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REVIEW
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Mutant Isocitrate Dehydrogenase Inhibitors as Targeted Cancer Therapeutics

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The identification of heterozygous neomorphic isocitrate dehydrogenase (IDH) mutations across multiple cancer types including both solid and hematologic malignancies has revolutionized our understanding of oncogenesis in these malignancies and the potential for targeted therapeutics using small molecule inhibitors. The neomorphic mutation in IDH generates an oncometabolite product, 2-hydroxyglutarate (2HG), which has been linked to the disruption of metabolic and epigenetic mechanisms responsible for cellular differentiation and is likely an early and critical contributor to oncogenesis. In the past 2 years, two mutant IDH (mutIDH) inhibitors, Enasidenib (AG-221), and Ivosidenib (AG-120), have been FDA-approved for IDH-mutant relapsed or refractory acute myeloid leukemia (AML) based on phase 1 safety and efficacy data and continue to be studied in trials in hematologic malignancies, as well as in glioma, cholangiocarcinoma, and chondrosarcoma. In this review, we will summarize the molecular pathways and oncogenic consequences associated with mutIDH with a particular emphasis on glioma and AML, and systematically review the development and preclinical testing of mutIDH inhibitors. Existing clinical data in both hematologic and solid tumors will likewise be reviewed followed by a discussion on the potential limitations of mutIDH inhibitor monotherapy and potential routes for treatment optimization using combination therapy.

Keywords: acute myeloid leukemia, enasidenib, glioma, IDH, isocitrate dehydrogenase, ivosidenib

INTRODUCTION

The discovery of mutations in isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) in over 80% of low-grade gliomas (LGGs) and secondary glioblastomas has revolutionized pharmaceutical approaches to targeted therapies and the overall glioma classification schema (1, 2). Driver mutations in IDH1 and IDH2 have been likewise identified in acute myeloid leukemia (AML), chondrosarcoma, myelodysplastic syndromes, and cholangiocarcinoma (3–6). Limitations in current treatment options, particularly in LGG and AML, due to both inefficacy and systemic toxicity, make mutant

IDH (mutIDH), and its associated molecular pathways attractive therapeutic targets (7–9). Major strides in developing and testing candidates for mutIDH inhibition have been made in the past few years with the FDA approvals of Ivosidenib (Tibsovo®) and Enasidenib (Idhifa®), selective mutIDH1 and mutIDH2 inhibitors, respectively (10, 11). While these agents have had some preliminary success in AML, utility in the treatment of IDH-mutant glioma or other IDH-mutated cancers has not been established (12, 13).

IDH1 and IDH2 are homodimeric isoenzymes involved in a major pathway for cellular NADPH generation through the oxidative decarboxylation of isocitrate to α-ketoglutarate. IDH1 is found in the cytosol and in peroxisomes, while IDH2 is a mitochondrial enzyme. Mutations in IDH3 isoforms, which form heterotetrameric complexes in mitochondria, are rarely seen in cancer, but there is some evidence that upregulation of wild-type IDH3 may contribute to various tumorigenic metabolic pathways (14, 15). The IDH1/2 mutations are heterozygous and neomorphic in that they establish a pathway for the NADPH-dependent conversion of the wild-type IDH product, α-ketoglutarate, to 2-hydroxyglutarate (2HG) (16). Simultaneously, significant decreases in NADPH production are also seen (17). Early structural and pharmacokinetic studies show that mutant IDH develops an increased affinity for both the cofactor NADPH and substrate α-ketoglutarate (16, 18). In the most common IDH1/2 mutants, the wild-type IDH function of oxidative decarboxylation of isocitrate to α-ketoglutarate is lost due to mutation of critical amino acid residues in the catalytic domain, IDH1 R132 and IDH2 R172, which are normally responsible for binding the β-carboxyl group of isocitrate and initiating catalysis (1, 16, 18). Interestingly, there is some evidence that, unlike the IDH1 mutant, the IDH2 mutant may not depend on heterodimerization with an IDH wild-type partner for 2HG production (19). Nevertheless, while the mutant IDH enzyme can exist either as a homodimer or as a heterodimer with the wild-type IDH within cancer cells, all reported oncogenic IDH mutations to date are genetically heterozygous, suggesting that the critical role of mutant IDH is related to its gain-of-function for conversion of the wild-type IDH product, α-ketoglutarate, to 2HG (20).

Accumulation of 2HG, increasingly well-characterized as an oncometabolite, disrupts multiple regulatory cellular pathways involving α-ketoglutarate-dependent dioxygenases including those involved in epigenetic remodeling and DNA repair (**Figure 1**) (21–23). Structural similarities between α-ketoglutarate and 2HG allow the latter to competitively occupy the same pockets as α-ketoglutarate in α-ketoglutarate-dependent dioxygenases (of which over 60 have been described in humans), without promoting enzymatic activation (22, 24–26). Changes in the epigenetic landscape brought on by 2HG-mediated disruption of the ten-eleven translocation (TET) family of 5-methylcytosine (5 mC) hydroxylases (DNA demethylases) and the JmJC domain-containing histone lysine demethylases (KDMs) are hypothesized to promote oncogenesis through DNA and histone hypermethylation and resultant transcriptional dysregulation (22, 27). The resulting global increase in DNA methylation in the mutIDH context is aptly named the CpG Island Methylator Phenotype (CIMP) (28, 29). Manipulating and reversing the oncogenic IDH-mutant methylome is the primary molecular endpoint for therapeutic IDH inhibition and 2HG reduction in both glioma and AML. It remains to be seen, however, if 2HG reduction alone will be sufficient to reverse oncogenic changes to the methylome, as epigenetic memory persists through daughter cells via methyltransferases, a topic we explore further in our discussion (30, 31).

Here, we provide an overview of the current literature on IDH mutations in cancer with a particular emphasis on glioma and AML and the potential for mutIDH as a therapeutic target in these contexts. We describe the current evidence for the various generations of mutIDH inhibitors through the drug-discovery, preclinical, and clinical stages and systematically review related past and ongoing clinical trials. We furthermore describe the possible adverse effects of IDH inhibitors, such as "differentiation syndrome," and conclude with a discussion on the potential for enhancing the efficacy of IDH inhibitors in combination with epigenetic modification-based therapies.

IDH Mutations in Glioma

Ten years ago, our understanding of the molecular landscape in glioma was transformed by the first genome-wide analysis of somatic mutations in glioblastoma (GBM) and the identification of recurrent mutations in IDH1 nearly exclusively in secondary GBM (2). Mutations in IDH1 and IDH2 are seen in over 80% of lower-grade gliomas (WHO grades II and III) and secondary GBMs that are thought to later develop from lower-grade lesions (2, 32, 33). The vast majority of somatic IDH mutations (>95%) are seen in IDH1, and the most commonly observed IDH1 mutation occurs at the R132 residue (1, 34). IDH2 mutations, which are mutually exclusive with those in IDH1 and found at a functionally analogous R172 residue, only represent a minority of somatic IDH mutations in glioma (35, 36).

IDH-mutant gliomas are generally further categorized into two major subtypes: those with chromosome 1p/19q co-deletion, historically termed oligodendrogliomas; and those without 1p/19q co-deletion, also known as astrocytomas (37). These two groups are biologically and clinically distinct. Up to 94% of IDH-mutant non-1p/19q co-deleted gliomas harbor loss-of-function TP53 mutations and 86% have inactivating ATRX mutations (37). Only few IDH mutant astrocytomas carry IDH wild-type driver mutations or copy number alterations, and those who do (for example CDKN2A or CDKN2B loss) are usually classified as IDH mutant GBM (1). These robust genomic differences are highly suggestive of a unique mechanism of oncogenesis in the IDH-mutant subgroup and furthermore imply that the IDH mutation is likely an early player in a cell-of-origin, which in its native state is capable of giving rise to both astrocyte and oligodendrocyte lineages. Clinically, IDH-mutant lesions present in a younger age group (median age in the fourth vs. the sixth decade of life), when compared to IDH-wild type gliomas (33). Furthermore, IDH mutations are well-known to be an independent favorable prognostic factor at all stages of glioma progression; for example, the median survival in IDH-mutant GBM is 31 months, over twice as long as the median 15-months survival in the wild-type counterpart (1).

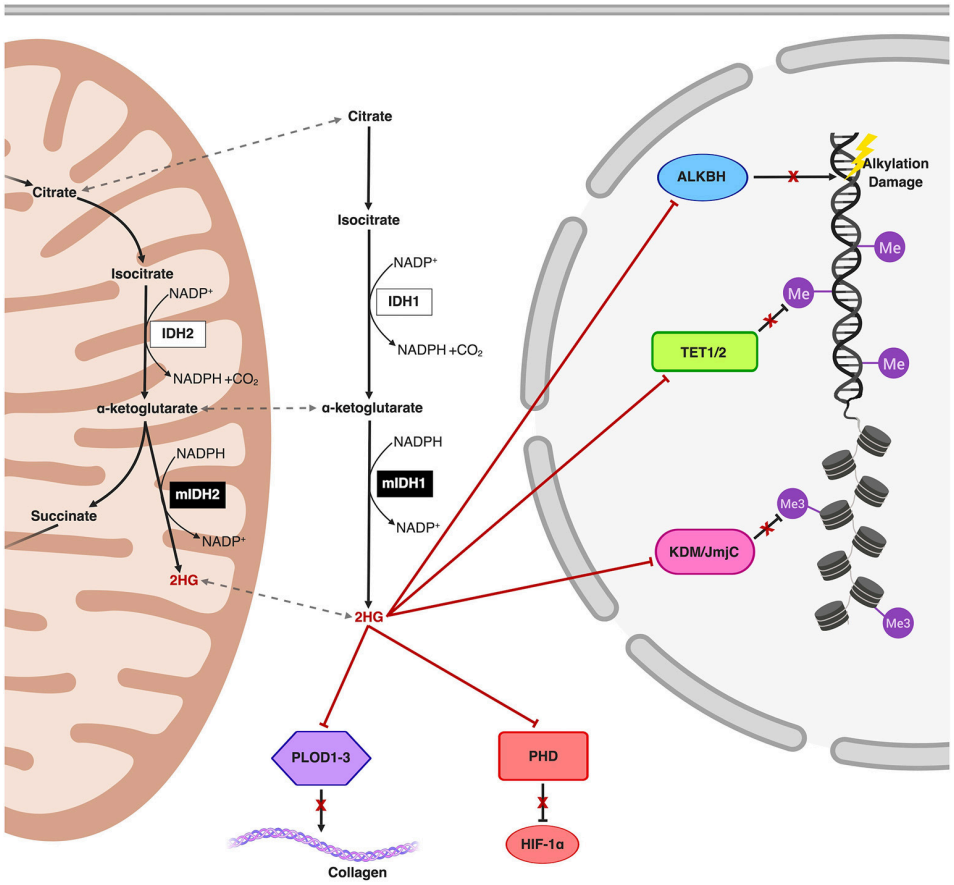

FIGURE 1 | Schematic representation of the mutIDH1 and mutIDH2 pathways and molecular mechanisms related to oncogenesis. The neomorphic enzyme, mutIDH1/2, converts the wild-type IDH product, α-ketoglutarate, to the oncometabolite, 2-hydroxyglutarate (2HG) both in the cytosol and in the mitochondria. 2HG competitively inhibits α-ketoglutarate-dependent dioxygenases both in the cytosol and in the nucleus. 2HG-mediated inhibition of the activity of Ten-Eleven Translocation (TET) enzymes and histone lysine demethylases (KDM) result in global epigenetic modifications on DNA and histones, respectively, resulting in a hypermethylator phenotype. Inhibition of prolyl hydroxylases and lysyl hydroxylases (such as PLOD1-3) interferes with both collagen maturation and with the degradation pathway of Hypoxia-Inducible Factor 1α (HIF-1α), thereby post-translationally stabilizing HIF-1α. Additionally, ALKBH, responsible for repair of oxidative DNA damage, is also inhibited by 2HG, an effect which potentially introduces risk for increased mutational burden.

Consistent with other IDH-mutant cancers, IDH-mutant glioma is characterized by high levels of 2HG and the resulting "CIMP" hypermethylator phenotype described previously. In glioma specifically, these genome-wide DNA methylation changes have been shown to establish "insulator dysfunction" or disruption of topologically-associated domains (TADs) and thereby directly influence key transcriptional regulatory pathways related to gliomagenesis (38, 39). As previously mentioned, analyses of clonality among glioma tumor samples suggests that the IDH mutation is a tumor-initiating event in a common progenitor cell, hypothesized by many to be derived from the subventricular zone stem cell niche (7, 40–42). Despite our enhanced understanding of the molecular pathogenesis of IDH-mutant glioma, however, effective treatments have yet to be developed and clinicians remain reliant on maximal safe surgical resection and various chemotherapeutic agents and radiation treatments to prolong survival (7). Furthermore, a unique characteristic of LGG is its diffuse and highly infiltrative phenotype, making surgical resection rarely curative in the long term. To compound the complexity of these tumors, and historically popular chemotherapeutic agents have been shown to induce hypermutant recurrent tumors (7). Recent efforts in developing small molecule inhibitors that target IDH mutation provide a new opportunity for progress in glioma treatment.

IDH Mutations in AML

Around the same time as the identification of recurrent IDH mutations in glioma, Mardis et al. published the results of a landmark study in which they sought to pinpoint recurrent mutations in AML that may be associated with the pathogenesis of the malignancy (43). In this study, the investigators identified for the first time the presence of IDH1 mutations in AML (43). 8.5% of analyzed samples had an IDH1 mutation at the R132 residue (mutated to either cysteine, histidine, or serine),

which is also the site of the overwhelming majority of somatic IDH mutations in glioma (1, 34). Shortly after the discovery of IDH1 mutations in AML, another landmark study reported the first case of IDH2-mutated AML, in which the R172 residue was mutated to lysine (18). Further investigation of AML DNA samples revealed the existence of several additional cases of AML where the $IDH2^{R172}$ residue was mutated (18). Interestingly, this study also found that a majority of the analyzed samples had IDH2 mutations (compared to IDH1 mutations) (18). This is in stark contrast to glioma, where the majority of IDH mutations are in IDH1.

Nearly one in five cases of AML is IDH-mutant, with IDH2-mutant AML being more prevalent than IDH1-mutant AML (11–13, 44–50). The $IDH2^{R140}$ mutation (in particular, the R140Q variant) is the most common, with the $IDH1^{R132}$ and $IDH2^{R172}$ mutations also appearing frequently in the literature (3, 12, 45, 46, 50–52). Other mutations include, but are not limited to, $IDH1^{V71}$ and IDH1 SNP rs11554137, a GGC to GGT transversion at the glycine residue at codon position 105 with unknown significance (47, 48, 53). Clinical and pathologic characteristics associated with IDH-mutant AML include normal karyotype (intermediate-risk cytogenetics), increased patient age, elevated platelet count, increased bone marrow blast percentage at initial presentation, increased peripheral blast percentage, decreased absolute neutrophil count (especially in IDH1-mutant AML), and concurrent mutations such as NPM1 and FLT3-ITD (44–47, 54). IDH1 and IDH2 mutations in AML are mutually exclusive, as in glioma. Likewise, in AML, IDH mutations are almost entirely mutually exclusive with TET2 mutations, suggesting that, mechanistically, these genes aref both involved in DNA hypermethylation as a driver of leukemogenesis (3, 45–47, 54).

It has been suggested that testing AML patients for IDH mutation status is simple and should be performed universally; however, the relationship between IDH mutation status and prognosis is considerably less clear and more controversial in AML than it is in other cancers such as glioma (46, 55). Most studies of IDH-mutant AML have suggested that mutIDH either foreshadows an adverse prognosis (given an association with increased blast percentage and older age at diagnosis) or is of little prognostic value (45, 48, 52, 55). Reported 2–3-years overall survival in IDH-mutant AML ranges between 51 and 89% in the literature; discrepancies are thought to be related to differences in cohort age, but some authors also argue that different specific IDH mutations may carry varied prognostic implications (3, 44, 45, 47, 54, 56, 57). Interestingly, IDH mutation status may also be useful for the detection of residual disease and prognostication following treatment; several studies investigating the value of serum 2HG during remission in AML have found that elevated serum 2HG levels actually predict shortened overall survival (55, 58, 59).

While induction/consolidation chemotherapy has revolutionized AML treatment strategy in the past 20 years, this standard-of-care universal treatment has evolved minimally since its introduction and is often contraindicated in elderly or otherwise frail patients (44). Given our enhanced understanding of the molecular and genetic subtypes of AML and the potential for targeted treatment, manipulation of these markers with small molecules may provide significant benefit. Drugs targeted to the mutIDH isotypes are one such example; for almost a decade, mutIDH inhibitors have been a focus of laboratory and clinical research in AML with great recent success leading to two FDA approvals specifically for AML indications.

Drug Development and Preclinical Studies

Multiple mutIDH inhibitors, including one pan-inhibitor and several specific to one mutIDH isoform, have been developed over the last several years. A handful of these are in use in clinical trials, but only two have been approved by the FDA; Enasidenib and Ivosidenib (10, 11). A detailed review of the structural and pharmacokinetic properties and relevant preclinical data for both FDA approved inhibitors will follow a brief discussion of other mutIDH inhibitors with demonstrated and repeated preclinical efficacy (**Table 1**).

Pan-Inhibitors

AG-881

AG-881 (Vorasidenib) is an orally available pan-inhibitor of both mutIDH1 and mutIDH2 and was the first pan-inhibitor developed under the Celgene and Agios Pharmaceuticals collaboration (**Figure 2**) (60–62). AG-881 contains a triazine moiety responsible for its allosteric inhibitory activity, and crystallography studies show that AG-881 binds mutIDH1 and mutIDH2 using the same allosteric pocket at the dimer interface, causing steric hindrance that locks the enzymes in an open, inactive conformation (61). Notably, the association of AG-881 with mutIDH1, in particular with $IDH1^{R132H}$, is more efficient than its interaction with mutIDH2 as it achieves maximal potency *in vitro* after significantly shorter incubation periods (61). IC_{50} for inhibition of 2-HG formation following 1 h of preincubation ranged from 6 to 34 nM in both patient-derived and genetically- engineered cell lines expressing $IDH1^{R132C}$, $IDH1^{R132G}$, $IDH1^{R132H}$, $IDH1^{R132L}$, or $IDH1^{R132S}$. For U87 and TF-1 cells transfected with $IDH2^{R140Q}$ or $IDH2^{R172K}$ by lentiviral vector, the IC_{50} values following 1 h of preincubation were 118 nM and 32 nM, respectively (62). In the same study, it was demonstrated that *ex vivo* treatment of primary human AML blasts with AG-881 induced myeloid differentiation (62). AG-881 has also been shown to effectively penetrate the blood-brain barrier in rodents, implicating its potential to treat both IDH-mutant AML and glioma patients (62). Based on this preclinical evidence, two multicenter clinical trials investigating the safety and efficacy of AG-881, one in solid tumors and the other in hematologic malignancies, are currently ongoing (60, 61).

Specific Inhibitors

BAY-1436032

One of the first mutIDH1-specific inhibitors to show preclinical efficacy in both AML and glioma models is BAY-1436032, developed by Bayer. An initial screen of over 3 million compounds based on mutIDH enzymatic activity generated a small group of compounds—with IC_{50} ranging from 0.6 to 17.1 μM—for further evaluation. Optimization of a lead compound based on differential inhibition of mutIDH1 and

TABLE 1 | MutIDH inhibitors.

Compound	Target	Mechanism	Susceptible mutations	IC_{50}	$T_{1/2}$	C_{max}	Time to C_{max}	Cl	AUC	Mode of elimination	Penetrates BBB	Metabolism	SPONSOR(S)
Ivosidenib (AG-120) (10, 13, 68)	mutIDH1	Reversible, allosteric, competitive inhibitor via cofactor (Mg) binding site	$IDH1^{R132H}$ $IDH1^{R132C}$ $IDH1^{R132G}$ $IDH1^{R132S}$ $IDH1^{R132L}$	Biochemical: $IDH1^{R132H}$: 12 nM $IDH1^{R132C}$: 13 nM $IDH1^{R132G}$: 8 nM $IDH1^{R132S}$: 12 nM $IDH1^{R132L}$: 13 nM Cell-Based: U87 ($IDH1^{R132H}$): 19 nM HT1080 ($IDH1^{R132C}$): 8 nM COR-L105 ($IDH1^{R132C}$): 15 nM HCCC-9810 ($IDH1^{R132S}$): 12 nM	93 h	6,551 ng/mL	~3 h	4.3 L/h	117, 348 ng•h/mL	77% fecal 17% renal	Yes (4.1% penetrance in rat model)	CYP3A4, *N*-dealkylation, hydrolytic pathways	Agios, Celgene
ENASIDENIB (AG-221)[1] (1, 12, 51)	mutIDH2	Allosteric, non-competitive inhibition via stabilization of open, inactive enzyme dimer conformation (steric hindrance)	$IDH2^{R140Q}$ $IDH2^{R172K}$	Biochemical (at 16 *h*): $IDH2^{R140Q}$: 100 nM $IDH2^{R172K}$: 400 nM $WT/IDH2^{R140Q}$: 30 nM $WT/IDH2^{R172K}$: 10 nM Cell-Based: HCT-116 ($IDH2^{R172K}$): 530 nM TF-1 ($IDH2^{R140Q}$): 20 nM TF-1 ($IDH2^{R172K}$): 980 nM U87 ($IDH2^{R140Q}$): 10 nM U87 ($IDH2^{R172K}$): 1,590 nM	137 h	1,300 ng/mL	~4 h	0.74 L/h	–	89% fecal 11% renal	-	CYP1A2, CYP2C9 CYP2C19, CYP2D6, CYP3A4, UGT1A1, UGT2B7, UGT2B15	Agios, Celgene
AG-881 (61, 62, 69)	Pan-inhibitor	Allosteric, non-competitive inhibition via stabilization of open, inactive enzyme dimer conformation (steric hindrance)	$IDH1^{R132H}$ $IDH1^{R132C}$ $IDH1^{R132G}$ $IDH1^{R132S}$ $IDH1^{R132L}$ $IDH2^{R140Q}$ $IDH2^{R172K}$	Biochemical: $IDH1^{R132H}$: 6 nM $IDH1^{R132C}$: 19 nM $IDH1^{R132G}$: 17 nM $IDH1^{R132S}$: 6 nM $IDH1^{R132L}$: 34 nM $WT/IDH1^{R132H}$: 4 nM $IDH2^{R140Q}$: 12 nM (16 h) $IDH2^{R172K}$: 94 nM (16 h) $WT/IDH2^{R140Q}$: 32 nM (16 h) $WT/IDH2^{R172K}$: 8 nM (16 h) Cell-Based: HCT-116 ($IDH1^{R132C}$): 22nM HCT-116 ($IDH1^{R132H}$): 3nM HCT-116 ($IDH2^{R172K}$): 130nM COR-L105 ($IDH1^{R132C}$): 3.8nM HCC-9810 ($IDH1^{R132S}$) 0.85nM HT1080 ($IDH1^{R132C}$): 4nM JJ012 ($IDH1^{R132G}$): 6.6nM TF-1 ($IDH1^{R132C}$): 3.2nM TF-1 ($IDH2^{R140Q}$): 14nM U87 ($IDH2^{R140Q}$): 7.1nM	67.2 h	–	–	–	2,746 ng•h/mL at 100 mg daily dose 7,020 ng•h/mL at 200 mg daily dose	–	Yes (brain-to-plasma ratio 0.62-1.96 in mouse model, 1.11-1.48 in rat model)	–	Agios, Celgene

(Continued)

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