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REVIEW

Isocitrate dehydrogenase mutations in myeloid malignancies

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Alterations to genes involved in cellular metabolism and epigenetic regulation are implicated in the pathogenesis of myeloid malignancies. Recurring mutations in *isocitrate dehydrogenase* (*IDH*) genes are detected in approximately 20% of adult patients with acute myeloid leukemia (AML) and 5% of adults with myelodysplastic syndromes (MDS). IDH proteins are homodimeric enzymes involved in diverse cellular processes, including adaptation to hypoxia, histone demethylation and DNA modification. The IDH2 protein is localized in the mitochondria and is a critical component of the tricarboxylic acid (also called the 'citric acid' or Krebs) cycle. Both IDH2 and IDH1 (localized in the cytoplasm) proteins catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG). Mutant IDH enzymes have neomorphic activity and catalyze reduction of α-KG to the (R) enantiomer of 2-hydroxyglutarate, which is associated with DNA and histone hypermethylation, altered gene expression and blocked differentiation of hematopoietic progenitor cells. The prognostic significance of mutant *IDH* (m*IDH*) is controversial but appears to be influenced by co-mutational status and the specific location of the mutation (*IDH1*-R132, *IDH2*-R140, *IDH2*-R172). Treatments specifically or indirectly targeted to m*IDH* are currently under clinical investigation; these therapies have been generally well tolerated and, when used as single agents, have shown promise for inducing responses in some m*IDH* patients when used as first-line treatment or in relapsed or refractory AML or MDS. Use of mIDH inhibitors in combination with drugs with non-overlapping mechanisms of action is especially promising, as such regimens may address the clonal heterogeneity and the multifactorial pathogenic processes involved in m*IDH* myeloid malignancies. Advances in mutational analysis have made testing more rapid and convenient, and less expensive; such testing should become part of routine diagnostic workup and repeated at relapse to identify patients who may benefit from treatments that target m*IDH*.

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INTRODUCTION

Acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) are heterogeneous myeloid disorders with multifactorial pathogenic mechanisms and a broad range of prognoses. AML is characterized by clonal proliferation of poorly differentiated cells of the myeloid lineage.[1] MDS reflects the presence of dysplasia and ineffective hematopoiesis commonly leading to bone marrow failure and insufficiency, with a resultant decrease in peripheral blood counts.[2] The pathogeneses of both involve recurrent genomic alterations, including somatic gene mutations and/or chromosomal abnormalities, that can define biologically distinct clinical subtypes.[3] Comprehensive genomic profiling at the time of diagnosis can inform disease classification, risk stratification and prognosis and ultimately allow for more selective therapeutic interventions.

Alterations to cellular metabolism, as well as somatic mutations of genes essential to epigenetic regulation, are implicated in the pathogenesis of several human malignancies.[4,5] Isocitrate dehydrogenases (IDHs) are homodimeric enzymes involved in diverse cellular processes, including adaptation to hypoxia, histone demethylation and DNA modification.[6] The IDH2 protein is a critical component of the tricarboxylic acid (also called the 'citric acid' or Krebs) cycle, and both IDH2 and IDH1 proteins catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) to produce reduced nicotinamide adenine dinucleotide phosphate (NADPH) from $NADP^+$ (Figure 1). Diverse dioxygenases depend on sufficient levels of α-KG for multiple cellular processes, as well as for epigenetic regulation.[7] IDH1 enzymes are localized to the cytoplasm and peroxisomes and IDH2 to the mitochondria.[6]

Somatic mutations in *IDH1* (m*IDH1*) and *IDH2* (m*IDH2*) genes have been described in both solid and hematological malignancies; m*IDH1* is more common in solid tumors and m*IDH2* is more common in hematological tumors.[8] *IDH1/2* mutations are heterozygous, retaining one wild-type (wt) allele, suggestive of an oncogenic gain of function. IDH proteins are encoded by the *IDH1* gene located at chromosome 2q33 and the *IDH2* gene residing at chromosome 15q26.[9] An *IDH3* isoform is also located in the mitochondria, but no oncogenic mutations in the *IDH3* gene have been reported to date.[9] Recurrent *IDH1/2* mutations are missense variants leading to a single amino-acid substitution of arginine residues at codon 132 in exon 4 of the *IDH1* gene and codons 140 or 172 in exon 4 of the *IDH2* gene.[10] Additionally, a germline-synonymous single-nucleotide polymorphism (rs11554137) located in codon 105 in exon 4 of the *IDH1* gene has been reported to have prognostic relevance in AML.[9,11]

IDH MUTATIONS IN AML AND MDS

Mutant IDH enzymes have neomorphic activity, catalyzing NADPH-dependent reduction of α-KG to an oncometabolite, the

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(R) enantiomer of 2-hydroxyglutarate ((R)-2-HG, also called 2-oxoglutarate) *in vitro* and *in vivo*.[12–14] Increased levels of the (S) enantiomer of 2-HG have not been reported in AML or MDS.[15] In an AML xenotransplantation model established from a patient with wt*IDH1* and mutant *nucleophosmin 1* (*NPM1*), (R)-2-HG but not (S)-2-HG acted as an oncometabolite and daily administration of (R)-2-HG was associated with significantly reduced platelet counts and shorter survival than (S)-2-HG-treated mice.[15] High concentrations of (R)-2-HG lead to enhanced proliferation and blocked differentiation of immature hematopoietic cells.[16] Cell lines with endogenous *IDH* mutations (for example, CS-1 chondrosarcoma) or engineered to express mutant IDH proteins (for example, TF-1 human erythroleukemia) show dramatically increased (R)-2-HG levels and impaired cellular differentiation.[7,16,17] Serum from patients with m*IDH* AML contains levels of (R)-2-HG that are more than 100-fold higher than expected under normal physiological conditions.[14,18]

(R)-2-HG is structurally similar to α-KG and has been shown to competitively inhibit α-KG-dependent enzymes, including members of the ten-eleven-translocation (TET) family of 5-methylcytosine hydroxylases and of the jumonji-domain-containing group of histone lysine demethylases.[6,19,20] TET2 protein is thought to be involved in both passive and active DNA demethylation by regulating genome-wide and locus-specific hydroxymethylation.[21] Similarly, histone demethylases regulate chromatin status, enabling activation or inhibition of gene transcription.[22] Inhibition of these epigenetic regulators by (R)-2-HG produces a hypermethylation 'signature', altering gene expression and leading to differentiation arrest of hematopoietic progenitors.[23,24] Figueroa *et al*.[25] evaluated the mutational and epigenetic profiles of 385 AML patients aged <60 years; patients with m*IDH1/2* AML exhibited a global hypermethylation phenotype associated with significant suppression of gene expression compared with patients with wt*IDH1/2* AML.

Although mIDH1 and mIDH2 enzymes both produce (R)-2-HG, they have different enzymatic activities. Cytoplasmic mIDH1 generates less (R)-2-HG than mitochondrial mIDH2 enzymes.[13] This may be due to differences in amounts of α-KG substrate, which is found in greater abundance in the mitochondrion than in the cytoplasm. (R)-2-HG production is enhanced in the presence of mIDH1/wtIDH1 heterodimers, suggesting that the retained wtIDH1 enzyme produces some of the α-KG that is reduced to (R)-2-HG.[26] In contrast, mIDH2 homodimers can produce abundant (R)-2-HG.[13] Mutated IDH2-R172 protein leads to greater accumulation of (R)-2-HG than mIDH2-R140 protein *in vitro*.[17]

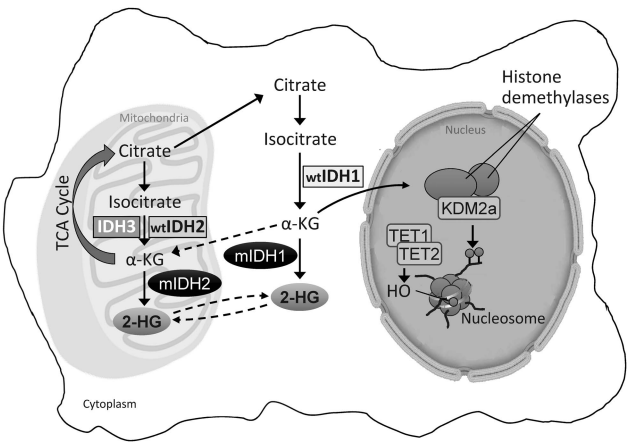

Figure 1. *IDH* mutations in cancer. Mutant IDH1 and IDH2 enzymes result in an increase of the oncometabolite, (R)-2-HG. (R)-2-HG induces a block of cell differentiation by inhibiting the activity of chromatin-modifying histone and DNA demethylases. Inhibition of these epigenetic regulators leads to a 'hypermethylation signature' that alters gene expression such that cells lose the ability to progress from immature progenitors to a fully differentiated state.[23] (Adapted by permission from Macmillan Publishers Ltd: Prensner and Chinnaiyan,[24] copyright 2011).

EPIDEMIOLOGY

Taken together, m*IDH1/2* are among the most common mutations in AML (~20% of patients combined, Table 1). *IDH* mutations increase in frequency with increasing age.[27] m*IDH* are less frequent in MDS (~5%) and myeloproliferative neoplasms, although the frequency increases to ~20% of patients with myeloproliferative neoplasms at leukemic transformation.[25,28,29] Mutant genes involved in epigenetic regulation, including m*IDH*, may exist in preleukemic stem cells, which retain the ability to differentiate into multiple lineages, can survive chemotherapy and proliferate during remission, eventually leading to relapse.[1,18,21,30,31]

IDH1 mutations are less common than *IDH2* mutations in AML and in MDS.[1,8,10,32,33] *IDH1* and *IDH2* mutations only rarely co-occur in the same patient.[34,35] In myeloid malignancies, *IDH1* mutations most often involve a cysteine (R132C) or histidine (R132H) substitution for arginine at R132. *IDH2*-R140 mutations are more common than *IDH2*-R172 mutations, representing ~80% of *IDH2* mutations in AML.[10,32] *IDH2*-R172 mutations may be more frequent in older patients.[10] With *IDH2* mutations, arginine is most often replaced by glutamine at residue 140 (R140Q) and by lysine at residue 172 (R172K).[36] Less frequently, other amino-acid substitutions are involved (for example, *IDH2*-R172W).[10,32]

Clinically, several studies suggest that, compared with wt*IDH*, patients with m*IDH* are older and tend to have higher platelet and bone marrow blast counts at diagnosis of AML or MDS.[10,35,37–39] m*IDH* are enriched in cytogenetically normal AML (CN-AML; 25–30% of CN-AML cases) and are also associated with cytogenetically intermediate-risk disease, and occur often with trisomy 8.[1,39,40] m*IDH* frequently co-occur with *NPM1* mutations but are almost always mutually exclusive with *TET2* mutations and with mutations in the *Wilms' tumor 1* (*WT1*) gene.[1,10,11,38–44] m*IDH* and mutations in *TET2* and *WT1* result in similar epigenetic alterations and may have overlapping roles in leukemogenesis; all block TET2 enzymatic function, resulting in dysregulated DNA methylation.[25,45] m*IDH* are frequently accompanied by mutations in the *serine/arginine-rich- splicing-factor-2* (*SRSF2*) gene, which is associated with abnormal splicing of mRNA.[1,46] These mutations have been reported to cluster in primary myelofibrosis, where they are associated with poorer overall survival (OS) and disease-free survival;[47] further investigation is needed to elucidate interactions between m*IDH* and *SRSF2* mutations in MDS and

Table 1. Frequencies of common recurrent gene mutations in adults with AML or MDS

Mutated gene	*Frequency in AML*[1]	*Frequency in MDS*[104,105]
NPM1	25–35%	2%
DNMT3A	18–22%	8%
FLT3-ITD	~20%	0–2%
TET2	7–25%	11–26%
IDH2	8–19%	~5%
AXLS1	5–17%	11–15%
RUNX1	5–15%	4–14%
NRAS	~15%	3–6%
IDH1	7–14%	3%

Abbreviations: AML, acute myeloid leukemia; MDS, myelodysplastic syndromes.

AML. Co-occurrence of m*IDH* and *Fms-related tyrosine kinase 3* (*FLT3*) mutations are less common.[44] Marcucci *et al.*[10] reported clinical outcomes for 358 patients with *de novo* CN-AML; results showed that patients with m*IDH1* were less likely to have *FLT3*-internal tandem duplication (*FLT3*-ITD). Interestingly, compared with other types of m*IDH*, m*IDH2*-R172 is less likely to be accompanied by additional frequently recurring mutations in AML (for example, *FLT3*-ITD, *CCAAT/enhancer-binding-protein-alpha (CEBPA)* or *NPM1*).[10,40,43]

PROGNOSIS

The prognostic impact of m*IDH1/2* in AML remains controversial. Several studies have suggested an association with adverse outcomes,[10,37,38,48,49] whereas others have failed to identify any clear influence on clinical response or survival,[11,35,50,51] and still others report improved survival (Table 2).[40,44] A meta-analysis that included 8121 patients with AML showed that those with m*IDH1* had inferior OS compared with patients without the mutation, and patients with m*IDH1* CN-AML had a lower rate of complete remission (CR) with cytotoxic induction chemotherapy.[48] Indeed, a preponderance of studies suggest m*IDH1* AML confers an adverse prognosis or has no prognostic value (Table 2). One study found no influence of m*IDH1*-R132 on OS, but the *IDH1* single-nucleotide polymorphism rs11554137 variant was associated with an adverse prognosis.[11] Analyses of the prognostic impact of *IDH2* mutations also show inconsistent results; for example, in one study of patients with CN-AML, m*IDH2* had no effect on OS or CR rate compared with wt*IDH2*,[52] but in another study, m*IDH2* was associated with lower rates of CR, higher relapse rates and shorter OS.[43]

Differences in prognostic findings may reflect variations in study methodologies. Some studies evaluate m*IDH* by point mutation and others combine m*IDH1/2* for analysis.[49,51,53] Specifically, with regard to m*IDH2*, R172 and R140 mutations are frequently analyzed together, although data suggest that these mutations have different effects on prognosis (Figure 2).[1,10,44,54–56] Recently, Papaemmanuil *et al.*[56] proposed new genomic classifications for AML, including (provisionally) 'AML with m*IDH2*-R172' as a distinct class, and recommended that *IDH2* testing be added to prognostic guidelines. In their study of 1540 AML patients, AML with m*IDH2*-R172 was not accompanied by other class-defining lesions (for example, *FLT3*, *NPM1*) and was associated with gene-expression and DNA-methylation profiles seen with more severe abnormalities in metabolic activity, compared with other *IDH* mutations. Nevertheless, unlike earlier reports,[10,54,55] investigators found m*IDH2*-R172 to have a relatively favorable effect on AML prognosis.[56] Mutational context may influence AML prognosis, but again, this is unclear. For example, there is conflicting evidence regarding prognosis of patients with m*IDH1/2* in the presence of *NPM1* mutations and *FLT3*-ITD^{-}; with some data suggesting a better prognosis and others reporting worsened outcomes or no influence of this mutational profile.[35,38,44,49,51,55,57] Patel *et al.*[44] conducted a large study ($N = 398$) to determine the prognostic relevance of frequent somatic mutations in younger patients (< 60 years) with AML, which showed a favorable effect of co-occurring mutated *NPM1* and m*IDH1* or m*IDH2*. These data were further supported using a proposed integrated prognostic model that combined cytogenetic risk and mutational status using retrospective data from younger patients with newly diagnosed *de novo* AML.[58] In this model (which has not yet been validated clinically), the presence of co-occurring *IDH1/2* and *NMP1* mutations in patients with intermediate-risk cytogenetics per Medical Research Council criteria was associated with median OS comparable to that of patients with Medical Research Council-defined favorable cytogenetic risk.[58] In contrast, Paschka *et al.*[38] reported that the subset of patients with CN-AML with a m*IDH*/*NPM1*$^{+}$/*FLT3*-ITD^{-} genotype in their study had significantly poorer

Table 2. *IDH* mutations in AML: frequency and clinical outcomes

	All m*IDH*		m*IDH1-R132*		m*IDH2 (all)*		m*IDH2-R140*		m*IDH2-R172*	
Study	*Frequency*	*Prognosis*	*Frequency*	*Prognosis*	*Frequency*	*Prognosis*	*Frequency*	*Prognosis*	*Frequency*	*Prognosis*
Abbas *et al.*[51] ($N = 893$)	17%	↔ OS	6%	↔ OS	11%	↔ OS	8.3%	↔ OS	2.6%	↔ OS
Aref *et al.*[37a] ($N = 211$)	19%	↓ OS ↔ CR	8.5%	NR	10.4%	NR	9.5%	NR	1%	NR
Boissel *et al.*[43] ($N = 520$)	NR	NR	9.6%	↑ RR[a]↓OS[a]	NR	NR	NR	NR	3.0%	↑ RR[a]↓OS[a]
Chotirat *et al.*[50] ($N = 230$)	19.1%	↔ OS	8.7	↔ OS	10.4%	↔ OS	8.7%	↔ OS	1.7%	↔ OS
Chou *et al.*[40] ($N = 446$)	18.2%	↑ OS (trend)	6.1%	↓ OS (trend)	12.1%	↑ OS ↔ DFS ↔ RFS	9.2%	NR	2.9%	NR
DiNardo *et al.*[35] ($N = 826$)	20%	↔ OS ↔ CR	7.1%	↔ OS ↔ CR	12.8%	↔ OS ↔ CR	10%	NR	2.7%	NR
Feng *et al.*[48] ($N = 8121$)	NR	NR	4.4–9.3%	↓ OS ↔ CR↓CR[a]	NR	NR	NR	NR	NR	NR
Green *et al.*[55] ($N = 1473$)	17%	NR	7%	↔ OS	10%	NR	8%	↑ OS	2.0%	↓ OS
Marcucci *et al.*[10a] ($N = 358$)	33.0%	NR	13.7%	↔ OS↓DFS	19.3%	↔ OS↓CR	15.6%	↔ OS	3.6%	↓ CR ↔ OS
Paschka *et al.*[38] ($N = 805$)	16%	↓ OS in NPM1^{mut}/no FLT3-ITD^{wta}	7.6%	↔ OS	8.7%	↔ OS	6.0%	↔ OS	2.7%	↔ OS
Patel *et al.*[42] ($N = 398$)	14.1%	↑ OS in NPM1^{mut}/FLT3-ITD^{wta}	5.8%	↑ OS	8.3%	↑ OS	6.0%	↑ OS	2.3%	NR
Ravandi *et al.*[57] ($N = 358$)	30%	↔ OS ↔ CR ↔ EFS	7%	↔ OS[b] ↔ CR ↔ EFS	14%	↔ OS ↔ CR ↔ EFS	NR	NR	NR	NR
Schnittger *et al.*[106] ($N = 1414$)	NR	NR	6.6%	↓ OS (trend)↓EFS↑RR	NR	NR	NR	NR	NR	NR
Thol *et al.*[52a] 153 MDS, 53 AML	NR	NR	NR	NR	12.1%	↔ OS ↔ CR	11%	NR	1.1%	NR
Wagner *et al.*[11a] ($N = 275$)	NR	NR	10.9%	↔ OS[c] ↔ CR ↔ RFS	NR	NR	NR	NR	NR	NR
Willander *et al.*[9] ($N = 189$)	21.7%	NR	7.9%	↔ OS[c]	13.7%	NR	11.1%	↓ OS	2.6%	↑ OS
Yamaguchi *et al.*[49] ($N = 233$)	16.7%	↓ OS↓CR ↔ RFS	8.6%	NR	8.2%	NR	7.3%	NR	0.9%	NR

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; DFS, disease-free survival; EFS, event-free survival; IDH, isocitrate dehydrogenase; m*IDH*, mutant *IDH*; mut, mutation; NR, not reported; OS, overall survival; RFS, relapse-free survival; RR, relapse rate; wt, wild type; '↔', no effect; ↑, improved; ↓, worsened. [a]Limited to cytogenetically normal AML. [b]*IDH1* G105 single-nucleotide polymorphism (SNP) had no effect on OS. [c]*IDH1* SNP rs11554137 was an adverse prognostic factor for OS.

OS than patients with m*IDH* CN-AML who did not have that genotype. Generally, m*IDH2*-R140 is much more likely to be accompanied by mutated *NPM1* or other frequently recurring mutation in AML or MDS than m*IDH2*-R172.[10,55,56]

IDH1/2 mutations appear to have a more consistently negative prognostic impact in MDS and myeloproliferative neoplasms than in AML.[59,60] A study of 193 patients with MDS showed that m*IDH1* was associated with shorter OS compared with patients with wt*IDH* and greater likelihood of leukemic transformation (67% vs 28%, respectively).[59] These findings were confirmed by results of a meta-analysis of seven studies that included a total of 1782 MDS patients.[60] The relative prognostic impact of type of *IDH* mutation in MDS is uncertain; Bejar *et al.*[61] reported that, based on survival data for 3200 patients with MDS, m*IDH2* was associated with significantly shorter OS, whereas m*IDH1* had only a marginal effect on OS. Other studies suggest m*IDH1* confers worse prognosis than m*IDH2* in MDS.[59,62] The prognostic influence of m*IDH* type may depend on patients' overall prognostic risk status.[46,61] Lin *et al.*[46] reported that m*IDH2* was a poor prognostic factor in patients with lower-risk MDS, based on International Prognostic Scoring System, revised International Prognostic Scoring System, French–American–British classification or World Health Orgaanization classification, but not in the higher-risk groups.

Serum (R)-2-HG concentration may also serve as a prognostic indicator.[63] Of 234 patients with CN-AML, a subgroup of patients who met an (R)-2-HG threshold at diagnosis that the investigators established as 'high' (>2.01 μg/ml, $\log_2$) were less likely to attain CR and had significantly poorer OS than patients in the 'normal' (R)-2-HG group (Figure 3). Posttreatment (R)-2-HG levels may also have prognostic implications. In a study of 223 younger patients with *de novo* AML treated with standard induction chemotherapy including 62 patients with m*IDH*, patients in CR who had higher serum levels of (R)-2-HG had poorer OS than patients in CR with lower levels of (R)-2-HG.[64] This study showed that (R)-2-HG levels were not significantly different among the different mutation types (m*IDH1*, m*IDH2*-R140 or m*IDH2*-R172); however, there was a trend for poorer OS for the small number of patients ($n=9$) with m*IDH2*-R172.[64] A different study also found a significant quantitative relationship between (R)-2-HG level and posttreatment clinical outcomes: serum (R)-2-HG concentrations of $\geqslant 2$ μmol/l were associated with poorer OS and disease-free survival.[65] In the latter study, *IDH2*-R172 mutations were associated with significantly higher levels of (R)-2-HG compared with the other *IDH* mutation types.

Further investigation is needed to more clearly elucidate the relationship between (R)-2-HG levels and clinical outcomes.

DETECTION

Because *IDH* mutations occur in approximately one in five patients with AML, and even more frequently in patients with CN-AML, mutational testing should be part of routine molecular assessment at diagnosis to identify patients who may in time benefit from targeted treatments currently under clinical study.[37] Identification of these mutations at diagnosis may also be pivotal for better risk stratification of MDS patients.[60]

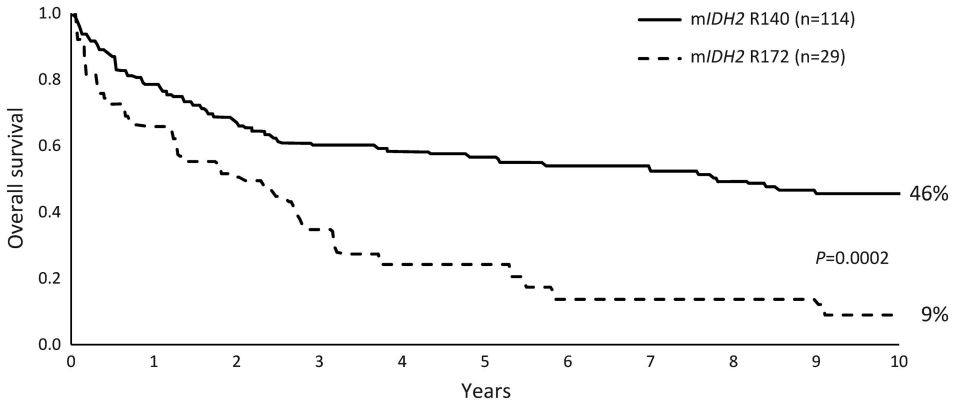

Figure 2. Location of *IDH2* mutation may influence prognosis in AML. OS in 148 adult patients with *IDH2*-mutation-positive AML treated in two Medical Research Council (MRC) trials[55] (Republished with permission of the American Society of Hematology; from Green *et al.*[55]

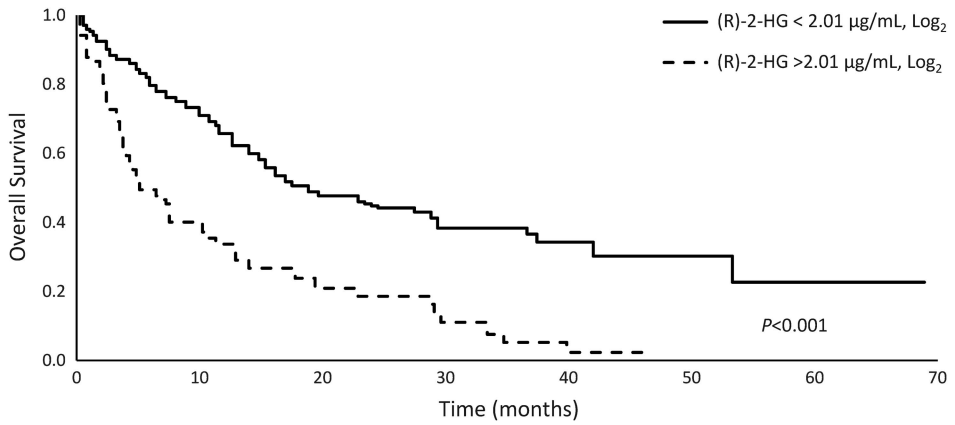

Figure 3. (R)-2-HG level may serve as a biomarker of prognosis and treatment effects. OS in patients with cytogenetically normal AML with high or normal levels of (R)-2-HG[63] (Adapted from Wang *et al.*[63]).

Testing for m*IDH* is straightforward, given that nearly all *IDH* mutations are located on exon 4, and affect IDH1 at a single residue, Arg132, or IDH2 at two residues, Arg140 and Arg172.[23] Several methods,[66–68] including PCR, Sanger or next-generation sequencing, are commonly used for m*IDH* detection.

High-resolution melting (HRM) analysis is a rapid, sensitive and cost-effective method of genotyping and mutational analysis.[69] HRM detects sequence differences that change the shape of the melting curve of DNA. A comparison between Sanger sequencing and HRM analysis showed 99–100% concordance of mutation detection but much greater sensitivity with the HRM technique, which detected mutations in samples diluted to only 10% of the mutated DNA.[69] As a heterozygous mutation, the highest detectable *IDH* variant allele fraction (VAF) is 50% and a recent report on 664 adult AML patients by Metzeler *et al.*[70] indicated that, for patients with m*IDH2*, VAF, on average, approached 50%. At this time, there is no established or standard VAF threshold to identify m*IDH* as a leukemogenic driver at diagnosis. A too-low VAF positivity threshold (for example, $< 2\%$) may have ambiguous clinical relevance and could be a signal of clonal hematopoiesis of indeterminate potential (CHIP). Prevalence of CHIP, a hematological malignancy-associated somatic mutation in the absence of other diagnostic features of MDS or AML, increases with age. Despite relatively high prevalence in older patients, the presence of leukemia-associated mutations is followed by a hematological malignancy in only a minority of cases.[71]

Many hospitals, particularly those affiliated with academic medical centers, perform mutational analyses with next-generation sequencing using MDS/AML gene panels. Private laboratories also perform these multiplex panels for diagnostic and prognostic purposes. A list of laboratories that conduct mutational analyses is available on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/gtr/).

IDH mutations detected at diagnosis tend to be stable during disease progression.[18,46] Sequential assessment of 151 patients with MDS demonstrated that all m*IDH* patients retained the mutation during disease evolution, while none of the wt*IDH* patients acquired an *IDH* mutation during follow-up.[46] However, variations in detection limits or expansion of the mutant clone over time can account for the presence of seemingly new mutations at relapse not previously detected at diagnosis. In one study, 5.7% of MDS patients were identified as having an *IDH* mutation at diagnosis, whereas 11.3% of patients had an *IDH* mutation at the time of leukemic transformation, demonstrating the value of comprehensive molecular profiling at disease progression.[39]

IDH1/2 mutations may also be suitable molecular markers of minimal residual disease with standard intensive chemotherapy approaches.[23] Paired diagnosis and relapse samples demonstrated that m*IDH1/2* cells can survive induction chemotherapy and contribute to relapse.[18] A study of patients with *NPM1*-mutant AML with concurrent *IDH1/2* ($n = 17$) or *DNMT3*A ($n = 15$) mutations revealed that *IDH1/2* mutations were reliable markers of minimal residual disease for 16 of the 17 patients: 7 of the 8 patients with detectable m*IDH1/2* in CR eventually relapsed, whereas all 9 patients with undetectable m*IDH1/2* remained in CR for the duration of the study.[72] This is distinct from treatment with targeted small-molecule mIDH inhibitors, where emerging data demonstrate CR in the setting of alleviation of maturation arrest–without chemo-ablation or destruction of the mutant clone.[73,74] Mutational persistence during remission and the potential that a low VAF is indicative of CHIP in older patients (a VAF threshold of $\geqslant 2\%$ in peripheral blood has been proposed as a diagnostic criterion of CHIP[75]) complicate the use of m*IDH* as a marker of minimal residual disease.

Supranormal levels of (R)-2-HG may serve as a noninvasive biomarker of *IDH* mutations.[64,65,76] (R)-2-HG in the serum or plasma can be measured by liquid or gas chromatography coupled with mass spectrometry. Additionally, there is an enzymatic (R)-2-HG assay based on conversion of (R)-2-HG to α-KG in the presence of (R)-2-hydroxyglutarate dehydrogenase and nicotinamide adenine dinucleotide (NAD^+) and subsequent detection of generated NADH. This assay was shown to distinguish between (R)-2-HG levels in tumor tissue of patients without m*IDH* and levels in patients with m*IDH*-positive AML.[77] Currently, no diagnostic or therapeutic (R)-2-HG 'threshold' level has been formally established; however, a discriminatory concentration of 700 ng/ml of (R)-2-HG in the serum has been proposed to identify patients with *IDH* mutations (serum (R)-2-hydroxyglutarate in healthy control subjects was < 200 ng/ml in this study).[64] At (R)-2-HG levels $\geqslant 700$ ng/ml, *IDH* mutations were detected that were previously missed by Sanger sequencing. Notably, the optimum compartment in which to measure (R)-2-HG has not been determined, although studies are underway to answer this question.[27,78]

TREATMENT

At this writing, there are no approved selective mIDH inhibitor drugs, and consistent with non-m*IDH* myeloid malignancies, treatment decisions are based on patients' age, performance status, use of prior treatment and other clinicopathological factors.[35] However, the treatment landscape may soon include targeted mIDH enzyme inhibitors and drugs that indirectly target m*IDH* leukemic cells. Multiple mIDH inhibitors are in preclinical stages of investigation, including HMS-101, which was shown to reduce (R)-2-HG and block colony formation in m*IDH1* human AML cells *in vitro;*[79] AGI-026, which reduced (R)-2-HG and was associated with improved survival in a m*IDH2*-R140 mouse model of R-2-hydroxyglutaric aciduria *in vivo;*[80] and AGI-5198, which reduced (R)-2-HG and induced apoptosis of m*IDH1* human chondrosarcoma cells *in vitro*.[81] In addition, several agents are now in various stages of clinical development (Table 3; includes ClinicalTrial.gov study registration information).

Induction chemotherapy

Induction chemotherapy has been the most commonly reported treatment for all AML patients who can tolerate such therapy, including those with m*IDH*. Compared with patients with wt*IDH*, rates of response and OS for m*IDH* patients treated with induction chemotherapy mirror general prognosis, that is, there are reports that outcomes are no different from[35,57] or worse than[10] those of patients with wt*IDH* or are dependent on the presence of *NPM1* or other co-mutations.[38,44] In a large retrospective study of patients with AML treated at a single site, patients with m*IDH* who received front-line induction or salvage chemotherapy had response rates comparable to those of patients with wt*IDH* regardless of co-mutational status.[35] In contrast, in the study by Marcucci *et al.*[10] patients with CN-AML and m*IDH1*/*NPM1*$^+$/*FLT3*-ITD$^-$ genotype had significantly shorter postinduction disease-free survival, and m*IDH2*-R172 patients were significantly less likely to attain CR, than wt*IDH* patients.

Hypomethylating agents (HMAs)

Because hypermethylation is a pathogenic hallmark of m*IDH1/2* in myeloid malignancies, there is a theoretical rationale for treatment with an HMA. Approximately 30–50% of all AML and MDS patients who receive an HMA attain a hematological response of some type.[82–84] However, evidence of increased effectiveness in patients with m*IDH* has been equivocal.[85,86] A retrospective cohort study that included 11 patients with m*IDH* MDS revealed that hypomethylating therapy with decitabine was associated with more favorable outcomes than chemotherapy or best supportive care.[60] In contrast, a study of 68 older patients (⩾60 years) with *de*

Table 3. Drugs currently in clinical development for treatment of m*IDH* AML and MDS

Drug	*Mechanism of action*	*Clinical phase*	*Patient type*	*Clinical activity*	*Registration*[a]
AG-221	Small-molecule allosteric inhibitor of mIDH2 protein; reduces the oncometabolite, 2-HG	2 and 3	Patients with R/R AML (phase 2); older patients (⩾60 years) with m*IDH2* R/R AML after second- or third-line therapy (phase 3)	41% ORR in phase 1 dose-escalation and expansion study[70]	NCT01915498 https://www.clinicaltrials.gov/ct2/show/NCT01915498?term=NCT01915498&rank=1NCT02577406 https://clinicaltrials.gov/ct2/show/NCT02577406
AG-120	Small-molecule allosteric inhibitor of mIDH1 protein; reduces the oncometabolite, 2-HG	2	m*IDH1* advanced hematological malignancies	35% ORR in phase 1 dose-escalation and expansion study[71]	NCT02074839 https://clinicaltrials.gov/ct2/show/NCT02074839?term=NCT02074839&rank=1
AG-881	Small-molecule mIDH1 and mIDH2 protein inhibitor; reduces the oncometabolite, 2-HG	1	m*IDH1* and m*IDH2* relapsed or refractory advanced hematological malignancies—after prior m*IDH* inhibitor failure	Unknown	NCT02492737 https://clinicaltrials.gov/ct2/show/NCT02492737?term=NCT02492737&rank=1
IDH305	Small-molecule m*IDH1* inhibitor	1	m*IDH1* advanced malignancies	Unknown	NCT02381886 https://clinicaltrials.gov/ct2/show/NCT02381886?term=NCT02381886&rank=1
FT-2102	No description available	1/1b	AML or high-risk MDS with m*IDH1*; under evaluation as monotherapy and in combination with azacitidine	Unknown	NCT02719574 https://clinicaltrials.gov/ct2/show/NCT02719574
ABT-199	Small-molecule BCL-2 inhibitor; works via synthetic lethality; that is, m*IDH* cells require BCL-2 to survive	2	R/R AML and patients unfit for chemotherapy	15.5% ORR (5/32); antileukemic activity (reduction of BM blasts >50%) was shown in 6/11 (54%) patients with m*IDH* AML[89]	NCT02203773 https://clinicaltrials.gov/ct2/show/NCT02203773?term=NCT02203773&rank=1 NCT02287233 https://clinicaltrials.gov/ct2/show/NCT02287233?term=NCT02287233&rank=1
CB-839	Glutaminase inhibitor	1	R/R AML and older patients (⩾60 years) unfit for IC (also includes patients with ALL)	Preliminary data showed 2/16 evaluable patients attained CRi[92] (prespecified end point to evaluate response in m*IDH* patients)	NCT02071927 https://clinicaltrials.gov/ct2/show/NCT02071927?term=NCT02071927&rank=1

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; ATRA, all-*trans* retinoic acid; BM, bone marrow; Comb, combination; CRi, complete remission with incomplete hematological recovery; MDS, myelodysplastic syndromes; m*IDH*, mutant isocitrate dehydrogenase; ORR, overall response rate; R/R, relapsed or refractory; 2-HG, 2-hydroxyglutarate. [a]ClinicalTrials.gov registration number.

novo, secondary or therapy-related AML found no association between clinical outcomes with front-line HMA treatment, with or without concomitant histone deacetylase inhibitor therapy, in the presence of m*IDH1/2*.[86] Similarly, in a retrospective study of 826 AML patients, 175 patients received front-line therapy with an HMA-based regimen. Of them, 48 m*IDH1/2* and 127 wt*IDH* AML patients showed no significant differences in overall response rate (ORR; 45% vs 58%, $P = 0.13$) or median OS (9.5 vs 10.3 months, $P = 0.8$).[35] The equivocal efficacy of HMAs in m*IDH* AML may be related to leukemogenic effects of excess (R)-2-HG other than hypermethylation of histone and DNA. Cytochrome *c* oxidase, an enzyme in the mitochondrial electron transport chain, is inhibited by (R)-2-HG and was associated with lowering the apoptotic threshold of THP-1 leukemia cells *in vitro*, making them dependent on the antiapoptotic effects of B-cell CLL/lymphoma (BCL-2).[18] However, Heuser *et al*.[87] predicted that an HMA in combination with an mIDH inhibitor may enhance and accelerate therapeutic response. Clinical trials of combinational therapy with the HMA, azacitidine, and small-molecule mIDH enzyme inhibitors are currently underway (see below).

Small-molecule mIDH inhibitors

Selective small-molecule mIDH1 and mIDH2 inhibitors are in clinical development;[8,79,88] these drugs bind within the active catalytic site of mIDH enzymes and prevent the conformational change necessary for mIDH to reduce α-KG to (R)-2-HG.[89–91] In preclinical studies, AGI-6780, a selective inhibitor of mIDH2-R140Q, was shown to rapidly reduce histone hypermethylation and reverse DNA hypermethylation over the course of weeks in TF-1 human erythroleukemia cells engineered to express mIDH2 protein and in *IDH2*-mutated primary human AML cells *in vitro*.[89,90] Reduced methylation levels were accompanied by evidence of cellular differentiation.

AG-120, enasidenib (AG-221/CC-90007), AG-881, IDH305 and FT-2102 are small-molecule allosteric inhibitors of mIDH proteins.[8,79,88] Both AG-120 and enasidenib have shown evidence of promoting differentiation of leukemic cells of AML patients.[73,88,92] These drugs are not thought to be cytotoxic and may confer lower rates of aplasia, neutropenia and thrombocytopenia than traditional chemotherapeutic agents. Therefore, in theory, these agents may be optimal as salvage therapy, alone or in rational combinations, in m*IDH* patients with relapsed or refractory (R/R) disease.[33]

AG-120

AG-120 is an oral inhibitor of mutant IDH1-R132 enzyme that is currently under study in a phase 1 dose-escalation and expansion trial in patients with m*IDH1* advanced hematological malignancies.[74] Plasma (R)-2-HG levels of patients with m*IDH1* AML receiving AG-120 are reduced to levels seen in healthy individuals (~99.7% inhibition).[93] AG-120 monotherapy has been generally well tolerated and associated with an ORR of 35% in a study in which the majority of patients (78%) had R/R AML.

Enasidenib

Further along in development, enasidenib is an oral inhibitor of IDH2-R140 and IDH2-R172 enzymes. Enasidenib also reduces (R)-2-HG levels in patients with m*IDH2* AML to levels detected in healthy subjects.[94] Interim results of a phase 1 dose-escalation and expansion study reported outcomes for 181 patients with advanced hematological malignancies, 128 of whom had R/R AML.[73] ORR with enasidenib was 41%, both overall and in the subset of patients with R/R AML.[73] There was no meaningful difference in response between R/R AML patients with *IDH2*-R140Q (36%) or *IDH2*-R172K (39%) mutations.[73] A subgroup of patients without a demonstrable hematological response but with prolonged stable disease showed neutrophil recovery during enasidenib treatment, despite persistence of blasts in peripheral blood and/or bone marrow.[73,95] Of interest, the m*IDH2* VAF was not reduced in the majority of patients who attained CR on study, indicating that eradication of the mutant clone was not necessary to attain a response.[73] A phase 2 expansion of this study is underway in patients with R/R AML,[73] as is the phase 3 IDHentify study, which compares enasidenib with conventional care regimens. At this writing, IDHentify is enrolling older (⩾60 years) patients with m*IDH2* AML who are refractory to, or in relapse after, second- or third-line AML therapy. A phase 1/2 study of AG-120 or enasidenib in combination with azacitidine vs azacitidine alone in patients with m*IDH1*- or m*IDH2*-positive newly diagnosed AML is underway at this writing.

AG-881

Oral AG-881 inhibits both mIDH1 and mIDH2 proteins and penetrates the blood–brain barrier.[23,96] AG-881 is under evaluation for use in solid tumors and in a phase 1, open-label, dose-escalation and expansion study in patients with advanced hematological malignancies that had progressed prior to mIDH inhibitor therapy.

IDH305 and FT-2102

A small-molecule mIDH1 inhibitor, IDH305 is under evaluation in a phase 1 dose-finding clinical trial for treatment of patients with m*IDH1* R/R advanced malignancies, including MDS and AML. Similarly, FT-2102 is an mIDH1 inhibitor under investigation in a phase 1 dose-finding study as a single agent or in combination with azacitidine in patients with AML or higher-risk MDS or who are R/R to prior treatment or ineligible for standard intensive therapy. At this writing, no clinical data associated with IDH305 or FT-2102 treatment have been reported.

Venetoclax (ABT-199)

Venetoclax is an oral, small-molecule *BCL-2* inhibitor under investigation for use in AML. Preclinical data demonstrated that expression of mIDH sensitized leukemic cells to venetoclax. This effect was mediated through the intracellular accumulation of (R)-2-HG.[18] These preclinical findings are supported by results of recent venetoclax clinical trials in patients with AML. In a phase 2 study of single-agent venetoclax in patients with R/R AML, a CR or CR with incomplete hematological recovery was observed in 3 of the 11 (27%) patients with an *IDH* mutation, compared with 3 of the 21 (14%) patients without the mutation.[97] Additionally, in a trial combining HMAs with venetoclax in elderly patients with untreated AML unfit for intensive chemotherapy, patients with an *IDH* mutation were more responsive.[98] These results suggest that *IDH* mutations may identify a patient subgroup that is likely to respond to pharmacological *BCL-2* inhibition. However, the duration of response was short for most patients, highlighting the need for combination strategies to enhance the efficacy of venetoclax.

CB-839

CB-839 is an oral inhibitor of glutaminase, the enzyme responsible for the production of glutamine. Neoplastic cells depend on glutamine to fuel the tricarboxylic acid cycle and to promote cell growth and proliferation, and glutamine is the primary source of α-KG in m*IDH* cells.[27,32] *In vitro*, CB-839 inhibited the growth of m*IDH* primary human AML cells, and preclinical data have demonstrated that CB-839 preferentially slowed the growth of AML cell lines that ectopically expressed mutant IDH1/2.[27,99] CB-839 is under investigation in a phase 1 trial as a single agent and in combination with azacitidine in patients with R/R AML; while an *IDH* mutation is not required to enroll, there is a

prespecified end point to evaluate response in the subset of patients with m*IDH*.[100]

All *trans*-retinoic acid (ATRA)

(R)-2-HG-related inhibition of lysine-specific demethylases may promote a response to the differentiating agent, ATRA, in non-acute promyelocytic leukemia AML.[101] *In vitro* data show that the combination of ATRA and the tyrosine kinase inhibitor, dasatinib, improved cell differentiation in primary AML samples and in AML cell lines harboring m*IDH1*-R132H and reduced tumor growth in mutant xenografted mice.[102]

CONCLUSION

IDH mutations are frequent in myeloid malignancies, particularly AML; are uniquely associated with elevated levels of the oncometabolite, (R)-2-HG; inhibit epigenetic regulators; and should be included in AML and MDS gene panels for prognostication. Advances in understanding of the genetics underlying myeloid malignancies are igniting an exciting era of development of promising and targeted treatments. Such approaches may be more effective and less toxic than conventional chemotherapy regimens.[103] Clinical trials of mIDH inhibitors as monotherapy in the R/R setting have shown much promise, although the emergence of resistant subclones has been observed;[73,74] investigations of use as front-line therapy and in combination regimens are now ongoing.

Given the genomic complexity of AML and MDS, and the observation that the founding clone can give rise to various subclones during disease evolution, selective agents that target a single mutation are unlikely to be curative in the large majority of patients. However, the use of targeted treatments in combination with drugs with non-overlapping mechanisms of action may address multifactorial pathogenic processes implicated in hematological malignancies and potentially have a revolutionary impact on patient outcomes.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

All authors contributed to, revised and approved the manuscript content and gave approval for submission to the journal.

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