REVIEW

Isocitrate dehydrogenase mutations in myeloid malignancies

BC Medeiros¹, AT Fathi², CD DiNardo³, DA Pollyea⁴, SM Chan⁵ and R Swords⁶

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* (*mIDH*) 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 *mIDH* 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 *mIDH* 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 *mIDH* 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 *mIDH*.

Leukemia (2017) 31, 272–281; doi:10.1038/leu.2016.275

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 pathogenesis 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 (*IDH*s) 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* (*mIDH1*) and *IDH2* (*mIDH2*) genes have been described in both solid and hematological malignancies; *mIDH1* is more common in solid tumors and *mIDH2* 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

¹Division of Hematology, Department of Medicine, Stanford University School of Medicine, Stanford Cancer Center, Stanford, CA, USA; ²Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA; ³Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; ⁴Division of Hematology, University of Colorado School of Medicine, Aurora, CO, USA; ⁵Princess Margaret Cancer Centre, Toronto, ON, Canada and ⁶Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, USA. Correspondence: Dr BC Medeiros, Division of Hematology, Department of Medicine, Stanford University School of Medicine Stanford, Stanford Cancer Center, 875 Blake Wilbur Drive, MC 5821, Stanford, CA 94305, USA.

E-mail: Bruno.medeiros@stanford.edu

Received 28 June 2016; revised 17 August 2016; accepted 25 August 2016; accepted article preview online 10 October 2016; advance online publication, 11 November 2016

(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 *wtIDH1* 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 *mIDH* 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 jumoni-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 *mIDH1/2* AML exhibited a global hypermethylation phenotype associated with significant suppression of gene expression compared with patients with *wtIDH1/2* 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

EPIDEMIOLOGY

Taken together, *mIDH1/2* are among the most common mutations in AML (~20% of patients combined, Table 1). *IDH* mutations increase in frequency with increasing age.²⁷ *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 *mIDH*, 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 *wtIDH*, patients with *mIDH* are older and tend to have higher platelet and bone marrow blast counts at diagnosis of AML or MDS.^{10,35,37–39} *mIDH* 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} *mIDH* 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} *mIDH* 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} *mIDH* 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 *mIDH* and *SRSF2* mutations in MDS and

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).

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}
<i>NPM1</i>	25–35%	2%
<i>DNMT3A</i>	18–22%	8%
<i>FLT3-ITD</i>	~20%	0–2%
<i>TET2</i>	7–25%	11–26%
<i>IDH2</i>	8–19%	~5%
<i>AXLS1</i>	5–17%	11–15%
<i>RUNX1</i>	5–15%	4–14%
<i>NRAS</i>	~15%	3–6%
<i>IDH1</i>	7–14%	3%

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

AML. Co-occurrence of *mIDH* 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 *mIDH1* were less likely to have *FLT3*-internal tandem duplication (*FLT3*-ITD). Interestingly, compared with other types of *mIDH*, *mIDH2*-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 *mIDH1/2* 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 *mIDH1* had inferior OS compared with patients without the mutation, and patients with *mIDH1* CN-AML had a lower rate of complete remission (CR) with cytotoxic induction chemotherapy.⁴⁸ Indeed, a preponderance of studies suggest *mIDH1* AML confers an adverse prognosis or has no prognostic value (Table 2). One study found no influence of *mIDH1*-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, *mIDH2* had no effect on OS or CR rate compared with *wtIDH2*,⁵² but in another study, *mIDH2* 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 *mIDH* by point mutation and others combine *mIDH1/2* for analysis.^{49,51,53} Specifically, with regard to *mIDH2*, 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 *mIDH2*-R172' as a distinct class, and recommended that *IDH2* testing be added to prognostic guidelines. In their study of 1540 AML patients, AML with *mIDH2*-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 *mIDH2*-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 *mIDH1/2* 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 *mIDH1* or *mIDH2*. 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 *NPM1* 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 *mIDH1*/*NPM1*^{+/FLT3}-ITD⁻ genotype in their study had significantly poorer

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

Study	All <i>mIDH</i>			<i>mIDH1</i> -R132			<i>mIDH2</i> (all)			<i>mIDH2</i> -R140			<i>mIDH2</i> -R172		
	Frequency	Prognosis		Frequency	Prognosis		Frequency	Prognosis		Frequency	Prognosis		Frequency	Prognosis	
Abbas et al. ⁵¹ (<i>N</i> = 893)	17%	↔ OS		6%	↔ OS		11%	↔ OS		8.3%	↔ OS		2.6%	↔ OS	
Aref et al. ^{37a} (<i>N</i> = 211)	19%	↓ OS ↔ CR		8.5%	↑ RR ^a ↓ OS ^a		10.4%	NR		9.5%	NR		1%	NR	
Boissel et al. ⁴³ (<i>N</i> = 520)	NR	↔ OS		9.6%	↔ OS		NR	NR		NR	NR		3.0%	↑ RR ^a ↓ OS ^a	
Chotirat et al. ⁵⁰ (<i>N</i> = 230)	19.1%	↔ OS		8.7	↓ OS (trend)		10.4%	↔ OS		8.7%	↔ OS		1.7%	↔ OS	
Chou et al. ⁴⁰ (<i>N</i> = 446)	18.2%	↑ OS (trend)		6.1%	↓ OS (trend)		12.1%	↑ OS ↔ DFS ↔ RFS		9.2%	NR		2.9%	NR	
DiNardo et al. ³⁵ (<i>N</i> = 826)	20%	↔ OS ↔ CR		7.1%	↔ OS ↔ CR		12.8%	↔ OS ↔ CR		10%	NR		2.7%	NR	
Feng et al. ⁴⁸ (<i>N</i> = 8121)	NR	NR		4.4–9.3%	↓ OS ↔ CR ↓ CR ^a		NR	NR		NR	NR		NR	NR	
Green et al. ⁵⁵ (<i>N</i> = 1473)	17%	NR		7%	↔ OS		10%	NR		8%	↑ OS		2.0%	↓ OS	
Marcucci et al. ^{10a} (<i>N</i> = 358)	33.0%	NR		13.7%	↔ OS ↓ DFS		19.3%	↔ OS ↓ CR		15.6%	↔ OS		3.6%	↓ CR ↔ OS	
Paschka et al. ³⁸ (<i>N</i> = 805)	16%	↓ OS in <i>NPM1</i> ^{mut} /no <i>FLT3</i> -ITD ^{wt}		7.6%	↔ OS		8.7%	↔ OS		6.0%	↔ OS		2.7%	↔ OS	
Patel et al. ⁴² (<i>N</i> = 398)	14.1%	↑ OS in <i>NPM1</i> ^{mut} /FLT3-ITD ^{wt}		5.8%	↑ OS		8.3%	↑ OS		6.0%	↑ OS		2.3%	NR	
Ravandi et al. ⁵⁷ (<i>N</i> = 358)	30%	↔ OS ↔ CR ↔ EFS		7%	↔ OS ^b ↔ CR ↔ EFS		14%	↔ OS ↔ CR ↔ EFS		NR	NR		NR	NR	
Schnittger et al. ¹⁰⁶ (<i>N</i> = 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} (<i>N</i> = 275)	NR	NR		10.9%	↔ OS ^c ↔ CR ↔ RFS		NR	NR		NR	NR		NR	NR	
Willander et al. ⁹ (<i>N</i> = 189)	21.7%	NR		7.9%	↔ OS ^c		13.7%	NR		11.1%	↓ OS		2.6%	↑ OS	
Yamaguchi et al. ⁴⁹ (<i>N</i> = 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; *mIDH*, mutant *IDH*; mut, mutation; NR, not reported; OS, overall survival; RFS, relapse-free survival; RR, relapse rate; wt, wild type; '↔', no effect; '↑', improved; '↓', worsened. ^aLimited 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 *mIDH* CN-AML who did not have that genotype. Generally, *mIDH2*-R140 is much more likely to be accompanied by mutated *NPM1* or other frequently recurring mutation in AML or MDS than *mIDH2*-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 *mIDH1* was associated with shorter OS compared with patients with *wtIDH* 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, *mIDH2* was associated with significantly shorter OS, whereas *mIDH1* had only a marginal effect on OS. Other studies suggest *mIDH1* confers worse prognosis than *mIDH2* in MDS.^{59,62} The prognostic influence of *mIDH* type may depend on patients' overall prognostic risk status.^{46,61} Lin *et al.*⁴⁶ reported that *mIDH2* 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 Organization 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, log₂) 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 *mIDH*, 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 (*mIDH1*, *mIDH2*-R140 or *mIDH2*-R172); however, there was a trend for poorer OS for the small number of patients (*n* = 9) with *mIDH2*-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 ≥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) trials⁵⁵ (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-HG⁶³ (Adapted from Wang *et al.*⁶³).

Testing for *mIDH* 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 *mIDH* 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 *mIDH2*, VAF, on average, approached 50%. At this time, there is no established or standard VAF threshold to identify *mIDH* 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 *mIDH* patients retained the mutation during disease evolution, while none of the *wtIDH* 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 *mIDH1/2* 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 *DNMT3A* ($n=15$) 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 *mIDH1/2* in CR eventually relapsed, whereas all 9 patients with undetectable *mIDH1/2* 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 $\geq 2\%$ in peripheral blood has been proposed as a diagnostic criterion of CHIP⁷⁵) complicate the use of *mIDH* 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 *mIDH* and levels in patients with *mIDH*-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 ≥ 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-*mIDH* 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 *mIDH* 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 *mIDH1* human AML cells *in vitro*;⁷⁹ AGI-026, which reduced (R)-2-HG and was associated with improved survival in a *mIDH2*-R140 mouse model of R-2-hydroxyglutaric aciduria *in vivo*;⁸⁰ and AGI-5198, which reduced (R)-2-HG and induced apoptosis of *mIDH1* human chondrosarcoma cells *in vitro*.⁸¹ In addition, several agents are now in various stages of clinical development (Table 3; includes ClinicalTrials.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 *mIDH*. Compared with patients with *wtIDH*, rates of response and OS for *mIDH* patients treated with induction chemotherapy mirror general prognosis, that is, there are reports that outcomes are no different from^{35,57} or worse than¹⁰ those of patients with *wtIDH* 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 *mIDH* who received front-line induction or salvage chemotherapy had response rates comparable to those of patients with *wtIDH* regardless of co-mutational status.³⁵ In contrast, in the study by Marcucci et al.¹⁰ patients with CN-AML and *mIDH1/NPM1⁺/FLT3-ITD⁻* genotype had significantly shorter postinduction disease-free survival, and *mIDH2*-R172 patients were significantly less likely to attain CR, than *wtIDH* patients.

Hypomethylating agents (HMAs)

Because hypermethylation is a pathogenic hallmark of *mIDH1/2* 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 *mIDH* has been equivocal.^{85,86} A retrospective cohort study that included 11 patients with *mIDH* 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 *mIDH* AML and MDS

Drug	Mechanism of action	Clinical phase	Patient type	Clinical activity	Registration ^a
AG-221	Small-molecule allosteric inhibitor of <i>mIDH2</i> protein; reduces the oncometabolite, 2-HG	2 and 3	Patients with R/R AML (phase 2); older patients (≥60 years) with <i>mIDH2</i> 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=1 NCT02577406 https://clinicaltrials.gov/ct2/show/NCT02577406 NCT02074839 https://clinicaltrials.gov/ct2/show/NCT02074839?term=NCT02074839&rank=1 NCT02492737 https://clinicaltrials.gov/ct2/show/NCT02492737?term=NCT02492737&rank=1 NCT02381886 https://clinicaltrials.gov/ct2/show/NCT02381886?term=NCT02381886&rank=1 NCT02719574 https://clinicaltrials.gov/ct2/show/NCT02719574 NCT02203773 https://clinicaltrials.gov/ct2/show/NCT02203773?term=NCT02203773&rank=1 NCT02287233 https://clinicaltrials.gov/ct2/show/NCT02287233?term=NCT02287233&rank=1
AG-120	Small-molecule allosteric inhibitor of <i>mIDH1</i> protein; reduces the oncometabolite, 2-HG	2	<i>mIDH1</i> advanced hematological malignancies	35% ORR in phase 1 dose-escalation and expansion study ⁷¹	
AG-881	Small-molecule <i>mIDH1</i> and <i>mIDH2</i> protein inhibitor; reduces the oncometabolite, 2-HG	1	<i>mIDH1</i> and <i>mIDH2</i> relapsed or refractory advanced hematological malignancies—after prior <i>mIDH</i> inhibitor failure	Unknown	
IDH305	Small-molecule <i>mIDH1</i> inhibitor	1	<i>mIDH1</i> advanced malignancies	Unknown	
FT-2102	No description available	1/1b	AML or high-risk MDS with <i>mIDH1</i> ; under evaluation as monotherapy and in combination with azacitidine	Unknown	
ABT-199	Small-molecule BCL-2 inhibitor; works via synthetic lethality; that is, <i>mIDH</i> 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 <i>mIDH</i> AML ⁸⁹	
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 <i>mIDH</i> 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; *mIDH*, mutant isocitrate dehydrogenase; ORR, overall response rate; R/R, relapsed or refractory; 2-HG, 2-hydroxyglutarate. ^aClinicalTrials.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 *mIDH1/2*.⁸⁶ Similarly, in a retrospective study of 826 AML patients, 175 patients received front-line therapy with an HMA-based regimen. Of them, 48 *mIDH1/2* and 127 *wtIDH* 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 *mIDH* 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 *mIDH* 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 *mIDH1* advanced hematological malignancies.⁷⁴ Plasma (R)-2-HG levels of patients with *mIDH1* 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 *mIDH2* 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 *mIDH2* 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 *mIDH2* 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 *mIDH1*- or *mIDH2*-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 *mIDH1* 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 *mIDH* cells.^{27,32} *In vitro*, CB-839 inhibited the growth of *mIDH* 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 *mIDH*.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 *mIDH1*-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

BCM has received research funding from Celgene and Agios and has received remuneration for Advisory Board participation from Celgene and Agios. ATF is a consultant for and receives clinical research funding from Celgene and declares Advisory Board participation for Agios. CDD has received research funding from Novartis, Celgene, Agios and Abbvie/Genentech and participates in Advisory Boards for Celgene and Agios. DAP has received research funding from Celgene and is a consultant for Celgene, Pfizer, Alexion, Ariad and Karyopharm. SMC has received research funding from Celgene, Agios and Abbvie/Genentech. RS declares Advisory Board participation for Novartis.

ACKNOWLEDGEMENTS

We received editorial support during manuscript development from Sheila Truten and Kelly Dittmore of MC2 Inc., Wynnewood, PA, USA who were funded by Celgene Corporation. Celgene did not contribute to content or participate in development of this paper. The authors are fully responsible for all content and editorial decisions.

AUTHOR CONTRIBUTIONS

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

REFERENCES

- 1 Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med* 2015; **373**: 1136–1152.
- 2 Albitar M, Manshouri T, Shen Y, Liu D, Beran M, Kantarjian HM et al. Myelodysplastic syndrome is not merely 'preleukemia'. *Blood* 2002; **100**: 791–798.
- 3 Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009; **114**: 937–951.
- 4 Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011; **11**: 85–95.
- 5 Conway O'Brien E, Prideaux S, Chevassut T. The epigenetic landscape of acute myeloid leukemia. *Adv Hematol* 2014; **2014**: 103175.
- 6 Clark O, Yen K, Mellinghoff IK. Molecular pathways: isocitrate dehydrogenase mutations in cancer. *Clin Cancer Res* 2016; **22**: 1–7.
- 7 Molenaar RJ, Radivoyevitch 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; **1846**: 326–341.
- 8 Stein EM. Molecular pathways: IDH2 mutations-co-opting cellular metabolism for malignant transformation. *Clin Cancer Res* 2016; **22**: 16–19.
- 9 Willander K, Falk IJ, Chairati R, Paul E, Hermansson M, Green H et al. Mutations in the isocitrate dehydrogenase 2 gene and IDH1 SNP 105C>T have a prognostic value in acute myeloid leukemia. *Biomark Res* 2014; **2**: 18.
- 10 Marcucci G, Mahary K, Wu YZ, Radmacher MD, Mrozek K, Margeson D et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol* 2010; **28**: 2348–2355.
- 11 Wagner K, Damm F, Gohring G, Gorlich K, Heuser M, Schafer I et al. Impact of IDH1 R132 mutations and an IDH1 single nucleotide polymorphism in cytogenetically normal acute myeloid leukemia: SNP rs11554137 is an adverse prognostic factor. *J Clin Oncol* 2010; **28**: 2356–2364.
- 12 Reitman ZJ, Parsons DW, Yan H. IDH1 and IDH2: not your typical oncogenes. *Cancer Cell* 2010; **17**: 215–216.
- 13 Losman JA, Kaelin WG Jr. What a difference a hydroxyl makes: mutant IDH, (R)-2-hydroxyglutarate, and cancer. *Genes Dev* 2013; **27**: 836–852.
- 14 Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 2009; **462**: 739–744.
- 15 Chaturvedi A, Araujo Cruz MM, Jyotsana N, Sharma A, Goparaju R, Schwarzer A et al. Enantiomer-specific and paracrine leukemogenicity of mutant IDH metabolite 2-hydroxyglutarate. *Leukemia* 2016; **30**: 1708–1715.
- 16 Losman JA, Looper RE, Koivunen P, Lee S, Schneider RK, McMahon C et al. (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science* 2013; **339**: 1621–1625.
- 17 Ward PS, Lu C, Cross JR, Abdel-Wahab O, Levine RL, Schwartz GK et al. The potential for isocitrate dehydrogenase mutations to produce 2-hydroxyglutarate depends on allele specificity and subcellular compartmentalization. *J Biol Chem* 2013; **288**: 3804–3815.
- 18 Chan SM, Thomas D, Corces-Zimmerman MR, Xavy S, Rastogi S, Hong WJ et al. Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nat Med* 2015; **21**: 178–184.
- 19 Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. *Cancer Cell* 2011; **19**: 17–30.
- 20 Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagg EA, Rose NR et al. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep* 2011; **12**: 463–469.
- 21 Chan SM, Majeti R. Role of DNMT3A, TET2, and IDH1/2 mutations in pre-leukemic stem cells in acute myeloid leukemia. *Int J Hematol* 2013; **98**: 648–657.
- 22 Accari SL, Fisher PR. Emerging roles of JmjC domain-containing proteins. *Int Rev Cell Mol Biol* 2015; **319**: 165–220.
- 23 Yang H, Ye D, Guan KL, Xiong Y. IDH1 and IDH2 mutations in tumorigenesis: mechanistic insights and clinical perspectives. *Clin Cancer Res* 2012; **18**: 5562–5571.
- 24 Prensner JR, Chinnaiyan AM. Metabolism unhinged: IDH mutations in cancer. *Nat Med* 2011; **17**: 291–293.
- 25 Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* 2010; **18**: 553–567.
- 26 Jin G, Reitman ZJ, Duncan CG, Spasojevic I, Gooden DM, Rasheed BA et al. Disruption of wild-type IDH1 suppresses D-2-hydroxyglutarate production in IDH1-mutated gliomas. *Cancer Res* 2013; **73**: 496–501.
- 27 Fathi AT, Wander SA, Faramand R, Emadi A. Biochemical, epigenetic, and metabolic approaches to target IDH mutations in acute myeloid leukemia. *Semin Hematol* 2015; **52**: 165–171.
- 28 Abdel-Wahab O, Levine RL. Mutations in epigenetic modifiers in the pathogenesis and therapy of acute myeloid leukemia. *Blood* 2013; **121**: 3563–3572.
- 29 Pardanani A, Lasho TL, Finke CM, Mai M, McClure RF, Tefferi A. IDH1 and IDH2 mutation analysis in chronic- and blast-phase myeloproliferative neoplasms. *Leukemia* 2010; **24**: 1146–1151.

- 30 Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. *Proc Natl Acad Sci USA* 2014; **111**: 2548–2553.
- 31 Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* 2014; **506**: 328–333.
- 32 Im AP, Sehgal AR, Carroll MP, Smith BD, Tefferi A, Johnson DE et al. DNMT3A and IDH mutations in acute myeloid leukemia and other myeloid malignancies: associations with prognosis and potential treatment strategies. *Leukemia* 2014; **28**: 1774–1783.
- 33 Stein EM. IDH2 inhibition in AML: finally progress? *Best Pract Res Clin Haematol* 2015; **28**: 112–115.
- 34 Platt MY, Fathi AT, Borger DR, Brunner AM, Hasserjian RP, Balaj L et al. Detection of dual IDH1 and IDH2 mutations by targeted next-generation sequencing in acute myeloid leukemia and myelodysplastic syndromes. *J Mol Diagn* 2015; **17**: 661–668.
- 35 DiNardo CD, Ravandi F, Agresta S, Konopleva M, Takahashi K, Kadia T et al. Characteristics, clinical outcome, and prognostic significance of IDH mutations in AML. *Am J Hematol* 2015; **90**: 732–736.
- 36 Molenaar RJ, Thota S, Nagata Y, Patel B, Clemente M, Hirsh C et al. Clinical and biological implications of ancestral and non-ancestral IDH1 and IDH2 mutations in myeloid neoplasms. *Leukemia* 2015; **29**: 2134–2142.
- 37 Aref S, Kamel Areida el S, Abdel Aal MF, Adam OM, El-Ghonemy MS, El-Baiomy MA et al. Prevalence and clinical effect of IDH1 and IDH2 mutations among cytogenetically normal acute myeloid leukemia patients. *Clin Lymphoma Myeloma Leuk* 2015; **15**: 550–555.
- 38 Paschka P, Schlenk RF, Gaidzik VI, Habdank M, Kronke J, Bullinger L et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. *J Clin Oncol* 2010; **28**: 3636–3643.
- 39 DiNardo CD, Jabbour E, Ravandi F, Takahashi K, Daver N, Routbort M et al. IDH1 and IDH2 mutations in myelodysplastic syndromes and role in disease progression. *Leukemia* 2016; **30**: 980–984.
- 40 Chou WC, Lei WC, Ko BS, Hou HA, Chen CY, Tang JL et al. The prognostic impact and stability of Isocitrate dehydrogenase 2 mutation in adult patients with acute myeloid leukemia. *Leukemia* 2011; **25**: 246–253.
- 41 Chou WC, Hou HA, Chen CY, Tang JL, Yao M, Tsay W et al. Distinct clinical and biologic characteristics in adult acute myeloid leukemia bearing the isocitrate dehydrogenase 1 mutation. *Blood* 2010; **115**: 2749–2754.
- 42 Patel KP, Ravandi F, Ma D, Paladugu A, Barkoh BA, Medeiros LJ et al. Acute myeloid leukemia with IDH1 or IDH2 mutation: frequency and clinicopathologic features. *Am J Clin Pathol* 2011; **135**: 35–45.
- 43 Boissel N, Nibourel O, Renneville A, Gardin C, Reman O, Contentin N et al. Prognostic impact of isocitrate dehydrogenase enzyme isoforms 1 and 2 mutations in acute myeloid leukemia: a study by the Acute Leukemia French Association group. *J Clin Oncol* 2010; **28**: 3717–3723.
- 44 Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med* 2012; **366**: 1079–1089.
- 45 Rampal R, Alkalai A, Madzo J, Vasanthakumar A, Pronier E, Patel J et al. DNA hydroxymethylation profiling reveals that WT1 mutations result in loss of TET2 function in acute myeloid leukemia. *Cell Rep* 2014; **9**: 1841–1855.
- 46 Lin CC, Hou HA, Chou WC, Kuo YY, Liu CY, Chen CY et al. IDH mutations are closely associated with mutations of DNMT3A, ASXL1 and SRSF2 in patients with myelodysplastic syndromes and are stable during disease evolution. *Am J Hematol* 2014; **89**: 137–144.
- 47 Lasho TL, Jimma T, Finke CM, Patnaik M, Hanson CA, Ketterling RP et al. SRSF2 mutations in primary myelofibrosis: significant clustering with IDH mutations and independent association with inferior overall and leukemia-free survival. *Blood* 2012; **120**: 4168–4171.
- 48 Feng JH, Guo XP, Chen YY, Wang ZJ, Cheng YP, Tang YM. Prognostic significance of IDH1 mutations in acute myeloid leukemia: a meta-analysis. *Am J Blood Res* 2012; **2**: 254–264.
- 49 Yamaguchi S, Iwanaga E, Tokunaga K, Nanri T, Shimomura T, Suzushima H et al. IDH1 and IDH2 mutations confer an adverse effect in patients with acute myeloid leukemia lacking the NPM1 mutation. *Eur J Haematol* 2014; **92**: 471–477.
- 50 Chotirat S, Thongnoppakhun W, Promsuwicha O, Boonthimat C, Auewarakul CU. Molecular alterations of isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) metabolic genes and additional genetic mutations in newly diagnosed acute myeloid leukemia patients. *J Hematol Oncol* 2012; **5**: 5.
- 51 Abbas S, Lugthart S, Kavelaars FG, Schelen A, Koenders JE, Zeilemaker A et al. Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value. *Blood* 2010; **116**: 2122–2126.
- 52 Thol F, Damm F, Wagner K, Gohring G, Schlegelberger B, Hoelzer D et al. Prognostic impact of IDH2 mutations in cytogenetically normal acute myeloid leukemia. *Blood* 2010; **116**: 614–616.
- 53 Abdel-Karim I, Plunkett Jr WK, O'Brien S, Giles F, Thomas D, Faderl S et al. A phase I study of pemetrexed in patients with relapsed or refractory acute leukemia. *Invest New Drugs* 2011; **29**: 323–331.
- 54 Boissel N, Nibourel O, Renneville A, Huchette P, Dombret H, Preudhomme C. Differential prognosis impact of IDH2 mutations in cytogenetically normal acute myeloid leukemia. *Blood* 2011; **117**: 3696–3697.
- 55 Green CL, Evans CM, Zhao L, Hills RK, Burnett AK, Linch DC et al. The prognostic significance of IDH2 mutations in AML depends on the location of the mutation. *Blood* 2011; **118**: 409–412.
- 56 Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med* 2016; **374**: 2209–2221.
- 57 Ravandi F, Patel K, Luthra R, Faderl S, Konopleva M, Kadia T et al. Prognostic significance of alterations in IDH enzyme isoforms in patients with AML treated with high-dose cytarabine and idarubicin. *Cancer* 2012; **118**: 2665–2673.
- 58 Sloan CE, Lusk MR, Boccuti AM, Sehgal AR, Zhao J, Dabner RD et al. A modified integrated genetic model for risk prediction in younger patients with acute myeloid leukemia. *PLoS One* 2016; **11**: e0153016.
- 59 Thol F, Weissinger EM, Krauter J, Wagner K, Damm F, Wichmann M et al. IDH1 mutations in patients with myelodysplastic syndromes are associated with an unfavorable prognosis. *Haematologica* 2010; **95**: 1668–1674.
- 60 Jin J, Hu C, Yu M, Chen F, Ye L, Yin X et al. Prognostic value of isocitrate dehydrogenase mutations in myelodysplastic syndromes: a retrospective cohort study and meta-analysis. *PLoS One* 2014; **9**: e100206.
- 61 Bejar R, Papaemmanuil E, Haferlach T, Garcia-Manero G, Maciejewski J, Sekeres M et al. Somatic mutations in MDS patients are associated with clinical features and predict prognosis independent of the IPSS-R: Analysis of combined datasets from the International Working Group for Prognosis in MDS-Molecular Committee. *Blood* 2015; **126**: 907.
- 62 Patnaik MM, Hanson CA, Hodnefield JM, Lasho TL, Finke CM, Knudson RA et al. Differential prognostic effect of IDH1 versus IDH2 mutations in myelodysplastic syndromes: a Mayo Clinic study of 277 patients. *Leukemia* 2012; **26**: 101–105.
- 63 Wang JH, Chen WL, Li JM, Wu SF, Chen TL, Zhu YM et al. Prognostic significance of 2-hydroxyglutarate levels in acute myeloid leukemia in China. *Proc Natl Acad Sci USA* 2013; **110**: 17017–17022.
- 64 DiNardo CD, Propert KJ, Loren AW, Paietta E, Sun Z, Levine RL et al. Serum 2-hydroxyglutarate levels predict isocitrate dehydrogenase mutations and clinical outcome in acute myeloid leukemia. *Blood* 2013; **121**: 4917–4924.
- 65 Janin M, Mylonas E, Saada V, Micol JB, Renneville A, Quivoron C et al. Serum 2-hydroxyglutarate production in IDH1- and IDH2-mutated de novo acute myeloid leukemia: a study by the Acute Leukemia French Association group. *J Clin Oncol* 2014; **32**: 297–305.
- 66 Mahdieh N, Rabbani B. An overview of mutation detection methods in genetic disorders. *Iran J Pediatr* 2013; **23**: 375–388.
- 67 Patel KP, Barkoh BA, Chen Z, Ma D, Reddy N, Medeiros LJ et al. Diagnostic testing for IDH1 and IDH2 variants in acute myeloid leukemia an algorithmic approach using high-resolution melting curve analysis. *J Mol Diagn* 2011; **13**: 678–686.
- 68 Horbinski C, Kelly L, Nikiforov YE, Durso MB, Nikiforova MN. Detection of IDH1 and IDH2 mutations by fluorescence melting curve analysis as a diagnostic tool for brain biopsies. *J Mol Diagn* 2010; **12**: 487–492.
- 69 Gorniak P, Ejduk A, Borg K, Makuch-Lasica H, Nowak G, Lech-Maranda E et al. Comparison of high-resolution melting analysis with direct sequencing for the detection of recurrent mutations in DNA methyltransferase 3A and isocitrate dehydrogenase 1 and 2 genes in acute myeloid leukemia patients. *Eur J Haematol* 2016; **96**: 181–187.
- 70 Metzeler KH, Herold T, Rothenberg-Thurley M, Amler S, Sauerland MC, Gorlich D et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood* 2016; **128**: 686–698.
- 71 McKerrell T, Park N, Moreno T, Grove CS, Ponstingl H, Stephens J et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Rep* 2015; **10**: 1239–1245.
- 72 Debarri H, Lebon D, Roumier C, Cheok M, Marceau-Renaut A, Nibourel O et al. IDH1/2 but not DNMT3A mutations are suitable targets for minimal residual disease monitoring in acute myeloid leukemia patients: a study by the Acute Leukemia French Association. *Oncotarget* 2015; **6**: 42345–42353.
- 73 Stein EM, DiNardo C, Altman JK, Collins R, DeAngelo DJ, Kantarjian HM et al. Safety and efficacy of AG-221, a potent inhibitor of mutant IDH2 that promotes differentiation of myeloid cells in patients with advanced hematologic malignancies: Results of a phase 1/2 trial. *Blood* 2015; **126**: 323.

- 74 DiNardo C, de Botton S, Pollyea DA, Stein EM, Fathi AT, Roboz GJ *et al*. Molecular profiling and relationship with clinical response in patients with IDH1 mutation-positive hematologic malignancies receiving AG-120, a first-in-class potent inhibitor of mutant IDH1, in addition to data from the completed dose escalation portion of the phase 1 study. *Blood* 2015; **126**: Abstract 1306.
- 75 Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP *et al*. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* 2015; **126**: 9–16.
- 76 Fathi AT, Sadrzadeh H, Borger DR, Ballen KK, Amrein PC, Attar EC *et al*. Prospective serial evaluation of 2-hydroxyglutarate, during treatment of newly diagnosed acute myeloid leukemia, to assess disease activity and therapeutic response. *Blood* 2012; **120**: 4649–4652.
- 77 Balss J, Pusch S, Beck AC, Herold-Mende C, Kramer A, Thiede C *et al*. Enzymatic assay for quantitative analysis of (D)-2-hydroxyglutarate. *Acta Neuropathol* 2012; **124**: 883–891.
- 78 Brunner AM, Neuberg D, Wander SA, Sadrzadeh H, Ballen KK, Amrein PC *et al*. Use of 2HG levels in the serum, urine, or bone marrow to predict IDH mutations in adults with acute myeloid leukemia. *Blood* 2015; **126**: 2597–2597.
- 79 Chaturvedi A, Araujo Cruz MM, Jyotsana N, Sharma A, Yun H, Gorlich K *et al*. Mutant IDH1 promotes leukemogenesis in vivo and can be specifically targeted in human AML. *Blood* 2013; **122**: 2877–2887.
- 80 Wang F, Travins J, Lin Z, Si Y, Chen Y, Powe J *et al*. A small molecule inhibitor of mutant IDH2 rescues cardiomyopathy in a D-2-hydroxyglutaric aciduria type II mouse model. *J Inher Metab Dis* 2016; **39**: 807–820.
- 81 Li L, Paz AC, Wilky BA, Johnson B, Galoian K, Rosenberg A *et al*. Treatment with a small molecule mutant IDH1 inhibitor suppresses tumorigenic activity and decreases production of the oncometabolite 2-hydroxyglutarate in human chondrosarcoma cells. *PLoS One* 2015; **10**: e0133813.
- 82 Dombret H, Seymour JF, Butrym A, Wierzbowska A, Selleslag D, Jang JH *et al*. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with > 30% blasts. *Blood* 2015; **126**: 291–299.
- 83 Kantarjian HM, Thomas XG, Dmoszynska A, Wierzbowska A, Mazur G, Mayer J *et al*. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. *J Clin Oncol* 2012; **30**: 2670–2677.
- 84 Fenaux P, Aides L. Review of azacitidine trials in Intermediate-2 and High-risk myelodysplastic syndromes. *Leuk Res* 2009; **33**(Suppl 2): S7–S11.
- 85 Emadi A, Faramand R, Carter-Cooper B, Tolu S, Ford LA, Lapidus RG *et al*. Presence of isocitrate dehydrogenase mutations may predict clinical response to hypomethylating agents in patients with acute myeloid leukemia. *Am J Hematol* 2015; **90**: E77–E79.
- 86 DiNardo CD, Patel KP, Garcia-Manero G, Luthra R, Pierce S, Borthakur G *et al*. Lack of association of IDH1, IDH2 and DNMT3A mutations with outcome in older patients with acute myeloid leukemia treated with hypomethylating agents. *Leuk Lymphoma* 2014; **55**: 1925–1929.
- 87 Heuser M, Araujo Cruz MM, Goparaju R, Chaturvedi A. Enigmas of IDH mutations in hematology/oncology. *Exp Hematol* 2015; **43**: 685–697.
- 88 Yaqub F. Inhibition of mutant IDH1 in acute myeloid leukaemia. *Lancet Oncol* 2015; **16**: e9.
- 89 Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E *et al*. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science* 2013; **340**: 622–626.
- 90 Kernysky A, Wang F, Hansen E, Schalm S, Straley K, Gliser C *et al*. IDH2 mutation-induced histone and DNA hypermethylation is progressively reversed by small-molecule inhibition. *Blood* 2015; **125**: 296–303.
- 91 Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C *et al*. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* 2013; **340**: 626–630.
- 92 de Botton S, Pollyea DA, Stein EM, Dinardo C, Fathi AT, Roboz GJ *et al*. Clinical safety and activity of AG-120, a first-in-class potent inhibitor of the IDH1 mutant protein, in a phase 1 study of patients with advanced, IDH1-mutant hematologic malignancies. *Haematologica* 2015; **100**: Abstract P563.
- 93 Fan B, Le K, Manyak E, Liu H, Pahl M, Bowden C *et al*. Longitudinal pharmacokinetic/pharmacodynamic profile of AG-120, a potent inhibitor of the IDH1 mutant protein, in a phase 1 study of IDH1-mutant advanced hematologic malignancies. *Blood* 2015; **126**: Abstract 1310.
- 94 Fan B, Chen Y, Wang F, Yen K, Utley L, Almon C *et al*. Pharmacokinetic/pharmacodynamic (PK/PD) evaluation of AG-221, a potent mutant IDH2 inhibitor, from a phase 1 trial of patients with IDH2 mutation-positive hematologic malignancies. *Haematologica* 2015; **100**: Abstract 379.
- 95 Stein EM, Tallman MS. Emerging therapeutic drugs for AML. *Blood* 2016; **127**: 71–78.
- 96 Agios Pharmaceuticals. Agios Pharmaceuticals selects third novel IDH mutant inhibitor, AG-881, for clinical development. (News release). 2015 (cited 28 March 2016); available from <http://investor.agios.com/phoenix.zhtml?c=251862&p=irol-newsArticle&ID=2041339>.
- 97 Konopleva M, Pollyea DA, Potluri J, Chyla BJ, Busman T, McKeegan E *et al*. A phase 2 study of ABT-199 (GDC-0199) in patients with acute myelogenous leukemia (AML). *Blood* 2014; **124**: Abstract 118.
- 98 Pollyea DA, DiNardo CD, thirman MJ, Letai A, Wei AH, Jonas BA *et al*. Results of a phase 1b study of venetoclax plus decitabine or azacitidine in untreated acute myeloid leukemia patients ≥ 65 years ineligible for standard induction therapy. *J Clin Oncol* 2016; **34**(Suppl): Abstract 7009.
- 99 Emadi A, Jun SA, Tsukamoto T, Fathi AT, Minden MD, Dang CV. Inhibition of glutaminase selectively suppresses the growth of primary acute myeloid leukemia cells with IDH mutations. *Exp Hematol* 2014; **42**: 247–251.
- 100 Wang ES, Frankfurt O, Orford KW, Bennett M, Flinn IW, Maris M *et al*. Phase 1 study of CB-839, a first-in-class, orally administered small molecule inhibitor of glutaminase in patients with relapsed/refractory leukemia. *Blood* 2015; **126**: Abstract 2566.
- 101 Schenk T, Chen WC, Gollner S, Howell L, Jin L, Hebestreit K *et al*. Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-trans-retinoic acid differentiation pathway in acute myeloid leukemia. *Nat Med* 2012; **18**: 605–611.
- 102 Boutzen H, Saland E, Cathebras M, Larrue C, Farge T, Serhan N *et al*. The combination of ATRA and dasatinib for differentiation therapy in acute myeloid leukemias with IDH mutations. *Blood* 2015; **126**: Abstract 2542.
- 103 Estey E, Levine RL, Lowenberg B. Current challenges in clinical development of 'targeted therapies': the case of acute myeloid leukemia. *Blood* 2015; **125**: 2461–2466.
- 104 Graubert T, Walter MJ. Genetics of myelodysplastic syndromes: new insights. *Hematology: The Education Program of the American Society of Hematology* 2011; **2011**: 543–549.
- 105 Chotirat S, Thongnoppakhun W, Wanachiwanawin W, Auewarakul CU. Acquired somatic mutations of isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) in preleukemic disorders. *Blood Cells Mol Dis* 2015; **54**: 286–291.
- 106 Schnittger S, Haferlach C, Ulke M, Alpermann T, Kern W, Haferlach T. IDH1 mutations are detected in 6.6% of 1414 AML patients and are associated with intermediate risk karyotype and unfavorable prognosis in adults younger than 60 years and unmutated NPM1 status. *Blood* 2010; **116**: 5486–5496.

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/4.0/>