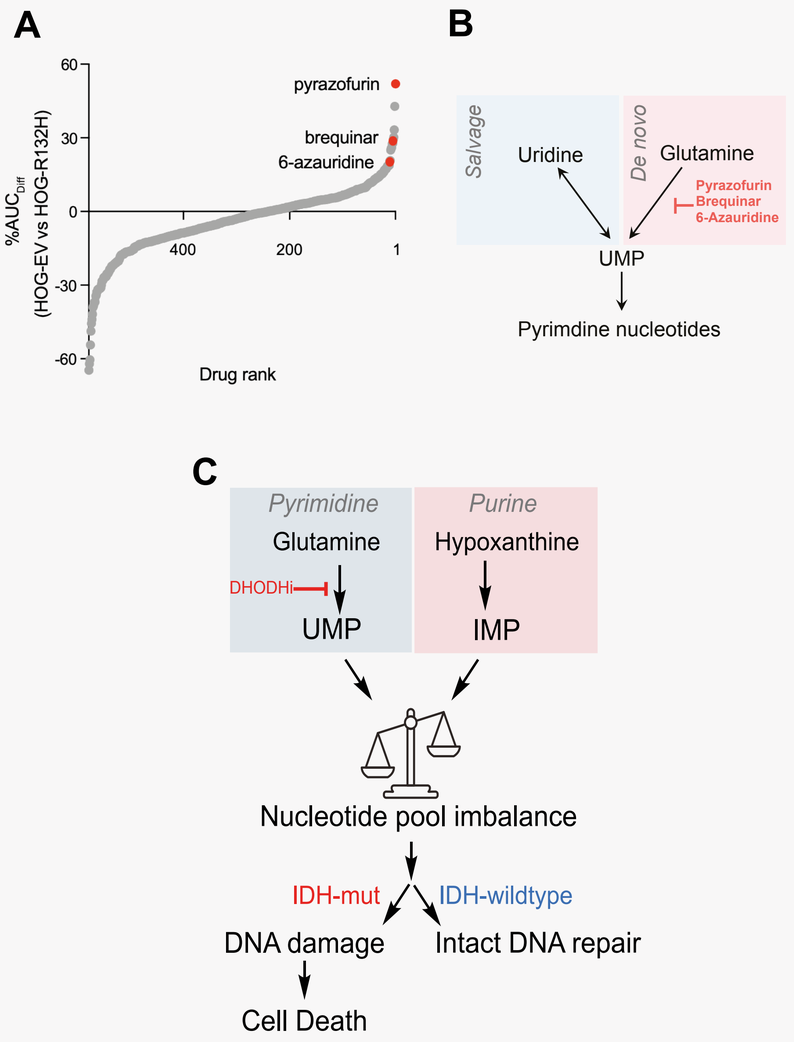

Fig. 1: (A) Waterfall plot from drug screen, indicating drugs that preferentially kill IDH-mutant glioma cells (HOG IDH1-R132H) compared to empty vector (EV)-expressing control cells. Percent difference in area under curve (AUC) values ($\%AUC_{Diff}$) in HOG-EV and HOG-R132H cells were calculated for all drugs. High $\%AUC_{Diff}$ indicates drugs that preferentially kill HOG-R132H cells over HOG-EV cells. (B) Schema of pyrimidine nucleotide synthesis and de novo pyrimidine synthesis pathway inhibited by the indicated drugs. (C) Schema depicting model by which DHODH inhibitors preferentially kill IDH-mutant glioma cells.

GLIO-1 is a blood brain barrier-penetrant DHODH inhibitor

- GLIO-1 is a blood-brain barrier penetrant inhibitor of DHODH with superior brain penetrance compared to BAY 2402234.
- GLIO-1 exhibits more potent inhibition of human and mouse DHODH enzymes.

A

Brain penetrance and potency

	BAY	GLIO-1
Mouse Brain : Blood	0.11	0.28
Human DHODH IC_{50}	9.2 ± 3.9 nM	2.9 ± 0.9 nM
Mouse DHODH IC_{50}	270 ± 40 nM	15.9 ± 7.8 nM

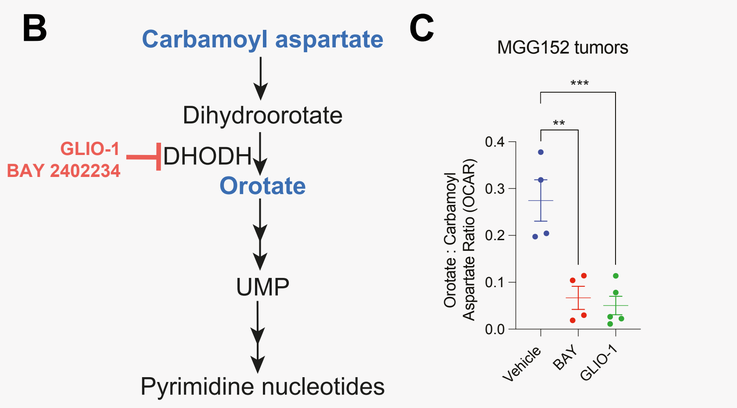

Fig. 2: (A) Brain penetrance (in mice after 2 hours 20 mg/kg PO dosing) and IC_{50} against human and mouse DHODH of BAY 2402234 and GLIO-1. (B) Schema of de novo pyrimidine synthesis. (C) Orotate to carbamoyl aspartate ratio of IDH-mutant orthotopic xenograft mice treated with BAY 2402234 (25 mg/kg diet), GLIO-1 (50 mg/kg diet), or vehicle.

GLIO-1 is on-target and effective in preclinical models of IDH-mutant glioma

- GLIO-1 preferentially kills IDH-mutant gliomas in preclinical studies
- Expression of a drug-resistant DHODH mutant (A58T) in IDH-mutant glioma cells fully rescues cytotoxicity induced by GLIO-1, indicating that GLIO-1 is acting on-target.
- GLIO-1 displayed similar efficacy and improved tolerability compared to BAY 2402234 in mouse models.

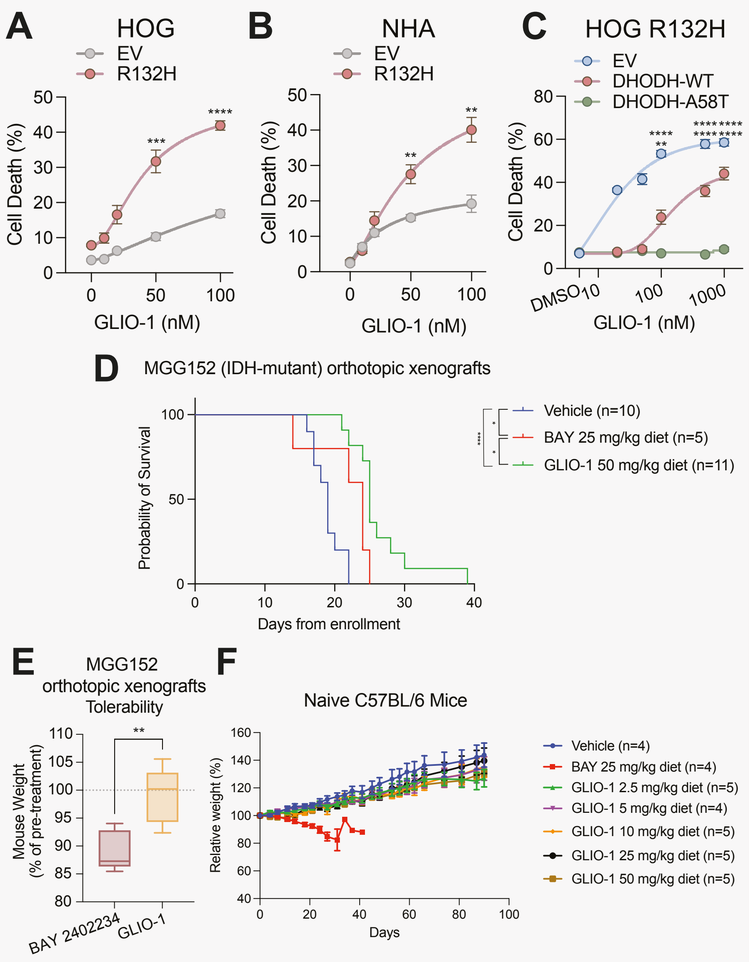

Fig. 3: (A–B) Cell death in human oligodendroglioma cells (A) or immortalized astrocytes (B) expressing mutant IDH1 (R132H) or an empty vector (EV) treated with GLIO-1. (C) Cell death assays of IDH-mutant HOG cells expressing a drug-resistant mutant of DHODH (DHODH-A58T), wildtype DHODH or an EV treated with GLIO-1. (D) Kaplan-Meier survival curves of MGG152 orthotopic xenografts treated with vehicle, BAY 2402234, or GLIO-1. (E) Average weights of mice in (D) 11 days of treatment . (F) Relative weights of C57BL/6 mice treated with the BAY 2402234 or GLIO-1.

KDM6-mutant bladder cancer models display sensitivity to GLIO-1

- We conducted a CRISPR knockout screen to identify mechanisms why IDH-mutant gliomas are sensitive to replication stress.
- Given that R2HG inhibits 2OG-dependent enzymes, we used a custom library with sgRNAs targeting all known 2OG-dependent enzymes.
- Six CRISPR screens across both a DHODHi and an ATRi identified knockout of the histone demethylase KDM6A as the top depleted hit, indicating that KDM6A loss sensitizes gliomas to replication stress.
- Knockout of KDM6A/B sensitized gliomas to DHODHi both in vitro and in vivo.

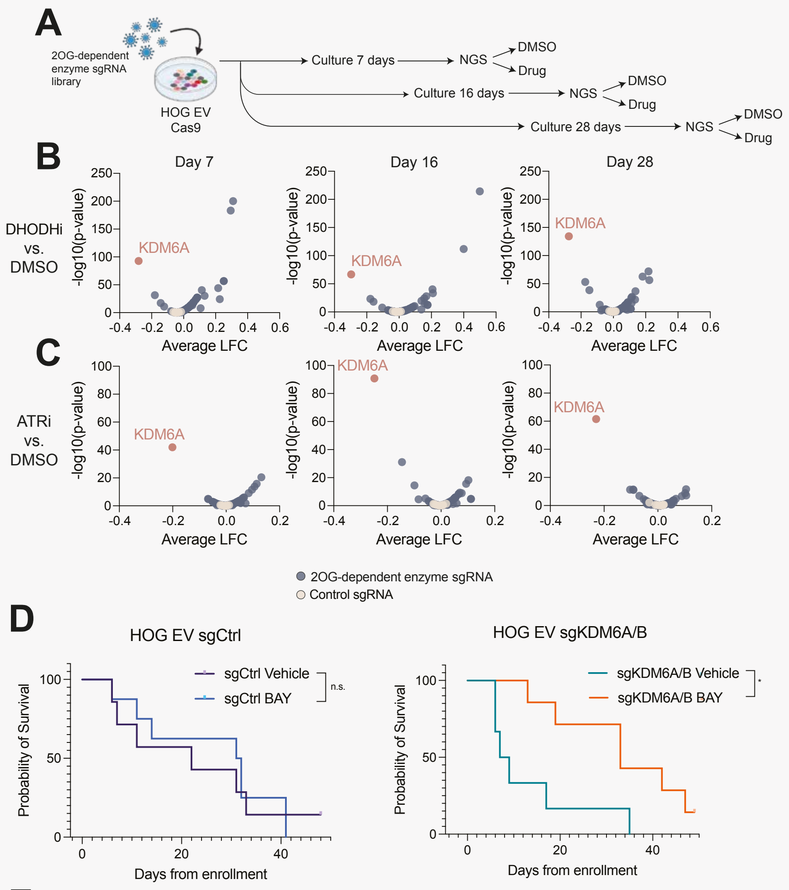

Fig. 4: (A–C) Schema and results of CRISPR screens. (D) Kaplan-Meier survival curves of IDH wildtype HOG cells with or without KDM6A/B knockout treated with vehicle or BAY 2402234. (E) Schema depicting model by which mutant IDH sensitizes gliomas to replication stress.

- Approximately one third of urothelial carcinomas harbor mutations in KDM6A or KDM6B.
- Endogenous KDM6-mutant bladder cancer cells are sensitive to DHODHi.
- KDM6A knockout sensitizes bladder cancer models to GLIO-1.

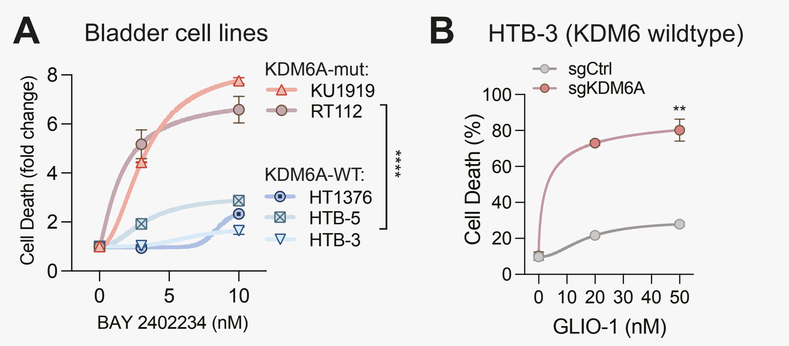

Fig. 5: (A) Cell death assays of endogenous KDM6A-mutant or KDM6A wildtype bladder cancer cell lines treated with BAY 2402234. (B) Cell death assays of HTB-3 cells harboring KDM6A knockout or control cells treated with GLIO-1.

Conclusions

- GLIO-1 is an on-target DHODHi that displays preclinical activity in IDH-mutant gliomas and KDM6-mutant bladder cancers, supporting further evaluation for translation to clinical testing.
- KDM6 activity is a determinant of replication stress sensitivity and serves as a potential pan-cancer, mechanism-based biomarker of DHODHi efficacy.