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Translational significance of CDKN2A/B homozygous deletion in isocitrate dehydrogenase-mutant astrocytoma

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Abstract

Isocitrate dehydrogenase (IDH) 1 or 2 mutations confer a favorable prognosis compared to IDH-wildtype in astrocytoma, frequently denoting a lower grade malignancy. However, recent molecular profiling has identified specific aggressive tumor subgroups with clear clinical prognostic implications that are independent of histologic grading. The homozygous deletion of CDKN2A/B is the strongest implicated independent indicator of the poor prognosis within IDH-mutant astrocytoma, and the identification of this alteration in these lower histologic grade tumors transforms their biology toward an aggressive grade 4 phenotype clinically. CDKN2A/B homozygous deletion is now sufficient to define a grade 4 tumor in IDH-mutant astrocytomas regardless of histologic appearance, yet there are currently no effective molecularly informed targeted therapies for these tumors. The biological impact of CDKN2A/B homozygous deletion in IDH-mutant tumors and the optimal treatment strategy for this molecular subgroup remains insufficiently explored. Here we review the current understanding of the translational significance of homozygous deletion of CDKN2A/B gene expression in IDH-mutant astrocytoma and associated diagnostic and therapeutic implications.

Keywords

CDKN2A/B | grade 4 glioma | IDH-mutant astrocytoma

Prognostic Significance of CDKN2A/B Homozygous Deletion in Isocitrate Dehydrogenase-Mutant Astrocytoma

Molecular diagnostics are increasingly incorporated into central nervous system (CNS) tumor classification to reflect the central role of genomic alterations in tumor biology and prognostic stratification. Historically, tumors of the CNS in adults have been defined based on histopathologic criteria alone, including the presumed cell of origin and degree of cell differentiation, with additional characterization based on the presence of high-grade features including microvascular proliferation and necrosis.[1,2] There is now an increasing recognition that tumor behavior is inadequately predicted from histologic grading alone and molecular profiling can characterize tumors into more favorable or poor prognostic subsets. The recognition of isocitrate dehydrogenase (IDH)-mutations in glioma as an early distinct prognostic alteration defines lower grade glioma including grade 2 and 3 astrocytoma and oligodendroglioma, with intact or codeleted 1p/19q,

respectively, and confers a favorable prognosis compared to the IDH-wildtype grade 4 glioblastoma (GBM).[3] Gliomas with IDH-mutation occur more frequently in younger adults compared with IDH-wildtype gliomas (median age 30–40 vs 50–60 years, respectively).[1,4] Despite the more favorable association, some IDH-mutant astrocytomas display a more rapid clinical progression and poor prognosis.[5–7] Moreover, a subset of IDH-mutant tumors displays an accelerated rate of tumor progression across timepoints of recurrence, indicating there is a biological evolution over time toward more aggressive disease.[8] Notably, the presence of the IDH-mutation is consistently retained across these timepoints of recurrence, highlighting the importance of a functional understanding of secondary drivers of malignancy including both the evolution of any preexisting molecular subclones and the emergence of acquired genomic alterations that drive a subset of IDH-mutant tumors toward more aggressive disease biology.[8,9] Among the alterations in IDH-mutant glioma profiled for an association with poor patient survival, the homozygous deletion of CDKN2A/B has emerged as the strongest predictor.[10]

CDKN2A/B is one of the most frequently altered genes found in human cancers and the loss of expression promotes malignant tumor behavior via cell-cycle dysregulation and increased cell proliferation.[11] CDKN2A/B homozygous deletion has been reported to occur in IDH-mutant astrocytomas in what would have previously been diagnosed histopathologically as World Health Organization (WHO) grades 2, 3, and 4 tumors at frequencies of 0%–12%, 6%–20%, and 16%–34%, respectively, highlighting the importance of an integrated molecular diagnosis for more accurate detection of aggressive tumors.[12] A recent systematic review of IDH-mutant gliomas (80% grade 2/3, 20% grade 4) reported a median incidence of CDKN2A/B homozygous deletion of 22% across studies, and when this alteration is present it represents the strongest known independent predictor of poor clinical outcome.[5–7,10,13] The adverse prognostic implication of CDKN2A/B homozygous deletion has been retrospectively profiled as conferring a median overall survival (OS) of 61 versus 154 months in histologic lower grade IDH-mutant glioma, and median OS of 38 versus 86 months in histologic grade 4 IDH-mutant glioma, respectively.[5–7,10] Given this clinical significance, the molecular signature of CDKN2A/B homozygous deletion in IDH-mutant astrocytoma has been deemed sufficient to define a grade 4 tumor regardless of histologic appearance as reflected in the 2021 WHO classification of tumors of the CNS.[1] IDH-mutant astrocytoma harboring a homozygous deletion of CDKN2A/B thus represents a clinically significant subgroup of grade 4 glioma. An improved understanding of the translational relevance that CDKN2A/B homozygous deletion confers within IDH-mutant astrocytomas represents an unmet need in addressing the paucity of treatment strategies for this subset of molecularly unique aggressive gliomas.

A Current Understanding of the Biologic Role of CDKN2A/B in IDH-Mutant Astrocytoma

CDKN2A/B maps to 9p21 and encodes the p14, p16 (CDKN2A), and p15 (CDKN2B) tumor suppressor proteins, which play a multifaceted role in recognizing cellular stress and in regulating cell senescence, differentiation, and apoptosis during stages of development and proliferation.[11,14] In healthy tissue, cell-cycle regulation is a critical component of homeostasis. Cyclin-dependent kinases are serine/threonine kinases that heterodimerize and form complexes with cyclins to direct mitogen-induced cell-cycle progression, and specific cyclin subtypes (D, E, A, and B) are dynamically induced throughout the cell-cycle reflecting their individual roles during cellular replication.[15] In the unstressed state, p16 inhibits activity of the cyclin D-CDK4/6 complex to prevent premature entry into S-phase. Moreover, in response to DNA damage, induction of p15 and p16 blocks early G1 to S-phase cell-cycle progression via cyclin D-CDK4/6 inhibition. P14 activity sequesters MDM2 activity to facilitate p53 accumulation in response to genotoxic stress, which then can drive p21 accumulation and inhibition of cyclin E-CDK2 further contributing to cell-cycle arrest.[14,16]

The adverse prognostic association of CDKN2A/B homozygous deletion in high-grade glioma may hold a differential significance across IDH-mutation status. Among the cohort of primary grade 4 gliomas analyzed in the Cancer Genome Atlas Program dataset, alterations in p53 and Rb signaling were found in 87% and 78% of cases, respectively, and the homozygous deletion of CDKN2A/B was the most frequent alteration within each of these cohorts, occurring in approximately half of all cases.[17] Despite this high frequency, the independent clinical prognostic significance of CDKN2A/B homozygous deletion among IDH-wildtype GBM may be limited to tumors with unmethylated O^6-methylguanine-DNA-methyltransferase (MGMT) status where there is a small but statistically significant decrease in OS compared to tumors without CDKN2A/B homozygous deletion (median OS 14.7 vs 16.9 months, respectively) that was not seen across MGMT-methylated tumors.[18] In contrast, given the strong prognostic implication in IDH-mutant astrocytoma, the functional relevance of CDKN2A/B homozygous deletion in glioma underlying aggressive tumor behavior may result from direct interference in IDH-regulated tumor biology.

The specific means by which homozygous deletion of CDKN2A/B in IDH-mutant astrocytoma confers an adverse clinical outcome remains to be fully elucidated, but several mechanisms can be proposed. The deletion of CDKN2A/B has been shown to accelerate glioma tumor progression faster than other cell-cycle pathway alterations,[19] yet the mitotic activity of IDH-mutant astrocytomas has not been shown to predict clinical prognosis.[20] This implies the role of CDKN2A/B homozygous deletion in promoting an aggressive phenotype is insufficiently explained by an increased proliferative potential alone. In a cohort of prospectively characterized gliomas, the homozygous deletion of CDKN2A/B in grades 2 and 3 IDH-mutant astrocytomas at tumor progression was associated with the development of imaging-based tumor enhancement, a predictor of aggressive behavior and acquired alterations in blood vessel physiology.[19] Together, alterations in cell-cycle control effectors were the most significantly associated aberrations with the development of enhancing disease in recurrent IDH-mutant tumors.[19] Both CDKN2A gene products p14 and p16 can inhibit angiogenesis in glioma cells

via upregulation of tissue inhibitor of metalloproteinase 3 or downregulation of vascular endothelial growth factor, respectively,[21,22] suggesting loss of CDKN2A may promote blood vessel growth in vivo.

IDH mutations also lead to altered tumor epigenetics, metabolism, and transcription via the mutant allele-directed conversion to an accumulation of the oncometabolite D-2-hydroxyglutarate (D-2HG) from α-ketoglutarate, which subsequently fosters histone methylation and inhibits cell differentiation and epigenetic reprogramming through the establishment of a glioma CpG island methylator phenotype (G-CIMP).[23,24] Interestingly, a subset of IDH-mutant astrocytomas displays globally reduced DNA methylation (G-CIMP-low), which correlates with a shorter OS than those with G-CIMP-high.[25,26] Most of the G-CIMP-low tumors (15 of 18) harbored alterations in RB pathway genes, including CDKN2A/B homozygous deletion and CDK4 amplification.[25] The association of poor prognosis in IDH-mutant astrocytoma to both G-CIMP-low status and CDKN2A/B homozygous deletion has subsequently been corroborated.[26,27] Moreover, amplification of mesenchymal-epithelial transition (MET) kinase also correlated to the poor OS in the G-CIMP-low subgroup of IDH-mutant tumors,[27] highlighting the potential for interaction among these signaling pathways to abrogate IDH-induced epigenetic regulation of these tumors toward a more aggressive phenotype. The D-2HG oncometabolite impairs homologous recombination and leads to depletion of NAD^+ required for poly(ADP-ribose)polymerase (PARP)-mediated DNA repair.[28] Thus given the frequent alterations in TP53 in IDH-mutant astrocytoma,[3] the additional loss of p14 in tumors harboring homozygous deletion of CDKN2A/B may act synergistically with D-2HG to further render DNA repair mechanisms ineffective and promote malignancy, but this remains to be shown conclusively.

Finally, the combination of IDH-mutation, ATRX mutation, and TP53 mutation generates defects in DNA repair and replication.[29–31] These defects generate chromosome copy number complexity which is associated with progression in IDH-mutant astrocytomas.[10,32] CDKN2A/B homozygous deletion is correlated with chromosome copy number complexity and it may be that DNA repair replication defects generate CDKN2A/B homozygous deletion.

Further Potential Biologic Considerations

The current grading of IDH-mutant astrocytoma defines homozygous deletion of CDKN2A/B as grade 4. However, alternative mechanisms of repression or silencing of CDKN2A/B gene expression also may have relevant implications in gliomas. The functional inactivation of CDKN2A/B has been shown to occur across tumor grades in glioma through promoter hypermethylation or mutation (reported in approximately 24% and less than 5%, respectively, across glioma cohorts).[13] These alternative mechanisms are associated with more variable repression of p16 expression, and the impact of this discrepancy on clinical outcomes remains to be clarified.[33] One prospective study identified two patients with acquired mutations of CDKN2A occurring in recurrent gliomas associated with disease acceleration and death within 11–15 months as compared to the more indolent clinical course of the primary tumors that were associated with a 5–6 year progression-free survival (PFS).[19] Given the rarity of this event, no definitive conclusions can be made on the role of CDKN2A/B mutations, still, these lesser described mechanisms of gene silencing should be considered for their potential to result in clinically meaningful loss of CDKN2A/B expression.

Treatment Implications

Molecular profiling has expanded the understanding of biologic subgroups within astrocytomas, and yet there are no effective targeted therapies that improve OS for these tumors to date in molecularly unselected cohorts.[34] Current treatment for grade 4 gliomas is similar regardless of molecular profile and includes maximal surgical resection, followed by temozolomide (TMZ) and radiotherapy (RT), as well as tumor treating fields (TTF). IDH-mutation status in these protocol-establishing studies is either unknown or IDH-mutant tumors comprised the minority of included patients (unknown and 7% of the high-grade gliomas studied in the TMZ/RT and TTF clinical trials, respectively).[35,36] Moreover, CDKN2A/B status was not evaluated as this was not relevant at that time. Additionally, while the benefit of adjuvant TMZ in IDH-mutant tumors is supported by the more recent CATNON trial in histopathologic grade 3 astrocytomas, the impact of CDKN2A/B homozygous deletion ($N = 43$) on this treatment response in IDH-mutant astrocytomas is yet to be reported.[26,37] Thus, there remains limited data to determine optimal front-line therapy for patients with IDH-mutant grade 4 tumors.

Successfully targeting grade 4 gliomas with novel molecular-based therapies has proven elusive in part due to intratumor heterogeneity and tumor cell plasticity, which have contributed to the current lack of clinically actionable molecular subgrouping of tumors. GBM has been extensively profiled into transcriptional subtypes (initially classified into proneural, classical, and mesenchymal), and IDH-mutant tumors are most closely linked to the proneural subtype.[38] However, these transcriptional subtypes are poor predictors of drug response.[34] Mounting data indicates resistant tumor subclones are present early in tumorigenesis and underlie tumor repopulation, with little associated selective pressure exerted by standard therapies on the evolution of genomic drivers of recurrent high-grade gliomas.[39,40] Tumors displaying subclonal selection across time were associated with shorter patient survival,[40] indicating the early identification of aggressive tumor subclones may better direct effective targeted therapy selection in this disease. CDKN2A/B homozygous deletion occurs early in glioma initiation and becomes enriched in recurrent IDH-mutant gliomas associated with genomic instability.[5–7,40] Thus the early use of therapies impacting pathway dysregulation in CDKN2A/B deleted clones represents an attractive strategy for the treatment of these tumors.

CDK 4/6 Inhibitors

Given the impact of CDKN2A/B homozygous deletion on cell-cycle promotion, CDK 4/6 inhibitors have represented an attractive strategy in glioma, yet most current trials are limited to GBM patients with stratification by IDH status not currently reported. The abemaciclib arm of the INSIGhT phase II trial randomizes patients with newly diagnosed MGMT-unmethylated GBM between either standard RT with concomitant and adjuvant TMZ or radiochemotherapy followed by adjuvant abemaciclib, a partial brain penetrant CDK4/6 inhibitor. The addition of abemaciclib to standard therapy was generally well tolerated and associated with a significantly longer PFS in all patients (hazard ratio [HR] 0.67) and a subset with CDK4 pathway activation with intact Rb protein (HR 0.64); however, there was no difference in the primary endpoint of OS.[41] The presence of intact Rb protein predicts CDK inhibitor response in preclinical models.[42] Abemaciclib has also been evaluated in combination with the selective ERK1/2 inhibitor LY3214996 in a phase 0 trial of recurrent GBM with intact Rb expression and either CDKN2A/B deletion or CDK4/6 amplification.[43] Both drugs achieved pharmacologically-relevant concentrations within the non-enhancing tissue following resection of GBM, and there was a corresponding decreased phosphorylation of downstream targets Rb and RSK, as well as decreased proliferation in the enhancing tumor.[43] Together, these studies suggest abemaciclib could be used early in glioma progression prior to overt MRI-based tumor enhancement if aggressive subclones with CDK pathway activation are identified. Notably, abemaciclib has been associated with a reduction in DNA-methyltransferase 1 expression, promoting tumor hypomethylation in several preclinical models.[44] Given that the aggressive biology of IDH-mutant astrocytoma with homozygous deletion of CDKN2A/B is associated with a G-CIMP-low status, the possibility that additional hypomethylation under CDK4/6 inhibition may potentiate this concern requires further study.

Additional CDK4/6 inhibitors have also been investigated in high-grade glioma, but without similar phase II trial success. Palbociclib showed good preclinical activity but was inefficient as monotherapy in phase II trials in recurrent GBM with detectable Rb expression.[45] Ribociclib has similarly shown promising phase 0 tissue penetration, but was ineffective as monotherapy in patients with recurrent GBM with intact Rb protein, potentially due to increased mTOR signaling underlying resistance.[46] The CDK9 inhibitor Zotiraciclib has been studied in phase I trials in patients with high-grade astrocytoma (independent of IDH status) with a tolerable toxicity profile when combined with TMZ, and the phase II study is underway (NCT02942264).[47] Neither CDKN2A/B homozygous deletion nor CDK pathway activation status was reported in the preliminary report, but the inclusion of grade 3 tumors in this study may provide more insight into the molecular subgroup of aggressive IDH-mutant astrocytoma.

The limited prognostic significance of CDKN2A/B homozygous deletion on IDH-wildtype glioma as compared to IDH-mutant glioma needs to be considered when extrapolating the utility of CDK4/6 inhibitors from studies in GBM. The CDKN2A/B homozygous deletion may be a more specific driver of aggressive behavior in IDH-mutant tumors compared to IDH-wildtype tumors, the latter of which are likely to behave aggressively due to many additional coexisting dysregulated pathways.[18] Notably, CDK4/6 inhibition has been shown to be more active in the proneural subtype of GBM where palbociclib targets the proneural glioma stem cell population and induces proneural-mesenchymal transition.[42] Since, IDH-mutant glioma is most closely associated with the proneural subtype,[38] this supports the continued study of CDK4/6 inhibitors specifically in the molecular subgroup of IDH-mutant astrocytoma with loss of CDKN2A/B.

IDH Inhibitors

IDH inhibitors are currently under investigation in clinical trials for patients with IDH-mutated gliomas, however, the differential response across molecular subgroups has not been well explored. IDH-mutant tumors generate D-2HG, an oncometabolite product linked to altered metabolic and epigenetic regulation of cellular differentiation presumed to underlie IDH-mutant tumorigenesis.[48,49] IDH inhibitors have already obtained FDA approval for use in other malignancies, including acute myeloid leukemia where these drugs induce differentiation. Current studies in glioma are based on the rationale that acquisition of IDH-mutation and formation of the D-2HG oncometabolite underlie early gliomagenesis, and therefore the early use of IDH inhibitors may delay progression to more aggressive recurrent tumors. The recent phase I studies of ivosidenib and vorasidenib, IDH1 or dual IDH1/2 mutation inhibitors, respectively, were well tolerated under doses of 500 mg or 100 mg, respectively, and associated with preliminary clinical activity in patients with non-enhancing gliomas. None of the patients with enhancing disease displayed radiographic response.[50,51] The phase III INDIGO trial has recently completed accrual and is evaluating the utility of early vorasidenib use in patients with residual or recurrent IDH-mutant grade 2 glioma without high-risk features and with only surgery as a previous therapy (NCT04164901). Since, these early data suggest IDH inhibitors may be ineffective once gliomas display more aggressive features, this implies a limited role for monotherapy use in IDH-mutant tumors harboring CDKN2A/B homozygous deletion. Moreover, inhibition of mutant IDH activity in solid tumors remains a controversial strategy. Tumors with IDH mutations are associated with an improved response to radiation and chemotherapy, hypothesized to occur through a compromised ability to repair DNA.[52–55] The oncometabolite D-2HG produced in IDH-mutant tumor cells has been shown to inhibit DNA repair enzymes as well as the homologous recombination DNA repair process.[52,54,55] Moreover, the D-2HG oncometabolite consumes NADPH levels, decreases NAD^+ availability, and also directly inhibits branched-chain amino acid (BCAA) transaminases decreasing glutamate levels. This leads to a depletion in glutathione and impaired free radical scavenging, further weakening DNA damage repair and sensitizing tumor cells to cytotoxic therapies.[28,56,57] Thus, IDH inhibition may thus confer increased resistance to these therapies in IDH-mutant tumors,[52,53] and IDH inhibitors, alone or in combination with RT and TMZ, may have a limited role in

aggressive grade 4 IDH-mutant astrocytomas. Instead, the potential utility of IDH inhibitors in combination with other targeted therapies in these tumors should be studied. These promising agents could include those that act on the dysregulated DNA repair, epigenetic and metabolomic reprogramming, and redox imbalance that were created under IDH-mutant direction.

Other Metabolic Regulators

The metabolic dysregulation of IDH-mutant astrocytoma has created interest in utilizing additional metabolic pathway modulators to control tumor cell growth in this disease, however, the differential response in IDH-mutant tumors across CDKN2A/B status is poorly studied to date. As earlier mentioned, IDH-mutant gliomas are glutamate depleted due to D-2HG-induced BCAA inhibition. Therefore, these gliomas are dependent on glutaminase to convert glutamine to glutamate and subsequently glutathione, making glutaminase inhibitors an attractive study drug.[57] Glutaminase inhibitors sensitize IDH-mutant glioma cells to oxidative stress and radiation in preclinical models of both oligodendrogliomas and IDH-mutant astrocytomas with CDKN2A/B homozygous deletion.[58] A phase Ib trial is currently evaluating the impact of glutaminase inhibitor CB-839 (telaglenastat) in combination with RT and TMZ in patients with previously untreated IDH-mutant grade 2/3 astrocytoma, and thus may provide further insight into the response across molecular subgroups in this disease.[57,59] Notably, in vitro studies have suggested that IDH-mutant glioma cells display metabolic plasticity in response to glutaminase inhibition, whereby glutamine is alternatively generated through upregulation of other enzyme pathways such as the conversion of aspartate via asparagine synthetase. These potential resistance pathways will need to be analyzed in the context of the clinical trial results.[60]

Mitochondrial metabolism is also altered in IDH-mutant tumors.[61] Treatment with ABT263 (navitoclax), a Bcl-2 family inhibitor, enhanced the killing of genetically engineered IDH-mutant GBM cells via impairment of mitochondrial function and induction of apoptosis, and prolonged survival in IDH1-mutated orthotopic GBM xenograft models.[62] Notably, the IDH-mutant models were generated from U87 and T98G cell lines which normally harbor loss of CDKN2A/B. Thus, these data support the further clinical study of this drug in IDH-mutant astrocytoma with CDKN2A/B homozygous deletion.

Tumors with the homozygous deletion of CDKN2A/B frequently also harbor loss of the methylthioadenosine phosphorylase (MTAP) due to the close proximity of the gene loci.[63] Tumors lacking expression of MTAP are particularly vulnerable to inhibition of the methionine adenosyltransferase 2α (MAT2A), due to a reduction in S-adenosylmethionine levels and the promotion of DNA damage and mitotic defects in tumor cells.[64] The first in class MAT2A inhibitor AG-270 was profiled across a panel of MTAP null PDX models including two gliomas (WHO grade 2 pleomorphic xanthroastrocytoma and WHO grade 3 oligodendroglioma) and exhibited tumor growth inhibition in both lines.[64] AG-270 is currently being studied in a phase I clinical trial in patients with tumors harboring the homozygous deletion of CDKN2A/B or MTAP, however, patients with CNS malignancies are currently excluded from this study (NCT03435250). A brain penetrant MAT2A inhibitor was dy of panobinosta recently designed, and thus merits further preclinical study in IDH-mutant astrocytoma with CDKN2A/B homozygous deletion.[65]

PARP Inhibitors

Given the role of the D-2HG oncometabolite in promoting homologous recombination repair defects as well as depletion of NAD^+ required for PARP-mediated DNA repair, strategies to create synergy through impairment of DNA damage repair processes represent an attractive strategy in IDH-mutant gliomas.[28] The use of PARP inhibitors (PARPi) in IDH-mutant glioma models has been shown as a promising strategy for example to confer an augmented response to cytotoxic therapy in several studies.[28,52,66] In these in vitro studies, IDH-wildtype GBM cell lines U87 or U251 were engineered to stably express IDH1 R132H mutant protein and subsequently displayed enhanced cell death when the PARPi olaparib was used in combination with TMZ compared to TMZ alone.[28] U87 and U251 cells do harbor loss of CDKN2A/B, and thus these data may provide a rationale for PARPiuse clinically in IDH-mutant gliomas with CDKN2A/B homozygous deletion. Moreover, PARPi induces a lethal telomere fusion in tumors that rely on the alternative lengthening of telomere (ALT) mechanism to circumvent growth limitations, and the majority of lower-grade astrocytomas are ALT dependent, suggesting an additional mechanistic basis for the use of PARPi in this tumor population.[31]

Several clinical trials are underway to assess the role of PARPi monotherapy or in combination with cytotoxic chemotherapy in IDH-mutant gliomas (NCT03749187, NCT03914742, and NCT03561870). Of these, the OLAGLI phase 2 study of olaparib monotherapy in recurrent IDH-mutant high-grade glioma recently reported early outcome data that the regimen was well tolerated and resulted in a modest activity with 2 patients (5%) deemed to have a partial response.[67] The study included both astrocytoma and oligodendrogliomas and molecular status including CDKN2A/B has not been reported, and thus no definitive conclusions can be drawn yet about the use of olaparib monotherapy in IDH-mutant astrocytoma with CDKN2A/B homozygous deletion. Ongoing follow-up of these studies will help to determine if the concurrent use of PARPi with cytotoxic therapy in IDH-mutant astrocytomas with CDKN2A/B homozygous deletion are effective strategies in this molecular subgroup.

Proteasome Inhibitors

A preclinical drug screen using IDH-mutant glioma stem cell lines identified two proteasome inhibitors (bortezomib and carfilzomib) among 147 candidate antineoplastic agents screened that displayed substantial antiproliferative activity.[68] More recently, a drug screening study using 107 FDA-approved agents in IDH-mutant patient-derived cell lines, all of which were characterized to have a loss of

CDKN2A/B, similarly identified bortezomib as the top drug candidate based on IC_{50} values.[71] However, preclinical data utilizing these drugs in gliomas indicated a limited ability to cross the blood-brain barrier, and confirmatory cell viability studies were thus undertaken with the brain penetrant proteasome inhibitor marizomib.[69–71] Marizomib has been evaluated in a phase III study in combination with standard TMZ-based radiochemotherapy regimens in newly diagnosed GBM. Although it did not meet the primary OS endpoint or a PFS benefit,[72] in vitro data indicates there is a differential response to proteasome inhibitors in GBM which may provide insight for use in IDH-mutant glioma. Drug resistance to proteasome inhibitors in GBM was associated with an inadequate level of the p53 apoptotic pathway activation, either due to mutations in p53 or pathway members including CDKN2A/B deletion, or due to increased antioxidant synthesis or induction and activation of the DNA damage repair response.[34] The concurrent use of agents that modulate the apoptotic threshold such as the Bcl-2 inhibitor navitoclax, noted earlier to enhance IDH-mutant GBM cell killing as monotherapy, or MDM2 inhibitors were able to potentiate the proteasome inhibitor response and synergistically kill GBM cells in these resistant lines.[34] Thus, given that p53 pathway dysregulation is lineage-defining in IDH-mutant astrocytoma, there may be a role for the use of proteasome inhibitors in combination with other apoptotic agents in IDH-mutant disease.[3,73]

A recent high-throughput combination drug screening study in aggressive pediatric diffuse midline gliomas (DMG) identified a lead combination of marizomib with the multi-histone deacetylase (HDAC) inhibitor panobinostat.[74] DMGs are characterized by broad epigenetic dysregulation secondary to a recurrent histone mutation (H3K27M), and this drug combination was found to mechanistically provide synergistic cytotoxicity via inhibition of tumor metabolism and decreased NAD+ that was only seen when the drugs were used in combination and not individually. A current phase I study of panobinostat, an HDAC inhibitor with marizomib in DIPG is ongoing (NCT04341311). Given the proposed cytotoxic mechanism of action for marizomib in this combination lies in enhancing a metabolic vulnerability within the tumors, the study of marizomib in IDH-mutant astrocytoma, a tumor similarly driven by metabolic and epigenetic dysregulation, also remains attractive for further study in combination with epigenetic regulators where hypomethylating agents (HMAs) may be preferable over HDAC inhibitors.

Hypomethylating Agents

The IDH mutation, through the production of the D-2HG oncometabolite, directs epigenetic reprogramming leading to a G-CIMP signature in IDH-mutant tumors.[24,75] Thus, therapies aimed at restoring an epigenetic phenotype have represented an attractive strategy in IDH-mutant tumors. 5-Azacytidine (5-aza), a cytidine analog that interferes with DNA synthesis and acts as an HMA by inhibiting methyltransferases, was shown to induce tumor regression in a patient-derived IDH-mutant grade 3 astrocytoma xenograft which correlated with induction of glial differentiation, however, CDKN2A/B status was not reported.[76,77] In addition, 5-aza was shown to enhance the therapeutic effect of TMZ in orthotopic IDH-mutant glioma models.[78] Clinically, 5-aza is currently being investigated in recurrent glioma with IDH1/2 mutation as monotherapy (NCT03666559). In addition, the combination drug decitabine with cedazuridine (ASTX727), a cytidine deaminase inhibitor in the gut and liver that allows higher plasma concentrations of decitabine, is currently in trial in IDH-mutant glioma patients (NCT03922555). The DNA-methyltransferase 1 inhibitor NTX-301 is currently being studied in IDH1 mutated high-grade glioma in combination with TMZ (NCT04851834). Importantly, however, given that the loss of CDKN2A/B in IDH-mutant astrocytoma is closely associated with a conversion from a high G-CIMP to a low G-CIMP profile, HMA monotherapy may be ineffective in this molecular subgroup and combination therapies would likely need to be considered.[25]

In vitro data have shown that treatment of glioma cells with 5-aza-2-deoxycytidine resulted in an increase in promoter accessibility and induction of p16 expression.[79] This may separately suggest a role for HMAs in gliomas with a wildtype CDKN2A/B locus but transcriptional repression through the epigenetic silencing mechanism of promoter hypermethylation. Promoter hypermethylation of CDKN2A/B has been found in a minority of gliomas as previously noted, however, whether these tumors recapitulate a similar grade 4 clinical phenotype to those tumors with CDKN2A/B locus homozygous deletion remains to be studied. Thus, the use of HMA in this cohort merits future preclinical studies to determine whether there exists a cohort of patients with loss of CDKN2A/B function via gene silencing that may benefit from this intervention and suggests the need to consider the p16 status in aggressive IDH-mutant tumors with otherwise apparent CDKN2A/B wildtype status.

MET Inhibitors

MET alterations have been well characterized to become enriched during the progression of low-grade glioma to high-grade glioma (formerly termed secondary GBM).[80,81] Many of these tumors with MET alteration were concurrently characterized as IDH-mutant tumors with deletion of CDKN2A/B.[80] Moreover, MET signaling amplification, similar to CDKN2A/B homozygous deletion, is associated with the G-CIMP-low subgroup of IDH-mutant tumors with a more aggressive phenotype and poor patient survival.[26,27] The CNS-penetrant MET inhibitor PLB-1001 has subsequently been tested in a phase I study of recurrent grade 3 or secondary GBM that progressed from a lower grade and with either PTPRZ1-MET fusion or MET-exon-14-skipping (METex14) mutation detected in the recurrent tumor.[80,82] Within the cohort of 6 secondary GBM patients who remained on trial, two had a partial response, two had stable disease, and two had disease progression. Stable disease was noted in five out of nine grade 3 glioma patients with progressive disease in the others, and pathway activation of PI3K-Akt-mTOR was noted in recurrent tumors following treatment with MET inhibition suggesting a potential mechanism of resistance.[80] Thus, the ongoing study of MET inhibitor monotherapy or in combination with PI3K

pathway inhibitors represents an opportunity for further study in IDH-mutant astrocytomas harboring CDKN2A/B homozygous deletion.

Other Targeted Therapies

Genomic profiling of IDH-mutant gliomas has identified further potential actionable alterations in a subset of these tumors. PDGFR alterations have been reported to occur in 19% of high-grade IDH-mutant gliomas (previously termed IDH-mutant GBM) and have been shown to be acquired alterations in the progression from low to high-grade IDH-mutant astrocytoma.[80,83] In representative patient-derived IDH-mutant glioma xenografts one tumor with PDGFR amplification displayed a response to sunitinib, whereas the drug was less potent in tumors without PDGFR amplification.[81] Given the indirect role of CDKN2A/B loss in promoting angiogenesis, the use of antiangiogenic therapies is an attractive strategy. Conversely, EGFR amplification has been rarely reported in IDH-mutant gliomas (3%), and moreover has been profiled to be nearly mutually exclusive with IDH-mutation in secondary GBM profiling, thus may unlikely or rarely represent a driver in these tumors.[80,83] Further molecular subtyping may help predict additional targetable alterations in this disease.

Future Directions and Conclusion

CDKN2A/B has been profiled clinically as an independent predictor of poor prognosis in IDH-mutant gliomas and defines a new molecular subgroup of grade 4 astrocytoma.[1] There currently remains limited functional data characterizing the mechanism of CDKN2A/B homozygous deletion in IDH-mutant astrocytoma pathophysiology, including a paucity of data on the impact for these coexisting alterations to result in synergistic signaling pathway dysregulation and their collective impact on additional aspects of tumor biology. Improved functional characterization of CDKN2A/B homozygous deletion in IDH-mutant tumors will elucidate the signaling mediators underlying their clinically aggressive behavior and provide a biological rationale for targeted therapy development for this grade 4 subgroup.

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Conflict of Interest Statement

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