INVITED REVIEW

Changing paradigms in oncology: Toward noncytotoxic treatments for advanced gliomas

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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 glioma 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...
In the adult mammalian brain, and NSC replicate rarely, for example, once every several months. Moreover, frequent deletion of CDKN2A is a replication-control program that senses cell stress (Grade 1—curable by surgery; Grade 2/3—overall survival durations of several years; Grade 4—overall survival durations months to few years\(^6\)).

Apoptosis is a replication-control program that senses cell stress (Grade 1—curable by surgery; Grade 2/3—overall survival durations of several years; Grade 4—overall survival durations months to few years\(^6\)). Moreover, frequent deletion of CDKN2A characterizes the 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 radiotherapy/chemotherapy, have rates of p53/p16-pathway inactivation exceeding 85%\(^5,10\) (Grade 1—curable by surgery; Grade 2/3—overall survival durations of several years; Grade 4—overall survival durations months to few years\(^6\)).

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 Reference 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.\(^7\) In the adult mammalian brain, neuronal stem cells (NSC) reside in neocortical-genic niches in the dentate gyrus of the hippocampus and in subventricular zones lining the lateral ventricles.\(^11-13\) NSC replicate rarely, for example, 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. Each replication is coupled to the 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).

3 | 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. 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 Reference 1B), as has the MTF circuit NFIA/ATF3/RUNX2 that commits NSC to glial-lineage fates.

What then is the MTF combination highly expressed in glioma cells? Gliomas express the MTF circuit SOX2/POU5F1/NANOG, which 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, 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).

4 | WHY DOES NEOPLASTIC EVOLUTION SELECT TO TRANSFORM IN LINEAGE-SPECIFYING MTF CIRCUIT CONTEXTS?

MYC, and its paralogues MYCN and MYCL, 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). The emergence of multicellularity (metazoa) occasioned MYC subordination to MTF circuits that create diverse lineages and hierarchies. MTF circuits that create tissue stem cells permit only low-grade MYC activity (shown in several tissue contexts, reviewed in Reference 3) presumably because quiescence protects the genomes of these cells vital to a multicellular organism overall life-span. In contrast, MTF circuits that commit cells into lineage activate and cooperate with MYC (or MYCN or MYCL) to propel replications every 1 to 2 days (reviewed in Reference 3), to in this way ensure the transit amplification needed to replenish specialized tissue cells lost to daily wear-and-tear. “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).

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 subtypes (Figure 2). Similar observations have been made by others,

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. 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. 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 in7). 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, for example, in familial gliomas, then propagate downstream into lineage-committed progenitors, wherein phenotypic consequences and clonal advantage most prominently emerge (reviewed in Reference 3). Upstream mutations can also skew downstream commitment decisions, for example, ATRX mutations skew NSC commitment decisions toward astro-over oligodendrogial or neuronal lineage fates.
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/MYCL. Several experimental conditions have been shown to correct this anomaly, resuming glioma lineage-maturation and hence terminating self-replication/tumor-initiating capacity. To develop such remedies for clinical use, it would be useful to understand how the failure occurs in the first-place.
The epigenetic landscape at late-glial genes enables their oncogenic repression

Here, the term “epigenetics” or chromatin refers to the 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, posttranslationally modify histones, exchange or reposition histones, to in this way facilitate (on) or obstruct (off) transcription of genes by the basal transcription factor machinery. 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 methytransferases DNMT1, DNMT3A and/or DNMT3B.

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).

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). 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). Elevated H3K27me3 at late-glial genes in ESC is erased with ontogeny into the normal brain (Figure 3C), 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), however, acquisition of the H3K27ac “on” mark, that occurs with ontogeny into the normal brain, fails to occur (Figure 3C). Failure to activate late-glial genes might be explained by different lineage-trajectory, for example, with H3F3A G34R/V-mutated gliomas that originate from GSX2/DLX-expressing interneuron-progenitors, however, these tumors also fail to activate late-neuronal programs. 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 the attenuated transition to late-glial genes is erased with ontogeny into the normal brain (Figure 3C).
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) (A) Public data GSE31848116. Median ± interquartile 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 vs clinicopathologic 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 GSE162976. 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.26 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 Atlas61 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 two-sided [Color figure can be viewed at wileyonlinelibrary.com]
programs, worse in higher grade disease (Figure 3E, F). The constitutive difference in the 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, have been shown for other tissue lineages also.⁶⁰,⁷³,⁷⁴

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).⁶⁶,⁷⁶-⁷⁹ 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.⁸⁰

Deletions of the H3K27me3 eraser KDM6B; KDM6A decrement in males. KDM6B is a H3K27me2 and H3K27me3-specific demethylase,⁶⁰,⁸¹,⁸² 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).⁸¹

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)⁸³ (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.⁸⁴

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.⁴,⁶,⁷⁶ ATRX is linked to the histone chaperone DAXX and to histone H3.3 exchange (reviewed in Reference 85)—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.⁸⁰ ATRX has also been implicated in telomere length regulation and DNA repair,⁵⁴,⁶⁶,⁷⁶ and cytogenetic instability resulting from ATRX loss might explain concordance of ATRX- with TP53-mutations.⁹⁰

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-Translocation (TET) family DNA methylcytosine dioxygenases that erase me-CpG.⁹¹,⁹² 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,⁶⁵,⁹³ 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.⁹⁴,⁹⁵

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).⁷⁷,⁷⁸,⁹⁷-⁹⁹ 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.¹⁰⁰,¹⁰¹

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,⁷⁰,⁷⁸ 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 coactivator recruited into lineage MTF protein hubs.⁵⁹,⁶²,⁷³,⁷⁴

Deletions of SWI/SNF-family ATP-dependent chromatin remodelers that repression 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% to 70% in grade 4 astrocytomas, and also in 20% to 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, for example, 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.⁵,⁷,¹⁰,⁷³

Alterations to lineage MTF. Genes for lineage MTF themselves, for example, 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 genes⁵⁰,⁷⁴,¹⁰¹,¹⁰³ (reviewed in Reference 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, replicatons and pace of disease in a merciless clinical reality\textsuperscript{17} (reviewed in Reference \textsuperscript{104}).

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).

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.

EZH2 downregulation with short hairpin RNA or with a small molecule (DZNep) impaired glioma self-replication in vitro and tumor-initiation in vivo.

Consistent with a nonapoptosis pathway, there was no significant effect on glioma cell viability, even as sphere morphology (a measure of self-replication) and proliferation were reduced.

In H3F3A K27M-mutated gliomas, EZH2 inhibition decreased proliferation in vitro and increased survival in mice. Another study looked specifically for a cytotoxic effect of EZH2 inhibition in the glioma cells to explain the cytoreduction, and did not find any.

ATP-dependent chromatin remodelers of the ISWI and CHD families, for example, 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, implying ISWI/CHD-family members are candidate targets for therapy.

Mitochondrial outputs other than AKG, for example, 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.

Implicating CTP specifically, CTP-restoration with exogenous cytidine prevented terminal-differentiation induction by the DHODH-inhibitor leflunomide. 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.

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.

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
| 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); noncontrast (n = 35) and contrast-enhancing (n = 31) | ORR 2.9% and growth rate reduction\(^b\) by 14% in 24 evaluable patients with noncontrast-enhancing gliomas |
| NCT02481154     | Vorasidenib (IDH1/2-inhibitor) | I     | Not recruiting | Yes | Glioma, IDH-mutant (n = 52); noncontrast (n = 22) and contrast-enhancing (n = 30) | ORR 18% in noncontrast-enhancing gliomas                                |
| NCT04164901     | Vorasidenib (IDH1/2-inhibitor) | III   | Recruiting   | No  | Glioma, IDH-mutant (WHO II); noncontrast-enhancing | Pending                                                                |
| NCT03343197     | Ivosidenib (n = 12); Vorasidenib (n = 13) | I     | Not recruiting | Yes | Glioma, IDH-mutant (n = 25); noncontrast 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); noncontrast (n = 9) and contrast-enhancing (n = 29) | ORR 22% in noncontrast-enhancing gliomas; ORR 14% in contrast-enhancing gliomas |
| NCT02746081     | BAY1436032 (IDH1-inhibitor) | I     | Not recruiting | Yes | Glioma, IDH-mutant (n = 49); noncontrast (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; noncontrast 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 6.8-28) and OS (median 5.7 months; range 0.7-28)\(^c\) |
| NCT00641706     | Vorinostat + bortezomib (proteasome inhibitor) | II    | Completed    | Yes  | GBM (n = 37) | ORR 3% (modified Mac Donald criteria); No improvement in PFS and OS\(^c\) |
| NCT01738646     | Vorinostat + bevacizumab (VEGF-antibody) | II    | Completed    | Yes  | GBM (n = 40) | No improvement in PFS and OS\(^c\) |
| NCT01266031     | Vorinostat + bevacizumab | II    | Completed    | Yes  | GBM (n = 49) | No improvement in PFS and OS\(^c\) |
| 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)\(^c\) |
| 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\(^c\) |
| NCT00268385     | Vorinostat + radiation therapy + temozolomide followed by vorinostat + temozolomide | I/II  | Not recruiting | No  | GBM (n = 107) | No improvement in PFS and OS\(^c\) |
| NCT01189266     | Vorinostat + radiation therapy followed by vorinostat | I/II  | Completed    | No  | Pediatric diffuse intrinsic pontine glioma (n = 76) | No improvement in PFS and OS\(^c\) |
5-azacytidine also suppressed IDH-wildtype GBM growth in vitro and in xenografts. 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). 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.

IDH1-mutated glioma cells were released to terminal differentiation by small molecule inhibitors of mutant-IDH1 in preclinical studies. Clinical trial results have been reported for the mutant-IDH1-inhibitorsivosidenib (Agios), olutasidenib (Forma Therapeutics), DS-1001b (Daichi Sankyo), BAY1436032 (Bayer), and the dual mutant-IDH1/IDH2-inhibitor vorasidenib, to treat relapsed/refractory gliomas with and without contrast enhancement on MRI. These low to modest response rates compare unfavorably to high response rates and regulatory approval of mutant-IDH-inhibitors to treat IDH-mutated 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. 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 in the normal brain. This measurement problem is not expected in a trial evaluating vorasidenib as a first-line treatment of IDH-mutant gliomas, results of which are pending (Table 1).

Histone deacetylases (HDAC) and lysine demethylase 1A (KDM1A) are implicated in the 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 preclinical in vivo studies. 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). Limited success in translating the preclinical observations into clinical therapy could reflect that HDACs and KDM1A have nonhistone 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 preclinically 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, for example, 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 noncytotoxic DNMT1-targeting regimens of decitabine or 5-azacytidine in patients with myeloid malignancies, including fragile elderly patients with the 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 preclinical data supports the 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 noncytotoxic drugs, all aiming to renew lineage-maturation, is routine treatment practice for some myeloid malignancies—p53/p16-attenuation and pathobiology of malignant self-replication recommends this approach to gliomas too.152

10 | 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: Nikolaus von Knebel Doeberitz and Yogen Saunthararajah. Funding: Daniel Paech and Yogen Saunthararajah. Collection and assembly of data: Nikolaus von Knebel Doeberitz and Yogen Saunthararajah. Visualization of data: Nikolaus von Knebel Doeberitz and Yogen Saunthararajah. Validation of data: All authors. Manuscript writing: Nikolaus von Knebel Doeberitz and Yogen Saunthararajah. 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.

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CONFLICT OF INTEREST

Ownership: Yogen Saunthararajah—EpiDestiny. Income: None. Research support: none. Intellectual property: Yogen Saunthararajah—patents around tetrahydrouridine, decitabine and 5-azacytidine (US 9259469 B2; US 9265785 B2; US 9895391 B2) and cancer differentiation therapy (US 9926316 B2). Stefan Pusch—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 our study are available from the corresponding author upon reasonable request.

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