VOLUME 28 · NUMBER 14 · MAY 10 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

IDH1 and *IDH2* Gene Mutations Identify Novel Molecular Subsets Within De Novo Cytogenetically Normal Acute Myeloid Leukemia: A Cancer and Leukemia Group B Study

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Submitted November 28, 2009; accepted January 27, 2010; published online ahead of print at www.jco.org on April 5, 2010.

Supported in part by National Cancer Institute Grants No. CA101140, CA114725, CA31946, CA33601, CA16058, CA77658, CA129657, and CA140158, by The Coleman Leukemia Research Foundation, and by the Deutsche Krebshilfe Dr Mildred Scheel Cancer Foundation (H.B.).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/10/2814-2348/$20.00

DOI: 10.1200/JCO.2009.27.3730

ABSTRACT

Purpose
To analyze the frequency and associations with prognostic markers and outcome of mutations in *IDH* genes encoding isocitrate dehydrogenases in adult de novo cytogenetically normal acute myeloid leukemia (CN-AML).

Patients and Methods
Diagnostic bone marrow or blood samples from 358 patients were analyzed for *IDH1* and *IDH2* mutations by DNA polymerase chain reaction amplification/sequencing. *FLT3*, *NPM1*, *CEBPA*, *WT1*, and *MLL* mutational analyses and gene- and microRNA-expression profiling were performed centrally.

Results
IDH mutations were found in 33% of the patients. *IDH1* mutations were detected in 49 patients (14%; 47 with R132). *IDH2* mutations, previously unreported in AML, were detected in 69 patients (19%; 13 with R172 and 56 with R140). R172 *IDH2* mutations were mutually exclusive with all other prognostic mutations analyzed. Younger age (< 60 years), molecular low-risk (*NPM1*-mutated/*FLT3*-internal tandem duplication–negative) *IDH1*-mutated patients had shorter disease-free survival than molecular low-risk *IDH1*/*IDH2*-wild-type (wt) patients ($P = .046$). R172 *IDH2*-mutated patients had lower complete remission rates than *IDH1*/*IDH2*wt patients ($P = .007$). Distinctive microarray gene- and microRNA-expression profiles accurately predicted R172 *IDH2* mutations. The highest expressed gene and microRNAs in R172 *IDH2*-mutated patients compared with the *IDH1*/*IDH2*wt patients were *APP* (previously associated with complex karyotype AML) and *miR-1* and *miR-133* (involved in embryonal stem-cell differentiation), respectively.

Conclusion
IDH1 and *IDH2* mutations are recurrent in CN-AML and have an unfavorable impact on outcome. The R172 *IDH2* mutations, previously unreported in AML, characterize a novel subset of CN-AML patients lacking other prognostic mutations and associate with unique gene- and microRNA-expression profiles that may lead to the discovery of novel, therapeutically targetable leukemogenic mechanisms.

J Clin Oncol 28:2348-2355. © 2010 by American Society of Clinical Oncology

INTRODUCTION

Despite progress in understanding mechanisms of leukemogenesis and improvement in treatment, only approximately 40% of younger (age < 60 years) and 10% of older (age ≥ 60 years) adults with acute myeloid leukemia (AML) achieve long-term survival.[1-4] These results underscore the need for novel therapeutic strategies that would improve outcome. To this end, identification of subsets of patients with distinct clinical and biologic features that would help to stratify them to specific risk-adapted and/or molecularly targeted therapies is imperative.[5,6]

Cytogenetically normal AML (CN-AML) is the largest group among both younger and older AML patients and the best characterized molecularly.[5-7] During the last 15 years, recurring mutations with prognostic significance in genes such as *FLT3*,[8,9] *NPM1*,[10,11] *CEBPA*,[12,13] *WT1*,[14,15] and *MLL*[16,17]

have been identified in de novo CN-AML. These markers are not mutually exclusive, and combinations of them may further refine prediction of the risk of adverse events. Thus, patients who carry an *NPM1* mutation but not an *FLT3* internal tandem duplication (ITD) are in the molecular low-risk group because they have a better outcome than patients who lack *NPM1* mutations and/or carry an *FLT3*-ITD and therefore are in the molecular high-risk group.[5,6] Among the latter, however, those who also harbor *CEBPA* mutations have outcomes similar to that of patients with mutated *NPM1* and no *FLT3*-ITD.[13] While the majority of CN-AML patients harbor one or more of the aforementioned mutations, in approximately 15% of the patients, no mutations have been detected,[18] suggesting the existence of hitherto undiscovered genetic alterations contributing to leukemogenesis and defining molecular risk for these patients.

Recent studies revealed that mutations in *IDH1* and *IDH2*, the genes encoding isoforms of the nicotinamide adenine dinucleotide phosphate–dependent isocitrate dehydrogenases, are recurrent in brain tumors, including WHO grade 4 gliomas and WHO grade 2 and 3 astrocytomas and oligodendrogliomas, and are associated with favorable outcome.[19,20] Importantly, an *IDH1* mutation was also discovered through massively parallel DNA sequencing analysis of the genome of a patient with CN-AML.[21] In the same study,[21] 15 *IDH1* mutations, but no *IDH2* mutations, were also found in a validation set of 187 AML patients. An analysis of overall survival (OS) of this patient population (n = 188), which was heterogeneous with regard to AML type (de novo *v* secondary), age, and cytogenetics, showed no independent prognostic significance of *IDH1* mutations. However, a subgroup analysis showed that *IDH1* mutations were associated with CN-AML, being detected in 13 (16%) of 80 such patients, and that they conferred adverse prognosis in the absence of *NPM1* mutations.[21]

To corroborate these preliminary data, we analyzed *IDH1* and *IDH2* mutations in a homogeneous cohort of 358 adults with de novo CN-AML treated with age-adapted intensive chemotherapy regimens on Cancer and Leukemia Group B (CALGB) first-line protocols and comprehensively characterized other gene mutations associated with outcome.

PATIENTS AND METHODS

Patients, Cytogenetic Analysis, and Treatment

We studied pretreatment bone marrow and blood samples with ≥ 20% blasts from 358 patients age 19 to 83 years with de novo CN-AML. Cytogenetic analyses at diagnosis were confirmed by central karyotype review.[22] To establish CN-AML, ≥ 20 metaphase cells from diagnostic bone marrow had to be analyzed and the karyotype had to be normal.[23] Institutional review board–approved informed consent for participation in the studies was obtained from all patients. Younger patients (age < 60 years; n = 159) were treated on CALGB 9621[24] and 19808[25] protocols and older patients (age ≥ 60 years; n = 199) were enrolled on protocols 8525,[26] 8923,[27] 9420,[28] 9720,[29] or 10201[30] (for treatment details, see Appendix, online only). No patient included in our analysis received allogeneic transplantation in first complete remission (CR). The median follow-up for younger and older patients alive and included in this analysis was 7.0 and 3.8 years, respectively.

Molecular Analyses

For *IDH1* and *IDH2* mutational analyses, DNA fragments spanning exons 4 of *IDH1* and *IDH2*, previously identified as "hot spots" for mutations in these genes,[20] were amplified by polymerase chain reaction and directly sequenced as detailed in the Appendix. Other molecular markers—*FLT3*-ITD,[9] *FLT3* tyrosine kinase domain (*FLT3*-TKD) mutations,[31] *MLL* partial tandem duplication (*MLL*-PTD),[17,32] and mutations in the *NPM1*,[33] *CEBPA*,[13] and *WT1*[14] genes—were assessed centrally as previously reported.

Genome-Wide Expression Analyses

Gene- and microRNA-expression profiling were conducted using the Affymetrix U133 Plus 2.0 array (Affymetrix, Santa Clara, CA) and the Ohio State University custom microRNA array (OSU_CCC version 4.0), respectively, as reported previously,[34,35] and described in the Appendix.

Statistical Analysis

Definitions of clinical end points—CR, disease-free survival (DFS), and OS—are provided in the Appendix. The differences among patients in baseline clinical and molecular features according to their *IDH1* and *IDH2* mutational status were tested using the Fisher's exact and Wilcoxon rank sum tests for categoric and continuous variables, respectively. Estimated probabilities of DFS and OS were calculated using the Kaplan-Meier method, and the log-rank test evaluated differences between survival distributions.

For expression profiling, summary measures of gene- and microRNA-expression levels were computed, normalized, and filtered (see Appendix). Expression signatures were derived by comparing gene- and microRNA-expression levels among patients with distinct types of *IDH* mutations and patients with wild-type *IDH1* and *IDH2* (*IDH1*/*IDH2*wt). Univariable significance levels of 0.001 for gene- and 0.005 for microRNA-expression profiling were used to determine the gene probe sets and microRNA probes comprising the signatures, respectively. Prediction of *IDH* mutation status using gene- and microRNA-expression profiles is described in the Appendix. All analyses were performed by the CALGB Statistical Center.

RESULTS

Frequency of IDH2 and IDH2 Mutations

Of the 358 AML patients analyzed, 118 (33%) harbored missense mutations in *IDH* genes. Forty-nine patients (14%) had *IDH1* mutations, including R132 mutations detected in 46 patients, V71 in two patients, and concurrent R132 and V71 mutations in one patient (Table 1). Sixty-nine patients (19%) had *IDH2* mutations: 56 were R140 and 13 were R172 mutations (Table 1). No patient had both *IDH1* and *IDH2* mutations.

Associations of IDH Mutations With Pretreatment Characteristics

Comparisons of pretreatment clinical characteristics of *IDH1*- or *IDH2*-mutated patients with those of *IDH1*/*IDH2*wt patients are reported in Table 2. All types of *IDH* mutations were significantly associated with higher platelet counts, *IDH2* mutations were associated with older age, and R172 *IDH2* mutations were associated with low WBC and a low percentage of circulating blasts.

At diagnosis, patients with *IDH1* mutations were less frequently *FLT3*-ITD–positive ($P = .02$), were more often categorized in the molecular low-risk group (*NPM1*-mutated/*FLT3*-ITD–negative; $P = .003$), and had a trend for lower frequencies of *WT1* ($P = .06$) and *CEBPA* mutations ($P = .08$) compared with *IDH1*/*IDH2*wt patients (Table 3).

Patients with R140 *IDH2* mutations had a lower frequency of *WT1* mutations ($P = .007$) than *IDH1*/*IDH2*wt patients. Strikingly, patients with R172 *IDH2* mutations did not carry any other prognostic mutations, including *FLT3*-ITD, *FLT3*-TKD, *MLL*-PTD, or mutations in the *NPM1*, *WT1*, or *CEBPA* genes (Table 3).

Table 1. Types of *IDH1* and *IDH2* Mutations in CN-AML

IDH1 Mutations			*IDH2* Mutations		
Nucleotide Change	Amino Acid Change	No. of Patients	Nucleotide Change	Amino Acid Change	No. of Patients
c.395G>A	R132H	23	c.419G>A	R140Q	53
c.394C>T	R132C	15	c.418C>T	R140W	1
c.394C>A	R132S	5			
c.394C>G		3	c.418G>T	R140L	2
c.211G>A	V71I	2	c.515G>A	R172K	13
c.395G>A and c.211G>A	R132H & V71I	1		—	—

NOTE. All mutations are missense mutations. The letters in the Amino Acid Change columns denote which amino acid from the wild-type sequence (first letter) is substituted by another amino acid in the mutated sequence (second letter). The number between the letters denotes the codon position. Amino acid abbreviations: C, cysteine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; Q, glutamine; R, arginine; S, serine; V, valine; W, tryptophan.
Abbreviations: CN, cytogenetically normal; AML, acute myeloid leukemia.

Associations of IDH *Mutations With Clinical Outcome*

Considering all patients with *IDH1* mutations, there was no difference in outcome compared with *IDH1*/*IDH2*wt patients (Appendix Table A1, online only). However, in an age group–stratified analysis, we observed a prognostic impact of *IDH1* mutations on the subset of younger (age < 60 years) patients in the molecular low-risk group (*NPM1*-mutated/*FLT3*-ITD–negative). *IDH1*-mutated patients (all with R132 *IDH1* mutation; see Appendix Table A2, online only for other clinical and molecular characteristics) had a significantly worse DFS ($P = .046$; 5-year DFS rates, 42% *v* 59%) and a trend for worse OS ($P = .14$; 5-year OS rates, 50% *v* 63%) compared with *IDH1*/*IDH2*wt patients (Figs 1A and 1B).

With regard to *IDH2* mutations, the outcome of patients with R140 *IDH2* mutations did not differ significantly from the outcome of *IDH1*/*IDH2*wt patients (Appendix Table A1). However, R172 *IDH2*-mutated patients had a significantly lower CR rate than those with *IDH1*/*IDH2*wt (38% *v* 75%; $P = .007$; Fig 1C). When analysis was limited to older patients, who represented most patients with this mutation (77%), those with R172 *IDH2* mutations had a lower CR rate (20% *v* 67%; $P = .005$) than *IDH1*/*IDH2*wt patients (Fig 1C). The estimated 3-year OS rates were 0% versus 17% for older patients with R172 *IDH2* mutations compared with *IDH1*/*IDH2*wt patients, but the difference in OS duration for the two groups was not significant.

Table 2. Clinical Characteristics According to *IDH* Mutational Status in De Novo Cytogenetically Normal Acute Myeloid Leukemia

Clinical Characteristic	*IDH1*-Mutated (n = 49) No.	%	R140 *IDH2*-Mutated (n = 56) No.	%	R172 *IDH2*-Mutated (n = 13) No.	%	*IDH1*/*IDH2*wt (n = 240) No.	%	*P* (*IDH1*-Mutated *v* *IDH1*/*IDH2*wt)	*P* (R140 *IDH2*-Mutated *v* *IDH1*/*IDH2*wt)	*P* (R172 *IDH2*-Mutated *v* *IDH1*/*IDH2*wt)
Age, years									.49	.006	.02
Median	62		64		70		60				
Range	21-82		29-83		38-83		19-81				
Male sex	23	47	32	57	7	54	118	49	.88	.30	.78
Race									.10	.78	.30
White	41	84	52	95	11	85	218	92			
Nonwhite	8	16	3	5	2	15	19	8			
Hemoglobin, g/dL									.73	.08	.77
Median	9.3		9.8		9.4		9.3				
Range	7.1-12.9		4.8-13.6		7.7-12.3		4.6-15.0				
Platelet count, $\times 10^9$/L									<.001	.008	<.001
Median	98		73		131		53				
Range	11-850		11-270		64-309		7-510				
WBC count, $\times 10^9$/L									.18	.30	<.001
Median	24.6		22.5		1.5		28.4				
Range	0.9-152.1		1.1-434.1		0.9-10.5		0.9-450.0				
Percentage of PB blasts									.26	.85	.002
Median	59		45		7		50				
Range	0-99		0-97		0-79		0-97				
Percentage of BM blasts									.06	.27	.58
Median	73		74		66		63				
Range	33-99		25-96		30-86		7-99				
Extramedullary involvement	11	24	12	22	1	8	75	32	.38	.19	.12

Abbreviations: wt, wild-type; PB, peripheral blood; BM, bone marrow.

Table 3. Molecular Characteristics According to *IDH* Mutational Status in De Novo Cytogenetically Normal Acute Myeloid Leukemia

Molecular Characteristic	*IDH1*-Mutated (n = 49)		R140 *IDH2*-Mutated (n = 56)		R172 *IDH2*-Mutated (n = 13)		*IDH1*/*IDH2*wt (n = 240)		*P* (*IDH1*-Mutated *v* *IDH1/IDH2*wt)	*P* (R140 *IDH2*-Mutated *v* *IDH1/IDH2*wt)	*P* (R172 *IDH2*-Mutated *v* *IDH1/IDH2*wt)
	No.	%	No.	%	No.	%	No.	%			
NPM1									.19	.76	< .001
Mutated	34	71	31	57	0	0	143	60			
Wild-type	14	29	23	43	13	100	96	40			
FLT3-ITD									.02	.12	.005
Present	10	20	15	27	0	0	91	38			
Absent	39	80	41	73	13	100	149	62			
Molecular risk group*									.003	.63	.01
Low	27	55	19	35	0	0	75	31			
High	22	45	35	65	13	100	164	69			
FLT3-TKD									.32	.47	.37
Present	3	6	4	7	0	0	29	12			
Absent	45	94	50	93	13	100	210	88			
WT1									.06	.007	.37
Mutated	1	2	0	0	0	0	27	11			
Wild-type	47	98	54	100	13	100	211	89			
CEBPA									.08	.13	.13
Mutated	2	6	4	9	0	0	38	18			
Wild-type	34	94	42	91	13	100	169	82			
MLL-PTD									.32	1.00	1.00
Present	1	2	3	6	0	0	16	7			
Absent	44	98	49	94	12	100	215	93			

Abbreviations: wt, wild-type; ITD, internal tandem duplication; TKD, tyrosine kinase domain; PTD, partial tandem duplication.
*Molecular low risk: *NPM1* mutated and *FLT3*-ITD-negative; high risk: *NPM1* wild-type and/or *FLT3*-ITD–positive.

Since R172 *IDH2* mutations were mutually exclusive with *NPM1* mutations, to account for the potentially favorable clinical impact of *NPM1* mutations on achievement of CR,[33] we compared the outcome of patients with R172 *IDH2* mutations with that of *IDH1*/*IDH2*wt patients without *NPM1* mutations. We focused only on older patients because they represented the vast majority of patients with R172 *IDH2* mutation. In this prognostically unfavorable subset, patients with R172 *IDH2* mutations showed a trend for a lower CR rate (20% *v* 56%; $P = .08$).

Biologic Insights Concerning R172 IDH2 *Mutations*

To gain biologic insights into the potentially unfavorable prognostic significance of R172 *IDH2* mutations, which are mutually exclusive with any other prognostically relevant mutations and therefore likely to identify a novel subset of CN-AML, we derived a gene-expression signature by comparing R172 *IDH2*-mutated patients with *IDH1/IDH2*wt patients. Because 77% of patients with R172 *IDH2* mutations were older, to eliminate age-dependent bias, we analyzed only patients age ≥ 60 years. Of the 451 differentially expressed probe sets ($P < .001$), 365 were upregulated and 86 were downregulated in patients with R172 *IDH2* mutations (Fig 2A; Appendix Table A3, online only).

To assess the accuracy of this gene-expression signature to correctly identify R172 *IDH2*-mutated patients versus those with *IDH1/IDH2*wt, we conducted a leave-one-out cross-validated prediction analysis. The mutation status of 95.5% of patients (including five of six R172 *IDH2*-mutated patients) was correctly predicted (Table 4).

Among the most upregulated probe sets in R172 *IDH2*-mutated patients were those representing *APP* (nine-fold), *CXCL12* (eight-fold), *PAWR* (eight-fold), *CDC42BPA* (eight-fold), and *SPARC* (seven-fold; Appendix Table A3). *APP* was previously reported to be upregulated in AML patients with complex karyotype.[36] Polymorphism in *CXCL12* (also known as *SDF1*) was associated with increased circulating blasts and extramedullary disease in AML.[37] *PAWR* was found to regulate *WT1* activity and to be overexpressed in myelodysplastic syndromes progressing to AML.[38] In addition, *CDC42BPA*, although not directly associated with AML, seemingly participates in tumor cell invasion.[39] In contrast, *SPARC*, encoding a matricellular glycoprotein with growth-inhibitory and antiangiogenic functions, was found to have lower expression in *MLL*-associated AML and tumor suppressor activity.[40] Other genes of interest upregulated in the R172 *IDH2*-mutated patients were *ID1* (four-fold), whose expression was recently correlated with poor outcome in AML[41]; *ABCB1* (*MDR1*; five-fold) mediating chemoresistance[42]; and *KRAS2* (2.6-fold), which is constitutively activated in several human cancers including AML.[43]

The downregulated genes we found include *KYNU*, which encodes a protein participating in the biosynthesis of NAD cofactors from tryptophan[44]; *SUCLG2*, involved in the Krebs cycle and mutated in Leigh-like syndrome[45]; *CD93*, involved in regulating phagocytosis of apoptotic cells and angiogenesis[46]; *LY86* and *LIST1*, associated with immune response pathways[47,48]; and *PTHR2*, a receptor for the parathyroid hormone.[49] To the best of our knowledge, none of these genes have previously been associated with AML.

Genome-wide profiling identifies aberrantly expressed microRNAs associated with distinct molecular subsets of CN-AML patients.[50] Therefore, we derived a microRNA-expression signature associated with older R172 *IDH2*-mutated CN-AML. The signature

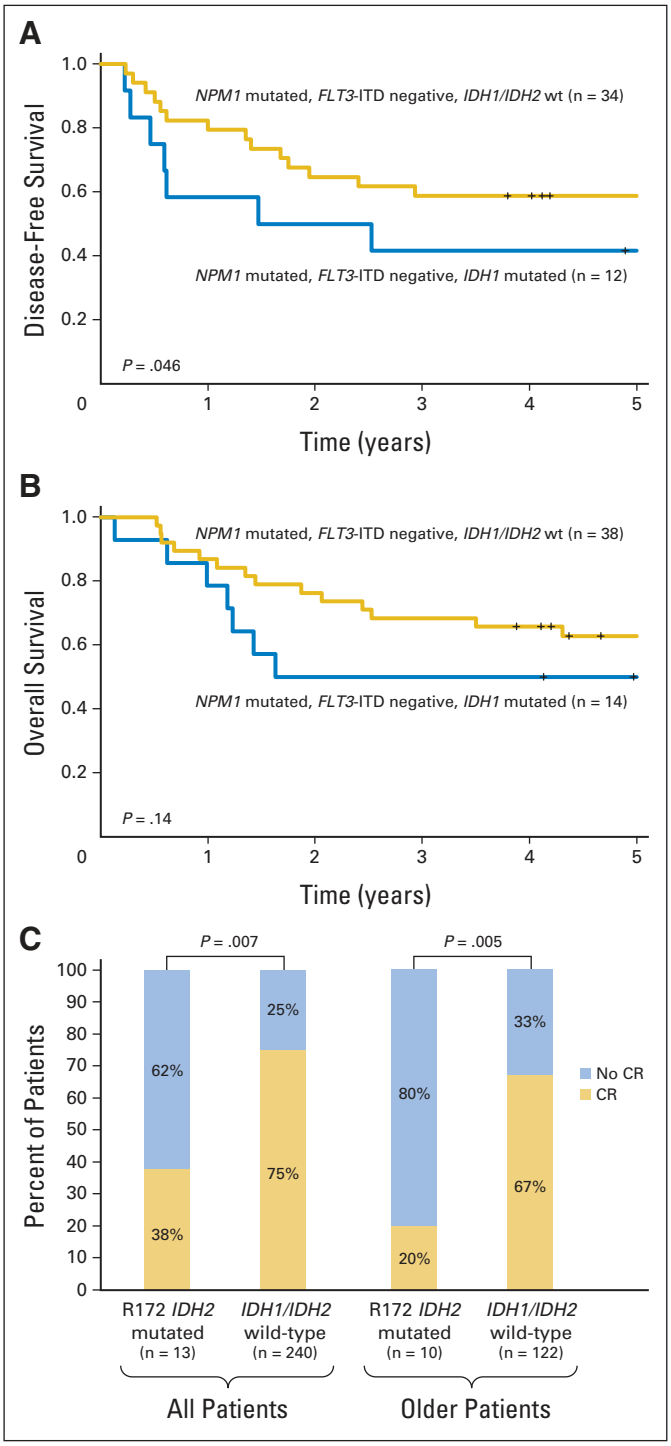

Fig 1. Impact of distinct *IDH* mutation types on clinical outcome of patients with cytogenetically normal acute myeloid leukemia. (A) Disease-free survival and (B) overall survival of younger molecular low-risk patients according to *IDH1* mutation status. (C) Complete remission rates according to R172 *IDH2* mutation status. ITD, internal tandem duplication; wt, wild-type; CR, complete remission.

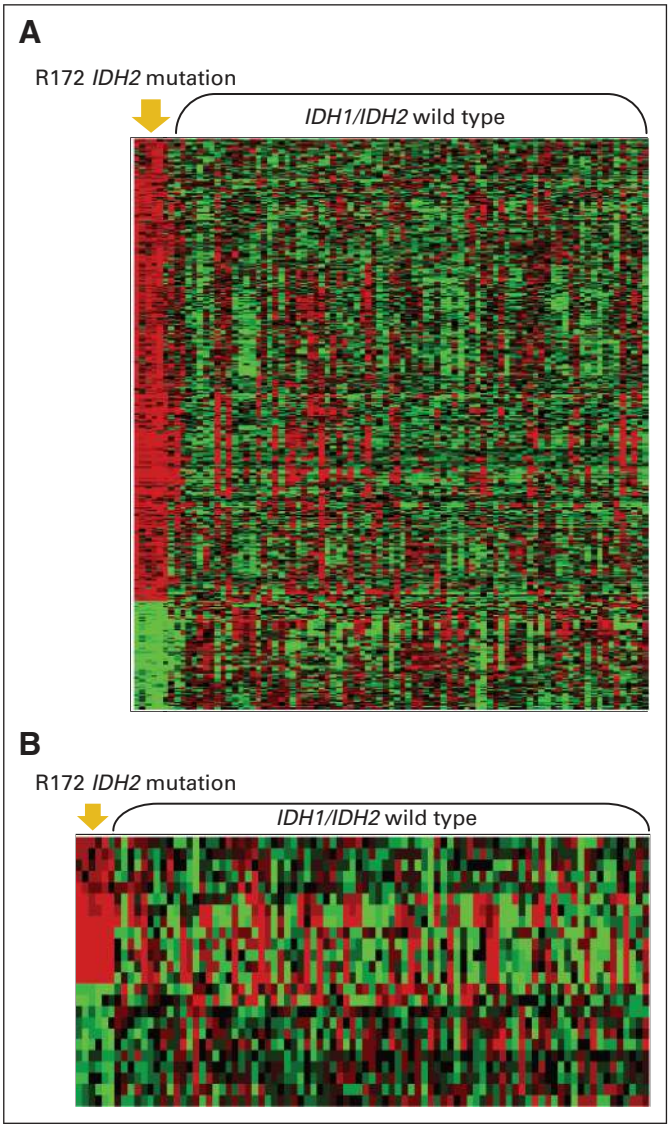

Fig 2. Genome-wide gene- and microRNA-expression profiles associated with R172 *IDH2* mutations. (A) Gene-expression and (B) microRNA-expression signatures, derived from comparing older patients with R172 *IDH2* mutations and those with the wild-type *IDH1/IDH2* genes. Rows represent gene probe sets (A) or microRNA probes (B), and columns represent patients. Patients are grouped by *IDH2* mutational status (R172 or wild-type). Expression values of the probe sets and microRNA probes are represented by color: green indicates expression less than the median value and red indicates expression greater than the median value for the given gene probe set or microRNA probe.

comprised 24 differentially expressed ($P < .005$) probes, 13 of which were upregulated and 11 of which were downregulated in R172 *IDH2*-mutated patients (Fig 2B; Appendix Table A4, online only). In leave-one-out cross-validated prediction analysis, the mutation status of 93.2% of patients (including five of six R172 *IDH2*-mutated patients) was correctly predicted (Table 4).

Among the microRNAs most upregulated (> four-fold) in R172 *IDH2*-mutated patients were members of the *miR-125* family (*miR-125a-5p* and *miR-125b*), *miR-1*, and *miR-133*. *miR-125b* has been shown to target the tumor suppressor gene *TP53* and inhibit myeloid differentiation,[51] whereas *miR-1* and *miR-133* have not been previously associated with human cancer, but they participate in cell fate decision mechanisms of pluripotent embryonic stem cells.[52] Among the most downregulated probes were those representing *mir-194-1*, *miR-526*, *miR-520a-3p*, and *mir-548b*, none of which have previously been associated with normal hematopoiesis or AML.

Table 4. Accuracy of Prediction of the R172 *IDH2* Mutational Status in Older CN-AML Using Leave-One-Out Cross-Validated Prediction Analysis From Gene- and MicroRNA-Expression Profiles

Classification	Overall Accuracy (%)	Sensitivity (%)	Specificity (%)
Gene-expression signature			
R172 (n = 6) *v* wild-type (n = 83)	95.5	83.3	96.4
MicroRNA-expression signature			
R172 (n = 6) *v* wild-type (n = 82)	93.2	83.3	93.9

Abbreviations: CN, cytogenetically normal; AML, acute myeloid leukemia.

DISCUSSION

Mutations in the *IDH1* and *IDH2* genes have been found in patients with glioma and predict favorable outcome.[19] Using whole-genome sequencing and validation analyses, Mardis et al[21] recently reported that *IDH1* mutations can also be found in AML and are associated with normal karyotypes. Therefore, we analyzed a larger, more homogeneous cohort of de novo CN-AML (n = 358), comprising both younger and older patients treated with age-adapted chemotherapy regimens on first-line CALGB clinical trials.

The first salient finding of our study was that we not only confirmed the presence of *IDH1* mutations but also found previously unreported *IDH2* mutations in CN-AML.[5] *IDH1* mutations were found in 14% of our patients, which is similar to the findings of Mardis et al.[21] In addition to the previously reported R132 *IDH1* mutations, we identified three patients with a V71I *IDH1* allele. Although this allele has been recently reported as a single nucleotide polymorphism (SNP), Bleeker et al[53] did not find it in any of the 672 tumor samples and 84 cell lines they sequenced. This suggests that if V71I *IDH1* is an SNP, it is rare and, therefore, the possibility that V71I *IDH1* represents a novel *IDH1* somatic or germline mutation associated with AML cannot be excluded.

Moreover, unlike Mardis et al,[21] we also detected two different types of mutations in the *IDH2* gene (ie, R140 and R172), which occurred with even greater frequency (19%) and, to the best of our knowledge, have not been previously reported in AML. Interestingly, while the R172 *IDH2* mutation was previously found in gliomas, to the best of our knowledge, the R140 *IDH2* mutation has not been previously reported in human cancer or normal tissue. Since changes in codon 140 detected in our patients led to the substitution of the arginine with three different amino acids (Table 1), it is likely that R140 *IDH2* represents a somatic mutation associated with AML rather than a newly discovered SNP. Studies of normal tissues from R140 *IDH2* AML patients are underway to confirm (or refute) the somatic nature of R140 *IDH2*. Both *IDH2* mutations were associated with older age but, remarkably, only R172 *IDH2* mutations were found in the absence of other recurrent mutations thereby identifying a novel subset of patients among those 15% of CN-AML patients for whom no prognostic gene mutation has been hitherto reported. When considered together, the frequency of mutations in genes encoding the isocitrate dehydrogenases is relatively high in CN-AML (33%), placing them among the most frequent mutations in CN-AML.

The second important finding relates to the prognostic significance of *IDH* mutations in specific age and molecular subsets of CN-AML. We showed that although *IDH1* mutations did not affect outcome in the whole cohort of CN-AML patients, they conferred worse prognosis in younger patients with molecular low-risk CN-AML. These results differ from two previous studies reporting that *IDH1* mutations conferred adverse outcome in *NPM1*wt patients with CN-AML[21] or various karyotypes.[54] Differences in sizes of patient cohorts analyzed, varying inclusion criteria (eg, we studied only de novo AML patients whereas Schnittger et al[54] also analyzed secondary AML), age, and treatment administered might contribute to these discrepancies among studies, which require further investigation for resolution.

Most patients with R172 *IDH2* mutations failed to achieve a CR following intensive cytarabine/anthracycline-based induction chemotherapy. Because *NPM1* mutations are a strong, favorable prognosticator in older CN-AML patients,[33] we also separately analyzed older patients without *NPM1* mutations; even then, the CR rate of patients with R172 *IDH2* mutations tended to be lower than that of *IDH1*/*IDH2*wt patients. These results suggest that it is the presence of the R172 *IDH2* mutation itself rather than the absence of *NPM1* mutations that decreases the odds of achieving CR. However, given the relatively small number of R172 *IDH2*-mutated patients in our cohort, larger studies should corroborate our results. Notably, in contrast with our data in CN-AML, R172 *IDH2* mutations were reported to predict a favorable outcome in patients with gliomas,[20] thereby supporting the notion that the prognostic significance of molecular markers may vary according to distinct biologic and/or therapeutic contexts in which they are evaluated. Furthermore, in contrast with R172 *IDH2* mutations, the outcome of patients with R140 *IDH2* mutations was not different from the outcome of patients with wt *IDH1* and *IDH2* genes, thereby suggesting different contributions to leukemogenesis from these two mutation types.

Finally, we showed that R172 *IDH2* mutations in CN-AML are associated with unique gene- and microRNA-expression signatures. Although the signature did not include previously reported unfavorable prognosticators in CN-AML (ie, *BAALC*, *ERG*, and *MN1*),[55-61] it comprised other upregulated genes associated with adverse karyotypes (*APP*),[36] unfavorable outcome (*ID1*),[41] increased rate of extramedullary disease (*CXCL12*),[37] or increased chemoresistance (*ABCB1*) in AML,[42] supporting the negative prognostic significance of this mutation type. Furthermore, among microRNAs differentially expressed in R172 *IDH2*-mutated patients, we noted upregulation of *miR-125b,* previously found to block myeloid differentiation,[51] and *miR-1* and *miR-133,* not reported previously in AML but involved in embryonal stem-cell differentiation.[52] Importantly, both gene- and microRNA-expression signatures appeared to predict the R172 *IDH2* mutational status with high accuracy, thus supporting the view that patients with R172 *IDH2* mutations profoundly differ biologically and clinically from patients with wt *IDH1* and *IDH2* alleles.

The mechanisms through which *IDH1* and *IDH2* mutations contribute to malignant transformation are under investigation. Thompson[62] postulated that *IDH1* and *IDH2* mutations result in gain rather than loss of function, given the high frequency of somatic mutations affecting a single codon and the absence of other mutations causing gene inactivation. Indeed, Dang et al[63] showed that the R132 *IDH1* mutation causes the encoded enzyme to acquire the ability to convert α-ketoglutarate to 2-hydroxy-glutarate, which accumulates in

the affected cells. This likely contributes to malignant transformation since inborn errors of 2-hydroxy-glutarate metabolism have been associated with an increased risk of brain tumors.[63] While similar mechanisms might be operative in patients harboring *IDH2* mutations, to the best of our knowledge, no functional study of the mutant proteins has been reported.

In summary, we report here that *IDH1* mutations predict shorter DFS in younger molecular low-risk CN-AML patients, R172 *IDH2* mutations are mutually exclusive with other known prognostic mutations and denote a novel subset of older CN-AML patients characterized by resistance to induction chemotherapy, and R140 *IDH2* mutations do not appear to confer prognostic significance. By deriving gene- and microRNA-expression signatures, we uncovered intriguing features in R172 *IDH2*-mutated patients that may lead to better understanding of the biologic role of this mutation and to the design of novel therapies targeting aberrant isocitrate dehydrogenase–driven activation of metabolic pathways.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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