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REVIEW

The regulatory mechanisms and inhibitors of isocitrate dehydrogenase 1 in cancer

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Abstract Reprogramming of energy metabolism is one of the basic characteristics of cancer and has been proved to be an important cancer treatment strategy. Isocitrate dehydrogenases (IDHs) are a class of key proteins in energy metabolism, including IDH1, IDH2, and IDH3, which are involved in the oxidative decarboxylation of isocitrate to yield α-ketoglutarate (α-KG). Mutants of IDH1 or IDH2 can produce D-2-hydroxyglutarate (D-2HG) with α-KG as the substrate, and then mediate the occurrence and development of cancer. At present, no *IDH3* mutation has been reported. The results of pan-cancer research showed that *IDH1* has a higher mutation frequency and involves more cancer types than *IDH2*, implying IDH1 as a promising anti-cancer target. Therefore, in this review, we summarized the regulatory mechanisms of IDH1 on cancer from four aspects: metabolic reprogramming, epigenetics, immune microenvironment, and phenotypic changes, which will provide guidance for the understanding of IDH1 and exploring leading-edge targeted treatment strategies. In addition, we also reviewed available IDH1 inhibitors so far. The detailed clinical trial results and diverse structures of preclinical candidates illustrated here will provide a deep insight into the research for the treatment of IDH1-related cancers.

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1. Introduction

Cancer seriously threatens human life and health. On January 6, 2022, the International Agency for Research on Cancer (IARC) under the World Health Organization released the *IARC Biennial Report 2020–2021*. The latest assessment shows that in this century, cancer is expected to overtake cardiovascular disease and become the main cause of premature death in most countries. From 2000 to 2022, Professor Robert A. Weinberg (Massachusetts Institute of Technology, USA) and Professor Douglas Hanahan (Agora Translational Cancer Research Center, Switzerland) summarized and developed the hallmarks of cancer every ten years to explain the mechanisms of the occurrence, development, and treatment response characteristics of malignant tumors[1–3]. Characteristics of cancer summarized in Fig. 1 will provide a reasonable explanation for the multi-level process of human tumor pathology, and will also have an outstanding impact on the development of cancer treatment pathways.

In normal tissues, glucose is converted to pyruvate by glycolysis, and then enters the mitochondria and is oxidized to supply energy for life activities through the tricarboxylic acid cycle, when the oxygen supply is sufficient. Only when the oxygen is deficient, pyruvate undergoes lactic acid fermentation for energy. However, tumor cells still preferentially obtain energy through glycolysis and produce lactic acid as the end product, even when the oxygen supply is sufficient. This phenomenon was first observed by Warburg in 1924, that is, the Warburg effect, which is an important feature of tumor energy metabolism[4,5] (Fig. 2A). Metabolic changes are thought to play an important role in the development of cancers, but the mechanism of metabolic changes in cancer cells is controversial. A study in 2006 revealed the molecular mechanism of the Warburg effect for the first time, proposing that hypoxia-inducible factor 1 (HIF-1) can cause the reprogramming of energy metabolism, including increased glucose uptake, glycolysis, and lactate production, under the condition of hypoxia or loss of Von Hippel-Lindau gene function[6]. In 2008, c-Myc and p53 were also proposed as key energy metabolism regulators, and it was pointed out that many oncogenes and tumor suppressor genes played corresponding roles through the above three transcription factors[7]. Since 2011, with the recognition of energy metabolism reprogramming as one of the top ten characteristics of cancer, energy metabolism-related targets, such as IDH1/2, Hexokinase 2, and M2-type pyruvate kinase 2, have become research hotspots in the field of cancer treatment[8]. In addition, the research on the molecular mechanism of energy metabolism reprogramming is gradually deepened[9,10]. From 2016 to 2022, the research on cancer energy metabolism reprogramming gradually entered the range of fatty acid, amino acid, and cholesterol metabolism[11,12]. More interestingly, studies on immunity, inflammation, microenvironment, ubiquitination system, and intestinal microbiota have increased people's understanding of cancer energy metabolism[13–17]. To meet the needs of rapid proliferation, cancer cells acquire the ability to rearrange their energy metabolism, which is the most fundamental manifestation of cancer adaptation to the environment. Therefore, targeting cancer energy metabolism is undoubtedly a very important cancer treatment method with broad potential.

As an important metabolic enzyme and tumor biomarker of many cancers, IDHs are considered as a valuable target for cancer treatment. Significant advances in cancer genetics have shown that genes encoding IDHs are frequently mutated in a variety of human malignancies. A series of groundbreaking studies further

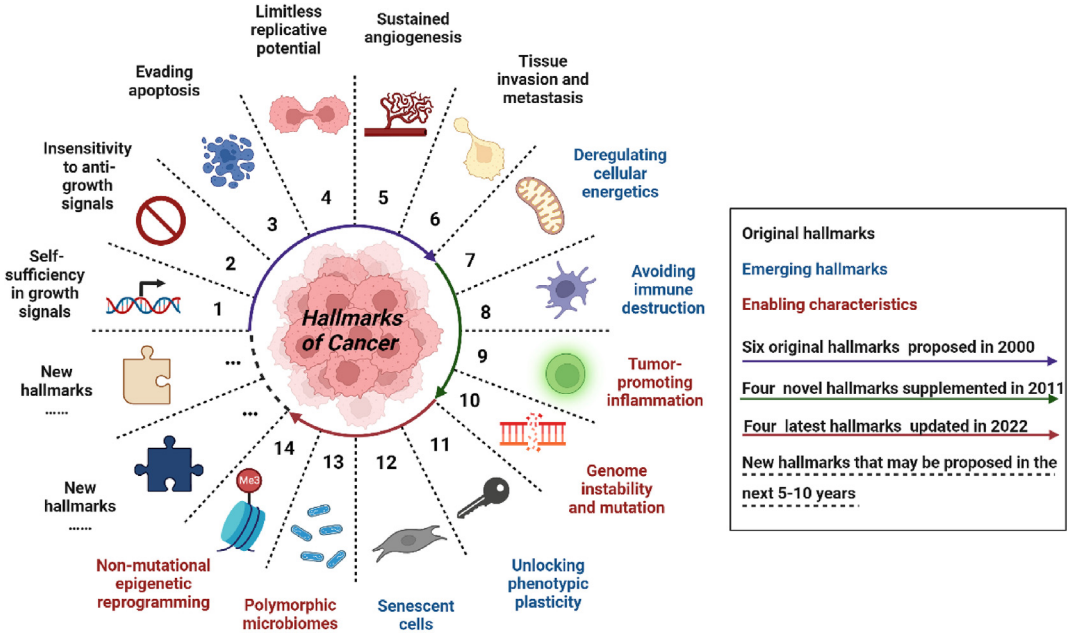


Figure 1 Hallmarks of cancer from 2000 to 2022. Original hallmarks are the initially identified cancer feature; emerging hallmarks are the features that have not been determined in the corresponding period and need further research and confirmation; enabling characteristics are the features that have been proposed in the corresponding period; colored circular arrows represent the time when the cancer hallmarks were presented; "…" represents the time when new cancer characteristics may be proposed in the future.

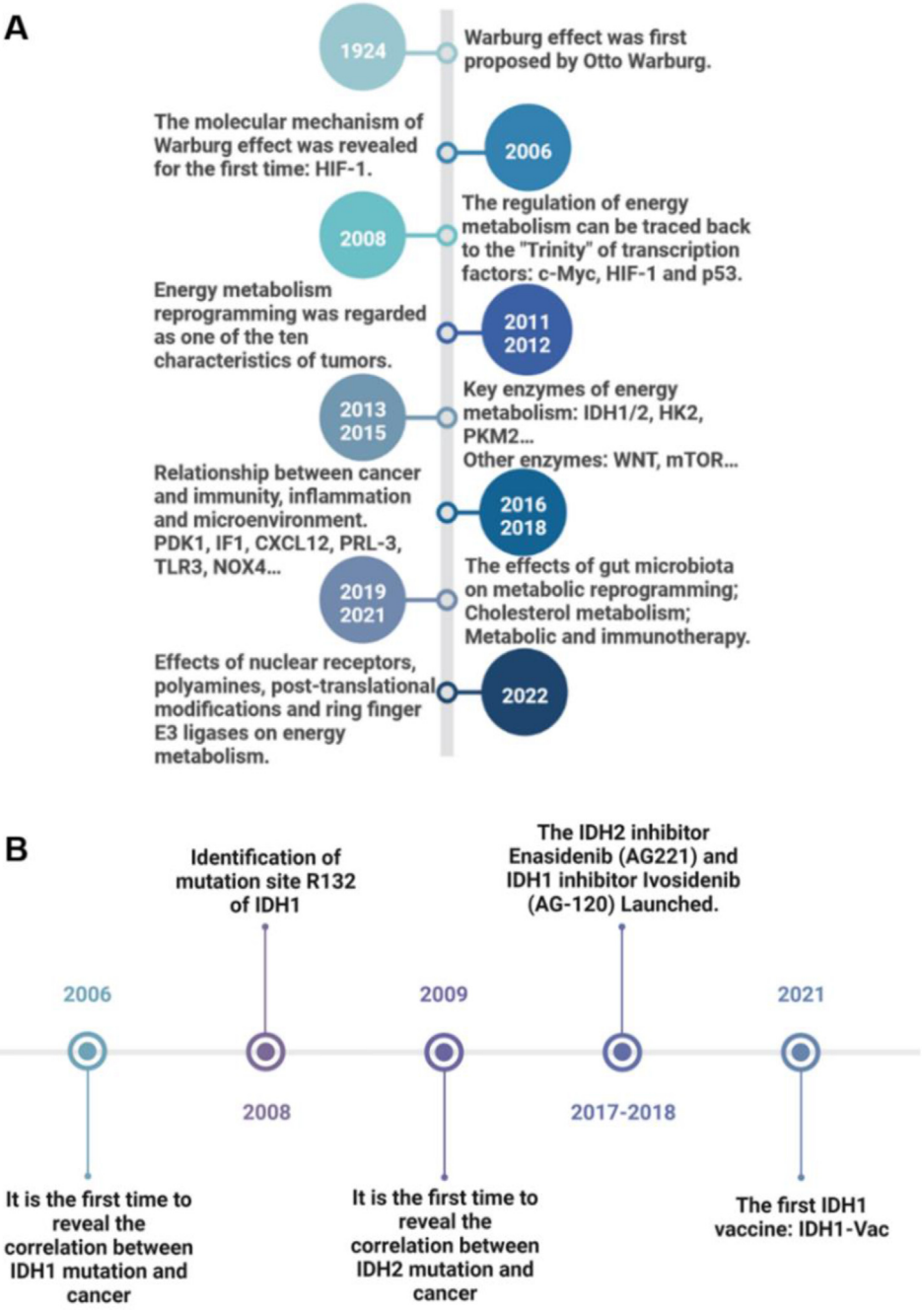


Figure 2 Research timeline of cancer energy metabolism reprogramming and IDH1/2. (A) Milestone events of cancer energy metabolism reprogramming from 1924 to 2022. (B) Milestone events of IDH1/2 research from 2006 to 2021. The description of key events is on the opposite side of the time.

elucidated the biological effects of *IDH* mutations, revealing the potential role of *IDH* mutations in tumorigenesis. In 2006, a sequencing study revealed for the first time that *IDH1* mutation was associated with tumors[18]. With the development of sequencing technology, mutation sites and frequencies of *IDH1/2* in different cancers have also been proposed one by one[19,20] (Fig. 2B). Up to now, no mutation of *IDH3* has been found. In a study of 5149 patients with solid tumors, 205 *IDH* mutations (3.78%) were found, including 145 *IDH1* mutations (2.68%) and 63 *IDH2* mutations (1.16%). Compared with *IDH2*, *IDH1* has a higher mutation frequency and involves more types of cancer[20]. In addition, according to the information provided by Cortellis Drug Discovery Intelligence database, there are 320 IDH1-related clinical trials, 137 IDH2-related clinical trials, and zero IDH3-related clinical trials, which further indicates that targeting IDH1 has a greater prospect in the field of clinical application. Therefore, in this review, we summarized the impacts of IDH1 on four aspects of cancer, including metabolic reprogramming, epigenetics, cancer immune microenvironment, and cancer phenotype. In addition, the IDH1 inhibitors and drug

combinations were summarized to provide ideas for IDH1-related cancer treatment and the development of drugs.

2. The cancer regulatory mechanisms of IDH1

2.1. *Physiological function and structure of IDH1*

IDHs are the rate-limiting enzyme in the tricarboxylic acid cycle involved in cellular energy metabolism, catalyzing the oxidative decarboxylation of isocitrate to α-KG and CO_2, and converts NAD(P)$^+$ into NAD(P)H. IDHs are divided into NADP$^+$-dependent cytoplasm/peroxisome IDH1, mitochondrial IDH2, and NAD$^+$-dependent mitochondrial IDH3[21,22]. IDH1 and IDH2 are involved in the metabolism of reduced glutamine during changes in electron transfer receptors and during hypoxia[23–25]. These two isomers also play an important role in cell resistance to oxidative damage through their forward oxidative decarboxylation reaction[26]. Furthermore, their reverse reductive carboxylation reaction plays a key role in the regulation of adipogenesis and glycolysis[27,28]. IDH3 catalyzes the irreversible conversion of isocitrate to α-KG during the tricarboxylic acid cycle[29] (Fig. 3).

IDH1 gene locates in zone 3, band 4 (2Q34) of chromosome 2, with a total length of 18,917 nucleotides. IDH1 is an asymmetric homodimer composed of two subunits, each with 414 amino acid residues. Each protein subunit is composed of 3 domains: large functional domain (located at AA1−103 and 286−414), and has a typical Rossmann fold; small domain (located at AA104−136 and 186−285), forming α/β sandwich structure; Clasp domain (located at AA137−185), and folds into two anti-parallel β-sheets[30]. The large and small domains are connected by β-sheets, and there are two cracks on their sides[31] (Fig. 4). The function was performed by forming two protein subunits into hydrophilic active sites. Deep fissures include NADP binding sites and isocitrate metal ion binding sites (Fig. 3), which can regulate the active and inactive state of IDH1 and the release of α-KG and NADPH[30]. After completion of catalysis, IDH1 can recombine NADP$^+$ and isocitrate, changing its conformation back to its inactive form[30]. Shallow fissures are involved in the conformational changes of the homodimer IDH1.

2.2. *IDH1 mutation is associated with cancer*

In 2006, a sequencing study on human breast cancer and colon cancer revealed for the first time that *IDH1* mutation was associated with cancer[18]. In 2008, the researchers set the research object as glioblastoma (GBM) and determined the point mutation of IDH1^{R132} by using the whole exome sequencing technology, suggesting that the mutation occurred mostly in young and secondary cancer patients[19]. Another group detected *IDH1* mutation in acute myeloid leukemia (AML) for the first time[32]. And *IDH2* mutation was found in some cancers[33] (Fig. 2B). Since the significance of *IDH1/2* mutation was clarified, there have been a lot of studies all over the world to analyze their mutation frequencies in different cancer species. The next-generation sequencing technology has greatly promoted research progress[20].

In AML with *IDH1/2* mutation, the mutation frequency is about 20%[34]. In gliomas, *IDH1/2* mutations are mainly seen in secondary GBM, oligodendroglioma, astrocytoma, and other low-grade malignant gliomas[35]. In 2021, a next-generation sequencing study involving 20 common solid tumors (more than 28,000 patients in total) showed that the incidence of *IDH1/2* mutation is only 1.3%,

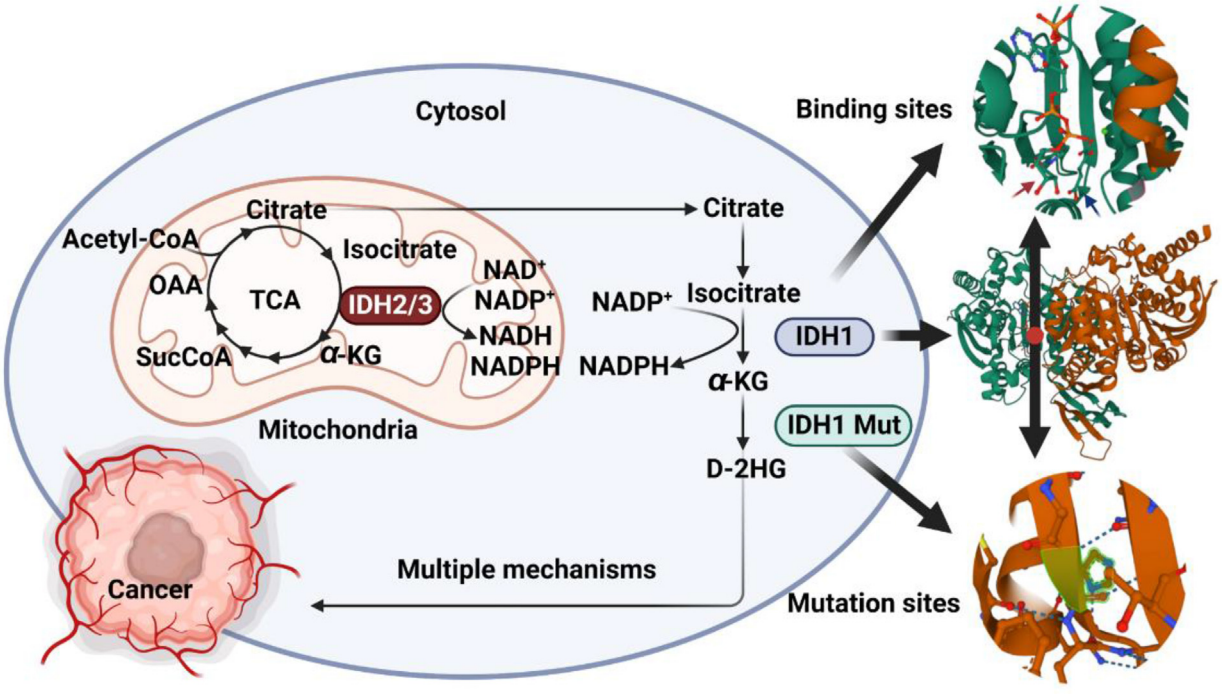


Figure 3 The selected function and structure of IDH1. The structure of IDH1^{WT} (PDB ID: 4KZO). The up circular picture is a magnified view of the binding site of IDH1^{WT}. The red arrow points to NADPH, and the blue arrow points to α-KG, green sphere is the divalent metal ion. The down circular picture is the enlarged view of the most common mutation site of IDH1^{WT}. IDH1^{R132H} is the most common mutation. IDH1 is composed of two subunits, heterozygous mutation (one subunit is mutated, and the other subunit is not mutated) of IDH1 can catalyze α-KG to yield D-2HG. Succinyl-coenzyme A (SucCoA); oxaloacetate (OAA); coenzyme A (CoA).

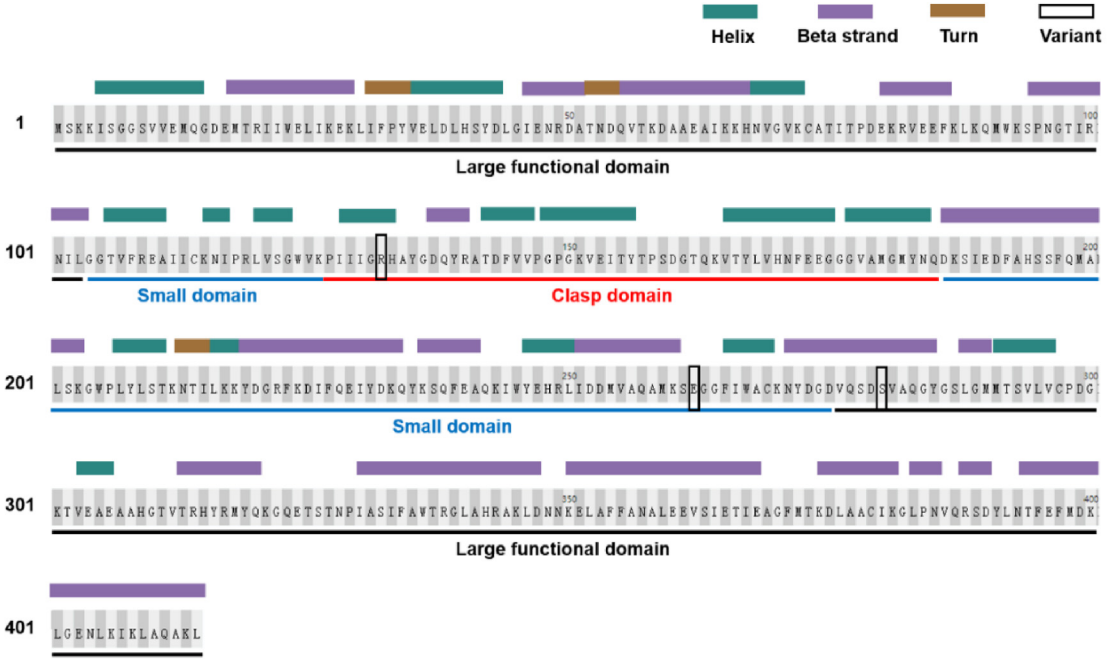
and the mutation frequency in lung cancer, colorectal cancer, liver cancer, stomach cancer, and other solid tumors is only 0.5%−3%[20]. The unique solid tumor with *IDH1/2* mutation frequency exceeding 5% is cholangiocarcinoma (low incidence)[36,37]. In addition, *IDH1/2* mutation frequency is also high in rare cancers such as chondrosarcoma (38%−86%), undifferentiated carcinoma of the paranasal sinuses (49%−82%), and angioimmunoblastic T-cell lymphoma (20%−30%)[38]. At present, *IDH1* mutation mainly occurs in the arginine132 residue, including R132H (the most common type), R132C, R132L, R132S, R132G, and R132P (Fig. 4). R172K is the most common IDH2 mutation. However, *IDH1* and *IDH2* mutations are mutually exclusive and rarely occur simultaneously. Currently, the conclusions of various studies are consistent, that is, cancers with *IDH1/2* mutations are not common. Even so, targeted IDH1 mutations have also brought precise treatment for AML, glioma, cholangiocarcinoma, and rare cancers[39].

2.3. *The regulation mechanism of IDH1 on cancer*

2.3.1. *IDH1-mediated metabolic reprogramming*

2.3.1.1. IDH1 mutation leads to accumulation of D-2HG. Under physiological conditions, IDH1 catalyzes the oxidative decarboxylation of isocitrate to α-KG[39] (Fig. 3). Once the gain of function mutation occurs, the affinity between mutant IDH1 and substrate decreases, and the activity of $IDH1^{WT}$ is inhibited by forming heterodimers[40] (Fig. 3). Further, α-KG is catalyzed by mutant IDH1 to generate a large amount of cancer-promoting metabolite D-2HG[41]. It has been reported that an amazing 30 mmol/L D-2HG can be found in *IDH1* mutated cancers[42]. D-2HG has a similar structure with α-KG. D-2HG can competitively suppress many α-KG-dependent enzymes, mainly including hydroxylation-related enzymes (such as prolyl-hydroxylase (PHD)), histone methylation-related enzymes, and DNA/RNA methylation-related enzymes, which will greatly disrupt normal physiological activities[43].

Hypoxia inducible factors-1α (HIF-1α) is a key component of the transcription factor HIF-1, which plays an important role in hypoxia conditions. It can sense low oxygen levels in cells, regulate the expression of genes related to angiogenesis, glucose metabolism, and other signaling pathways critical for tumor growth. HIF-1α is also prevalent in solid tumors[44]. PHD can adjust the stability of HIF-1α. Under normal partial pressure of oxygen, proline residues of HIF-1α were hydroxylated by PHD, then ubiquitinated, and finally degraded by the proteasome. Under anoxic conditions, however, hydroxylation will not occur, which will eventually lead to HIF-1α accumulation[43]. The HIF-1α



	AML	AdCC	A	BN	E	L	GBM	GM	GLM1	HCC	LUAD	CMM	MDB	MC	MM	MDS	NB	NLI	ODGM	PAAD
S280F	+																			
E262K						+														
R132P	+																			
R132L	+	+					+			+	+	+	+		+	+	+	+		+
R132S	+	+	+				+			+	+	+	+		+	+	+	+		+
R132G	+	+		+			+			+	+	+	+		+	+	+	+		+
R132C	+	+	+	+	+		+			+	+	+	+		+	+	+	+		+
R132H	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+

Figure 4 Amino acid sequence, mutation site, and mutation-related disease of IDH1. The human IDH1 is composed of 414 amino acids. In this figure, rectangles of different colors are used to show the structural characteristics of IDH1. The black rectangle marks the mutation site of IDH1, and the table below shows the disease corresponding to the mutation. Acute myeloid leukemia (AML); adenoid cystic carcinoma (AdCC); astrocytoma (A); breast neoplasm (BN); enchondromatosis (E); lymphoma (L); glioblastoma (GBM); glioblastoma multiforme (GM); glioma susceptibility 1 (GLM1); hepatocellular carcinoma (HCC); lung adenocarcinoma (LUAD); malignant melanoma of skin (CMM); medulloblastoma (MDB); metaphyseal chondromatosis (MC); multiple myeloma (MM); myelodysplastic syndrome (MDS); neoplasm of brain (NB); neoplasm of the large intestine (NLI); oligodendroglioma (ODGM); prostate adenocarcinoma (PAAD). The above information cited from the Uniport Database. Update [2020]. URL: https://www.uniprot.org/uniprotkb/O75874/feature-viewer.

subunit is transferred to the nucleus to form a heterodimer with the HIF-1β ligand subunit (aryl hydrocarbon receptor nuclear translocator), and then specifically binds to the hypoxia-responsive elements, in turn, induces the transcription of hypoxia-related genes, and promotes tumorigenesis[45,46] (Fig. 5). Therefore, mutant IDH1 inhibits the activity of PHD and improves the expression level of HIF-1α, and then, expression of target genes such as vascular endothelial growth factor, hexokinase, glucose transporter-1, phosphofructokinase, and phosphoglycerate kinase 1 increases, which may promote tumor cell growth, invasion, angiogenesis, and metastasis[40,47].

Electron transport chain is composed of five main complexes. Complexes I, III, and IV establish proton gradients by transferring electrons to oxygen molecules, and finally, complex V uses this proton gradient to promote ATP synthesis. It has been reported that D-2HG can inhibit complex IV[48] and complex V[49], which will greatly reduce the proportion of energy supply through oxidative phosphorylation. In order to better meet the energy demand, cancer cells will adjust the energy supply mode to aerobic glycolysis (Fig. 5).

Recently, it has been pointed out that mutant IDH1 can also cause heme synthesis disorder and reduce the level of heme catabolites (biliverdin and bilirubin). Mice with *Idh1* mutation will suffer from erythrocytic dysplasia[50]. D-2HG produced by mutant IDH1 can directly inhibit the activity of α-KG dehydrogenase, which in turn, leads to the reduction of succinyl-coenzyme A, causes the disorder of heme synthesis, and finally blocks the differentiation of erythroblasts in the late stage. At the same time, heme synthesis disorder affects the expression of heme oxygenase-1 and reduces the level of heme catabolite. Finally, it will promote the excessive accumulation of reactive oxygen species in cells, induce the death of *IDH1* mutant erythroid cells, lead to the imbalance of myeloid erythroid development of bone marrow precursor cells, and participate in the occurrence of myeloid tumors[50] (Fig. 5).

2.3.1.2. IDH1 mutation leads to the decrease of NADPH level. NADPH is a necessary co-factor for cell functions such as lipid metabolism, glucose metabolism, and anti-oxidative stress[28,51,52]. NADPH is an important electron donor for glutathione, thioredoxin, and other transcription factors. Moreover, NADPH plays an important role in regulating the redox state of cells. IDH1 catalyzes the conversion of isocitrate to α-KG is accompanied by the production of NADPH, which has the function of maintaining the balance of cell redox reaction and regulating the level of reactive oxygen species (ROS). NADPH converts glutathione disulfide into glutathione (GSH), and GSH is the main antioxidant of ROS[43] (Fig. 5). *IDH1* mutation leads to the decrease of NADPH level, which makes cells more susceptible to damage by ROS, causing cell membrane damage and enzyme activity changes. At the same time, DNA damage leads to genome instability, which ultimately leads to carcinogenesis. Research by Shi et al.[53] showed that in glioma cells overexpression of the *IDH1* mutant gene, the

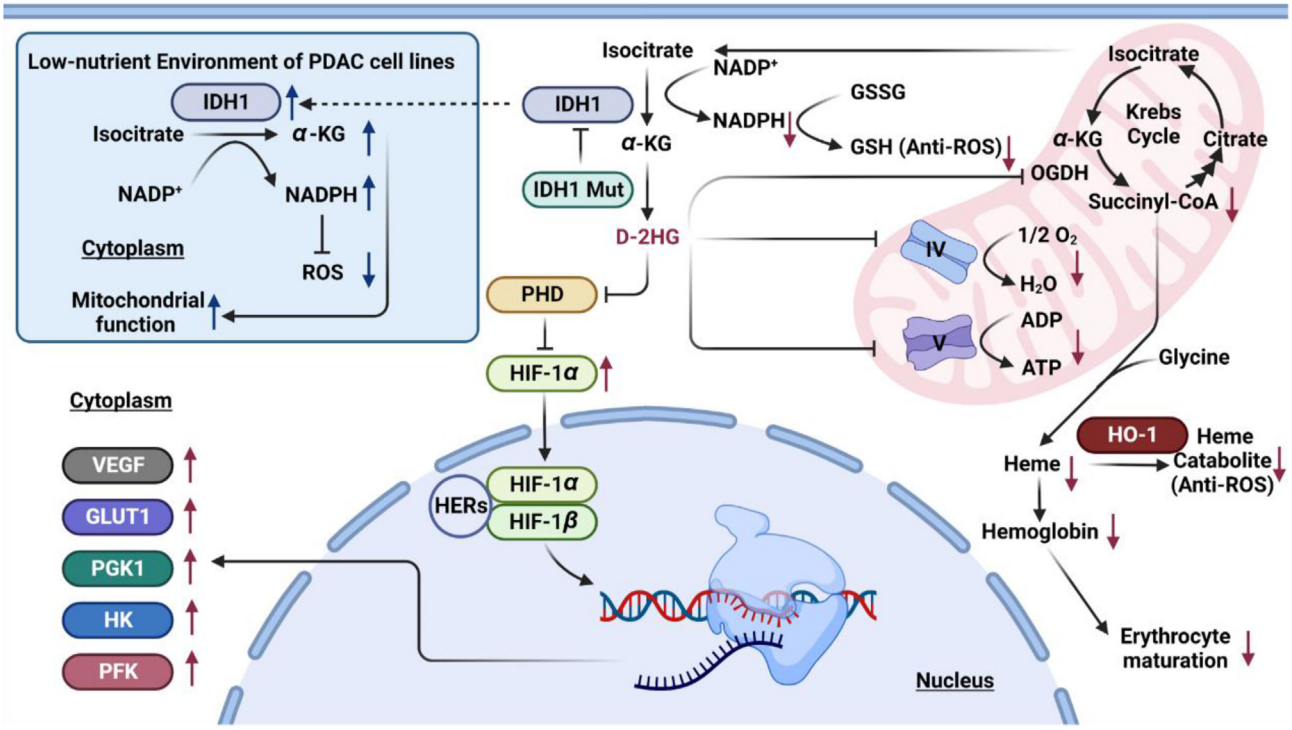


Figure 5 IDH1-mediated metabolic reprogramming. In general, IDH1 mutation increases the level of D-2HG and the expression of cancer-promoting proteins through the HIF-1α signal pathway. In addition, IDH1 mutation also inhibits the synthesis of ATP, results in a decline in antioxidant capacity, and causes erythroid cell maturation disorder *via* the inhibition of α-KG dehydrogenase. In the low-nutritional stated PDAC cells, the levels of IDH1 and NADPH increased, enhancing the mitochondrial function to maintain the growth of cancer cells. The black arrow means the promotion effects. The black T-shaped arrow means inhibition effects. The red arrow means the final effect caused by IDH1 mutations. The blue arrow means the change of IDH1 level in the low-nutritional stated PDAC cells and the resulting final effect. Vascular endothelial growth factor (VEGF), hexokinase (HK), glucose transporter-1 (GLUT1), phosphofructokinase (PFK), phosphoglycerate kinase 1 (PGK1), prolyl-hydroxylase (PHD), hypoxia inducible factors-1α (HIF-1α), hypoxia-responsive elements (HERs), heme oxygenase-1 (HO-1), reactive oxygen species (ROS), glutathione (GSH).

intracellular NADPH level is reduced, which in turn leads to inhibition of the growth of glioma cells. Studies have reported that the treatment of glioblastoma can be enhanced by targeting IDH1-mediated NADPH biosynthesis[54].

ROS is the main molecule produced by the body during oxidative stress and has long been considered as an important factor for the development and recurrence of cancers. ROS accumulation can induce cell apoptosis, but moderate ROS production is one of the important components of inflammatory characteristics of innate immune response[55]. Lipopolysaccharide (LPS) extracted from Gram-negative bacteria are typical stimuli that trigger inflammatory cascades *in vitro* and *in vivo*. IDH1 helps to reduce the ROS induced by LPS or H_2O_2 treatment[56,57]. *In vitro* studies have found that LPS can induce the expression of IDH1 and reduce the ROS induced by LPS or H_2O_2 in rat murine macrophages RAW 264.7, at the same time, the overexpression of IDH1 can reduce the level of intracellular peroxides, which may reduce ROS level in this way, thereby inhibiting tumor development[57]. *In vivo* studies have shown that IDH1 protects mouse liver cells from damage caused by endotoxin-induced oxidative stress by regulating the ratio of $NADP^+$/NADPH in the cell[58]. This suggests that stimulating IDH1 activity in inflammatory responses, including in the early stages of septic shock, may be an effective therapeutic strategy to reduce oxidative stress. Under the induction of tumor promoter Tissue polypeptide antigen and UVC, IDH2 in JB6 P+ was not significantly changed. IDH1 knockout and overexpression enhances and inhibits Tissue polypeptide antigen-induced tumor-like transformation of cells, respectively[59]. Therefore, regulating the activity of IDH1 may be one of the effective ways to reduce inflammatory oxidative stress during tumorigenesis and development.

2.3.1.3. Metabolic remodeling induced by wild-type IDH1. Researchers found an RNA-binding protein, human antigen R (HuR), which can enhance mitochondrial function and antioxidant capacity when nutrition is deficient[60]. After HuR knockdown, the level of IDH1 is also significantly reduced. In addition, some studies have shown that HuR participates in the pre-translation modification of IDH1. Therefore, researchers believe that IDH1 and HuR are closely related and are also crucial to the survival of cells in a low-nutrient environment[61]. Researchers found that ROS in tumor cells accumulated in the early stage under low glucose culture conditions, but decreased on the third day, accompanied by a compensatory increase in NADPH over time. Only when $IDH1^{WT}$ is knocked down, the survival rate of cancer cells in low glucose culture will be reduced. In addition, the $IDH1^{WT}$-knockout PDAC cell line lost its antioxidant capacity, suggesting that $IDH1^{WT}$ plays an important role in the survival of tumor cells in a low glucose environment[62]. After exogenous supplement of α-KG, the level of related metabolites in cells recovered, and mitochondria were able to continue to maintain the survival of cells, suggesting that the IDH1 metabolite α-KG influences the survival of cells under hypoxia by mediating mitochondrial function[62] (Fig. 5). More importantly, this study pointed out that IDH1 mutant inhibitors can inhibit wild-type IDH1 in the cell environment with low magnesium ion concentration, which will provide new therapeutic ideas for the treatment of IDH1-related cancers. At the same time, it also puts forward higher requirements for clinical diagnosis and detection technology.

2.3.2. *Effect of IDH1 on epigenetics*

D-2HG can occupy the same binding pocket of α-KG and competitively inhibit many α-KG-dependent enzymes, such as DNA demethylase and histone demethylase, which can lead to DNA and histone hypermethylation, and then make the abnormal epigenetic regulation, block cell differentiation, cause abnormal expression of a series of oncogenes, suppressor oncogene, and signal transduction genes, resulting in the occurrence of cancers[63–68].

2.3.2.1. D-2HG inhibits DNA demethylase. TET oncogene family member 2 (TET2) plays an important role in stem cell differentiation, epigenetic regulation, and the occurrence of hematopoietic malignancies. TET2 achieves DNA demethylation by converting 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC)[69]. The loss of TET2 function can cause DNA hypermethylation of hematopoietic stem cells, which in turn leads to abnormal gene expression[70]. The *TET2* gene is mutated in myeloid diseases including acute myeloid leukemia[71]. So far, more and more evidences show that TET2 mutations play an important role in AML. The study of Figueroa et al.[72] showed that abnormal DNA methylation is a hallmark of AML. Clinical observations have demonstrated that IDH1 and IDH2 mutations lead to a hypermethylated phenotype, destroy the function of TET2, and impair hematopoietic differentiation[72]. In addition, IDH1/2 mutations in AML and TET2 mutations have similar DNA methylation phenotypes. Importantly, they are mutually exclusive[72], implying that they have the same pathway of action. The research further supports the latest data on this cooperative mechanism[73]. The oncogenome map is used to detect 207 patients with glioblastoma multiforme and found that IDH1 mutation is closely related to glioma-CpG island methylator phenotype[74]. Among patients with DNA hypermethylation, 78% of patients have *IDH1* gene mutations, while no IDH1 mutations are found in patients without DNA hypermethylation[74]. These studies show that the mutant $IDH1^{R132H}$ and its product D-2HG can inhibit TET2 from catalyzing the production of 5hmC, leading to DNA hypermethylation, which in turn leads to cancer formation (Fig. 6).

In addition to inhibiting the DNA hypermethylation of IDH1/2 mutant cells by mediated TET2 activity, D-2HG can also regulate DNA methylation through DNA methyltransferase 1 (DNMT1). D-2HG binds to DNMT1 and promotes its separation from the receptor-interacting protein 3 (RIP3) promoter, induces hypermethylation, inhibits RIP3 protein, thereby inhibits RIP3-dependent cell necrosis, and promotes tumorigenesis[75]. The D-2HG produced by IDH1 mutant cells promotes the binding of DNMT1 to the Fibulin-5 promoter, leading to methylation, and ultimately enhancing the migration and proliferation of non-small cell lung cancer cells[76] (Fig. 6).

2.3.2.2. D-2HG inhibits histone demethylase. Histone methylation is an important form of epigenetic modification. JmjC domain containing histone demethylase (JHDM) is an important histone demethylase, which mainly catalyzes the demethylation of histones H3K4, H3K9, H3K27, H3K36, and H4K20[77–82]. Histone demethylases play an important role in human diseases such as neurological disorders and cancer[83,84]. *In vitro* studies have shown that D-2HG inhibits the activity of histone demethylase in cells[65]. Xu et al.[65] further used *in vivo* studies to verify that D-2HG can inhibit the activity of a variety of histone demethylases. Lu et al.[66] found that a variety of histone methylation markers increased in the cultured cell models expressing $IDH1^{R132H}$ mutation or treated with cell-permeable D-2HG. Interestingly, as the number of cell passages increased, DNA methylation also appeared in cells. It is worth noting that the appearance of histone methylation is always

earlier than DNA methylation[85]. The study indicated that astrocytes expressing mutant IDH1^{R132H} showed increased H3K9me2, H3K27me3 and H3K36me3 levels, as well as decreased 5hmC and hypermethylation, further proving that IDH1 mutation is the molecular basis of G-CIMP[86]. Research also showed that the expression of IDH1 and IDH2 mutants inhibited 5mC hydroxylation and histone demethylation[65]. In the azoxymethane mouse bowel cancer model, the level of D-2HG in the tumor is elevated[87]. Colvin et al.[88] found that D-2HG can induce histone modifications, leading to increased gene expression in the promoter region of Zinc finger E-box binding homeobox 1 (*ZEB1*) gene, which is the main regulator of epithelial−mesenchymal transition (EMT). D-2HG can also directly induce EMT of colorectal cancer cells[88]. EMT promotes cancer cells to invade local tissues and enter the blood, leading to distant organ metastasis[89]. D-2HG levels are elevated in clinical samples of colorectal cancer, especially those related to distant metastasis, suggesting the role of D-2HG in tumor metastasis[88] (Fig. 6).

2.3.3. *Effect of IDH1 mutation on cancer immune microenvironment*

At present, many studies have shown that abnormal accumulation of metabolites can lead to tumorigenesis. More and more studies show that IDH1 is involved in the regulation of the cancer microenvironment (acquired immunity and natural immunity), and the combination of IDH1 inhibitors and cancer immunotherapy drugs shows good therapeutic effects.

2.3.3.1. Regulation of IDH1 on acquired immune system. D-2HG produced by mutant IDH1 may cause the immune microenvironment of GBM and other cancers to be suppressed[90]. By inhibiting the expression and activation of signal transducer and activator of transcription 1, D-2HG reduces the secretion of chemokine CXC chemokineligand-10 in glioma cell lines, thereby inhibiting cytotoxic T lymphocyte infiltration at tumor sites[91]. The transporter solute carrier family 13 member 3 assists T lymphocytes in uptake of D-2HG. Excess D-2HG inhibits ATPase, reduces ATP production, and weakens phospholipase C gamma phosphorylation, both leading to the reduction of nuclear translocation of activated T cell nuclear factor, and eventually reducing the activation of T lymphocytes[92]. In high-grade glioma, D-2HG can enhance the tryptophan-2,3-dioxygenase activity in macrophages, thus promoting the metabolism of L-tryptophan to the aryl hydrocarbon receptor ligand kynurine. Kynurine induced aryl hydrocarbon receptor translocation to the nucleus, where it increased interleukin 10 production and decreased the expression of costimulatory molecules cluster of differentiation 86, cluster of differentiation 80, and major histocompatibility complex II. This leads to reduced antigen presentation and increased T cell inhibition, thus driving a more

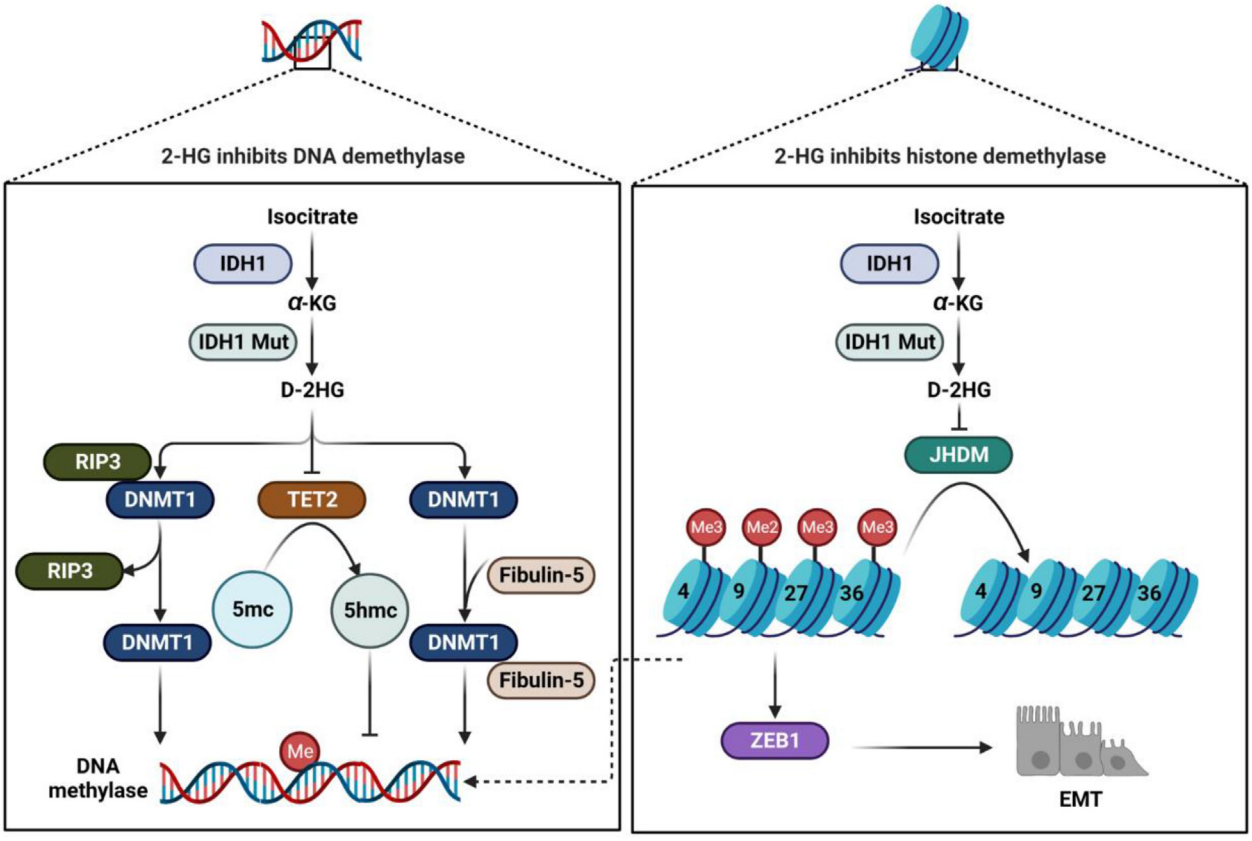


Figure 6 Effect of IDH1 on Epigenetics. IDH1 mutation causes the level of D-2HG to increase, which in turn promotes the hypermethylation of DNA and histones, and eventually induces cancer. The black arrow means the promotion effects. The black T-shaped arrow means inhibition effects. The black dotted arrow means the time sequence. "Me" means methylation. Receptor-interacting protein 3 (RIP3), DNA methyltransferase 1 (DNMT1), TET oncogene family member 2 (TET2), 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), JmjC domain containing histone demethylase (JHDM), zinc finger E-box binding homeobox 1 (ZEB1), epithelial−mesenchymal transition (EMT).

immunosuppressive tumor microenvironment[93]. In addition, D-2HG can also induce transient hypermethylation of programmed cell death-ligand 1 promoter, thereby reducing the expression of PD-L1[94]. Some studies have shown that the combination of IDH1 inhibitor and anti-PD-L1 can significantly prolong the survival period of IDH1 mutant glioma mice[95]. In general, D-2HG inhibits anti-tumor T cell immunity. Inspired by this, targeted mutant IDH may have a synergistic effect with immunotherapy (Fig. 7).

2.3.3.2. Regulation of IDH1 on natural immune system. D-2HG also inhibits the natural immune function and inactivates complement in IDH1 mutant astrocytes. The mechanism is that D-2HG inhibits the assembly of C5 convertase in the classical pathway of complement activation and inactivates the assembled C3/C5 convertase. At the same time, it inhibits the assembly of C3/C5 convertase in the alternative pathway[96]. In these ways, glioma cells can resist complement mediated lysis and phagocytosis. The receptor natural killer cell group 2D (NKG2D) on natural killer cells activates NK cells when it connects with the NKG2D ligand (NKG2DL) on the surface of target cells (such as tumor cells), thereby killing tumor cells. The expression level of NKG2D on the surface of IDH1 mutant astrocytes is lower than that of wild-type IDH1, which may be related to the hypermethylation of NKG2D promoter[97]. The down-regulation of NKG2D helps tumor cells escape the cytolysis of NK cells (Fig. 7).

2.3.4. Effect of IDH1 on cancer phenotype

2.3.4.1. IDH1 induces apoptosis or autophagy depending on cell type. The study of Gilbert et al.[98] showed that D-2HG can trigger the apoptosis of LN18 cells, while the apoptosis of U87MG cells did not change. This indicates that the apoptotic response to D-2HG is cell type specific. In addition, both cell lines did not show significant changes in the activity of caspase 8-dependent exogenous pathways[98]. D-2HG also increased the formation of autophagosomes in U87MG cells, which is a sign of autophagosome formation[98]. Their research showed that IDH1 mutations can induce apoptosis and autophagy, but these effects vary greatly with cell types.

2.3.4.2. IDH1 induces apoptosis through multiple signaling pathways. Prostate apoptosis response-4 (Par-4) is a tumor suppressor protein. Par-4 can promote the apoptosis of a variety of cancer cells. Par-4 can kill human cancer cells from pancreas[99], cervix[100], lung[100], prostate[101], kidney[101], endometrium[102], and colon[103]. D-2HG can inhibit the transcription of Par-4 *in vitro* by inhibiting promoter activity and enhancing mRNA degradation[104]. The apoptosis-inducing selectivity in the cancer cell domain within Par-4 is highly active on glioma cells. Among IDH1 wild-type high-grade gliomas, gliomas expressing more Par-4 have a significantly longer median survival[104] (Fig. 8).

Anti-apoptotic B-cell lymphoma-2 (Bcl-2) family members, such as B-cell lymphoma-X_L (Bcl-X_L) and myeloid cell leukemia 1 (Mcl-1), are highly expressed in human glioblastoma. Compared with wild-type IDH1 cells, the apoptosis induced by Bcl-X_L inhibition was significantly more in IDH1 mutant cells[105]. In anaplastic astrocytoma, the level of Mcl-1 in IDH1 mutant cells is lower than that in IDH1 wild-type cells. The specific knockdown of Mcl-1 makes glioblastoma cells sensitive to apoptosis mediated by Bcl-X_L inhibition[105]. The energy expenditure mediated by D-2HG activates adenosine 5′-monophosphate-activated protein kinase (AMPK), which leads to weakening of protein synthesis and mechanistic target of rapamycin (mTOR) signal, and ultimately to the decrease of Mcl-1[105]. These data indicate that IDH1 mutant gliomas are susceptible to Bcl-X_L inhibition (Fig. 8).

The C/EBP homologous protein (CHOP) is a member of the C/EBP family, and its expression level is relatively low during the growth of normal cells, but it is significantly increased when the endoplasmic reticulum is stressed[106]. Since CHOP lacks a stable DNA binding domain, it needs to be heterodimerized with other members of the C/EBP family to transcriptionally regulate the expression of the response gene[107]. Research by Yang et al.[106] found that in melanoma cells, endoplasmic reticulum stress increases the expression and activity of CHOP, which directly binds to the IDH1 promoter region after forming a heterodimer with C/EBPβ, transactivating IDH1 expression. The activated IDH1 promotes the degradation of HIF-1α and down-regulates it, which in turn makes melanoma cells apoptosis induced by hypoxia (Fig. 8).

Research by Li et al.[108] showed that D-2HG competitively inhibits succinate dehydrogenase (SDH), preferentially inducing succinyl-CoA accumulation and excessive succinylation in mitochondria. IDH1 mutation or SDH inactivation can cause excessive succinylation, cause respiratory depression, and induce cancerous metabolism and mitochondrial depolarization[108]. These mitochondrial dysfunctions cause Bcl-2 to accumulate on the mitochondrial membrane and cause apoptosis resistance in hypersuccinylated cells[108] (Fig. 8).

c-Jun N-terminal kinase (JNK), belonging to the family of mitogen-activated protein kinases, responds to stress stimuli such as serum starvation, and also plays an important role in the apoptosis pathway. Jiang et al.[109] knocked *IDH1*^{R132Q} into mutant mouse cells and found that D-2HG inhibited JNK activation induced by serum starvation and prevented cell apoptosis. During starvation, cell division cycle 42 (Cdc42) usually destroys the self-inhibition of mixed lineage kinase 3 (MLK3) and triggers the MLK3−MKK4/7 (mitogen-activated protein kinase kinase, MKK) −JNK−Bim (Bcl-2 interacting mediator of cell death) apoptosis cascade[109]. D-2HG binds to Cdc42 and eliminates its binding to MLK3, causing MLK3 inactivation and apoptosis[109] (Fig. 8).

In the study of Rosiak et al.[110], IDH1^{R132H}-positive neural stem cells and their derivatives have a high percentage of apoptotic cells. By analyzing the activity of poly ADP-ribose polymerase (PARP) and caspase-3, it was confirmed that the expression of IDH1^{R132H} increased the apoptosis sensitivity of neural stem cells and their derivatives[110]. Strong apoptosis causes insufficient differentiation of cells expressing IDH1^{R132H} (Fig. 8).

According to reports, long non-coding RNAs (lncRNAs) are important regulators in tumorigenesis. IDH1 antisense RNA 1 (IDH1-AS1) is an lncRNA that can interact with genes to regulate the Warburg effect. The study by Zhang et al.[111] found that paired box 5 is a transcriptional activator of IDH1-AS1, and the up-regulation of IDH1-AS1 induced by paired box 5 promotes the proliferation and apoptosis of prostate cancer by regulating autophagy related protein 5 (ATG5)-mediated autophagy. Wang et al.[112] used primary glioblastoma cell lines U251 and U87-MG to study the effect of IDH1-AS1 on the growth of glioma cells, and found that IDH1-AS1 overexpression inhibited cell proliferation and blocked the cell cycle in the G1 phase, and the protein expression levels of cyclinD1, cyclin A, cyclin E, CDK2 and CDK4 (cyclin dependent kinase, CDK) decrease, and cell apoptosis increases (Fig. 8).

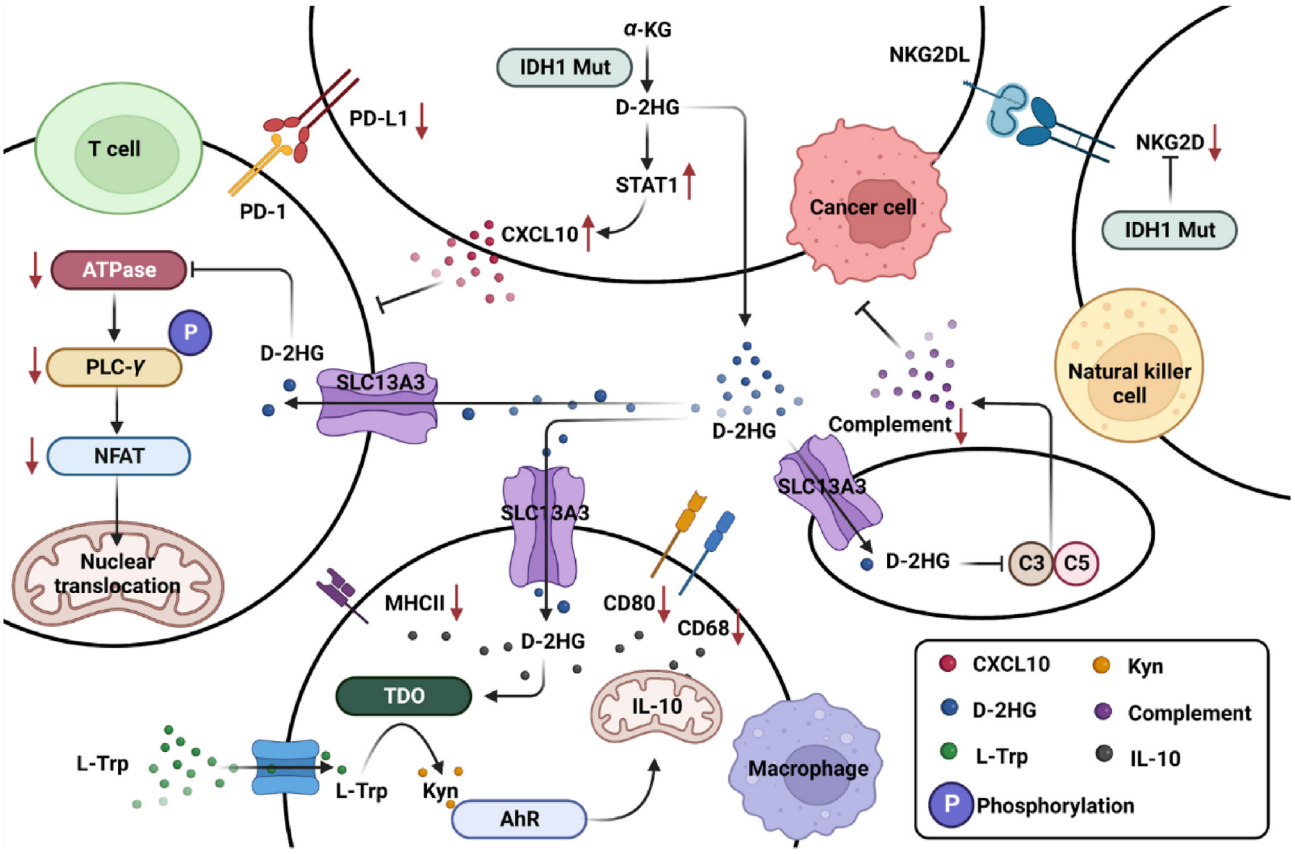


Figure 7 Effect of IDH1 mutation on cancer immune microenvironment. IDH1 mutation will cause the cancer microenvironment to be in a state of immunosuppression. The black arrow means the promotion effects. The black T-shaped arrow means inhibition effects. The red arrow means the final effects caused by IDH1 mutation. Signal transducer and activator of transcription 1 (STAT1), CXC chemokine ligand-10 (CXCL10), solute carrier family 13 member 3 (SLC13A3), phospholipase C gamma (PLC-γ), nuclear translocation of activated T cell nuclear factor (NFAT), tryptophan-2,3-dioxygenase (TDO), L-tryptophan (L-Trp), aryl hydrocarbon receptor (AhR), kynurine (Kyn), interleukin 10 (IL-10), cluster of differentiation 86 (CD86), cluster of differentiation 80 (CD80), major histocompatibility complex II (MHCII), programmed cell death-ligand 1 (PD-L1), natural killer cell group 2D (NKG2D), NKG2D ligand (NKG2DL), natural killer (NK).

p53 is an important tumor suppressor protein. On one hand, it can activate various responses, including arresting the cell cycle and promoting apoptosis[113]. These all seem to help suppress cancers. On the other hand, p53 responds to stress caused by factors such as oncogene activation, hypoxia or starvation[113]. These can promote the development of cancers. In addition, p53 can also play a role in normal development. The activation of p53 may also have adverse effects and may cause disease. Parsons et al.[19] found that the frequency of *p53* mutations in human glioblastoma with *IDH1* mutation is very high. The study by Hu et al.[114] showed that patients with osteosarcoma with high IDH1 expression have very high p53 expression. Therefore, IDH1 may be related to p53. Subsequent studies have shown that IDH^{R132H} mutation is significantly related to p53, but is negatively related to epidermal growth factor receptor (*EGFR*) mutation[115]. Next, we should investigate whether these three molecules participate in a common signal pathway (Fig. 8).

2.3.4.3. Effects of IDH1 on the proliferation, migration, and drug resistance of cancer. The Wnt/β-catenin signaling pathway plays a key role in normal embryonic development and promotes the metastasis of several cancers[116–120]. Research by Cui et al.[121] showed that the R132H mutation in IDH1 negatively regulates Wnt/β-catenin signaling, thereby reducing the proliferation, cell survival and invasion of human gliomas. IDH1 mutation causes cell cycle arrest in G1 phase, reduces the ratio of G2/M phase, inhibits cell proliferation, down-regulates cell invasion, and improves the prognosis of patients with glioma[122]. Research by Wang et al.[123] also showed that IDH1 mutations can cause cell cycle arrest in G1 phase, reduce cell proliferation and invasion, and increase sensitivity to chemotherapy (Fig. 8).

Shen et al.[124] studied the function of IDH1 in cell migration. Studies have shown that IDH1 regulates the migration of primary GBM cells by changing the level of α-KG. The function of the IDH1/α-KG axis may depend on the regulation of phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mTOR pathway. Overexpression of IDH1 or α-KG treatment both promoted the PI3K/AKT/mTOR pathway[124]. Treatment with mTOR-specific inhibitor rapamycin can reverse the increase in cell migration caused by overexpression of IDH1 and α-KG treatment (Fig. 8).

Studies have found that overexpression of the wild-type *IDH1* gene does not affect cell cycle, apoptosis, and invasion ability. However, it leads to resistance to high-dose temozolomide (TMZ) chemotherapy *in vivo* and *in vitro*. The mechanism is that overexpression of wild-type IDH1 reduces the Bcl-2-associated X (Bax)/Bcl-2 ratio and caspase-3 activity, and inhibits TMZ-

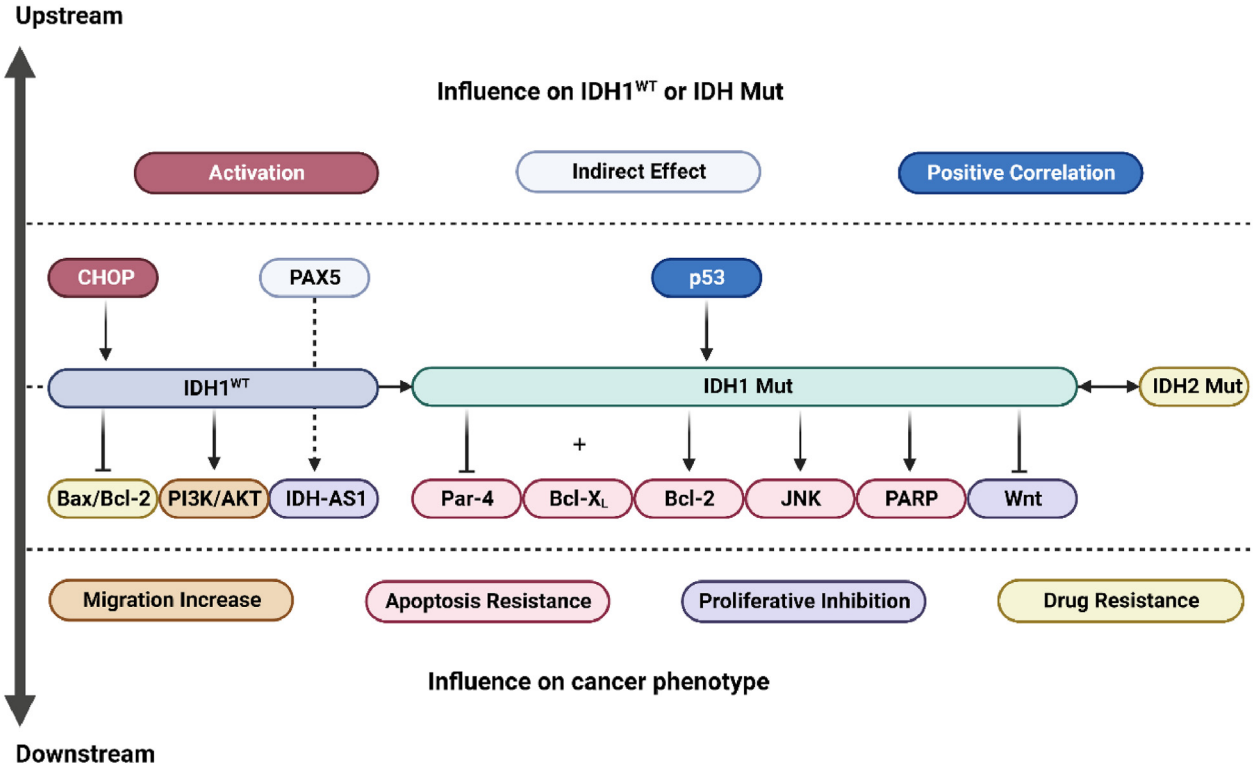


Figure 8 The effect of IDH1 on cancer phenotype and its molecular mechanism. The black arrow means the promotion effects. The black T-shaped arrow means inhibition effects. "+" means synergistic effect. The colors of targets represent the influence on IDH1^{WT} and IDH1 (upstream), or the influence on cancer cell phenotype (downstream). Prostate apoptosis response-4 (Par-4), B-cell lymphoma-2 (Bcl-2), B-cell lymphoma-XL (Bcl-XL), myeloid cell leukemia 1 (Mcl-1), adenosine 5′-monophosphate-activated protein kinase (AMPK), mechanistic target of rapamycin (mTOR), C/EBP homologous protein (CHOP), c-Jun N-terminal kinase (JNK), poly ADP-ribose polymerase (PARP), paired box 5 (PAX5), phosphoinositide 3-kinase (PI3K), protein kinase B (AKT).

induced apoptosis[123]. Calvert et al.[125] demonstrated that wild-type IDH1 is overexpressed in primary GBM, and the genetic or pharmacological inhibition of IDH1 activity reduces the growth of tumor cells, part of the mechanism is to increase the drug's sensitivity to apoptosis. This finding indicates that the up-regulation of IDH1 represents a common metabolic adaptation of GBM for macromolecule synthesis and treatment of drug resistance. In addition, some researchers first proposed the mechanism of acquired drug resistance of IDH through clinical case studies. The research results show that when receiving the same type of inhibitor treatment, the conversion between IDH1 and IDH2 mutants will occur (IDH1 inhibitor will cause mutant IDH1 to change into mutant IDH2), and cancer cells can continue to produce D-2HG[126]. These findings confirm the roles of continuous D-2HG production in cancer progression and propose a therapeutic strategy to prevent or overcome drug resistance, that is, the combination of IDH1 and IDH2 inhibitors. Therefore, DNA sequencing and D-2HG content determination are necessary for the early stage of IDH-targeted therapy (Fig. 8).

3. IDH1 inhibitors

In Table 1, we listed IDH1 inhibitors in the clinical research stage and showed their clinical therapeutic potential. In Table 2, we summarized IDH1 inhibitors in the preclinical research stage based on the core skeleton. The structural diversity of these compounds laid the foundation for obtaining inhibitors with higher activity and better selectivity. In addition, in Table 3, we have collected the IDH1 combination drug cases currently in the clinical research stage, which may provide new treatment methods for the treatment of refractory and acquired drug-resistant cancers.

3.1. *IDH1 inhibitors in the clinical trial*

AG-120 (ivosidenib) is a potent inhibitor of mutated IDH1, which has been shown to significantly inhibit the production of D-2HG in tumor models[127]. In phase I clinical trials of solid and hematological malignancies, AG-120 showed promising clinical activity[127]. Ivosidenib is a promising new drug for the treatment of AML with *IDH1* mutation. Ivosidenib can permanently relieve relapsed or refractory AML with *IDH1* mutations[128]. From a phase I trial, Choe et al.[129] investigated the molecular mechanism of resistance to ivosidenib in 174 patients with mIDH1 relapsed/refractory AML. Studies have found that mutations in the receptor tyrosine kinase pathway are associated with primary resistance to ivosidenib, and multiple mechanisms lead to acquired resistance, especially the disappearance of receptor tyrosine kinase pathway mutations and D-2HG recovery mutations[129]. Ivosidenib may also slow down the progression of IDH1 mutant gliomas[130]. In patients with advanced glioma, ivosidenib (500 mg) once a day is associated with prolonged disease control, good safety, and decreased growth of non-enhancing tumors[131]. The results of a phase III study of IDH1 mutations and chemotherapy-refractory

cholangiocarcinoma showed that ivosidenib significantly improved progression-free survival, and it was well tolerated[132]. A phase I clinical study of ivosidenib in patients with advanced chondrosarcoma showed that ivosidenib has good safety and clinical activity[133] (Table 1 and Fig. 9).

AG-221 is a dual inhibitor of IDH1 and IDH2, its conditions are leukemia, myelodysplastic syndrome, and solid tumor. The product was first marketed in the United States in 2017 for the treatment of adult patients with recurrent or refractory AML. In addition, phase III clinical trials are ongoing at celgene for the treatment of patients 60 years or older with AML refractory to or relapsed after second or third-line AML therapy. The drug is also in phase II clinical development at the company for the treatment of patients with high-risk IDH2-mutant myelodysplastic syndrome, as monotherapy or in combination with azacytidine[144] (Table 1 and Fig. 9).

IDH-305 (**13**) is an oral inhibitor of IDH1 mutants that can effectively inhibit the production of D-2HG in a variety of xenograft models[145]. IDH305 (**13**) has entered human clinical trials for the treatment of cancers with IDH1 mutations, such as AML, chondrosarcoma, and cholangiocarcinoma. IDH305 (**13**) also showed good brain penetration[145], indicating its potential in the treatment of IDH1 mutant brain cancer (Table 1 and Fig. 9).

DS-1001b is a selective, orally bioavailable, mutant IDH1 inhibitor that can destroy the proliferation of chondrosarcoma cells with IDH1 mutations *in vitro* and *in vivo*, and reduce the level of D-2HG[146]. DS-1001b also reduced the levels of H3K4me3 and H3K9me3, restored the abnormal histone modifications induced by D-2HG[146]. Inhibition of mutant IDH1 by DS-1001b is a promising treatment for chondrosarcoma (Table 1 and Fig. 9).

BAY-1436032 is a pan-inhibitor of IDH1 protein with different codon 132 mutations[147]. It works through an allosteric inhibition mechanism. Except for the inhibition of angiotensin 2 at an IC_{50} of 4.2 μmol/L, the researchers did not detect any relevant off-target effects. In other words, BAY-1436032 is a highly specific and effective inhibitor against IDH1 proteins with R132H or R132C mutations. BAY-1436032 can strongly reduce D-2HG levels in cells carrying $IDH1^{R132H}$, $IDH1^{R132C}$, $IDH1^{R132G}$, $IDH1^{R132S}$, and $IDH1^{R132L}$ mutations[147]. *In vitro* or *in vivo*, BAY-1436032 showed no toxicity[147]. BAY-1436032 can also be taken orally. BAY-1436032 also significantly prolonged the survival of mice transplanted with human astrocytomas carrying the $IDH1^{R132H}$ mutation[147]. Unfortunately, researchers have not yet obtained the co-crystallization of BAY-1436032 and mutant IDH1 protein. BAY-1436032 is very effective against all major types of IDH1 mutant AML[148]. In IDH1 mutant AML, the results of a phase I clinical study showed that BAY-1436032 is safe and moderately effective as a monotherapy[149]. But at the highest dose tested, BAY-1436032 still has a low overall remission rate and incomplete target inhibition[149], so it is not suitable for further clinical development in AML. In another study, in a patient-derived IDH1 mutant AML xenograft model *in vivo*, BAY-1436032 combined with chemotherapy can delay the transplantation of leukemia cells[150]. BAY-1436032 can also play a strong synergistic effect with azacitidine by inhibiting mitogen-activated protein kinase/extracellular regulated protein kinase and Retinoblastoma gene/E2F transcription factor 2 signaling, significantly prolonging the survival period of AML patients with IDH1 mutations[151] (Table 1 and Fig. 9).

Vorasidenib (AG881) is an effective, oral, blood−brain barrier permeable, dual inhibitor of mIDH1 and mIDH2, and is the first dual mIDH1/2 inhibitor reported so far[152]. Konteatis et al.[152] determined the crystal structure of the complex formed by $IDH1^{R132H}$ and $IDH2^{R140Q}$ homodimers and Vorasinib in combination with NADPH, with a resolution of 2.1 and 1.99 Å, respectively. This increases our understanding of double mutant suppression. In 2018, Ma et al.[153] resolved the crystal structures of $IDH1^{R132H}$/NADPH/AG881 and $IDH2^{R140Q}$/NADPH/AG881 complexes. In addition, in the orthotopic glioma mouse model, vorasinib can penetrate the brains of several preclinical species and greatly inhibit the production of D-2HG in glioma tissues[153]. Vorasidenib is currently in clinical development and has shown promising clinical activity in early clinical trials[154] (Table 1 and Fig. 9).

FT-2102 is an effective, brain-permeable, orally selective mIDH1 inhibitor, which can effectively inhibit the production of D-2HG in a xenograft model *in vivo*[155]. Caravella et al.[155] analyzed the crystal structure of FT-2102 coordinated with $mIDH1^{R132H}$. FT-2102 has brain permeability, so it can be used to treat mIDH1-driven central nervous system cancers. Currently, FT-2102 is undergoing clinical research in hematological malignancies, solid tumors, and gliomas with mIDH1[155]. In summary, two IDH1 inhibitors (Idhifa and Tibsovo) have been approved for marketing worldwide. In addition to being effective for acute myeloid leukemia, it is also expected to become a new targeted therapy for patients with IDH1 mutations in cholangiocarcinoma. More drugs targeting at IDH1 remain to be discovered (Fig. 9 and Table 1).

HMPL-306 is a new small molecule dual inhibitor of IDH1 and IDH2. At present, three international phase I clinical studies of HMPL-306, led by MD Anderson Cancer Center, have been launched for the treatment of patients with advanced solid tumors and malignant blood tumors. All patients will receive drug treatment in March 2021. The HMPL-306 structure has not been disclosed at present. Hehuang Pharmaceutical (Chi-Med) holds global ownership. HMPL-306 is expected to become the first IDH1/IDH2 dual-targeting inhibitor in the world[156] (Table 1).

PEPIDH1M is a peptide vaccine in phase I clinical trials at Duke University for the intradermal treatment of patients with IDH1 positive recurrent grade II glioma. PEPIDH1M vaccine is made up of a peptide that spans the mutated region of $IDH1^{R132H}$. The peptide is administrated with granulocyte macrophage colony stimulating factor mixed with montanide ISA51 (vaccine adjuvant). According to the research results released in March 2021, in the clinical trial of 24 people (ClinicalTrials.gov Identifier: NCT02193347), the vaccine had a great impact on the cardiovascular system, causing hypertension (100%) and anemia (83.3%). The main adverse reactions of the gastrointestinal tract were constipation (45.83%) and nausea (41.67%). In addition, it caused the increase of alanine aminotransferase and aspartate aminotransferase, with a probability of about 30%[157] (Table 1).

3.2. *IDH1 inhibitors in the preclinical study stage*

3.2.1. *IDH1 inhibitors based on phenyl-glycine scaffold*

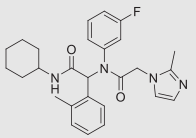
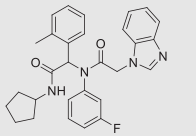
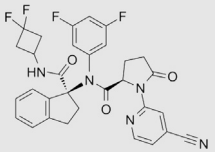
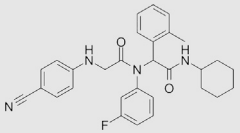
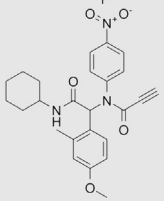
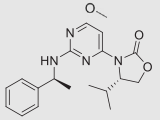
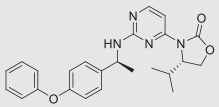
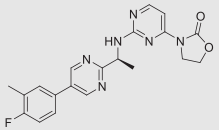
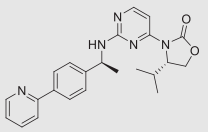
AGI-5198 is the first reported $IDH1^{R132H}$ inhibitor, showing strong D-2HG inhibition in tumor xenograft models[158]. Since high levels of D-2HG have been shown to change the epigenetic state and biology of cells, the utility of this molecule is very important to evaluate the biological consequences of IDH mutations and the potential of IDH inhibitors to treat IDH mutant tumors. In addition, it can induce the demethylation of histone H3K9me3 and the re-expression of genes related to differentiation[159]. ML309 is an effective inhibitor targeting $IDH1^{R132H}$, which can reduce the

Table 1 IDH1 inhibitors in the clinical research stage.

Code name	NCT No.	Condition	Clinical efficacy
AG-120 (ivosidenib) Launched, 2018	NCT02989857 (Phase III) NCT03839771 (Phase III) NCT03173248 (Phase III)	Cholangiocarcinoma; Gliomas; AML; Myelodysplastic syndrome	Cholangiocarcinoma[132]: mPFS: 6.9 months, SAE: 30%; Advanced cholangiocarcinoma with IDH1 mutation[134]: mOS: 10.3 months, SAE: 2%; Gliomas[135]: ORR: 2.9%, mPFS: 13.6 months; AML[128]: ORR: 41.6%, CRR: 21.6%.
AG-221 (enasidenib) Launched, 2017	NCT03839771 (Phase III) NCT02577406 (Phase III) NCT04822766 (Phase III)	Myeloid leukemia; AML; Myelodysplastic syndrome	AML[136]: ORR: 40.3%, mOS: 19.7 months; Myelodysplastic syndromes[137]: ORR: 53%, mOS: 16.9 months.
AG-881 (vorasidenib)	NCT04164901 (Phase III)	Glioma	Glioma[138]: ORR: 18%, mPFS: 36.8 months.
DS-1001b	NCT04458272 (Phase II) NCT05303519 (Phase II)	Glioma	Glioma[139]: ORR: 17%, mPFS: 10.4 months.
FT-2102 (olutasidenib)	NCT04013880 (Phase I/II) NCT03684811 (Phase I/II) NCT02719574 (Phase I/II)	AML; Myelodysplasia; Glioma; Cholangiocarcinoma	Glioma[140]: ORR: 48%.
BAY-1436032	NCT03127735 (Phase I) NCT02746081 (Phase I)	AML; Metastatic cancer; Glioma	AML[149]: ORR: 15%; Solid Tumors[141]: ORR: 11%.
IDH-305	NCT02977689 (Phase II) NCT02987010 (Phase II)	Glioma; AML; myelodysplastic syndrome	AML[142]: CR/CRi: 32%.
LSN-3410738 (LY-3410738)	NCT04521686 (Phase I) NCT04603001 (Phase I)	Metastatic cancer; AML; Chronic myelomonocytic leukemia; Myelodysplasia	–
HMPL-306	NCT04272957 (Phase I) NCT04764474 (Phase I) NCT04762602 (Phase I)	Leukemia; Myelodysplastic syndrome; Myeloid leukemia	–
IDH1RpepvaccH (vaccine)	NCT02771301 (Phase II)	Neurologic cancer Glioma	Glioma[143]: ORR: 84.4%, Three-year survival rate: 84%, TRAE: 90.6%, SAE: 3.1%.
PEPIDH1M (vaccine)	NCT02193347 (Phase I)	Glioma	–

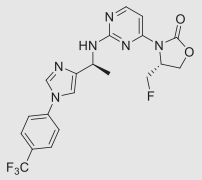
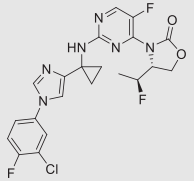
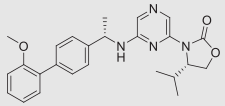
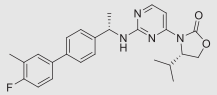
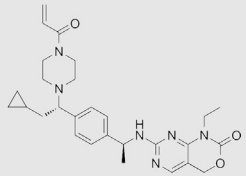
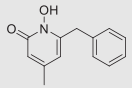
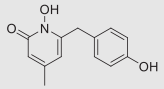
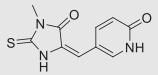
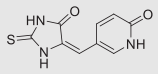
ORR, overall response rate; mOS, median overall survival; mPFS, median progression-free survival; SAE, serious adverse events; CRR, rate of complete remission; CR/Cri, complete remission/complete remission with incomplete recovery; TRAE, treatment related adverse events.

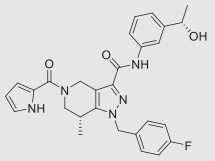
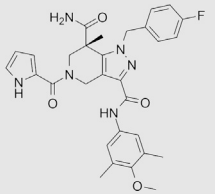
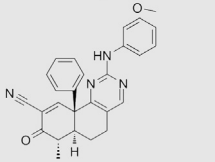
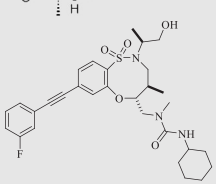
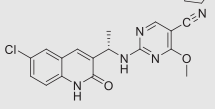
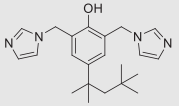
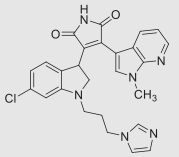
Table 2 Inhibitors targeting IDH1 in the preclinical study stage.

Compound	Structure	*In vitro* activity	*In vivo* activity/PK/ADME	Ref.
AGI-5198		$IDH1^{R132H}$ (enzyme): IC_{50} = 0.07 μmol/L; U87 (cell lines): IC_{50} = 0.07 μmol/L	U87 R132H tumor xenograft mouse model: 2-HG inhibition: 89.4% (BID) and 69% (Single)	158
ML309		$IDH1^{R132H}$ (enzyme): IC_{50} = 96 nmol/L; U87 MG cells: 2-HG assay: EC_{50} = 509 nmol/L	Male BALB/c nude mice: T_{max} = 1.0 h; C_{max} = 3625 ng/mL; $t_{1/2}$ = 3.76 h	160
(*R*)-1-(4-Cyanopyridin-2-yl)-*N*-((*S*)-1-((3,3-difluorocyclobutyl)carbamoyl)-2,3-dihydro-1*H*-inden-1-yl)-*N*-(3,5-difluorophenyl)-5-oxopyrrolidine-2-carboxamide **(6f)**		$IDH1^{R132H}$ (enzyme): IC_{50} = 45 nmol/L; $IDH1^{R132C}$ HT-1080 cells: 2-HG inhibition: IC_{50} < 5 nmol/L	–	161
IDH-C227		IC_{50} < 0.1 μmol/L against HT-1080 and 0.25 μmol/L against U87MG cells.	–	187
N-(2-(Cyclohexylamino)-1-(4-methoxy-2-methylphenyl)-2-oxoethyl)-*N*-(4-nitrophenyl)propiolamide **(43)**		$IDH1^{R132H}$ (enzyme): IC_{50} = 961.5 nmol/L $IDH1^{R132H}$ U87 cells 2-HG inhibition: EC_{50} = 208.6 nmol/L	–	162
IDH125		$IDH1^{R132H}$ (enzyme): IC_{50} = 0.22 μmol/L; $IDH1^{R132H}$ HCT116 cells 2-HG inhibition: IC_{50} = 0.66 μmol/L	–	163
IDH662		$IDH1^{R132H}$ (enzyme): IC_{50} = 0.01 μmol/L; $IDH1^{R132H}$ HCT116 cells 2-HG inhibition: IC_{50} = 0.022 μmol/L	Plasma protein binding >99%	163
IDH889		$IDH1^{R132H}$ (enzyme): IC_{50} = 0.02 μmol/L; $IDH1^{R132H}$ HCT116 cells: 2-HG inhibition: IC_{50} = 0.014 μmol/L	HCT116 R132H tumor xenograft mouse model (10 mg/kg, Oral) AUC: 3.6 μmol/(L·h), C_{max}: 1.7 μmol/L Brain/plasma ratio: 1.4	163
NI-1		$IDH1^{R132H}$ (enzyme): IC_{50} = 96 nmol/L	–	166

(*continued on next page*)

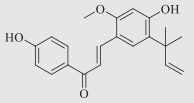
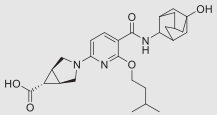
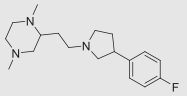
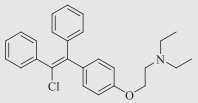
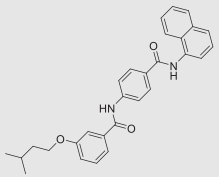
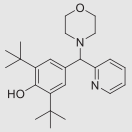
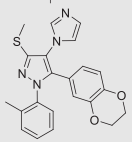
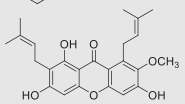
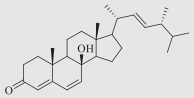
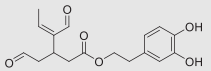
Table 2 (*continued*)

Compound	Structure	*In vitro* activity	*In vivo* activity/PK/ADME	Ref.
(*R*)-4-(Fluoromethyl)-3-(2-(((*S*)-1-(1-(4-(trifluoromethyl)phenyl)-1*H*-imidazole-4-yl)ethyl)amino)pyrimidin-4-yl) oxazolidin-2-one **(19)**		IDH1^{R132H} HCT116 cells: 2-HG inhibition: IC_{50} = 0.039 μmol/L	Rat liver microsomal (10 mg/kg, Oral) Cl_{int}: 7 μL/min/mg AUC: 180 μmol/(L·h)	164
(*R*)-3-(2-((1-(1-(3-Chloro-4-fluorophenyl)-1*H*-imidazole-4-yl)cyclopropyl)amino)-5-fluoropyrimidin-4-yl)-4-((*S*)-1-fluoroethyl)oxazolidin-2-one **(5t)**		IDH1^{R132H} (enzyme): IC_{50} = 35 nmol/L; IDH1^{R132H} HT-1080 cells 2-HG inhibition: IC_{50} = 18 nmol/L	Rat liver microsomal: Cl_{int}: 45 mL/min/g	165
(*S*)-4-Isopropyl-3-(6-(((*S*)-1-(2′-methoxy-[1,1′-biphenyl]-4-yl)ethyl)amino) pyrazin-2-yl)oxazolidin-2-one **(3g)**		2-HG levels (%, IDH1 R132H): 31.9% (10 μmol/L) 2-HG levels (%, IDH1 R132C): 40.6% (10 μmol/L)	PAMPA-BBB assay[a]: Permeability ($\times 10^{-6}$ cm/s): 6.65 ± 0.42	166
I-8		IDH1^{R132H} (enzyme): IC_{50} = 135.6 ± 17.9 nmol/L IDH1^{R132C} (enzyme): IC_{50} = 174.2 ± 22.1 μmol/L	HT-1080 R132C tumor xenograft BALB/c mice 150 mg/kg could induce 30% inhibitory of 2-HG production	167
IDH1-IN-6		IDH1^{R132H} (enzyme): IC_{50} = 6.27 nmol/L; IDH1^{R132C} (enzyme): IC_{50} = 36.9 nmol/L; IDH1^{R132C} HT-1080 cells: 2-HG inhibition: IC_{50} = 1.28 nmol/L	–	168
SYC-435 (Compound **2**)		IDH1^{R132H} (enzyme): *Ki* = 0.19 μmol/L; IDH1^{R132C} (enzyme): *Ki* = 0.12 μmol/L	–	169
1-Hydroxy-6-(4-hydroxybenzyl)-4-methylpyridin-2(1*H*)-one (**3**)		IDH1^{R132H} (enzyme): *Ki* = 0.28 μmol/L; IDH1^{R132C} (enzyme): *Ki* = 0.27 μmol/L	–	169
Thiohydantoin16 (**16**)		IDH1^{R132H} (enzyme): *Ki* = 0.75 μmol/L; IDH1^{R132C} (enzyme): *Ki* = 1.2 μmol/L	–	171
(*E*)-5-((5-oxo-2-Thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1*H*)-one **(18)**		IDH1^{R132H} (enzyme): *Ki* = 0.42 μmol/L; IDH1^{R132C} (enzyme): *Ki* = 2.3 μmol/L	–	171

GSK321		HT-1080 cells: 2-HG inhibition: EC_{50} = 85 nmol/L; $IDH1^{R132H}$ (enzyme): IC_{50} = 4.6 nmol/L; $IDH1^{R132C}$ (enzyme): IC_{50} = 3.9 nmol/L; $IDH1^{R132G}$ (enzyme): IC_{50} = 2.9 nmol/L	2-HG inhibition: ($IDH1^{R132C}$ HT-1080 fibrosarcoma cells): EC_{50} = 320 nmol/L	172
GSK864		$IDH1^{R132H}$ (enzyme): IC_{50} = 15.2 nmol/L; $IDH1^{R132C}$ (enzyme): IC_{50} = 8.8 nmol/L; $IDH1^{R132G}$ (enzyme): IC_{50} = 16.6 nmol/L	–	172
(6a*S*,7*S*,10a*R*)-7-Methyl-8-oxo-10a-phenyl-2-(phenylamino)-5,6,6a,7,8,10a-hexahydrobenzo[*h*]quinazoline-9-carbonitrile (**1**)		$IDH1^{WT}$ (enzyme): IC_{50} = 410 nmol/L	–	172
BRD2879		$IDH1^{R132H}$ (enzyme): IC_{50} = 50 nmol/L; $IDH1^{R132C}$ (enzyme): IC_{50} = 2.5 μmol/L	Human plasma protein binding: 99.5%	173
(*S*)-2-((1-(6-Chloro-2-oxo-1,2-dihydroquinolin-3-yl)ethyl)amino)-4-methoxypyrimidine-5-carbonitrile (**63**)		$IDH1^{R132H}$ (enzyme): IC_{50} = 18 nmol/L; $IDH1^{R132C}$ (enzyme): IC_{50} = 130 nmol/L; $IDH1^{R132H}$ HCT116 cells: 2-HG inhibition: IC_{50} = 45 nmol/L; $IDH1^{R132C}$ HCT116 cells: 2-HG inhibition: IC_{50} = 233 nmol/L	HCT116 R132H/R132C xenograft bearing female BALB/c nude mice: 2-HG inhibition: IC_{50} = 49 nmol/L (R132H); IC_{50} = 46 nmol/L (R132C).	174
Bisimidazole 3		$IDH1^{R132H}$ (enzyme): IC_{50} = 13 ± 5 nmol/L; $IDH1^{R132H}$ HEK-293 cells: 2-HG inhibition: IC_{50} = 81.5 nmol/L	–	175
3-(1-(3-(1*H*-Imidazole-1-yl)propyl)-6-chloroindolin-3-yl)-4-(1-methyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)-1*H*-pyrrole-2,5-dione (**11e**)		$IDH1^{R132H}$ (enzyme): IC_{50} = 0.16 ± 0.04 μmol/L	–	176

(*continued on next page*)

Table 2 (*continued*)

Compound	Structure	*In vitro* activity	*In vivo* activity/PK/ADME	Ref.
Licochalcone A		$IDH1^{R132H}$ (enzyme): IC_{50} = 76.87 μmol/L; $IDH1^{R132C}$ (enzyme): IC_{50} = 5.176 μmol/L	–	165
CRUK-MI 20a (**20a**)		$IDH1^{WT}$ (enzyme): IC_{50} = 0.27 ± 0.22 μmol/L	–	177
HMS-101		$IDH1^{R132H}$ (enzyme): IC_{50} = 5 μmol/L; $IDH1^{R132C}$ (enzyme): IC_{50} = 4 μmol/L	–	178
Clomifene		$IDH1^{R132H}$ (enzyme): IC_{50} = 50.20 ± 0.12 μmol/L; $IDH1^{R132C}$ (enzyme): IC_{50} = 42.33 ± 0.31 μmol/L; $IDH1^{R132H}$ HT-1080 cells: 2-HG inhibition: IC_{50} = 37.86 ± 0.32 μmol/L	HT-1080 R132H tumor xenograft mouse model: 100 mg/kg Clomifene could induce 57.38% inhibitory of D-2HG production.	179
ZX06		2-HG levels (%, IDH1 R132H): 54.9% (10 μmol/L); 2-HG levels (%, IDH1 R132C): 48.4% (10 μmol/L)	PAMPA-BBB assay: Permeability ($\times 10^{-6}$ cm/s): 8.15 ± 0.29	180
DC_H31		$IDH1^{R132H}$ (enzyme): IC_{50} = 0.41 μmol/L; $IDH1^{R132C}$ (enzyme): IC_{50} = 2.7 μmol/L	–	181
KRC-09		$IDH1^{R132H}$ (enzyme): IC_{50} = 76.87 μmol/L; 2-HG levels: (IDH1 R132H): 45% (50 μmol/L)	–	182
α-Mangostin		$IDH1^{R132H}$ (enzyme): K_i = 2.85 μmol/L	–	183
(8*R*,10*R*,13*R*)-17-((2*R*,5*R*,*E*)-5,6-Dimethylhept-3-en-2-yl)-8-hydroxy-10,13-dimethyl-1,2,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*] phenanthren-3-one (**3**)		$IDH1^{R132H}$ HT-1080 cells: 2-HG inhibition: IC_{50} = 35.97 μmol/L	–	184
DOA		$IDH1^{R132H}$ (enzyme): IC_{50} = 12 μmol/L	–	185

[a]PAMPA-BBB: blood–brain barrier specific parallel artificial membrane permeability assay.

Table 3 The combination therapy of IDH1 inhibitors and other targeted drugs.

IDH1 inhibitor	Combined drug	NCT No.	Condition	Conclusion/objective
Ivosidenib (AG-120)	Azacytidine (DNMT1 inhibitor)	NCT03173248 (Phase III)	AML	Recovery of blood counts; rates of febrile neutropenia and infections were reduced
Ivosidenib (AG-120)	Azacytidine (DNMT1 inhibitor)	NCT03503409 (Phase II)	AML; Myelodysplasia	Evaluate the efficacy and safety in patient with IDH1 mutation-positive myelodysplastic syndrome
Ivosidenib (AG-120)	Nivolumab (anti-PD-1)	NCT04056910 (Phase II)	Metastatic cancer; Glioma	Evaluate response to treatment, survival and safety events
Ivosidenib (AG-120)	Nivolumab (anti-PD-1)	NCT04044209 (Phase II)	AML; Myelodysplasia	Evaluate safety and efficacy
Ivosidenib (AG-120)	Enasidenib (IDH2 inhibitor)	NCT02632708 (Phase I)	AML	Decrease in plasma and bone marrow 2-HG concentrations; CCR: 18.4%
Ivosidenib (AG-120)	Enasidenib (IDH2 inhibitor)	NCT03839771 (Phase III)	AML; Myelodysplasia	Evaluate safety and efficacy
Ivosidenib (AG-120)	Enasidenib (IDH2 inhibitor)	NCT02677922 (Phase I/II)	AML	Treatment-emergent adverse events (TEAEs) including nausea, anemia and thrombocytopenia were reported
Ivosidenib (AG-120)	Vorasidenib (AG-881)	NCT03343197 (Phase I)	Glioma	Activation of IFN signaling and increased T-cell infiltration was observed
Ivosidenib (AG-120)	Venetoclax (Bcl-2 inhibitor)	NCT03471260 (Phase I/II)	AML; Hematologic cancer; Myelodysplasia; Myeloproliferative diseases	Evaluate the safety and efficacy
Ivosidenib (AG-120)	Itraconazole (Hedgehog inhibitor)	NCT02831972 (Phase I)	Healthy volunteers	Ivosidenib alone or with itraconazole were well-tolerated with similar favorable safety profiles
Ivosidenib (AG-120)	Enasidenib (IDH2 inhibitor) Fedratinib (JAK2 inhibitor)	NCT04955938 (Phase 1b)	Chronic myeloid leukemia; Myelodysplasia; Myelofibrosis; Polycythemia vera; Thrombocythemia	Evaluate the safety and efficacy
Ivosidenib (AG-120)	Azacytidine (DNMT1 inhibitor) Venetoclax (Bcl-2 Inhibitor)	NCI-2018-00921 (Phase I/II)	Myeloid leukemia	One-year overall survival were 75%, 50% and 100% in newly diagnosed AML, relapsed/refractory (R/R) AML and myelodysplatic syndrome, CRR: 67%
Ivosidenib (AG-120)	Enasidenib (IDH2 inhibitor) Azacytidine (DNMT1 inhibitor)	NCT02677922 (Phase I/II)	AML; Myelodysplasia; Myeloproliferative diseases	ORR: 78%
Enasidenib (AG-221)	Azacytidine (DNMT1 inhibitor)	NCT02677922 (Phase I/II)	AML	TRAE: 44%
Enasidenib (AG-221)	Azacytidine (DNMT1 inhibitor)	NCT03683433 (Phase II)	AML	TEAE: 85%
Enasidenib (AG-221)	Azacytidine (DNMT1 inhibitor)	NCT03383575 (Phase II)	AML	All patients reported leukocytosis.
Enasidenib (AG-221)	Azacytidine (DNMT1 inhibitor)	AG221-AML-005 (Phase I/II)	AML	mOS remained unchanged (22.0 months)
Enasidenib (AG-221)	Azacytidine (DNMT1 inhibitor)	NCT03013998 (Phase II)	AML	Low early death rate; High CR/CRi: 47%; yielded durable remissions

(*continued on next page*)

Table 3 (*continued*)

IDH1 inhibitor	Combined drug	NCT No.	Condition	Conclusion/objective
Enasidenib (AG-221)	Azacytidine (DNMT1 inhibitor)	NCT03683433 (Phase II)	AML; Chronic myelomonocytic leukemia; Myelodysplasia	Evaluate the clinical activity of enasidenib in combination with azacitidine for patients with relapsed/refractory acute myeloid leukemia
Enasidenib (AG-221)	Cobimetinib (MEK1 inhibitor)	NCT05441514 (Phase Ib)	AML	Evaluate the efficacy and safety
Enasidenib (AG-221)	Venetoclax (Bcl-2 inhibitor)	19-5939 (Phase I/II)	AML; Myelodysplasia; Myeloproliferative diseases	Evaluate the safety, tolerability, efficacy, and best dose of venetoclax administered in combination with enasidenib in patients with blood cancers
Enasidenib (AG-221)	Daunorubicin (topoisomerase II) Cytarabine (DNA polymerase)	NCT03825796 (Phase II)	AML	Evaluate the efficacy and safety
Enasidenib (AG-221)	Azacytidine (DNMT1 inhibitor) Venetoclax (Bcl-2 inhibitor)	NCT03683433 (Phase II)	AML	6-month OS was 70%; CR/CRi: 100% (ND AML); CR/CRi: 58% (R/R AML)
Olutasidenib (FT-2102)	Cedazuridine (cytidine deaminase (CDA) inhibitor) Decitabine (deoxycytidine analog antimetabolite and a DNA methyltransferase inhibitor)	NCT04013880 (Phase I/II)	AML; Myelodysplasia	Evaluate the efficacy and safety
Olutasidenib (FT-2102)	Azacytidine (DNMT1 inhibitor)	NCT03684811 (Phase Ib/II)	Glioma	Dose-limiting toxicities ($\geq$grade 3 transaminase elevations) were noted in combination group, meeting stopping criteria
Olutasidenib (FT-2102)	Azacytidine (DNMT1 inhibitor)	NCT02719574 (Phase I/II)	AML; Myelodysplasia	mOS: 37.7 (monotherapy) versus 52.5 (combination therapy) weeks
Vorasidenib (AG-881)	Omeprazole (PPI)	NCT04128787 (Phase I)	Healthy volunteers	Evaluate the safety and tolerability
Vorasidenib (AG-881)	Lamotrigine (anticonvulsant agent)	NCT04015687 (Phase I)	Healthy volunteers	Evaluate the safety and pharmacokinetics
IDH1RpepvaccH (vaccine)	Avelumab (anti-PD-L1)	NCT03893903 (Phase I)	Glioma	Evaluate safety, tolerability and immunogenicity
IDH1RpepvaccH (vaccine)	Temozolomide (DNA alkylating)	NCT02454634 (Phase I)	Astrocytoma; Glioma; Oligodendroglioma	TRAE: 90.6%
PEPIDH1M (vaccine)	Temozolomide (DNA alkylating)	NCT02193347 (Phase I)	Glioma	Evaluate the safety

ND, new diagnosis; ORR, overall response rate; mOS, median overall survival; CRR, rate of complete remission; CR/Cri, complete remission/complete remission with incomplete recovery; TRAE, treatment related adverse events; TEAEs, treatment-emergent adverse events.

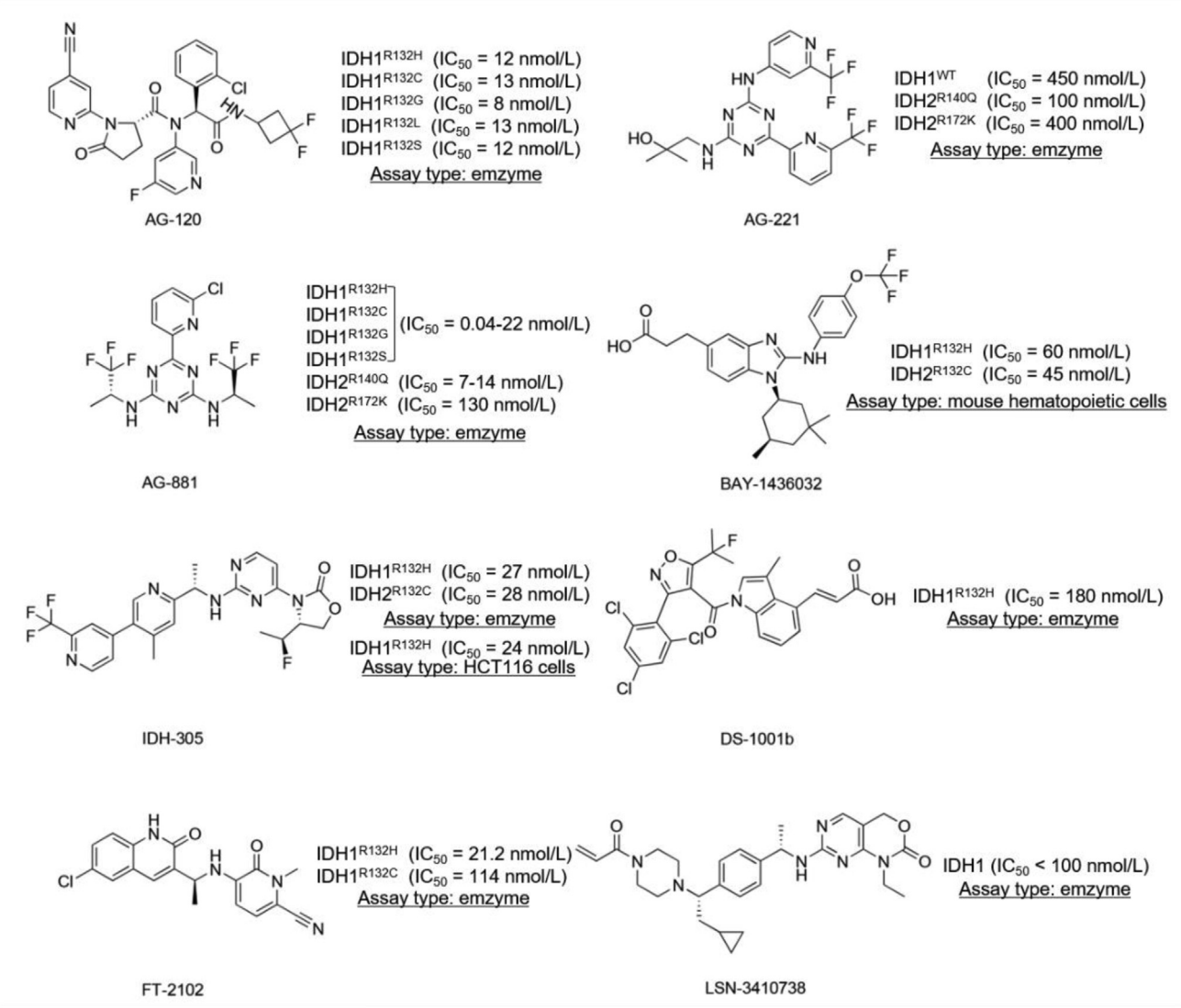


Figure 9 Structures of IDH1 inhibitor in the clinical trial stage. Assay type and activity data are displayed on the right side of the compound's structure.

production of D-2HG in U87MG glioblastoma cells[160]. ML309 possesses a good *in vitro* ADME and *in vivo* PK profile[160]. But it has no obvious blood−brain barrier penetration ability in healthy mice[160]. AGI-5198 and ML309 are $IDH1^{R132H}$ inhibitors obtained by hit compound optimization. They have the same phenyl glycine scaffold, but they are synthesized by different routes. AGI-5198 adopted the Ugi reaction, which greatly reduced the difficulty of synthesis. A detailed structure−activity relationship analysis was carried out in the study of ML309. The above methods and conclusion will provide valuable information for the research of IDH1 inhibitors based on this core structure. AG-120 was designed and synthesized based on this scaffold. After that, a series of new IDH1 inhibitors were obtained by optimizing AG-120 through a reasonable structure-based design. Compound **6F** is of excellent cellular potency ($IC_{50} < 10$ nmol/L) and also shows selectivity for wild-type IDH1 (79-fold) and mutant $IDH2^{R140Q}$ (>2000-fold). Pharmacokinetic studies showed low clearance and high bioavailability (>30%)[161]. Compound **43** also derives from the optimization of AG-120, but its activity still needs further optimization[162] (Table 2).

3.2.2. IDH1 inhibitors based on 3-pyrimidin-4-yl-oxazolidin-2-one scaffold

IDH125 was identified as a potential $IDH1^{R132H}$ inhibitor by high-throughput screening and pharmacochemical methods ($IDH1^{R132H}$ enzyme inhibition assay: $IC_{50} = 0.22$ μmol/L). To obtain highly potent compounds, 20 compounds were synthesized. Among them, IDH662 and IDH889 are the compounds with the best *in vitro* activity. However, IDH662 has a high plasma protein binding rate of 99%, which limits its *in vivo* activity. In the HCT116 $IDH1^{R132H}$ tumor xenograft model, IDH889 can significantly reduce D-2HG[163]. It is noteworthy that, in addition to the possible treatment of AML, chondrosarcoma, cholangiocarcinoma, and other forms of mutant IDH1-driven cancers, IDH889 also shows brain penetration exposure[163], suggesting its potential in the treatment of IDH1 mutant brain cancer patients. IDH305 was further optimized by IDH889. It is currently in phase II clinical trials for the treatment of glioma and AML. In addition, compound **19** was also obtained based on IDH889 optimization[164]. Through rational design based on structure, Zheng et al.[165] discovered and optimized imidazole cyclopropyl amide analogs.

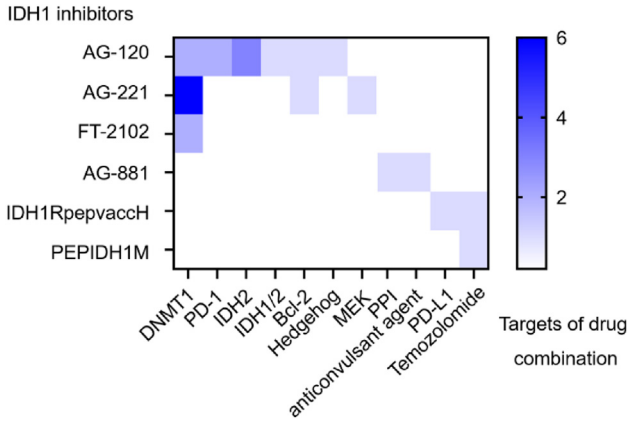


Figure 10 Combined application of IDH1 inhibitors and other target drugs in the clinical research. Different IDH1 inhibitors are listed on the left side of the heatmap. Targets of drug combination are located below the heat map. The color depth represents the number of clinical experiments.

The best compound **5t** can effectively inhibit the activity of $IDH1^{R132H}$, reduce the production of D-2HG, and has moderate liver microsome stability and PK characteristics[165]. Although the efficacy of compound **5t** is twice that of IDH305, the oral exposure of compound **5t** is not enough for an efficacy study. More efforts will be made to improve *in vivo* exposure to expand its further development. Ma et al.[166] synthesized a series of mIDH1 inhibitors containing the backbone of 3-pyrazine-2-yl-oxazolidin-2-one. Further evaluation found that compound **3g** and the positive drug NI-1 have similar inhibitory activity, and neither concentration of compound **3g** shows significant toxicity[166]. Compound **3g** has a strong inhibitory effect on $IDH1^{132H}$ and $IDH1^{R132C}$, and higher selectivity for $IDH1^{WT}$. In addition, compound **3g** shows a good ability to penetrate the blood−brain barrier[166]. These findings indicate that compound **3g** is worthy of further optimization, looking for inhibitors with lower toxicity for the treatment of IDH1 mutant brain cancer patients. Compound I-8 specifically inhibits the production of D-2HG in IDH1 mutant cells, reduces histone methylation levels, and induces differentiation[167]. In addition, I-8 can also be taken orally[167]. IDH1-IN-6 is a potent, selective, and orally active mutant isocitrate dehydrogenase (IDH) inhibitor with IC_{50} of 6.27 and 3.71 nmol/L for $IDH1^{R132H}$ and $IDH1^{R132C}$, respectively. IN-6 is less active on inhibiting the IDH wild-type enzymes. IN-6 inhibits the production of D-2HG in HT-1080 cells with an IC_{50} of 1.28 nmol/L, indicating the inhibition of mutant $IDHl^{R132C}$ in cells[168] (Table 2).

3.2.3. IDH1 inhibitors based on 1-hydroxypyridin-2-one scaffold

Zheng et al.[169] discovered two 1-hydroxypyridine-2-one (compounds **2** and **3**), which are effective inhibitors of $IDH1^{R132H}$ and $IDH1^{R132C}$, with K_i values as low as 120 nmol/L. These compounds can inhibit the production of D-2HG in IDH1 mutant cells[169]. They are 60 times more selective than $IDH1^{WT}$ and are not cytotoxic to human cells. The researchers also determined the X-ray structure of $IDH1^{R132H}$ that forms a complex with compound **2** or **3**, revealing the exact combination of these two compounds and the structural basis for high selectivity. The crystal structure shows that these inhibitors bind with $IDH1^{R132H}$ through hydrogen bonds, electrostatic and hydrophobic interactions[169]. Liu et al.[170] reported the discovery, design, synthesis, and structure−activity relationship of a series of 1-hydroxypyridine-2-one type compounds against IDH1 mutants. 1-Hydroxypyridine-2-one type compounds **4** and **7** are low micromolar inhibitors of $IDH1^{R132H}$. Under the guidance of structure−activity relationship and the X-ray structure of the $IDH1^{R132H}$/IDH2 complex, Liu et al.[170] designed and synthesized 61 derivatives, of which several effective inhibitors have K_i values of 140−270 nmol/L. 1-Hydroxypyridine-2-one type inhibitors have blood−brain barrier permeability[170]. It is necessary to further develop this type of inhibitors (Table 2).

3.2.4. IDH1 inhibitors based on 2-thiohydantoins scaffold

Wu et al.[171] synthesized a series of novel 2-thiohydantoins and related compounds. These compounds can reduce the cell concentration of D-2HG in BT142 gliomas with $IDH1^{R132H}$ mutation, reduce histone methylation levels, and selectively inhibit the self-renewal ability of glioma stem-like cells with $IDH1^{R132H}$ mutation[171]. The author also analyzed the crystal structure of the complex formed by $IDH1^{R132H}$ and compound **16** or **22**, showing the inhibitor−protein interaction, and laying the foundation for further structure-based inhibitor design[171]. In addition, the most effective inhibitor **18** is a competitive inhibitor relative to α-KG, which exerts a non-competitive mode of action on NADPH[171]. These compounds are new chemical probes for studying IDH1 mutations in cancer and provide new scaffolds for drug discovery against IDH1 mutation cancers (Table 2).

3.2.5. IDH1 inhibitors based on tetrahydropyrazolopyridine scaffold

Okoye-Okafor et al.[172] analyzed the crystal structure of GSK321 bound to $IDH1^{R132H}$ homodimer in the presence of $NADP^+$. The results of crystal and biochemical studies indicate that GSK321 binds to the allosteric site of IDH1, making the enzyme in a catalytically inactive conformation[172]. GSK321 stably reduced the production of D-2HG in several different IDH1 mutant AML cells[172]. It induces the differentiation of granulocytes and can reverse the methylation of DNA cytosine[172]. In addition, GSK864 also has nanomolar inhibitory activity against IDH1 mutants, showing that tetrahydropyrazolopyridine scaffold is of great research value. Compound **1** is a hit compound obtained by high-throughput screening together with GSK321. It shows inhibitory activity against $IDH1^{WT}$ with an IC_{50} value of 410 nmol/L. Three compounds **11**, **13**, and **15** with better activity were obtained through further structure optimization[172]. The above results provide a very potential core structure for the development of IDH1 inhibitors (Table 2).

3.2.6. IDH1 inhibitors based on 8-membered ring sulfonamides scaffold

BRD2879 inhibits the production of D-2HG in cells without significant toxicity[173]. However, the high molecular weight, lipophilicity, and low solubility of the specific inhibitor BRD2879 limit its use *in vivo*. BRD2879 represents a new structural class of mutant IDH1 inhibitors that, with optimization, may prove useful in the study of this enzyme and its role in cancer (Table 2).

3.2.7. IDH1 inhibitors based on quinolinone scaffold

Lin et al.[174] discovered and optimized a series of quinolinones. Through rational design based on the structure, the researchers identified compound **63**. It can effectively inhibit IDH1 mutants R132H, R132C, R132G, and R132L[174] with good cell permeability, ADME/PK properties, and oral bioavailability. In the

HCT116-IDH1^{R132H} or HCT116-IDH1^{R132C} xenograft BALB/c nude mice model, compound **63** can significantly reduce the level of D-2HG[174]. Preclinical studies have shown that compound **63** may have the potential to treat GBM, AML, or other forms of mIDH1-driven cancer (Table 2).

3.2.8. Other IDH1 inhibitors

3.2.8.1. IDH1 inhibitors with other core structures. Bisimidazole 3 (bisimidazoline scaffold) non-competitively inhibits IDH1^{R132H} relative to NADPH and α-KG and the production of D-2HG in cells[175]. Studies have shown that bisimidazole 3 selectively inhibits IDH1 mutations by binding to allosteric sites, and the inhibition is competitive with Mg^{2+} [175]. Hu et al.[176] designed and synthesized a series of 3-(7-azaindolyl)-4-indolyl maleimides. Among them, **11a**, **11c**, **11e**, **11g**, and **11s** showed a good inhibitory effect on IDH1^{R132H} with high selectivity for wild-type IDH1[176]. Compounds **11a**, **11c**, **11e**, **11g**, and **11s** can effectively inhibit the production of D-2HG in U87MG cells expressing IDH1^{R132H} [176]. Their research provides new information for the design of new IDH1^{R132H} inhibitors.

Licochalcone A (imidazole cyclopropyl amine) is a selective inhibitor of IDH1^{R132C} with IC_{50} value of 5.176 μmol/L[186]. Compared with the R132C mutation, the R132H mutation is not conducive to the binding of licochalcone A to the IDH1 protein[186]. Licochalcone A can induce apoptosis and cell cycle arrest in HT-1080 cells[186]. Jones et al.[177] obtained a series of inhibitors targeting IDH1^{R132H} through high-throughput screening. Among them, compound **20a** promoted the differentiation of human IDH1^{R132H} AML cells derived from patients, but did not promote differentiation in IDH1 wild-type AML cells[177]. In addition, the researchers also clarified the crystal structure of the complex formed by IDH1^{R132H} and compound **20a**, and the position of the previously unresolved protein loop can be observed[177]. More complete structure lays the foundation for the future development of IDH1^{R132H} inhibitors. HMS-101 binds to the active site of mutant IDH1, inhibits cell proliferation, and induces differentiation of IDH1 mutant leukemia cells[178]. HMS-101 can also inhibit the production of D-2HG in syngeneic mutant IDH1 mouse models and human AML xenograft models in patients[178]. In addition, in cells treated with HMS-101, differentiation-related transcription factors CEBPA and PU.1 were significantly increased, while cell cycle regulator cyclin A2 decreased[178]. Besides, it also weakens the hypermethylation of histones[178]. This study provides clinical evidence for the further development of IDH1 mutant competitive inhibitors and the treatment of IDH1 mutant AML patients (Table 2).

3.2.8.2. IDH1 hit compounds found based on virtual screening. Virtual screening technology provides a fast and economical method for discovering new active substances by selecting compounds in a large database for screening. This method also provides many potential hit compounds for the discovery of IDH1 inhibitors. Through virtual screening, it was found that clomiphene can selectively inhibit the activity of IDH1^{R132H} in a non-competitive manner and through an allosteric inhibition mechanism[179]. *In vivo* studies have shown that oral clomiphene can significantly inhibit tumor growth in HT-1080-bearing CB-17/Icr-scid mice[179]. An in-depth study of mechanism of clomiphene has great benefits for the treatment of many patients with glioma or AML. Zou et al.[180] obtained 7 compounds through virtual screening based on cross-docking. They have a moderate mIDH1 inhibitory effect, of which ZX06 is the most effective and safest[180]. ZX06 can also penetrate the blood−brain barrier[180]. Therefore, ZX06 should be used as a lead compound to be further optimized for the treatment of IDH1 mutant brain cancer patients. In addition, this new virtual screening strategy should be further optimized to introduce more novel scaffold mIDH1 inhibitors. DC_H31 is a new type of IDH1$^{R132H/C}$ inhibitor, which acts through an allosteric mechanism[181]. At the cellular level, DC_H31 can inhibit cell proliferation in HT-1080 cells, promote cell differentiation, and reduce the production of D-2HG[181]. *In vivo* and *in vitro*, DC_H31 can promote the development of more effective pan-inhibitors against IDH1$^{R132H/C}$ through further structural optimization. KRC-09 can effectively inhibit the activity of IDH1^{R132H} mutants and reduce the concentration of D-2HG in the U-87 MG cell line containing IDH1^{R132H} [182]. Although it is not as good as known inhibitors (such as AGI-5198), it has a novel scaffold that provides an idea for the future development of effective IDH1^{R132H} inhibitors (Table 2).

3.2.8.3. Natural inhibitors of IDH1. α-Mangostin, a new selective inhibitor of IDH1^{R132H}, competitively inhibits the binding of α-KG to IDH1^{R132H}, but has no inhibitory effect on IDH1[183]. It can selectively promote the demethylation of 5-methylcytosine and histone H3 trimethylated lysine residues in IDH1^{R132H} MCF10A cells[183]. It should be noted that there is no structural similarity between α-mangostin and the previous IDH1^{R132H} selective inhibitors. Through structure-based virtual screening, it was found that compound **3** inhibited the mutant IDH1 in a non-competitive manner[184]. Compound **3** treatment can reduce the concentration of D-2HG in HT-1080 cells and reduce the level of histone H3K9me3 methylation[184]. Compound **3** may be the lead compound for anticancer drug candidates. The decarboxymethyl oleuropein aglycone (DOA) present in extra-virgin olive oil (EVOO) can specifically inhibit IDH1^{R132H} and reduce the production of D-2HG[185]. DOA can restore the activity of histone demethylase inhibited by D-2HG, and also restore the expression of PD-L1 epigenetics[185]. This study shows that phenolic compounds in olive oil are inhibitors of IDH1 mutant and can be used as scaffolds for drug discovery (Table 2).

3.3. The combination therapy of IDH1 inhibitors and other targeted drugs

Cancer cells often upregulate different growth-promoting factors, which can act independently or interact in cells through signal networks. Cancer cells can easily acquire drug resistance by upregulating alternative factors or converting other signal pathways that promote proliferation. Therefore, there are lots of limitations in the treatment targeting only a single target. To overcome the shortcomings of single target drugs, it has become a recognized method to combine two (or more) different targets related to cancer development to achieve synergistic anticancer efficacy. At present, there are 31 IDH1 combined drug projects in the clinical research stage. AG-120 and AG-221 have the largest number of combined drug studies, accounting for 74.2% of all studies. Among them, AG-120 has the largest number of combined targets, and AG-221 and Azacitidine (DNMT1 inhibitor) are studied the most widely. In addition, there were 6 experiments (19.4%) in which the three drugs were combined for treating cancer (Fig. 10).

3.3.1. Combination of IDH1 inhibitors and DNMT1 inhibitors

The combination of IDH1 inhibitors and DNMT1 inhibitor (azacitidine) is the most common. The clinical trial results showed that AG-120 combined with azacitidine significantly improved the

event-free survival (EFS, 33%) of patients compared with placebo and azacitidine group in the treatment of IDH1 mutant acute myeloid leukemia. The median overall survival (mOS) was 24.0 months with AG-120 and azacitidine, but only 7.9 months with placebo and azacitidine. More importantly, the rate of grade 3 and higher-level adverse events in this combined drug strategy was lower than that in the control group, 28% *vs*. 34%[187]. In addition, the combined application of FT-2102 and azacitidine also showed good clinical effects on the treatment of acute mycoid leukemia, mOS: 37.7 (monotherapy) *vs*. 52.5 (combination therapy) weeks, but this combined application needs to adjust the dosage to eliminate the toxicity of dose limitation. In the clinical trial of AG-221 combined with azacitidine, it is necessary to further consider how to reduce treatment-emergent adverse events to achieve higher safety (Table 3).

3.3.2. Combination of IDH1 inhibitors and anti-PD-1/anti-PD-L1

Since programmed cell death protein 1 (PD-1) antibody did not prolong the survival of glioblastoma patients in phase III clinical study, in order to further explore effective immunotherapy strategies, the researchers analyzed the immune regulatory targets. The results showed that adenosine a2a receptor/CD73/CD39 (cluster of differentiation, CD) pathway had the highest expression frequency in glioma patients, followed by the PD-1 pathway. Mechanism studies have shown that in IDH1 mutant glioma patients, D-2HG can upregulate CD73 expression on immune cells[188]. These results explain the phase III clinical results of PD-1 antibody to a certain extent, and also provide a basis for the combined application of IDH1 and PD-1/PD-L1 antibodies. At present, three studies on the combination of IDH1 inhibitors and PD-1 (including PD-L1) antibodies are conducting safety evaluation, which may provide a new and effective means for glioma immunotherap (Table 3).

3.3.3. Combination of IDH1 and IDH2 inhibitors

The mutual conversion of IDH1 and IDH2 is one of the mechanisms of acquired drug resistance caused by IDH1 or IDH2 inhibitor single drug application[126], which makes the combination of IDH1 and IDH2 inhibitors have great therapeutic potential. The most common combination of IDH1 and IDH2 inhibitors is AG-120 and AG-221, as well as AG-120 and AG-881. In the three clinical studies of AG-120 and AG-221, the subjects were all patients with acute myeloid leukemia. The results showed that the combination of the two drugs could reduce the concentration of D-2HG in bone marrow and plasma, and the rate of complete remission was 18.4%. However, treatment-emergent adverse events (TEAEs) including nausea, anemia, and thrombocytopenia were reported. NCT03343197 is a phase I clinical study with glioma patients as the research object. After AG-120 and AG-881 were administrated in combination, activation of interferon signaling and increased T-cell infection were observed. Some studies have pointed out that differentiation syndrome is a serious adverse reaction of IDH inhibitors ivosidenib and enasidenib in patients with IDH1 and IDH2-mutated AML, respectively[189]. Therefore, during the combined application of IDH1 and IDH2 inhibitors, we need to pay high attention to this serious drug-related adverse reaction (Table 3).

3.3.4. Combination of IDH1 inhibitors and Bcl-2 inhibitors

AML is a highly heterogeneous disease, and multiple factors can affect the prognosis, including age, cytogenetic abnormalities, and molecular genetic abnormalities (IDH1/2, nucleophosmin 1, p53, and Fms-like tyrosine kinase 3). The over-expression of Bcl-2 is associated with the formation of drug resistance. Through large-scale RNAi screening, some researchers found that in acute myeloid leukemia, $IDH1^{R132H}$ mutation has a strong dependence on Bcl-2, an anti-apoptotic gene. Treatment with Venetoclax (ABT-199), a specific inhibitor of Bcl-2, will lead to more apoptosis of cells containing $IDH1^{R132H}$ mutation. This research result indicated that patients with acute myeloid leukemia carrying IDH1/2 mutations may respond to Bcl-2 inhibitors, which provides a clear molecular basis for the combined application of drugs that block the activity of mitochondrial electron transport chain in the treatment of AML[48]. At present, the clinical study of ABT-199, AG-120 and azacytidine show great therapeutic potential, one-year overall survival is 75%, 50%, and 100% in newly diagnosed AML, relapsed/refractory AML, and myelodysplastic syndrome, respectively, and rate of complete remission is 67% (Table 3).

3.3.5. Combination of IDH1 inhibitors and other target inhibitors

Itraconazole (R51211) is a triazole antifungal agent and an effective oral active hedgehog signal pathway antagonist with an IC_{50} about 800 nmol/L, which has anticancer and antiangiogenic effects[190]. At present, the combined application of AG-120 and itraconazole has shown good tolerance in healthy volunteers. The combined application of other target inhibitors such as cobimetinib (MEK1 inhibitor), omeprazole (proton pump inhibitor), lamotrigine (anticonvulsant agent), temozolomide (DNA alkylating), and corresponding IDH1 inhibitors is mostly at the stage of safety evaluation. These combinations will expand the application of IDH1 inhibitors in the field of cancer treatment (Table 3).

4. Conclusions

Cancer is the main cause of human death, and its morbidity and mortality are increasing year by year. The occurrence and development of cancer are closely watched by researchers, but the mechanism of cancer occurrence is quite complicated, and it is still a major problem that needs to be solved urgently in today's society. Abnormal energy and metabolism are important characteristics of malignant tumors, which play an important role in the progression of cancers. In 2011, Robert A. Weinberg proposed ten characteristics of cancer, including the abnormal energy metabolism. Isocitrate dehydrogenase (IDH) plays a very important role in the process of energy metabolism. In the tricarboxylic acid cycle, isocitrate dehydrogenase can catalyze the conversion of isocitrate to α-KG. After IDH is mutated, its enzymatic activity changes, which can convert α-KG into D-2HG, leading to high levels of D-2HG in the body. D-2HG is a recognized cancer metabolite and can promote cancer cell proliferation through a variety of mechanisms. Therefore, IDH gene mutations are closely related to the occurrence and development of cancers.

IDH1 plays an important role in the process of life activities including glutamine metabolism, cell active oxygen regulation, fat synthesis, phospholipid metabolism, insulin secretion, etc. Recent studies have found that the changes in IDH1 expression and the mutation of amino acid 132 are closely related to many cancers. IDH1 is one of the most widely mutated metabolic enzymes in human cancers discovered so far. The relationship between IDH1 (including its mutants) and cancer development was first discovered in gliomas. With the deepening of research, it is found that it

has a certain correlation with many cancers. IDH1 mutations have been found in various cancers such as cholangiocarcinoma, acute myeloid leukemia, brain glioma, and chondrosarcoma. Mutant IDH1 acquires a new catalytic function, which can catalyze the production of D-2HG from α-KG, and then regulate the induction, growth, metastasis, metabolism, and drug resistance of cancer through various mechanisms. Inhibitors against IDH1 mutations can significantly reduce the level of D-2HG, which has become a hot spot in the development of some oncology drugs.

In this paper, we discussed the impacts of IDH1 on the occurrence and development of cancer from four perspectives. In the respect of metabolic reprogramming, IDH1 mutation causes a large amount of D-2HG accumulation, inhibits mitochondrial function, and promotes aerobic glycolysis to provide energy for cells. In addition, mutant IDH1 inhibits wild-type IDH1, causing depletion of NADPH, making cells unable to resist oxidative stress, and thus causing DNA damage. We also noticed that in some cancers, a low nutrition statement will increase the expression of $IDH1^{WT}$, increase the level of NADPH, and enhance the function of mitochondria to ensure the normal growth of cancer cells. Cancer cells have a strong adaptive capacity. For cancer treatment based on energy metabolism, it is necessary to assess the nutritional status of patients, detect the expression of energy metabolism targets and their metabolite levels, formulate stricter drug indications, and achieve individualized treatment. D-2HG produced by IDH1 mutant can mediate hypermethylation of DNA and histones and promote the occurrence of cancer. Compared with genetic factors of cancer, reversible epigenetic variation can be regulated by chemical drugs, showing broad prospects in cancer treatment. At present, the drugs targeting epigenetics cannot be widely used in cancer treatment because of their low efficacy, low bioavailability, poor stability, high toxicity, poor drug compliance, etc. Therefore, the combination of IDH1/2 inhibitors and DNMT1 inhibitors may be a potential therapeutic strategy. Many clinical studies have been carried out on the combination of IDH1/2 inhibitors and DNMT1 inhibitors. With the deepening of research, epigenetic drugs will be able to meet the clinical needs better. Moreover, IDH1 mutation will cause the immune microenvironment to be in a state of inhibition. The analysis of this state will provide a basis for the combined application of IDH1 inhibitors and cancer immunotherapy methods. Cancer is a multidimensional and complex biological system. The impacts of IDH1 on cancer phenotype can further verify this point of view. IDH1 affects the proliferation, apoptosis, migration, and drug resistance of cancer cells through different signal pathways. Therefore, the interpretation of IDH1-related cancer signal pathway network will provide all-around guidance for cancer treatment based on IDH1.

Currently, the research and development of IDH1 inhibitors are mainly concentrated in the field of small molecules. In addition, vaccines targeting mutant IDH1 are in the clinical research stage. The clinical trials of combination drugs and dual-targeted drugs foreshadowed the trend of IDH1 inhibitors and further confirmed the limitations of single-target therapy. Inspired by the dual-targeted drugs and proteolysis-targeting chimeras (PROTAC) technology, we initiated the concept of "dual PROTAC", which achieved the simultaneous degradation of EGFR and PARP by a single drug[191], providing a new idea for the development of IDH1 inhibitors. Many studies have shown that PROTACs have excellent anti-cancer activity, which can solve the problem of drug resistance, and reduce the toxic side effects through selectively targeted degradation. The design of IDH1 mutant small molecules with better selectivity will greatly promote the research of IDH1 degraders and provide more treatment options for IDH1-related cancer.

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Author contributions

Lixia Chen, Lidian Chen and Hua Li: Conceptualization, Writing-Reviewing and Editing. Yang Liu, Wei Xu: Writing-Original draft preparation and Visualization. Mingxue Li, Yueying Yang, and Dejuan Sun: Writing-Original draft preparation. All authors have approved the final version of the article.

Conflicts of interest

All the authors declared that they have no conflicts of interest.

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