Review

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Mutant IDH in Gliomas: Role in Cancer and Treatment Options

Georgios Solomou [1,2,3] *,†, Alina Finch1 †, Asim Asghar [1] and Chiara Bardella [1]

[1] Institute of Cancer Genome Sciences, University of Birmingham, Birmingham, UK. Division of Academic Neurosurgery, Department of Clinical Neurosciences, University of Cambridge, Cambridge CB2 0QQ, UK

[2] Wellcome MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge CB2 0AW, UK

* Correspondence: giorgossolomou@hotmail.com

† These authors have contributed equally.

Abstract: Altered metabolism is a common feature of many cancers and in some cases is a consequence of mutation in metabolic genes, such as the ones involved in the Krebs cycle. The Isocitrate dehydrogenase (*IDH*) gene is mutated in many gliomas and other cancers. Physiologically IDH converts isocitrate to αketoglutarate (αKG), but when mutated, IDH reduces αKG to D2-hydroxyglutarate (D2-HG). D2-HG accumulates at high levels in IDH mutant cancers, and in the last decade, a massive effort has been done to develop small inhibitors targeting mutant IDH. In this review, we summarize the current knowledge concerning the cellular and molecular consequences of IDH mutations, and the therapeutic approaches developed to target IDH mutant tumors, with a focus on gliomas.

Keywords: isocitrate dehydrogenase; cancer metabolism; hydroxyglutarate; gliomas; oncometabolites

Introduction

The isocitrate dehydrogenase (IDH) family includes three enzymes, IDH1, IDH2, and IDH3 that are ubiquitously expressed in human cells. Although all these enzymes catalyze the same chemical reaction, i.e., the oxidative decarboxylation of isocitrate (ICT) to α-ketoglutarate (αKG), each enzyme has unique biological properties. IDH1 is localized to the cytoplasm and peroxisomes of the cells, where it has a role in glucose and lipid metabolisms and protects against reactive oxygen species (ROS) [1] [2] [3] [4]. IDH2 and IDH3 are both found in the mitochondrial matrix [5], where IDH2 exerts its regulatory function on the tricarboxylic acid (TCA) cycle and protects against oxidative stress [6], while IDH3 functions as a central enzyme of the TCA cycle [6]. IDH1 and IDH2 are homodimers, while IDH3 is a heterotetrameric protein, composed of two catalytic α subunits required for ICT binding, a β subunit, which has a structural role to facilitate the enzyme subunits assembly, and the γ subunit, that through binding of citrate and ADP, functions as an allosteric modulator [7] [8]. The chemical reaction catalyzed by IDH1 and IDH2 is reversible and requires the binding of nicotinamide adenine dinucleotide phosphate ($NADP^+$) as a co-factor, which is converted into NADPH [9], while the chemical reaction of IDH3 is irreversible, and involves binding of NAD^+, which is reduced to NADH. Moreover, unlike IDH1 and 2, the chemical reaction catalyzed by IDH3 is tightly regulated by substrate availability and allosteric effectors (citrate, ICT, adenosine 5′- diphosphate (ADP), nicotinamide adenine dinucleotide (NAD^+), Mg^{2+}/$Mn^{2+}$and Ca^{2+}) product inhibition (NADH NADPH and αKG) and competitive feedback inhibition (adenosine 5′- triphosphate (ATP)) [10] to avoid unnecessary usage of ICT and increase of αKG.

In the last decades, recurrent somatic mutations in *IDH1* and *IDH2* genes have been identified in many cancers, such as low-grade gliomas (LGG) (>70%), secondary glioblastoma (diffuse astrocytoma, WHO CNS grade 4 according to the 2021 guidelines) (55%-88%) [11–16], chondrosarcoma (56%) [17], intrahepatic cholangiocarcinoma (23%) [18], acute myeloid leukemia

(16%), as well as to a lower extent, myelodysplastic syndrome and angioimmunoblastic T-cell lymphoma [19], and other solid tumors.

In contrast to *IDH1* and *IDH2*, no tumor-associated mutations for any of the *IDH3* genes (*IDH3A*, *IDH3B*, and *IDH3G*) have been reported [20].

IDH changes are missense mutations, which affect predominantly the arginine residues R132 in IDH1, R17,2, and R140 in IDH2 [11]. These arginine residues are crucial for the recognition of isocitrate. In the mutant IDH1, the highly positively charged arginine residue at position 132 is replaced by lower polarity amino acids such as histidine, cysteine, glycine, or lysine. This missense mutation intervenes with the alpha and beta carboxyl sites of isocitrate [21] [22]. This results in a weak affinity for isocitrate in addition to raised NADPH levels, since less is going to be used as a co-factor for a forward reaction. IDH mutations are monoallelic changes, which result in an enzyme composed of wild-type and mutant monomers. Consequently, in IDH mutant cells, the wildtype part of the dimer leads to the conversion of isocitrate to α-KG producing NADPH, whereas the mutant monomer, by using NADPH as a co-factor, converts α-KG to the D2-enantiomer of hydroxyglutarate (D2-HG; the L2- enantiomer, L2-HG, is not significantly produced), exhibiting a neomorphic activity [23]. Consequently, in IDH-mutant tumors, D2-HG accumulates to millimolar concentrations, while due to the enzymatic loss of function, the αKG decreases [24] [25]. Besides its role as a biomarker in IDH-mutant malignancies, D2-HG is thought to be an oncometabolite [24], mainly for its role in competitively inhibiting the enzymatic activities of αKG-dependent dioxygenases, such as histone lysine demethylases (KDMs), or ten-eleven translocation methylcytosine (TET) family enzymes [26] [27]. Consequently, IDH mutations result in the hypermethylation of DNA and histones, which is considered one of the leading mechanisms of tumorigenesis.

IDH1 and *IDH2* mutations appear to be mutually exclusive [28] [29] [30], with some rare exceptions [13] [28] [19]. Interestingly, the nature of the various IDH substitutions differ between the cancer types, such as IDH1 is more frequently mutated in solid tumours, while IDH2 is mainly mutated in some blood cancers. Moreover, specific IDH variants seem to be more frequent in a particular type of tumor. For instance, in LGG, the majority of IDH mutations occur in the IDH1 gene, which is mainly converted to the R132H variant. In chondrosarcoma and cholangiocarcinoma, IDH1 is most frequently mutated and specifically to the variant R132C [31]. Interestingly, patients harboring $IDH1^{R132H}$ mutated tumors have lower levels of genome-wide DNA methylation, an increased gene expression, and a worst prognosis, compared to tumors with other IDH1/2 mutations [31].

The reason for the tissue specificity of the various IDH mutations in different cancers is still not clear. However, it looks like that specific IDH mutations differ in their capacity of producing D2-HG [32] [33]. For instance, $IDH1^{R132H}$ has a relatively low capacity to produce D2-HG compared to other mutations, such as $IDH1^{R132C}$, which is highly more efficient [32] [33]. Potentially each tumor type may require a certain amount of D2-HG to initiate tumor formation, as different levels of D2-HG might be required to impact tissue-specific enzymes/pathways driving cancer initiation and progression. For instance, not all αKG- dependent dioxygenases are equally well inhibited by the D2-HG, and some of them, such as TET2 enzymes require high levels of D2-HG to be inhibited [34].

It is well recognized that IDH mutations have a biological impact both intracellularly, as well as part of the tumor microenvironment, favoring tumor formation and recurrence, and in the last decades, many functional studies have been focused on understanding the molecular mechanisms responsible for the formation IDH-mutant tumor. These studies mainly focus on the functional significance of the D2-HG accumulation, but despite many efforts, the biological mechanism driving tumor formation is not completely clear. Taking into consideration that current therapies fail to demonstrate improvement in outcomes, IDH-induced biochemical alterations should be adequately understood and assessed as a potential target. Here we summarize the current knowledge of IDH-mutated human malignancies, with a focus on glioma, discussing the oncogenic role of mutant IDH in tumor formation and the therapeutic opportunities.

The Oncogenic Role of Mutant Idh in Tumour Formation and Progression

Metabolic Alterations

IDH mutations result in significant alterations and reprogramming of the cellular metabolic pathways. Indeed, the production of D2-HG leads to significant reduction and drainage of Krebs cycle substrates, and thus carbohydrate sources [24] [35]. Consequently, the Krebs cycle is forced to adjust and drain other carbohydrate sources to yield ATP [36–38].

Glutamine derivatives can serve as key substrates to the Krebs cycle compensating for the exhaustion of isocitrate by mutant IDH. Glutamate dehydrogenase 2, the enzyme responsible for the conversion of glutamate to α-KG has been found highly expressed in IDH mutant brain cells, to possibly compensate for the reduction of α-KG consequent to IDH mutation [39]. In addition, IDH-mutated glioma cells are highly sensitive to the inhibition of glutaminase, an enzyme that contributes to the lysis of glutamate, further adding to the notion that IDH-mutated cells are glutamate-dependent [40]. Additionally, the depletion of NADPH caused by the formation of D2-HG, leads to the reduction of intracellular lipogenesis, resulting in great dependence on exogenous lipid sources [2] and as such, partly explaining the slow but steady growth of IDH mutant gliomas.

Compared to other tumors, IDH mutant gliomas show a distinctive metabolic behavior. In the majority of cancers, the need for an adequate supply of energy is mediated by the increased expression of lactate dehydrogenase (LDH) [41]. LDH catalyzes the transformation of pyruvate, the end product of glycolysis, to L-lactate [42]. L-lactate can serve as immediate fuel for the increased demands for energy, to match the rapid proliferative potential of the cancer cells. On the contrary, in IDH-mutated glioma patient-derived samples, the LDH is silenced [43,44]. Hypermethylation of the promoter region of the LDH gene was found to be the main reason for the lack of LDH expression [43,44]. This epigenetic silencing might explain the slow-growing nature of IDH mutants compared to IDH wild-type gliomas [44,45].

Moreover, IDH mutant gliomas seem to rely less on the glycolytic pathway, and more on oxidative phosphorylation to produce energy compared to the WT IDH1 gliomas [46]. A reduction in the glycolytic pathway, might not only partially explains the slow-growing nature of IDH mutant gliomas, but provides useful metabolic targets for future IDH mutant glioma therapies.

Redox Imbalance

IDH mutations lead to the increased affinity for NADPH and α-KG, leading to the conclusion that the mutant glioma cells will prefer using NADPH and not NADP+ [24] [47]. The high consumption of NADPH disrupts the reducing equivalents of biochemical reactions leading to the accumulation of reactive oxygen species (ROS) [48] [49]. ROS are involved in genomic instability, cellular motility, and acquisition of invasive characteristics [50]. Excessive ROS leads to DNA and protein damage, disrupting enzymatic reactions and gene expression. The alteration of gene expression might lead to new oncogenic mutations. Consequently, ROS accumulation is fundamental and a hallmark of cancer biology, especially for IDH-mutated gliomas. Cells derived from IDH1 mutated gliomas exhibit strong oxidative stress, evident by the increased expression of manganese superoxide dismutase [51]. The elevated stress was confirmed with further evaluation shown that IDH mutated cells are prone to oxidative damage [52] [53]. In the face of the raised oxidative profile enhancing antioxidant pathways, such as the synthesis of glutathione, maybe be a valuable strategy to downplay the oncogenic effects of ROS [54].

Epigenetic Modifications

Epigenetic alterations are mechanisms that affect gene expression without causing permanent changes to the DNA sequence as mutations would. Epigenetic changes range from DNA methylation, non-coding RNA molecules, and covalent modifications to histones, such as acetylation, methylation, and phosphorylation [55] [56]. IDH mutations are very tightly linked with changes to the epigenetic landscape in cells and this is due to their abnormal production of D2-HG, which competitively inhibits

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a range of enzymes involved in maintaining "normal" DNA methylation. The resulting disruption leads to genome-wide changes in epigenetics called hypermethylation and leads to widespread alterations in gene expression [57]. Understanding the pathogenesis of IDH mutant gliomas concerning the hypermethylation patterns might lead to the identification of rational therapeutic targets.

Analysis of DNA methylation changes of GBM samples data from The Cancer Genome Atlas (TCGA) database found a strict correlation between IDH mutation and the development of a unique DNA methylation signature [58,59]. This came to be known as the glioma cytosine-phosphate-guanine (CpG) island methylator phenotype (G-CIMP). The G-CIMP phenotype is considered a favorable marker of prognosis only in patients affected by IDH mutant gliomas [60], although increased hypermethylation on CpG sites of the genome, has been partially found in other IDH mutant tumors compared to their wildtype countertypes [57]. A study by Unruh and colleagues demonstrated that the extent and targets of the DNA hypermethylation induced by mutant IDH are highly variable between various IDH mutant cancers (AML, cholangiocarcinomas, melanomas, gliomas), depending on the cellular contexts and this might explain why mutant IDH is a favorable prognostic marker specifically in gliomas and not in other IDH mutant tumors [57].

Methyltransferases and demethylases can control the DNA methylation pattern. Within the demethylation process, 5-methylcytosine is converted to 5- hydroxymethyl cytosine (5-hmC) catalyzed by methylcytosine dioxygenase TET, in an α-KG and iron-dependent manner. In addition, TET concomitantly catalyzes cytosine demethylation steps by converting 5-hmC to 5-carboxylcytosine (5-caC) and -formylcytosine. Thymine DNA glycosylase BER enzymes will eventually convert 5ca-C to cytosine [61]. However, in IDH mutant gliomas, the TET activity is inhibited due to D2-HG, which has a structural similarity to α-KG [34] [62]. Therefore, the DNA demethylation process cannot take place, and consequently, a hypermethylated phenotype is present in mutant IDH gliomas [63] [64]. Follow-up studies have shown that once hypermethylation happens, it is irreversible and thus plays a pivotal role in malignant transformation and recurrence [65].

Furthermore, D2-HG is aiding histone methylation by inhibiting histone demethylases. A notable example will be lysine-specific demethylase KDM [34] [27]. Histone methylation is predominantly regulated via histone methyltransferases, which add the methyl group as well as demethylases (such as KDM), which remove the methyl group. Because α-KG is playing a catalytic role in these reactions, in a similar way as with the aforementioned TET, the increased levels of D2-HG competitively block the reactions [38]. It is now recognized that histone and CpG island hypermethylation patterns are predominantly found in IDH mutant glioma stem cells (GSCs) [29]. Studies have shown that CpG hypermethylation leads to the inactivation of tumor suppressor genes (TSG) as well as altered gene expression related to cell differentiation [66]. Thus, the IDH mutations ultimately block cell differentiation and cell cycle regulation, leading to uncontrolled proliferation, with concomitant accumulation of new somatic mutations, which are acquired across time and remain undetected. However, the development of glioma not only requires seeds (GSCs) with uncontrolled proliferation but also fertile soil (tumor microenvironment).

Tumour Microenvironment

The D2-HG produced by mutant IDH accumulates at mM ranges both intracellularly [24] [25] and extracellularly [67], causing not only a biochemical cascade of events inside the cell, but also important metabolic alterations in the surrounding cellular microenvironment. These metabolic changes may affect the behaviour of the non-neoplastic cells present in the tissue in a multitude of ways to ultimately promote tumor growth and progression through non-cell intrinsic mechanisms.

IDH mutation and Tumor-Specific Immune Cells

The microenvironment surrounding glioma is rife with a variety of immune cells including tumor-infiltrating lymphocytes (TILs), natural killer (NK) cells, tumor-associated macrophages (TAMs), microglia, myeloid-derived suppressor cells (MDSCs), neutrophils, dendritic cells, and fibroblasts [68].

Increasing evidence has indicated that mutant IDH can decrease the levels of tumor immune surveillance, enabling cells to escape it. Various glioma studies have shown that mutant IDH affects tumor immune-cell infiltration and function by modulating chemokine secretion. For instance, IDH1 mutation has been shown to reduce leukocyte chemotaxis into gliomas in a syngenic mouse model, by repressing the expression of key chemoattractant cytokine genes including *Cxcl-2*, *Ccl-2*, and *C5* [69]. Other studies demonstrated that IDH mutant human gliomas have reduced levels of CD8+T lymphocytes compared to their WT counterpart; this was also confirmed by using a syngenic mouse model of glioma, where expression of mutant IDH or a treatment with 2-HG reduced infiltration of cytotoxic CD8+T lymphocytes and levels of T cells-associated chemokines within tumors. Interestingly, these effects were reversible by using a specific inhibitor of mutant IDH1 [70]. Accordingly, other studies reported that glioma stem cells and astrocytes expressing IDH mutations can escape immune surveillance, by causing epigenetic repression of natural killer cells' ligand genes [71]. The inhibition of the immune response caused by mutant IDH might explain at least in part, the difference in the aggressiveness of IDH-mutant gliomas compared to the wild-type samples.

Many recent studies have also indicated that IDH mutation modifies TAM phenotypes to influence glioma growth. TAMs are the main innate immune effector cells in malignant gliomas and can be separated into two categories, M_1 with a pro-inflammatory signature and M_2 with an anti-inflammatory signature [72]. Mutant IDH has been found to reduce the infiltration of TAMs in both IDH1-mutant mouse models and human glioma tissues [69]. Another study found that although the total number of TAMs is lower in IDH1-mutant compared to the WT IDH GBM samples, the remaining TAMs are more pro-inflammatory [73]. Conversely, another recent study reported that TAMs from patients with IDH-mutant gliomas exhibit a more immunosuppressive phenotype than IDH-wild-type samples [74].

Mutation in IDH1 plays an important role in TAM activation. D2-HG can be taken up by TAMs using membrane transporters and favors the activation of the M1 state of TAMs [75]. An in vitro assay combining human TAMs with glioma cells for 24 hours showed that the presence of IDH1 mutation within the glioma cells caused an increase in the expression of M1 genes and a decrease in that of the M2 genes [75]. Moreover, a mutation in IDH1 also promotes TAM migration in vitro and *in vivo*; conditioned medium derived from IDH1-mutant glioma cells significantly promotes TAM migration compared with that from IDH1-wild-type glioma cells. Accordingly, *in vivo*, data showed that the IDH1-mutant glioma cells have increased TAM recruitment, which promotes the anti-tumor functions of TAMs in vivo [75]. Migration of both $CD4^+$ and $CD8^+$ T cells is inhibited by the application of D2-HG whilst T cell proliferation and production of IFNγ were both inhibited in a dose-dependent manner [76]. This provides direct evidence that D2-HG produced by mutant IDH has a vital role in immune suppression.

The role of D2-HG was further elucidated using genetically-engineered CAR-T cells. CAR-T cells were generated that were either a KO or OE of the D2-HGDH enzyme – D2-HG dehydrogenase – which takes D2-HG as its substrate and produces α-ketoglutarate. The KO and OE CAR-T cells were tested using the cell line NALM6 overexpressing mutant IDH1. The results show that D2-HGDH-OE CAR-T cells had a profound rate of tumor cell death compared to that of the D2-HGDH-KO CAR-T cells. Mice bearing mutant IDH1 tumors showed a significant increase in their survival following treatment with D2-HGDH-OE CAR-T cells. It was later shown that the mechanism behind this was down to the reduction in D2-HG, and therefore the removal of immune suppression, allowing for an increase in memory cell differentiation and cytokine production [77].

A recent study provides new evidence for mechanistic insight into the effects of D2-HG on $CD8^+$ T-cell inhibition [78]. Notarangelo *et al.* showed that D2-HG acts directly on CD8+ T-cells to impair cytokine production and proliferation in a reversible and dose-dependent manner leading to the suppression of this immune cell group. The study went further to show that D2-HG inhibits the ability of CD8+ T-cells to perform glycolysis by direct inhibition of lactate dehydrogenase (LDH). In vitro studies using purified, recombinant LDH protein and exogenous D2-HG showed a non-competitive inhibition of the enzyme leading to altered glycolytic flux in the CD8+ T-cells. Application of D2-HG to CD8+ T-cells led to a reduction in the production of IFN-γ and therefore also in its autocrine effects

on gene expression. Encouragingly, the effects seen in vitro are also found in vivo when analyzing samples from IDH-mutant tumors. These glioma samples demonstrate reduced IFN-γ related gene expression as well as reduced lactate levels due to the inhibition by D2-HG [78].

Together, this data shows that mutant IDH enzyme, via the aberrant production of D2-HG, leads to suppression of the immune system both in terms of activation and infiltration. Overall, this leads to an immunologically "cold" tumor microenvironment, which improves overall patient survival but does limit treatment options as immune-targeted therapies are rendered ineffective. Further understanding the difference in the role of the immune system in both IDH WT and mutant gliomas will be essential to finding ways to effectively target these tumors.

IDH Mutation, Intra-Tumor Hypoxia, and Angiogenesis

Contrasting data exist in the literature regarding the ability of mutant IDH to increase the levels of hypoxia and the formation of new blood vessels in the tumor. It was originally hypothesized that 2-HG produced by mutant IDH, by competing with α-ketoglutarate may inhibit the prolyl hydroxylase (HIF-PHD) responsible for the ubiquitination and proteasomal degradation of hypoxia-inducible factor (HIF), enabling HIF to become stable in normoxic conditions, and thus leading the expression of HIF-target genes, such as vascular endothelial growth factor (VEGF) [79] [27]. Accordingly, HIF1α was found upregulated in cells overexpressing mutant *IDH1*, in cells treated with exogenous 2-HG and in IDH mutant tumors, and in brains of mouse embryos expressing *idh1* R132H [79] [27] [80]. However, D2-HG is only a relatively weak inhibitor of HIF-PHD [27], and the following studies found no correlation between IDH mutation status and the expression of HIF1α in patients with glioma [81,82], or in the brains of adult mice where mutant *idh1* has been expressed [83]. Additionally, other studies demonstrated that D2-HG can stimulate rather than inhibit the activity of HIF-PHD, and in some cellular contexts, such as human astrocyte, colorectal, and erythroleukemia cell lines, the expression of IDH1 R132H reduced HIF levels [47,84]. Recently other studies found decreased levels of VEGF in IDH mutants compared to WT GBM samples and that the expression of HIF2α and microvessel density were independent of IDH mutation status [85]. On the same line, a gene set variation analysis of a cohort of TCGA patients has shown that IDH1/2 mutations were associated with an inhibition of angiogenesis signaling pathways. The same study found also that IDH mutant gliomas tend to have a lower relative cerebral blood volume compared to the IDH WT counterpart, indicative of a decreased angiogenesis of IDH mutant samples compared to the WT [86]. However, other findings reported that in primary GBM, intra-tumoral levels of HIF1α and peripheral serum levels of VEGF were significantly higher in IDH1 mutated versus IDH WT samples [87]. Thus, these discrepancies may be due to experiments being conducted in cells originating from different tissues and organs but might also arise from the subset of genetic changes characterizing the different types of tumors analyzed. Consequently, a link between IDH mutation status and hypoxia-induced angiogenesis is not a straightforward phenomenon, and the difference in cellular contexts and the associated genetic changes between different tumors may cause contrasting experimental results. However, albeit controversial, these findings are interesting as highlight the possibility that HIF, despite being traditionally viewed as an oncogene, may also act as a tumor suppressor under certain conditions.

IDH Mutation and Tumor-Associated Epilepsy

Patients affected by IDH mutant gliomas present a more frequent epileptic activity compared to IDH wildtype patients [88] [89]. Unfortunately, the seizures shown by IDH mutant patients are frequently resistant to first-line epilepsy treatment [90]. Nevertheless, recently it has been reported that a patient affected by IDH1 mutant oligodendroglioma showed reduced tumor-associated epilepsy after treatment with the IDH1 mutant inhibitor AG-120 (ivosidenib) [91]. To explain the higher incidence of seizures in IDH mutant patients compared to the IDH wildtype patients, the neuroexcitatory properties of the D2-HG need to be considered. D2- HG is released by IDH mutant glioma cells into the tissue microenvironment, and it has structural similarities to glutamate, thus it may communicate to the surrounding neurons via the interaction with the NMDA receptor [92].

Indeed, it has been demonstrated that activation of NMDA receptors, whether by glutamate or D2-HG, leads to an increase in membrane excitation and thus, epilepsy [93] [94]. The epileptogenic activity elicited by IDH mutation on the neighboring neurons has been recently demonstrated to be triggered by the mTOR pathway and it can be blocked by the addition of rapamycin [77]. However, the concentrations of extracellular D2-HG produced by mutant IDH can reach the mM range [95], whilst the normal extracellular concentration of glutamate is estimated to be between the nM and μM range [96]. This suggests that levels of D2-HG in mutant IDH gliomas, by leading to an exacerbated or prolonged activation of glutamate receptors may also be excitotoxic.

The Role of IDH Mutation in Tumor Invasion

Glioma cell invasion is one of the main reasons for tumor recurrence and thus poor prognosis. The exact mechanisms underlying invasion are yet to be elucidated but mathematical modeling using data obtained from MRI scans has demonstrated that IDH mutant tumors are less aggressive but more diffuse and invasive than their WT counterparts [97]. To explain this phenomenon, it has been proposed that IDH mutant tumors can modulate their microenvironment, by increasing the extracellular levels of D2-HG. High levels of D2-HG might acidify the stroma of the tumor to a degree that is toxic to normal cells, causing the degeneration of the tissue and formation of space for the migration of glioma cells [97]. However, since no evidence of extensive acidosis and tissue damage is observed in the majority of grade II and grade III gliomas, where normally D2-HG production occurs, alternative explanations have also emerged. It has been proposed that glioma cells, and especially IDH mutant cells, in which αKG and NADPH levels are decreased [98], import glutamate from the tumor microenvironment, which is used to produce αKG in the anaplerotic reaction glutaminolysis. Therefore, in IDH-mutated glioma cells, the neurotransmitter glutamate could act as a chemotactic compound [99].

In colorectal cancer cells, exogenous administration of D2-HG was found to directly induce epithelial-mesenchymal transition (EMT) by increasing the genetic expression of ZEB1, a master regulator of EMT; consequently, D2-HG was found to promote cancer cells motility and invasion. Interestingly D2-HG was applied at 250μM whilst endogenous production by IDH mutation has been known to reach the mM range [95]. This would suggest that this phenomenon may be present even at an enhanced level in human disease. The same study found that elevated levels of D2-HG were present in some colorectal cancer cells and specimens in absence of mutations in IDH. In particular, colorectal cancers with high levels of D-2-HG were the samples with an increased frequency of distant organ metastasis [100]. Another study involving glioma cell lines T98 and U87 also showed that exogenous application of D2-HG led to an increase in cell proliferation and invasion. This study went one step further by introducing the IDH1 R132H mutation via a lentiviral transduction method. These cells - along with non-transduced, D2-HG treated cells – had an increase in protein expression of β- catenin, which is involved in the invasion-associated epithelial-mesenchymal transition [101]. Interestingly, another study utilizing overexpression of IDH1 R132H in U87 and U251 cells demonstrated the opposite effect on cell proliferation and invasion whilst also having a negative effect on β-catenin [102]. This contradictory evidence suggests that IDH mutation, subsequent production of D2-HG, and cell invasion mechanisms are not linear and may have many other factors involved which can be influenced by experimental conditions. Interestingly, *in vivo,* studies in which IDH1 R132H was selectively induced in the brain subventricular zone (SVZ) of the adult mouse demonstrated that SVZ cells gained the ability to proliferate ectopically and spread outside of the SVZ into the surrounding brain regions, a process which in healthy mice does not occur [83]. This indicates that IDH1 mutation grants cells the capability of invasion into the nearby parenchyma in the early stages of tumor development.

The Emerging Role of IDH3 in Cancer and Beyond

IDH3 is the third member of the IDH family and a key enzyme of the TCA cycle. As mentioned previously, IDH3 catalyzes the irreversible conversion of isocitrate to αKG, meanwhile reducing

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NAD+ to NADH [103]. NADH produced during the TCA cycle is then used to generate ATP by the electron transport chain [104].

Increasing experimental evidence indicates that IDH3 is involved in the epigenetic regulation of the genome. The αKG is an obligatory cofactor of the αKG-dependent dioxygenases, a superfamily of enzymes involved in many biological processes including epigenetic regulation of gene transcription [105]. Consequently IDH3, by producing αKG, may modulate the enzymatic function of the αKG-dependent dioxygenases, and consequently influence the demethylation of DNA and histones. It has been demonstrated that like several other TCA enzymes, such as pyruvate decarboxylase and pyruvate dehydrogenase, IDH3A localizes temporarily to the nucleus during the zygotic development, and it contributes to the expression of genes important for zygotic genome activation and developmental progression [106]. Another recent study demonstrated that mitochondrial metabolites, including TCA cycle intermediates, play critical roles in regulating cell pluripotency. During somatic cell reprogramming to pluripotency acquisition, many TCA cycle enzymes, including Idh3a localized to the nucleus to induce epigenetic remodeling [107]. Moreover, recently, a nonclassical and incomplete TCA cycle was discovered in the nucleus of eukaryotic cells, to possibly generate and consume metabolic intermediates used not for energy production, but for epigenetic regulation [108].

Loss-of-function and missense mutations in both *IDH3A* and *IDH3B* have been linked to inherited retinal diseases (IRDs), which are a leading cause of blindness in children and adults [109] [110] [111]. Besides, like many other TCA cycles enzymes, whose genetic mutations have been implicated in neurological disorders [112–120], a homozygous missense mutation (p.Pro304His) in *IDH3A* has been associated with a severe form of encephalopathy, which causes neurological defects from birth and retinal degeneration [121].

Currently, *IDH3* is not mutated in any tumors. In a screen of 47 glioblastoma samples, no *IDH3* mutations were found [122]. However, abnormal expression of IDH3 has been implicated in the cause and development of several cancers [123–126].

IDH3α is overexpressed in various human cancer cell lines [123] and cancers [126], and in patients affected by human hepatocellular carcinoma (HCC) [126], lung and breast cancers [123] high levels of IDH3α expression were correlated with poor prognosis. Interestingly, IDH3α was identified as a novel upstream activator of HIF-1α. *In vitro,* data showed that IDH3α overexpression results in an upregulation of HIF1α in both normoxic and hypoxic conditions. By reducing the level of intracellular α-KG, aberrant expression of IDH3α was found to suppress the hydroxylation of the HIF-1α protein, and consequently to upregulate HIF-1α stability and transactivating activity. Accordingly, *in vivo,* data showed that overexpression of IDH3α significantly accelerated the growth of HeLa/IDH3α xenograft tumors, while silencing IDH3α was observed to obstruct tumor growth [123]. IDH3 was found to be overexpressed also in GBM and it was found to promote GBM progression, through its essential role in the one-carbon metabolism, which regulates nucleotide production as well as DNA methylation through its effect on cytosolic serine hydroxymethyltransferase (cSHMT) [124]. Interestingly, it was found that cells on the leading edge of the tumor expressed IDH3α more than those in the center of the tumor.

Recently, IDH3α has been found to promote the progression of HCC by inducing expression of metastasis-associated 1 (*MTA1*), an oncogene regulating cancer progression and metastasis [126]. Aberrant expression of IDH3α in HCC cells has been found to promote epithelial-mesenchymal transition by inducing MTA1 expression and thereby increasing cell migration and invasion [126].

In contrast, it was found that the downregulation of IDH3α promotes the transformation of fibroblasts into cancer-associated fibroblasts by up-regulating HIF-1α, which in turn caused a switch from oxidative phosphorylation to glycolysis [125].

All these studies demonstrated that although the role of IDH3 in cancer is still not clear, the impact of IDH3 on tumor initiation and progression is definitively context dependent; while in some cell types, aberrant expression of IDH3 is advantageous, in others downmodulation of IDH3 is important to promote cell transformation. The various effects of IDHα on different cell types could be

explained by the specific metabolic requirements of each different tissue during malignant transformation.

Novel Therapeutic Options for IDH Mutant Glioma Direct Inhibition of Mutant IDH

Over the last decade, there has been a rapid development of direct inhibitors of mutant IDH, aimed to decrease the levels of D2-HG or 2-HG in IDH-mutant tumors. AGI-5198 has been reported as the first novel, synthetic, direct enzyme inhibitor of IDH [127]. Celgene Corporation and Agios Pharmaceuticals was the first collaboration to generate IDH mutant inhibitors via a high-throughput screening campaign. AGI-5198 is a phenyl-arginine-based compound able to block the generation of 2-HG produced by IDH mutant cells by 90% and to impair the growth of IDH mutant xenograft in vivo [128]. As reported by Rohle and colleagues AGI-5198 showed efficacy in patient-derived glioma xenograft models but the drug was also able to induce the differentiation of glioma cells, to reduce cell proliferation and histone methylation. However, it is worth noting that the global DNA methylation leading to the glioma CIMP phenotype was notably unchanged after the administration of the drug [127]. A study using IDH1 mutant knock-in human astrocyte models later suggested that the IDH1 inhibition has a small effective time frame because the IDH1 mutation is likely to change from a 'driver' to a 'passenger' mutation [129]. Despite the reduced proliferation and histone modification, 4 days after the oncogenic insult, the drug was found to be incapable of blocking or reversing the IDH1 mutation phenotypic changes. Further, work by Tateishi and colleagues showed that treating patient-derived $IDH1^{R132H}$ glioma tumor spheres with the S- enantiomer of the AGI-5198 does not block cell proliferation despite the decrease of 2-HG levels, and in vivo data using an orthotopic xenograft glioblastoma IDH mutant model showed similar survival of mice treated with the AGI-5198 S-enantiomer compared to the mice treated with vehicle [130]. These mixed results in glioma models can be explained by the fact that IDH mutations are an early event in gliomagenesis and an oncogenic driver in LGGs, however during tumor evolution IDH mutant gliomas acquire a plethora of other subsequent mutations, which might render gliomas, and especially high-grade gliomas, almost ineffective to IDH mutant inhibitors. Another hypothesis is that there might be an epigenetic memory that is indelible by the IDH mutation enzymatic inhibition. Thus, a combinatorial approach to treatment may be needed. In addition, the fact that AGI-5198 is rapidly metabolized and cleared leads to the conclusion that it might be a poor candidate for use of clinical trials [131].

AG-120 (ivosidenib), AG-881 (vorasidenib), and AG-221 (enasidenib) are the second generations of selective, reversible drug inhibitors produced, which are approved by F.D.A. for the treatment of acute myeloid leukemia [132,133]. AG-221 was the first selective IDH mutant inhibitor drug to be approved by F.D.An in 2017, **Table 1**. Initially, via high-throughput screening, a precursor of the drug was chosen, being the most prevalent form of IDH2 in AML, $IDH2^{R140Q}$ [134] [135]. The precursor was able to bind to an allosteric site of IDH2 within the heterodimer interface of the enzyme, much like its predecessor AG-6780 [136]. However, it was shown via x-ray crystallography that the heterodimer interface binding forces $IDH2R^{140Q}$ to adopt a non-catalytic open conformation leading to inhibition consistent with thefu nctional changes described in $IDH1^{R123H}$ inhibition [137]. Subsequent modifications optimized the potency, solubility, clearance, and bioavailability of the drug leading to the development of AG-221 [134]. AG-221 showed time-dependent potency leading to the reduction of 2-HG in biochemical assays [134] [134] [138] with subsequent 2-HG depletion in transgenic $IDH2^{R14OQ}$ TF-1 erythroleukemia cells and in patient-derived primary AML cells in both $IDH2^{R140Q}$ and $IDH2^{R172K}$ [134]. *In vivo,* within 20 days the mouse models with patient-derived $IDH2^{R140Q}$ AML produced cells specific markers of differentiation such as CD11b, CD14, and 15 with a reduction in the CD17 immature marker with an increase in survival. Regarding the use of AG-221, no information yet exists regarding its penetrance through the BBB in addition the fact that IDH2 mutations are less abundant in gliomas did not lead to many clinical studies for glial tumors. Currently, there is a single trial of dose escalation for gliomas (NCT02273739, **Table 1** and other IDH mutant tumors, which started in 2014). The drug showed inhibitory effects for these tumors. However, appropriate dosing was an issue.

AG-120 (Ivosidenib) was developed by the Celegene/Agios aiming to optimize the AGI-5198 for human therapeutic applications [139]. Broad structure activity profiling led to the replacement of the cyclohexyl moieties with the fluorinated cycloalkyl groups, preventing oxidation by the liver [131], plus the addition of the pyrimidine ring allowed for better potency leading to the drug called AG-14100. However, AG-14100 was a potent liver enzyme inducer. Subsequent modification led to AG-120 with good potency and clearance. AG-120 is a specific allosteric, reversible inhibitor of IDH1m, competing with magnesium, an essential co-factor for the enzyme, preventing the formation of the catalytic site [140]. AG-120 was shown to inhibit a plethora of IDH^{R132H} mutants cell lines selectively [141]. A significant disadvantage is a low penetrance (4.1%) in mouse models with intact BBB, which may be increased with a disrupted BBB in glioma models [127]. More animal studies are needed to evaluate its penetrance. Regarding mouse models, a dose-dependent 2-HG depletion has been observed, across cell types including an $IDH1^{R132H}$ mutant glioma xenograft mouse model [142] [127] [143]. In addition, the same articles reported that AG-120 was able to modulate some oncogenic properties of cancer cells such as inducing differentiation in AML myeloblasts and inhibiting invasion and migration in chondrosarcoma cell lines [142] [143] [144]. Thereafter, the drug was F.D.A. approved for refractory/relapsed AML and currently under investigation in 2 clinical glioma trials evaluating the efficacy and safety profile, **Table 1**. In 2015, the phase 1 data on the safety of AG-120 in patients with advanced gliomas amongst other solid tumors showed that the drug is well-tolerated with a promising pharmacokinetic profile [145]. In the trial, AG-120 was initially administered in a dose-escalation manner over 28 days ranging from 100mg to 1200mg. The maximum dose resulting in greater D2-HG reduction was 500mg. Therefore, 500mg was chosen for the dose expansion phase of the trial for both enhancing and non-enhancing IDH mutate gliomas [146]. The latest data showed that the AG-120 optimal dosing regime of 500mg once a day led to a 98% reduction of the 2-HG in cholangiocarcinoma and chondrosarcoma patients [146]. However, the plasma 2-HG levels for the glioma did not seem to be a robust pharmacokinetic marker. Therefore, a second multicentre trial is underway on recurrent LGG non-enhancing gliomas with IDH mutation using an AG-120 and AG-881, with the primary outcome to compare the D2-HG concentrations in surgically removed tumors, which were treated versus not treated with the drug inhibitors [147]. Clinical safety, dosage, tolerance, and pharmacokinetics are also to be studied. This safe, feasibility trial will provide appropriate dosing of AG-120 for future randomized studies. It is worth noting that the new inhibitors exhibit a good CSF-plasma ratio [131]. On the contrary, the main issue with the most common chemotherapeutic agent for HGGS, the TMZ, was whether the amount of the drug reaching the tumor might not be enough to eliminate the remaining cells. It is worth noting that no serious adverse events have been reported to date with the use of AG-120.

AG-881 also named Vorasidenib is the only oral pan-inhibitor for both IDH1 and IDH2 mutations, developed by Celgene and Agios Pharmaceuticals [148] [149] [150]. AG-881 was shown to bind the allosteric pocket at the dimer interface, leading to steroid hindrance and subsequent conformational change to an open and inactive state [149]. AG-881 though achieved more efficient inhibition in vitro with shorter incubation periods to the $IDH1^{R132H}$ mutation [149]. Experiments showed the inhibition of 2-HG formation following 1h incubation in genetically engineered patient-derived TS603 glioma cell lines expressing $IDH1^{R132C, G, H, L, S}$ [151]. In TF-1 and U87 transfected $IDH2^{R140Q}$ and $IDH2^{R172K}$ cells 2-HG inhibition was also shown, as well as inducing differentiation [150]. AG-881 was shown to penetrate the BBB in healthy rodents maybe showing promise to be used in humans. Indeed, AG-881 is currently under a phase 1, open-label dose-escalation and expansion trial for the safety and pharmacokinetic properties to be investigated in both IDH1 and IDH2 mutated gliomas [152] [152]. According to the data presented in 2021, the drug shows a favorable pharmacokinetic profile with no severe adverse effects and an increase in survival to 24 months in 60% of the patients [153]. The median progression-free survival was 36.8 months [95% confidence interval (CI), 11.2–40.8] for patients with non-enhancing glioma and 3.6 months (95% CI, 1.8– 6.5) for patients with enhancing glioma. Previously, a dose range of 25-300mg was tested in the dose escalation arm and 10 or 50mg in the dose expansion arm [152]. Doses >100mg lead to toxicity. The most advanced clinical trial design is a phase 3 multicenter, randomized, double-blind, placebo-

controlled study of AG-881 in subjects with residual or recurrent grade 2 glioma with an IDH1 or IDH2 mutation **Table 1** [154]. Approximately 366 participants are planned to be randomized 1:1 to receive orally administered AG-881 50 mg QD or placebo, **Table 1**. There is now (March 2023) a press release out awaiting official results to be published.

BAY-1436032 is a relatively new species, non-competitive IDH1m inhibitor with adequate pre-clinical results in AML and glioma models (**Table 1**). This compound is an allosteric IDH1 enzyme inhibitor, which binds to the dimer interface of mutant IDH [155]. BAY-1436032 was selected across a selection of over 3milllion compounds based on IDH enzymatic activity with IC50 ranging from 0.6 to 17.1 micrometers [155]. The drug showed equivocal inhibition to all $IDH1^{R132}$ mutants in AML human-derived cell lines and genetically engineered lines of solid tumors with reduced proliferation and induction of differentiation. In astrocytoma, xenograft $IDH1^{R132H}$ mouse models the drug showed effective penetration through the BBB with prolonged survival. There are two ongoing trials for AML and solid tumors reviewed here [156]. The glioma trial is an open-label, non-randomized, multicenter phase I study to determine the maximum tolerated or recommended phase II dose of oral mutant IDH1 Inhibitor BAY1436032 and to characterize its safety, tolerability, pharmacokinetics, and preliminary pharmacodynamic and anti-tumor activity in patients with $IDH1^{R132X}$ mutant advanced solid tumors including gliomas [157], **Table 1**. In dose escalation, 29 subjects with various tumor types across doses in the dose escalation ranging from 150 to 1500mg demonstrated a 76% reduction in D2-HG levels. A dose of 1,500mg was chosen for the dose expansion phase. 35 glioma patients showed an 11% objective response rate and 43% stability of the disease [157]. The full results are to be published. Another new inhibitor, the MRK-A was able to achieve a robust intracranial 2-HG inhibition in the orthotopic mouse brain tumor models generated using BT142 and GB10 glioma cells, in which IDH mutation has naturally occurred. However, even with near complete inhibition of intratumoral 2-HG production, not all IDH mutant glioma models responded to treatment, but only BT142 displayed significant tumor growth inhibition resulting in a measurable survival benefit. Pronounced differences in the gene expression patterns between BT142 and GB10 tumors were also observed following MKR-A treatment [158].

IDH-305 is an allosteric non-competitive inhibitor of $IDH1^{R132C, H}$ developed by Novartis. IDH-305 stabilizes the enzyme via an inactive conformational change [159] showing efficacy in reducing 2-HG levels with substantial blood-brain barrier penetrance in murine models. A current phase 1, single group assignment, open-label for advanced malignancies that harbor IDH^{R132H} mutations trial has just been published, NCT02381886, Table 1 [160]. 35/41 patients demonstrated target engagement with a reduction in 2-HG concentration at all doses -75-750 mg twice daily. Complete remission (CR) or CR with incomplete count recovery occurred in 10/37 (27%) patients with AML and 1/ 4 patients with myelodysplastic syndrome. Adverse events (AEs) suspected to be related to the study drug were reported in 53.7% of patients. There are a number of new IDH1-specific inhibitors such as the FT-2102 IDH^{R132C} competitive inhibitor, which is in clinical trial monotherapy, **Table 1**. The Ds 1001b direct $IDH1^{R132X}$ inhibitor with evidence of penetrance through the BBB both in humans and xenograft mouse models is also highly promising with the phase 1 single group assignment trial showing tolerance at 1400mg with a progression to phase 2 trial, [161] **Table 1**. Phase 1 showed that twice-daily oral administration resulted in antitumor activity in patients with recurrent/progressive IDH1-mutated glioma. A phase II study of DS-1001 in patients with chemotherapy- and radiotherapy-naïve IDH1-mutant WHO grade 2 gliomas are ongoing to verify the efficacy of DS-1001 as a single agent (NCT04458272).

A number of other new inhibitors are noted in the table with some promising results in other malignancies such as the AML outlined in another comprehensive review [156]. Despite the positive results, the success of IDH-mutant inhibitors is found to have a plethora of limitations in IDH-mutant gliomas. A study showed that although AGI-5198 reduces neomorphic activity, it also does not alleviate the DNA and histone hypermethylation phenotype since histone methylation was found to be high [129]. Further, Sulkowski and colleagues demonstrated that AGI-5198 is preventing DNA damage in cancer cells, leading to the conclusion that this might allow for resistance to DNA damage agents like current chemo and radiotherapeutic options [162]. This has also been confirmed by another

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study, which suspected that IDH1 mutated cells under the action of AGI-5198 gain radioprotective abilities [163]. Currently, a number of other novel molecular inhibitors are being tested. Those can be combined with IDH inhibitors to overcome possible drawbacks of each, through umbrella and platform trials ongoing such as the Tessa Jowell BRAIN MATRIX Platform (TJBM) [164].

IDH Vaccine

IDH mutations are an early event in gliomagenesis [165], and are present in recurrent gliomas [166] [167]. This makes mutant IDH an excellent target for potential immune therapies. Initial attempts to produce vaccines against mutant IDH were performed by Schumacher *et al.* where they inoculated mice with a 20 amino acid peptide spanning part of the mutated catalytic the pocket of the IDH enzyme. This resulted in mice mounting a robust immune response from $CD4^+$ T-helper cells, which were specific to the IDH mutation. The growth of subcutaneous sarcomas carrying either IDH1 WT or $IDH1^{R132H}$ was investigated following preventative vaccination. Tumor-positive for $IDH1^{R132H}$ grew more slowly than those expressing the WT protein, following vaccination with the mutant IDH peptide vaccine [168], showing the efficacy of the anti-tumor therapy. A direct follow-up to this initial vaccine research was the NOA16 trial, a first-in-human, single-arm phase 1 trial in which newly diagnosed patients with grades III and IV $IDH1^{R132H}$ astrocytomas were recruited [169]. The presence of IDH mutation confers a survival advantage to patients diagnosed with grade III or IV gliomas and so acts as a unique target for immune therapy [170] [171]. The trial comprised 8 vaccinations in total and 90.6% of participants experienced non-severe adverse effects. A greater percentage of trial participants experienced pseudoprogression – a phenomenon by which a tumor appears to have increased in size when viewed via imaging tests, but where no such growth has occurred – compared to a molecularly matched control group which was indicative of an immune response induced by the $IDH1^{R132H}$ vaccine. However, the authors do concede that the pseudoprogression seen may have been a delayed response from previous rounds of radiotherapy as they excluded patients presenting with pseudoprogression from the trial [169].

In summary, research conducted so far indicates that mutant IDH is a potential target for immunotherapy. The phase 1 NOA16 trial shows that the vaccine is well tolerated and safe thus paving the way for phase 2 trials to assess the vaccine's effectiveness. Patients included in the NOA16 trial were diagnosed with either WHO grade III or IV astrocytomas, leaving the door open for IDH vaccination research in oligodendroglioma to understand if IDH vaccination can be of benefit to this group. The IDH^{R132H} vaccine has made promising steps towards becoming a novel therapeutic option for glioma, however, other cancers regularly display mutations in either IDH1 or IDH2, thus opening the possibility of also being susceptible to targeted IDH mutant vaccination. Glioma most commonly contains the IDH^{R132H} point mutation [172], however, this is not strictly conserved in other cancers which have been shown to express variants including $IDH1^{R132C}$ and $IDH1^{R132G}$ but also mutations in IDH2, commonly, $IDH2^{R140Q}$ and $IDH2^{R172K}$ [173]. This means there is a wide range of potential targets for IDH-specific vaccination treatments across different cancers.

Modulating Epigenetic Alterations in IDH Mutant Gliomas

As previously mentioned, the IDH mutation leads to histone and DNA hypermethylation patterns [26,27,29,57–59,62–66,174].

The hypermethylation phenotype might lead to oncogenic activities in IDH mutant cells, thus intervening with the epigenetic changes has been postulated as a potential therapeutic option for IDH mutant gliomas. Flavahan and colleagues have demonstrated that glioma CpG island methylator phenotype (G-CIMP) is linked to hypermethylation at sites for cohesion and CCCTC-binding factor (CTCF), leading to the reduced affinity of this protein [175]. The CTFC-reduced binding affinity allows for the enhancer-mediated expression of PDGFR-A. PDGFR-A is a known mitogen that has been linked to gliomagenesis [176]. By administrating a demethylating agent, they showed that the CTCF binding is partially restored and the PDGFR- A expression is reduced. The notion that inhibiting hypermethylation might be beneficial has also been documented by another study. Decitabine, a DNA methyltransferase inhibitor was able to suppress the proliferation both in vitro

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and *in vivo*, of IDH mutant glioma cells [177]. Concomitantly, the usage of 5-azacytidine, an analog that controls the DNA methyltransferase activity, has led to reduced proliferation of the IDH-mutated xenograft glioma model [178]. However, epigenetic changes are only a piece of the puzzle. Combinatorial therapies might be needed to tackle the oncogenic potential induced by IDH mutations.

Inhibiting DNA Repair

DNA is susceptible to a range of damaging agents both from within the body, such as replication errors and toxins, and from outside the body, such as UV and ionizing radiation [179]. Because of this, we have evolved an impressive array of mechanisms to counteract this damage including homologous repair (HR) and non-homologous end joining (NHEJ) [180].

As already mentioned, the tumorigenic effects of D2-HG are proposed to derive from modulating αKG-dependent dioxygenases, many of which are involved in histone and DNA methylation, but also enzymes playing key roles in the DNA damage response, such as alkB homolog (ALKBH) enzyme [181,182]. Wang et al. found that glioma cells expressing mutant IDH have reduced repair kinetics, accumulated more DNA damage, and were sensible to alkylating agents. The sensitization to alkylating agents could be reversed by the overexpression of ALKBH2 or AKLBH3. This data indicates that alkylating agents may represent a valuable therapeutic option for treating IDH-mutated cancer patients [181].

D2-HG is also able to compromise the HR of DNA damage response [162]. Sulkowsky et al. found that the application of a 2-HG analog – 2R-octyl-α-hydroxyglutarate – to IDH1 WT cells caused an increase in double-strand breaks, suggesting that D2-HG was able to inhibit the HR pathway [162]. Alongside HR there is another pathway of DNA repair with relies on the poly(ADP-ribose) polymerase (PARP) family of enzymes. PARP enzymes play a role in the repair of single-strand breaks where they perform base-excision and nucleotide-excision repair [183]. Interestingly, when the HR pathway is inhibited in mutant IDH1 cell lines due to the D2-HG accumulation, PARP enzymes become a point of failure in the system. Specifically inhibiting PARP in these IDH1 mutant cells leads to the selective death of mutant but not WT cells. Conversely, inhibiting the neomorphic activity of mutant IDH1 with specific small molecule inhibitors reversed the deficiency in HR, with the number of double-strand breaks almost returning to that seen in IDH1 WT cells. Interestingly, the PARP inhibitor sensitivity induced by mutant IDH1 is present and functional in both patient-derived AML cells and glioma cells [162]. Taken together, this data indicates that IDH1 mutant cells are sensitive to the activity of PARP inhibitors, and this has the potential to be exploited in IDH mutant malignancies. In another study, it was demonstrated that depleting NAD+, which is needed for PARP during TMZ-induced BER, using GMX1778 as well as inhibiting Nicotinamide phosphoribosyltransferase (NAMPT) using FK866, eliminates the remaining PARP of repair activity [130] [184]. This induces a specific metabolic stress response to TMZ-induced DNA damage and improves the duration of the therapy response. Additionally, there is a currently active phase II/III trial – NCT02152982 – investigating whether the combination of TMZ and PARP inhibitor veliparib is more effective at treating newly diagnosed glioblastoma compared to TMZ alone. Recruited patients are required to have MGMT promoter methylation as this indicates that tumors are sensitive to TMZ and that the added inhibition of PARP DNA repair would further exacerbate DNA damage leading to a higher rate of cell death [185]. At the time of writing, this trial reached its primary completion date on the 1st of December 2021, with the estimated study completion date being the 15th of December 2024 (https://clinicaltrials.gov/ct2/show/study/NCT02152982).

Another type of DNA repair comes in form of telomere lengthening, which is a mechanism employed by cancer cells to maintain their growth over time [186]. Lower-grade astrocytomas as well as secondary glioblastomas frequently have a loss of TERT which would normally mean that cells wouldn't be able to maintain the length of their telomeres thus resulting in stunted cell growth. However, TERT loss is usually accompanied by loss of ATRX and IDH1 mutation which when combined in cells is associated with alternative lengthening of telomeres (ALT) [187]. ALT is a poorly understood mechanism related to homologous recombination that allows tumor cells to continue

growing whilst protecting their telomeres from replicative shortening [188]. In low-grade gliomas, it may be possible for IDH inhibitors to ameliorate the ALT phenotype since the triad of loss of TERT, loss of ATRX, and IDH mutation are required to produce the ALT phenotype [187]. However, given IDH mutation is such an early event in gliomagenesis it may not be clinically realistic to administer inhibitors in time to prevent tumor formation. In light of this, there have been studies into inhibitors of the ALT mechanism itself including an inhibitor of ATR kinase which leads to an increase in the fragility of telomeres [188]. In conclusion, genomic instability and glioma metabolism is interrelated and thus offer a unique area to explore therapeutic strategies.

Inhibiting Metabolic Pathways

IDH mutant enzymes cause the accumulation of D2-HG and thus drain some of Krebs's cycle intermediates. Therefore anaplerotic pathways are recruited to compensate for the loss of Krebs cycles metabolites, such as α-KG. Understanding and delineating the new metabolic alterations will allow us to evaluate potential druggable targets as novel therapeutic options. In IDH mutated glioma, the *de novo* production of NAD is reduced due to epigenetic silencing of nicotinamide phosphoribosyltransferase *(NAPRT1*). NAD is an important co-factor, pertinent to electron transport and metabolism of redox reactions, as it can carry H+ ions and is derived from *de novo* and salvage pathways [189]. In IDH mutant glioma cells the *de novo* production of NAD is reduced. Therefore the only option for these cells is to generate NAD through salvage pathways [189] [130]. Thus, blocking the salvage pathway, by inhibiting the enzyme Nicotine phosphoribosyltransferase (NAMPT) can inhibit NAD production [190]. Thus in IDH-mutant glioma cells, where the *de novo* production of NAD is reduced by the silencing of the *NAPRT1* gene, the inhibition of NAMPT leads to the reduction of NAD+ salvage pathways (NAMPT), resulting in a lack of metabolic substrate. This leads to a biochemical crisis activating the energy sensor AMPK initiating autophagy. In contrast, the IDH wildtype cells had an increased expression of NAPRT1 and alternative pathways to maintain the energy supply of NAD+. In, summary the study concluded that IDH1 mutation can make cells NAD+ dependent, thus it is a rationale therapeutic target in metabolic pathways.

Moreover, in IDH mutant cells glutaminolysis is the major pathway of metabolic compensation due to a lack of isocitrate [39]. Thus, targeting glutamine/ate metabolism might deplete the energy sources and thus inhibit major anabolic functions of the IDH mutant cell. For instance, bis-2-[5-9phenylacetamide)-1,3,4-thiadiazol-2y]ethyl sulfide has been shown to block glutaminase and thus hampers the glutamate metabolism and reduces proliferation and growth in IDH mutant AML cell [40] [191]. Further, another drug called Zaprinast was able to block glutaminase and reduce the proliferation of IDH-mutated glioma cells [192]. Moreover, another glutamine inhibitor, a drug called telaglenastat (CB-839) was shown to cause reduced D2-HG production and induce glioma differentiation [193]. A phase 1 RCT combining TMZ, radiotherapy, and the glutaminase blocker CB-839 is about to start recruiting patients affected by IDH-mutated diffuse and anaplastic astrocytomas [194]. By suppressing glutaminolysis, tumor growth might cease, and differentiation might take place.

Modulating Redox Homeostasis

ROS are predominantly elevated in IDH-mutated tumors [51] [195]. It was found that glutamine/ate and glutathione are reduced in IDH-mutated glioma cells compared to adjacent normal tissues. D2-HG is negatively correlated with the levels of glutathione. Therefore, glutathione may be essential for IDH mutant maintenance of redox homeostasis [196]. Increased consumption and thus, reduction of glutathione, suggests the increased burden of ROS scavenging. Understanding these relationships will allow for therapies able to intervene with redox homeostasis. Limiting ROS scavenging, which is driven by glutathione, could be an add-on therapy to the existing or under-trial therapies. As previously mentioned, CB-839 can lead to blockage of glutamine metabolism and thus impair redox homeostasis as well as sensitization to radiotherapy [197].

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Differentiation Therapy

One of the main consequences arising from IDH mutation is its ability to block normal cellular differentiation. Studies performed in a variety of IDH mutant malignancies have demonstrated that mutant IDH can halt normal cell differentiation, causing an expansion of progenitor cells, which is thought to be an important step in cancer development [29] [198] [199] [200] [80]. Inhibition of mutant IDH with small molecule inhibitors can release the differentiation block, and possibly hinder tumor growth.

In the glioma context, Rohle et al. demonstrated that inhibition of mutant IDH by using a small IDH mutant selective inhibitor, AGI-5198 hampered the in vitro and in vivo growth of an oligodendroglioma cell line (TS603) harboring an endogenous IDH1-R132H mutation. Treatment of mice xenografts with AGI-5198, caused an upregulation of many several genes involved in glial differentiation, while the in vitro treatment of TS603 cells with AGI-5198, showed a loss of repressive histone marks at the promoters of astrocytic marker genes. This study demonstrated that, at least in this model, targeting mutant IDH1 can impair glioma development *in vivo*, which is related to changes in cellular differentiation [127].

More recently, treatment of undifferentiated glioma cells, which ectopically express IDH mutation (BT142) with an IDH mutant inhibitor (MRK-A), has an undifferentiated cell state and showed in vitro a decreased expression of some key stem cell markers. Treatment of mice bearing orthotopic BT142 tumors with MRK-A showed a reduction in the 5mC DNA signature, indicating a release of the hypermethylation phenotype seen in IDH mutant tumors. Following treatment with MRK-A, showed a significant upregulation of 245 genes, which is in line with the removal of the hypermethylated phenotype. Mice that received MRK- Treatment show an extended survival and reduced Ki67 staining compared to their vehicle-treated control group [158]. This study has begun to explain how differentiation therapy may be of benefit in the treatment of IDH mutant brain tumors, as it removes the 2-HG- dependent hypermethylation phenotype and alters the expression of a wide range of genes, which may enable differentiation of the cells. Further work is needed to understand whether there are any long-term effects of releasing these genes from their suppression via methylation and also to understand if differentiation therapy can be combined with other treatment modalities.

The use of IDH inhibitors as differentiation therapy has been also shown in hematological malignancies such as acute myeloid leukemia (AML). Wang et al. developed a specific inhibitor for the most commonly occurring IDH mutation in AML, IDH2-R140Q, AGI-6780. Treatment with this inhibitor of an erythroleukemia cell line which ectopically expressed IDH2-R140Q decreased the levels of 2-HG and released these cells from the block to differentiation. Similar studies on IDH2-mutated primary human AML cells similarly demonstrated showed a reduction in 2-HG levels and an increased differentiation of mutated AML cells [136]. This data suggests that mutant IDH causes a block to differentiation that is released upon treatment with an inhibitor.

More recent studies have shown that AG-120 (ivosidenib) treatment decreased intracellular levels of 2-hydroxyglutarate and induced differentiation in models of *IDH1*-mutated tumors [131] [201]. Recently, AG-120 has been licensed for use in relapsed or refractory AML [202]. AG-120 was tested in phase I clinical trial where it was shown to induce a substantial remission in patients with mutant IDH relapsed-refractory AML [132].

The combination use of an IDH1 inhibitor, BAY1436032, and a DNA methyltransferase inhibitor, azacitidine, has proven to be a novel, clinically relevant drug combination in the treatment of AML. The frequency of leukemia stem cells (LSCs) in PDX models was analyzed and found that they were decreased 4.1-fold with azacytidine alone and 117-fold with BAY1436032 alone. However, the frequency of LSCs was reduced by 470-fold when the drugs were given sequentially and by 33,150-fold when given simultaneously. This data shows that it is important to continue evaluating the synergistic effects of different drugs. Similarly, azacitidine has also been shown to reverse the differentiation block caused by mutations in IDH2 in chondrosarcoma cell lines [203]. This raises the possibility that IDH mutant chondrosarcoma could be targeted by dual therapy in a similar way to AML outlined above. The use of ivosedenib or enasidenib in the treatment of mutant

IDH AML presents a risk of the patient developing differentiation syndrome (DS) [204]. Whilst the exact cause of DS has yet to be established, it is currently thought to arise from the sudden release of cytokines from myeloid cells as they are forced to differentiate [205]. With this in mind, care must be taken when studying the effects of differentiation therapy in the treatment of brain tumors as there may be unintended consequences and symptoms for the patient.

Conclusion and Future Directions

IDH1 and IDH2 mutations are found in approximately 80% of World Health Organisation (WHO) grade II/III cases. We outline key metabolic alterations, as well as epigenetic changes, and redox imbalance, which might not be uniform across IDH mutant gliomas and ongoing targeted therapeutic options, with a number of clinical trials specific to IDH inhibition recently published. However, whether the metabolic effect holds a key role in oncogenesis and tumor progression or it is merely a consequence of initial gene mutations remains largely unclear. Here we outline pathways and treatments in support of a deranged metabolism driving the disease process. New IDH1 mutant glioma models are needed to examine the spectrum of responses to treatment that may be observed clinically following the administration of IDH1 mutant inhibitors. Taking into consideration that current therapies fail to demonstrate improvement in glioma treatments, IDH-induced biochemical alterations should be adequately understood and assessed as a potential target.

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