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Brief report

Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value

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Somatic mutations in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) were recently demonstrated in acute myeloid leukemia (AML), but their prevalence and prognostic impact remain to be explored in large extensively characterized AML series, and also in various other hematologic malignancies. Here, we demonstrate in 893 newly diagnosed cases of AML mutations in the *IDH1* (6%) and *IDH2* (11%) genes. Moreover, we identified *IDH* mutations in 2 JAK2 V617F myeloproliferative neoplasias (n = 96), a single case of acute lymphoblastic leukemia (n = 96), and none in chronic myeloid leukemias (n = 81). In AML, *IDH1* and *IDH2* mutations are more common among AML with normal karyotype and *NPM1*^{mutant} genotypes. *IDH1* mutation status is an unfavorable prognostic factor as regards survival in a composite genotypic subset lacking *FLT3*^{ITD} and *NPM1*^{mutant}. Thus, *IDH1* and *IDH2* mutations are common genetic aberrations in AML, and *IDH1* mutations may carry prognostic value in distinct subtypes of AML. (*Blood*. 2010;116(12): 2122-2126)

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Introduction

Somatic mutations in the genes encoding the isocitrate dehydrogenases IDH1 and IDH2 were revealed in more than 70% of World Health Organization grade 2 and 3 astrocytomas, oligodendrogliomas, and glioblastomas.[1-3] Mutations in *IDH1* and *IDH2* were mutually exclusive and affected the arginines on position 132 of IDH1 and position 172 of IDH2.[3] Patients with malignant gliomas with IDH1 or IDH2 mutations showed a better response to therapy than those with wild-type *IDH* genes.[3] Mutations in these residues of IDH significantly disturb the function of both isocitrate dehydrogenases, as demonstrated by impaired production of nicotinamide adenine dinucleotide phosphate.[3,4] In acute myeloid leukemia (AML), mutant IDH enzyme activity results in accumulation of the cancer-associated metabolite 2-hydroxyglutarate.[5,6]

Recently, acquired mutations in the gene encoding IDH1 were identified in 8%[7] and 5.5%[8] of newly diagnosed AML cases. *IDH1* mutations were significantly associated with normal karyotype and *NPM1* mutations.[7,8] Overall, the *IDH1* mutation status did not suggest a relationship with overall survival (OS), but the sample sizes were limited in these studies.[7,8] However, a trend for an adverse effect on OS was suggested in normal karyotype AML with *NPM1*$^{wild\text{-}type}$.[7]

The prevalence and prognostic value of *IDH* mutations in AML, as well as other hematologic malignancies, remain to be further established. In this study, we determined the frequencies of both *IDH1* and *IDH2* mutations in cohorts of AML, acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and *JAK2* V617F myeloproliferative neoplasia (MPN). In a cohort of 893 cases of AML, we investigated their distribution in relationship with cytogenetic and molecular risk categories as well as recurrent gene mutations commonly apparent in AML, and we evaluated the impact of *IDH* mutations on treatment outcome.

Methods

Bone marrow aspirates or peripheral blood samples of cohorts of patients with various hematologic malignancies were collected after written informed consent in accordance with the Declaration of Helsinki. All experiments described were approved by the Erasmus University Medical Center Institutional Review Board. AML, ALL, and CML patients were treated according to the HOVON (Dutch-Belgian Hematology-Oncology Cooperative Group) AML protocols HO04, HO04A, HO29, HO42, HO42A, and HO43, ALL protocols HO18, HO37, HO70, and HO71, and CML protocol HO51 (http://www.hovon.nl). The MPN samples were collected, and the JAK2 V617F mutation was determined in our routine molecular diagnostics facility.

IDH1 and *IDH2* mutations in AML, refractory anemia with excess blasts, ALL, CML, and JAK2 V617F MPN were determined by cDNA amplifications using FW1-IDH1 cDNA WAVE 5′-CTTCAGAGAAGCCATTATCTG-3′ and REV2-IDH1 cDNA WAVE 5′-TCACTTGGTGTGTAGGTTATC-3′ (IDH1 R132), FW1-IDH2 cDNA WAVE 5′-GAACTATCCGGAACATCCTG-3′ and REV2-IDH2 cDNA WAVE 5′-CTTGACACCACTGCCATC-3′ (IDH2 R172), or FW-IDH2-Ex4 5′-GTTCAAGCTGAAGAAGATGTG-3′ and REV-IDH2-Ex5-6 cDNA WAVE 5′-TGAGATGGACTCGTCGGTG-3′ (IDH2 R140). All polymerase chain reaction (PCR) reactions were carried out at an annealing temperature of 60°C in the presence of 25mM deoxynucleoside triphosphate, 15 pmol primers, 2mM $MgCl_2$, Taq polymerase, and 1 times buffer (Invitrogen). Cycling conditions were as follows: 1 cycle 5 minutes at 94°C, 30 cycles 1 minute at 94°C, 1 minute at annealing temperature, 1 minute at 72°C, and 1 cycle 7 minutes at 72°C. All *IDH1* and *IDH2* reverse-transcribed PCR products were subjected to denaturing high performance liquid chromatography (dHPLC) analyses using a Transgenomic WAVE system. Samples were run at 61.4°C (IDH1 R132), 57.7°C (IDH2 R172), or 6.1°C (IDH2 R140). PCR products showing aberrant dHPLC profiles were purified using the Multiscreen-PCR 96-well system (Millipore) followed by direct sequencing with the appropriate forward and reversed primers using an ABI-PRISM3100 genetic analyzer (Applied Biosystems). PCR products were sequenced with

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Table 1. Distribution of *IDH1* and *IDH2* mutations in 893 cases of AML

	IDH1 mutant	*IDH2 mutant*	Wild-type	*P*
Mean age at diagnosis, years (range)	50 (20-71)	50 (18-72)	45 (15-77)	.002*
Mean WBC at diagnosis, $\times 10^9$/L (range)	48 (1-400)	42 (18-72)	46 (0-510)	
Mean platelets at diagnosis, $\times 10^9$/L (range)	131 (14-494)	104 (11-884)	83 (3-998)	.05*
Mean blasts at diagnosis, percentage (range)	64 (0-96)	65 (15-95)	57 (0-98)	
Total,† no. (%)	55 (6)	97 (11)	743	
Female, no. (%)	30 (7)	52 (12)	384	
Male, no. (%)	25 (6)	45 (10)	359	
FAB, no. (%)				
M0	—	5 (11)	40	
M1	21 (12)	36 (21)	113	
M2	14 (6)	27 (11)	195	
M3	—	—	22	
M4	7 (5)	10 (7)	130	
M5	8 (5)	14 (8)	151	
M6	—	1 (6)	17	
M7	—	—	1	
RAEB	3 (15)	—	17	
RAEB-t‡	1 (2)	1 (2)	43	
Unknown	1 (6)	3 (17)	14	
Karyotype classification,§ no. (%)				
t(8;21)	—	2 (4)	51	
inv(16)	—	—	50	
t(15;17)	—	—	21	
CA unfavorable	2 (2)	6 (7)	74	
MK	—	3 (4)	72	
CN	39 (10)	58 (15)	283	< .001*
CA rest	11 (6)	23 (13)	141	
Unknown	3 (5)	5 (8)	51	
Mutations‖, no. (%)				
FLT3 ITD	15 (7)	19 (9)	177	
FLT3 TKD	9 (10)	12 (13)	69	
NPM1	35 (13)	40 (15)	191	.001*
$NPM1^{wt}FLT3^{wt}$	19 (3)	50 (9)	475	
$NPM1^{mut}FLT3^{wt}$	21 (15)	28 (20)	91	
$NPM1^{mut}FLT3^{ITD}$	14 (11)	12 (10)	100	
$NPM1^{wt}FLT3^{ITD}$	1 (1)	7 (8)	77	
N-*RAS*¶	3 (6)	3 (6)	43	
K-*RAS*¶	1 (17)	—	4	
CEBPA¶	1 (3)	4 (11)	30	

WBC indicates white blood cell count at diagnosis; FAB, French-American-British classification; —, not applicable; RAEB, refractory anemia with excess blasts; and RAEB-t, refractory anemia with excess blasts in transformation.

*IDH^{mut} vs $IDH^{wild\text{-}type}$.

†Includes 2 AML patients with *IDH1* and *IDH2* mutation.

‡At the time of diagnosis, these cases were classified as RAEB-t but would now be classified as AML.

§Karyotypes were centrally reviewed. CA unfavorable: inv(3)/t(3;3), t(6;9), 11q23 abnormalities except t(9;11), −5, 5q−, −7, 7q− or t(9;22); MK: monosomal karyotypes (very unfavorable); CN: normal cytogenetics or -X or -Y as single abnormalities only (intermediate-risk I); CA rest: any other abnormal cytogenetics not included in any of the other categories (intermediate-risk II).

‖Mutation detection in *FLT3* (ITD or TKD), *NPM1*, N-*RAS*, K-*RAS*, and *CEBPA* was performed as described previously.[10-13]

¶A total of 518 cases were analyzed.

FW-IDH1 cDNA WAVE (IDH1 R132), FW-IDH2 cDNA WAVE (R172 IDH2), or FW-IDH2-Ex4 (R140 IDH2). We validated this strategy using 350 cases of de novo AML that were previously analyzed using PCR on genomic DNA followed by direct sequencing.

Information on the *IDH1* and *IDH2* mutation status of all AML cases is available as supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article) and of all AML cases that were previously gene expression–profiled at the Gene Expression Omnibus (National Center for Biotechnology Information; www.ncbi.nlm-.nih.gov/geo, accession no. GSE6891).

The relation between *IDH* mutations and various patient characteristics was determined by the Student *t* test, equal variances not assumed (continuous variables) and the Fisher exact test (categorical variables).

We distinguished the following cytogenetic risk categories: (1) favorable: t(8;21), inv(16) or t(15;17); (2) unfavorable: inv(3)/t,3,3 t(6;9), 11q23 abnormalities other than t(9;11), −5, 5q−, −7, 7q−, or t(9;22) (cytogenetically abnormal [CA] unfavorable); (3) very unfavorable: monosomal karyotypes[9]; (4) intermediate-risk I: cytogenetically normal (CN) and (V); intermediate-risk II: the remaining AML cases (CA rest).

OS endpoints were death (failure) and alive at last follow-up (censored), as measured from entry onto trial. Event-free survival (EFS) endpoints were remission induction failure, disease relapse, or death from any cause, measured from entry onto trial. Distribution estimations and survival distributions of OS and EFS were calculated by the Kaplan-Meier method and the log-rank test.

Results and discussion

To determine the frequencies of *IDH1* and *IDH2* mutations in AML, we screened cDNA of 893 newly diagnosed AMLs by

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reverse-transcribed PCR/dHPLC followed by direct sequencing (Table 1). *IDH1* mutations were identified in 55 AML cases (6%) and *IDH2* mutations in 97 cases (11%). A total of 152 (17%) mutations in either *IDH1* or *IDH2* were apparent in 150 cases. *IDH1* and *IDH2* mutations were mutually exclusive except in 2 cases of AML (nos. 7272 and 10400) with dual mutations in *IDH1* and *IDH2*. The R132H mutation was the most prevalent mutation in IDH1 (n = 31, 56%). In addition, various other IDH1 protein mutations were identified (R132C, n = 15, 28%; R132G, n = 6, 11%; R132L, n = 3, 6%). We identified 74 IDH2 R140Q mutations,[6,14] 22 cases with an IDH2 R172K mutation, and a single case with a R172M substitution (no. 7309).

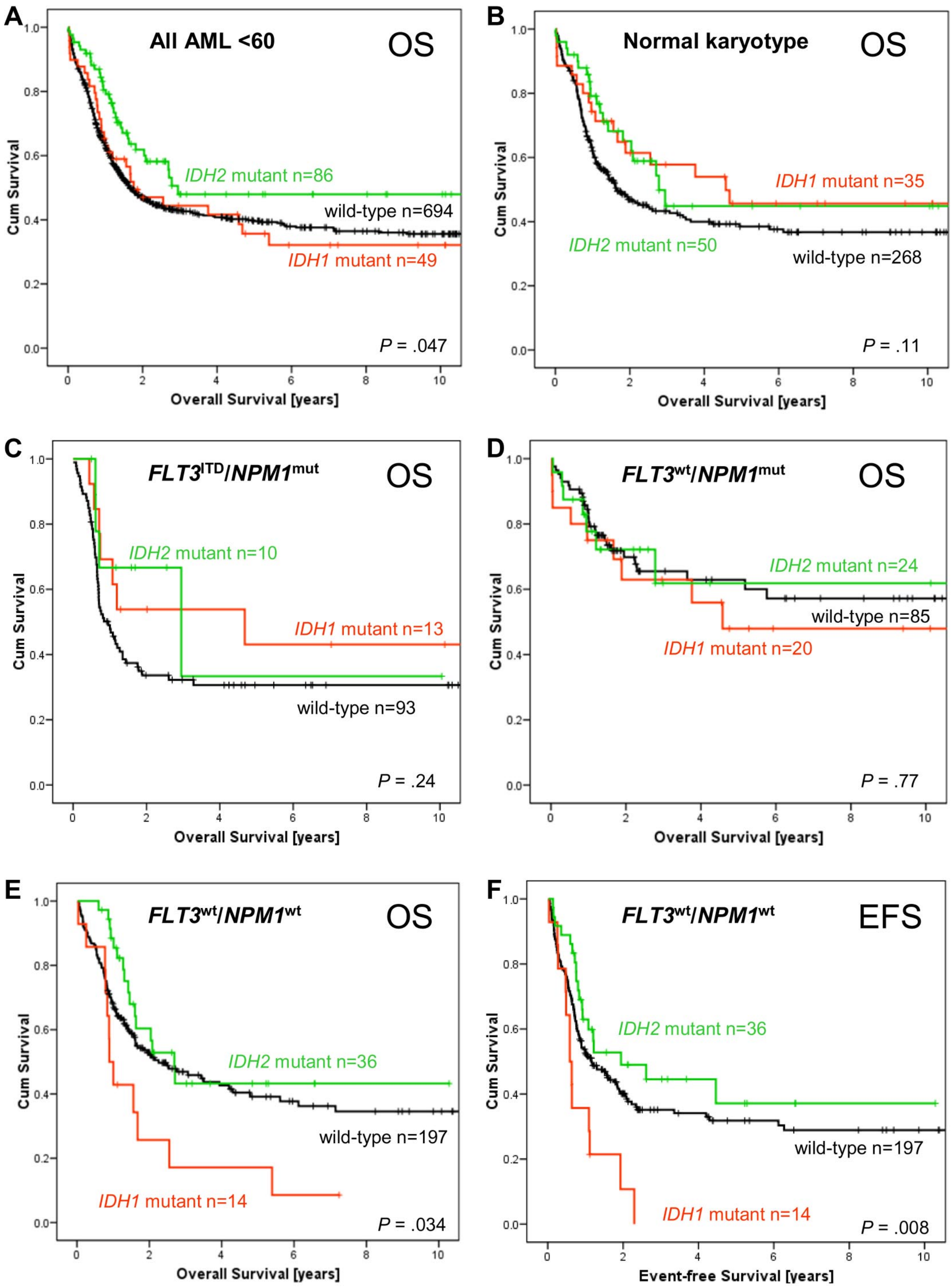

Figure 1. Survival analyses of patients with AML with or without *IDH1* and *IDH2* mutations. (A) Kaplan-Meier estimates of OS for all AML patients. (B) OS for AML patients with normal karyotypes. (C) OS for patients with intermediate-risk AML and *FLT3*^{mutant} and *NPM1*^{mutant}. (D) OS for patients with intermediate-risk AML and *FLT3*$^{wild\text{-}type}$ and *NPM1*^{mutant}. (E) OS for patients with intermediate-risk AML and *FLT3*$^{wild\text{-}type}$ and *NPM1*$^{wild\text{-}type}$. (F) EFS for patients with intermediate-risk AML and *FLT3*$^{wild\text{-}type}$ and *NPM1*$^{wild\text{-}type}$. Survival curves in red represent cases with *IDH1*^{mutant}; those in green, *IDH2*^{mutant}; and those in black, cases with *IDH1*$^{wild\text{-}type}$ and *IDH2*$^{wild\text{-}type}$, respectively. The log-rank *P* value is indicated per Kaplan-Meier analysis.

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In addition to AML, we investigated the prevalence of *IDH1* and *IDH2* mutations in JAK2 V617F MPN (n = 96), ALL (n = 96), including cases with *BCR-ABL* (n = 21), *MLL* fusions (*MLL-AF4*, *MLL-AF9*, or *MLL-ENL*) (n = 6), *SIL-TAL* (n = 2), *E2A-PBX* (n = 2), and *SET-NUP* (n = 1) and CML in chronic phase (n = 81). We identified a mutation in IDH1 (R132C) and IDH2 (R140Q) in 2 independent cases of JAK2 V617F MPN, indicating that these mutations can be present before leukemic transformation.[14] In addition, we identified an IDH2 R140Q mutation in a single case of ALL. No *IDH* mutations were present in CML.

AML with *IDH1*^{mut} and *IDH2*^{mut} are more prevalent at older age and present with significantly higher average platelet counts at diagnosis compared with AML with *IDH*$^{wild\text{-}type}$ (Table 1). *IDH1* and *IDH2* mutations were significantly more frequently present among cytogenetically normal AML ($P < .001$, CN; Table 1). In addition, *IDH* mutations appear to be significantly associated with *NPM1*^{mutant} ($P < .001$; Table 1). The specificity of the pathogenetic involvement of *IDH* gene mutations in AML is also suggested by the observations that they did not significantly associate with various other recurrent mutations (ie, *FLT3*^{ITD} [internal tandem duplication] *FLT3*^{TKD} [tyrosine kinase domain], N-*RAS*, K-*RAS*, or *CEBPA* gene mutations).

To investigate the prognostic value of *IDH1* mutations, 829 AML patients younger than 60 years were considered for survival analysis. The median follow-up of these patients is 33.2 months. The OS of patients with AML with or without *IDH1*^{mutant} or *IDH2*^{mutant} genotypes among the entire series of patients with AML did not differ ($P = .05$; Figure 1A). OS of *IDH*^{mutant} patients in the subgroups with intermediate-risk cytogenetics ($P = .13$), normal karyotypes (Figure 1B, $P = .11$), and intermediate-risk cytogenetics with *FLT3*$^{wild\text{-}type}$ ($P = .32$), *FLT3*^{ITD} ($P = .09$), *NPM1*$^{wild\text{-}type}$ ($P = .06$), or *NPM1*^{mutant} ($P = .25$) genotypes were not significantly different from those with *IDH*$^{wild\text{-}type}$. Similar results were obtained in analyses as regards EFS. Of note, *IDH*^{mutant} patients within the AML subtype *NPM1*$^{wild\text{-}type}$ were associated with an inferior EFS ($P = .02$).

Because there is significant overlap in the occurrence of mutations in *NPM1*^{mutant} and *FLT3*^{ITD}, we also assessed the value of *IDH* gene mutations in each of the 4 composite variants, but no significant prognostic effect of *IDH* mutations was apparent as regards OS or EFS among *FLT3*^{ITD}/*NPM1*^{mutant} (OS, $P = .24$; EFS, $P = .24$) and *FLT3*$^{wild\text{-}type}$/*NPM1*^{mutant} (OS, $P = .77$; EFS, $P = .75$; Figure 1C and 1D, respectively). Only 8 AML patients with *IDH* mutations were identified among *FLT3*^{ITD}/*NPM1*$^{wild\text{-}type}$, which prevents reliable survival analysis. However, among the *FLT3*$^{wild\text{-}type}$/*NPM1*$^{wild\text{-}type}$ AML subtype, the presence of *IDH1* mutations (n = 14 cases) predicted for both significantly reduced OS (Figure 1E, $P = .032$) and EFS ($P = .005$). These data suggest an only moderate prognostic effect of *IDH1*^{mut} because it is not evident in genetically heterogeneous series of AML, but only in intermediate-risk AML in the absence of *NPM1*^{mut} and *FLT3*^{ITD}. Apparently, the *NPM1*^{mutant} and *FLT3*^{ITD} markers override the prognostic effect of *IDH1*^{mut}. In this regard, we wish to note that the numbers of the 4 composite subgroups, even though this study was performed in a relatively large series of AML, become obviously increasingly small, which limits the statistical power of these analyses and prohibits the interesting exploratory analysis for *IDH1* and *IDH2* mutations separately.

Acquired *IDH* gene mutations (ie, not only in *IDH1* but also *IDH2*[6]) are common abnormalities in AML. The results of the current study demonstrate that the frequency of *IDH2* mutations exceeds those of *IDH1*. Together, *IDH1* and *IDH2* mutations account for a considerable frequency of approximately 17% in adult AML. The presence of *IDH* gene mutations appears to be associated with normal karyotypes and *NPM1* mutations. The observation that *IDH1* mutations appear to correlate with significantly inferior outcome in patients *FLT3*$^{wild\text{-}type}$/*NPM1*$^{wild\text{-}type}$ AML requires confirmation in future studies.

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Authorship

Contribution: S.A. performed research, analyzed data, and wrote the paper; S.L. performed research and analyzed data; F.G.K., A.S., J.E.K., A.Z., and A.W.R. performed research; W.J.L.v.P. analyzed data; B.L. designed research, analyzed data, and wrote the paper; and P.J.M.V. designed and performed research, analyzed data, and wrote the paper.

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