von Knebel Doeberitz Nikolaus (Orcid ID: 0000-0002-8453-3220)

Changing paradigms in oncology: toward non-cytotoxic treatments for advanced gliomas

Nikolaus von Knebel Doeberitz[1], Daniel Paech[1,2], Dominik Sturm[3,4,5], Stefan Pusch[6,7], Sevin Turcan[8], Yogen Saunthararajah[9]

[1]Division of Radiology, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany

[2]Department of Neuroradiology, Bonn University Hospital, 53127 Bonn, Germany

[3]Hopp Children's Cancer Center (KiTZ) Heidelberg, 69120 Heidelberg, Germany.

[4]Division of Pediatric Glioma Research, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), 69120 Heidelberg, Germany.

[5]Department of Pediatric Oncology, Hematology & Immunology, Heidelberg University Hospital, 69120 Heidelberg, Germany

[6]Department of Neuropathology, Institute of Pathology, Ruprecht-Karls-University Heidelberg, 69120 Heidelberg, Germany

[7]German Cancer Consortium (DKTK), Clinical Cooperation Unit (CCU) Neuropathology, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany

[8]Department of Neurology, Heidelberg University Hospital, 69120 Heidelberg, Germany

[9]Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA

Correspondence: Yogen Saunthararajah, 9500 Euclid Avenue, NE6, Cleveland, OH 44195, tel: 216 444 8170, email: saunthy@ccf.org

Word Counts: Abstract – 152; Words - 3973; References: 152; Tables: 1; Figures: 5

Key Words: Cancer Epigenetics, Neurooncology, Glioma, Glioma Therapy, Epigenetic Glioma Therapy

Article Category: Cancer Genetics and Epigenetics; Cancer Therapy and Prevention

LIST of ABBREVIATIONS

2-HG – 2-Hydroxyglutarate

AKG – Alpha-ketoglutarate

ARID1A/B – AT-rich interactive domain-containing protein 1A/B (MTFs)

ATF3 – Activating transcription factor 3 (MTF)

ATP – Adenosine triphosphate

ATRX – Alpha thalassemia x-linked mental retardation (SWI/SNF protein)

BAF – ATP-dependent BRG1/BRM associated factor (SWI/SNF complex)

CBP – CREB binding protein (HDAC)

CDA – Cytidine deaminase

CDKN2A – Cyclin-dependent kinase inhibitor 2A (gene encoding p21)

CHD4 – Chromodomain helicase DNA binding protein 4 (ISWI protein)

CPEC – Cyclopentenyl cytosine

CTPS2 – CTP synthase 2

DAXX – Death domain associated protein 6

DHODH – Dehydroorotoate dehydrogenase

DLX – Distal less X (family of developmental transcription factors)

DNA – Deoxyribonucleic acid

DNMT1/3A/3B – DNA methyl transferase 1/3A/3B

EANO – European Association of Neuro-Oncology

EGFR – Epidermal growth factor receptor

EP300 – Gene encoding HDAC p300

ESC – Embryonic stem cell

EZH2 – Enhancer of zeste 2 (PRC2 subcomponent)

G34R/V – Mutation resulting in an exchange of glycine residue 34 on histone 3.3 for arginine or valine

GBM – Glioblastoma multiforme

GFAP – Glial fibrillary acidic protein

GSX2 – GS homebox 2 (MTF)

GTP – Guanosine triphosphate

H3F3A – Gene encoding histone variant 3.3

H3K27ac – Acetylated lysine residue 27 on histone 3

H3K27me2/3 – Di/tri-methylated lysine residue 27 on histone 3

H3K4me3 – Tri-methylated lysine residue 4 on histone 3

HDAC – Histone deacetylases

HIST1H3B/C – Genes encoding histone variant 3.1

IDH 1/2 - Isocitrat dehydrogenase 1/2

ISWI – Imitation switch (gene family of ATP dependent chromatin remodelers)

K27M – Mutation resulting in an exchange of lysine residue 27 on histone 3.1 or histone 3.3 for methionine

KDM1A – Jumonji histone lysine demethylase 1A (H3K4me3 demethylase)

KDM6A/B – Jumonji histone lysine demethylase 6A/B (H3K27me3 demethylases)

KMT – Histone lysine methyl transferase

meCpG – Methylated deoxycytosine residues preceding deoxyguanine residues

MRI – Magnetic resonance imaging

MTF – Master transcription factor

MYC – Derived from the name of the avian virus "myelocytomatosis" (MTF)

MYCL1 – MYC lung carcinoma derived 1

MYCN – MYC neuroblastoma derived

NANOG – Of irish origin "Tir na nÓg", meaning "land of eternal youth" (MTF)

NFIA – Nuclear factor 1 A (MTF)

NSC – Neuronal stem cell

OLIG2 – Oligodendrocyte transcription factor 2 (MTF)

PDGFR – Platelet-derived growth factor receptor

POU5F1 – POU class homebox 1 (gene encoding the MTF Oct-4)

PRC2 – Polycomb repressor complex 2

RANO – Response assessment in neuro-oncology

RUNX2 – RUNX family transcription factor 2 (MTF)

SET-domain – Su(var)3-9, Enhancer-of-zest and Trithorax (protein domain of specific KMTs)

shRNA – Short hairpin ribonucleic acid

SMARC - SWI/SNF related, matrix associated, actin dependent regulators of chromatin (different SWI/SNF and ISWI family proteins involved in chromatin remodeling)

SOX2/10 – Sex determining region Y (SRY) box 2/10 (MTFs)

SUZ12 – Suppressor of zeste 12 (PRC2 subcomponent)

SWI/SNF – Switch/sucrose non-fermentable (family of ATP dependent chromatin remodelers)

TET1/2 – Ten-eleven translocation methylcytosine dioxygenase 1/2

TP53 – Tumor protein p53 (gene encoding protein p53)

UTP – Uridine triphosphate

WHO – World health organization

ABSTRACT

Glial-lineage malignancies (gliomas) recurrently mutate and/or delete the master regulators of apoptosis p53 and/or p16/CDKN2A, undermining apoptosis-intending (cytotoxic) treatments. By contrast to disrupted p53/p16, glioma cells are live-wired with the master transcription factor circuits that specify and drive glial lineage-fates: these transcription factors activate early-glial and replication programs as expected, but fail in their other usual function of forcing onward glial lineage-maturation – late-glial genes have constitutively 'closed' chromatin requiring chromatin-remodeling for activation - glioma-genesis disrupts several epigenetic components needed to perform this work, and simultaneously amplifies repressing epigenetic machinery instead. Pharmacologic inhibition of repressing epigenetic enzymes thus allows activation of late-glial genes and terminates glioma self-replication (self-replication = replication without lineage-maturation), independent of p53/p16/apoptosis. Lineage-specifying master transcription factors therefore contrast with p53/p16 in being enriched in self-replicating glioma cells, reveal a cause-effect relationship between aberrant epigenetic repression of late-lineage programs and malignant self-replication, and point to specific epigenetic targets for non-cytotoxic glioma-therapy.

1. ***The pathway objective of conventional oncotherapy: p53/p16-dependent apoptosis***

A time-traveling oncologist from the 1970s would readily recognize the principles and practice of much current oncotherapy (reviewed in[1]), since pharmacology-pathway goals have remained steadfast: terminate malignant replications/self-replications by activating the metazoan program of apoptosis (also known as cytotoxicity)[2]. Apoptosis is a replication-control program that senses cell stress or injury as stimuli to upregulate the master regulator p53 (*TP53*) and its key co-factor p16 (*CDKN2A*); these in turn activate gene expression modules that force cell cycle exits as an interlude for repair, then if the stress persists, compel orderly cell self-destructions. Some tissue-lineages, mainly testicular, lymphoid and myeloid, can transform and advance to disseminated malignancy without *TP53* or *CDKN2A* deletions and/or mutations (reviewed in[3]). The apoptosis master switch is therefore available for activation, enabling cures even of advanced cases. Underscoring the centrality of p53/p16 to these treatment successes, p53/p16 loss-of-function or dominant-negative alterations typify relapsed/refractory testicular, lymphoid and myeloid malignancies[3].

Transformation of most other lineages into advanced malignancy, however, usually does entail attenuation or deep inactivation of the p53/p16-system. Cancers derived from these lineages thus demonstrate **a)** anticipatory resistance to cytotoxic therapy (*primary refractory*), or **b)** ready selection by cytotoxic therapy for the most apoptosis-attenuated malignant sub-clones, that resist subsequent treatments (*relapsed-refractory*)[3], a clinical reality demonstrated by glial malignancies (gliomas). The World Health Organization (WHO) and European Association of Neuro-Oncology (EANO) use histological features such as microvascular proliferation and necrosis, and molecular features such as *IDH1, IDH2, ATRX* mutations and *CDKN2A* deletions, to categorize gliomas into grades with predictable, different natural histories and treatment outcomes[4, 5]: *Oligodendroglioma, IDH-mutant, 1p/19q-codeleted, WHO grade 2/3* are oligodendroglial-lineage transformations in which *TP53* or *CDKN2A* inactivating mutations/deletions are infrequent, and these malignancies have better overall survivals than astroglial-lineage malignancies *astrocytoma, IDH-mutant, WHO grade 2/3* that routinely incorporate *TP53* inactivating mutations (>99% of cases)[6-8]. Moreover, frequent deletion of *CDKN2A* characterizes progression of astrocytomas from grade 2/3 to grade 4[4, 9], and *glioblastomas* (*GBM, IDH wild-type, WHO grade 4*) that have poor prognoses despite surgery and intense radio-/chemo-therapy, have rates of p53/p16-pathway inactivation exceeding 85%[6, 10] (Grade 1- curable by surgery; Grade 2/3 - overall survival durations of several years; Grade 4 - overall survival durations months to few years[4, 5]).

Thus, durable remissions as pay-offs for short- and long-term toxicities of aggressive chemotherapy and radiation are expected only for the few glioma types containing genetically intact p53/p16-systems (reviewed in[3]), and it would seem logical to develop new treatments not reliant on p53/p16/apoptosis.

2. ***Replication: the heart of evolution, including neoplastic evolution***

All cancers share an essential foundation of relentless replication, the engine that drives evolution. Synchronized duplication of life's materials and machines is inexplicably complex. Neoplastic evolution is unlikely to reinvent such complexity, refined over millennia, and therefore hijacks it from the normal cellular contexts in which it occurs: **a)** tissue stem cells, and/or **b)** lineage-committed progenitors[3]. In the adult mammalian brain neuronal stem cells (NSC) reside in neuroglial-genic niches in the dentate gyrus of the hippocampus and in subventricular zones lining the lateral ventricles[11-13]. NSC replicate rarely, e.g. once every several months, and these replications can be naturally decoupled from onward lineage-differentiation for self-replications needed to maintain NSC pools through life-span (linear proliferation kinetics over long time-scales)[11, 14, 15]. Instead of self-replication, daughter cells may commit toward neuronal or glial lineage-fates, to produce lineage-committed progenitors[12-14]. Lineage-committed progenitors replicate every day for exponential proliferation kinetics over short time-scales[14]. Each replication is coupled to acquisition of neuronal- or glial-lineage programs (lineage-maturation), culminating after 4 or more cell divisions in activation of final neuronal or glial lineage-fate programs that terminate proliferation (terminal-differentiation)[11, 14, 16].

Which of these normal replication contexts do gliomas hijack?

Malignant clonal expansion requires a substantial proportion of replications to be self-replications, a biological and mathematical requirement that can be computationally modeled[17]. Since NSC are the only normal brain cells to self-replicate, an intuitive assumption is that glioma self-replications derive from NSC (**Figure 1A**). To examine this assumption it is useful to recap the role of 'master transcription factors' (MTFs) in cell fate determination. MTFs are sequence-specific, DNA-binding proteins that cooperate in specific combinations (MTF circuits) to activate the hundreds to thousands of genes that define specific cell fates and functions. The MTF circuit that creates NSC, SOX2/POU5F1/NANOG, has been reproducibly identified (reviewed in[18]), as has the MTF circuit NFIA/ATF3/RUNX2 that commits NSC to glial-lineage fates[19, 20].

What then is the MTF combination highly expressed in glioma cells? Gliomas express the MTF circuit SOX2/POU5F1/NANOG, that converts other somatic cells into NSC (and also into embryonic stem cells, ESC) in the same pattern as that observed in normal whole brain (**Figure 1A, B**): SOX2 is highly expressed in gliomas, but SOX2 is normally highly expressed through glial-lineage maturation[20], and accordingly, is also highly expressed in normal brain that consists mainly of terminally-matured cells. The other two MTF in the NSC circuit POU5F1 and NANOG are expressed at barely detectable levels and are not more elevated in aggressive GBMs than less aggressive grade 2/3 oligodendrogliomas or astrocytomas (**Figure 1B**). Instead, the MTF circuit that forces NSC commitment into the glial-lineage NFIA/ATF3/RUNX2 *is expressed at log-levels higher than POU5F1 or NANOG, with further upregulation in GBM vs grade 2/3 oligodendrogliomas or astrocytomas* (**Figure 1B**).

Why does neoplastic evolution select to transform in lineage-specifying MTF circuit contexts?

MYC, and its paralogues MYCN and MYCL1, are the ancient MTF that regulate hundreds of genes essential for nutrient supply, energy production, provision of cellular building blocks, cell cycle entry and progression (replication)[21]. The emergence of multi-cellularity (metazoa) occasioned MYC subordination to MTF circuits that create diverse lineages and hierarchies[22]: MTF circuits that create tissue stem cells permit only low grade MYC activity (shown in several tissue contexts, reviewed in[3]) presumably because quiescence protects the genomes of these cells vital to a multi-cellular organisms overall life-span. By contrast, MTF circuits that commit cells into lineage activate and cooperate with MYC (or MYCN or MYCL1) to propel replications every 1-2 days (reviewed in[3]), to in this way ensure the transit amplification needed to replenish specialized tissue cells lost to daily wear-and-tear[3, 15, 23, 24]. 'Transit' amplification is transitory because lineage-specifying MTF circuits simultaneously activate lineage-differentiation programs that cascade (lineage-maturation) toward activation of final specialized-fate programs which antagonize MYC and terminate replication (terminal-differentiation)[3, 16, 24]. Gliomas thus express: **a)** high levels of both glial lineage-specifying MTF and MYC (or MYC paralogues)(**Figure 1B**); **b)** high levels of early glial-lineage programs (https://biologic.crick.ac.uk/astrocyte) and MYC-target genes (**Figure 1C**); **c)** strong positive correlation between early-glial and MYC-target gene expression (**Figure 1D, E**); **d)** strong negative correlation between late-glial and MYC-target gene expression (**Figure 1D, E**); **e)** more aggressive glioma subtypes display a left-shift away from late-glial toward early-glial gene expression (**Figure 1C, D**); and **f)** this left-shift independently predicts and stratifies for worse overall survival, even within WHO/EANO glioma sub-types

(**Figure 2**). Similar observations have been made by others[25-29], and glial-lineage of gliomas is evident also from: **a)** histo-morphological examination; **b)** biomarkers measured by immunohistochemistry or other methods, e.g., OLIG2, GFAP, NES, PDGFR and EGFR[30]; **c)** functional properties, e.g., responses to neurotransmitters like glutamate[31-33]; **d)** global and/or single-cell comprehensive gene expression profiles[28, 34, 35].

5. *Clarifying cancer 'stem' cell terminology*

Self-replicating cancer cells are often referred to in the literature as 'stem' or 'stem-like' (cancer 'stem' cells), which is true in so far as they self-replicate, but the terminology obscures that by MTF content, dependency, and many other parameters, these cells phenocopy lineage-committed progenitors[36-43]. Tens of glioma cell lines that indefinitely self-replicate in vitro, as well as other human and murine glioma cells shown to initiate GBM in mice, faithfully recapitulate the high lineage-specifying MTF circuit/early-glial/MYC-target gene expression configuration observed in bulk glioma samples[44-50]. Self-replicating malignant cells in other tissues are also characterized by high expression and dependency on lineage-specifying MTF circuits that activate and cooperate with MYC for transit-amplification (reviewed in[3]). Thus, *normal self-replication is restricted to tissue stem cells, but malignant self-replication is not* (self-replication = replication without lineage-maturation) (**Figure 1A**).

Oncogenic mutations, however, can originate in stem cells as far upstream as germ-line, e.g., in familial gliomas, then propagate downstream into lineage-committed progenitors, wherein phenotypic consequences and clonal advantage most prominently emerge[51] (reviewed in[3]). Upstream mutations can also skew downstream commitment decisions, e.g., *ATRX* mutations skew NSC commitment decisions toward astro- over oligodendroglial or neuronal lineage-fates[52].

6. *A core failure driving glioma-genesis*

Gliomas thus coordinately upregulate early-glial and MYC-target genes as expected from their lineage MTF content, *the failure is to not then activate late-glial programs that suppress early-glial genes/MYC/MYCN/MYCL1.* Several experimental conditions have been shown to correct this anomaly, *resuming glioma lineage-maturation and hence terminating self-replication/tumor-initiating capacity*[44, 45, 53, 54]. To develop such remedies for clinical use, it would be useful to understand how the failure occurs in the first-place.

7. *The epigenetic landscape at late-glial genes enables their oncogenic repression*

Here, the term 'epigenetics' or chromatin refers to three-dimensional organization of DNA around histone protein octamers (nucleosomes), that is configured and reconfigured by enzyme-containing multi-protein complexes that methylate or demethylate DNA bases, post-translationally modify histones, exchange or reposition histones, to in this way facilitate ('on') or obstruct ('off') transcription of genes by the basal transcription factor machinery[3, 24]. Gene repression ('off') for example is favored by tri-methylation of lysine 27 on histone 3 (H3K27me3), a histone modification executed by Polycomb Repressor Complex 2 (PRC2) in which EZH2 is the enzyme component, and/or mono-methylation of DNA deoxycytidine residues that precede deoxyguanine residues (me-CpG) by DNA methyltransferases DNMT1, DNMT3A and/or DNMT3B[55-57]. Gene activation ('on') on the other hand is favored by H3K27 acetylation (H3K27ac) executed by CBP/p300, and H3K4 trimethylation (H3K4me3) executed by SET-domain containing histone methyltransferases (KMTs)[56, 58].

How are me-CpG, H3K27me3 and H3K27ac marks distributed at MYC-target, early- and late-glial genes? Me-CpG is minimal at MYC-target and early-glial but elevated at late-glial genes in the ultimate tissue baseline of ESC (**Figure 3A**)[59]. Relatively high me-CpG at late-glial genes is even higher in grade 2/3 oligodendrogliomas or astrocytomas (*IDH1*- or *IDH2*-mutated gliomas) *vs* normal brain (**Figure 3B**)[60-63]. Elevated H3K27me3 at late-glial genes in ESC is erased with ontogeny into normal brain (**Figure 3C**)[57, 59], but this erasure does not occur in analyzed GBM (**Figure 3C**); H3K27me3 is depleted from late-glial genes in gliomas containing *H3F3A* K27M or G34V mutations (**Figure 3C**)[64, 65], however, acquisition of the H3K27ac mark, that occurs with ontogeny into normal brain, fails to occur (**Figure 3C**)[38, 66-68]. Failure to activate late-glial genes might be explained by different lineage-trajectory, e.g., with *H3F3A* G34R/V-mutated gliomas that originate from GSX2/DLX-expressing interneuron-progenitors, however, these tumors also fail to activate late-neuronal programs[69]. Thus, pediatric gliomas, including H3F3A K27M-mutated gliomas, recapitulate key features of adult gliomas: *wiring with the glial lineage-commitment MTF circuit* (**Figure 3D**), *early-glial and MYC-target gene activation as expected from this* (**Figure 3E**), *but attenuated transition to late-glial programs, worse in higher grade disease* (**Figure 3E, F**). Constitutive difference in epigenetic landscape at replication/early-lineage *vs* late-lineage genes, and exploitation of this epigenetic gradient by neoplastic evolution to decouple replication from lineage-maturation, has been shown for other tissue lineages also[38, 70, 71].

8. *Mechanisms underlying chromatin-remodeling failure at late-glial genes*

The following genetic alterations are highly recurrent or pathognomonic of gliomas, and are implicated in chromatin-remodeling failure at late-glial genes:

Amplifications of EZH2 or SUZ12, key components of the PRC2 complex that writes H3K27me3. Almost all GBMs recurrently amplify *EZH2* via whole chromosome 7 gains, and ~10% also amplify *SUZ12*, driving higher expression (**Figure 4A, B**)[44, 72-75]. Supporting functional consequences, immunohistochemical quantification found >95% H3K27me3-positive cells in 41/72 (57%) GBM samples analyzed, and >50% H3K27me3-positive cells in most of the remainder[76].

Deletions of the H3K27me3 eraser KDM6B; KDM6A decrement in males. KDM6B is a H3K27me2 and H3K27me3-specific demethylase[58, 77, 78], and its gene locus at chromosome 17p13.1 is deleted in ~10% of GBMs with correspondingly suppressed expression (**Figure 4A, B**)(17p13.1 is also the *TP53* locality). *KDM6A* at Xp11.3 produces another PRC2-counteracting demethylase - substantially lower KDM6A levels in males *vs* females may contribute to male-bias in glioma incidence (**Figure 4C**)[77].

Missense mutations H3F3A K27M or HIST1H3B/C K27M. These heterozygous histone 3 (H3) gene mutations occur in ~30% of pediatric high-grade gliomas and ~100% of diffuse midline gliomas. The resulting amino-acid substitution precludes writing H3K27me3 ('off') or H3K27ac ('on') marks (dominant negative effect)[79](**Figure 3C**), and late-glial genes are as repressed in these gliomas as gliomas without the mutations (**Figure 3D-F**). That is, *H3 K27M functional impact is aberrant repression of late-glial genes, with depletion of H3K27me3, but also H3K27ac*[80].

Recurrent deletions of EP300 (p300) that writes H3K27ac. The histone acetyltransferase p300, encoded by *EP300* at 22q13.2, is deleted in ~10% of grade 2/3 astrocytomas, increasing to >40% of grade 4 astrocytomas or GBMs, with correspondingly suppressed expression (**Figure 4A, B**). H3K27ac deposition at late-glial genes is decreased in gliomas compared to normal brain (**Figure 3C**).

Inactivating mutations/deletions of alpha-thalassemia X-linked mental retardation (ATRX) that mediates H3 exchange: Inactivating mutations in *ATRX* at Xq21.1 characterize astrocytomas and *H3F3A* G34R/V-mutated pediatric gliomas[4, 7, 60]. ATRX is linked to the histone chaperone DAXX and to histone H3.3 exchange (reviewed in[81]) - histone turnover may regulate H3K27me3 amounts, since in immunohistochemical analyses of a series of astrocytoma samples (n=41), all had >50%, and most had >95%, H3K27me3-positive cells, while oligodendrogliomas that do not have ATRX inactivating mutations had

<5% H3K27me3-positive cells[76]. ATRX has also been implicated in telomere length regulation and DNA repair[52, 82, 83] and cytogenetic instability resulting from ATRX loss might explain concordance of *ATRX*- with *TP53*-mutations[27, 84-86].

Missense IDH1 or IDH2 mutations that compromise me-CpG erasure by TETs. IDH1/2 mutations are pathognomonic of oligodendrogliomas and astrocytomas (**Figure 4B**). Cytoplasmic wildtype IDH1 and mitochondrial IDH2 produce alpha-ketoglutarate (AKG), a mandatory cofactor for Jumonji histone demethylases (KDMs) and Ten Eleven Translocation (TET) family DNA methylcytosine dioxygenases that erase me-CpG[87, 88]. Mutant-IDHs produce an oncometabolite 2-hydroxyglutarate (2HG) that competes with AKG, inhibits TET family enzymes, increases me-CpG and represses late-glial genes (**Figure 3B**): mutant-IDH1 or 2HG introduction into differentiating glial cells stalled lineage-maturation[62, 89], and *IDH*- and *ATRX*-mutation in neural precursors increased neuro-glial precursor proliferation and immortalized astrocytes - the increased proliferation was controlled by apoptosis, thus, subsequent *TP53*-mutations caused glioma-genesis[52, 90-92].

TET1 deletions. *TET1* deletions via chromosome 10 losses, or minimal deletion of the *TET1* locus at 10q21.3, typify GBMs, and also occur in astrocytomas, increasing from <10% in grade 2/3 astrocytomas to ~40% in grade 4 (**Figure 4A, B**)[73, 74, 93-95]. Interestingly, me-CpG at late-glial genes is much higher in IDH-mutant gliomas, even though these have intact *TET1*, than in GBMs with *TET1* haploinsufficiency (**Figure 3B**) - possibly, reduction in TET1 protein amounts disrupts multiprotein coactivator complexes to hence impede gene activation in ways beyond disrupted erasure of me-CpG[96, 97].

Amplifications of DNA methyltransferase 1 (DNMT1) that writes me-CpG. *DNMT1* at 19p13.2 is amplified in ~40% of oligodendrogliomas and in ~40% of GBMs[27, 74], driving higher DNMT1 expression (**Figure 4A, B**). DNMT1 writes/maintains me-CpG onto the newly synthesized DNA strand during S-phase (maintenance methyltransferase) and is also a corepressor recruited into lineage MTF protein hubs[57, 59, 70, 71].

Deletions of SWI/SNF-family ATP-dependent chromatin remodelers that reposition nucleosomes to allow basal transcription factor access to genes. *ARID1A* (chromosome 1p36.11), *ARID1B* (6q25.3), *SMARCA2* (9p24.3) and *SMARCB1* (22q11) are components of the BAF coactivator complex that repositions nucleosomes for gene activation. Oligodendrogliomas are characterized by 1p deletions and hence *ARID1A* haploinsufficiency (**Figure 4A, B**). Deletions of *ARID1B* and *SMARCA2* are found in 20-30% of grade 2/3 astrocytomas, increasing to 30-70% in grade 4 astrocytomas, and also in 20-40% in GBMs, driving lower expression (**Figure 4A, B**).

Deletions/translocations of genes for other activating machinery. Genes for other key components in the machinery needed to activate genes, e.g., cohesins, splicing factors, mediator family members and histone methyltransferases containing SET-domains (KMTs), are frequently deleted and sometimes translocated in gliomas, as for cancers in general[6, 7, 10, 70].

Alterations to lineage MTF. Genes for lineage MTF themselves, e.g., *SOX10*, can be mutated, translocated or deleted, to thereby disrupt mutual cooperation in MTF circuits that mediates exchange of corepressors for coactivators – corepressor/coactivator imbalance in lineage MTF hubs represses instead of activates late-lineage genes[38, 71, 98, 99] (reviewed in [3]).

Glioma-genesis thus impedes, in several orthogonal ways, the epigenetic work that replicating glial-precursors must exercise to transition to terminal glial-fates. Each ectopic replication caused by this friction against lineage-maturation is an opportunity to select another mutation or copy number alteration to further hinder epigenetic work needed to mature, thereby escalating grade, replications and pace of disease in a merciless clinical reality[17] (reviewed in[100]).

9. *Resuming lineage-maturation, instead of activating apoptosis, to terminate malignant self-replications*

Demonstrating cause-effect, inhibiting corepressors terminates glioma self-replication via lineage-maturation, shown by several groups (**Figure 5**): small molecule inhibitors of EZH2 (MC4040, MC4041, tazemetostat) decreased glioma cell proliferation without apoptosis-induction, but by resumed onward lineage-differentiation (upregulation of p27 and E-cadherin)[101]. Tazemetostat also decreased glioma self-replication in vitro and in vivo in other studies, again not by apoptosis, although terminal-differentiation was not specifically analyzed[102]. EZH2 downregulation with short hairpin RNA or with a small molecule (DZNep) impaired glioma self-replication in vitro and tumor-initiation in vivo[44]. Consistent with a non-apoptosis pathway, there was no significant effect on glioma cell viability, even as sphere morphology (a measure of self-replication) and proliferation were reduced[44]. In *H3F3A* K27M-mutated gliomas, EZH2 inhibition decreased proliferation in vitro and increased survival in mice[103]. Another study looked specifically for a cytotoxic effect of EZH2 inhibition in the glioma cells to explain the cytoreduction, and did not find any[104].

ATP-dependent chromatin remodelers of the ISWI family, e.g., CHD4, SMARCA5, oppose SWI/SNF ATP-dependent chromatin remodelers. That is, they execute a linchpin epigenetic repression event of re-positioning nucleosomes to obstruct access to genes by basal transcription factor machinery. CHD4

depletion using shRNA promoted astrocyte differentiation in vitro[105], implying the ISWI-family are candidate targets for therapy.

Mitochondrial outputs other than AKG, e.g., cytidine triphosphate (CTP), also facilitate lineage-differentiation: small molecules that inhibit de novo pyrimidine synthesis and decrease CTP, including dihydroorotate dehydrogenase (DHODH)-inhibitors (several available) and the cytidine triphosphate synthase 2 (CTPS2)-inhibitor cyclopentenyl cytosine (CPEC), release cancer cells including glioma cells to terminal-differentiation[106-108]. Implicating CTP specifically, CTP-restoration with exogenous cytidine prevented terminal-differentiation induction by the DHODH-inhibitor leflunomide[109]. These results imply that CTP operates as a cofactor in a corepressor complex, and interestingly, key DNA packaging proteins in prokaryotes are CTP-dependent and related to eukaryotic condensins[110].

The deoxycytidine analog decitabine, a clinical pro-drug approved to treat myeloid malignancies, inhibits and depletes DNMT1 from dividing cells. Self-replication of *IDH1*-mutated glioma cells was terminated by decitabine treatment in vitro, without activation of apoptosis, but with activation of neuronal/glial lineage-differentiation genes, and with morphology changes of terminal-differentiation[111]. The cytidine analog 5-azacytidine also inhibits/depletes DNMT1: long-term administration of 5-azacytidine to mice with *IDH1*-mutated anaplastic astrocytoma significantly decreased tumor growth; histological examination indicated terminal-differentiation was the pathway of tumor cytoreduction[112]. 5-azacytidine also suppressed IDH-wildtype GBM growth in vitro and in xenografts[113]. 5-azacytidine and decitabine unfortunately have pharmacology limitations for treating gliomas (or other solid tumors) in humans, one being that both are rapidly inactivated in solid tissues by the catabolic enzyme cytidine deaminase (CDA)[114, 115]. To address this limitation, a combination of decitabine with the CDA-inhibitor cedazuridine is in glioma clinical trials (**Table 1**). Another limitation is that both are pro-drugs that require activation by uridine cytidine kinase 2 and deoxycytidine kinase respectively, pyrimidine metabolism enzymes that are intrinsically much more highly expressed in hematopoietic cells - neutropenia can thus clinically pre-empt achievement of DNMT1-targeting in solid tumor tissue, and methods to overcome this limitation are being explored[114, 116, 117].

IDH1-mutated glioma cells were released to terminal-differentiation by small molecule inhibitors of mutant-IDH1 in pre-clinical studies[118-121]. Clinical trial results have been reported for the mutant-IDH1-inhibitors ivosidenib (Agios), olutasidenib (Forma Therapeutics), DS-1001b (Daichi Sankyo), BAY1436032 (Bayer), and the dual mutant-IDH1/IDH2-inhibitor vorasidenib, to treat relapsed/refractory gliomas[122-126]: objective response rates by Response Assessment in Neuro-Oncology (RANO) criteria ranged from ~3-29%

(**Table 1**). These low to modest response-rates compare unfavorably to high response-rates and regulatory approval of mutant-IDH-inhibitors to treat IDH-mutated myeloid malignancies. By way of possible explanation, IDH-mutant gliomas contain numerous mutations and copy number alterations impacting several classes of epigenetic enzymes (**Figure 4**), compared to few such alterations in IDH-mutant myeloid malignancies. Clinical treatment narrowly specific for mutated-IDH may therefore have less impact on relieving aberrant repression of late-lineage genes in glioma *vs* myeloid cancer cells[84-86, 127-130]. In this regard, even in IDH-mutant myeloid malignancies, clinical practice often combines mutant-IDH-inhibitors with the DNMT1-targeting agents 5-azacytidine or decitabine.

One caveat with magnetic resonance imaging (MRI) assessment of glioma-response is that advancing glioma can be difficult to distinguish from radiation-induced changes to normal brain[128, 131]. This measurement problem is not expected in a trial evaluating vorasidenib as first-line treatment of IDH-mutant gliomas, results of which are pending[132] (**Table 1**).

Histone deacetylases (HDAC) and lysine demethylase 1A (KDM1A) are implicated in repression of lineage-differentiation programs in cancer cells broadly including glioma cells, and accordingly, HDAC- and KDM1A-inhibitors induce terminal-differentiation in vitro and in pre-clinical in vivo studies[133-137]. Several HDAC inhibitors are approved to treat peripheral T-cell lymphomas, but none are approved to treat cancers of other lineages: vorinostat, panobinostat and valproic acid combined with standard treatments have been evaluated in glioma clinical trials, but without clear evidence of added benefit (**Table 1**)[138-147]. Limited success in translating the pre-clinical observations into clinical therapy could reflect that HDACs and KDM1A have non-histone substrates such that even on-target drug-effects produce clinical toxicities that restrict exposures needed to achieve intended epigenetic pharmacodynamic effects in solid tumor tissue.

Next steps? HDACs, KDM1A, DNMT1, CHD4, EZH2, DHODH (or CTPS2) and mutant-IDH1/2 are thus validated pre-clinically as targets for inhibition to compel p53/p16-independent glioma cell cycling exits. However, no major successes have occurred with limited attempts at clinical translation to date (**Table 1**). Reasons for this, and thus potential solutions, can be determined: some targets, e.g., HDACs, KDM1A, have wide cell-physiology roles such that even specific, on-target actions of small molecule inhibitors cause toxicities, including cytotoxicity, that limits feasible clinical exposures needed to achieve tumor pharmacodynamic effect. DNMT1 is a target that can in principle be safely engaged, shown by safety and effectiveness of non-cytotoxic DNMT1-targeting regimens of decitabine or 5-azacytidine in patients with myeloid malignancies, including fragile elderly patients with p53-inactivated disease. However, for drug-

metabolism reasons, these pro-drugs have very limited distribution and activation in glioma and other solid tumor tissue - potential solutions for this have been proposed but need clinical evaluation. CHD4 does not yet have a small molecule inhibitor for clinical evaluation, although at least one is in pre-clinical development. Safe clinical inhibitors for EZH2 and DHODH are available, but results from glioma clinical trials are not available – the pre-clinical data supports pursuit of clinical trials. Arguably, clinical trials with inhibitors of mutant-IDH are the only ones in which intended molecular pharmacodynamic effects were sufficiently achieved in glioma-tissue, but even so responses were minimal to modest - glioma-genesis selects to alter epigenetic enzymes from several classes, and oncotherapy should counter like-wise; combining non-cytotoxic drugs, all aiming to renew lineage-maturation, is routine treatment practice for some myeloid malignancies – p53/p16-attenuation and patho-biology of malignant self-replication recommends this approach to gliomas too[148].

CONCLUSION

Genetic attenuation of the p53/p16-apoptosis pathway in glioma cells contributes to poor outcomes with apoptosis-intending (cytotoxic) treatments. Normal p53-intact cells are meanwhile destroyed, causing significant toxicity. Contrasting with attenuated p53/p16, self-replicating glioma cells highly express glial lineage-specifying MTF circuits that cooperate with MYC to activate exponential proliferation, but fail in their other usual function of also driving maturation along lineage-axes: late-glial genes have constitutively 'closed' chromatin requiring chromatin-remodeling for activation, and neoplastic evolution selects to disrupt the epigenetic machinery that performs this work. Pharmacologic inhibition of repressing epigenetic enzymes recouples to lineage-maturation and hence terminates malignant self-replication, independent of p53/p16/apoptosis, justifying clinical development oriented to epigenetic molecular pharmacodynamic effects without cytotoxicity.

Author Contributions: Conceptualization and design: NvKD and YS. Funding: DP and YS. Collection and assembly of data: NvKD and YS. Visualization of data: NvKD and YS. Validation of data: All authors. Manuscript writing: NvKD and YS. Review and editing of the manuscript: All authors. Final approval of the manuscript: All authors. Accountable for all aspects of the manuscript: All authors. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

Conflicts of Interest: Ownership: YS – EpiDestiny. Income: none. Research support: none. Intellectual property: YS - patents around tetrahydrouridine, decitabine and 5-azacytidine (US 9,259,469 B2; US 9,265,785 B2; US 9,895,391 B2) and cancer differentiation therapy (US 9,926,316 B2). S.P. - eligible to royalties as co-inventor of BAY1436032. The corresponding patents are under the administrative supervision of the DKFZ technology transfer office. All other authors declare no conflicts of interest.

Data Availability Statement: All data analyzed are in public databases - The Cancer Genome Atlas (TCGA), Encode, GEO Database, Cancer Cell Line Encyclopedia (CCLE), European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) - as specifically indicated in each figure legend. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding: YS: National Heart, Lung and Blood Institute PO1 HL146372; National Cancer Institute P30 CA043703; RO1 CA204373; R21 CA263430. DP and NvKD: German Research Foundation (DFG), Grant No. 445704496.

REFERENCES

1. Mukherjee S. *The emperor of all maladies : a biography of cancer*, 1st Scribner hardcover ed. New York: Scribner, 2010. xiv, 571 p., 8 p. of plates.

2. Domazet-Loso T, Tautz D. Phylostratigraphic tracking of cancer genes suggests a link to the emergence of multicellularity in metazoa. *BMC Biol* 2010;**8**: 66.

3. Velcheti V, Schrump D, Saunthararajah Y. Ultimate Precision: Targeting Cancer but Not Normal Self-replication. *American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting* 2018: 950-63.

4. Weller M, van den Bent M, Preusser M, Le Rhun E, Tonn JC, Minniti G, Bendszus M, Balana C, Chinot O, Dirven L, French P, Hegi ME, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nat Rev Clin Oncol* 2021;**18**: 170-86.

5. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, Soffietti R, von Deimling A, et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol* 2021;**23**: 1231-51.

6. Suzuki H, Aoki K, Chiba K, Sato Y, Shiozawa Y, Shiraishi Y, Shimamura T, Niida A, Motomura K, Ohka F, Yamamoto T, Tanahashi K, et al. Mutational landscape and clonal architecture in grade II and III gliomas. *Nat Genet* 2015;**47**: 458-68.

7. Cancer Genome Atlas Research N, Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, Cooper LA, Rheinbay E, Miller CR, Vitucci M, Morozova O, Robertson AG, et al. Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med* 2015;**372**: 2481-98.

8. Wick W, Roth P, Hartmann C, Hau P, Nakamura M, Stockhammer F, Sabel MC, Wick A, Koeppen S, Ketter R, Vajkoczy P, Eyupoglu I, et al. Long-term analysis of the NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with PCV or temozolomide. *Neuro Oncol* 2016;**18**: 1529-37.

9. Reis GF, Pekmezci M, Hansen HM, Rice T, Marshall RE, Molinaro AM, Phillips JJ, Vogel H, Wiencke JK, Wrensch MR, Walsh KM, Perry A. CDKN2A loss is associated with shortened overall survival in lower-grade (World Health Organization Grades II-III) astrocytomas. *J Neuropathol Exp Neurol* 2015;**74**: 442-52.

10. Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, Beroukhim R, Bernard B, et al. The somatic genomic landscape of glioblastoma. *Cell* 2013;**155**: 462-77.

11. Gotz M, Nakafuku M, Petrik D. Neurogenesis in the Developing and Adult Brain-Similarities and Key Differences. *Cold Spring Harb Perspect Biol* 2016;**8**.

12. Dillen Y, Kemps H, Gervois P, Wolfs E, Bronckaers A. Adult Neurogenesis in the Subventricular Zone and Its Regulation After Ischemic Stroke: Implications for Therapeutic Approaches. *Transl Stroke Res* 2020;**11**: 60-79.

13. Kempermann G, Song H, Gage FH. Neurogenesis in the Adult Hippocampus. *Cold Spring Harb Perspect Biol* 2015;**7**: a018812.

14. Obernier K, Cebrian-Silla A, Thomson M, Parraguez JI, Anderson R, Guinto C, Rodas Rodriguez J, Garcia-Verdugo JM, Alvarez-Buylla A. Adult Neurogenesis Is Sustained by Symmetric Self-Renewal and Differentiation. *Cell Stem Cell* 2018;**22**: 221-34 e8.

15. Mu Y, Gage FH. Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Mol Neurodegener* 2011;**6**: 85.

16. Galderisi U, Jori FP, Giordano A. Cell cycle regulation and neural differentiation. *Oncogene* 2003;**22**: 5208-19.

17. Ashkenazi R, Gentry SN, Jackson TL. Pathways to tumorigenesis--modeling mutation acquisition in stem cells and their progeny. *Neoplasia* 2008;**10**: 1170-82.

18. Flitsch LJ, Brustle O. Evolving principles underlying neural lineage conversion and their relevance for biomedical translation. *F1000Res* 2019;**8**.

19. Tiwari N, Pataskar A, Peron S, Thakurela S, Sahu SK, Figueres-Onate M, Marichal N, Lopez-Mascaraque L, Tiwari VK, Berninger B. Stage-Specific Transcription Factors Drive Astrogliogenesis by Remodeling Gene Regulatory Landscapes. *Cell Stem Cell* 2018;**23**: 557-71 e8.

20. Lattke M, Goldstone R, Ellis JK, Boeing S, Jurado-Arjona J, Marichal N, MacRae JI, Berninger B, Guillemot F. Extensive transcriptional and chromatin changes underlie astrocyte maturation in vivo and in culture. *Nat Commun* 2021;**12**: 4335.

21. Stine ZE, Walton ZE, Altman BJ, Hsieh AL, Dang CV. MYC, Metabolism, and Cancer. *Cancer discovery* 2015;**5**: 1024-39.

22. Srivastava M, Simakov O, Chapman J, Fahey B, Gauthier ME, Mitros T, Richards GS, Conaco C, Dacre M, Hellsten U, Larroux C, Putnam NH, et al. The Amphimedon queenslandica genome and the evolution of animal complexity. *Nature* 2010;**466**: 720-6.

23. Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, Chen X, Taipale J, Hughes TR, Weirauch MT. The Human Transcription Factors. *Cell* 2018;**172**: 650-65.

24. Enane FO, Saunthararajah Y, Korc M. Differentiation therapy and the mechanisms that terminate cancer cell proliferation without harming normal cells. *Cell Death Dis* 2018;**9**: 912.

25. Bai H, Harmanci AS, Erson-Omay EZ, Li J, Coskun S, Simon M, Krischek B, Ozduman K, Omay SB, Sorensen EA, Turcan S, Bakirciglu M, et al. Integrated genomic characterization of IDH1-mutant glioma malignant progression. *Nat Genet* 2016;**48**: 59-66.

26. Mazor T, Pankov A, Johnson BE, Hong C, Hamilton EG, Bell RJA, Smirnov IV, Reis GF, Phillips JJ, Barnes MJ, Idbaih A, Alentorn A, et al. DNA Methylation and Somatic Mutations Converge on the Cell Cycle and Define Similar Evolutionary Histories in Brain Tumors. *Cancer Cell* 2015;**28**: 307-17.

27. Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, Morozova O, Newton Y, Radenbaugh A, Pagnotta SM, Anjum S, Wang J, et al. Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. *Cell* 2016;**164**: 550-63.

28. Venteicher AS, Tirosh I, Hebert C, Yizhak K, Neftel C, Filbin MG, Hovestadt V, Escalante LE, Shaw ML, Rodman C, Gillespie SM, Dionne D, et al. Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq. *Science* 2017;**355**.

29. de Souza CF, Sabedot TS, Malta TM, Stetson L, Morozova O, Sokolov A, Laird PW, Wiznerowicz M, Iavarone A, Snyder J, deCarvalho A, Sanborn Z, et al. A Distinct DNA Methylation Shift in a Subset of Glioma CpG Island Methylator Phenotypes during Tumor Recurrence. *Cell Rep* 2018;**23**: 637-51.

30. Perry A, Wesseling P. Histologic classification of gliomas. *Handb Clin Neurol* 2016;**134**: 71-95.

31. Brazel CY, Nunez JL, Yang Z, Levison SW. Glutamate enhances survival and proliferation of neural progenitors derived from the subventricular zone. *Neuroscience* 2005;**131**: 55-65.

32. Jansson LC, Akerman KE. The role of glutamate and its receptors in the proliferation, migration, differentiation and survival of neural progenitor cells. *J Neural Transm (Vienna)* 2014;**121**: 819-36.

33. Venkataramani V, Tanev DI, Strahle C, Studier-Fischer A, Fankhauser L, Kessler T, Korber C, Kardorff M, Ratliff M, Xie R, Horstmann H, Messer M, et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. *Nature* 2019;**573**: 532-8.

34. Couturier CP, Ayyadhury S, Le PU, Nadaf J, Monlong J, Riva G, Allache R, Baig S, Yan X, Bourgey M, Lee C, Wang YCD, et al. Single-cell RNA-seq reveals that glioblastoma recapitulates a normal neurodevelopmental hierarchy. *Nat Commun* 2020;**11**: 3406.

35. Neftel C, Laffy J, Filbin MG, Hara T, Shore ME, Rahme GJ, Richman AR, Silverbush D, Shaw ML, Hebert CM, Dewitt J, Gritsch S, et al. An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. *Cell* 2019;**178**: 835-49 e21.

36. Will B, Vogler TO, Narayanagari S, Bartholdy B, Todorova TI, da Silva Ferreira M, Chen J, Yu Y, Mayer J, Barreyro L, Carvajal L, Neriah DB, et al. Minimal PU.1 reduction induces a preleukemic state and promotes development of acute myeloid leukemia. *Nature medicine* 2015;**21**: 1172-81.

37. Ng KP, Hu Z, Ebrahem Q, Negrotto S, Lausen J, Saunthararajah Y. Runx1 deficiency permits granulocyte lineage commitment but impairs subsequent maturation. *Oncogenesis* 2013;**2**: e78.

38. Enane FO, Shuen WH, Gu X, Quteba E, Przychodzen B, Makishima H, Bodo J, Ng J, Chee CL, Ba R, Seng Koh L, Lim J, et al. GATA4 loss of function in liver cancer impedes precursor to hepatocyte transition. *The Journal of clinical investigation* 2017.

39. Tsherniak A, Vazquez F, Montgomery PG, Weir BA, Kryukov G, Cowley GS, Gill S, Harrington WF, Pantel S, Krill-Burger JM, Meyers RM, Ali L, et al. Defining a Cancer Dependency Map. *Cell* 2017;**170**: 564-76 e16.

40. Sack LM, Davoli T, Li MZ, Li Y, Xu Q, Naxerova K, Wooten EC, Bernardi RJ, Martin TD, Chen T, Leng Y, Liang AC, et al. Profound Tissue Specificity in Proliferation Control Underlies Cancer Drivers and Aneuploidy Patterns. *Cell* 2018;**173**: 499-514 e23.

41. Hoadley KA, Yau C, Wolf DM, Cherniack AD, Tamborero D, Ng S, Leiserson MD, Niu B, McLellan MD, Uzunangelov V, Zhang J, Kandoth C, et al. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell* 2014;**158**: 929-44.

42. Garraway LA, Sellers WR. Lineage dependency and lineage-survival oncogenes in human cancer. *NatRevCancer* 2006;**6**: 593-602.

43. Bradner JE, Hnisz D, Young RA. Transcriptional Addiction in Cancer. *Cell* 2017;**168**: 629-43.

44. Suva ML, Riggi N, Janiszewska M, Radovanovic I, Provero P, Stehle JC, Baumer K, Le Bitoux MA, Marino D, Cironi L, Marquez VE, Clement V, et al. EZH2 is essential for glioblastoma cancer stem cell maintenance. *Cancer Res* 2009;**69**: 9211-8.

45. Filbin MG, Tirosh I, Hovestadt V, Shaw ML, Escalante LE, Mathewson ND, Neftel C, Frank N, Pelton K, Hebert CM, Haberler C, Yizhak K, et al. Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. *Science* 2018;**360**: 331-5.

46. Ma S, Pang C, Song L, Guo F, Sun H. Activating transcription factor 3 is overexpressed in human glioma and its knockdown in glioblastoma cells causes growth inhibition both in vitro and in vivo. *Int J Mol Med* 2015;**35**: 1561-73.

47. Vladimirova V, Waha A, Luckerath K, Pesheva P, Probstmeier R. Runx2 is expressed in human glioma cells and mediates the expression of galectin-3. *J Neurosci Res* 2008;**86**: 2450-61.

48. Lee JS, Xiao J, Patel P, Schade J, Wang J, Deneen B, Erdreich-Epstein A, Song HR. A novel tumor-promoting role for nuclear factor IA in glioblastomas is mediated through negative regulation of p53, p21, and PAI1. *Neuro Oncol* 2014;**16**: 191-203.

49. Kang P, Lee HK, Glasgow SM, Finley M, Donti T, Gaber ZB, Graham BH, Foster AE, Novitch BG, Gronostajski RM, Deneen B. Sox9 and NFIA coordinate a transcriptional regulatory cascade during the initiation of gliogenesis. *Neuron* 2012;**74**: 79-94.

50. Lan X, Jorg DJ, Cavalli FMG, Richards LM, Nguyen LV, Vanner RJ, Guilhamon P, Lee L, Kushida MM, Pellacani D, Park NI, Coutinho FJ, et al. Fate mapping of human glioblastoma reveals an invariant stem cell hierarchy. *Nature* 2017;**549**: 227-32.

51. Lee JH, Lee JE, Kahng JY, Kim SH, Park JS, Yoon SJ, Um JY, Kim WK, Lee JK, Park J, Kim EH, Lee JH, et al. Human glioblastoma arises from subventricular zone cells with low-level driver mutations. *Nature* 2018;**560**: 243-7.

52. Danussi C, Bose P, Parthasarathy PT, Silberman PC, Van Arnam JS, Vitucci M, Tang OY, Heguy A, Wang Y, Chan TA, Riggins GJ, Sulman EP, et al. Atrx inactivation drives disease-defining phenotypes in glioma cells of origin through global epigenomic remodeling. *Nat Commun* 2018;**9**: 1057.

53. Suva ML, Rheinbay E, Gillespie SM, Patel AP, Wakimoto H, Rabkin SD, Riggi N, Chi AS, Cahill DP, Nahed BV, Curry WT, Martuza RL, et al. Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. *Cell* 2014;**157**: 580-94.

54. Foltz G, Yoon JG, Lee H, Ryken TC, Sibenaller Z, Ehrich M, Hood L, Madan A. DNA methyltransferase-mediated transcriptional silencing in malignant glioma: a combined whole-genome microarray and promoter array analysis. *Oncogene* 2009;**28**: 2667-77.

55. Di Croce L, Helin K. Transcriptional regulation by Polycomb group proteins. *Nat Struct Mol Biol* 2013;**20**: 1147-55.

56. Hyun K, Jeon J, Park K, Kim J. Writing, erasing and reading histone lysine methylations. *Exp Mol Med* 2017;**49**: e324.

57. Greenberg MVC, Bourc'his D. The diverse roles of DNA methylation in mammalian development and disease. *Nat Rev Mol Cell Biol* 2019;**20**: 590-607.

58. Schuettengruber B, Bourbon HM, Di Croce L, Cavalli G. Genome Regulation by Polycomb and Trithorax: 70 Years and Counting. *Cell* 2017;**171**: 34-57.

59. Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nusbaum C, Jaffe DB, Gnirke A, Jaenisch R, et al. Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* 2008;**454**: 766-70.

60. Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, Pfaff E, Tonjes M, Sill M, Bender S, Kool M, Zapatka M, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell* 2012;**22**: 425-37.

61. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* 2010;**17**: 510-22.

62. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AW, Lu C, Ward PS, Thompson CB, Kaufman A, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 2012;**483**: 479-83.

63. Flavahan WA, Drier Y, Liau BB, Gillespie SM, Venteicher AS, Stemmer-Rachamimov AO, Suva ML, Bernstein BE. Insulator dysfunction and oncogene activation in IDH mutant gliomas. *Nature* 2016;**529**: 110-4.

64. Harutyunyan AS, Chen H, Lu T, Horth C, Nikbakht H, Krug B, Russo C, Bareke E, Marchione DM, Coradin M, Garcia BA, Jabado N, et al. H3K27M in Gliomas Causes a One-Step Decrease in H3K27 Methylation and Reduced Spreading within the Constraints of H3K36 Methylation. *Cell Rep* 2020;**33**: 108390.

65. Chan KM, Fang D, Gan H, Hashizume R, Yu C, Schroeder M, Gupta N, Mueller S, James CD, Jenkins R, Sarkaria J, Zhang Z. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev* 2013;**27**: 985-90.

66. Velcheti V, Radivoyevitch T, Saunthararajah Y. Higher-Level Pathway Objectives of Epigenetic Therapy: A Solution to the p53 Problem in Cancer. *American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting* 2017;**37**: 812-24.

67. Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, Epstein CB, Zhang X, Wang L, Issner R, Coyne M, Ku M, Durham T, et al. Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature* 2011;**473**: 43-9.

68. Gallegos DA, Chan U, Chen LF, West AE. Chromatin Regulation of Neuronal Maturation and Plasticity. *Trends Neurosci* 2018;**41**: 311-24.

69. Chen CCL, Deshmukh S, Jessa S, Hadjadj D, Lisi V, Andrade AF, Faury D, Jawhar W, Dali R, Suzuki H, Pathania M, A D, et al. Histone H3.3G34-Mutant Interneuron Progenitors Co-opt PDGFRA for Gliomagenesis. *Cell* 2020;**183**: 1617-33 e22.

70. Gu X, Enane F, Tohme R, Schuerger C, Radivoyevitch T, Parker Y, Zuberi E, Przychodzen B, Jha BK, Lindner D, Rini B, Saunthararajah Y. PBRM1 loss in kidney cancer unbalances the proximal tubule master transcription factor hub to repress proximal tubule differentiation. *Cell Rep* 2021;**36**: 109747.

71. Gu X, Ebrahem Q, Mahfouz RZ, Hasipek M, Enane F, Radivoyevitch T, Rapin N, Przychodzen B, Hu Z, Balusu R, Cotta CV, Wald D, et al. Leukemogenic nucleophosmin mutation disrupts the transcription factor hub that regulates granulomonocytic fates. *The Journal of clinical investigation* 2018;**128**: 4260-79.

72. Rao RA, Dhele N, Cheemadan S, Ketkar A, Jayandharan GR, Palakodeti D, Rampalli S. Ezh2 mediated H3K27me3 activity facilitates somatic transition during human pluripotent reprogramming. *Sci Rep* 2015;**5**: 8229.

73. Ozawa T, Riester M, Cheng YK, Huse JT, Squatrito M, Helmy K, Charles N, Michor F, Holland EC. Most human non-GCIMP glioblastoma subtypes evolve from a common proneural-like precursor glioma. *Cancer Cell* 2014;**26**: 288-300.

74. Korber V, Yang J, Barah P, Wu Y, Stichel D, Gu Z, Fletcher MNC, Jones D, Hentschel B, Lamszus K, Tonn JC, Schackert G, et al. Evolutionary Trajectories of IDH(WT) Glioblastomas Reveal a Common Path of Early Tumorigenesis Instigated Years ahead of Initial Diagnosis. *Cancer Cell* 2019;**35**: 692-704 e12.

75. Orzan F, Pellegatta S, Poliani PL, Pisati F, Caldera V, Menghi F, Kapetis D, Marras C, Schiffer D, Finocchiaro G. Enhancer of Zeste 2 (EZH2) is up-regulated in malignant gliomas and in glioma stem-like cells. *Neuropathol Appl Neurobiol* 2011;**37**: 381-94.

76. Kitahama K, Iijima S, Sumiishi A, Hayashi A, Nagahama K, Saito K, Sasaki N, Kobayashi K, Shimizu S, Nagane M, Shibahara J. Reduced H3K27me3 levels in diffuse gliomas: association with 1p/19q codeletion and difference from H3K27me3 loss in malignant peripheral nerve sheath tumors. *Brain Tumor Pathol* 2021;**38**: 23-9.

77. Chang S, Yim S, Park H. The cancer driver genes IDH1/2, JARID1C/ KDM5C, and UTX/ KDM6A: crosstalk between histone demethylation and hypoxic reprogramming in cancer metabolism. *Exp Mol Med* 2019;**51**: 1-17.

78. Sui A, Xu Y, Yang J, Pan B, Wu J, Guo T, Shen Y, Guo X. The histone H3 Lys 27 demethylase KDM6B promotes migration and invasion of glioma cells partly by regulating the expression of SNAI1. *Neurochem Int* 2019;**124**: 123-9.

79. Sturm D, Bender S, Jones DT, Lichter P, Grill J, Becher O, Hawkins C, Majewski J, Jones C, Costello JF, Iavarone A, Aldape K, et al. Paediatric and adult glioblastoma: multiform (epi)genomic culprits emerge. *Nat Rev Cancer* 2014;**14**: 92-107.

80. Harutyunyan AS, Krug B, Chen H, Papillon-Cavanagh S, Zeinieh M, De Jay N, Deshmukh S, Chen CCL, Belle J, Mikael LG, Marchione DM, Li R, et al. H3K27M induces defective chromatin spread of

PRC2-mediated repressive H3K27me2/me3 and is essential for glioma tumorigenesis. *Nat Commun* 2019;**10**: 1262.

81. Voon HP, Wong LH. New players in heterochromatin silencing: histone variant H3.3 and the ATRX/DAXX chaperone. *Nucleic Acids Res* 2016;**44**: 1496-501.

82. Nandakumar P, Mansouri A, Das S. The Role of ATRX in Glioma Biology. *Front Oncol* 2017;**7**: 236.

83. Juhasz S, Elbakry A, Mathes A, Lobrich M. ATRX Promotes DNA Repair Synthesis and Sister Chromatid Exchange during Homologous Recombination. *Mol Cell* 2018;**71**: 11-24 e7.

84. Cohen A, Sato M, Aldape K, Mason CC, Alfaro-Munoz K, Heathcock L, South ST, Abegglen LM, Schiffman JD, Colman H. DNA copy number analysis of Grade II-III and Grade IV gliomas reveals differences in molecular ontogeny including chromothripsis associated with IDH mutation status. *Acta Neuropathol Commun* 2015;**3**: 34.

85. Jonsson P, Lin AL, Young RJ, DiStefano NM, Hyman DM, Li BT, Berger MF, Zehir A, Ladanyi M, Solit DB, Arnold AG, Stadler ZK, et al. Genomic Correlates of Disease Progression and Treatment Response in Prospectively Characterized Gliomas. *Clin Cancer Res* 2019;**25**: 5537-47.

86. Barthel FP, Johnson KC, Varn FS, Moskalik AD, Tanner G, Kocakavuk E, Anderson KJ, Abiola O, Aldape K, Alfaro KD, Alpar D, Amin SB, et al. Longitudinal molecular trajectories of diffuse glioma in adults. *Nature* 2019;**576**: 112-20.

87. Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagg EA, Rose NR, Leung IK, Li XS, Woon EC, Yang M, McDonough MA, King ON, et al. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO reports* 2011;**12**: 463-9.

88. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT, Liu LX, Jiang WQ, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. *Cancer Cell* 2011;**19**: 17-30.

89. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, Edwards CR, Khanin R, Figueroa ME, Melnick A, Wellen KE, O'Rourke DM, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature* 2012;**483**: 474-8.

90. Bardella C, Al-Dalahmah O, Krell D, Brazauskas P, Al-Qahtani K, Tomkova M, Adam J, Serres S, Lockstone H, Freeman-Mills L, Pfeffer I, Sibson N, et al. Expression of Idh1(R132H) in the Murine Subventricular Zone Stem Cell Niche Recapitulates Features of Early Gliomagenesis. *Cancer Cell* 2016;**30**: 578-94.

91. Modrek AS, Golub D, Khan T, Bready D, Prado J, Bowman C, Deng J, Zhang G, Rocha PP, Raviram R, Lazaris C, Stafford JM, et al. Low-Grade Astrocytoma Mutations in IDH1, P53, and ATRX Cooperate to Block Differentiation of Human Neural Stem Cells via Repression of SOX2. *Cell Rep* 2017;**21**: 1267-80.

92. Philip B, Yu DX, Silvis MR, Shin CH, Robinson JP, Robinson GL, Welker AE, Angel SN, Tripp SR, Sonnen JA, VanBrocklin MW, Gibbons RJ, et al. Mutant IDH1 Promotes Glioma Formation In Vivo. *Cell Rep* 2018;**23**: 1553-64.

93. Stasik S, Juratli TA, Petzold A, Richter S, Zolal A, Schackert G, Dahl A, Krex D, Thiede C. Exome sequencing identifies frequent genomic loss of TET1 in IDH-wild-type glioblastoma. *Neoplasia* 2020;**22**: 800-8.

94. Baysan M, Woolard K, Cam MC, Zhang W, Song H, Kotliarova S, Balamatsias D, Linkous A, Ahn S, Walling J, Belova GI, Fine HA. Detailed longitudinal sampling of glioma stem cells in situ reveals Chr7 gain and Chr10 loss as repeated events in primary tumor formation and recurrence. *Int J Cancer* 2017;**141**: 2002-13.

95. Fu R, Ding Y, Luo J, Yu L, Li CL, Li DS, Guo SW. TET1 exerts its tumour suppressor function by regulating autophagy in glioma cells. *Biosci Rep* 2017;**37**.

96. Tsai YP, Chen HF, Chen SY, Cheng WC, Wang HW, Shen ZJ, Song C, Teng SC, He C, Wu KJ. TET1 regulates hypoxia-induced epithelial-mesenchymal transition by acting as a co-activator. *Genome Biol* 2014;**15**: 513.

97. Deplus R, Delatte B, Schwinn MK, Defrance M, Mendez J, Murphy N, Dawson MA, Volkmar M, Putmans P, Calonne E, Shih AH, Levine RL, et al. TET2 and TET3 regulate GlcNAcylation and H3K4 methylation through OGT and SET1/COMPASS. *EMBO J* 2013;**32**: 645-55.

98. Hu Z, Gu X, Baraoidan K, Ibanez V, Sharma A, Kadkol S, Munker R, Ackerman S, Nucifora G, Sauntharajah Y. RUNX1 regulates corepressor interactions of PU.1. *Blood* 2011;**117**: 6498-508.

99. Gu X, Hu Z, Ebrahem Q, Crabb JS, Mahfouz RZ, Radivoyevitch T, Crabb JW, Sauntharajah Y. Runx1 regulation of Pu.1 corepressor/coactivator exchange identifies specific molecular targets for leukemia differentiation therapy. *The Journal of biological chemistry* 2014;**289**: 14881-95.

100. Velcheti V, Wong KK, Sauntharajah Y. EZH2 Inhibitors: Take It EZy, It Is All About Context. *Cancer discovery* 2019;**9**: 472-5.

101. Stazi G, Taglieri L, Nicolai A, Romanelli A, Fioravanti R, Morrone S, Sabatino M, Ragno R, Tarrone S, Nebbioso M, Carletti R, Artico M, et al. Dissecting the role of novel EZH2 inhibitors in primary glioblastoma cell cultures: effects on proliferation, epithelial-mesenchymal transition, migration, and on the pro-inflammatory phenotype. *Clin Epigenetics* 2019;**11**: 173.

102. Jin X, Kim LJY, Wu Q, Wallace LC, Prager BC, Sanvoranart T, Gimple RC, Wang X, Mack SC, Miller TE, Huang P, Valentim CL, et al. Targeting glioma stem cells through combined BMI1 and EZH2 inhibition. *Nature medicine* 2017;**23**: 1352-61.

103. Mohammad F, Weissmann S, Leblanc B, Pandey DP, Hojfeldt JW, Comet I, Zheng C, Johansen JV, Rapin N, Porse BT, Tvardovskiy A, Jensen ON, et al. EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas. *Nature medicine* 2017;**23**: 483-92.

104. Wiese M, Schill F, Sturm D, Pfister S, Hulleman E, Johnsen SA, Kramm CM. No Significant Cytotoxic Effect of the EZH2 Inhibitor Tazemetostat (EPZ-6438) on Pediatric Glioma Cells with Wildtype Histone 3 or Mutated Histone 3.3. *Klin Padiatr* 2016;**228**: 113-7.

105. Sparmann A, Xie Y, Verhoeven E, Vermeulen M, Lancini C, Gargiulo G, Hulsman D, Mann M, Knoblich JA, van Lohuizen M. The chromodomain helicase Chd4 is required for Polycomb-mediated inhibition of astroglial differentiation. *EMBO J* 2013;**32**: 1598-612.

106. Huang M, Wang Y, Collins M, Graves LM. CPEC induces erythroid differentiation of human myeloid leukemia K562 cells through CTP depletion and p38 MAP kinase. *Leukemia* 2004;**18**: 1857-63.

107. Bierau J, van Gennip AH, Helleman J, van Kuilenburg AB. The cytostatic- and differentiation-inducing effects of cyclopentenyl cytosine on neuroblastoma cell lines. *Biochem Pharmacol* 2001;**62**: 1099-10[illegible].

108. Wang X, Yang K, Wu Q, Kim LJY, Morton AR, Gimple RC, Prager BC, Shi Y, Zhou W, Bhargava S, Zhu Z, Jiang L, et al. Targeting pyrimidine synthesis accentuates molecular therapy response in glioblastoma stem cells. *Sci Transl Med* 2019;**11**.

109. Huang M, Wang Y, Collins M, Mitchell BS, Graves LM. A77 1726 induces differentiation of human myeloid leukemia K562 cells by depletion of intracellular CTP pools. *Mol Pharmacol* 2002;**62**: 463-72.

110. Soh YM, Davidson IF, Zamuner S, Basquin J, Bock FP, Taschner M, Veening JW, De Los Rios P, Peters JM, Gruber S. Self-organization of parS centromeres by the ParB CTP hydrolase. *Science* 2019;**366**: 1129-33.

111. Turcan S, Fabius AW, Borodovsky A, Pedraza A, Brennan C, Huse J, Viale A, Riggins GJ, Chan TA. Efficient induction of differentiation and growth inhibition in IDH1 mutant glioma cells by the DNMT Inhibitor Decitabine. *Oncotarget* 2013;**4**: 1729-36.

112. Borodovsky A, Salmasi V, Turcan S, Fabius AW, Baia GS, Eberhart CG, Weingart JD, Gallia GL, Baylin SB, Chan TA, Riggins GJ. 5-azacytidine reduces methylation, promotes differentiation and induces tumor regression in a patient-derived IDH1 mutant glioma xenograft. *Oncotarget* 2013;**4**: 1737-47.

113. Kratzsch T, Kuhn SA, Joedicke A, Hanisch UK, Vajkoczy P, Hoffmann J, Fichtner I. Treatment with 5-azacitidine delay growth of glioblastoma xenografts: a potential new treatment approach for glioblastomas. *J Cancer Res Clin Oncol* 2018;**144**: 809-19.

114. Gu X, Tohme R, Tomlinson B, Sakre N, Hasipek M, Durkin L, Schuerger C, Grabowski D, Zidan AM, Radivoyevitch T, Hong C, Carraway H, et al. Decitabine- and 5-azacytidine resistance emerges from adaptive responses of the pyrimidine metabolism network. *Leukemia* 2020.

115. Ramsey HE, Oganesian A, Gorska AE, Fuller L, Arrate M, Boyd K, Keer H, Azab M, Savona MR. Oral Azacitidine and Cedazuridine Approximate Parenteral Azacitidine Efficacy in Murine Model. *Target Oncol* 2020;**15**: 231-40.

116. Hill B, Jagadeesh D, Pohlman B, Dean R, Parameswaran N, Chen J, Radivoyevitch T, Morrison A, Fada S, Dever M, Robinson S, Lindner D, et al. A pilot clinical trial of oral tetrahydrouridine/decitabine for noncytotoxic epigenetic therapy of chemoresistant lymphoid malignancies. *Semin Hematol* 2021;**58**: 35-44.

117. Sohal D, Krishnamurthi S, Tohme R, Gu X, Lindner D, Landowski TH, Pink J, Radivoyevitch T, Fada S, Lee Z, Shepard D, Khorana A, et al. A pilot clinical trial of the cytidine deaminase inhibitor tetrahydrouridine combined with decitabine to target DNMT1 in advanced, chemorefractory pancreatic cancer. *Am J Cancer Res* 2020;**10**: 3047-60.

118. Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, Tsoi J, Clark O, Oldrini B, Komisopoulou E, Kunii K, Pedraza A, et al. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* 2013;**340**: 626-30.

119. Pusch S, Krausert S, Fischer V, Balss J, Ott M, Schrimpf D, Capper D, Sahm F, Eisel J, Beck AC, Jugold M, Eichwald V, et al. Pan-mutant IDH1 inhibitor BAY 1436032 for effective treatment of IDH1 mutant astrocytoma in vivo. *Acta Neuropathol* 2017;**133**: 629-44.

120. Konteatis Z, Artin E, Nicolay B, Straley K, Padyana AK, Jin L, Chen Y, Narayaraswamy R, Tong S, Wang F, Zhou D, Cui D, et al. Vorasidenib (AG-881): A First-in-Class, Brain-Penetrant Dual Inhibitor of Mutant IDH1 and 2 for Treatment of Glioma. *ACS Med Chem Lett* 2020;**11**: 101-7.

121. Machida Y, Nakagawa M, Matsunaga H, Yamaguchi M, Ogawara Y, Shima Y, Yamagata K, Matsumoto T, Hattori A, Itoh M, Seki T, Nishiya Y, et al. A Potent Blood-Brain Barrier-Permeable Mutant IDH1 Inhibitor Suppresses the Growth of Glioblastoma with IDH1 Mutation in a Patient-Derived Orthotopic Xenograft Model. *Mol Cancer Ther* 2020;**19**: 375-83.

122. Mellinghoff IK, Ellingson BM, Touat M, Maher E, De La Fuente MI, Holdhoff M, Cote GM, Burris H, Janku F, Young RJ, Huang R, Jiang L, et al. Ivosidenib in Isocitrate Dehydrogenase 1-Mutated Advanced Glioma. *J Clin Oncol* 2020;**38**: 3398-406.

123. Natsume A, Wakabayashi T, Miyakita Y, Narita Y, Mineharu Y, Arakawa Y, Yamasaki F, Sugiyama K, Hata N, Muragaki Y. Phase I study of a brain penetrant mutant IDH1 inhibitor DS-1001b in patients with recurrent or progressive IDH1 mutant gliomas: American Society of Clinical Oncology, 2019.

124. De La Fuente MI, Colman H, Rosenthal M, Van Tine BA, Levaci D, Walbert T, Gan HK, Vieito M, Milhem MM, Lipford K. A phase Ib/II study of olutasidenib in patients with relapsed/refractory IDH1 mutant gliomas: Safety and efficacy as single agent and in combination with azacitidine: American Society of Clinical Oncology, 2020.

125. Wick A, Bahr O, Schuler M, Rohrberg K, Chawla SP, Janku F, Schiff D, Heinemann V, Narita Y, Lenz HJ, Ikeda M, Ando Y, et al. Phase I Assessment of Safety and Therapeutic Activity of BAY1436032 in Patients with IDH1-Mutant Solid Tumors. *Clin Cancer Res* 2021;**27**: 2723-33.

126. Mellinghoff IK, Penas-Prado M, Peters KB, Burris HA, 3rd, Maher EA, Janku F, Cote GM, de la Fuente MI, Clarke JL, Ellingson BM, Chun S, Young RJ, et al. Vorasidenib, a Dual Inhibitor of Mutant IDH1/2, in Recurrent or Progressive Glioma; Results of a First-in-Human Phase I Trial. *Clin Cancer Res* 2021;**27**: 4491-9.

127. Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, McLean CY, Fouse SD, Yamamoto S, Ueda H, Tatsuno K, Asthana S, Jalbert LE, et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science* 2014;**343**: 189-93.

128. van den Bent MJ, Mellinghoff IK, Bindra RS. Gray Areas in the Gray Matter: IDH1/2 Mutations in Glioma. *American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting* 2020;**40**: 1-8.

129. Mathur R, Zhang Y, Grimmer MR, Hong C, Zhang M, Bollam S, Petrecca K, Clarke J, Berger MS, Phillips JJ, Oberheim-Bush NA, Molinaro AM, et al. MGMT promoter methylation level in newly diagnosed low-grade glioma is a predictor of hypermutation at recurrence. *Neuro Oncol* 2020;**22**: 1580-90.

130. van Thuijl HF, Mazor T, Johnson BE, Fouse SD, Aihara K, Hong C, Malmstrom A, Hallbeck M, Heimans JJ, Kloezeman JJ, Stenmark-Askmalm M, Lamfers ML, et al. Evolution of DNA repair defects during malignant progression of low-grade gliomas after temozolomide treatment. *Acta Neuropathol* 2015;**129**: 597-607.

131. Mellinghoff IK, Chang SM, Jaeckle KA, van den Bent M. Isocitrate Dehydrogenase Mutant Grade II and III Glial Neoplasms. *Hematol Oncol Clin North Am* 2021.

132. Mellinghoff IK, Van Den Bent MJ, Clarke JL, Maher EA, Peters KB, Touat M, De Groot JF, De La Fuente MI, Arrillaga-Romany I, Wick W. INDIGO: A global, randomized, double-blind, phase III study of vorasidenib (VOR; AG-881) vs placebo in patients (pts) with residual or recurrent grade II glioma with an isocitrate dehydrogenase 1/2 (IDH1/2) mutation: American Society of Clinical Oncology, 2020.

133. Anastas JN, Zee BM, Kalin JH, Kim M, Guo R, Alexandrescu S, Blanco MA, Giera S, Gillespie SM, Das J, Wu M, Nocco S, et al. Re-programing Chromatin with a Bifunctional LSD1/HDAC Inhibitor Induces Therapeutic Differentiation in DIPG. *Cancer Cell* 2019;**36**: 528-44 e10.

134. Xu J, Sampath D, Lang FF, Prabhu S, Rao G, Fuller GN, Liu Y, Puduvalli VK. Vorinostat modulates cell cycle regulatory proteins in glioma cells and human glioma slice cultures. *J Neurooncol* 2011;**105**: 241-51.

135. Cornago M, Garcia-Alberich C, Blasco-Angulo N, Vall-Llaura N, Nager M, Herreros J, Comella JX, Sanchis D, Llovera M. Histone deacetylase inhibitors promote glioma cell death by G2 checkpoint abrogation leading to mitotic catastrophe. *Cell Death Dis* 2014;**5**: e1435.

136. Grasso CS, Tang Y, Truffaux N, Berlow NE, Liu L, Debily MA, Quist MJ, Davis LE, Huang EC, Woo PJ, Ponnuswami A, Chen S, et al. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nature medicine* 2015;**21**: 555-9.

137. Sareddy GR, Viswanadhapalli S, Surapaneni P, Suzuki T, Brenner A, Vadlamudi RK. Novel KDM1A inhibitors induce differentiation and apoptosis of glioma stem cells via unfolded protein response pathway. *Oncogene* 2017;**36**: 2423-34.

138. Puduvalli VK, Wu J, Yuan Y, Armstrong TS, Vera E, Wu J, Xu J, Giglio P, Colman H, Walbert T, Raizer J, Groves MD, et al. A Bayesian adaptive randomized phase II multicenter trial of bevacizumab with or without vorinostat in adults with recurrent glioblastoma. *Neuro Oncol* 2020;**22**: 1505-15.

139. Peters KB, Lipp ES, Miller E, Herndon JE, 2nd, McSherry F, Desjardins A, Reardon DA, Friedman HS. Phase I/II trial of vorinostat, bevacizumab, and daily temozolomide for recurrent malignant gliomas. *J Neurooncol* 2018;**137**: 349-56.

140. Ghiaseddin A, Reardon D, Massey W, Mannerino A, Lipp ES, Herndon JE, 2nd, McSherry F, Desjardins A, Randazzo D, Friedman HS, Peters KB. Phase II Study of Bevacizumab and Vorinostat for Patients with Recurrent World Health Organization Grade 4 Malignant Glioma. *Oncologist* 2018;**23**: 157-e21.

141. Su JM, Kilburn LB, Mansur DB, Krailo M, Buxton A, Adekunle A, Gajjar A, Adamson PC, Weigel B, Fox E, Blaney SM, Fouladi M. Phase 1/2 Trial of Vorinostat and Radiation and Maintenance Vorinostat in Children with Diffuse Intrinsic Pontine Glioma: A Children's Oncology Group Report. *Neuro Oncol* 2021.

142. Galanis E, Anderson SK, Miller CR, Sarkaria JN, Jaeckle K, Buckner JC, Ligon KL, Ballman KV, Moore DF, Jr., Nebozhyn M, Loboda A, Schiff D, et al. Phase I/II trial of vorinostat combined with temozolomide and radiation therapy for newly diagnosed glioblastoma: results of Alliance N0874/ABTC 02. *Neuro Oncol* 2018;**20**: 546-56.

143. Galanis E, Jaeckle KA, Maurer MJ, Reid JM, Ames MM, Hardwick JS, Reilly JF, Loboda A, Nebozhyn M, Fantin VR, Richon VM, Scheithauer B, et al. Phase II trial of vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study. *J Clin Oncol* 2009;**27**: 2052-8.

144. Friday BB, Anderson SK, Buckner J, Yu C, Giannini C, Geoffroy F, Schwerkoske J, Mazurczak M, Gross H, Pajon E, Jaeckle K, Galanis E. Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma: a north central cancer treatment group study. *Neuro Oncol* 2012;**14**: 215-21.

145. Su JM, Murray JC, McNall-Knapp RY, Bowers DC, Shah S, Adesina AM, Paulino AC, Jo E, Mo C, Baxter PA, Blaney SM. A phase 2 study of valproic acid and radiation, followed by maintenance valproic acid and bevacizumab in children with newly diagnosed diffuse intrinsic pontine glioma or high-grade glioma. *Pediatr Blood Cancer* 2020;**67**: e28283.

146. Krauze AV, Myrehaug SD, Chang MG, Holdford DJ, Smith S, Shih J, Tofilon PJ, Fine HA, Camphausen K. A Phase 2 Study of Concurrent Radiation Therapy, Temozolomide, and the Histone Deacetylase Inhibitor Valproic Acid for Patients With Glioblastoma. *Int J Radiat Oncol Biol Phys* 2015;**92**: 986-92.

147. Lee EQ, Reardon DA, Schiff D, Drappatz J, Muzikansky A, Grimm SA, Norden AD, Nayak L, Beroukhim R, Rinne ML, Chi AS, Batchelor TT, et al. Phase II study of panobinostat in combination with bevacizumab for recurrent glioblastoma and anaplastic glioma. *Neuro Oncol* 2015;**17**: 862-7.

148. Zavras PD, Shastri A, Goldfinger M, Verma AK, Sauntharajah Y. Clinical Trials Assessing Hypomethylating Agents Combined with Other Therapies: Causes for Failure and Potential Solutions. *Clin Cancer Res* 2021;**27**: 6653-61.

149. Sakamoto D, Takagi T, Fujita M, Omura S, Yoshida Y, Iida T, Yoshimura S. Basic Gene Expression Characteristics of Glioma Stem Cells and Human Glioblastoma. *Anticancer Res* 2019;**39**: 597-607.

150. Kim J, Woo AJ, Chu J, Snow JW, Fujiwara Y, Kim CG, Cantor AB, Orkin SH. A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. *Cell* 2010;**143**: 313-24.

151. Zhang Y, Chen F, Donehower LA, Scheurer ME, Creighton CJ. A pediatric brain tumor atlas of genes deregulated by somatic genomic rearrangement. *Nat Commun* 2021;**12**: 937.

152. Mermel CH, Schumacher SE, Hill B, Meyerson ML, Beroukhim R, Getz G. GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol* 2011;**12**: R41.

TABLE and FIGURE LEGENDS

Table 1. Clinical trials of non-cytotoxic epigenetic drugs (or pro-drugs) to treat gliomas. Overall objective response rate (ORR) per Radiology Assessment in Neuro-Oncology (RANO) criteria for gliomas with and without contrast-enhancement on MRI unless indicated otherwise. *Yes = Patients received one or more rounds of radio-/chemotherapy prior to study inclusion. **Growth rate reduction assessed by longitudinal MRI measurements of three-dimensional tumor volume before and after treatment. ***Compared to intervention without HDAC-inhibitor or historic control. OS = overall survival. PFS = progression free survival. No glioma clinical trial data is available at this time for EZH2-, KDM1A- or DHODH-inhibition.

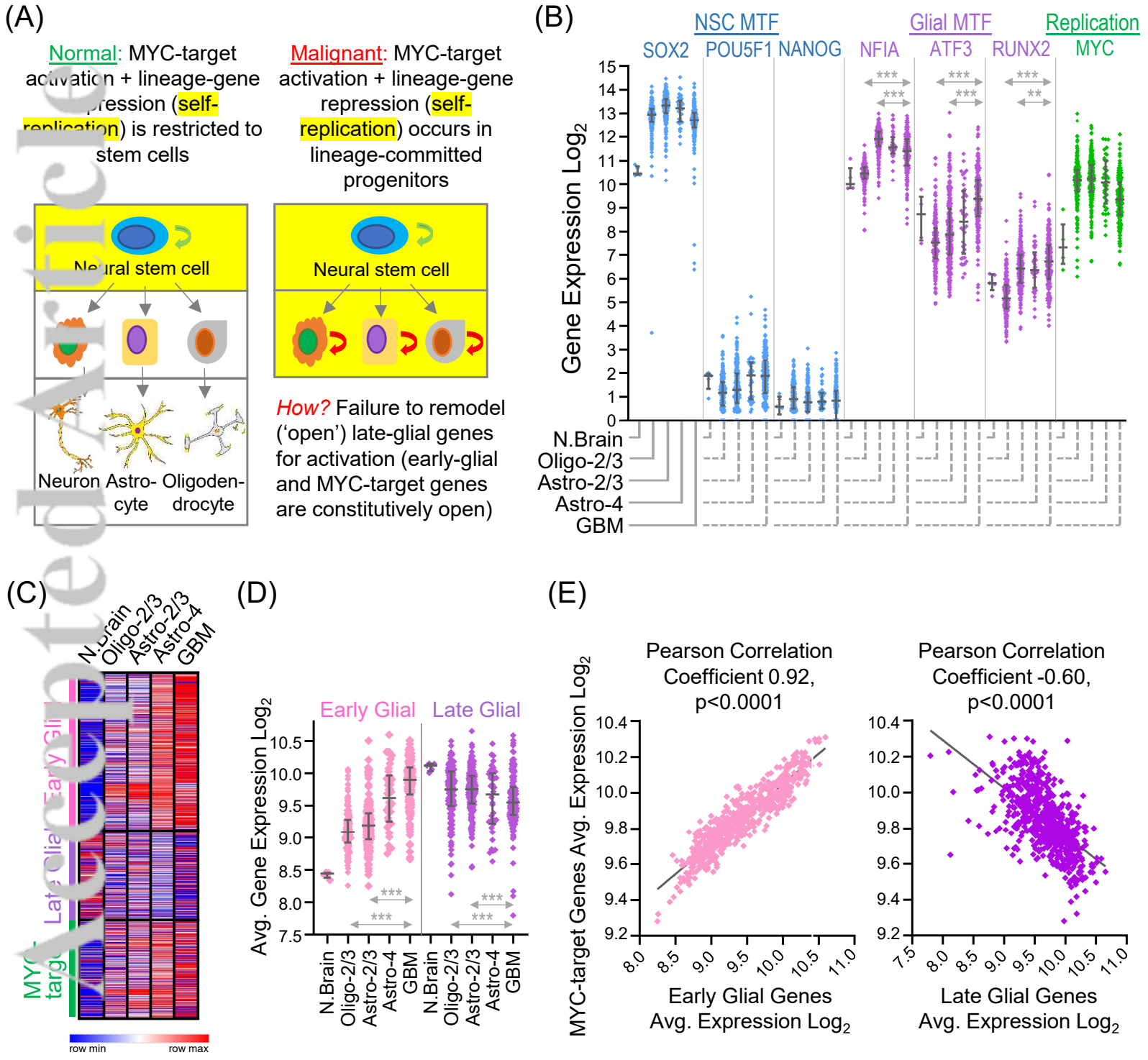
Figure 1. A) Normal self-replication (replication without onward lineage-differentiation) is restricted to tissue stem cells, but malignant self-replication is not. **B) Of the master transcription factor (MTF) circuit SOX2/POU5F1/NANOG** [18, 149] **that produces neural stem cells (NSC), only SOX2 that is stably expressed through glial lineage-maturation[20] is also highly expressed in gliomas, however, the MTF circuit NFIA/ATF3/RUNX2**[19] **that compels NSC commitment into glioma lineage-precursors is highly expressed.** Normal brain n=5; Oligo-2/3 = oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade 2 or 3, n=176; Astro-2/3 = astrocytoma, IDH-mutant, WHO grade 2 or 3, n=241; Astro-4 = astrocytoma, IDH-mutant, WHO grade 4, n=38; GBM = glioblastoma, IDH wild-type, WHO grade 4, n=196. TCGA RNA-seq public data, RSEM values (counts normalized by RNA-Seq by Expectation Maximization). Mann-Whitney 2-sided test ***p<0.0001, **p<0.005. **C) Consistent with lineage MTF circuit wiring, gliomas significantly upregulate 539 genes that characterize astroglial lineage-commitment/early-maturation (early glial)**[20](67 known MYC-target genes excluded), **and 337 MYC-target genes identified by chromatin-immunoprecipitation**[150], but there is anomalous suppression of 310 astroglial late lineage-differentiation (late glial) genes[20]**.** Average expression of each gene in glioma samples of each glioma sub-type (samples as per panel B). **D) More aggressive gliomas demonstrate deeper suppression of late-glial, and more upregulation of early-glial, genes**. Average expression per sample of all genes in a category. Lines=median±IQR, ***p<0.0001 Mann-Whitney 2-sided test (samples as per panels B). **E) Early-glial and MYC-target gene expression positively correlate (67 known MYC-target genes were excluded from early glial genes analyzed); Late-glial and MYC-target gene expression negatively correlate**.

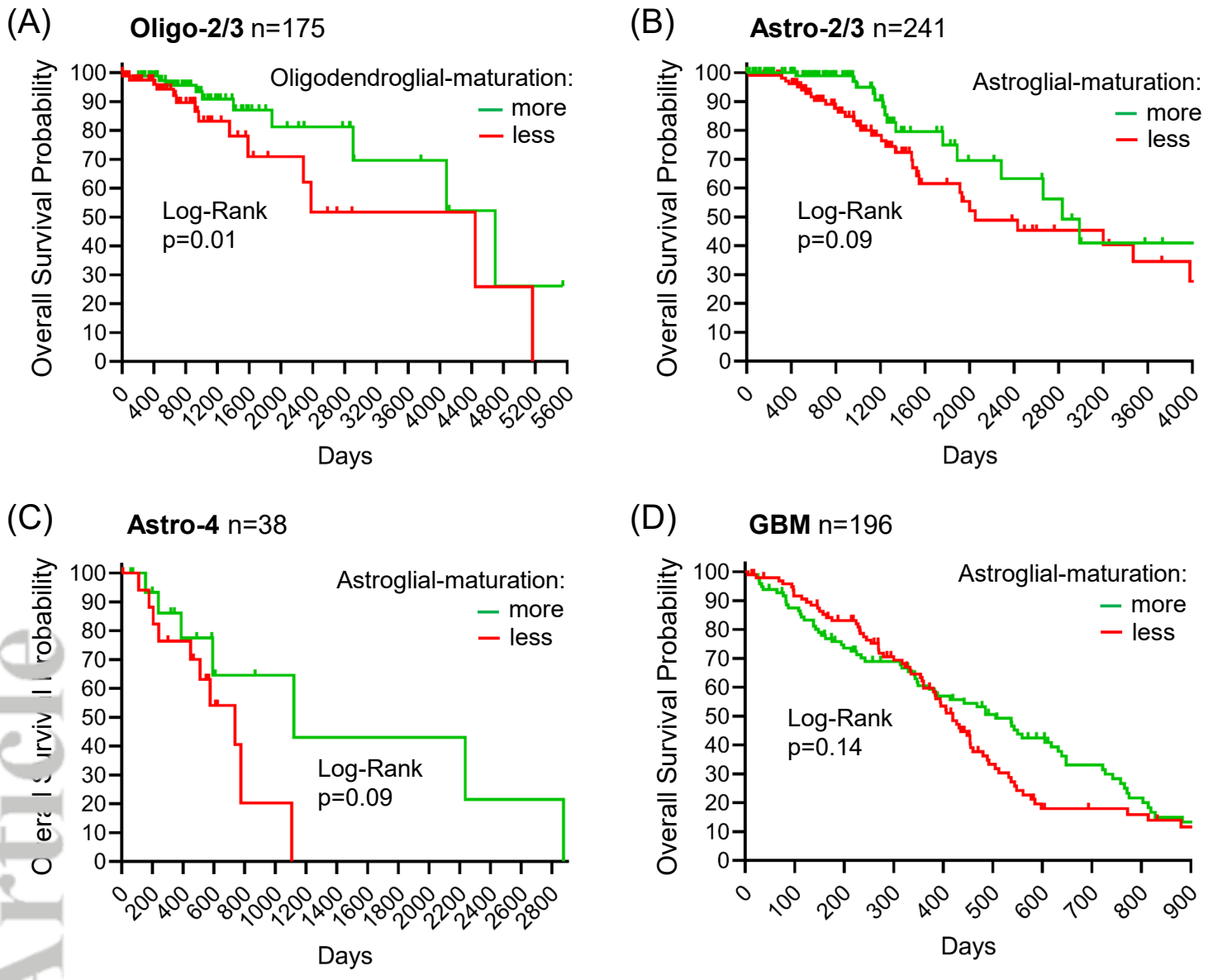
Figure 2. Less onward maturation of cells committed into the glial lineage (higher expression of commitment/early-glial genes, lower expression of late-glial genes) **independently predicts and stratifies for worse overall survival within well-established EANO/WHO glioma sub-types.** Cases within pathologic subgroups were stratified around the median average expression of late oligodendroglial lineage-genes (as identified in[28]) for Oligo-2/3, or early astroglial lineage-genes (as identified in [20]) for Astro-2/3, Astro-4 and GBM. Overall survival data TCGA. **A) Oligo-2/3. B) Astro-2/3. C) Astro-4. D) GBM.**

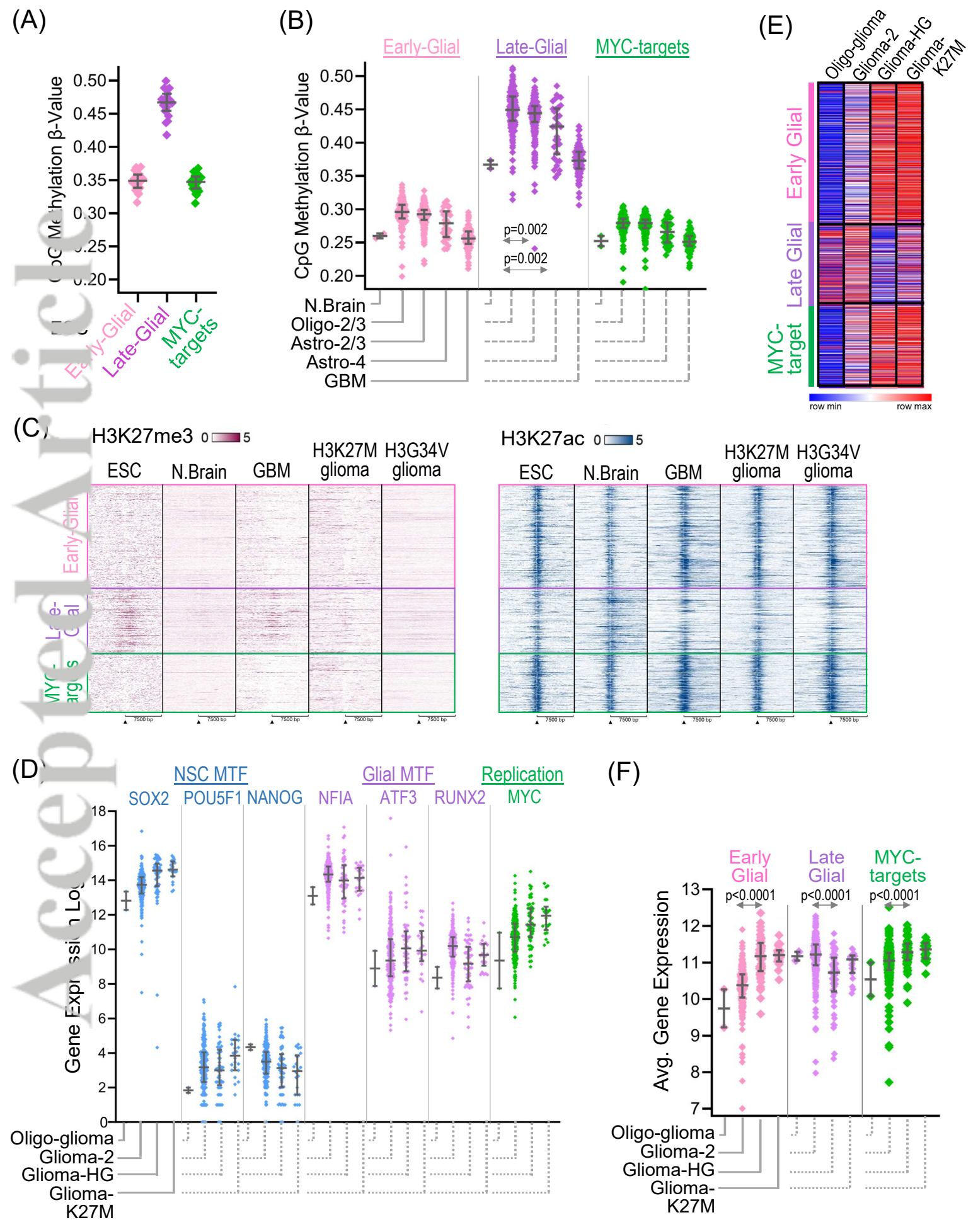
Figure 3. Glioma-genesis exploits differences in epigenetic landscape: MYC-target and early-glial genes have a constitutively accessible epigenetic configuration but late-glial genes do not. A) me-CpG at early-glial, late-glial and MYC-target genes in embryonic stem cells (ESC) (gene groups as per figure 1). Public data GSE31848116. Median ± inter-quartile range (IQR). ESC n=19. me-CpG measured by Illumina 450K array. **B) me-CpG at early-glial, late-glial and MYC-target genes in normal cerebral cortex versus clinico-pathologic types of glioma.** me-CpG measured by Illumina 450K array, TCGA public data as per figure 1. P-value Mann-Whitney test 2-sided. **C) H3K27me3 and H3K27ac distributions at early-glial, late-glial and MYC-target genes in ESC, normal brain cortex and gliomas without and with histone 3 gene (H3F3A) mutations.** Public ChIP-seq data (FastQ files processed by UseGalaxy suite of tools): ESC H3K27me3 – GSM428295 (Encode); Normal cerebral cortex H3K27me3 – GSM772833 (Encode); ESC H3K27ac – GSM466732 (Encode); Normal cerebral cortex H3K27ac – GSM1112812 (Encode); GBM (SF9402), H3K27M glioma (SF7761) and H3G34V glioma (KNS42) H3K27me3 and H3K27ac GSE162976117. Plots using EASEQ. **D) Pediatric gliomas recapitulate the glial lineage-specifying MTF (NFIA, ATF3, RUNX2) wiring observed in adult gliomas (Figure 1). Of NSC-specifying MTF, only SOX2 is highly expressed, again as also seen in adult gliomas, and as expected from stable SOX2 expression through normal glial lineage-maturation[20].** Oligo-glioma = oligodendroglioma n=2; Glioma-2 = glioma grade 2 n=236; Glioma-HG = glioma high-grade n=53; Glioma-K27M = glioma containing H3F3A K27M mutation n=22. Pediatric Brain Tumor Atlas [151] public data, RSEM values (counts normalized by RNA-Seq by Expectation Maximization). **E) More aggressive pediatric gliomas display deeper late-glial gene suppression, accompanied by more upregulation of early-glial and MYC-target genes.** Heat map collapsed on average expression per gene in all the samples in each subtype (samples as per panel D). **F) Average expression of all early-glial, late-glial and MYC-target genes in each pediatric glioma sample (samples as per panel D).** P-values Mann-Whitney test 2-sided.

Figure 4. Gliomas contain recurrent genetic alterations expected to preserve or increase H3K27me3 and me-CpG, and simultaneously decrease H3K27ac and other chromatin remodeling needed to activate late-glial genes. TCGA public data, n=651. **A) Significant correlation between gene copy number and expression of the chromatin remodelers.** The GISTIC2 method[152] produced segmented copy number variant data mapped to genes to produce gene-level estimates. Gene-level transcription estimates by RNA-Sequencing were analyzed as $\log_2(x+1)$ transformed RSEM normalized counts. Pearson correlation coefficients, p-value 2-sided. **B) Glioma-genesis alters several classes of chromatin remodelers, at frequencies that increase with aggression of disease.** Percentage of cases in each WHO/EANO glioma sub-group with the indicated gene copy number or mutation changes. Gene-level copy number estimates were generated by the GISTIC2 method were thresholded to estimated values -2,-1,0,1,2 representing homozygous or single copy deletion (del), diploid normal copy, or low-level or high-level copy number amplification (amp). **C) The H3K27 demethylase KDM6A is significantly less expressed in gliomas from males versus females**. ***p<0.0001, Mann-Whitney test, 2-sided.

Figure 5. Summary. Glioma-genesis selects to impede chromatin-remodeling needed to activate late-glial lineage genes, thus converting the exponential replications of glial-lineage committed progenitors into self-replications (glioma 'stem' cells). Inhibiting repressing epigenetic enzymes enables glial-lineage transcription factors, already highly expressed in glioma stem cells, to activate late-glial genes and hence terminate malignant self-replications, without a need for an intact apoptosis program.



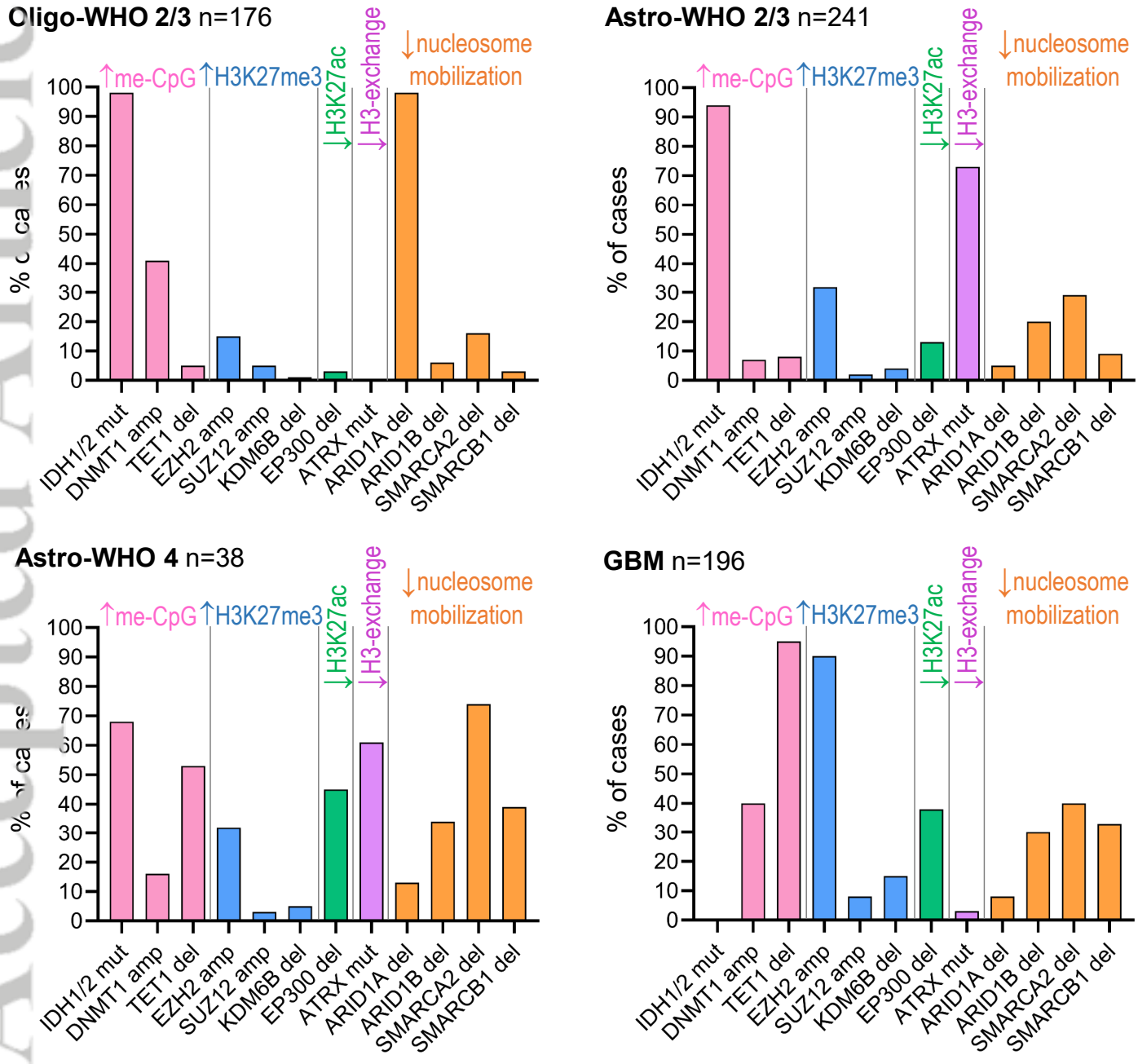

(A)
Oligo-2/3 n=175
Oligodendroglial-maturation:
more
less
Log-Rank
p=0.01
Overall Survival Probability
Days
(B)
Astro-2/3 n=241
Astroglial-maturation:
more
less
Log-Rank
p=0.09
Overall Survival Probability
Days
(C)
Astro-4 n=38
Astroglial-maturation:
more
less
Log-Rank
p=0.09
Days
(D)
GBM n=196
Astroglial-maturation:
more
less
Log-Rank
p=0.14
Overall Survival Probability
Days



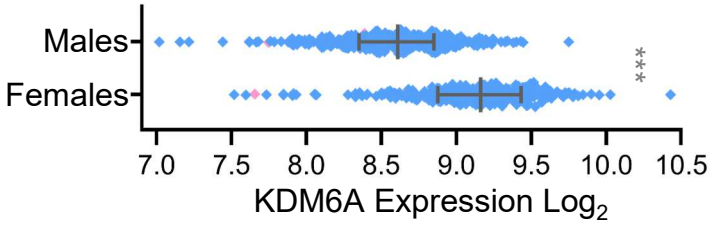
(A)

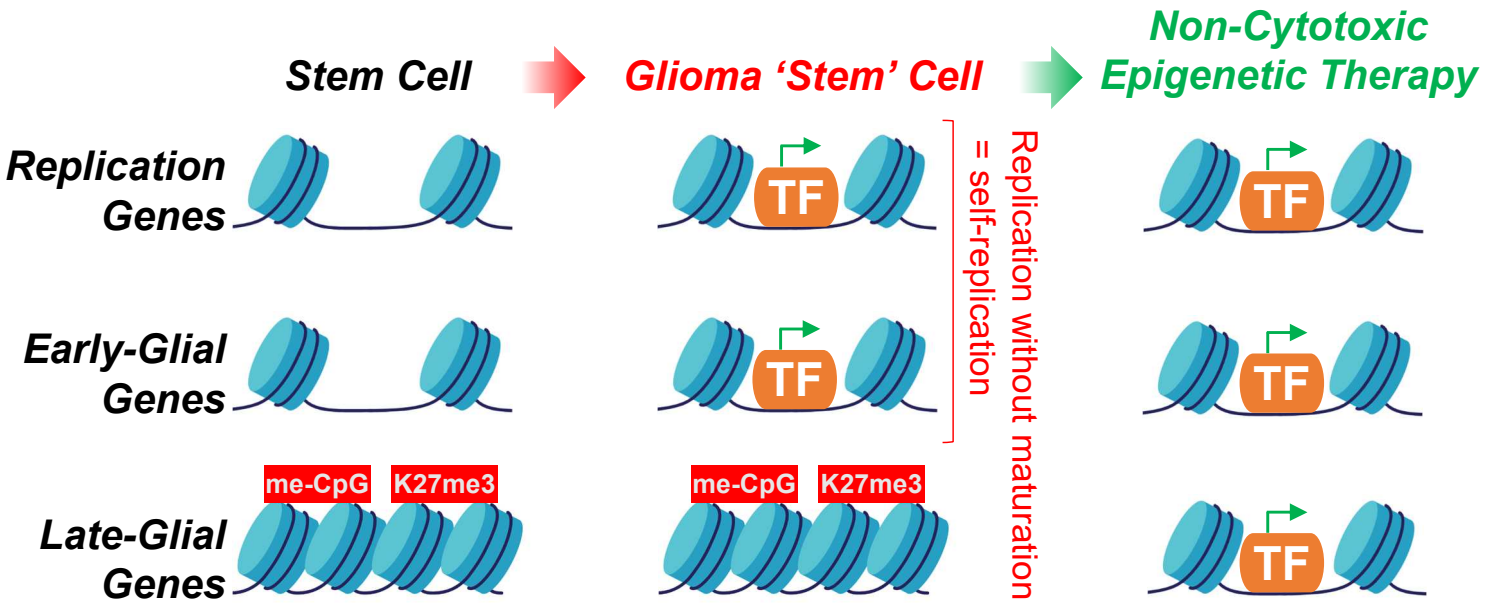
	DNMT1	TET1	EZH2	SUZ12	KDM6B	EP300	ARID1A	ARID1B	SMARCA2	SMARCB1
Pearson's r	0.31	0.64	0.48	0.22	0.20	0.46	0.45	0.43	0.49	0.55
P-value	<0.0001									

(B)



(C)



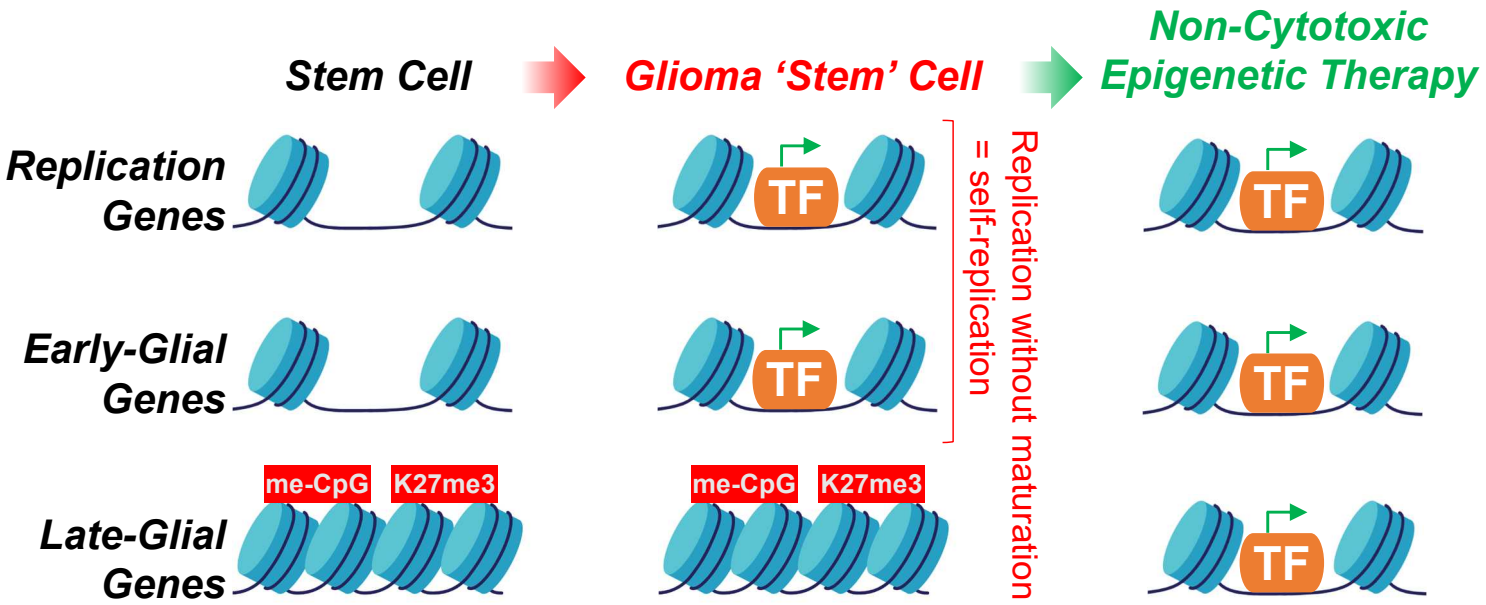


1. Chromatin is constitutively 'open' at ***Replication*** and ***Early-Glial*** genes, but 'closed' at ***Late-Glial*** genes

2. **Glioma-genesis** selects to disrupt machinery that opens chromatin. **Glial-lineage TF** thus activate replication and early-glial, but not late-glial genes (**malignant self-replication**)

3. Drugs to inhibit chromatin-closing enzymes allow glial-lineage TF to activate **terminal glial-fates**

TF = transcription factor me-CpG = methylated CpG K27me3 = histone 3 lysine 27 trimethylation



1. Chromatin is constitutively 'open' at ***Replication*** and ***Early-Glial*** genes, but 'closed' at ***Late-Glial*** genes

2. **Glioma-genesis** selects to disrupt machinery that opens chromatin. **Glial-lineage TF** thus activate replication and early-glial, but not late-glial genes (**malignant self-replication**)

3. Drugs to inhibit chromatin-closing enzymes allow glial-lineage TF to activate **terminal glial-fates**

TF = transcription factor me-CpG = methylated CpG K27me3 = histone 3 lysine 27 trimethylation

Table 1. Clinical trials of non-cytotoxic epigenetic drugs (or pro-drugs) to treat gliomas. Overall objective response rate (ORR) per Radiology Assessment in Neuro-Oncology (RANO) criteria for gliomas with and without contrast-enhancement on MRI unless indicated otherwise. *Yes = Patients received one or more rounds of radio-/chemotherapy prior to study inclusion. **Growth rate reduction assessed by longitudinal MRI measurements of three-dimensional tumor volume before and after treatment. ***Compared to intervention without HDAC-inhibitor or historic control. OS = overall survival. PFS = progression free survival. No glioma clinical trial data is available at this time for EZH2-, KDM1A- or DHODH-inhibition.

Trial Identifier	Drug	Phase	Status	Prior XRT/ Chemo*	Glioma subtype/contrast-enhancement on MRI	Results
NCT02073994	Ivosidenib (IDH1-inhibitor)	I	Not recruiting	Yes	Glioma, IDH-mutant (n=66); non-contrast (n=35) and contrast-enhancing (n=31)	ORR 2.9% and growth rate reduction** by 14% in 24 evaluable patients with non-contrast-enhancing gliomas
NCT02481154	Vorasidenib (IDH1/2-inhibitor)	I	Not recruiting	Yes	Glioma, IDH-mutant (n=52); non-contrast (n=22) and contrast-enhancing (n=30)	ORR 18% in non-contrast-enhancing gliomas
NCT04164901	Vorasidenib (IDH1/2-inhibitor)	III	Recruiting	No	Glioma, IDH-mutant (WHO II); non-contrast-enhancing	Pending
NCT03343197	Ivosidenib (n=12);Vorasidenib (n=13)	I	Not recruiting	Yes	Glioma, IDH-mutant (n=25); non-contrast enhancing	2-HG levels in resected glioma tissue substantially lowered by both ivosidenib and vorasidenib
NCT03684811	Olutasidenib (IDH1-inhibitor)	Ib/II	Not recruiting	Yes	Glioma, IDH-mutant (n=24); contrast-enhancement not described in interim results	ORR 4%
NCT04458272	DS-1001b (IDH1-inhibitor)	II	Not recruiting	Yes	Glioma, IDH-mutant (n=38); non-contrast (n=9) and contrast-enhancing (n=29)	ORR 22% in non-contrast-enhancing gliomas; ORR 14% in contrast-enhancing gliomas
NCT02746081	BAY1436032 (IDH1-inhibitor)	I	Not recruiting	Yes	Glioma, IDH mutant (n=49); non-contrast (n=2) and contrast-enhancing (n=33); GBM (n=14)	ORR 11% in gliomas (RANO criteria for contrast-enhancing gliomas)
NCT03666559	5-azacytidine (DNMT1-inhibitor)	II	Recruiting	Yes	Glioma, IDH-mutant; contrast-enhancement not described	Pending
NCT03922555	Decitabine (DNMT1-inhibitor) + cedazuridine (CDA-inhibitor)	I	Recruiting	Yes	Glioma, IDH-mutant; non-contrast enhancing	Pending
NCT00238303	Vorinostat (HDAC-inhibitor)	II	Completed	Yes	GBM (n=52)	ORR 4% (Mac Donald criteria); Modest increase in PFS (median 11.2 months; range 6.8 to 28) and OS (median 5.7 months; range 0.7 to 28)***
NCT00641706	Vorinostat + bortezomib (proteasome inhibitor)	II	Completed	Yes	GBM (n=37)	ORR 3% (modified Mac Donald criteria); No improvement in PFS and OS***
NCT01738646	Vorinostat + bevacizumab (VEGF-antibody)	II	Completed	Yes	GBM (n=40)	No improvement in PFS and OS***
NCT01266031	Vorinostat + bevacizumab	II	Completed	Yes	GBM (n=49)	No improvement in PFS and OS***

NCT00762255	Vorinostat + bevacizumab + irinotecan	I	Completed	Yes	GBM (n=19)	Significantly increased OS in patients receiving higher doses of vorinostat compared to patients receiving lower doses (10.1 vs. 5.7 months respectively)***
NCT00939991	Vorinostat + bevacizumab + temozolomide	I/II	Completed	Yes	GBM (n= 39)	ORR 43.6% (RANO criteria for contrast-enhancing gliomas); No improvement in PFS and OS***
NCT00268385	Vorinostat + radiation therapy + temozolomide followed by vorinostat + temozolomide	I/II	Not recruiting	No	GBM (n=107)	No improvement in PFS and OS***
NCT01189266	Vorinostat + radiation therapy followed by vorinostat	I/II	Completed	No	Pediatric diffuse intrinsic pontine glioma (n=76)	No improvement in PFS and OS***
NCT00859222	Panobinostat (HDAC-inhibitor) + bevacizumab	I/II	Completed	Yes	GBM (n=24) and anaplastic astrocytoma (WHO III; n= 15)	ORR 29.2% for GBM cohort and 26.7% for astrocytoma (WHO III) cohort (RANO criteria for contrast-enhancing gliomas); No improvement in PFS and OS***
NCT00302159	Valproic acid (HDAC-inhibitor) + radiation therapy + temozolomide	II	Completed	No	GBM (n=30)	ORR 0% (RANO criteria for contrast-enhancing gliomas); Improved OS (70% after 12 months; CI 76 – 98) and PFS (70% after 6 months; CI 57 – 87)***
NCT00879437	Valproic acid + radiation therapy followed by valproic acid and bevacizumab	II	Completed	No	Pediatric diffuse intrinsic pontine glioma (DIPG; n=18) and high grade glioma (HGG; n=14)	ORR 55% for DIPG cohort and 42% for HGG cohort (RANO criteria for contrast-enhancing gliomas); No improvement in PFS and OS***