Acute Myeloid Leukemia With *IDH1* or *IDH2* Mutation

Frequency and Clinicopathologic Features

Keyur P. Patel, MD, PhD,[1] Farhad Ravandi, MD,[2] Deqin Ma, MD, PhD,[1] Abhaya Paladugu,[1] Bedia A. Barkoh,[1] L. Jeffrey Medeiros, MD,[1] and Rajyalakshmi Luthra, PhD[1]

Key Words: Acute myeloid leukemia; Isocitrate dehydrogenase; *IDH1*; *IDH2*; Mutation

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Upon completion of this activity you will be able to:
- describe the physiologic role of *IDH1* and *IDH2*.
- describe the nature of *IDH1* and *IDH2* mutations.
- discuss the characteristics of acute myeloid leukemia with *IDH1* and *IDH2* mutations.

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Questions appear on p 163. Exam is located at www.ascp.org/ajcpcme.

Abstract

*Mutations in the isocitrate dehydrogenase 1 (*IDH1*) and* IDH2 *genes are reported in acute myeloid leukemia (AML). We studied the frequency and the clinicopathologic features of* IDH1 *and* IDH2 *mutations in AML. Mutations in* IDH1 *(*IDH1^{R132}*) and* IDH2 *(*IDH2^{R172}*) were assessed by Sanger sequencing in 199 AML cases. Point mutations in* IDH1^{R132} *were detected in 12 (6.0%) of 199 cases and in* IDH2^{R172} *in 4 (2.0%) of 196 cases. Of the 16 mutated cases, 15 (94%) were cytogenetically normal, for an overall frequency in this group of 11.8%.* IDH1^{R132} *and* IDH2^{R172} *mutations were mutually exclusive. Concurrent mutations in* NPM1, FLT3, CEBPA, *and* NRAS *were detected only in AML with the* IDH1^{R132} *mutation. The clinical and laboratory variables of patients with AML with* IDH *mutations showed no significant differences compared with patients with wild-type* IDH. *We conclude that* IDH1^{R132} *and* IDH2^{R172} *mutations occur most often in cytogenetically normal AML cases with an overall frequency of approximately 11.8%.*

Identification of somatically acquired gene mutations has provided critical insights into the pathogenesis of acute myeloid leukemia (AML).[1] Gene mutations in AML provide useful markers for diagnosis and for monitoring response to therapy and also provide information useful in assessing prognosis and making therapeutic decisions.[2-6] The most recent World Health Organization (WHO) classification of myeloid neoplasms acknowledges the clinical significance of gene mutations in AML and has proposed separate entities for AML with mutations in *NPM1* and *CEBPA*.[7] It is interesting that mutations in AML are detected commonly in cytogenetically normal (CN) cases, which account for 40% to 50% of all AMLs.[1,8] Currently, no known mutations are identified in about 20% to 30% of CN AML cases, suggesting the possibility that more mutations likely exist.

Recently, the entire genome of a patient with CN AML was sequenced, and a total of 64 somatic mutations, 12 within coding sequences of genes and 52 in conserved or regulatory regions, were identified.[9] In particular, a novel mutation was detected in isocitrate dehydrogenase 1 (*IDH1*), a metabolic gene frequently mutated in gliomas.[10-12] The mutation occurred consistently at an evolutionary conserved arginine residue at codon 132 (R132) within the substrate binding site of the enzyme and was strongly associated with normal cytogenetic status. A limited number of studies examining the frequency of the *IDH1* mutation in AML were performed subsequently.[13-16] In addition to *IDH1^{R132}*, mutations in codon 172 of *IDH2* (*IDH2^{R172}*), a mitochondrial isoform of *IDH1*, have been documented in AML.[14,17]

Published studies have focused on examining the frequency and correlation with mutational status and clinical outcome. A very limited amount of information is available on the histomorphologic features and immunophenotypic profiles

associated with *IDH1*^{R132} and *IDH2*^{R172} mutations in AML. Only 1 study, involving a Chinese population, reported clinical and biologic features of AML with the *IDH1*^{R132} mutation.[16] There are no such reports available for the Western population or for AML with the *IDH2*^{R172} mutation.

We report the frequency of *IDH1*^{R132} and *IDH2*^{R172} mutations in 199 AML cases with clinical, histologic, and immunologic characterization of the mutated cases. We also performed a meta-analysis of available studies that have assessed AML cases for *IDH1*^{R132} mutations.

Materials and Methods

Study Group

DNA was extracted from diagnostic bone marrow aspirate samples of AML with 20% or more blasts using methods described previously.[18] All samples had been sent to the clinical Molecular Diagnostics Laboratory, The University of Texas M.D. Anderson Cancer Center, Houston, at the time of diagnosis. Residual DNA was used under an approved institutional review board protocol. Cases of AML with favorable-risk cytogenetics were excluded from analysis based on data from an earlier study that showed absence of *IDH1* mutations in this group.[9] Pertinent laboratory information was obtained from the laboratory information system. The patient characteristics are listed in **Table 1** and **Table 2**.

IDH1 and *IDH2* Mutation Detection

Exon 4 mutations in codon R132 of *IDH1* and codon R172 of *IDH2* were detected by using polymerase chain reaction (PCR) amplification followed by Sanger sequencing. Previously described PCR primers were modified

Table 1
Clinical Features of AML With *IDH1*^{R132} Mutation*

	All Cases	*IDH1*^{R132} Mutant Cases (n = 12)	*IDH1* Wild-Type Cases (n = 187)	*P*†
No. of cases	199	12/199 (6.0)	187/199 (94.0)	
Mean (range) age (y)	55 (17-89)	55 (37-77)	54 (17-89)	.917‡
No. of males/females (M/F ratio)	95/104 (1:1.1)	3/9 (1:3)	92/95 (1:1.03)	.134
WHO classification				
AML, NOS	163	10 (83)	153 (81.8)	1.00
AML, MLD	30	2 (17)	28 (15.0)	1.00
t-AML	4	0 (0)	4 (2.1)	1.00
AML, biphenotypic	2	0 (0)	2 (1.1)	1.00
FAB classification				
M0	11	0 (0)	11 (5.9)	1.00
M1	32	4 (33)	28 (15.0)	.106
M2	46	4 (33)	42 (22.5)	.482
M4	42	0 (0)	42 (22.5)	.074
M5	20	3 (25)	17 (9.1)	.106
M6	5	0 (0)	5 (2.7)	1.00
M7	1	0 (0)	1 (0.5)	1.00
RAEB-T	14	1 (9)	13 (7.0)	.596
NA	28	0 (0)	28 (15.0)	.223
Mean (range) WBC count (× 10^9/L)	19.4 (0.3-204)	14.7 (0.3-83.1)	19.7 (0.3-204)	.515‡
Mean (range) blasts (%)	53 (20-98)	55 (22-88)	53 (20-98)	.732‡
Karyotype				.059
Normal	127	11 (92)	116 (62.0)	
Abnormal	72	1 (8)	71 (38.0)	
Cytogenetic risk group				.223
Intermediate	166	12 (100)	154 (82.4)	
Poor	33	0 (0)	33 (17.6)	
Additional mutations				
NPM1	—	5/11 (45)	13/54 (24)	.161
FLT3-ITD	—	3/12 (25)	43/179 (24.0)	1.00
FLT3-D835	—	1/12 (8)	9/179 (5.0)	.486
CEBPA	—	2/10 (20)	0/4 (0)	1.00
NRAS	—	2/12 (17)	12/154 (7.8)	.267
KRAS	—	0/12 (0)	7/154 (4.5)	1.00
KIT	—	1/9 (11)	0/78 (0)	1.00
IDH2	—	0/11 (0)	4/185 (2.2)	1.00
Molecular risk group§				.114
Low	11	3/11 (27)	8/84 (10)	
High	84	8/11 (73)	76/84 (90)	

AML, acute myeloid leukemia; AML, MLD, AML with myelodysplasia-related changes; AML, NOS, AML not otherwise specified; FAB, French-American-British; NA, not available; RAEB-T, refractory anemia with excess blasts in transformation; t-AML, therapy-related AML; WHO, World Health Organization.
* Data are given as number (percentage) or number positive/number tested (percentage) unless otherwise indicated. The WBC count is given in Système International units; to convert to conventional units (/μL), divide by 0.001.
† Fisher exact test unless otherwise indicated.
‡ 2-tailed *t* test.
§ Molecular low risk, *NPM1* mutated and *FLT3-ITD* wild type; high risk, *NPM1* wild type and/or *FLT3-ITD*+.

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with the addition of M13 sequence.[19] The PCR primers used were forward, 5'- TGTAAAACGACGGCCAGTC GGTCTTCAGAGAAGCCATT-3' and reverse, 5'- CA GGAAACAGCTATGACCGCAAAATCACATTATT GCCAAC-3' for *IDH1*^{R132} and forward, 5'-TGTAAAA CGACGGCCAGTAGCCCATCATCTGCAAAAAC-3' and reverse, 5'-CAGGAAACAGCTATGACCCTAGGCG AGGAGCTCCAGT-3' for *IDH2*^{R172}. All primers were purchased from Integrated DNA Technologies, Coralville, IA.

For *IDH1*^{R132} and *IDH2*^{R172}, 2 μL of patient DNA (100 ng/μL) was added to 48 μL of PCR master mix that consisted of 31.7 μL of molecular grade water, 5 mL of 10× PCR buffer II (Applied Biosystems, Carlsbad, CA), 4 μL of 25 mmol/L magnesium chloride, 10 mmol/L deoxynucleoside triphosphate (dNTP) mix, 1 μL each of M13-tagged forward and reverse primers (10 μmol/L), and 0.3 μL of Amplitaq Gold (5 U/μL; Applied Biosystems). PCR conditions for *IDH1*^{R132} and *IDH2*^{R172} included initial denaturing at 95°C for 10 minutes; 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds; and final extension at 72°C for 7 minutes.

Post-PCR purification of the products was performed using QIAquick kit (Qiagen, Valencia, CA). PCR products were analyzed on a 2% agarose gel (115 V for 30-40 minutes) using 8 μL of PCR product and 2 μL of 1× gel-loading dye. Sanger sequencing of *IDH1*^{R132} and *IDH2*^{R172} amplicons was performed using M13-tagged primers in a 20-μL final volume that contained 4 μL of PCR product, 6.8 μL of water,

Table 2
Clinical Features of AML With *IDH2*^{R172} Mutation*

	All Cases	*IDH2*^{R172} Mutant Cases (n = 4)	*IDH2* Wild-Type Cases (n = 192)	*P*†
No. of cases	196	4/196 (2.0)	192/196 (98.0)	
Mean (range) age in (y)	55 (17-89)	51 (22-76)	55 (17-89)	.778‡
No. of males/females (M/F) ratio	93/103 (1:1.1)	1/3 (1:3)	92/99 (1:1.1)	.623
WHO classification				
AML, NOS	160	4 (100)	156 (81.3)	1.000
AML, MLD	30	0 (0)	30 (15.6)	1.000
t-AML	4	0 (0)	4 (2.1)	1.000
AML, biphenotypic	2	0 (0)	2 (1.0)	1.000
FAB classification				
M0	11	0 (0)	11 (5.6)	1.000
M1	30	3 (75)	27 (14.1)	.010
M2	45	1 (25)	44 (22.9)	1.000
M4	42	0 (0)	42 (21.9)	.579
M5	19	0 (0)	19 (9.9)	1.000
M6	5	0 (0)	5 (2.6)	1.000
M7	1	0 (0)	1 (0.5)	1.000
RAEB-T	15	0 (0)	15 (7.8)	1.000
NA	28	0 (0)	28 (14.6)	1.000
Mean (range) WBC count (× 10^9/L)	19.7 (0.3-204.2)	4.3 (1.7-11.8)	20.0 (0.3-204.2)	.001‡
Mean (range) blasts (%)	53 (20-98)	59 (42-73)	53 (20-98)	.390‡
Karyotype				.299
Normal	124	4 (100)	120 (62.5)	
Abnormal	72	0 (0)	72 (37.5)	
Cytogenetic risk group				1.000
Intermediate	163	4 (100)	159 (82.8)	
Poor	33	0 (0)	33 (17.2)	
Additional mutations				
NPM1	—	0/4 (0)	17/60 (28.3)	.566
FLT3-ITD	—	0/4 (0)	46/185 (24.9)	.574
FLT3-D835	—	0/4 (0)	10/185 (5.4)	1.000
CEBPA	—	0/4 (0)	2/10 (20)	1.000
NRAS	—	0/4 (0)	13/159 (8.2)	1.000
KRAS	—	0/4 (0)	7/159 (4.4)	1.000
KIT	—	0/4 (0)	1/81 (1.2)	1.000
IDH1	—	0/4 (0)	11/192 (5.7)	1.000
Molecular risk group§				1.000
Low	11	0/4 (0)	11/91 (12)	
High	84	4/4 (100)	80/91 (88)	

AML, acute myeloid leukemia; AML, MLD, AML with myelodysplasia-related changes; AML, NOS, AML not otherwise specified; FAB, French-American-British; NA, not available; RAEB-T, refractory anemia with excess blasts in transformation; t-AML, therapy-related AML; WHO, World Health Organization.
* Data are given as number (percentage) or number positive/number tested (percentage) unless otherwise indicated. The WBC count is given in Système International units; to convert to conventional units (/μL), divide by 0.001.
† Fisher exact test unless otherwise indicated.
‡ 2-tailed *t* test.
§ Molecular low risk, *NPM1* mutated and *FLT3-ITD* wild type; high risk, *NPM1* wild type and/or *FLT3-ITD*+.

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2 μL of 5× sequencing buffer, 3.2 μL of M13 forward or reverse primer (1 μmol/L), and 4 μL of BigDye v1.1 (Applied Biosystems). Sanger sequencing was performed using M13 forward, 5'-TGTAAAACGACGGCCAGT-3', and M13 reverse, 5'-CAGGAAACAGCTATGACC-3', primers.

Sequencing conditions included initial denaturing at 96°C for 1 minute and 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 seconds. Sequencing products were purified using the DyeEx 96 Spin Kit (Qiagen) and analyzed by capillary gel electrophoresis on the 3130 genetic analyzer (Applied Biosystems).

NPM1 Mutation Detection

Mutations in coding regions of exon 12 of *NPM1* were detected using PCR amplification of a 168-base-pair segment followed by capillary gel electrophoresis. PCR primers included forward, 5'-FAM-GATGTCTATGAAGTGTTGTGGTTCC-3' and reverse, 5'- GGACAGCCAGATCAACTG-3'. PCR was performed in a 50-μL reaction volume that contained 2 μL of patient DNA (100 ng/μL), 5 μL of 10× ThermoPol Buffer (New England BioLabs, Ipswich, MA) with magnesium sulfate, 5 μL of 10 mmol/L dNTP, 1 μL of *NPM1* forward primer (10 μmol/L), 1 μL of *NPM1* reverse primer (10 μmol/L), 35.2 μL of water, and 0.75 μL of Vent DNA polymerase (New England Biolabs) (2 U/μL). PCR conditions included initial denaturing at 95°C for 10 minutes, 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and final extension at 72°C for 7 minutes. PCR products were analyzed by capillary electrophoresis on a 3100 or 3130 genetic analyzer (Applied Biosystems).

RAS Mutation Detection

Mutations in codons 12, 13, and 61 of *KRAS* were detected by using pyrosequencing as described earlier.[18] Mutations in codons 12, 13, and 61 of *NRAS* were detected with the same protocol but using the following primers: codons 12 and 13, forward, 5'-GTTCTTGCTGGTGTGAAATGA-3'; reverse, 5'-BIOTIN-CTCTATGGTGGGATCATATTC-3'; and sequencing, 5'-CAAACTGGTGGTGGTTGGAGCA-3'; and codon 61, forward, 5'-GGACATACTGGATACAGCT-3'; reverse, 5'-BIOTIN-CTGTAGAGGTTAATATCCGCA-3'; and sequencing, 5'-GGACATACTGGATACAGCT-3'.

Detection of *KIT, FLT3-ITD, FLT3-D835, CEBPA,* and *TP53* Mutations

Mutation analysis for *KIT*, *FLT3-ITD*, *FLT3-D835*, *CEBPA*, and *TP53* mutations was performed using Sanger sequencing as described previously.[20-23] For *CEBPA* and *TP53*, an M13 sequence was added to the previously described PCR primers for Sanger sequencing. M13-tagged primers were used for Sanger sequencing.

Morphologic, Immunophenotypic, and Cytogenetic Analysis

Bone marrow aspirate smears were stained with Wright-Giemsa, and aspirate clot and biopsy specimens were stained with H&E. Flow cytometric immunophenotypic analysis was performed using 4-color staining, and conventional G-banded karyotyping was performed as described previously.[24]

Results

IDH1^{R132} and *IDH2*^{R172} Mutations in AML

A total of 199 AML cases were tested for *IDH1*^{R132} mutation, and 196 cases were tested for *IDH2*^{R172} mutation (Tables 1 and 2). The *IDH1*^{R132} mutation was detected in 12 cases (6.0%), and the *IDH2*^{R172} mutation was detected in 4 (2.0%) of 196 cases **Figure 1**. No mutated cases had both *IDH1* and *IDH2* mutations, suggesting that these mutations are mutually exclusive.

Cytogenetic Features of *IDH*-Mutated AML Cases

The AMLs tested included 127 (63.8%) with normal and 72 (36.2%) with abnormal cytogenetics. Subdivided by cytogenetic risk group, 166 cases (83.4%) were intermediate risk and 33 (16.6%) were poor risk. Of 12 AML cases with an *IDH1* mutation, 11 (92%) had normal cytogenetics and all 12 (100%) were in the intermediate-risk cytogenetic group. The association between *IDH1* mutation and normal cytogenetics approached statistical significance (P = .059; Fisher exact test). All 4 cases with an *IDH2* mutation had normal cytogenetics and belonged to the intermediate-risk group. Of the 16 mutated cases (12 *IDH1* and 4 *IDH2*), 15 (94%) were cytogenetically normal, for an overall frequency in this group of 11.8% (15/127).

Molecular Features of *IDH*-Mutated AML Cases

The 12 AMLs with *IDH1*^{R132} mutations were equally distributed between R132H and R132C substitutions **Table 3**. All *IDH2*^{R172} mutations resulted in R172K substitutions (Table 3). *IDH1*^{R132}-mutated cases showed a higher frequency of concurrent *NPM1* mutation compared with wild-type cases (5/11 [45%] vs 13/54 [24%]), which did not achieve statistical significance (P = .161; Fisher exact test). Additional gene mutations were identified in *IDH1*-mutated cases, including *NPM1*, *FLT3-ITD*, *CEBPA*, *NRAS*, *KIT*, and *FLT3-D835* (Table 1). Of 11 *IDH1*-mutated cases, 8 (73%) met the criteria for the high-risk molecular group. There was no significant association of the *IDH1*^{R132} mutation with *FLT3-ITD*, *FLT3-D835*, *CEBPA*, *NRAS*, *KRAS*, or *KIT* mutations or a high-risk molecular profile (Tables 1

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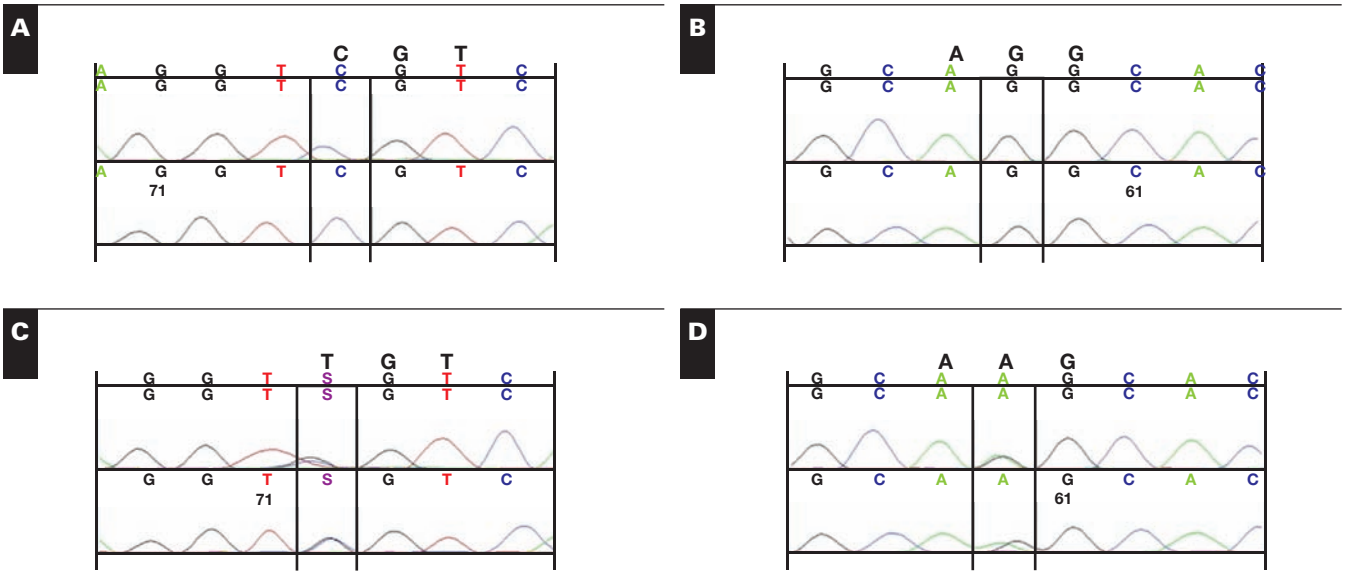

Figure 1 Detection of *IDH1* and *IDH2* mutations by Sanger sequencing. **A**, Wild-type *IDH1* codon 132: CGT, R132. **B**, Wild-type *IDH2* codon 172: AGG, R172. **C**, Mutant *IDH1* codon 132: TGT, R132C. **D**, Mutant *IDH2* codon 172: AAG, R172K. Note that both $IDH1^{R132}$ and $IDH2^{R172}$ mutations are heterozygous missense point mutations.

and 3).[1,25] None of the 4 $IDH2^{R172}$-mutated cases showed a concurrent mutation in *NPM1*, *FLT3-ITD*, *FLT3-D835*, *CEBPA*, *NRAS*, *KRAS*, *KIT*, or *IDH1* (Tables 2 and 3). All 4 $IDH2^{R172}$-mutated AMLs met the criteria for the high-risk molecular group based on the absence of the *NPM1* mutation.

TP53 Mutations in AML Cases With the $IDH1^{R132}$ Mutation

In glioma, non-R132H mutations of *IDH1* are associated with a higher frequency of *TP53* mutations and a distinct gene expression profile compared with the R132H mutations. We therefore tested 11 available $IDH1^{R132}$-mutant AML cases for

Table 3
Cytogenetic and Molecular Features of AML Cases With $IDH1^{R132}$ and $IDH2^{R172}$ Mutations

Case No./ Sex/Age (y)	Classification		Karyotype	Cytogenetic Risk Group	Mutation	Nucleotide Change	Amino Acid Change	Other Mutations
	WHO	FAB						
1/F/37	AML, NOS	M1	N	I	*IDH1*	CGT-CAT	R132H	*NPM1, FLT3-ITD, CEBPA*
2/F/37	AML, NOS	M1	N	I	*IDH1*	CGT-TGT	R132C	*N-RAS, KIT*
3/F/51	AML, MLD	M1	N	I	*IDH1*	CGT-CAT	R132H	*NPM1, FLT3-ITD*
4/F/73	AML, NOS	M1	N	I	*IDH1*	CGT-TGT	R132C	*FLT3-D835*
5/F/52	AML, NOS	M2	N	I	*IDH1*	CGT-TGT	R132C	*FLT3-ITD*
6/M/52	AML, NOS	M2	N	I	*IDH1*	CGT-CAT	R132H	*NPM1*
7/M/53	AML, NOS	M2	N	I	*IDH1*	CGT-TGT	R132C	—
8/F/55	AML, NOS	M2	N	I	*IDH1*	CGT-CAT	R132H	*CEBPA*
9/F/52	AML, NOS	M5	N	I	*IDH1*	CGT-TGT	R132C	—
10/F/58	AML, NOS	M5	N	I	*IDH1*	CGT-CAT	R132H	*NPM1, RAS*
11/F/77	AML, NOS	M5	N	I	*IDH1*	CGT-CAT	R132H	*NPM1*
12/M/62	AML, MLD	RAEB-T	A*	I	*IDH1*	CGT-TGT	R132C	—
13/F/48	AML, NOS	M1	N	I	*IDH2*	AGG-AAG	R172K	—
14/M/59	AML, NOS	M1	N	I	*IDH2*	AGG-AAG	R172K	—
15/F/76	AML, NOS	M1	N	I	*IDH2*	AGG-AAG	R172K	—
16/F/22	AML, NOS	M2	N	I	*IDH2*	AGG-AAG	R172K	—

AML, acute myeloid leukemia; AML, MLD, AML with myelodysplasia-related changes; AML, NOS, AML not otherwise specified; C, cysteine; FAB, French-American-British; H, histidine; I, intermediate risk; K, lysine; N, normal; R, arginine; RAEB-T, refractory anemia with excess blast in transformation; WHO, World Health Organization.
* Abnormal karyotype, 46,XY,del(5)(q13q33),inv(12)(p11.2q24.1)[6]/46,XY[14].

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TP53 mutations. We detected a P72R polymorphism, known to predispose to a variety of human cancers, in 4 of 5 R132C cases and 5 of 6 R132H cases. We did not find any significant difference in the frequency of *TP53* mutations in R132C and R132H subgroups, as each subgroup had only 1 case with *TP53* mutation.

Morphologic and Immunophenotypic Features of AML With *IDH* Mutations

For AMLs with the $IDH1^{R132}$ mutation, the mean blast count was 55% (range, 22%-88%). Using the WHO classification, the mutated cases were classified as AML, not otherwise specified (NOS; n = 10) or AML with myelodysplasia-related changes (n = 2). The AML, NOS group was further classified as AML without maturation (French-American-British classification [FAB] M1), AML with maturation (FAB M2), and acute monocytic leukemia (FAB M5). Auer rods were detected in 5 (50%) of 10 cases. Dysplastic features in 1 or more hematopoietic lineages were evident in 10 (83%) of 12 AML cases with the $IDH1^{R132}$ mutation and 3 (75%) of 4 AMLs with the *IDH2* mutation **Table 4**. Bone marrow cellularity ranged from 25% to 100% (mean, 71%).

For AMLs with $IDH2^{R172}$ mutations, the mean blast count was 53% (range, 20%-98%). These 4 mutated cases were classified as AML without maturation (n = 3) and AML with maturation (n = 1). *IDH2*-mutated cases were significantly more often classified as AML without maturation compared with *IDH2* wild-type AML cases (3/4 [75%] vs 27/192 [14.1%]; $P < .01$; Fisher exact test). Auer rods were detected in 3 (75%) of 4 cases. Of 4 AML cases with the *IDH2* mutation, 3 (75%) showed dysplastic features in 1 or more hematopoietic lineages (Table 4). Bone marrow cellularity ranged from 60% to 80% (mean, 70%).

Flow cytometric Immunophenotypes of AML with $IDH1^{R132}$ and $IDH2^{R172}$ mutations are similar and are listed in **Table 5**. Overall AMLs with the $IDH1^{R132}$ or $IDH2^{R172}$ mutation showed a myeloid immunophenotype: CD117+, HLA-DR+/– (more cases positive than negative), CD34+/–, CD38+, CD13+, CD33+, and myeloperoxidase+/–. CD64 was present in AML M5, consistent with monocytic differentiation.

Clinical Features of *IDH*-Mutated AML Cases

For the 12 *IDH1*-mutated AMLs, the mean age was 55 years (range, 37-77 years), and the male/female ratio was 1:3. The mean WBC count was 15,000/μL (15.0×10^9/L). For the 4 *IDH2*-mutated cases, the mean age was 51 years (range, 22-76 years), and the male/female ratio was 1:3. The mean WBC count was 4,000/μL (4.0×10^9/L). There was no significant difference in age, sex, WBC count, platelet count, hemoglobin level, bone marrow blast count and cellularity, dysplastic features, or morphologic classification between the *IDH1*- and *IDH2*-mutated AML cases (Table 1).

Table 4
Hematologic and Morphologic Features of AML Cases With $IDH1^{R132}$ and $IDH2^{R172}$ Mutations*

Mutation/Case No./ Sex/Age (y)	Classification		WBC Count ($\times 10^9$/L)	Hemoglobin (g/dL)	Platelet Count ($\times 10^9$/L)	Marrow Cellularity (%)	Blasts (%)	Auer Rods	Dysplastic Features
	WHO	FAB							
IDH1									
1/F/37	AML, NOS	M1	45.9	7.9	180	100	84	3+	Absent
2/F/37	AML, NOS	M1	4.9	9.6	42	100	84	1+	Mega
3/F/51	AML, MLD	M1	4.9	9.6	31	80	53	NA	E, Mega
4/F/73	AML, NOS	M1	16.9	10	92	95	67	2+	E
5/F/52	AML, NOS	M2	6.7	11.2	254	25	37	–	E (slight), Mega
6/M/52	AML, NOS	M2	0.5	13.9	127	30	22	1+	M, E (slight)
7/M/53	AML, NOS	M2	0.3	12.5	45	90	27	–	M, E, Mega
8/F/55	AML, NOS	M2	2.6	13.5	213	30	56	1+	M
9/F/52	AML, NOS	M5	1.4	13.6	119	80	24	–	M, E (slight), Mega
10/F/58	AML, NOS	M5	83.1	9.3	69	90	86	–	M, Mega
11/F/77	AML, NOS	M5	8.6	12.8	225	90	80	NA	Mega
12/M/62	AML, MLD	RAEB-T	0.7	10	291	40	44	–	Absent
IDH2									
13/F/48	AML, NOS	M1	1.7	8.2	99	80	61	1+	M, E
14/M/59	AML, NOS	M1	1.8	9.7	238	60	61	1+	E
15/F/76	AML, NOS	M1	11.8	9.4	18	70	73	–	Absent
16/F/22	AML, NOS	M2	1.7	8.4	79	70	42	3+	M, E

AML, acute myeloid leukemia; AML, MLD, AML with myelodysplasia-related changes; AML, NOS, AML not otherwise specified; E, erythroid; FAB, French-American-British; M, myeloid; Mega, megakaryocytes; NA, not available; RAEB-T, refractory anemia with excess blast in transformation; WHO, World Health Organization; –, negative; 1+, rare; 2+, intermediate; 3+, frequent.

* The WBC and platelet counts are given in Système International (SI) units; conversion to conventional units is as follows: WBC count (/μL), divide by 0.001; platelet count ($\times 10^3$/μL), divide by 1.0. Hemoglobin values are given in conventional units; to convert to SI units (g/L), multiply by 10.0.

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Table 5
Flow Cytometric Immunophenotype of AML Cases With *IDH1*^{R132} and *IDH2*^{R172} Mutations

Mutation/Case No./ Sex/Age (y)	Classification		CD34	CD117	HLA-DR	CD38	CD13	CD33	CD14	Other
	WHO	FAB								
IDH1										
1/F/37	AML, NOS	M1	–	+	+	+	+	+	–	CD9+
2/F/37	AML, NOS	M1	+	+	+	+	+	+	–	MPO+
3/F/51	AML, MLD	M1	+	+	+	+	+	+	–	NA
4/F/73	AML, NOS	M1	–	+	–	+	+	+	–	NA
5/F/52	AML, NOS	M2	+	+	+	+	+	+	NA	MPO+
6/M/52	AML, NOS	M2	–	NA	NA	+	NA	+	–	MPO+, CD16(dim)
7/M/53	AML, NOS	M2	–	+	–	+	+	NA	NA	MPO+
8/F/55	AML, NOS	M2	+	+	+	+	+	+	–	CD4(partial), CD7(partial)
9/F/52	AML, NOS	M5	NA	NA	+	NA	+	+	Dim	CD64+
10/F/58	AML, NOS	M5	–	+	+	+	+	+	NA	CD9+, CD64(dim), MPO+
11/F/77	AML, NOS	M5	–	NA	+	+	–	+	–	CD56+, CD64+
12/M/62	AML, MLD	RAEB-T	+	+	+	–	+	+	–	CD56+, CD64–, MPO–
IDH2										
13/F/48	AML, NOS	M1	+	+	+	+	+	+	NA	MPO+
14/M/59	AML, NOS	M1	+	+	+	+	+	+	NA	MPO+, TdT–
15/F/76	AML, NOS	M1	+	+	+	+	+	+	–	MPO+
16/F/22	AML, NOS	M2	+	+	+	+	+	+	–	MPO+

AML, acute myeloid leukemia; AML, MLD, AML with myelodysplasia-related changes; AML, NOS, AML not otherwise specified; FAB, French-American-British; MPO, myeloperoxidase; NA, not available; RAEB-T, refractory anemia with excess blasts in transformation; TdT, terminal deoxyribonucleotide transferase; WHO, World Health Organization.

These clinical variables in the *IDH1*- and *IDH2*-mutated AML cases were also compared with wild-type AMLs. *IDH2*^{R172}-mutant AMLs were also significantly associated with a lower WBC count compared with wild-type AMLs (4,300/μL vs 20,000/μL [4.3 vs 20.0 × 10^9/L]; $P < .001$; Student *t* test). There was no significant difference in age, sex, and blast count between *IDH1*-mutated and unmutated AML cases or between *IDH2*^{R172}-mutated and unmutated cases (Table 2).

Meta-analysis of AMLs With *IDH1*^{R132} Mutation

We performed a meta-analysis of all available studies that assessed for *IDH* mutations in AML cases. To date, virtually all studies have focused on *IDH1* mutations in AML, with a total of 1,787 AMLs reported, with 146 cases (8.2%) being mutated **Table 6**. Because the *IDH1*^{R132} mutation is predominantly restricted to CN AML, inclusion of AML cases with abnormal karyotypes in earlier studies most likely underrepresents the true frequency of *IDH1* mutations in CN AML. We extracted the fraction of CN AMLs with the *IDH1*^{R132} mutation from the studies reported. Of 1,152 CN AMLs tested, 129 (11.2%) showed the *IDH1*^{R132} mutation compared with 17 (4.7%) of 360 AMLs with an abnormal karyotype. Review of cytogenetic studies showed that all 66 *IDH1*-mutated AML cases were limited to the intermediate-risk category. *IDH1*^{R132} mutations resulted in a higher R132H substitution (73/146 [50.0%]) than R132C substitution (45/143 [31.5%]).

Analysis of available test results for coexisting mutations showed *NPM1* to be the most common concurrent mutation, found in 87 (60.4%) of 144, followed by *FLT3-ITD* (37/146 [25.3%]) and *CEBPA* (14/155 [9.0%]). None of the 60 AMLs with an *IDH1*^{R132} mutation showed a concurrent *IDH2* mutation. The number of AML cases assessed for the *IDH2* mutation was too few to perform a similar meta-analysis.

Discussion

IDH1 and *IDH2*, nicotinamide adenine dinucleotide phosphate+-dependent isocitrate dehydrogenases, catalyze the oxidative carboxylation of isocitrate to α-ketoglutarate. *IDH1* has a key role in the production of the cytosolic reduced form of nicotinamide adenine dinucleotide phosphate necessary for the regeneration of reduced glutathione, a main antioxidant in mammalian cells.[26] Recurring mutations in *IDH1* and *IDH2* occur at a very high frequency in many different types of glioma, especially secondary glioblastoma.[12,27]

IDH mutations in gliomas are always reported at the same arginine residues, R132 in *IDH1* and R172 in *IDH2*, that are responsible for hydrophilic interactions with isocitrate.[28] Initial analyses showed that *IDH* mutations were restricted primarily to gliomas, with rare cases of prostate cancer and B-lymphoblastic leukemia showing *IDH* mutations.[12,29,30]

Recently, Mardis and colleagues[9] sequenced the entire genome of a cytogenetically normal case of AML and detected a novel *IDH1*^{R132} mutation. They subsequently screened 187 AML cases and showed a heterozygous *IDH1*^{R132} mutation in 15 cases (8.0%). Sixteen (original case plus 15

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Table 6
Meta-analysis of AML With *IDH1*^{R132} Mutation*

	Present Study (n = 12)	Mardis et al,[9] 2009 (n = 16)	Chou et al,[16] 2010 (n = 27)	Marcucci et al,[14] 2010 (n = 49)†	Wagner et al,[13] 2010 (n = 30)	Ho et al,[15] 2010 (n = 12)	All Cases (n = 146)
All cases	12/199 (6.0)	16/188 (8.5)	27/493 (5.5)	49/358 (13.7)	30/275 (10.9)	12/274 (4.4)	146/1,787 (8.2)
Mean (range) age (y)	55 (37-77)	48.9 ± 15.4‡	52.5 (25-75)	62§ (21-82)	50§ (33-80)	61§ (34-81)	52‖
M/F ratio	1:3	1.3:1	1.1:1	1:1.1	1:1.7	2:1	1:1.1
FAB classification				NA		NA	
M1	4 (33)	10 (63)	14 (52)		6 (20)		34/82 (41)
M2	4 (33)	3 (19)	9 (33)		9 (30)		25/82 (30)
M4	0 (0)	3 (19)	2 (7)		8 (27)		13/82 (16)
M5	2 (17)	0 (0)	2 (7)		4 (13)		8/82 (10)
RAEB-T	2 (17)	0 (0)	0 (0)		0 (0)		2/82 (2)
Mean (range) blasts (%)	55 (22-88)	76.7 ± 16.4‡	NA	73* (33-99)	80* (20-99)	80* (38-99)	67‖
Cytogenetic findings				NA¶	NA¶		
Normal	11 (92)	13 (81)	20/26 (77)			6/10 (60)	50/64 (78)
Abnormal	1 (8)	3 (19)	6/26 (23)			4/10 (40)	14/64 (22)
Cytogenetic risk group				NA†	NA†		
Favorable	0 (0)	0 (0)	0 (0)			0 (0)	0 (0)
Intermediate	12 (100)	16 (100)	26/26 (100)			12 (100)	66/66 (100)
Poor	0 (0)	0 (0)	0 (0)			0 (0)	0 (0)
Frequency in CN AML	11/127 (8.7)	13/80 (16)	20/227 (8.8)	49/358 (13.7)	30/275 (10.9)	6/85 (7)	129/1,152 (11.2)
Type of *IDH1* mutation							
R132H	6 (50)	7 (44)	7 (26)	24 (49)	21 (70)	8 (67)	73/146 (50.0)
R132C	6 (50)	8 (50)	10 (37)	15 (31)	5 (17)	1 (8)	45/146 (30.8)
R132S	0 (0)	1 (6)	5 (19)	5 (10)	3 (10)	0 (0)	14/146 (9.6)
R132L	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	2 (17)	3/146 (2.1)
R132G	0 (0)	0 (0)	4 (15)	0 (0)	1 (3)	1 (8)	6/146 (4.1)
Coexisting mutations							
NPM1	5/11 (45)	7/16 (44)	15/27 (56)	34/48 (71)	17/30 (57)	9/12 (75)	87/144 (60.4)
FLT3-ITD	3/12 (25)	4/16 (25)	10/27 (37)	10/49 (20)	4/30 (13)	6/12 (50)	37/146 (25.3)
FLT3-D835	1/12 (8)	1/16 (6)	3/27 (11)	3/48 (6)	NA	NA	8/103 (7.8)
CEBPA	2/10 (20)	NA	1/27 (4)	2/36 (6)	8/30 (27)	1/12 (8)	14/115 (12.2)
NPM1+FLT3-ITD	2/12 (17)	NA	9/27 (33)	NA	NA	NA	11/39 (28)
NRAS	2/12 (17)	1/16 (6)	4/27 (15)	NA	NA	2/12 (17)	9/67 (13)
KRAS	0/12 (0)	NA	0/27 (0)	NA	NA	NA	0/39 (0)
KIT	1/9 (11)	NA	0/27 (0)	NA	NA	NA	1/36 (3)
IDH2	0/11 (0)	NA	NA	0/49 (0)	NA	NA	0/60 (0)

C, cysteine; CN, cytogenetically normal; FAB, French-American-British; G, glycine; H, histidine; L, leucine; NA, not available; RAEB-T, refractory anemia with excess blasts in transformation.
* Data are given as number (percentage) or number positive/number tested (percentage) unless otherwise indicated.
† Includes 2 non-R132 mutations.
‡ Mean ± SD.
§ Median age.
‖ Average of mean values only.
¶ Only cytogenetically normal cases tested in the study.

additional cases) *IDH1*^{R132}-mutated cases had available cytogenetic data, and 13 (81%) were CN AML. In addition, all 16 *IDH1*-mutated AMLs had intermediate-risk cytogenetics. These findings were in contrast with previous studies that did not detect the *IDH1*^{R132} mutation in 145 AML cases.[12,30] Differences in the cytogenetic findings in the study groups and sensitivities of the detection assays could underlie these discrepancies. For this reason, we chose to undertake this study reviewing our experience in approximately 200 cases of AML at our institution.

Based on the available data in the literature, we specifically studied AML cases with intermediate- or poor-risk cytogenetic findings.[9] Our results show that the frequency of *IDH1* mutations is lower in CN AML cases. We detected *IDH1*^{R132} mutations in 11 (8.7%) of 127 CN AMLs compared with the initial report of 16%.[9] We showed that all AML cases with the *IDH1* mutation have intermediate-risk cytogenetics. In addition, we identified *IDH2*^{R172} mutations in 4 (2.0%) of 196 AML cases, all of which were CN AML. In no case did we find both *IDH1* and *IDH2* mutations, strongly suggesting that *IDH1*^{R132} and *IDH2*^{R172} are mutually exclusive. Only 1 other study has assessed for both *IDH1* and *IDH2* mutations in a large group of CN AML cases. Marcucci and colleagues[14] showed that *IDH* mutations occur in 33% of cases, with *IDH1* in 14% and *IDH2* in 19%. The explanation for the higher rate of *IDH1* and particularly *IDH2* mutations in CN AML cases in this study, compared with our own data, is explained in large part because Marcucci and colleagues[14] assessed for *IDH2* mutations involving the arginine residue at codon 140 (R140). More than 80% of the *IDH2* mutations they detected were R140 mutations. We did not test for *IDH2*^{R140} mutations in our study because the significance of mutations at this codon is unknown. These mutations have not been shown to have prognostic significance in AML and are not seen in other

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human cancers. Other authors have suggested that further studies are needed to determine whether R140 substitutions represent a true pathogenic mutation or a polymorphism.[14]

Because *IDH1*^{R132} is mainly limited to CN AML, the frequency of the *IDH1*^{R132} mutation in AMLs is likely to vary from one study to the next, depending on the percentage of AML cases with abnormal karyotypes. For example, the *IDH1*^{R132} mutation was detected in 7% of CN AMLs but in only 4% of all AML cases in a study by Ho and colleagues.[15] It is therefore important to account for the cytogenetic composition of the study group when interpreting the frequency of *IDH1*^{R132} in AML. We performed meta-analysis of our study and other available studies to obtain a more global view of *IDH1* mutations in AML. A total of 146 AMLs with the *IDH1*^{R132} mutation have been reported. Most cases with the *IDH1* mutation have been CN AMLs and restricted to the intermediate-risk cytogenetic group. The overall frequency of the *IDH1*^{R132} mutation in CN AML is 11.0% (141/1,279). The male/female ratio is 1:1.1.

Despite the recent interest in *IDH* mutations in AML, a limited amount of information is available on morphologic and immunophenotypic features. Morphologic classification using the FAB system is available for 82 cases. The most common morphologic types were M1 in 34 cases (41%) and M2 in 25 cases (30%). Very few cases have been classified using the 2008 WHO system. The classification of cases in our study closely matches what has been reported. The *IDH1*-mutated cases were classified as AML, NOS (n = 10) or AML with myelodysplasia-related changes (n = 2). It is interesting that the *IDH2*-mutated cases were all AML, NOS. In this category, 7 cases were AML without maturation (FAB M1), 5 cases were AML with maturation (FAB M2), and 3 cases were acute monocytic leukemia (FAB M5). The immunophenotype of most cases in our study was typical myeloid, with expression of CD13, CD33, and CD117 in most cases and expression of CD34 in 9 of 15 assessed. These findings are generally consistent with a recent report in a Chinese population by Chou and colleagues.[16] However, Chou et al[16] reported that monocytic differentiation (FAB M4) and expression of CD13, CD14, and HLA-DR are unusual in AML with the *IDH* mutation. Our experience differs because 3 cases showed monocytic differentiation (FAB M5), and most cases expressed CD13 and HLA-DR.

Our study is the first to report bone marrow aspirate and biopsy findings of AML with *IDH* mutations in some detail. It is interesting to note that more than 80% of AML cases with the *IDH1* or *IDH2* mutation showed varying degrees of dysplastic findings in erythroid, myeloid, and megakaryocytic lineages. *IDH1* mutations are shown to be early events in the development of astrocytomas and oligodendrogliomas. It seems plausible that *IDH1* mutations are also an early event in myeloid neoplasia. A recent article reported *IDH1* mutations in early myelodysplastic syndrome (MDS) and in secondary AML arising from MDS or MDS/myeloproliferative neoplasms (MPNs).[31] In our study, 1 patient with AML with an *IDH1*^{R132} mutation had a history of MDS (case 12, Tables 3, 4, and 5). In 2 available studies on *IDH1* mutations in MPN, one showed an *IDH1* mutation with a coexisting *JAK2* mutation, and the other failed to detect an *IDH1* mutation in an MPN that transformed to leukemia.[32,33] The involvement of *IDH1* in myeloid neoplasms could be explained by a high requirement of glutamate, an essential amino acid that is converted to α-ketoglutarate and acts as a substrate for the mutant *IDH1*, as described subsequently, in myeloid cells.[17,26,34] Indeed, acivicin, a glutamine antagonist, treatment decreased the growth and viability of a variety of leukemia cell lines.[35]

We detected equal distribution of R132H and R132C mutations in 12 AMLs with the *IDH1*^{R132} mutation. In the meta-analysis, R132H (73/146 [50.0%]) and R132C (45/146 [30.8%]) were the most frequent mutations detected. These findings suggest a different pattern of distribution of *IDH1*^{R132} mutations in AMLs than in gliomas, in which the R132H mutation constitutes about 90% of *IDH1*^{R132} mutations.[12] In addition, we did not detect differences in *TP53* mutation profiles of R132H and R132C AML cases, unlike those shown in gliomas.[36] These findings suggest possible differences in the role of *IDH1* mutations between glioma and AML. In fact, *IDH1* mutations are associated with a good prognosis in glioma, whereas the limited amount of literature in AML suggests shorter disease-free survival in young patients with the *IDH1*-mutant/*NPM1*-mutant/*FLT3-ITD*–wild-type group.[14,37]

IDH1^{R132} mutations are frequently accompanied by *NPM1*, *FLT3*, *CEBPA*, *RAS*, and *KIT* mutations. We found *NPM1* (5/11 [45%]) and *FLT3-ITD* (3/12 [25%]) mutations to be the 2 most frequent. Comparable frequencies of *NPM1* (87/144 [60.4%]) and *FLT3-ITD* (29/146 [19.9%]) were detected in the meta-analysis. Unlike a recent study, we did not find statistically significant correlations of *IDH1*^{R132} and *IDH2*^{R172} mutations with *NPM1*-mutated and an *FLT3-ITD*–negative low-risk molecular profile.[14] The frequent presence of coexisting mutations suggests that *IDH1* mutations may act cooperatively in leukemogenesis. In contrast, no additional mutations were detected with *IDH2*^{R172} mutations in 4 cases. This finding is consistent with 13 cases in the only other available study.[14]

The details of a possible pathogenic role of *IDH* mutations are just beginning to emerge. Traditionally, up-regulation of a cancer-associated transcription factor, hypoxia-induced factor, has been considered to be a major pathogenic mechanism.[26,38] More recently, accumulation of 2-hydroxyglutarate in the cells and the serum of patients with glioma and with AML with the *IDH1* mutation has been shown.[17,28,39,40] This could be used as a potential diagnostic test in the management of patients with *IDH* mutations.

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All reported studies have used Sanger sequencing–based assays, which have a sensitivity of about 20%.[9,13-17] Because *IDH1* and *IDH2* are heterozygous mutations, a minimum blast count of 40% and/or enrichment of myeloblasts will be needed for the Sanger sequencing–based detection of *IDH* mutations. In practice, however, we have detected *IDH* mutations in samples with a minimum blast count of 22%. Because there is an indication that the mutation is retained at relapse, highly sensitive laboratory assays will be needed for monitoring therapy response and early relapses.[16,39] The involvement of specific codons allows the use of sensitive approaches such as high-resolution melt curve analysis and pyrosequencing-based assays, which are currently under development in our laboratory.

IDH1^{R132} and *IDH2*^{R172} mutations represent a novel class of point mutations in CN AML. Both mutations occur predominantly in CN AML, leading to overproduction of an oncometabolite, 2-hydroxyglutarate. These mutations are heterozygous in nature and mutually exclusive. Despite many similarities, it is possible that molecularly and clinically, they represent distinct subgroups. *IDH1*^{R132} is frequently accompanied by other mutations, whereas *IDH2*^{R172} is commonly the only mutation detected. Most AML cases with an *IDH* mutation, *IDH1* or *IDH2*, are morphologically classified as AML with or without myeloid maturation (FAB M1 or M2), have morphologic evidence of dysplasia, and have a nondistinctive myeloid immunophenotype.

From the Departments of [1]Hematopathology and [2]Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston.

Address correspondence to Dr Luthra: Dept of Hematopathology, The University of Texas M.D. Anderson Cancer Center, 8515 Fannin St, Unit 149, Houston, TX 77030.

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