IDH1/2 Mutations in Cancer Stem Cells and Their Implications for Differentiation Therapy

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Summary
Isocitrate dehydrogenase 1 and 2 (IDH1/2) are enzymes recurrently mutated in various types of cancer, including glioma, cholangiocarcinoma, chondrosarcoma, and acute myeloid leukemia. Mutant IDH1/2 induce a block in differentiation and thereby contribute to the stemness and oncogenesis of their cells of origin. Recently, small-molecule inhibitors of mutant IDH1/2 have been Food and Drug Administration–approved for the treatment of IDH1/2-mutated acute myeloid leukemia. These inhibitors decrease the stemness of the targeted IDH1/2-mutated cancer cells and induce their differentiation to more mature cells. In this review, we elucidate the mechanisms by which mutant IDH1/2 induce a block in differentiation and the biological and clinical effects of the release into differentiation by mutant-IDH1/2 inhibitors. (J Histochem Cytochem 70:83–97, 2022)

Keywords
cancer stem cells, chemotherapy, differentiation, enasidenib, isocitrate dehydrogenase, ivosidenib, targeted therapy, therapy responses, 2-hydroxyglutarate

Introduction
Cancer stem-like cells (CSCs) are malignant cells with stem cell characteristics, specifically the potential for self-renewal and differentiation. Similar to stem cells in benign tissues that support the renewal of more mature cells, CSCs are hypothesized to replace malignant cells with a more limited lifespan and therefore enable the persistence of cancer in the presence or absence of cancer therapy and the metastasis of tumors. The CSC hypothesis is based on experimental models with immunodeficient mice in whom a small subset of cells from human tumors can be engrafted and populate the full diversity of malignant cells that were present in the original tumor, whereas other subsets of cancer cells do not have this ability. Although this definition of CSCs is thus largely based on the ability to engraft a tumor in an experimental mouse model and not necessarily clinical observations in human patients, the development of novel cancer therapies is increasingly impacted by the rationale that deep and durable remissions can best be achieved by targeting the CSCs and not just the tumor bulk.1

Isocitrate dehydrogenase 1 and 2 (IDH1/2) are enzymes that perform key roles in various cellular functions, including the regulation of carbohydrate metabolism, epigenetics, differentiation, DNA repair, and redox states. Wild-type IDH1 and IDH2 (wtIDH1/2) oxidize and decarboxylate isocitrate to α-ketoglutarate (α-KG) in the cytoplasm and mitochondria, respectively, and simultaneously reduce NADP⁺ to NADPH. Through these metabolites, wtIDH1/2 function in the aforementioned myriad of cellular processes because α-KG is a core metabolite of the tricarboxylic acid

Received for publication August 23, 2021; accepted September 16, 2021.

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(TCA) cycle and is also necessary for the function of α-KG-dependent dioxygenases that are involved in epigenetic regulation and DNA repair.\(^2\) In addition, NADPH is one of the most important sources of reducing power in most human tissues and is therefore essential in maintaining cellular redox states.\(^3\) \(^4\) \(IDH1/2\) mutations (\(IDH1/2\)mt) are almost always heterozygous and occur in a hotspot fashion in the enzymatically active sites, for example, \(IDH1^{R132}\), \(IDH2^{R140}\), and \(IDH2^{R172}\), to enable a neomorphic reaction by mutant \(IDH1/2\) (mtIDH1/2) that converts α-KG into D-2-hydroxyglutarate (D-2-HG).\(^5\) Human cells have a very limited capacity to metabolize D-2-HG, which subsequently accumulates to millimolar concentrations and competitively inhibits α-KG-dependent dioxygenases.\(^5\)–\(^9\) Therefore, D-2-HG is considered to be an oncometabolite. In addition, \(IDH1/2\)mt cancer cells have a decreased NADPH production capacity because mtIDH1/2 enzymes have lost the ability to reduce NADP\(^+\) to NADPH but instead oxidize NADPH to NADP\(^+\) to catalyze the conversion from α-KG to D-2-HG.\(^4\)\(^5\)\(^10\) Together, the D-2-HG accumulation and the decreased NADPH production capacity affect the abovementioned plethora of cellular functions, which may all contribute to oncogenesis and have been extensively reviewed before.\(^2\)\(^9\)\(^11\)

\(IDH1/2\)mt are found in multiple cancer types, such as glioma and glioblastoma,\(^10\)\(^12\)\(^13\) chondrosarcoma,\(^14\) cholangiocarcinoma,\(^15\) myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML).\(^16\)–\(^18\) In the origin of these types of cancer, \(IDH1/2\)mt are considered to be inaugural or at least early events.\(^14\)\(^19\)–\(^22\) In the case of MDS and AML, the data are conflicting because it has been postulated that \(IDH1/2\)mt are not necessarily early events in the formation of AML, but rather drive progression from precursor states such as MDS to full-blown AML.\(^18\) In tumors where \(IDH1/2\)mt are indeed inaugural or early genetic events, they are present in all, or at least the large majority of, cancer cells including the CSCs.\(^22\) As a consequence, \(IDH1/2\)mt that occur as early genetic events are important targets for cancer therapy because then all subclones contain the \(IDH1/2\)mt and are sensitive to mtIDH1/2 inhibitors. This appreciation of the contribution of \(IDH1/2\)mt to oncogenesis motivated the development of mtIDH1/2 inhibitors.\(^23\) These small-molecule inhibitors effectively reduce the production of D-2-HG by mtIDH1/2 and are also known as sidenis.\(^24\)\(^25\) Examples include the mtIDH1 inhibitor ivosidenib, which is Food and Drug Administration (FDA)-approved for the treatment of \(IDH1\)mt newly diagnosed (ND), refractory or relapsed (R/R) AML and \(IDH1\)mt previously treated, locally advanced, or metastatic cholangiocarcinoma\(^26\)–\(^29\); the mtIDH2 inhibitor enasidenib, which is FDA-approved for the treatment of \(IDH2\)mt R/R AML\(^30\)\(^31\); the dual mtIDH1/2 inhibitor vorasidenib, which is being investigated for \(IDH1/2\)mt recurrent or progressive glioma\(^32\); and various other mtIDH1/2 inhibitors that are still in clinical trials.\(^33\) In addition to the aforementioned indications, ivosidenib is currently being investigated for the treatment of \(IDH1\)mt glioma and chondrosarcoma in early-phase clinical trials\(^34\)\(^35\) and enasidenib is studied in \(IDH2\)mt MDS.\(^36\) Most clinical trials were conducted with mtIDH1/2 inhibitors as monotherapy, but ivosidenib and enasidenib have also been investigated in combination with intensive chemotherapy or azacitidine for AML.\(^37\)\(^39\)

**IDH1/2mt in Cancer Cell Stemness and Differentiation**

During the early preclinical investigation of the biological effects of mtIDH1/2 and their inhibitors, the increased stemness of \(IDH1/2\)mt cancer cells and its underlying mechanisms were already appreciated. First, several hallmarks of cellular dedifferentiation were observed when \(IDH1/2\)mt were introduced into vitro and in vivo experimental models. Second, preclinical, translational, and clinical studies with mtIDH1/2 inhibitors consistently showed that these agents reverse this stemness and promote cellular differentiation of \(IDH1/2\)mt cancer cells. Both will be discussed in this review.

**Epigenetic Hypermethylation and Cellular Dedifferentiation in mtIDH1/2 Cells**

DNA and histone hypermethylation is a feature that is observed across most investigated types of cancer that frequently contain \(IDH1/2\)mt, such as glioma,\(^40\) chondrosarcoma,\(^41\)\(^42\) cholangiocarcinoma,\(^20\) and AML.\(^16\) Furthermore, genomic hypermethylation and an associated block of differentiation are induced by the expression of mtIDH1/2 or the administration of D-2-HG in cell models related to these types of cancer, such as primary human astrocytes,\(^43\)\(^44\) human and mouse neural stem cells,\(^45\)–\(^47\) human and mouse mesenchymal stem cells,\(^42\)\(^48\) mouse hepatoblasts,\(^49\) and mouse hematopoietic cells,\(^50\)\(^51\) but also human embryonic cells and mouse adipocytes.\(^52\) These findings were associated with an increased number of \(IDH1/2\)mt cancerous stem cells when these mutations were induced in benign stem or progenitor cells of these tissues.\(^16\)\(^42\)\(^44\)\(^46\)–\(^50\)\(^52\)\(^53\) DNA and histone hypermethylation occurs because the accumulated D-2-HG in \(IDH1/2\)mt cells inhibits α-KG-dependent demethylases, such as the DNA demethylase TET2 and the family of histone lysine demethylases (KDMs).\(^6\)\(^16\)\(^43\)\(^52\)
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The need for IDH1/2mt cancer cells to induce epigenetic hypermethylation and suppress TET2 function is emphasized by the finding that in several IDH1/2mt AML patients who were successfully treated with an mtIDH1/2 inhibitor but whose AML subsequently relapsed, a novel TET2 mutation that was not present at baseline emerged and DNA hypermethylation was sustained despite low D-2-HG levels. In addition to epigenetic mechanisms, IDH1/2mt also blocks differentiation via metabolic pathways. For example, mtIDH1 inhibits α-KG-dependent dioxygenases necessary for succinyl-CoA production, a critical component for heme production. The resulting succinyl-CoA deficiency attenuates heme biosynthesis in IDH1mt hematopoietic cells, blocking erythroid differentiation at the late erythroblast stage and the erythroid commitment of hematopoietic stem cells.

Induction of Differentiation by mtIDH1/2

In preclinical mechanistic studies, inhibition of mtIDH1 in primary IDH1mt glioma xenografts in mice induced the expression of genes associated with both astrocytic and oligodendrocytic differentiation (GFAP, AQP4, ATP1A2, PTGDS, and ZBTB16), and this effect was associated with reduced repressive H3K9 and H3K27 methylation on the promoters of these genes. In addition, mtIDH1/2 inhibition in IDH1/2mt AML cells induces differentiation. This was observed in an IDH2mt erythroleukemia cell line, in which erythropoietin (EPO)-induced differentiation to red blood cells was restored by mtIDH2 inhibition, and this was associated with the reversion of DNA and histone hypermethylation. Crucially, treatment of these IDH2mt erythroleukemia cells with an mtIDH2 inhibitor did not induce apoptosis even at high dosages, suggesting that the antileukemic efficacy of mtIDH2 inhibitors is not based on cytotoxicity but on releasing the brakes on differentiation of IDH2mt AML cells that are trapped in the stem and progenitor cell compartment. Similar findings were seen in primary human IDH1mt or IDH2mt AML cells in vitro and in mice xenografts, in which mtIDH1 or mtIDH2 inhibition induced blast differentiation as shown by an increase in cells that were positive for cell surface markers associated with monocytic and granulocytic differentiation (CD11b, CD14, CD15) and intracellular myeloperoxidase (MPO) as marker of maturation into the neutrophilic pathway. Quantitatively, 20 days of mtIDH2 inhibition in primary human IDH2mt AML mouse xenografts induced differentiation of 70% of the human blood cells into the monocytic/macrophage and granulocytic lineages, which was associated with a mild to marked decrease in the percentage of human blasts (2- to 35-fold). Furthermore, functional assays during mtIDH2 inhibition in primary human IDH2mt AML cells in vitro yielded the presence of mature, functional neutrophils with phagocytic activity. Differentiation induction of primary human IDH1mt AML cells by mtIDH1 inhibition was associated with reduced histone trimethylation levels at the H3K4, H3K9, H3K27, and H3K36 loci, as well as reduced global DNA methylation.

However, not all epigenetic effects of IDH1/2mt are reversible. In primary IDH1mt glioma xenografts grown in mice, an mtIDH1 inhibitor induced demethylation of histone markers (see above) without appreciable changes in genome-wide DNA methylation. Furthermore, in some preclinical glioma models, mtIDH1 inhibition had no effect on tumor growth, histone or genome-wide DNA methylation, or the expression of genes associated with stemness or glial differentiation. In addition, long-term studies on inducible IDH1mt in immortalized human astrocytes revealed that mtIDH1 results in progressive and sometimes irreversible accumulation of methylation marks on DNA and histones because the epigenome and transcriptome do not completely return to the original state after the subsequent long-term discontinuation of mtIDH1 expression. In agreement with these findings, mouse xenografts continuously expressing mtIDH1 exhibited a tumor growth rate that was comparable to mouse xenografts with discontinued expression of mtIDH1. On the molecular level, L1CAM, a marker for glioma stem cells, was among the genes that was persistently upregulated despite long-term loss of mtIDH1 expression, and L1CAM is associated with gliomagenesis in xenograft models. In clinical studies with mtIDH1/2 inhibitors in IDH1/2mt AML patients, potent suppression of D-2-HG is sometimes observed without epigenetic demethylation. This phenomenon was associated with primary resistance against mtIDH1/2 inhibitors.

Induction of Differentiation by mtIDH1/2

Inhibitors in Translational Studies

In support of these preclinical findings, induction of differentiation has also been shown in patients with IDH1/2mt malignancies that were treated with mtIDH1/2 inhibitors. Translationally, the induction of differentiation has been most thoroughly studied in the context of enasidenib treatment for IDH2mt R/R AML. Direct and morphologic evidence of myeloid differentiation during enasidenib treatment was shown in a patient with IDH2R140Q R/R AML with trisomy 8 in the majority of myeloblasts, with the persistence of trisomy 8 in promyelocytes and mature granulocytes after 4 weeks of enasidenib treatment.
Of note, ivosidenib or enasidenib induces myeloid differentiation and trilineage hematopoietic recovery in \( IDH1/2 \)mt R/R AML without an intercurrent period of bone marrow aplasia or hypoplasia, which is frequently seen during treatment with cytotoxic chemotherapeutic agents, consistent with differentiation as the mechanism of action.\(^{29–31}\) This is corroborated by molecular data showing that in many \( IDH2 \)mt R/R AML patients achieving a complete remission (CR) on enasidenib, the \( IDH2 \)mt variant allelic frequencies (VAFs) were unchanged between pre-therapy leukemic cells and neutrophils at the time of CR, consistent with the differentiation of \( IDH2 \)mt leukemia cells into mature neutrophils.\(^62\) Of note, some patients do achieve a decrease in \( IDH2 \)mt VAF, and these patients have the most durable responses.\(^30\) This was further confirmed by functional assays, wherein these R/R AML patients in CR on enasidenib had \( IDH2 \)mt-differentiated leukemic neutrophils with intact phagocytic activity, consistent with the restoration of normal granulocyte function.\(^62\) In additional translational analyses in patient samples, flow cytometry–based immunophenotyping analyses revealed that enasidenib treatment promoted differentiation of not only \( IDH2 \)mt cells, but also \( IDH2 \)wt cells into mature cells.\(^63\) The differentiation effect of enasidenib treatment on \( IDH2 \)wt cells probably occurs via the abrogation of paracrine D-2-HG inhibition on differentiation of \( IDH2 \)wt cells. This evidence of differentiation induction by \( mtIDH1/2 \) inhibitors can also be related to decreased DNA hypermethylation after such therapies. Genome-wide methylation is reduced in \( IDH1/2 \)mt AML cells after patients have been treated with \( mtIDH1/2 \) inhibitors, but a gene expression profile associated with AML stemness persisted, suggesting that a complete reversal of the \( mtIDH1/2 \)-induced DNA hypermethylation is not necessary for a clinical response.\(^54\) In addition to these data, a longitudinal study of patients with \( IDH1 \)mt cholangiocarcinoma that was treated with ivosidenib was performed. Before-treatment and on-treatment biopsies were compared, revealing that \( mtIDH1 \) inhibition induced a more differentiated morphology of cholangiocarcinoma cells on H&E staining and the expression of hepatocyte lineage markers \( HNF-4\alpha \), \( FOXA1 \) (\( HNF-3\alpha \)), \( FOXA2 \) (\( HNF-3\beta \)), and \( PPAR\alpha \). These signs of differentiation were associated with a better clinical response to ivosidenib and longer progression-free survival.\(^64\) Translational data from \( IDH1/2 \)mt glioma treated with \( mtIDH1/2 \) inhibitors are not available, probably due to the inherent difficulties of obtaining multiple tumor samples over time in glioma patients.

**Induction of Differentiation by mtIDH1/2 Inhibitors in Patients**

In addition, there is supporting evidence for the induction of differentiation by \( mtIDH1/2 \) inhibitors from the clinical characteristics of these agents in the treatment of \( IDH1/2 \)mt AML. The most tangible clinical proof of this hypothesis is that ivosidenib and enasidenib can induce a differentiation syndrome in ~20% of patients with \( IDH1/2 \)mt AML, which can even be fatal if left untreated. This adverse effect is called IDH-inhibitor-associated differentiation syndrome (IDH-DS) and is a boxed warning for these drugs. Patients and clinicians should therefore be alert for fever, dyspnea, hypoxia, pulmonary infiltrates, pleural or pericardial effusions, rapid weight gain or peripheral edema, hypotension, lymphadenopathy, bone pain, and hepatic, renal, or multiorgan dysfunction. IDH-DS has many clinical similarities to the differentiation syndrome, or retinoic acid syndrome, that is observed during differentiation therapy with all-trans retinoic acid (ATRA) and arsenic trioxide for acute promyelocytic leukemia (APL).\(^65\) Once IDH-DS is suspected, corticosteroid therapy and hemodynamic monitoring should be initiated until symptom resolution.\(^66,67\)

Ivosidenib and enasidenib monotherapy lead to a CR in 19–30% and to any response (CR with or without hematologic recovery, partial remission, or a morphologic remission without hematologic recovery) in 40–55% of patients with \( IDH1/2 \)mt AML, with the highest CR and response rates being achieved in ND AML and the lowest rates in R/R AML (see Table 1).\(^28–31\) Even higher response rates have been observed when \( mtIDH1/2 \) inhibitors were combined with intensive chemotherapy or azacitidine in \( IDH1/2 \)mt ND AML,\(^37–39\) but to be able to isolate the differentiation effects of \( mtIDH1/2 \) inhibition we will focus on studies with \( mtIDH1/2 \) monotherapy. Among transfusion-dependent patients, ivosidenib and enasidenib facilitated transfusion independence for red blood cells and/or platelets in ~40% of patients.\(^28–30\) These transfusion independence rates are considerably higher than the CR rate which supports that the mechanism of action of \( mtIDH1/2 \) inhibitors relies more on the induction of differentiation rather than cytotoxicity. This is also emphasized by the finding that more than half of the patients who had stable disease after 3 months of treatment, but who did not achieve a reduction in blast counts, reached red blood cell transfusion independence,\(^30\) whereas transfusion independence is rarely reached without the achievement of a response in other therapies for AML.

In addition, the median time to first response (~2 months) and the median time to best response (~3–4 months) of ivosidenib and enasidenib are more similar.
to the response patterns of hypomethylating therapy with decitabine, azacitidine, or low-dose cytarabine than with intensive chemotherapy.29–31,39,73,74 This similarity may be relevant because at low doses, hypomethylation agents also mainly work via the induction of differentiation and only limitedly via cytotoxic effects and can require 4–6 months to induce a response.74 Furthermore, IDH1/2 mt VAFs were studied before and during treatment with mtIDH1/2 inhibitors. Responses to mtIDH1/2 inhibitors were observed in patients with clonal IDH1/2 mt and patients with subclonal IDH1/2 mt, suggesting that ancestral or non-ancestral IDH1/2 mt clones are both amenable to respond to mtIDH1/2 inhibitors.33,62,75 This is relevant because in ancestral IDH1/2 mt clones the IDH1/2 mt is likely present in the CSC compartment, whereas in non-ancestral IDH1/2 mt clones the IDH1/2 mt may not be present in the CSC compartment.18 Ivosidenib and enasidenib induced an IDH1/2 mt molecular remission, defined as an IDH1/2 mt VAF below the limit of detection (0.02–0.04%), in ~10–30% of patients, and all of them had a hematologic response, often a CR (~80–100%).28–30 As expected, patients with an IDH1/2 mt molecular remission were more likely to achieve a CR and a significantly longer overall survival than patients without a molecular remission (15 months vs 10 months for ivosidenib and 23 months vs 9 months for enasidenib), suggesting that IDH1/2 mt clearance from all AML cells, including the CSC compartment, is necessary for a deep and durable remission.29,30,54

Table 1. Clinical Efficacy of mtIDH1/2 Inhibitors in Prospective Clinical Trials in Patients With IDH1/2mt Cancer.

| Treatment                     | Patient Population | SD Rate (%) | OR Rate (%) | CR Rate (%) | Ref.                |
|-------------------------------|--------------------|-------------|-------------|-------------|---------------------|
| mtIDH1 inhibitors             |                    |             |             |             |                     |
| Ivosidenib monotherapy        | IDH1 mt ND AML     | 55          | 30          |             | Roboz et al.28      |
| Ivosidenib with azacitidine   | IDH1 mt ND AML     | 78          | 61          |             | DiNardo et al.37    |
| Ivosidenib with 7+3           | IDH1 mt ND AML     | 87          | 68          |             | Stein et al.38      |
| Ivosidenib monotherapy        | IDH1 mt R/R AML    | 42          | 22          |             | DiNardo et al.29    |
| BAY1436032 monotherapy        | IDH1 mt AML        | 15          | 4           |             | Heuser et al.31     |
| Ivosidenib monotherapy        | IDH1 mt cholangiocarcinoma | 51 | 2 | 0 | Abou-Alfa et al.26 |
| Ivosidenib monotherapy        | IDH1 mt glioma     | 67          | 3           | 0           | Mellinghoff et al.34|
| Ivosidenib monotherapy        | IDH1 mt chondrosarcoma | 52 | 0 | 0 | Tap et al.35        |
| mtIDH2 inhibitor              |                    |             |             |             |                     |
| Enasidenib with azacitidine   | IDH2mt ND AML      | 74          | 54          |             | DiNardo et al.39    |
| Enasidenib with 7+3           | IDH2mt ND AML      | 87          | 55          |             | Stein et al.38      |
| Enasidenib monotherapy        | IDH2mt R/R AML     | 40          | 19          |             | Stein et al.30      |
| Enasidenib monotherapy        | IDH2mt MDS         | 53          | 0           |             | Stein et al.36      |
| Vorasidenib monotherapy       | IDH1/2mt glioma    | 73          | 18          | 0           | Mellinghoff et al.32|
| Other agents                  |                    |             |             |             |                     |
| Azacitidine with venetoclax   | IDH1/2mt ND AML, ineligible for 7+3 | 75 | | 75 | DiNardo et al.68    |
| Venetoclax with HMA           | IDH1/2mt ND AML, ineligible for 7+3 | 86 | | 86 | Pollyea et al.69    |
| Venetoclax with HMA/LDAC      | IDH1 mt ND AML, ineligible for 7+3 | 82 | | 82 | DiNardo et al.70    |
| Venetoclax with HMA/LDAC      | IDH2mt ND AML, ineligible for 7+3 | 100 | | 100 | DiNardo et al.70    |
| Venetoclax with LDAC          | IDH1/2mt ND AML, ineligible for 7+3 | 57 | | 57 | Wei et al.71        |
| Venetoclax monotherapy        | IDH1/2mt R/R AML   | 50          | 33          |             | Konopleva et al.72  |

Abbreviations: SD, stable disease; OR, overall response; CR, complete remission (for hematologic malignancies) or complete response (for solid tumors); ND, newly diagnosed; AML, acute myeloid leukemia; 7+3, intensive chemotherapy with 7 days of cytarabine and 3 days of an anthracycline; R/R, relapsed or refractory; MDS, myelodysplastic syndromes; HMA, hypomethylating agent/DNA methyltransferase inhibitor, that is, azacitidine or decitabine; LDAC, low-dose cytarabine.
D-2-HG was reduced by a median of 95% in patients who achieved a response and an almost equal median of 94% in those who did not achieve a response. On the contrary, this discordance between suppression of plasma D-2-HG levels and clinical benefit might be disease-specific to AML because in cholangiocarcinoma, treatment duration, which may be a surrogate for clinical efficacy, appeared to be associated with suppressed plasma D-2-HG levels after one cycle of ivosidenib treatment. In the context of enasidenib, a more potent reduction of plasma D-2-HG levels was achieved in IDH2R140Q than in IDH2R172K R/R AML patients (median −93% vs median −47%). In patients with IDH2R140G R/R AML, the level of plasma D-2-HG reduction by enasidenib was not associated with a clinical response, with plasma D-2-HG reductions of −82%, −44%, and −38% in the abovementioned categories, respectively. These D-2-HG reductions are not complete, not even in patients achieving a CR, because of background D-2-HG production in non-malignant cells by other enzymes than mtIDH1/2. In addition, these data show that near-complete abrogation of D-2-HG production by IDH2mt AML cells is not always sufficient to induce a CR. This suggests that IDH1/2mt AML can develop resistance mechanisms that confer D-2-HG independence or that D-2-HG production is not homogeneously stopped in all malignant cells within IDH1/2mt AML with residual D-2-HG production still occurring in the CSC compartment.

Resistance Mechanisms to mtIDH1/2 Inhibitors

Several mechanisms of primary and secondary resistance to mtIDH1/2 inhibitors have been described. In primary resistance, a patient fails to respond to a therapy. In secondary resistance, relapse occurs after an earlier response to a therapy. In both cases, the CSC compartment may play a crucial role.

With respect to primary resistance to mtIDH1/2 inhibitors, an universal mechanism across various types of IDH1/2mt cancers has not yet been described. In IDH1/2mt AML, baseline co-mutations in genes of the receptor tyrosine kinase (RTK) pathway (NRAS, FLT3, and PTPN11) and hematopoietic differentiation transcription factors (RUNX1, CEBPA, and GATA2) are negatively associated with the occurrence of a response to mtIDH1/2 inhibitors. These findings can be partially extended to IDH1/2mt cholangiocarcinoma, in which gene signatures associated with activated RTK and PI3K/AKT-activity are associated with early progression upon treatment with ivosidenib. In glioma, the presence of genetic alterations in cell cycle pathway genes was associated with a shorter progression-free survival. In addition to these genetic hallmarks of mtIDH1/2-inhibitor primary resistance, leukemia stemness is associated with poor responses to mtIDH1/2 inhibitors in IDH1/2mt AML. In primary samples of patients with IDH1/2mt AML treated with mtIDH1/2 inhibitors, resistance to mtIDH1/2 inhibitors was more frequently observed in patients with versus patients without a hypermethylated genotype with a gene expression profile associated with AML stemness. In addition, a genetic CSC risk score, the LSC17, was a better predictor for response to mtIDH1/2 inhibition than the established and aforementioned molecular risk factors such as cytogenetics, RUNX1 mutation status, or RAS/RTK mutation status.

With respect to secondary resistance to mtIDH1/2 inhibitors, several mechanisms have been described, and they may be more universal across the various types of IDH1/2mt cancers. First, there is the emergence of downstream mutations, such as TET2, BCR, differentiation genes (RUNX1, GATA2, CEPBA), and RTK genes (RAS, FLT3, PTPN11, KIT). Second, IDH1/2mt patients who relapse on mtIDH1/2 inhibitor therapy may do so because of D-2-HG-restoring mutations. Restoration of D-2-HG production during mtIDH1 or mtIDH2 inhibition can result either from a second-site mutation in the inhibited IDH1/2 homolog that prevents binding of the mtIDH1/2 inhibitor or from a process termed isofom switching or homolog switching, in which an IDH2mt emerges during mtIDH1 inhibition or vice versa. In patients with IDH1/2mt R/R AML, 35% of patients who achieved a response on enasidenib developed such a D-2-HG-restoring mutation, with second-site mutations and homolog switching occurring in equal proportions of patients (~20%). Second-site mutations, such as IDH1S280F, IDH1R119P, IDH2Q316E, and IDH2R1319M occur outside of the catalytically active site of the IDH1/2 enzymes and are modeled to directly or indirectly prevent the binding of allosteric mtIDH1/2 inhibitors such as ivosidenib and enasidenib. Among relapsing IDH1/2mt R/R AML patients who exhibited isofrom switching, most developed an IDH2mt that was not yet detected at baseline, either in the same clone that was previously IDH1mt or in a separate clone. A smaller proportion of patients had two co-existing clones, one IDH1mt and one IDH2mt, with the relapse driven by the IDH2mt clone during mtIDH1 inhibition.
A final mechanism of acquired mtIDH1 inhibitor resistance was described in IDH1 mt cholangiocarcinoma in which an IDH1R132C mutation converted to an IDH1R132F mutation, suggesting that the phenylalanine instead of the cysteine at residue 132 confers resistance to ivosidenib.77

Currently, second-generation mtIDH1/2 inhibitors are in development, which may decrease the secondary resistance rates to mtIDH1/2 inhibition. Vorasidenib inhibits both mtIDH1 and mtIDH2 at nanomolar concentrations80 and is currently under investigation for IDH1/2mt glioma.52 Its dual mtIDH1/2 inhibition will likely reduce the possibility for IDH1/2mt cells to escape mtIDH1/2 inhibition via isoform switching. In addition, a novel type of inhibitor that is in preclinical testing does not target the allosteric site, but instead binds the active site of mtIDH1,81 thereby decreasing the possibility for IDH1/2mt to restore their D-2-HG production via a second-site mutation at the allosteric site.

**Combination Therapies With mtIDH1/2 Inhibitors**

Another therapeutic strategy that may increase the clinical benefit of mtIDH1/2 inhibitors or decrease the risk of resistance is combining these drugs with other anti-cancer agents. For example, ivosidenib and enasidenib have been combined with intensive chemotherapy or azacitidine in IDH1/2mt ND AML,37–39 and these strategies achieved higher response rates than ivosidenib or enasidenib monotherapy (see Table 1). This supports the notion that the best clinical outcomes in patients with IDH1/2mt cancer may be achieved by a combination of an mtIDH1/2 inhibitor with another targeted agent or chemotherapy.77–39 Although preclinical evidence suggested that mtIDH1/2 inhibitors decrease the effect of cytotoxic therapy in IDH1/2mt cancer cells,82–84 impressive response rates have been achieved with ivosidenib or enasidenib combined with induction and consolidation chemotherapy in patients with IDH1/2mt ND AML.38 However, this study was not randomized and thus cannot confirm or contradict the aforementioned preclinical warnings against combining mtIDH1/2 inhibitors with cytotoxic therapy. Whether or not adding mtIDH1/2 inhibitors to intensive chemotherapy has clinical benefit in patients with IDH1/2mt ND AML is currently being studied in a randomized clinical trial.38

Combining azacitidine and enasidenib in vitro results in greater reductions in DNA methylation and enhanced EPO-induced erythroid differentiation in an erythroleukemia cell line overexpressing mtIDH2, compared with azacitidine or enasidenib alone. This is consistent with a model wherein enasidenib-induced reactivation of TET DNA demethylase enzymes contributes to azacitidine-induced inhibition of DNA methyltransferase enzymes.85 In agreement with these preclinical results, a high CR rate of 61% was achieved with ivosidenib plus azacitidine in patients with IDH1mt ND AML ineligible for intensive chemotherapy in a non-randomized clinical trial.37 Moreover, in a randomized clinical trial, the CR rate was significantly higher in the enasidenib plus azacitidine group compared with the azacitidine-only group (54% vs 12%).39

Another potential powerful combination is that of an mtIDH1/2 inhibitor and the BCL2 inhibitor venetoclax. IDH1/2mt cancers are sensitive to BCL2 inhibition because D-2-HG accumulation induces BCL2 dependence via inhibition of cytochrome c oxidase, also known as complex IV of the electron transport chain. This effect lowered the threshold to trigger mitochondrial apoptosis upon BCL2 inhibition by venetoclax and rendered IDH1/2mt AML cells 13-fold more sensitive to venetoclax compared with IDH1/2wt AML cells.86 This observation is corroborated by clinical data, in which venetoclax achieved a modest 33% CR rate when administered as monotherapy to IDH1/2mt R/R AML patients,72 but high and durable CR rates (57–100%) when administered in combination with azacitidine, decitabine, or low-dose cytarabine in patients with IDH1/2mt ND AML who were ineligible for intensive chemotherapy (see Table 1).58–71 However, the finding that D-2-HG increases BCL2 dependency may also impose a limitation on combining mtIDH1/2 inhibitors and venetoclax. Suppression of D-2-HG production by an mtIDH1/2 inhibitor may decrease the BCL2 dependency of IDH1/2mt cancer cells and instead facilitate resistance against venetoclax. On the contrary, mtIDH1/2 inhibitor-induced differentiation may further increase venetoclax activity on IDH1/2mt cells by lowering the apoptotic threshold, as has been observed with other agents that promote differentiation.87 In vitro and in vivo experiments with an erythroleukemia cell line and primary AML samples suggested that enasidenib-induced differentiation further sensitizes IDH2mt AML cells to venetoclax, but that venetoclax might be antagonized by enasidenib when it fails to induce differentiation when administered as enasidenib monotherapy. A conundrum here is that in all three primary AML samples studied as mouse xenografts, the combination of enasidenib and venetoclax induced more differentiation as measured by CD15 expression than enasidenib monotherapy alone, even when enasidenib seemed to antagonize the antiproliferative effect of venetoclax.87 Clinical trials on the combination of an mtIDH1/2 inhibitor and venetoclax are currently ongoing.
Other Differentiation Strategies Directly or Indirectly Targeting mtIDH1/2

In addition to mtIDH1/2 inhibitors, other differentiation agents may be used to induce differentiation of IDH1/2mt AML cells. For example, IDH1/2mt induces H3K4 trimethylation of the CEBPA promoter and thereby increases the expression of this transcription factor in AML cells. In the context of normal hematopoiesis and AML, CEBPα expression is associated with the granulocyte-monocytic progenitor step and IDH1/2mt AML cells are therefore locked in this stage. This block in differentiation was associated with a gene expression signature that was enriched for genes responsive to treatment with ATRA, analogous to promyelocytic leukemia/retinoic acid receptor α (PML/RARα)-driven APL. This was supported by experiments in primary IDH1/2mt AML mouse xenografts, in which ATRA-induced differentiation of AML blasts to more differentiated granulocyte-monocytic cells was achieved in a similar fashion as APL, but not primary IDH1/2wt AML xenografts.

As mentioned above, mtIDH1/2 inhibitors can induce transfusion independence for red blood cells and/or platelets in IDH1/2mt AML patients. An interesting observation is that enasidenib can also induce hematopoietic differentiation independently of wtIDH2 or mtIDH2. In IDH2-deficient hematopoietic-progenitor cells, enasidenib but no other mtIDH1/2 inhibitors induced differentiation to mature erythrocytes. This process was mediated by accumulation of protoporphyrin IX, the direct precursor of heme, by virtue of off-target inhibition of enasidenib on the ATP-binding cassette subfamily G member 2 (ABCG2), a transporter highly expressed in erythroid progenitors which is responsible for efflux of protoporphyrin IX. Therefore, enasidenib may be a promising therapeutic agent for improvement of anemia in a wide array of clinical contexts outside of IDH1/2mt cancers. This mechanism is supplementary to the mtIDH1/2-inhibitor-induced restoration of the abovementioned deficiency of succinyl-CoA in IDH1/2mt myeloid progenitor cells, a critical component for heme biosynthesis.

Concluding Remarks and Future Perspectives

IDH1/2mt are attractive therapeutic targets for various reasons, but most prominently because they are early events in oncogenesis and are therefore present in a large proportion of cancer cells, including the CSC compartment. These mutations induce a block in differentiation early in the maturation of cells in glial, cartilaginous, biliary, and myeloid tissues, and mtIDH1/2 inhibitors potently reverse the metabolic effects of mtIDH1/2 and release differentiation of IDH1/2mt cells into more mature cells. Because the mechanism of action is different and/or complementary to that of cytotoxic agents, other hypomethylating agents and other targeted agents such as venetoclax, we envisage that mtIDH1/2 inhibitors can be most efficaciously used in combination with these agents and that multiple classes of drugs can collaborate in the optimal eradication of the CSC compartment and, therefore, long-term remissions for IDH1/2mt cancer patients.

Competing interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

RJM wrote the first draft, and JWW supervised the writing. Both authors have read and approved the manuscript prior to submission.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Dutch Cancer Society (KWF; grant 10460) and the Fondation pour la Recherche Nuovo-Soldati (2019).

Literature Cited

1. Rosen JM, Jordan CT. The increasing complexity of the cancer stem cell paradigm. Science. 2009;324(5935):1670–3. doi:10.1126/science.1171837.
2. Molenaar RJ, Radivojevitch T, Maciejewski JP, van Noorden CJ, Bleeker FE. The driver and passenger effects of isocitrate dehydrogenase 1 and 2 mutations in oncogenesis and survival prolongation. Biochim Biophys Acta. 2014 Dec;1846(2):326–41. doi:10.1016/j.bbagrm.2014.05.004.
3. Atai NA, Renkema-Mills NA, Bosman J, Schmidt N, Rijkeboer D, Tigchelaar W, Bosch KS, Troost D, Jonker A, Bleeker FE, Miletic H, Bjerkvig R, De Witt Hamer PC, Van Noorden CJ. Differential activity of NADPH-producing dehydrogenases renders rodents unsuitable models to study IDH1R132 mutation effects in human glioblastoma. J Histoch Chim. 2011 May;59(5):489–503. doi:10.1369/0022155411400606.
4. Bleeker FE, Atai NA, Lamba S, Jonker A, Rijkeboer D, Bosch KS, Tigchelaar W, Troost D, Vandertop WP, Bardelli A, Van Noorden CJ. The prognostic IDH1(R132) mutation is associated with reduced NADP+-dependent IDH activity in glioblastoma. Acta Neuropathol. 2010 Apr;119(4):487–94. doi:10.1007/s00401-010-0645-6.
5. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM,
Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, Su SM. Cancer-associated IDH1 mutations produce 2-hydroxylutarate. Nature. 2009 Dec 10;462(7274):739–44. doi:10.1038/nature08617.

6. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT, Liu LX, Jiang WG, Liu J, Zhang JY, Wang B, Frye S, Zhang Y, Xu YH, Lei QY, Guan KL, Zhao SM, Xiong Y. Oncometabolite 2-hydroxylutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell. 2011 Jan 18;19(1):17–30. doi:10.1016/j.ccr.2010.12.014.

7. Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkisson S, Losman JA, Joensuu P, Bergmann U, Gross S, Travins J, Weiss S, Looper R, Ligon KL, Verhaak RG, Yan H, Kaelin WG Jr. Transformation by the (R)-enantiomer of 2-hydroxylutarate linked to EGLN activation. Nature. 2012 Feb 15;483(7390):484–8. doi:10.1038/nature10898.

8. Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagg EA, Rose NR, Leung IK, Li XS, Woon EC, van Eijk R, d’Adamo P, van Ruler JW, Boots-Sprenger SH, Wesseling P, Hulsebos TJ. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med. 2009 Sep 10;360(8):765–73. doi:10.1056/NEJMoa0808710.

9. Losman JA, Koivunen P, Kaelin WG Jr. 2-Oxoglutamate-dependent oxygenases in cancer. Nat Rev Cancer. 2020 Dec;20(12):710–26. doi:10.1038/s41568-020-00303-3.

10. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009 Feb 19;360(8):765–73. doi:10.1056/NEJMoa0808710.

11. Pirozzi CJ, Yan H. The implications of IDH mutations for cancer development and therapy. Nat Rev Clin Oncol. 2021 Jun 15;18:645–61. doi:10.1038/s41571-021-00521-0.

12. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008 Sep 26;321(5897):1807–12. doi:10.1126/science.1164382.

13. Molenaar RJ, Verbaan D, Lamba S, Zanon C, Jeuken JW, Boots-Sprenger SH, Wesseling P, Hulsebos TJ, Troost D, van Tilborg AA, Leenstra S, Vandervort WP, Bardelli A, van Noorden CJ, Bleecker FE. The combination of IDH1 mutations and MGMT methylation status predicts survival in glioblastoma better than either IDH1 or MGMT alone. Neuro Oncol. 2014 Sep;16(9):1263–73. doi:10.1093/neuonc/nou065.

14. Pansuriya TC, van Eijk R, d’Adamo P, van Ruler MA, Kuijjer ML, Oosting J, Cleon-Jansen AM, van Oosterwijk JG, Verbeke SL, Meijer D, van Wezel T, Nord KH, Sangiorgi L, Toker B, Liegl-Atzwanger B, San-Julian M, Sciot R, Limaye N, Kindblom LG, Daugaard S, Godfraind C, Boon LM, Vikkula M, Kurek KC, Szuhaï K, French PJ, Bovee JV. Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. Nat Genet. 2011 Nov 06;43(12):1256–61. doi:10.1038/ng.1004.

15. Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, Schenkein DP, Hezel AF, Ancukiewicz M, Liebman HM, Kwak EL, Clark JW, Ryan DP, Deshpande V, Dias-Santagata D, Ellisen LW, Zhu AX, Lafortre AJ. Frequent mutation of isocitrate dehydrogenase-1 (IDH1) and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. Oncologist. 2012;17(1):72–9. doi:10.1634/theoncologist.2011-0386.

16. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanathakumar A, Fernandez HF, Tallman MS, Sun Z, Wolniak K, Peeters JK, Liu W, Choe SE, Fantin VR, Paitetta E, Lowenberg B, Licht JD, Godley LA, Delwel R, Valk PJ, Thompson CB, Levine RL, Melnick A. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell. 2010 Dec 14;18(6):553–67. doi:10.1016/j.ccr.2010.11.015.

17. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, Koboldt DC, Fulton RS, Delehaunty KD, McGrath SD, Fulton LA, Locke DP, Magrini VJ, Abbott RM, Vickery TL, Reed JS, Robinson JS, Wylie T, Smith SM, Carmichael L, Eldred JM, Harris CC, Walker J, Peck JB, Du F, Dukes AF, Sanderson GE, Brummell AM, Clark E, McMichael JF, Meyer RJ, Schindler JK, Pohl CS, Wallis JW, Shi X, Lin L, Schmidt H, Tang Y, Haipek C, Wiechert ME, Ivy J, Kalicki J, Elliott G, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson MA, Baty J, Heath S, Shannon WD, Nagarajan R, Link DC, Walter MJ, Graubert TA, DiPersio JF, Wilson RK, Ley TJ. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med. 2009 Sep 10;361(11):1058–66. doi:10.1056/NEJMoa0903840.

18. Molenaar NJ, Thota S, Nagata Y, Patel B, Clemente M, Przychodzen B, Hirsh C, Viny AD, Hosano N, Bleecker FE, Meggendorfer M, Alpermann T, Shiraishi Y, Chiba K, Tanaka H, van Noorden CJ, Radiroyevitch T, Carraway HE, Makishima H, Miyano S, Sekeres MA, Ogawa S, Haferlach T, Maciejewski JP. Clinical and biological implications of ancestral and non-ancestral IDH1 and IDH2 mutations in myeloid neoplasms. Leukemia. 2015 Mar;29(11):2134–42. doi:10.1038/leu.2015.91.

19. Schumacher T, Bunse L, Pusch S, Sahm F, Wiestler B, Quandt J, Menn O, Osswald M, Oezen I, Ott M, Keil M, Balss J, Rauschenbach K, Grabowska AK, Vogler I, Diekmann J, Trautwein N, Eichmüller SB, Okun J, Stevanovic S, Riemer AB, Sahin U, Friese MA, Beckhove P, von Deimling A, Wick W, Platten M. A vaccine targeting mutant IDH1 induces antitumour immunity. Nature. 2014 Aug 21;512(7514):324–7. doi:10.1038/nature13387.
20. Farshidifar F, Zheng S, Gingrich NC, Newton Y, Shih J, Robertson AG, Hinoue T, Hoadley KA, Gibb EA, Roszik J, Covington KR, Wu CC, Chinbrot E, Stransky N, Hegde A, Yang JD, Reznik E, Sadeghi S, Pedamallu CS, Ojesina AI, Hess JM, Auman JT, Riehe SK, Bowiby R, Borad MJ, Cancer Genome Atlas N, Zhu AX, Stuart JM, Sander C, Akbari R, Cherniack AD, Deshpande V, Mounajied T, Foo WC, Torbenson MS, Klein DE, Laird PW, Wheeler DA, McRee AJ, Bathe OF, Andersen JB, Bardeesy N, Roberts LR, Kwong LN. Integrative genomic analysis of cholangiocarcinoma identifies distinct IDH-mutant molecular profiles. Cell Rep. 2017 Mar 14;18(11):2780–94. doi:10.1016/j.celrep.2017.02.033.

21. Papammanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Tolf F, Bolli N, Gundem G, Van Loo P, Martincorena I, Ganly P, Mudie L, McLaren S, O’Meara S, Raine K, Jones DR, Teague JW, Butler AP, Greaves MF, Ganser A, Dohner K, Schlenck RF, Dohner H, Campbell PJ. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016 Jun 9;374(23):2209–21. doi:10.1056/NEJMoa1516192.

22. Venteicher AS, Tirosh I, Hebert C, Yizhak K, Nettel C, Filbin MG, Hovestadt V, Escalante LE, Shaw ML, Rodman C, Gillespie SM, Dionne D, Luo CC, Ravichandran H, Mylvaganam R, Mount C, Onozato ML, Nahed BV, Wakimoto H, Curry WT, Iafrieti AJ, Rivera MN, Frosch MP, Golub TR, Brastianos PK, Getz G, Patel AP, Monje M, Cahill DP, Rozenblatt-Rosen O, Louis DN, Bernstein BE, Regev A, Suva ML. Decoupling lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq. Science. 2017 Mar 31;355(6332):eaai8478. doi:10.1126/science.aai8478.

23. Popovici-Muller J, Saunders JO, Salituro FG, Travins JM, Yan S, Zhao F, Gross S, Dang L, Yen KE, Yang H, Straley KS, Jin S, Kunii K, Fantin VR, Zhang S, Pan Q, Shi D, Biller SA, Su SM. Discovery of the first potent inhibitors of mutant IDH1 that lower tumor 2-HG in vivo. ACS Med Chem Lett. 2012 Oct 11;3(10):850–5. doi:10.1021/ml300225h.

24. Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, Tsoi J, Clark O, Oldrini B, Komissopoulou E, Kuni K, Pedraza A, Schalm S, Silverman L, Miller A, Wang F, Yang H, Chen Y, Kermetsky A, Rosenblum MK, Liu W, Biller SA, Su SM, Brennan CW, Chan TA, Graeber TG, Yen KE, Mellinghoff IK. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science. 2013 May 3;340(6132):626–30. doi:10.1126/science.1236062.

25. Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E, Straley K, Kermetsky A, Liu W, Gliser C, Yang H, Gross S, Artin E, Saada V, Mylonas E, Quivoron C, Popovici-Muller J, Saunders JO, Salituro FG, Yan S, Murray S, Wei W, Gao Y, Dang L, Dorsch M, Agresta S, Schenkin DP, Biller SA, Su SM, de Botton S, Yen KE. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. Science. 2013 May 3;340(6132):622–6. doi:10.1126/science.1234769.

26. Abou-Alfa GK, Macarulla T, Javle MM, Kelley RK, Lubner SJ, Adeva J, Cleary JM, Catenacci DV, Borad MJ, Bridgewater J, Harris WP, Murphy AG, Oh DY, Whisenant J, Lowery MA, Goyal L, Shroft RT, El-Khoueiry AB, Fan B, Wu B, Chamberlain CX, Jiang L, Gliser C, Pandya SS, Valle JW, Zhu AX. Ivosidenib in IDH1-mutant, chemotherapy-refractory cholangiocarcinoma (ClarIDHy): a multicentre, randomised, double-blind, placebo-controlled, phase 3 study. Lancet Oncol. 2020 Jun;21(6):796–807. doi:10.1016/S1470-2045(20)30157-1.

27. Zhu AX, Macarulla T, Javle MM, Kelley RK, Lubner SJ, Adeva J, Cleary JM, Catenacci DVT, Borad MJ, Bridgewater JA, Harris WP, Murphy AG, Oh DY, Whisenant JR, Lowery MA, Goyal L, Shroft RT, El-Khoueiry AB, Chamberlain CX, Agudo-Fraile E, Choe S, Wu B, Liu H, Gliser C, Pandya SS, Valle JW, Abou-Alfa GK. Final overall survival efficacy results of ivosidenib for patients with advanced cholangiocarcinoma with IDH1 mutation: the phase 3 randomised clinical ClarIDHy trial. JAMA Oncol. 2021 Sep 23. doi:10.1001/jamaoncol.2021.3836.

28. Roboz GJ, DiNardo CD, Stein EM, de Botton S, Mims AS, Prince GT, Altman JK, Arellano ML, Donnellan W, Erba HP, Mannis GN, Pollyea DA, Stein AS, Uy GL, Watts JM, Fathi AT, Kantarjian HM, Tallman MS, Choe S, Dai D, Fan B, Wang H, Zhang V, Yen KE, Kapaislis SM, Hickman D, Liu H, Agresta SV, Wu B, Attar EC, Stone RM. Ivosidenib induces deep durable remissions in patients with newly diagnosed IDH1-mutant acute myeloid leukemia. Blood. 2020 Feb 13;135(7):463–71. doi:10.1182/blood.2019002140.

29. DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, Swords R, Collins RH, Mannis GN, Pollyea DA, Donnellan W, Fathi AT, Pigneur A, Erba HP, Prince GT, Stein AS, Uy GL, Foran JM, Traer E, Stuart RK, Arellano ML, Slack JL, Sekeres MA, Willekens C, Choe S, Wang H, Zhang V, Yen KE, Kapaislis SM, Yang H, Dai D, Fan B, Goldmesser M, Liu H, Agresta S, Wu B, Attar EC, Tallman MS, Stone RM, Kantarjian HM. Durable remissions with ivosidenib in IDH1-mutated relapsed or refractory AML. N Engl J Med. 2018 Jun 21;378(25):2386–98. doi:10.1056/NEJMoa1716984.

30. Stein EM, DiNardo CD, Fathi AT, Pollyea DA, Stone RM, Altman JK, Roboz GJ, Patel MR, Collins R, Flinn IW, Sekeres MA, Stein AS, Kantarjian HM, Levine RL, Vyas P, MacBeth KJ, Tosolini A, Xu Q, Gupta I, Lila T, Risueno A, Yen KE, Wu B, Attar EC, Tallman MS, de Botton S. Molecular remission and response patterns in patients with mutant-IDH2 acute myeloid leukemia treated with enasidenib. Blood. 2020 Feb 13;135(7):463–71. doi:10.1182/blood.2019002140.
32. Mellinghoff IK, Penas-Prado M, Peters KB, Burris HA III, Maher EA, Janku F, Cote GM, de la Fuente M, Clarke JL, Ellingson BM, Chun S, Young RJ, Liu H, Choe S, Lu M, Le K, Hassan I, Steelman L, Pandya SS, Cloughesy TF, Wen PY. Vorosidinib, a dual inhibitor of mutant IDH1/2, in recurrent or progressive glioma; results of a first-in-human phase I trial. Clin Cancer Res. 2021 Aug 15;27(16):4491–9. doi:10.1158/1078-0432.CCR-21-0611.

33. Heuser M, Palmisiano N, Mantzaris I, Mims A, DiNardo C, Silverman LR, Wang ES, Fiedler W, Baldus C, Schwid S, Pardee T, Perl AE, Cai C, Kaufluss S, Lagkadinou E, Rentzsch C, Wagner M, Wilkinson G, Wu B, Jeffers M, Genvresse I, Kramer A. Safety and efficacy of BAY1436032 in IDH1-mutant AML: a phase I study results. Leukemia. 2020 Nov;34(11):2903–13. doi:10.1038/s41375-020-0996-5.

34. Mellinghoff IK, Ellingson BM, Touat M, Maher E, De La Fuente MI, Holdhoff M, Cote GM, Burris H, Janku F, Young RJ, Huang R, Jiang L, Choe S, Fan B, Yen K, Lu M, Bowden C, Steelman L, Pandya SS, Cloughesy TF, Wen PY. Ivosidenib in isocitrate dehydrogenase 1-mutated advanced glioma. J Clin Oncol. 2020 Oct 10;38(29):3398–406. doi:10.1200/JCO.19.02492.

35. Tap WD, Villalobos VM, Cote GM, Burris H, Janku F, Mir O, Beeram M, Wagner AJ, Jiang L, Lu W, B, Choe S, Yen K, Gliser C, Fan B, Agresta S, Pandya SS, Trent JC. Phase I study of the mutant IDH1 inhibitor ivosidenib: safety and clinical activity in patients with advanced chondrosarcoma. J Clin Oncol. 2020 Mar 31;38(10):1693–701. doi:10.1200/JCO.19.02492.

36. Stein EM, Fathi AT, DiNardo CD, Pollyea DA, Roboz GJ, Collins R, Sekeres MA, Stone RM, Attar EC, Frattini MG, Tosolini A, Xu Q, See WL, MacBeth KJ, de Botton S, Tallman MS, Kantarjian HM. Enasidenib in patients with mutant IDH2 myelodysplastic syndromes: a phase 1 subgroup analysis of the multicentre, AG221-C-001 trial. Lancet Haematol. 2020 Apr;7(4):e309–19. doi:10.1016/s41375-020-00494-0.

37. DiNardo CD, Stein AS, Stein EM, Fathi AT, Frankfort O, Schuh AC, Dohner H, Martinelli G, Patel PA, Raffoux E, Tan P, Zeidam AM, de Botton S, Kantarjian HM, Stone RM, Frattini MG, Lersch F, Gong J, Gianolio DA, Zhang V, Franovic A, Fan B, Goldwasser M, Daigle S, Choe S, Wu B, Winker T, Vyas P. Mutant isocitrate dehydrogenase 1 inhibitor ivosidenib in combination with azacitidine for newly diagnosed acute myeloid leukemia. J Clin Oncol. 2021 Jan 1;39(1):57–65. doi:10.1200/JCO.20.01632.

38. Stein EM, DiNardo CD, Fathi AT, Mims AS, Pratz KW, Savona MR, Stein AS, Stone RM, Winer ES, Seet CS, Dohner H, Pollyea DA, McCloskey JK, Odenike O, Lowenberg B, Ossenkoppele GJ, Patel PA, Roshal M, Frattini MG, Lersch F, Franovic A, Nabhan S, Fan B, Choe S, Wang H, Wu B, Hua L, Almon C, Cooper M, Kantarjian HM, Tallman MS. Ivosidenib or enasidenib combined with intensive chemotherapy in patients with newly diagnosed AML: a phase 1 study. Blood. 2021 Apr 1;137(13):1792–803. doi:10.1182/blood.2020007233.

39. DiNardo CD, Schuh AC, Stein EM, Montesinos P, Wei AH, de Botton S, Zeidam AM, Fathi AT, Kantarjian HM, Bennett JM, Frattini MG, Martin-Regueira P, Lersch F, Gong J, Hasan M, Vyas P, Dohner H. Enasidenib plus azacitidine versus azacitidine alone in patients with newly diagnosed, mutant-IDH2 acute myeloid leukemia (AG221-AML-005): a single-arm, phase 1b and randomised, phase 2 trial. Lancet Oncol. 2021 Nov;22(11):1597–608. doi:10.1016/s1470-2241(21)00494-0.

40. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW, Aldape K, Cancer Genome Atlas Research Network. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell. 2010 May 18;17(5):510–22. doi:10.1016/j.ccr.2010.03.017.

41. Nicole R, Ayadi M, Gomez-Brouchett A, Armenoullot L, Banneau G, Elarouci N, Tallegas M, Docouvelaes AV, Aubert S, Redini F, Marie B, Labit-Bouvier C, Reina N, Karanian M, Le Nail LR, Anract P, Gouin F, Larousserie F, de Reynies A, de Pineux I. Integrated molecular characterization of chondrosarcoma reveals critical determinants of disease progression. Nat Commun. 2019 Oct 11;10(1):4622. doi:10.1038/s41467-019-12525-7.

42. Lu C, Venneti S, Akalin A, Fang F, Ward PS, Dematteo RG, Intlekofer AM, Chen C, Ye J, Hamed M, Nafa K, Agaram NP, Cross JR, Khanin R, Mason CE, Healey JH, Lowe SW, Schwartz GK, Melnick A, Thompson CB. Induction of sarcomas by mutant IDH2. Genes Dev. 2013 Sep 15;27(18):1986–98. doi:10.1101/gad.198200.112.

43. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AW, Lu C, Ward PS, Thompson CB, Kaufman A, Guryanova O, Levine R, Heguy A, Viale A, Morris LG, Huse JT, Mellinghoff IK, Chan TA. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature. 2012 Sep 15;483(7390):479–83. doi:10.1038/nature10866.

44. Nicell PV, Schumacher SA, Adams J, van Ruymbeke M, Lee P, Fong J, Chyu J, Le K, Hassan I, Steelman L, Pandya SS, Cloughesy TF, Wen PY. Vorosidinib, a dual inhibitor of mutant IDH1/2, in recurrent or progressive glioma; results of a first-in-human phase I trial. Clin Cancer Res. 2021 Aug 15;27(16):4491–9. doi:10.1158/1078-0432.CCR-21-0611.
Lazaris C, Stafford JM, LeRoy G, Kader M, Dhaliwal J, Bayin NS, Frenster JD, Serrano J, Chiriboga L, Baitalmal R, Nanjagud G, Chi AS, Golfinos JG, Wang J, Karajannis MA, Bonneau RA, Reinberg D, Tsirigos A, Zagzag D, Snuderl M, Skok JA, Neubert TA, Piacentonakis DG. Low-grade astrocytoma mutations in IDH1, P53, and ATRX cooperate to block differentiation of human neural stem cells via repression of SOX2. Cell Rep. 2017 Oct 31;21(5):1267–80. doi:10.1016/j.celrep.2017.10.009.

47. Bardella C, Al-Dalahmah O, Krell D, Al-Qahtani K, Tomkova M, Adam J, Serres S, Lockstone H, Freeman-Mills L, Pfefter I, Sibson N, Goldrin D, Schuster-Boeckler B, Pollard PJ, Soga T, McCullagh JS, Schofield CJ, Mulolland P, Ansorge O, Kriaucionis S, Ratcliffe PJ, Szele FG, Tomlinson I. Expression of Idh1(R132H) in the murine subventricular zone stem cell niche recapitulates features of early gliomagenesis. Cancer Cell. 2016 Oct 10;30(4):578–94. doi:10.1016/j.ccell.2016.08.017.

48. Suikj J, Baelde HJ, Roelofs H, Clenton-Jansen AM, Bovee JV. The oncometabolite D-2-hydroxyglutarate induced by mutant IDH1 or -2 blocks osteoblast differentiation in vitro and in vivo. Oncotarget. 2015 Jun 20;6(17):14832–42. doi:10.18632/oncotarget.4024.

49. Saha SK, Parachoniak CA, Ghanta KS, Fitamant J, Ross KN, Najem MS, Gurumurthy S, Abbay EA, Sia D, Cornella H, Miltiadous O, Walesky C, Deshpande A, Brustle A, Harris IS, Holmes R, Wakeham A, Haight JS, Schofield CJ, Mulkholland P, Ansorge O, Kriaucionis S, Ratcliffe PJ, Szele FG, Tomlinson I. Expression of Idh1(R132H) in the murine subventricular zone stem cell niche recapitulates features of early gliomagenesis. Cancer Cell. 2016 Oct 10;30(4):578–94. doi:10.1016/j.ccell.2016.08.017.

50. Sasaki M, Knobbe CB, Munger JC, Lind EF, Brenner D, Brustle A, Harris IS, Holmes R, Wakeham A, Haight J, You-Ten A, Li WY, Schalm S, Su SM, Virtanen C, Reifenberger G, Ohashi PS, Barber DL, Figueroa ME, Melnick A, Zuniga-Pflucker JC, Mak TW. IDH1(R132H) mutation increases murine hematopoietic progenitors and alters epigenetics. Nature. 2012 Aug 30;488(7413):656–9. doi:10.1038/nature11323.

51. Gu Y, Yang R, Yang Y, Zhao Y, Wakeham A, Li WY, Tseng A, Leca J, Berger T, Saunders M, Fortin J, Gao X, Yuan Y, Xiao L, Zhang F, Zhang L, Gao G, Zhou W, Wang Z, Mak TW, Ye J. IDH1 mutation contributes to myeloid dysplasia in mice by disturbing heme biosynthesis and erythropoiesis. Blood. 2021 Feb 18;137(7):945–58. doi:10.1182/blood.2020007075.

52. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, Edwards CR, Kahanin R, Figueroa ME, Melnick A, Wellen KE, O’Rourke DM, Berger SL, Chan TA, Levine RL, Mellinghoff IK, Thompson CB. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature. 2012 Feb 15;483(7390):474–8. doi:10.1038/nature10860.

53. McKenney AS, Lau AN, Somasundara AVH, Spitzer B, Intliekofer AM, Ahn J, Shank K, Rapaport FT, Patel MA, Papalexi E, Shih AH, Chiu A, Freinkman E, Akbay EA, Steadman M, Nagaraj R, Yen K, Teruya-Feldstein J, Wong KK, Rampal R, Vander Heiden MG, Thompson CB, Levine RL. JAK2/IDH-mutant-driven myeloproliferative neoplasm is sensitive to combined targeted inhibition. J Clin Invest. 2018 Feb 1;128(2):789–804. doi:10.1172/JCI94516.

54. Wang F, Morita K, DiNardo CD, Furudate K, Tanaka T, Yan Y, Patel KP, MacBeth KJ, Wu B, Liu G, Frattini M, Matthews JA, Little LD, Gumbs C, Song X, Zhang J, Thompson EJ, Kadia TM, Garcia-Manero G, Jabbour E, Ravandi F, Bhalla KN, Konopleva M, Kantarjian HM, Andrew Futreal P, Takahashi K. Leukemia stemness and co-occurring mutations drive resistance to IDH inhibitors in acute myeloid leukemia. Nat Commun. 2021 May 10;12(1):2607. doi:10.1038/s41467-021-22874-x.

55. Pusch S, Kraeutsch S, Fischer V, Balss J, Ott M, Schirmer D, Capper D, Sahm F, Eisel J, Beck AC, Jugold M, Eichwald V, Kafuluss S, Panknin O, Rehwinkel H, Zimmermann K, Hillig RC, Gruenther J, Toschi L, Neuhaus R, Haegertab A, Hess-Stumpf H, Baurer M, Wick W, Unterberg A, Herold-Mende C, Platten M, von Deimling A. Pan-mutant IDH1 inhibitor BAY 1436032 for effective treatment of IDH1 mutant astrocytoma in vivo. Acta Neuropathol. 2017 Apr;133(4):629–44. doi:10.1007/s00401-017-1677-y.

56. Machida Y, Nakagawa M, Matsunaga H, Yamasu Y, Ogawara Y, Shimaa Y, Yamagata K, Katsumoto T, Hattori A, Itoh M, SekiT, Nishiyaa Y, Nakamura K, Suzuki a, Imaoka a, Baba D, Suzuki a, Sampaetraan O, Saya H, Ichimurab K, Kitabayashibi K. A potent blood-brain barrier-permeable mutant IDH1 inhibitor suppresses the growth of glioblastoma with IDH1 mutation in a patient-derived orthotopic xenograft model. Mol Cancer Ther. 2020 Feb;19(2):375–83. doi:10.1158/1535-7163.MCT-18-1349.

57. Yen K, Travins J, Wang F, David MD, Artin E, Straley K, Padyana A, Gross S, DeLaBarre B, Tobin E, Chen Y, Nagaraj b, Choe S, Jin L, Konteatis Z, Cianchetta G, Saunders JD, Salituro FG, Quivoron E, Opolon P, Bawa OA, Saada V, Paci A, Broutin S, Bernard OA, de Botton B, Marteyn BS, Pilichowska M, Xu Y, Fang C, Jiang F, Wei W, Jin S, Silverman L, Liu W, Yang H, Dang L, Dorsch M, Penard-Lacronique V, Biller SA, Su SM. AG-221, a first-in-class therapy targeting acute myeloid leukemia harboring oncogenic IDH2 mutations. Cancer Discov. 2017 May 10;7(5):478–93. doi:10.1158/2159-8290.CD-16-1034.

58. Kernytsky A, Wang F, Hansen E, Schalm S, Straley K, Gliser C, Yang H, Travins J, Murray S, Dorsch M, Agresta S, Schenkein DP, Biller SA, Su SM. AG-221, a first-in-class therapy targeting acute myeloid leukemia harboring oncogenic IDH2 mutations. Cancer Discov. 2017 May 10;7(5):478–93. doi:10.1158/2159-8290.CD-16-1034.

59. Cheruvadi A, Herbst L, Pusch S, Klett L, Goparaju R, Stichel D, Kaufuss S, Panknin O, Zimmermann K, Toschi L, Neuhaus R, Haegebarth A, Rehwinkel H,
Hess-Stumpp H, Bauser M, Bochtler T, Struys EA, Sharma A, Bakkali A, Geffers R, Araujo-Cruz MM, Thol F, Gabdoulline R, Ganser A, Ho AD, von Deiming A, Rippe K, Heuser M, Kramer A. Pan-mutant-IDH1 inhibitor BAY1436032 is highly effective against human IDH1 mutant acute myeloid leukemia in vivo. Leukemia. 2017 Oct;31(10):2020–8. doi:10.1038/leu.2017.46.

60. Okoye-Okafor UC, Bartholdy B, Cartier J, Gao EN, Pietrak B, Rendina AR, Rominger C, Quinn C, Smallwood A, Wiggall KL, Reif AJ, Schmidt SJ, Qi H, Zhao H, Jobery G, Faethyl-Savitski M, Bantscheff M, Drewes G, Durauswami C, Brady P, Groy A, Narayananagi SR, Antony-Debre I, Mitchell K, Wang HR, Kao YR, Christopel M, Carvajal L, Barreyro E, Paita E, Makishima H, Will B, Concha N, Adams ND, Schwartz B, McCabe ST, Maciejewski J, Verm A, Steidl U. New IDH1 mutant inhibitors for treatment of acute myeloid leukemia. Nature Chem Biol. 2015 Nov;11(11):878–86. doi:10.1038/nchembio.1930.

61. Tateishi K, Wakimoto H, Iafraje AJ, Tanaka S, Loebel F, Nelnic L, Wiederschain D, Bedel O, Deng G, Zhang B, He T, Shi X, Gerstzen RE, Zhang Y, Yeh JJ, Curry WT, Zhao D, Sundaram S, Nigim F, Koemer MA, Ho Q, Fisher DE, Roider EM, Kemeny LV, Samuels Y, Flaherty KT, Batchelor TT, Chi AS, Cahill DP. Extreme vulnerability of IDH1 mutant cancers to NAD+ depletion. Cancer Cell. 2015 Dec 14;28(6):773–84. doi:10.1016/j.ccell.2015.11.006.

62. Amatangelo MD, Quek L, Shih A, Stein EM, Roshal M, David MD, Marney B, Farnoud NR, de Botton S, Bernard OA, Wu B, Yen KE, Tallman MS, Papamannuel E, Penard-Lacronique V, Thakurta A, Vyas P, Levine RL. Enasidenib induces acute myeloid leukemia cell differentiation to promote clinical response. Blood. 2017 Aug 10;130(6):732–41. doi:10.1182/blood.2016-077944.

63. Quek L, David MD, Kennedy A, Metzner A, Amatangelo M, Shih A, Stoilova B, Quivoron C, Heiblig M, Willekens C, Saada V, Alsafadi S, Vijayabaskar MS, Peniket A, Bernard OA, Agresta S, Yen Y, MacBeth S, Stein E, Vassilious GS, Levine R, De Botton S, Thakurta A, Penard-Lacronique V, Vyas P. Clonal heterogeneity of acute myeloid leukemia treated with the IDH2 inhibitor enasidenib. Nat Med. 2018 Aug;24(8):1167–77. doi:10.1038/s41591-018-0115-6.

64. Aguado-Fraile E, Tassinari A, Isishiy Y, Sigel C, Lowery MA, Goyal L, Gilsen C, Jiang L, Pandya SS, Wu B, Bardeesy N, Choe S, Deshpande V. Molecular and morphological changes induced by idoxifin correlate with efficacy in mutant-IDH1 cholangiocarcinoma. Future Oncol. 2021 Jun;17(16):2057–74. doi:10.2217/fon-2020-1274.

65. Stahl M, Tallman MS. Differentiation syndrome in acute promyelocytic leukaemia. Br J Haematol. 2019 Oct;187(2):157–62. doi:10.1111/bjh.16151.

66. Norsworthy KJ, Mulkey F, Scott EC, Ward AF, Przepiorka D, Charlamb R, Dorff SE, Deerrooth A, Kazandjian D, Sridhara R, Beaver JA, Farrell AT, de Ciaro RA, Pazdur R. Differentiation syndrome with idoxifin and enasidenib treatment in patients with relapsed or refractory IDH-mutated AML: a U.S. Food and Drug Administration systematic analysis. Clin Cancer Res. 2020 Aug 15;26(16):4280–88. doi:10.1158/1078-0432.CCR-20-0834.

67. Fathi AT, Dinardo CD, Kline I, Kenvin L, Gupta I, Attar EC, Stein EM, de Botton S, Investigators ACS. Differentiation syndrome associated with enasidenib, a selective inhibitor of mutant isocitrate dehydrogenase 2: analysis of a phase 1/2 study. JAMA Oncol. 2018 Aug 1;4(8):1106–10. doi:10.1001/jamaoncol.2017.4695.

68. Dinardo CD, Jonas BA, Pullerat V, Thirman MJ, Garcia JS, Wei AH, Konopleva M, Dohner H, Letai F, Fenaux P, Koller E, Havelange V, Leber B, Esteve J, Wang J, Pejza S, Hajej R, Porka K, Illes A, Lavie D, Lemoli RM, Yamamoto K, Yoon SS, Jiang JH, Yeh SP, Turgut M, Hong WJ, Zhou Y, Potluri J, Pratz KW. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. N Engl J Med. 2020 Aug 13;383(7):617–29. doi:10.1056/NEJMoa2012971.

69. Pollyea DA, Pratz K, Letai F, Jonas BA, Wei AH, Pullerat V, Konopleva M, Thirman MJ, Arelano M, Becker PS, Chyla B, Hong WJ, Jiang Q, Potluri J, DiNardo CD. Venetoclax with azacitidine or decitabine in patients with newly diagnosed acute myeloid leukemia: long term follow-up from a phase 1b study. Am J Hematol. 2021 Feb 16;96(2):208–17. doi:10.1002/ajh.26039.

70. DiNardo CD, Tiong IS, Quagliari A, MacRaiil S, Loghavi S, Young FC, Thijssen R, Pomilio G, Ivey A, Salmon JM, Glysou C, Fleming SA, Zhang Q, Ma H, Patel KP, Kornblau SM, Xu Z, Chua CC, Chen X, Blombery P, Flensburg C, Cummings N, Alfantis I, Kantarjian H, Huang DC, Roberts AW, Majewski IJ, Konopleva M, Wei AH. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. Blood. 2020 Mar 12;135(11):791–803. doi:10.1182/blood.2019003988.

71. Wei AH, Montesinos P, Ivanov V, DiNardo CD, Novak J, Lanbi K, Kim I, Stevens DA, Fiedler W, Pagoni M, Samolova O, Hu Y, Anagnostopoulos A, Bergeron J, Hou JZ, Murthy V, Yamauchi T, McDonald A, Chyla B, Gopakalrashnan S, Jiang Q, Mendes W, Hayslip J, Panayiotides P. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: a phase 3 randomized placebo-controlled trial. Blood. 2020 Jun 11;135(24):2137–45. doi:10.1182/blood.2020004856.

72. Konopleva M, Pollyea DA, Potluri J, Chyla B, Hodgal L, Busman T, McKeegan E, Salem AH, Zhu M, Ricker JL, Blum W, DiNardo CD, Kadia T, Dunbar M, Kirby R, Falotico N, Leverson J, Humerickhouse R, Mabry M, Stone R, Kantarjian H, Letai F. Efficacy and biological correlates of response in a phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia. Cancer Discov. 2016 Oct;6(10):1106–17. doi:10.1158/2159-8290.CD-16-0313.
74. Al-Ali HK, Jaekel N, Niederwieser D. The role of hypomethylating agents in the treatment of elderly patients with AML. J Geriatr Oncol. 2014 Jan;5(1):89–105. doi:10.1016/j.jgo.2013.08.004.

75. Choe S, Wang H, DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, Watts JM, Pollyea DA, Fathie AT, Tallman MS, Kantarjian HM, Stone RM, Quek L, Konteatis Z, Dang L, Nicolay B, Nejad P, Liu G, Zhang V, Liu H, Goldwasser M, Liu W, Marks K, Bowden C, Biller SA, Attar EC, Wu B. Molecular mechanisms mediating relapse following ivosidenib monotherapy in IDH1-mutant relapsed or refractory AML. Blood Adv. 2020 May 12;4(9):1894–905. doi:10.1016/bloodadvances.202001503.

76. Ng SW, Mitchell A, Kennedy JA, Chen WC, McLeod J, Ibrahimova N, Arruda A, Popescu A, Gupta V, Schimmer AD, Schuh AC, Yee KW, Bullinger L, Herold T, Gorlich D, Buchner T, Hiddemann W, Berdel WE, Wormann B, Cheok M, Preudhomme C, Dombret H, Metzeler K, Buske C, Lowenberg B, Valk PJ, Zandstra PW, Minden MD, Dick JE, Wang JC. A 17-gene stemness score for rapid determination of risk in acute leukaemia. Nature. 2016 Dec 15;540(7633):433–7. doi:10.1038/nature20598.

77. Lowery MA, Burris HA III, Janku F, Biller SA, Attar EC, Wu B. Molecular mechanisms mediating relapse following ivosidenib monotherapy in IDH1-mutant relapsed or refractory AML. Blood Adv. 2020 May 12;4(9):1894–905. doi:10.1016/bloodadvances.202001503.

78. Molenaar RJ, Botman D, Smits MA, Hira VV, van Lith SA, Stap J, Hennemann P, Khursheed M, Lenting K, Mul AN, Dimitrakopoulou D, van Drunen CM, Hoebe RA, Radiroyevitch T, Wilmink JW, Maciejewski JP, Vandertop WP, Leenders WP, Bleeker FE, van Noorden CJ. Radioprotection of IDH1-mutated cancer cells by the IDH1-mutant inhibitor AGI-5198. Cancer Res. 2015 Nov 15;75(22):4790–802. doi:10.1158/0008-5472.CAN-14-3603.

79. Molenaar RJ, Radiroyevitch T, Nagata Y, Khursheed M, Przychodzen B, Makishima H, Xu M, Bleeker FE, Wilmink JW, Carraway HE, Mukherjee S, Sekeres MA, van Noorden CJF, Maciejewski JP. IDH1/2 mutations sensitize acute myeloid leukemia to PARP inhibition and this is reversed by IDH1/2-mutant inhibitors. Clin Cancer Res. 2018 Apr 1;24(7):1705–15. doi:10.1158/1078-0432.CCR-17-2796.

80. Sulkowski PL, Corso CD, Robinson ND, Scanlon SE, Purshouse KR, Bai H, Liu Y, Sundaram RK, Hegn DC, Fons NR, Breuer GA, Song Y, Mishra-Gorur K, De Feyter PJ. Neoadjuvant IDH1 and 2 for treatment of glioma. ACS Med Chem Lett. 2020 Feb 13;11(2):101–7. doi:10.1021/acsmedchemlett.9b00509.

81. Chaturvedi A, Goparaju R, Gupta C, Weder J, Kluinmann T, Araujo Cruz MM, Kloos A, Goerlich K, Schottmann R, Othman B, Struys EA, Bahre H, Grote-Koska D, Brand G, Ganser A, Preller M, Heuser M. In vivo efficacy of mutant IDH1 inhibitor HMS-101 and structural resolution of distinct binding site. Leukemia. 2020 Feb;34(2):416–26. doi:10.1038/s41375-019-0582-x.

82. Molenaar RJ, Radiroyevitch T, Nagata Y, Khursheed M, Przychodzen B, Makishima H, Xu M, Bleeker FE, Wilmink JW, Carraway HE, Mukherjee S, Sekeres MA, van Noorden CJF, Maciejewski JP. IDH1/2 mutations sensitize acute myeloid leukemia to PARP inhibition and this is reversed by IDH1/2-mutant inhibitors. Clin Cancer Res. 2018 Apr 1;24(7):1705–15. doi:10.1158/1078-0432.CCR-17-2796.

83. Molenaar RJ, Radiroyevitch T, Nagata Y, Khursheed M, Przychodzen B, Makishima H, Xu M, Bleeker FE, Wilmink JW, Carraway HE, Mukherjee S, Sekeres MA, van Noorden CJF, Maciejewski JP. IDH1/2 mutations sensitize acute myeloid leukemia to PARP inhibition and this is reversed by IDH1/2-mutant inhibitors. Clin Cancer Res. 2018 Apr 1;24(7):1705–15. doi:10.1158/1078-0432.CCR-17-2796.

84. Sulkowski PL, Corso CD, Robinson ND, Scanlon SE, Purshouse KR, Bai H, Liu Y, Sundaram RK, Hegn DC, Fons NR, Breuer GA, Song Y, Mishra-Gorur K, De Feyter PJ. Neoadjuvant IDH1 and 2 for treatment of glioma. ACS Med Chem Lett. 2020 Feb 13;11(2):101–7. doi:10.1021/acsmedchemlett.9b00509.

85. Molenaar RJ, Botman D, Smits MA, Hira VV, van Lith SA, Stap J, Hennemann P, Khursheed M, Lenting K, Mul AN, Dimitrakopoulou D, van Drunen CM, Hoebe RA, Radiroyevitch T, Wilmink JW, Maciejewski JP, Vandertop WP, Leenders WP, Bleeker FE, van Noorden CJ. Radioprotection of IDH1-mutated cancer cells by the IDH1-mutant inhibitor AGI-5198. Cancer Res. 2015 Nov 15;75(22):4790–802. doi:10.1158/0008-5472.CAN-14-3603.

86. Molenaar RJ, Radiroyevitch T, Nagata Y, Khursheed M, Przychodzen B, Makishima H, Xu M, Bleeker FE, Wilmink JW, Carraway HE, Mukherjee S, Sekeres MA, van Noorden CJF, Maciejewski JP. IDH1/2 mutations sensitize acute myeloid leukemia to PARP inhibition and this is reversed by IDH1/2-mutant inhibitors. Clin Cancer Res. 2018 Apr 1;24(7):1705–15. doi:10.1158/1078-0432.CCR-17-2796.

87. Sulkowski PL, Corso CD, Robinson ND, Scanlon SE, Purshouse KR, Bai H, Liu Y, Sundaram RK, Hegn DC, Fons NR, Breuer GA, Song Y, Mishra-Gorur K, De Feyter PJ. Neoadjuvant IDH1 and 2 for treatment of glioma. ACS Med Chem Lett. 2020 Feb 13;11(2):101–7. doi:10.1021/acsmedchemlett.9b00509.
87. Cathelin S, Sharon D, Subedi A, Cojocari D, Phillips DC, Leverson JD, MacBeth KJ, Nicolay B, Narayanaswamy R, Ronseaux S, Liu G, Chan SM. Enasidenib-induced differentiation promotes sensitivity to venetoclax in IDH2-mutated acute myeloid leukemia. Leukemia. Epub 2021 Nov 6. doi:10.1038/s41375-021-01468-y.

88. Boutzen H, Saland E, Larrue C, de Toni F, Gales L, Castelli FA, Cathebas M, Zaghdoudi S, Stuani L, Kaoma T, Riscal R, Yang G, Hirsch P, David M, De Mas-Mansat V, Delabesse E, Valler L, Delhommeau F, Jouanin I, Ouelfelli O, Le Cam L, Linares LK, Junot C, Portais JC, Vergez F, Recher C, Sarry JE. Isocitrate dehydrogenase 1 mutations prime the all-trans retinoic acid myeloid differentiation pathway in acute myeloid leukemia. J Exp Med. 2016 Apr 4;213(4):483–97. doi:10.1084/jem.20150736.

89. Dutta R, Zhang TY, Kohnke T, Thomas D, Linde M, Gars E, Stafford M, Kaur S, Nakauchi Y, Yin R, Azizi A, Narla A, Majeti R. Enasidenib drives human erythroid differentiation independently of isocitrate dehydrogenase 2. J Clin Invest. 2020 Apr 1;130(4):1843–9. doi:10.1172/JCI133344.