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REVIEW ARTICLE

Corrected: Correction

# Wild-type and mutated IDH1/2 enzymes and therapy responses

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## Abstract

Isocitrate dehydrogenase 1 and 2 (IDH1/2) are key enzymes in cellular metabolism, epigenetic regulation, redox states, and DNA repair. *IDH1/2* mutations are causal in the development and/or progression of various types of cancer due to supraphysiological production of D-2-hydroxyglutarate. In various tumor types, *IDH1/2*-mutated cancers predict for improved responses to treatment with irradiation or chemotherapy. The present review discusses the molecular basis of the sensitivity of *IDH1/2*-mutated cancers with respect to the function of mutated *IDH1/2* in cellular processes and their interactions with novel IDH1/2-mutant inhibitors. Finally, lessons learned from *IDH1/2* mutations for future clinical applications in *IDH1/2* wild-type cancers are discussed.

## Introduction

Isocitrate dehydrogenase 1 and 2 (IDH1/2) are key enzymes that function at a crossroads of cellular metabolism, epigenetic regulation, redox states, and DNA repair. Mutations in the genes encoding for these enzymes occur in various types of malignancies, including >80% of low-grade gliomas and secondary glioblastomas [1–3], ~60% of chondrosarcomas [4], ~20% of intrahepatic cholangiocarcinomas (ICC) [5] and ~10% of acute myeloid leukemias (AML) [6–8]. These mutations occur in a hotspot fashion in the catalytically active sites of these enzymes and the main driver of oncogenesis is the neomorphic production of D-2-hydroxyglutarate (D-2HG; Fig. 1) [9]. The resulting D-2HG accumulation competitively inhibits α-ketoglutarate (αKG)-dependent enzymes, causing cellular alterations in the above-mentioned plethora of cellular metabolism, epigenetic regulation, redox states, and DNA repair, all of which may contribute to carcinogenesis which has been extensively reviewed elsewhere [10–12]. As the neomorphic production of D-2HG is essentially a gain of function that is exclusive to mutant IDH1/2 enzymes, it was quickly realized that these frequently-occurring genetic alterations were promising targets for personalized anti-cancer therapy with small-molecule inhibitors [13]. Within 5 years after the initial development of these compounds, the IDH2-mutant inhibitor enasidenib was approved by the FDA as a first-in-class inhibitor for the treatment of relapsed or refractory *IDH2*-mutated AML [14, 15].

Soon after their discovery, it was appreciated that *IDH1/2* mutations were associated with a relatively prolonged patient survival for glioma [3] and glioblastoma [2] but not for AML [16, 8] or chondrosarcoma [4]. For ICC, some studies reported that *IDH1/2* mutations were independent predictive factors for prolonged progression-free and overall survival [17], whereas other studies reported no difference between the survival of *IDH1/2*-mutated vs. IDH1/2 wild-type ICC [18, 19] and one study (reporting only six *IDH1/2*-mutated cases) even found a worse prognosis of *IDH1/2*-mutated ICC compared to wild-type counterparts [20]. The assumption that *IDH1/2* mutations are causal for the improved clinical outcome in glioma was supported by clinical evidence, as *IDH1/2* mutations predicted for improved tumor responses to chemotherapy and/or irradiation in clinical trials [21, 22] and retrospective analyses [23–27]. Furthermore, cancer cells are sensitized to radiation and chemotherapy by the introduction of mutant *IDH1/2* or by silencing of wild-type *IDH1/2* (Table 1). *IDH1/2* mutations or the absence of IDH1/2 wild-type enzymes create downstream vulnerabilities in cancer that can be therapeutically targeted with small-molecule inhibitors,

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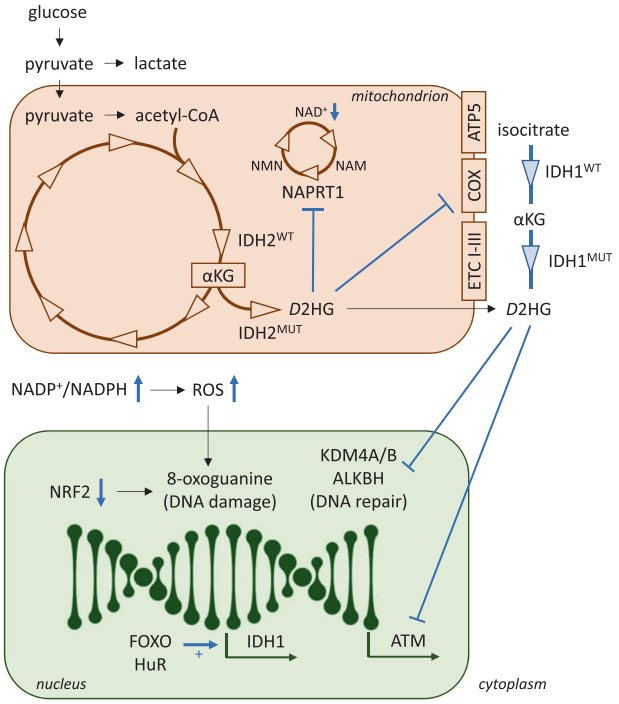


**Fig. 1** Effects of *IDH1/2* mutations and D-2HG accumulation on cellular metabolism, redox states, and DNA damage repair. *ALKBH* alkylation repair homolog, *ATM* ataxia-telangiectasia mutated, *ATP5* adenosine triphosphate synthase, *CoA* coenzyme A, *COX* cytochrome *c* oxidase, *D-2HG* D-2-hydroxyglutarate, *ETC* electron transport chain, *FOXO* forkhead box proteins, *HuR* human antigen R, *IDH* isocitrate dehydrogenase, *KDM* lysine histone demethylase, *NAD(P)* nicotinamide dinucleotide (phosphate), *NAD(P)H* nicotinamide dinucleotide (phosphate), reduced, *NAM* nicotinamide, *NAMPT* nicotinamide phosphoribosyltransferase, *NMN* nicotinamide mononucleotide, *NRF2* nuclear factor (erythroid-derived 2)-like, *ROS* reactive oxygen species

such as poly(ADP-ribose) polymerase (PARP) inhibitors, nicotinamide phosphoribosyltransferase (NAMPT) inhibitors, BCL-2 inhibitors and biguanides. A better understanding of the mechanisms of these vulnerabilities may aid to improve personalized therapy for patients with or without *IDH1/2*-mutated cancers and is the subject of this review.

## IDH1/2 enzymes in metabolism

IDH1 and IDH2 catalyze the reversible oxidative decarboxylation of isocitrate to αKG in the cytoplasm and mitochondria, respectively, with concomitant reduction of $NADP^+$ to NADPH (Fig. 2). Although IDH1/2 do not generate NADH, the canonical product of the TCA cycle, IDH1/2 perform the same isocitrate-to-αKG conversion as the traditional TCA cycle enzyme IDH3. *IDH1/2* mutations are neomorphic [9], but also confer a loss of function of wild-type IDH1/2 kinetics [28, 29] and redirect carbon metabolites away from the TCA cycle and oxidative phosphorylation towards D-2HG production. This is evidenced by decreased expression of TCA cycle enzymes downstream of IDH [30] and decreased oxidative metabolism in Seahorse metabolic assays using *IDH1/2*-mutated cancer cells [30, 31]. *IDH1*-mutant-induced mitochondrial dysfunction is also compensated by an increase in the number of mitochondria in *IDH1/2*-mutated cells [32–35]. As a result, *IDH1/2*-mutated cancer cells are vulnerable to inhibition of the residual oxidative metabolism with inhibitors of the electron transport chain (ETC), such as the biguanides metformin and phenformin that inhibit NADH dehydrogenase (complex I) of the ETC [29, 36, 37]. Metformin is currently investigated for safety and efficacy in a clinical trial of patients with *IDH1/2*-mutated solid tumors [38].

A consequence of rewired metabolism in *IDH1/2*-mutated cells is the dependence on the glutaminolysis pathway, which provides anaplerosis to the TCA cycle at the level of αKG. *IDH1/2*-mutated cells need αKG to produce D-2HG but at the same time they restrict αKG production by impairing glycolytic influx and TCA cycle metabolism [30]. αKG production from glutamine provides an alternative source of fuel to satisfy *IDH1/2*-mutated cells in their D-2HG production, but also render these cells vulnerable to pharmacological inhibition of glutaminolysis with the use of aminooxyacetic acid, BPTES, zaprinast, or chloroquine [35, 39–42]. It has been hypothesized that *IDH1*-mutated glioma depend on glutamate rather than glutamine for TCA cycle anaplerosis [43, 44].

*IDH1/2* mutations or IDH1/2 knockdown disable the oxidative decarboxylation reaction that converts αKG to isocitrate [37]. This reaction occurs predominantly in hypoxia, when glycolytic influx of pyruvate in the TCA cycle is compromised and cells use the reverse IDH1/2 reaction to generate citrate and acetyl-CoA from glutamine and glutamate in order to preserve the capacity to synthesize lipids in hypoxic contexts [37,45–47]. Knockdown of *IDH1* sensitized glioma-initiating cells with *EGFR* amplifications to treatment with erlotinib through decreased fatty acid and cholesterol biosynthesis. This vulnerable phenotype was rescued by treatment with cell membrane-permeable αKG or the fatty acid palmitate plus the cholesterol precursor mevalonate [48].

Finally, the metabolic rewiring by *IDH1/2* mutations renders cells vulnerable to inhibitors that exploit a synthetic lethality of this *IDH1/2*-mutant metabolism. One is that D-2HG inhibits cytochrome *c* oxidase (complex IV of the ETC) and prevents cytochrome *c* release into the mitochondrial matrix. Besides restricting oxidative mitochondrial metabolism, cytochrome *c* release puts *IDH1/2*-mutated cells on the brink of apoptosis through BAX/BAK-mediated permeabilization of the outer mitochondrial membrane. Under steady-state conditions, this is prevented

**Table 1** Overview of therapeutic agents to which cells with mutated or knocked down IDH1/2 are sensitized

| Agent | IDH1/IDH2 | Model or patient population | Protection by IDH1/2$^{MUT}$ inhibitor? | References |
|---|---|---|---|---|
| Irradiation | *IDH1*$^{WT/R132H}$ isogenic | HCT116 colorectal cancer cells; U251 glioblastoma cells; HeLa cells, murine HSCs | Yes | [29, 74, 75] |
| | IDH1$^{R132H}$ IDH2$^{R172K}$ overexpression | U87 and U373 glioblastoma cells | No | [62] |
| | *IDH1*$^{R132H}$ and *IDH2*$^{R140Q}$ endogenous | Primary human AML cells | No | [75] |
| | IDH1 knockdown | U87 glioblastoma cells in vivo, U138 and A172 glioblastoma cells | N/A | [63] |
| Chemotherapy | | | | |
| 5-Fluoruracil | IDH1$^{R132H}$ overexpression | U87 glioblastoma cells | No | [66] |
| Busulfan | IDH1$^{R132H}$ overexpression | U87 glioblastoma cells | No | [81] |
| Carmustine (BCNU) | IDH1$^{R132C/H/L}$ overexpression and IDH1 knockdown | LN229 glioblastoma cells and HEK293 cells | No | [41] |
| Cisplatin (CDDP) | IDH1$^{R132H}$ overexpression | U87 and U251 glioblastoma cells | No | [64] |
| | *IDH1*$^{WT/R132H}$ isogenic | HeLa cells | No | [75] |
| Daunorubicin | *IDH1*$^{WT/R132H}$ isogenic | Murine HSCs | No | [74] |
| Gemcitabine | IDH1 knockdown | Mia pancreatic cancer cells | N/A | [73] |
| Lomustine (CCNU) | IDH1$^{R132H}$ overexpression | U87 glioblastoma cells | No | [81] |
| Procarbazine, lomustine (CCNU) and vincristine (PCV) | *IDH1*$^{MUT}$ endogenous | WHO grade II and III glioma patients | No | [21, 22] |
| Temozolomide | IDH1$^{R132H}$ overexpression | U87 and U251 glioblastoma cells in vivo, UACC257 melanoma cells | No | [31, 60] |
| | *IDH1*$^{MUT}$ endogenous | Primary glioma neurospheres, HT1080 chondrosarcoma cells in vivo, low-grade glioma patients | No | [26, 88] |
| Targeted therapy | | | | |
| ABT-263 | IDH1$^{R132H}$ overexpression | U87 and T98G glioblastoma cells, glioma stem-like cells in vitro, U87 glioblastoma cells in vivo | No | [51] |
| | *IDH1*$^{WT/R132H}$ isogenic | HCT116 colorectal cancer cells in vitro and in vivo | No | [51] |
| | IDH1$^{R132H}$ endogenous | Patient-derived glioblastoma xenograft | No | [51] |
| All-*trans* retinoic acid | *IDH1/2*$^{MUT}$ endogenous | Primary AML cells in vitro and in vivo | No | [51] |
| | IDH1$^{R132H}$ overexpression | HL60 APL cells in vitro and MOLM14 AML cells in vitro and in vivo | Yes | [61] |
| Berzosertib | *IDH1*$^{WT/R132H}$ isogenic | HeLa cells | No | [75] |
| Erlotinib | IDH1 knockdown | Glioma stem cells | N/A | [48] |
| Dasatinib | *IDH1*$^{R132C}$, *IDH1*$^{R132S}$, *IDH1*$^{R132V}$ endogenous | ICC cells in vitro and in vivo | No | [53] |
| | IDH2$^{R172K}$ overexpression | Murine intrahepatic cholangiocarcoma cells in vitro and in vivo | No | [53] |
| FK866 and GMX1778 | *IDH1/2*$^{MUT}$ endogenous | Primary glioma neurospheres, SW1353 chondrosarcoma cells, HT1080 chondrosarcoma cells in vivo | No | [52, 88] |

**Table 1** (continued)

| Agent | IDH1/IDH2 | Model or patient population | Protection by IDH1/2$^{MUT}$ inhibitor? | References |
|---|---|---|---|---|
| | IDH1$^{R132H}$ overexpression | U87 glioblastoma cells in vivo, UACC257 melanoma cells | | |
| Niraparib (MK-4827) | *IDH1*$^{WT/R132H}$ isogenic | HeLa cells | No | [75] |
| Rucaparib | *IDH1*$^{WT/R132H}$ isogenic | HeLa cells | No | [75] |
| Olaparib | *IDH1*$^{WT/R132H}$ isogenic | HCT116 colorectal cancer in vivo, HeLa cells in vivo, THP-1 AML cells, HT1080 chondrosarcoma in vivo, primary glioma neurospheres | Yes | [75, 88] |
| Sacratinib | *IDH1*$^{R132C}$, *IDH1*$^{R132S}$, *IDH1*$^{R132V}$ endogenous | ICC cells in vitro | No | [53] |
| | IDH2$^{R172K}$ overexpression | Murine ICC cells in vitro | No | [53] |
| Talazoparib (BMN-673) | *IDH1*$^{WT/R132H}$ isogenic | HCT116 colorectal cancer, THP-1 AML cells, HeLa cells | Yes | [75] |
| | *IDH1*$^{WT/R132H}$ endogenous | Primary human glioma cells | No | [75] |
| Venetoclax (ABT-199) | IDH1$^{R132H}$, IDH2$^{R140Q}$, IDH2$^{R172K}$ overexpression | THP-1 acute myeloid leukemia cells | Yes | [49] |
| | *IDH1*$^{MUT}$ endogenous | Primary human AML cells | No | [49] |
| | *IDH1*$^{R132}$, *IDH2*$^{R140}$, *IDH2*$^{R172K}$ endogenous | AML patients | No | [50] |
| Metabolic therapy | | | | |
| Aminooxyacetic acid | IDH1$^{R132H}$ overexpression and IDH1 knockdown | LN229 glioblastoma cells | No | [41] |
| BPTES | IDH1$^{R132H}$ overexpression | D54 glioblastoma cells | No | [42] |
| | *IDH1*$^{R132C/G/H}$, *IDH2*$^{R140Q}$ endogenous | Primary AML cells | No | [40] |
| Metformin | *IDH1*$^{WT/R132H}$ isogenic | HCT116 colorectal cancer cells | Yes | [29, 36] |
| Phenformin | *IDH1*$^{WT/R132H}$ isogenic | HCT116 colorectal cancer cells | No | [37] |
| Zaprinast | IDH1$^{R132H}$ overexpression | Normal human astrocytes | No | [39] |

ABT-263 and venetoclax are BH3 mimetics, of which venetoclax is more selective towards BCL-2 inhibition whereas ABT-263 inhibits both BCL-2 and BCL-XL, all-*trans* retinoic acid induces differentiation by promoting retinoic acid-responsive gene expression; berzosertib is an ATR/ATM inhibitor; dasatinib and sacratinib are multikinase inhibitors, FK866 and GMX1778 are NAMPT inhibitors; niraparib, rucaparib, olaparib, and talazoparib are PARP inhibitors. *AML* acute myeloid leukemia, *APL* acute promyelocytic leukemia, *ICC* intrahepatic cholangiocarcinoma, *HSC* hematopoietic stem cell, *IDH1/2*$^{MUT}$ mutated *IDH1/2*

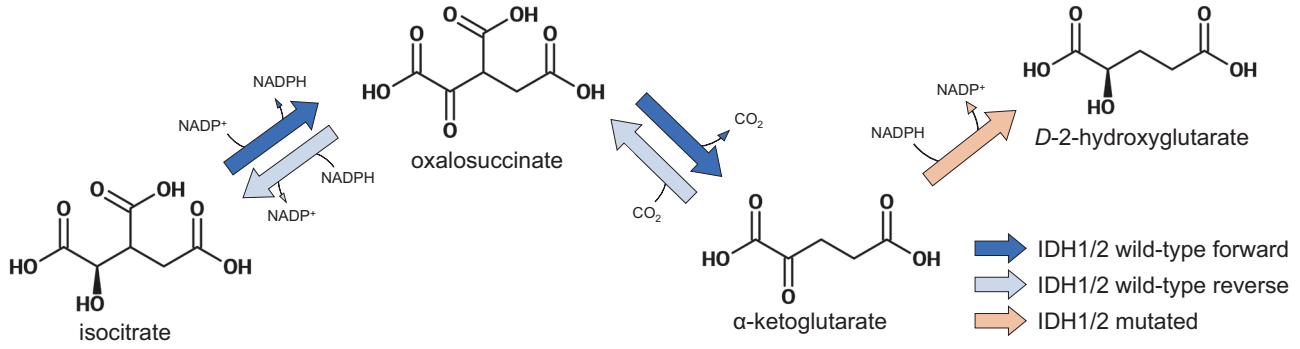


**Fig. 2** Biochemical reactions of IDH1 and IDH2 wild type and mutant enzymes. The forward reaction is an oxidative decarboxylation, while the reverse reaction is a reductive carboxylation

by BCL-2 binding to the proapoptotic proteins BAX/BAK, but disruption of this binding by the BH3 mimetic ABT-199 (venetoclax, FDA-approved for the treatment of chronic lymphocytic leukemia) results in apoptosis of *IDH1/2*-mutated cells while *IDH1/2* wild-type cells are relatively insensitive [49]. In a clinical study among AML patients, those with IDH1/2 mutations had a higher response rate (36%) to venetoclax than those with IDH1/2 wild type (9%) [50]. A similar effect was observed in glioblastoma models, although BCL-xL and not BCL-2 seemed to be the primary target of the BH mimetic ABT-263 in these glioblastoma models [51]. Another example is that D-2HG downregulates nicotinate phosphoribosyltransferase (NAPRT1), an enzyme in the $NAD^+$ salvage pathway, which causes sensitivity towards depletion of $NAD^+$ by pharmacological inhibition of NAMPT with the preclinical compounds FK866 and GMX1778 leading to AMP kinase-initiated autophagy and cell death in *IDH1*-mutated cancer cells [52]. Finally, *IDH1/2*-mutated ICC cells are dependent on the kinase SRC for mTOR-mediated proliferation and survival and these cells are highly sensitive to treatment with the multikinase inhibitor dasatinib, which also has affinity for SRC [53]. These synthetic lethalities are probably the result of complex crosstalk between *IDH1/2* mutations and tissue-specific intracellular processes, because among *IDH1/2*-mutated models, AML and glioblastoma but not ICC was sensitized to BCL-2/BCL-xL inhibition [49, 51, 53], glioma but not ICC was sensitized to NAMPT inhibition [52, 53], and ICC but not chondrosarcoma or lung cancer was sensitized to dasatinib [53].

## IDH1/2 enzymes in redox states

Since the reductive carboxylation of isocitrate to αKG by IDH1/2 is redundant besides the function of IDH3 in the TCA cycle, IDH1 and IDH2 presumably arose in evolution for the purpose of NADPH production in the cytoplasm and mitochondria, respectively [12]. NADPH is an important source of cellular reducing power and is required to recharge, activate, or generate reduced glutathione (GSH) [54], thioredoxin [55], catalase tetramers [56], and cytochrome P450 [54, 57], all of which are involved in the reduction of reactive oxygen species (ROS). Moreover, NADPH is necessary for the synthesis of deoxynucleotides and thus for DNA damage repair [58]. *IDH1/2* mutations cause loss of IDH1/2 wild-type functions [28, 29], resulting in decreased NADPH and GSH levels and increased ROS levels, both in steady-state conditions and after induction of ROS production [29, 41, 48, 52, 59, 60]. In addition, D-2HG accumulation induces oxidative stress independently of *IDH1/2* mutations [32–34]. In AML, the $IDH1^{R132H}$ gene expression signature was enriched in genes that result in a cellular phenotype that is responsive to treatment with small molecules that target ROS and $NADP^+$/NADPH signaling and metabolism [61]. Oxidative stress is possibly induced via inhibition of wild-type IDH1/2 activity due to αKG mimicry of D-2HG, which results in a pseudo-product inhibition of wild-type IDH1/2 [55] or via increased mitochondrial transmembrane proton leakage due to cytochrome *c* retention in the mitochondrial intermembrane space as described above [49]. Further evidence of increased ROS levels as mediator of increased therapy sensitivity of *IDH1/2*-mutated cells is shown by the almost complete reversal of this sensitivity in various cell models by the antioxidant and GSH surrogate *N*-acetyl-cysteine. This reversal of sensitivity has been shown in the presence of carmustine (BCNU) [41], irradiation [29, 62, 63], cisplatin, temozolomide [64], and erlotinib in glioma-initiating cells with *EGFR* amplification, where increased ROS levels increase erlotinib-induced apoptosis after *IDH1* knockdown [48]. Some in vitro studies showed depleted GSH levels and increased ROS levels in cancer cells with *IDH1* mutations [29, 64–66] but *IDH1/2* mutations did not alter ROS levels in brains and hematopoietic cells of $IDH1^{R132H}$ knock-in mice [67, 68] or immortalized human astrocytes [69] in other studies. However, these studies only interrogated steady-state conditions and used a ROS marker (CM-

$H_2$DCFDA) that is insensitive to $H_2O_2$ [70], the oxidant that is most probably elevated in cells that are depleted of NADPH and have limited peroxidase and peroxiredoxin activity.

IDH1/2 are the most important NADPH producers in most human organs, including the brain [28, 71], and *IDH1* mutations lead to depleted NADPH levels in colorectal cancer cells [29] and glioma cells [64, 66]. In myeloid cells, glucose-6-phosphate dehydrogenase (G6PDH) of the pentose phosphate pathway is the major NADPH provider [12]. IDH1 is the highest upregulated NADPH-producing enzyme when glioblastoma and normal brain tissue are compared and IDH1 mRNA and protein expression is upregulated following radiation, suggesting a role for IDH1 in cellular responses to radiation [48, 63], possibly by induction of IDH1 expression via Forkhead box O (FOXO) transcription factors [72]. In pancreatic cancer cells, IDH1 expression is induced by HuR (*ELAVL1*) after treatment with gemcitabine and the HuR–IDH1 regulatory axis is essential for adaptive pancreatic cancer cell survival under acute stress [73]. Furthermore, the introduction of a mutant IDH1/2 protein radiosensitizes glioblastoma cells and other cancerous and noncancerous cells [29, 74, 75]. Radiosensitization also occurs after knockdown of IDH1 [63, 77, 78], whereas overexpression of IDH1 protects cancer cells against chemotherapy [73, 79]. These latter two findings are important, because it isolates the role of IDH1 loss-of-function in cellular radiosensitization and rules out D-2HG from being solely responsible for this phenomenon. Radiosensitivity of $IDH1^{-/-}$ cells was related to increased cellular senescence due to depletion of antioxidants and deoxynucleotides in $IDH1^{-/-}$ cells following irradiation, whereas apoptosis, necrosis, autophagy, unrepaired DNA double-strand breaks, and homologous recombination repair remained unchanged [63]. This partly corroborates and partly contrasts the situation in *IDH1/2*-mutated cells, where radiosensitivity is caused by depletion of antioxidants [29], and by reduced DNA damage responses and double-strand break repair [29, 75]. Since D-2HG accumulation is strongly linked to perturbed DNA damage repair (see below), it seems plausible that ROS-associated therapy sensitivity of *IDH1/2*-mutated cells mainly occurs via increased cellular senescence due to depleted levels of antioxidants and deoxynucleotides, whereas D-2HG-associated therapy sensitivity of *IDH1/2*-mutated cells is predominantly associated with inhibition of DNA damage response proteins.

Finally, *IDH1* mutations are implicated in the downregulation and aberrant subcellular localization of nuclear factor-erythroid 2-related factor 2 (NRF2) and NAD(P)H quinine oxidoreductase 1 (NQO1), which are important cellular defense proteins against oxidative stress. Their downregulation and dysfunction is associated with increased sensitivity to chemotherapy with temozolomide, but a regulatory relationship between IDH1 and NRF2 remains unclear.

## IDH1/2 enzymes in DNA repair

Besides the indirect effects of *IDH1/2* mutations on DNA repair via redox state perturbations, IDH1/2 and DNA repair are interwoven via direct inhibition by D-2HG of αKG-dependent dioxygenases involved in DNA repair. For example, D-2HG inhibits the DNA repair enzyme alkB homolog (ALKBH) [80, 81] and the DNA damage response proteins lysine-specific demethylase 4A/B (KDM4A/B) [75, 82, 83] and suppresses the expression of the DNA damage response protein ATM [75]. These findings are linked with increased DNA damage in *IDH1/2*-mutated cells as compared to *IDH1/2* wild-type cells, either in steady-state conditions or after treatment with cytotoxic or targeted agents [29, 31, 74, 75]. Perturbed steady-state DNA repair may contribute to oncogenesis of *IDH1/2*-mutated cancers, but a perturbed DNA damage response is even more likely to be related to the increased susceptibility of *IDH1/2*-mutated cancers to DNA damage-inducing cytotoxic agents.

Inhibition of ALKBH by D-2HG results in sensitization of *IDH1/2*-mutated cancer cells to alkylating agents such as busulfan and CCNU [81]. These findings provide a molecular basis for the sensitivity of *IDH1/2*-mutated glioma towards a regimen of radiotherapy in the presence or absence of procarbazine, CCNU, and vincristine (PCV) [21, 22], of which the first two are DNA alkylators. Moreover, temozolomide is another DNA-alkylating agent that is the standard of care first-line therapy for glioblastoma and *IDH1/2* mutations predict for glioblastoma responses to temozolomide [26].

*IDH1/2*-mutated cancers are known to confer defects in homologous recombination, whereas the other major DNA double-strand break repair pathway, non-homologous end-joining, remains intact [75]. The result is increased levels of DNA damage [29, 31, 75], which prompted researchers to perform a focused high-throughput screen of DNA repair inhibitors [75]. This resulted in the observation that *IDH1/2*-mutated cancers are sensitive to PARP inhibitors in vivo and this sensitivity for PARP inhibitors synergizes with temozolomide or cisplatin treatment in vitro, but there is disagreement on the underlying mechanisms [31, 75, 76]. One study implicated $NAD^+$ deficiency in *IDH1/2*-mutated cells in PARP dysfunction, because $NAD^+$ is an essential cofactor for PARP-mediated single-strand DNA repair [31]. However, this conclusion was based on associative evidence obtained from findings that the PARP DNA repair machinery was intact in *IDH1/2*-mutated cells whereas $NAD^+$ levels were more depleted in *IDH1/2*-mutated cells

than in *IDH1/2* wild-type cells after DNA-damaging temozolomide treatment in vitro. However, mechanistic experiments to pinpoint $NAD^+$ levels as the critical factor for *IDH1/2* mutation-mediated PARP dysfunction were not performed [31]. Another study showed that $NAD^+$ levels have no role in *IDH1/2* mutation-induced PARP sensitivity but instead convincingly showed that D-2HG inhibits the αKG-dependent dioxygenases KDM4A/B. Inhibition of KDM4A/B induces a homologous recombination defect that creates a "BRCAness" phenotype in *IDH1/2*-mutant cells, which results in PARP inhibitor sensitivity in vivo [75]. Of note, *IDH1/2* mutations and D-2HG accumulation were already linked to KDM4 inhibition several years earlier but thus far this observation was only linked to global histone hypermethylation and not to decreased DNA damage responses [84]. The mechanism behind the latter phenomenon is that histone methylation, such as demethylation of histone H3 lysine 20 (H3K20) and trimethylation of H3K9, is a barrier to DNA double-strand break repair that can be relieved by KDM4A [82] and KDM4B [83], respectively. Moreover, these demethylases cooperate with or sometimes orchestrate the activity of canonical DNA damage response proteins, such as 53BP1 in the case of KDM4A [82] and PARP1 in the case of KDM4B [83]. TET2 is a major downstream target of D-2HG accumulation and is considered to be a major mediator of *IDH1/2*-mutant-mediated oncogenesis [11, 12, 85]. However, it is unlikely that TET2 inhibition contributes to sensitization of *IDH1/2*-mutant cells to PARP inhibitors, because restoration of TET2 function sensitizes rather than protects TET2 haploinsufficient AML cells to PARP inhibitors [86]. This is an intriguing finding that also questions how IDH1/2-mutant inhibitors reverse the PARP inhibitor sensitization of *IDH1/2*-mutated cells, as IDH1/2-mutant inhibitors reduce D-2HG levels and should restore TET2 function [87]. One study speculated that perturbed DNA damage repair and increased temozolomide sensitivity of *IDH1/2*-mutated cells are caused by impaired oxidative metabolism because administration of αKG protected *IDH1/2*-mutated cells against temozolomide treatment. However, it now seems more plausible that αKG administration reduces the competitive inhibition of D-2HG of ALKBH and/or KDM4A/B and restores the activity of these DNA damage repair enzymes [31]. It was also demonstrated that temozolomide treatment made a bigger dent in $NAD^+$ levels in *IDH1/2*-mutated than in *IDH1/2* wild-type cells which was driven by $NAD^+$ consumption by PARP [88]. As a result, combined treatment with temozolomide and NAMPT inhibitors had a synergistic effect in *IDH1/2*-mutated cancers in vivo and may represent a promising therapeutic avenue for *IDH1/2*-mutated cancer patients.

Whole-proteome analyses of *Idh1* wild-type and *Idh1*-mutated murine hematopoietic stem cells revealed that the latter had lower levels of the (phosphorylated) DNA damage response proteins phospho-ATM, phospho-CHEK2, and γH2AX. Mutant IDH1 downregulates the DNA damage response protein ATM via an epigenetic mechanism that involves chromatin modifications via histone lysine demethylation. Mechanistic experiments involving inhibitors of epigenetic modifiers affecting H3K9 and H3K27 showed a direct link between repressive trimethylation at these histone marks and downregulation of ATM. The authors speculated that KDM4 inhibition by D-2HG is responsible for ATM suppression and excluded TET2 as a mechanistic link between D-2HG accumulation and epigenetic suppression of ATM expression, because $Tet2^{-/-}$ mice had normal ATM levels. At the therapeutic level, reduced ATM activity was associated with increased sensitivity to irradiation and the DNA-damaging chemotherapeutic agent daunorubicin [74]. Reduced ATM expression has also been observed in human primary *IDH1/2*-mutated AML cells as compared to their wild-type counterparts and was rescued by using an IDH1/2-mutant inhibitor. In these human cell models, *IDH1/2* mutations and ATM suppression caused sensitivity to irradiation and daunorubicin [76].

## IDH1/2 enzymes in epigenetic regulation

αKG and D-2HG, the products of wild-type and mutant IDH1/2, respectively, are closely involved in epigenetic regulation of gene expression. Via this mechanism, IDH1/2 form a link between metabolism and epigenetics, as has been extensively reviewed elsewhere [89]. D-2HG is an inhibitor of various αKG-dependent dioxygenases necessary for DNA and histone demethylation, such as TET2 and Jumonji-domain containing lysine histone demethylases [10, 11]. The downstream epigenetic effects of *IDH1/2* mutations have been predominantly investigated in the context of *IDH1/2*-mutant-mediated oncogenesis but not as targets for personalized therapy. However, *IDH1/2*-mutant-induced epigenetic modifications do have potential as therapeutic targets. For example, *IDH1* mutations induce a gene expression signature that render AML cells vulnerable to all-*trans* retinoic acid (ATRA/tretinoin), which promotes the expression of genes that induce differentiation of *IDH1*-mutated AML cells [61]. Furthermore, the epigenetic effects of *IDH1/2* mutations may underlie many of the synthetic lethalities already described in this review, including the downregulation of NAPRT1 to sensitize cells to NAMPT inhibition [52], the downregulation of NRF2 and ATM to sensitize cells to chemo/radiotherapy [60, 74], the downregulation of BCAT1 to increase the dependency on glutaminolysis [90], and the induction of homologous recombination defects to sensitize cells to PARP inhibitors, as described above in more detail [75].

## IDH1/2-mutant inhibitors and therapy responses

*IDH1/2* mutations are inaugural or at least early events in the formation of glioma [91], chondrosarcoma [4], ICC [92], and AML [93] (although in that case the data are conflicting [8]) and are thus present in the large majority, if not all, cancer cells. This makes *IDH1/2* mutations attractive therapeutic targets, because such tumor homogeneity decreases the risk of therapy resistance since targeting IDH1/2 mutations affects all cancer cells. The appreciation of the role of *IDH1/2* mutations in oncogenesis and their early occurrence prompted the development of IDH1/2-mutant inhibitors [13]. Enasidenib (AG-221/CC-90007) is now registered for the treatment of refractory/relapsed *IDH2*-mutated AML and the IDH1-mutant inhibitor ivosidenib (AG-120) is in clinical trials. Enasidenib suppresses D-2HG production, reverses epigenetic dysregulation, and induces cellular differentiation in *IDH2*-mutated AML, where it achieved an overall response rate of 40% and a complete remission rate of 19% [15, 94, 95]. MDS patients with ancestral *IDH1/2* mutations had a worse survival than patients with subclonal *IDH1/2* mutations [8], which may suggest that patients with ancestral *IDH1/2* mutations are the best candidates for therapy with IDH1/2-mutant inhibitors. However, an association between the mutant *IDH2* variant allelic frequency and clinical response to enasidenib was not observed in AML patients [94].

These results with enasidenib monotherapy are promising for the difficult-to-treat population of patients with refractory/relapsed AML, but even before the first clinical trials with IDH1/2-mutant inhibitors started it was doubted whether combination regimens of IDH1/2-mutant inhibitors and conventional chemotherapy or targeted DNA damage-inducing agents would be safe and efficacious [12]. In the context of our increasing understanding of the therapy response-modulating effects of *IDH1/2* mutations, which almost exclusively point at an increased sensitization to cytotoxic agents of most types of cancer, it is plausible that concomitant administration of such cytotoxic agents and IDH1/2-mutant inhibitors counteract each other. For example, IDH1/2-mutant inhibitors protect *IDH1/2*-mutated glioma, AML, chondrosarcoma, and colorectal carcinoma cells against irradiation, daunorubicin, and PARP inhibitors [29, 75, 76]. In all cases, the mechanism of therapy protection by IDH1/2-mutant inhibitors was based on the reversal of the mechanism that rendered *IDH1/2*-mutated cancer cells sensitive to the therapeutic agent. For example, pretreatment with the IDH1-mutant inhibitor AGI-5198 (the preclinical version of ivosidenib/AG-120) decreased D-2HG levels, restored NADPH production, and decreased ROS levels and these phenomena collectively or ultimately resulted in less *IDH1/2*-mutated cell death after irradiation [29]. In the case of protection of *IDH1*-mutated cells against pharmacological PARP inhibition, pretreatment with AGI-5198 decreased D-2HG levels and the number of DNA double-strand breaks and reverted the PARP inhibitor sensitivity of *IDH1*-mutated cells to levels observed in *IDH1* wild-type cells [75]. In both instances, the therapy-protective effects of AGI-5198 was overcome by administration of exogenous D-2HG to increase D-2HG levels independently of the inhibited mutant IDH1 enzyme [29, 75].

## Concluding remarks and future perspectives

*IDH1/2* mutations are attractive therapeutic targets for various reasons, but most prominently because they are early events in oncogenesis. As a consequence, this tumor homogeneity ensures that the chance of relapse of *IDH1/2*-mutated cancers is theoretically small after a complete response/remission obtained by the application of targeted therapeutic agents. Another prediction on the basis of the early occurrence of *IDH1/2* mutations in oncogenesis and the plethora of downstream cellular effects of *IDH1/2* mutations is the profoundly altered tumor biology during oncogenesis. Consequently, many research efforts have been devoted to the discovery of specific vulnerabilities, especially in the domains of metabolism and DNA damage responses. This has resulted in a better understanding of the sensitization of cancer cells by *IDH1/2* mutations to conventional chemo/radiotherapy, but also of the susceptibility to targeted agents that have maximal efficacy and minimal side effects. We expect that pharmacological inhibition of BCL-2, NAMPT, and PARP are the most promising therapeutic avenues in this category [49, 52, 75], especially in combination with each other or with chemo/radiotherapy. Olaparib, rucaparib, and niraparib are PARP inhibitors [95], and venetoclax is a BCL-2 inhibitor [97] that are all FDA-approved for the treatment of other types of cancer, which may ease future clinical trials with these drugs for the treatment of *IDH1/2*-mutated cancers. Because of the inherent counteracting nature of IDH1/2-mutant inhibitors on the one hand and personalized targeted therapy of *IDH1/2*-mutated cancers on the other hand, it is doubtful whether there will ever be clinically safe and effective combinations of IDH1/2-mutant inhibitors and cytotoxic agents to which a particular *IDH1/2*-mutated cancer is sensitized [29, 75]. The future looks bright for preclinical and clinical research on *IDH1/2*-mutated cancers and its advances may eventually trickle down to the much larger populations of patients with *IDH1/2* wild-type cancers. It is becoming increasingly clear that inhibition of wild-type IDH1/2 to mimic therapy responses of IDH1/2-mutant cancer biology may have therapeutic potential. This was recently

demonstrated in three preclinical models [48, 63, 73]. Another example of the trickle-down effect of the increased research on *IDH1/2* mutations is that reasonably potent pharmacological inhibitors of wild-type IDH1 such as GSK864 ($IC_{50}$: ~470 nM) have become available whereas we previously only had RNA interference, genetic modification, or the unspecific and impotent oxalomalate [78] to inhibit wild-type IDH1. GSK864 was originally developed as IDH1-mutant inhibitor but also showed activity against wild-type IDH1 [98]. In this way, we learn from lessons from nature so that ultimately *IDH1/2* wild-type patients may benefit as well from our understanding of the increased therapy sensitivity of *IDH1/2*-mutated cancers.

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## Compliance with ethical standards

**Conflict of interest** J.P.M. has received honoraria, has performed consultancy, and has served as a speaker on behalf of Celgene. The remaining authors declare that they have no competing interests.



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