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Review

Bridging Cancer Biology with the Clinic: Comprehending and Exploiting IDH Gene Mutations in Gliomas

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Abstract. *Isocitrate dehydrogenases 1 and 2 (IDH1/2) are enzymes that play a major role in the Krebs cycle. Mutations in these enzymes are found in the majority of lower gliomas and secondary glioblastomas, but also in myeloid malignancies and other cancers. IDH1 and IDH2 mutations are restricted to specific arginine residues in the active site of the enzymes and are gain-of-function, i.e. they confer a neomorphic enzyme activity resulting in the accumulation of D-2-hydroxyglutarate (2-HG). 2-HG is an oncometabolite causing profound metabolic dysregulation which, among others, results in methylator phenotypes and in defects in homologous recombination repair. In this review, we summarize current knowledge regarding the function of normal and mutated IDH, explain the possible mechanisms through which these mutations might drive malignant transformation of progenitor cells in the central nervous system, and provide a comprehensive review of potential treatment strategies for IDH-mutated malignancies, focusing on gliomas.*

In the past decade, our knowledge regarding the molecular events that drive malignant transformation in diffuse gliomas has increased significantly. This knowledge led to a major revision of the 2007 edition of the World Health Organization Classification of Tumors of the Central Nervous System that was published 2 years ago, the 2016 Classification of Tumors of the Central Nervous System. In the 2016 revision, molecular parameters are integrated with histology in order to clearly define glioma subtypes, providing at the same time, in some cases, prognostic information. IDH mutations are undoubtedly one of the most important molecular parameters in the current glioma classification (1-3).

IDH mutations in gliomas were first reported in 2008. Parsons *et al*., using next-generation sequencing technology, found *IDH1* mutations to be present in 12% of patients with glioblastoma, mainly in younger patients and in patients with secondary glioblastoma. In this initial publication, it was reported for the first time that the presence of *IDH1* mutations is also associated with improved prognosis in terms of overall survival, relative to patients with glioblastoma with wild-type *IDH1* (4). Following this pivotal study, Yan *et al*. reported that *IDH1* and *IDH2* mutations occur in the majority (>70%) of WHO grade II/III astrocytomas, oligodendrogliomas, oligoastrocytomas and secondary glioblastomas, and are associated with improved prognosis (5). Multiple subsequent studies have confirmed these findings (6-13). Interestingly, two studies have shown that the presence of an IDH mutation in patients with astrocytoma/glioblastoma is a stronger predictor for overall survival than the histological type and grade of the tumor (14, 15).

IDH1 and *IDH2* mutations have also been found in patients with hematological malignancies and in a number of solid tumor types, including acute myeloid leukemia (AML), myelodysplastic syndromes, myeloproliferative neoplasms, cholangiocarcinoma, melanoma and cartilaginous tumors (16-24). In AML, *IDH1*/*IDH2* mutations have been reported in 16-17% of patients, while the frequencies in all other entities remain much lower (23, 25). Furthermore, IDH mutations have also been observed in intrahepatic

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cholangiocarcinomas (26), chondrosarcomas (16) and sporadically in other tumors as well (Table I).

In this review, we summarize current knowledge regarding the function of normal and mutated IDH, explain the possible mechanisms through which these mutations might drive malignant transformation of progenitor cells in the central nervous system, and provide a comprehensive review of potential treatment strategies for IDH-mutated malignancies, focusing on gliomas.

Table I. *Frequency of isocitrate dehydrogenase (IDH) mutations in different tumor types.*

Gene	Tumor type	Frequency	Reference
IDH1	Astrocytoma	54-72%	(6, 9, 41)
	Oligodendroglioma	65-70%	(6, 9, 41)
	Primary glioblastoma	3%	(9)
	Secondary glioblastoma	50-88%	(6, 9)
	AML	6-16%	(65)
	MDS	2-3%	(65)
	MPN	<1%	(65)
	Chondrosarcoma	40%	(16)
	Intrahepatic cholangiocarcinoma	7-20%	(17)
IDH2	Low-grade gliomas and glioblastoma	<5%	(5, 51)
	AML	8-19%	(65)
	MDS	2-7%	(130)
	MPN	2%	(65)
	Chrondrosarcoma	16%	(16)
	Intrahepatic cholangiocarcinoma	3%	(17)

AML, Acute myeloid leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms.

Biochemistry and Function of Wild-type IDH

In biochemistry, IDH enzymes are known for their role in the Krebs cycle (tricarboxylic or citric acid cycle). The IDH family in humans consists of three IDH isoenzymes, IDH1, IDH2 and IDH3, which all catalyze the oxidative decarboxylation of isocitrate into alpha-ketoglutarate (aKG) and carbon dioxide. IDH1 and IDH2 are homodimeric enzymes with high structural similarity (69%) that are encoded by two distinct genes, *IDH1* on 2q33.3 and *IDH2* on 15q26.1, respectively. The protein IDH1 is located in the cytoplasm and peroxisomes, while IDH2 is located in the mitochondria. They are both nitotinamide adenine dinucleotide phosphate ($NADP^+$)-dependent enzymes that use $NADP^+$ as a cofactor, producing NADPH during their enzymatic activity (27, 28). IDH1 and IDH2 are the major sources of NADPH in the cytosol and mitochondria respectively (20).

IDH3 is a heterotetrameric enzyme consisting of two alpha, one beta and one gamma subunit that are encoded by the genes *IDH3A* on 15q25.1-2, *IDH3B* on 20p13 and *IDH3G* on Xq28, respectively. IDH3 is located in the mitochondria and is one of the most critical enzymes in the Krebs cycle. IDH3 uses NAD^+ as a cofactor and produces NADH, which is necessary for energy production (29, 30). Despite its important role in cellular metabolism, mutations in *IDH3* genes have not as yet been linked to tumorigenesis.

IDH1 and IDH2 are necessary for the production of aKG and NADPH. NADPH is an important cofactor in many cellular functions, *e.g.* lipid metabolism (31), glucose metabolism (32) and oxidative stress defense, by reducing glutathione and thioredoxin, and by activating catalase (33-35). Interestingly, IDH1 is the main source of NADPH in the brain and is also thought to be a main source of NADPH in other tissuesl (36, 37).

aKG is also an important molecule in diverse cellular processes, by being an essential cofactor for the family of aKG-dependent dioxygenases. This family consists of more than 60 enzymes that require aKG as a cofactor. aKG-dependent dioxygenases are present in all living organisms and affect multiple cellular functions: they catalyze hydroxylation reactions on a diverse set of substrates, including collagen, lipids, proteins, histones, transcription factors, alkylated DNA and RNA, 5-methylcytosine of genomic DNA and 6-methyladenine of RNA, as well as antibiotics. Members of the aKG-dependent dioxygenase family are propyl hydroxylases, jumonji-C domain-containing histone demethylases, ten-eleven translocation (TET) enzymes, 5-methylcytosine hydroxylases, collagen propyl-4-hydroxylases 1, 2 and 3, and many more (37, 38). Many of these enzymes play a pivotal role in regulating histone and DNA demethylation in normal cells (39, 40).

Biochemistry and Function of Mutant IDH

In gliomas, mutations in the *IDH1* and *IDH2* genes are somatic and invariably heterozygous, missense mutations that result in a single amino acid substitution. Interestingly, these mutations involve specific conserved arginine residues in the active site of the IDH enzymes that play a key role in substrate binding (4).

For the *IDH1* gene, mutations are found at the arginine codon 132 (R132) and the most common mutation is the substitution of arginine by histidine (R132H), which occurs in more than 90% of all *IDH1* mutants. Far less common are R132C substitutions (arginine→cysteine), R132S (arginine→serine), R132G (arginine→glycine) and R132L mutations (arginine→leucine), which occur in approximately 5% of cases. For the *IDH2* gene, the homologous conserved arginine residue is at codon 172 (R172) and the reported mutations are R172K (arginine→lysine), R172M (arginine→methionine), R172G (arginine→glycine) and R172W (arginine→tryptophan) (5, 41), as well as R172S (arginine→serine). All these mutations are

registered in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (https://cancer.sanger.ac.uk/cosmic). The incidence of mutations of each IDH gene differs among tumor types, as summarized in Table I.

The fact that *IDH1/2* mutations affect the active site of the enzyme led to the initial theory that these mutations cause loss of enzymatic activity of wild-type IDH. Initial *in vitro* studies had shown that mutant IDH proteins impair normal IDH catalytic action in a dominant-negative fashion by heterodimerizing to the wild-type enzyme and altering its activity, leading to reduced levels of aKG and NADPH (42-44). Reduced levels of NADPH and aKG could then lead to cellular damage and genomic instability, through impairment of the normal cellular detoxification mechanisms, as well as other important cellular processes described above. This theory was supported by biochemical studies suggesting that mutant IDH1/2 was unable to catalyze the oxidative decarboxylation of isocitrate. However, subsequent studies revealed that mutant IDH enzymes were not catalytically inactive, but rather enzymes with a gain-of-function activity [summarized in (45)].

A landmark report by Dang *et al*. showed that the mutant IDH1 protein is not a non-functional enzyme, but rather an enzyme with a neomorphic activity, which converts aKG to 2-hydroxyglutarate (2-HG) with the simultaneous consumption of NADPH (46). 2-HG is a byproduct in normal mitochondrial metabolism that is normally found at very low levels in the cell and has two enantiomers, D-2HG and L-2HG (or, R-2HG and S-2HG, respectively). *IDH1*-mutant cells exclusively produce D-2HG at very high levels, up to 100-fold higher than in normal tissues. In the same manner, *IDH2* mutations have been found to result in high levels of D-2HG (47). Jin *et al*. showed that accumulation of high levels of D-2HG takes place *in vivo* and *in vitro* only when both wild-type and mutant *IDH1* alleles are expressed (48), indicating that the wild-type–mutant IDH1 heterodimer is critical for the rapid conversion of aKG to D-2HG. Indeed, loss of wild-type IDH1 expression in *IDH1*-mutated glioblastoma cells led to reduced levels of D-2HG. This may explain why loss of heterozygosity is rare in IDH-mutated gliomas (48, 49). Interestingly, *in vitro* studies have additionally shown that L-2HG is increased in human neuroblastoma, pediatric glioblastoma, glioblastoma, renal cell carcinoma, and embryhonic kidney cell lines in the setting of hypoxia *via* enzymatic reduction of aKG (50).

It is worth noting that 2-HG accumulation in human cells has also been described in 2-HG acidurias that are rare inherited metabolic disorders characterized by abnormally high levels of hydroxyglutaric acid in almost all organ systems and body fluids (51). 2-HG aciduria is caused by inherited loss-of-function mutations in the genes encoding enantiomer specific flavin adenine dinucleotide-dependent 2-hydroxyglutarate dehydrogenases (*D-2HGDH* and *L-2HGDH* for D and L enantiomers, respectively) that normally convert 2-HG to aKG preventing its accumulation in cells (52, 53). D-2HG aciduria has also been reported in association with inherited gain-of-function mutations in *IDH2* and is characterized by developmental delay, epilepsy and cardiomyopathy and short life-span, while L-2HG is characterized by a variable clinical course and by increased risk of developing brain tumors in longer survivors (51, 54).

The right-enantiomer, D-2HG, is structurally similar to aKG. The two molecules differ only in the presence of a C2 hydroxyl group in D-2HG instead of the C2 carbonyl group in aKG. D-2HG has been shown to act *in vitro* as a competitive inhibitor of aKG-dependent enzymes, including jumonji-C domain-containing histone demethylases and TET family of hydroxylases. These enzymes, as mentioned earlier, are key regulators of histone and DNA demethylation in cells and therefore their inhibition through D-2HG results in a profile of cellular hypermethylation (38, 55-57). D-2HG is considered an 'oncometabolite' not only because it increases dramatically in cells as a consequence of *IDH1/2* mutations (46), but also because it was shown to transform hematopoetic into leukemia cells (58) and promote epithelial–mesenchymal transition in colorectal cancer cells even in the absence of IDH mutation (59); by contrast, L-2HG does not appear to have such phenotype-aggravating properties.

The intracellular accumulation of 2HG in IDH-mutated gliomas has been used as a non-invasive tool for accurate diagnosis using magnetic resonance spectroscopic (MRS) technology (60).

Recently, a more specific technique has been described, Fourier-transform infrared spectroscopy, which seems promising for rapid and intraoperative glioma classification. This technique can provide prognostic information to the surgeon at the time of the surgery for optimal treatment decisions (61), but needs further development for clinical applications. In the same vein, *in vivo* 3D MRS may be used for patient surveillance in the context of clinical trials for assessing treatment efficiency (62).

Role of Mutant IDH in Oncogenesis

The originally proposed oncogenic effect of IDH mutations through increasing the expression of hypoxia-inducible factor-1 alpha (HIF1α) and its target genes (44) was later rejected since IDH mutations in fact result in increased degradation of HIF1α (63). The exact role of IDH mutations in tumorigenesis is still not fully understood, although much progress has been made in this direction (64).

The described gain-of-function IDH mutations giving rise to gliomas have traditionally been considered as being directly responsible for malignant transformation (13, 30, 65), since these are established in neural progenitors in the

brain (30) before lineage-specific genomic alterations that are described in IDH-mutated gliomas (Figure 1). Corresponding knowledge on the manifestation of IDH mutations in myeloid malignancies is less concise; in leukemia, for example, the biological effects of IDH mutations, including a favorable prognostic impact of *IDH2* mutations, seem to depend on coexisting genomic alterations (66); this may support a previous hypothesis that IDH mutations may develop in later steps of malignant transformation in this context (67).

The early establishment of IDH mutation seems to provide the common origin and perturbed metabolic and molecular environment for the development of IDH-mutation-positive gliomas (68-70) that diverge into specific oligodendrocytic and astrocytic lineages based on additional genomic alterations aided by changes in their microenvironment (71). Thus, oligodendrogliomas acquire changes such as 1p/19q co-deletion and mutations or fusions in key genes located in 1p, such as the capicua transcriptional repressor gene (*CIC*) and far upstream element binding protein 1 gene (*FUBP1*), but also in the promoter of telomerase reverse transcriptase (*TERT*), the gene encoding the catalytic subunit of telomerase (72-74). In comparison, astrocytomas are characterized by the addition of mutations in tumor protein 53 (*TP53*) in 80% of cases and in alpha thalassemia/mental retardation syndrome X-linked gene (*ATRX*); the latter is involved in alternative lengthening of telomeres (75-77), while *ATRX* mutations are mutually exclusive from mutations in the *TERT* promoter. As shown with single-cell RNA sequencing, lineage determination in IDH mutation-positive gliomas is non-overlapping (71), thus justifying the WHO 2016 classification that recommends avoiding use of the histological term 'mixed oligoastrocytoma'.

Additional genomic changes determine the malignant potential of anaplastic gliomas (Figure 1). It remains obscure however, how IDH mutations and the resulting oncometabolite D-2HG contribute to the acquisition of the changes necessary for the development of each glioma lineage, and whether the same mutations drive the transition of low-into high-grade glioma.

The main global metabolic consequence in IDH-mutated cells seems to be energy deprivation: IDH-mutated tumors have a low-energy requirement, with dozens of metabolites contributing to this pro-survival feature (68, 70). The low-energy requirement of IDH mutation-positive tumors may in fact be related to the methylator phenotype that has been proposed as the oncogenic mechanism of IDH mutations. Noushmehr *et al.* in 2010 described a specific hypermethylation pattern in CpG islands in a subset of patients with glioma referred to as a glioma CpG island methylator phenotype (G-CIMP). This pattern is associated with altered expression of several genes through transcriptional silencing (78). Furthermore, G-CIMP has been found to be tightly associated with a specific gene-expression profile called the proneural subtype; the latter is always characterized by the presence of IDH mutations (79). Following these studies, Turcan *et al.* were able to replicate the G-CIMP signature in human astrocytic cells by expressing *IDH1* R132H mutations, establishing in this way the attractive theory that IDH mutations constitute the molecular basis of G-CIMP (80). However, not all IDH mutation-positive gliomas exhibit the methylator phenotype (69), while the same phenotype may result from mutations in other genes.

As previously mentioned, aKG-dependent dioxygenases are directly involved in histone and DNA methylation and their inhibition through D-2HG can result in a hypermethylation pattern consistent with the G-CIMP signature. This interesting scenario is supported by multiple preclinical data. DNA demethylation is in part being regulated by TETs, enzymes that catalyze the conversion of 5-methylcytosine to its unmethylated form. It has been shown that *in vitro* expression of IDH mutations inhibits TET activity, resulting in DNA hypermethylation, and this inhibition can be reversed by the exogenous addition of aKG (38). In patients with AML, *IDH1/2* mutations have been found to result in a DNA hypermethylation pattern similar to G-CIMP through impairment of normal *TET* catalytic activity. Interestingly, a subset of patients with AML harbor TET loss-of-function mutations and have the same epigenetic defects as patients with IDH mutations. It is worth noting that IDH and *TET* mutations are mutually exclusive (81).

Main implications of the methylator phenotype associated with IDH mutations include the altered expression of genes participating in practically all cellular functions, some of which have been studied in detail. IDH mutations have been shown to inhibit aKG-dependent histone jumonji-C demethylases that regulate histone methylation. Chowdhury *et al.* showed that IDH mutations inhibit members of the jumonji-C family with different potencies, with the 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) family of histone demethylases being the most sensitive to inhibition, leading to an increase in histone methylation marks (55). These findings have been replicated by other investigators (38). Lu *et al.* showed that this increase in histone methylation leads to a block in cellular differentiation, probably due to hypermethylation of genes associated with differentiation (82). Flavahan *et al.* showed that the methylator phenotype associated with IDH mutations alters normal chromosomal topology *via* disruption of CCCTC-binding factor (CTCF) insulator protein binding. This protein is responsible for organizing DNA into chromatin loops and boundaries. Methylation of CTCF-binding sites disrupts normal binding of the CTCF proteins, allowing aberrant gene expression. It has been shown that in IDH-mutant cells, loss of CTCF binding at a specific domain boundary results in

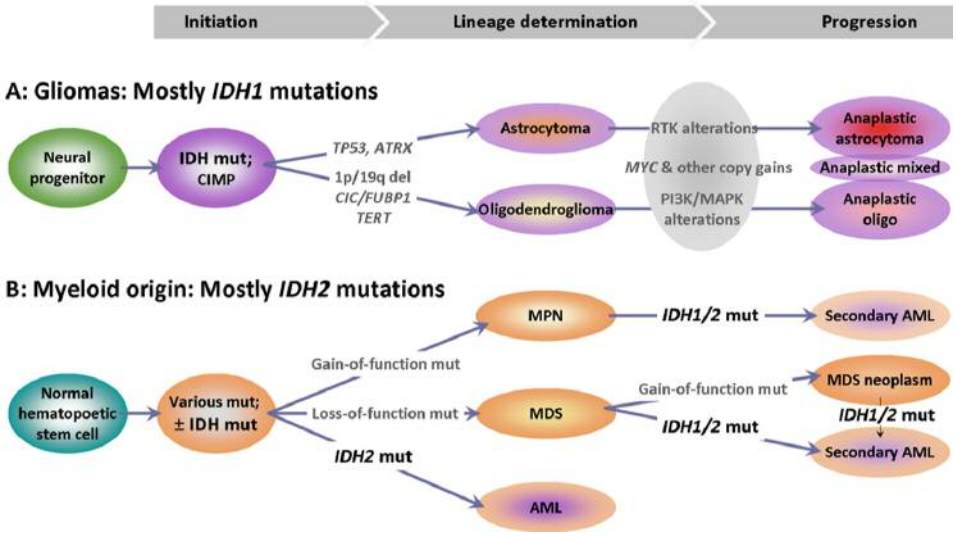

Figure 1. *Potential differences in the development of isocitrate dehydrogenase (IDH) mutations in the course of oncogenesis in the two main systems with IDH-mutant malignancies. A: The IDH-dependent pathway in glioma development is well characterized: IDH mutations are established in local progenitors that give rise to two glioma lineages with specific genotypic characteristics, as described in the text. B: The sequence of events with respect to IDH mutations in myeloid malignancies remains hypothetical. In cytogenetically normal (primary) acute myeloid leukemia (AML), IDH mutations occur early and are clonal. In most other cases and in secondary AML, IDH mutations may be subclonal, indicating a later establishment. MPN: Myeloproliferative neoplasm; MDS: myelodysplastic syndrome; RTK: Receptor tyrosine kinase pathway; PI3K/MAPK: phosphoinositide 3-kinase/mitogen-activated protein kinase pathway.*

overexpression of a prominent glioma oncogene, platelet-derived growth factor receptor A (*PDGFRA*). Moreover, treatment of these cells with a demethylating agent resulted in down-regulation of *PDGFRA* and partial restoration of the CTCF function (83). Turcan *et al*. also proposed that histone and chromatin modifications are the main consequences of the metabolic environment in IDH-mutant cells (84). Chesnelong *et al*. found that in IDH-mutant cells there is silencing of lactate dehydrogenase A gene (*LDHA*) *via* methylation of its promoter. LDHA is a subunit of LDH essential for glycolysis and silencing of *LDHA* in these cells results in a defective glycolytic pathway. This could perhaps explain the slow growth reported for IDH-mutant tumors compared to their wild-type IDH counterparts (85). The methylation-based silencing of genes encoding for immune checkpoint inhibitors, such as programmed cell death protein 1 (PDCD1/PD1) and programmed cell death-ligand 1 (PDL1) (86-88) is also worth mentioning in the context of the above described IDH-related methylator phenotype.

Two consequences directly attributed to the high levels of 2-HG resulting from IDH mutations include endoplasmic reticulum stress in murine glioma progenitors (89) and the stalled DNA-damage repair demonstrated in various IDH mutation-positive tumors, including gliomas (90). Compared to IDH mutation-negative gliomas, IDH mutation-positive gliomas exhibit higher apoptotic death rates that can be further increased with endoplasmic reticulum stress-inducing agents such as tunicamycin and thapsigargin (89) and with inhibition of B-cell lymphoma-extra large protein (BCL-xL) (91). IDH mutations through high 2-HG levels induce homologous recombination repair deficiency, which was accompanied by a high sensitivity of IDH-mutated tumors to poly (ADP-ribose) polymerase (PARP) inhibitors in preclinical models (90, 92). The importance of the above features in IDH mutation-positive tumors lies in the fact that IDH mutations can be exploited with synthetic lethality strategies using existing drugs.

Prognostic and Predictive Role of IDH Mutations

Many studies have shown that patients with IDH mutation-positive gliomas have better survival compared to their wild-type IDH counterparts irrespective of histology and grade, making IDH mutation the most important prognostic factor for survival, followed by age, tumor grade and O^6-methylguanine-DNA methyltransferase gene (*MGMT*) status, as summarized in (20). The favorable prognostic effect of IDH mutations compared to wild-type IDH has been reported in patients with glioblastoma (median overall survival: 31 *vs*. 15 months) and anaplastic astrocytoma (median overall survival: 65 *vs*. 38 months) (5, 14, 93, 94) and with anaplastic oligodendroglioma (95). In line with the

above findings is recent work by Yao *et al*. in which it was shown in glioma stem cells *in vitro* that the presence of IDH mutation was associated with a less aggressive phenotype compared to wild-type IDH (96).

Patients with glioma with IDH mutations have distinct clinical features. They are usually younger in age (4, 5) and most commonly the lesion is found in the frontal lobes compared to patients with wild-type IDH tumors (97-101). Moreover, these tumors are associated with different characteristics on radiological imaging, such as less contrast enhancement, less necrosis and increased stiffness compared to gliomas with wild-type IDH (102, 103).

As mentioned, the above findings have made it clear that histology and grading are not enough to fully characterize a glioma tumor and led to the 2016 WHO CNS classification that incorporates molecular features in addition to histology and grade. Traditionally, mutations, IDH included, have been detected with wet (polymerase chain reaction-based) molecular methods such as Sanger sequencing (Figure 2) and these are included in most next-generation sequencing panels. The development of an antibody that recognizes the most frequent IDH alteration in gliomas, namely *IDH1* p.R132H (104), and its validation for diagnostic immunohistochemistry application in 2014, has greatly facilitated the integration of IDH testing in routine pathology diagnostic practice (Figure 3).

Similarly to patients with IDH-mutated glioma, patients with IDH-mutated cholangiocarcinoma have been shown to have better overall survival and longer time to recurrence after surgical resection (105).

In patients with AML, mutation of *IDH2,* but not *IDH1* has been shown to be associated with better overall survival compared to patients with wild-type IDH (106). However, conflicting results have been reported (64, 66) and the effect of IDH mutations in leukemia seems to depend on the genomic context and coexisting alterations rather than standing independently (66).

IDH mutations have been shown to be predictive of response to chemotherapy. This has been shown in patients with low-grade gliomas and secondary glioblastomas treated with temozolomide (107, 108), as well as in patients with anaplastic oligodendroglioma treated with procarbazine, lomustine and vincristine (109).

In their study, Lu *et al*. showed that a possible mechanism for chemosensitivity in patients with *IDH1* mutation-positive gliomas is through 2-HG production and oxidative defects, which result in impairment of PARP1-mediated DNA repair (92).

Targeted Treatment of IDH-mutant Tumors

Based on the unique characteristics of IDH-mutant tumors, it is intriguing to assume that these tumors may need to be treated differently from their wild-type IDH counterparts.

The mostly tested strategy in this context is with compounds acting as IDH inhibitors. The fact that IDH mutations occur early in gliomagenesis and that such mutations are tumor specific and are expressed uniformly in all tumor cells (13) resulted in the development of drugs targeting IDH1/2 enzymes. Data from preclinical studies showed that IDH inhibitors can reduce 2-HG production, reverse histone and DNA methylation, and induce cell differentiation (110-112). The clinical trials testing the efficiency of IDH inhibitors in gliomas and hematological malignancies are shown in Table II.

Popovici-Muller *et al*. in 2012 were the first to develop an IDH1/2 inhibitor that lowered the 2-HG level in a glioblastoma xenograft mouse model (113). Following these findings, Chatuverdi *et al*. showed that the use of another IDH1 inhibitor in leukemia cells from patients with *IDH1* mutations blocked colony formation without affecting normal cells (114). AGI-5198, also an IDH1 inhibitor, was shown in two *in vitro* studies to inhibit 2-HG production and induce cell differentiation (110, 115). Similarly, AGI-6780, an IDH2 inhibitor, was shown to induce differentiation of *IDH2*-mutated erythroleukemia and primary human AML cell lines (111).

IDH inhibitors were first studied in patients in 2013. The first agent to be tested was AG-221 (enasidenib), a selective IDH2 inhibitor. Yen *et al*. showed *in vitro* that enasidenib suppressed 2-HG production in and induced cellular differentiation of primary human *IDH2* mutation-positive AML cells *ex vivo* and in xenograft mouse models (116). On this basis, enasidenib was initially tested in a phase I/II clinical trials in patients with advanced hematological malignancies and a known *IDH2* mutation (NCT01915498). Results from this trial were encouraging. The drug was well tolerated and resulted in 98% reduction of 2-HG levels. The objective response rate among 181 patients with relapsed or refractory AML was 41%, with 28% of patients achieving complete response and a median response duration of 5.8 months. Grade 3 or 4 drug-related side-effects were observed in 21% of patients and included indirect hyperbilirubinemia (12%) and IDH-inhibitor differentiation syndrome (7%) (117-119). Subsequent translational studies from the trial population have shown that cellular differentiation is the main mechanism of action of enasidenib (120). Interestingly, in the trial population, 23% of patients taking enasidenib at a dose of 100 mg once daily had a complete response lasting a median of 8.2 months (121).

These results led to the approval of enasidenib by the Food and Drug Administration (FDA) (August 2017) for use in patients with refractory or relapsed AML along with a companion diagnostic, the RealTime IDH2 assay to detect *IDH2* mutation (USFDA Approved Drugs—FDA Granted Regular Approval to Enasidenib for the Treatment of Relapsed or Refractory AML; https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm569482.htm),

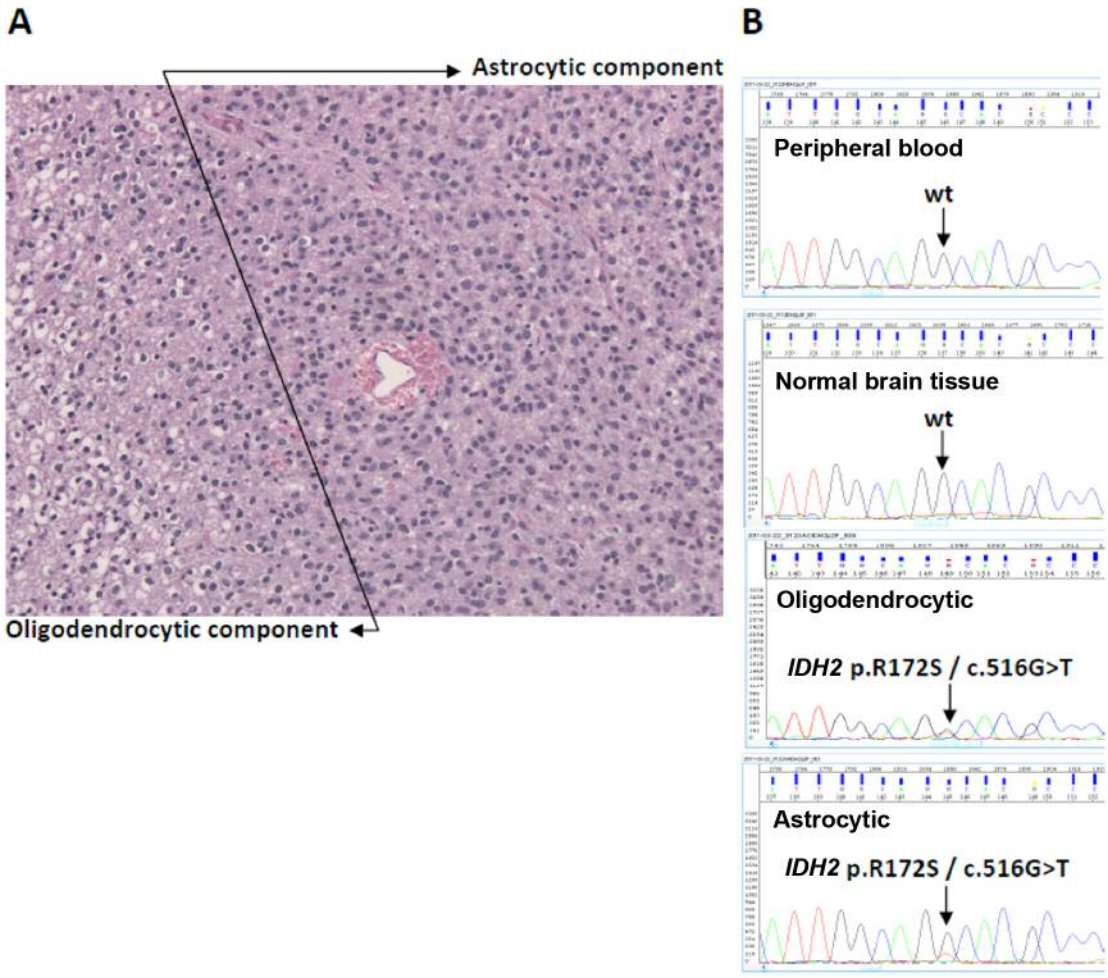

Figure 2. *Example of an isocitrate dehydrogenase-2 (IDH2)-mutant grade III glioma. The patient was a 22-year-old male with a central nervous system tumor that recurred within 3 years. The case was examined before the introduction of diagnostic IDH1 R132H antibody. A: As shown in the hematoxylin- and eosin-stained section, morphologically the tumor exhibited both an oligodendrocytic and an astrocytic component (original magnification ×200). B: Sanger sequencing targeting IDH1 exon 2 and IDH2 exon 4 (CCDS 10359.1) was applied to the patient's germline DNA from peripheral blood, on a normal brain sample, and on the indicated tumor DNA samples that were macrodissected from the same paraffin section. The pathogenic IDH2 mutation p.R172S/c.516G>T (arginine→ serine; COSM34090) was tumor-specific and was found in both the oligodendrocytic and astrocytic components. Courtesy: Department of Pathology, School of Medicine, Aristotle University of Thessaloniki, Greece (archived in 2011).*

which is also being tested in many clinical trials. A phase III trial evaluating enasidenib versus best supportive care in elderly patients with AML harboring *IDH2* mutation is currently recruiting (NCT02577406). Enasidenib has also been evaluated in a phase I/II trial in patients with advanced solid tumors harboring an *IDH2* mutation (NCT02273739).

Similarly, AG 120, an IDH1 inhibitor, has been tested in patients with advanced hematologic malignancies (NCT02074839) and solid tumors harboring *IDH1* mutations (NCT02073994). Preliminary results have been presented and AG 120 has been reported to have a clinical benefit rate of 37% among all patients and 25% among those with glioma (122).

Furthermore, additional IDH inhibitors have been designed and have entered clinical trials, including IDH-305, DS-1001b and BAY-1436032, which are all *IDH1*-mutant inhibitors (122, 123), and AG-881 (vorasidenib), a pan inhibitor of mutant IDH1 and IDH2 enzymes, which has been shown to fully penetrate the blood–brain barrier (NCT02381886, NCT03030066, NCT02746081). AG-881 is currently being tested in two clinical trials, NCT02481154 and NCT02492737, involving patients with advanced solid tumors including gliomas with *IDH1*/*IDH2* mutations and advanced hematological malignancies, respectively (20, 49).

Table II. *Summary of clinical trials evaluating isocitrate dehydrogenase (IDH) inhibitors, alone and in combination, in patients with glioma or myeloproliferative malignancies.*

IDH inhibitor	Disease/patient population	Somatic IDH status	Trial phase	Status (May 2018)	CT.GOV ID
AG-221 (enasidenib) vs. conventional regimen	Advanced hematological malignancies	*IDH2* mut	1/2	Active, not recruiting	NCT01915498
	Advanced solid tumors including glioma	*IDH2* mut	1/2	Completed, results awaited	NCT02273739
	Older patients with late-stage AML	*IDH2* mut	3	Recruiting	NCT02577406
AG-881 (vorasidenib)	Advanced hematological malignancies	*IDH1* or *IDH2* mut	1	Active, not recruiting	NCT02492737
	Advanced solid tumors including gliomas	*IDH1* or *IDH2* mut	1	Active, not recruiting	NCT02481154
AG-120 (ivosidenib)	Advanced solid tumors, including gliomas	*IDH1* mut	1	Active, not recruiting	NCT02073994
	Advanced hematological malignancies	*IDH1* mut	1	Active, not recruiting	NCT02074839
	Grade II or III gliomas prior to surgery	*IDH1* mut	1	Recruiting	NCT03343197
	Previously treated cholangiocarcinoma	*IDH1* mut	3	Recruiting	NCT02989857
IDH305	Advanced malignancies	*IDH1* mut (IDH-R132)	1	Active, not recruiting	NCT02381886
BAY1436032	Advanced AML	*IDH1* mut	1	Recruiting	NCT03127735
	Advanced solid tumors	*IDH1* mut	1	Recruiting	NCT02746081
DS-1001b	Gliomas relapsed after standard radiotherapy	*IDH1* mut	1	Recruiting	NCT03030066
AG-221 plus AG-120 plus azacitidine	Newly diagnosed AML	*IDH1* or *IDH2* mut	1/2	Recruiting	NCT02677922
AG-120 or AG-221 with chemotherapy	Newly diagnosed AML	*IDH1* or *IDH2* mut	1	Recruiting	NCT02632708
AG-221 alone or AG-221 plus azacitidine	High-risk MDS	*IDH2* mut	2	Recruiting	NCT03383575
Azacitidine alone or AG-120 plus azacitidine	Untreated AML	*IDH1* mut	3	Recruiting	NCT03173248
AG-120 plus venetoclax	Relapsed or refractory AML	*IDH1* mut	1/2	Recruiting	NCT03471260
AG-120 or AG-881	Grade II or III gliomas prior to surgery	*IDH1* mut	1	Recruiting	NCT03343197

AML, Acute myeloid leukemia; MDS, myelodysplastic syndrome; mut, mutant; CT.GOV.ID, clinicaltrials.gov identifier.

A different treatment approach, synthetic lethality for IDH-mutant solid tumors, was recently suggested by Sulkowski *et al.* (90) based on the observation that 2-HG results in homologous recombination repair deficiency, rendering such cells highly sensitive to PARP inhibition. Importantly, they also observed that this sensitivity is reversed by IDH inhibitors. In the same context, Lu *et al.* (92) reported on synergistic effects between temozolomide and the PARP inhibitor olaparib, in patients with IDH mutation, providing the possibility to achieve improved cytotoxic effects with minimal use of alkylating agents in order to reduce bone marrow cytotoxicity (109). Based on these results, a phase I trial with the above combination in patients with relapsed glioblastoma recently completed accrual and results are awaited (NCT01390571) (124).

Another potential pathway to be targeted in IDH-mutant gliomas was identified based on the specific hypermethylation patterns associated with such tumors. As previously described, histone and DNA hypermethylation reported in IDH-mutant tumors leads to an arrest of cellular differentiation and malignant transformation. The use of decitabine and azacytidine, both DNA-demethylating agents approved by the FDA for myelodysplastic syndromes, has been shown in preclinical models to reverse the hypermethylation phenotype, induce cellular differentiation and delay tumor growth in *IDH1*-mutated xenograft models (125, 126).

As immunotherapy constantly gains ground in the battle against cancer, many researchers have tried to use different immunotherapy approaches against IDH-mutant tumors. The fact that IDH mutations are events that occur early and

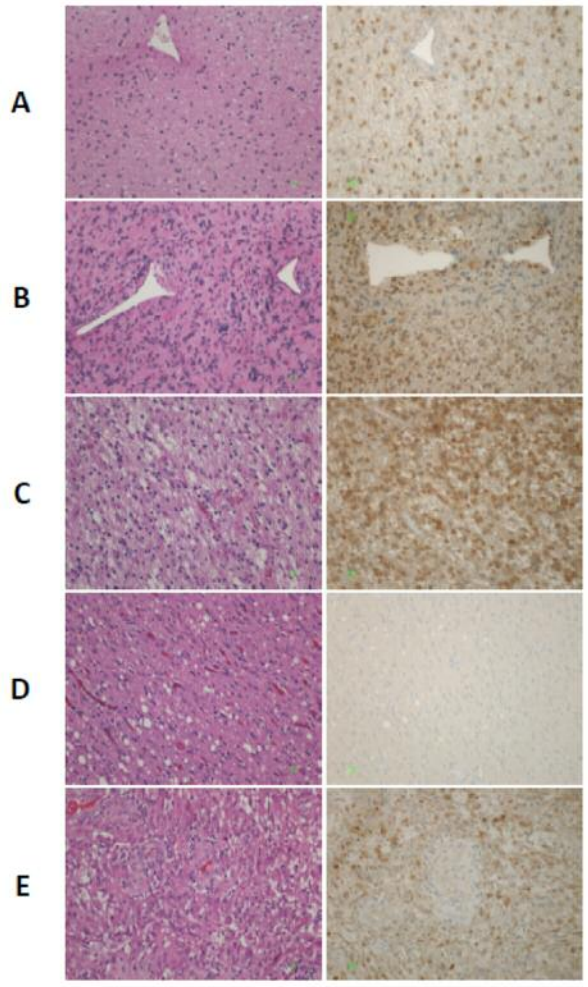

Figure 3. *Isocitrate dehydrogenase-1 (IDH1)-R132H immunohistochemistry in gliomas of different histological grade. Left panel: Hematoxylin- and eosin-stained sections; Right panel: Sections stained using the diagnostic antibody to IDH1 R132H (clone DIA-H09). The antibody detects the most common IDH1 mutation, arginine→histidine at codon 132, but may also detect further amino acid changes in the same codon. Staining is cytoplasmic. A: Diffuse astrocytoma (grade II). B: Anaplastic astrocytoma (grade III). C: Anaplastic oligoastrocytoma (grade III). The area corresponds to the oligodendrocytic component. D: Same case as in C, IDH1 R132H-negative area in the periphery of the tumor. Reactive gliosis (or astrocytosis) is expected to be stain negatively with this antibody, which is of great value for distinguishing between non-tumor and tumor in areas with high cellularity in diagnostic CNS pathology. E: Glioblastoma (grade IV). Note that vascular endothelial cells are negative for this marker and serve as an internal negative control. In A, B, C and E, negative residual cells are present in the tumor bed. Original magnification ×200. Courtesy: Dr. Thomas Zaramboukas; Dept. of Pathology; School of Medicine; Aristotle University of Thessaloniki, 54124 Thessaloniki.*

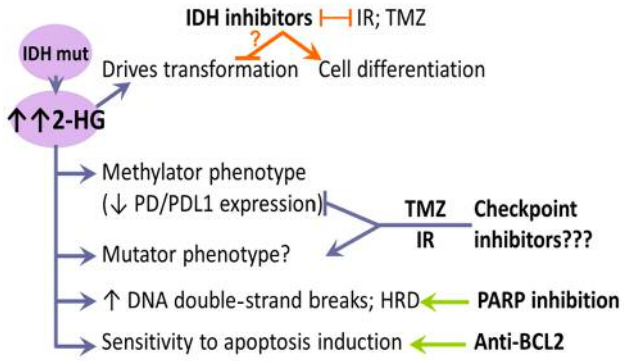

Figure 4. *Features of isocitrate dehydrogenase (IDH) mutations in gliomas and potential treatment approaches. IDH inhibitors effectively reduce the level of 2-hydroxyglutarate (the oncometabolite in IDH-mutated tumors) and induce cell differentiation, but are incompatible with major treatment modalities for gliomas. In addition, these inherently counteract any of the molecular treatment options presented (right panel). In the clinical setting, when tumors are diagnosed, transformation has been accomplished and tumors have already acquired additional molecular/genomic characteristics, questioning the value of targeting IDH for effective tumor growth inhibition. The three drug classes on the right target the effects of 2-HG and not 2-HG itself. Checkpoint inhibitors are under trial, but these need their target molecules programmed cell death protein 1 (PD1) and programmed death ligand 1 (PDL1) to be expressed or overexpressed in order to be effective. These molecules are underexpressed in IDH-mutant tumors due to promoter hypermethylation. Temozolomide (TMZ) may reverse the low expression of checkpoint molecules in IDH-mutant gliomas and induce the hyper-mutator phenotype, but this has still to be proven in the clinical setting. The straightforward options seem to be poly-ADP-ribose polymerase (PARP) targeting in the context of synthetic lethality, and apoptosis inhibition with already available drugs. Combinations of the three classes of drugs on the right may be considered. BCL2: B-cell lymphoma 2 protein; IR: irradiation; HRD: homologous recombination repair deficiency.*

steadily through time in gliomagenesis, in combination with the fact that mutant IDH enzymes are expressed only in glioma cells, have made them intriguing antigens for activating the immune system. A mutation-specific anti-IDH1(R132H)-specific peptide vaccine has been produced and been shown to generate an immune reaction in preclinical studies and to reduce tumor growth in a mouse model (127, 128). Currently there are three IDH1 peptide and dendritic vaccines targeting the *IDH1*R132H mutation that are being evaluated in phase I clinical trials (NCT02193347, NCT02454634 and NCT02771301). Since *PD1* and *PDL1* genes were found to be methylated and underexpressed in IDH-mutant tumors (86-88), inhibitors of PD1 (pembrolizumab) and PDL1 (avelumab) are currently being evaluated in pretreated patients with gliomas, either alone in patients with glioma grade II-IV that harbor hypermutator phenotype, or in combination with radiotherapy in secondary glioblastoma (NCT02658279 and NCT02968940, respectively). Pretreatment with irradiation with/without temozolomide may interfere with the

methylator phenotype and hence alter the expression of targets of pembrolizumab and avelumab, while temozolomide may induce a mutator phenotype in gliomas, through inactivation of DNA mismatch repair genes (129).

In conclusion, several therapeutic approaches are being tested in patients with IDH mutant tumors based on preclinical evidence (summarized in Figure 4). The first approach involves IDH inhibitors, which target the effect of IDH mutations, *i.e.*, the oncometabolite 2-HG, and reduce its levels. The second tries to exploit the effects of the oncometabolite, *i.e.* deficient DNA strand breaks and apoptosis. The third is in the context of immunotherapy. Because IDH inhibitors do not kill cells but induce them to differentiate or drive them into a reversible autophagic state, these inherently counteract existing treatment modalities, such as radiotherapy, cytotoxic chemotherapeutics, as well as most molecularly targeted agents. Thus, despite approval, this class of molecules is not expected to be effectively integrated into clinical practice, for gliomas at least. In comparison, approaches of synthetic lethality appear more promising based on the rationale of combination possibilities and repurposing of existing drugs. IDH-mutant tumors are progressively being understood as an individual class of malignancies across tumors of different origins, the reason being the oncometabolite (D-)2-HG. Deepening our knowledge on how this metabolite is influenced by currently applied treatments in patients with IDH mutation-positive tumors is warranted for the efficient design of clinical trials targeting its consequences directly or after initial treatment failure, particularly in patients with glioma.

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