

Persistent *IDH1/2* mutations in remission can predict relapse in patients with acute myeloid leukemia



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Haematologica 2019
Volume 104(2):305-311

ABSTRACT

Persistence of *IDH1* or *IDH2* mutations in remission bone marrow specimens of patients with acute myeloid leukemia has been observed, but the clinical impact of these mutations is not well known. In this study, we evaluated 80 acute myeloid leukemia patients with known *IDH1* R132 or *IDH2* R140/R172 mutations and assessed their bone marrow at the time of remission to determine the potential impact of persistent *IDH1/2* mutations. Approximately 40% of acute myeloid leukemia patients given standard treatment in this cohort had persistent mutations in *IDH1/2*. Patients with an *IDH1/2* mutation had an increased risk of relapse after 1 year of follow-up compared to patients without a detectable *IDH1/2* mutation (59% versus 24%; $P < 0.01$). However, a persistent mutation was not associated with a shorter time to relapse. High *IDH1/2* mutation burden (mutant allelic frequency $\geq 10\%$) did not correlate with relapse rate (77% versus 86% for patients with a low burden, i.e., mutant allelic frequency $< 10\%$; $P = 0.66$). Persistent mutations were also observed in *NPM1*, *DNMT3A* and *FLT3* during remission, but *IDH1/2* mutations remained significant in predicting relapse by multivariate analysis. Flow cytometry was comparable and complementary to next-generation sequencing-based assay for predicting relapse. Monitoring for persistent *IDH1/2* mutations in patients with acute myeloid leukemia in remission can provide information that could be used to justify early interventions, with the hope of facilitating longer remissions and better outcomes in these patients.

Introduction

Acute myeloid leukemia (AML), defined as more than 20% of myeloblasts in blood and/or bone marrow, is heterogeneous and complex at the genomic level. Data from The Cancer Genome Atlas show that many genes are recurrently mutated in patients with AML, including *NPM1*, *FLT3*, *DNMT3A*, *IDH1/2*, and *KRAS/NRAS*.¹ *IDH1* and *IDH2* mutations are found in 6-16% and 8-19% of AML patients, respectively.^{2,7} Collectively, *IDH1/2* mutations are observed in 16-20% of AML patients and are enriched (25-30%) in cases of AML with a normal karyotype.^{6,8,9} *IDH1/2* mutations are acquired early in the natural history of AML and can be present in the founding clone.¹⁰ There are known mutational hot spots in these genes: codon 132 (Arg) in *IDH1* and codons 140 (Arg) and 172 (Arg) in *IDH2*. *IDH2* R140 mutations occur more commonly than R172 mutations in AML.⁵ *IDH1* and *IDH2* mutations can also infrequently occur together at presentation.¹¹ The presence of an *IDH1/2* mutation alone is not sufficient for the development of AML.¹² *IDH2* mutations can be associated with clonal hematopoiesis of indeterminate potential (CHIP) in the older population.¹⁵ Moreover, *IDH1/2* mutations occur

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Received: February 12, 2018.

Accepted: August 24, 2018.

Pre-published: August 31, 2018.

doi:10.3324/haematol.2018.191148

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/104/2/305

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together with mutations of other genes, at frequencies that depend on the *IDH* allele, suggesting that additional genomic insults are needed for AML to develop fully. For example, *IDH2* R140 mutations are strongly associated with *NPM1* mutations.¹⁰

IDH1 and *IDH2* encode NADP⁺-dependent isocitrate dehydrogenases, converting isocitrate to α -ketoglutarate, while reducing NADP⁺ to NADPH with the production of CO₂. *IDH1* is present in the cytoplasm and peroxisome, whereas *IDH2* resides in mitochondria and is a component of the Krebs' cycle.¹⁴ *IDH1* R132 mutation and *IDH2* R140/R172 mutations reduce α -ketoglutarate to the oncometabolite D-2-hydroxyglutarate (also known as R-2-hydroxyglutarate).^{14,15} D-2-hydroxyglutarate has structural similarities to α -ketoglutarate and can competitively inhibit enzymes dependent on α -ketoglutarate, such as the TET enzyme family and histone lysine demethylases, and indeed *IDH1/2* mutations in AML are associated with global DNA hypermethylation and impaired hematopoietic differentiation.^{16,17}

Persistent *IDH1* or *IDH2* mutations have been observed in AML patients at the time of clinical and morphological remission.^{18,19} Debarri *et al.* reported that persistent *IDH1/2* mutations in AML at the time of remission could predict relapse.¹⁹ However, their study cohort was small with only eight patients in complete remission with persistent *IDH1/2* mutations, precluding a definitive conclusion. In this study, we explored the utility of mutant *IDH1* and *IDH2* as minimal residual disease markers in predicting relapse in a large cohort of AML patients.

Methods

Patients

We searched the database of The University of Texas MD Anderson Cancer Center from November 1, 2012 to December 31, 2017 and identified 80 newly diagnosed AML patients with *IDH1* R132 or *IDH2* R140/R172 mutations who achieved complete remission (CR) or CR with incomplete hematologic recovery (CRi), according to the 2017 European LeukemiaNet (ELN) recommendations for the diagnosis and management of AML,²⁰ in bone marrow at any time-point of their treatment. To investigate the effect of predominant and well-established mutant *IDH1/2* clones in AML, only cases with a mutant allelic frequency (MAF) $\geq 10\%$ in a pre-treatment sample were included. All cases were collected consecutively and classified according to the 2017 World Health Organization (WHO) classification system.²¹ Patients with therapy-related AML were excluded from this study. Clinical, laboratory and cytogenetic data were collected from the patients' electronic medical records. This study was approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center (Houston, TX, USA) and was conducted in accordance with the Declaration of Helsinki.²²

IDH1/2 sequencing

IDH1/2 sequencing was performed on all patients as a part of clinically validated next-generation sequencing-based (NGS) assay (a 53-gene panel, a 28-gene panel or an 81-gene panel) as described previously.²³ The limit of detection was 1% for the NGS assay. A sequencing library was prepared using 250 ng of genomic DNA and respective sequencing libraries were subjected to a MiSeq sequencer (Illumina Inc.). NGS data were analyzed using MiSeq Reporter (TruSeq) or SureCall (Haloplex). The Integrative Genomics Viewer (IGV, Broad Institute) was used to visualize read

alignment and confirm variant calls.²⁴ A custom-developed, in-house software package (OncoSeek) was used to annotate sequence variants and to interface the data with the IGV. Nomenclature of genetic variants was designated following the Human Genome Variation Society recommendations.²⁵

FLT3 analysis

The presence of internal tandem duplications or point mutations at codon 835 or 836 in *FLT3* was determined as described previously.²⁶

Cytogenetic analysis

Conventional chromosome analysis (karyotyping) was performed on G-banded metaphase cells prepared from unstimulated 24-hour and 48-hour bone marrow cultures as described previously.²⁷ Twenty metaphases were analyzed in most cases, but fewer than 20 metaphases were analyzed in some cases when inadequate metaphases were available for complete analysis. The results were reported using the current International System for Human Cytogenetic Nomenclature.²⁸ Cytogenetic risk stratification was assessed in each patient using the United Kingdom Medical Research Council (UKMRC) system.²⁹

Statistical analysis

A Fisher exact test was used when comparing categorical variables. Mann-Whitney and Kruskal-Wallis tests were used when comparing numerical variables in two groups or three or more groups, respectively. The cumulative incidence rate of relapse was determined using the competing risk method. The association between an *IDH1/2* mutation and the cumulative incidence outcome was determined using a proportional subdistribution hazards regression model (Fine and Gray regression model).³⁰ Differences in the cumulative incidence among patients with different mutations were assessed using the Gray test.³¹ Time to relapse was calculated from the date of morphological remission to the date of relapse. All variables with a *P* value < 0.05 (two-tailed) were considered to be statistically significant. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA) and SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Patients

The study group included 80 patients (37 men and 43 women) with a median age of 59 years (range, 31 to 90) at diagnosis. The median hemoglobin concentration, white blood cell count and platelet count were 9.0 g/dL (range, 6.3 to 13.9), $7.9 \times 10^9/L$ (range, 0.4 to $263.1 \times 10^9/L$) and $58 \times 10^9/L$ (range, 1 to $1,069 \times 10^9/L$), respectively (Table 1). The median bone marrow blast count was 62% (range, 21 to 95%). Among 76 patients with cytogenetic information available, 88% (n=67) and 12% (n=9) had intermediate and adverse cytogenetic risk, respectively. There were no patients with favorable cytogenetic risk. A diploid karyotype was seen in 52 (68%) patients. Various frontline therapies were administered to this cohort of patients, but no patients received an IDH inhibitor as frontline therapy. All patients younger than 60 years of age (n=41) were treated with intensive chemotherapy including 7+3 (idarubicin and cytarabine), CIA (clofarabine, idarubicin and cytarabine), FIA (fludarabine, idarubicin and cytarabine), or CLIA (cladribine, idarubicin and cytarabine with or without sorafenib). The patients over 60 years old (n=39)

were treated with intensive chemotherapy (n=10), hypomethylating agents (n=23) or low-dose cytarabine with or without nucleoside analogs (n=6). The median clinical follow-up was 17.5 months (range, 4.9 to 77.5 months).

IDH mutations in pretreatment samples

All 80 patients harbored *IDH1* and/or *IDH2* mutations: 78 patients had a single *IDH1* or *IDH2* mutation and two patients had both *IDH1* and *IDH2* mutations. The two patients who had two different *IDH1/2* mutations had major (20~34%) and minor (1~14%) clones represented by MAF. As a single mutation, *IDH2* R140 mutations were most common (n=46), followed by *IDH1* R132 (n=24) and *IDH2* R172 (n=7) mutations. *IDH2* R172_H173delinsSA was found in one patient. Detailed information regarding the *IDH1/2* mutations is presented in Table 1. The median MAF of *IDH1/2* mutations in pretreatment samples was 43.8% (range, 12.3% to 62.7%). The median MAF of the *IDH1* R132 mutation (39.2%) was similar to that of *IDH2* R140 (44.1%) and *IDH2* R172 (42.5%) mutation ($P=0.31$). There were no significant differences in median bone marrow blast count among the three groups ($P=0.54$).

Persistent IDH mutations in complete remission or complete remission with incomplete hematologic remission

The mutational status of *IDH1/2* was available in first CR (n=36) or CRi (n=44) for all patients. In 51 patients treated with intensive chemotherapy, analysis of *IDH1/2* was performed after the first, second and third or beyond cycles of therapy in 21, 16 and 14 patients, respectively. In 23 patients treated with hypomethylating agents, analysis of *IDH1/2* was performed before and after the fourth cycle of therapy in nine and 15 patients, respectively. The latter also included six patients who had received six or more cycles of therapy. In patients treated with low-dose cytarabine with or without additional drugs (n=6), one, one and four patients were tested for *IDH1/2* after, respectively, their first, second and fourth or beyond cycles of therapy. A total of 31 (39%) patients had persistent *IDH1/2* mutations in CR or CRi (CR^{IDH+}). Among the patients in CR (n=36), 12 (33%) had persistent *IDH1/2* mutations. Similarly, among patients in CRi (n=44), 19 (43%) had persistent *IDH1/2* mutations ($P=0.49$). *IDH1* R132, *IDH2* R140 and *IDH2* R172 mutations were observed in, respectively, ten (38.5%), 19 (41.3%), and two (25%) patients with mutations in a pretreatment bone marrow specimen ($P=0.68$) (Figure 1A). Compared to the MAF values in pretreatment samples, the MAF of *IDH1/2* mutations in CR or CRi were reduced in all patients (median MAF: 10.2%, range, 1% to 34.3%) (Figure 1B). CR^{IDH+} was not correlated with cytogenetic abnormalities. CR^{IDH+} was observed in patients with diploid karyotype and those with any cytogenetic abnormalities with similar frequency (40.4% and 37.5%, respectively; $P=0.99$). Of 24 patients with cytogenetic abnormalities in pretreatment samples, only two had persistent cytogenetic abnormalities.

IDH mutations in remission are associated with an increased risk of relapse

The cumulative incidence rate of relapse in patients with CR^{IDH+} was 59% at 12 months and 80% at 24 months. The cumulative incidence rate was significantly

higher in patients with CR^{IDH+} than in patients without a detectable *IDH1/2* mutation in remission (CR^{IDH-}) (Figure 2A). Using the Fine and Gray regression model, the risk of relapse at 1 year of follow-up was higher for patients with CR^{IDH+} than patients in the CR^{IDH-} group (59% versus 24%; hazard ratio, 3.89; 95% confidence interval: 1.98 to 7.62; $P<0.01$) (Table 2). Regarding mutation type, 90%, 74% and 100% of patients with persistent *IDH1* R132, *IDH2* R140 and *IDH2* R172 clones relapsed, respectively ($P=0.44$). There were no differences regarding relapse between patients treated with intensive chemotherapy and hypomethylating agents (47.1% and 43.5%, respectively) ($P=0.77$). The median time to relapse in patients with CR^{IDH+} was not significantly different from that of CR^{IDH-} patients (median: 8.1 and 6.9 months, respectively) ($P=0.71$).

IDH1/2 mutation burden does not correlate with relapse

Among patients with CR^{IDH+} , the MAF values for CR^{IDH+} were not significantly different between relapsed (median MAF: 10.0%) and non-relapsed patients (median MAF: 20.5%) ($P=0.19$). To further evaluate the correlation between *IDH1/2* mutation burden and relapse, we arbitrarily divided patients according to whether they had a high MAF ($\geq 10\%$) or low MAF ($<10\%$). Among 17 patients with a high MAF, 13 (77%) patients relapsed, whereas 12 of 14 (86%) patients with a low MAF relapsed ($P=0.66$). The difference in the median time to relapse

Table 1. Laboratory, cytogenetic and *IDH1/2* mutation data of patients with acute myeloid leukemia (n=80).

	Median	Range
Age (years)	59	31 to 90
Hemoglobin (g/dL)	9.0	6.3 to 13.9
White blood cells, $\times 10^9$ L	7.9	0.4 to 263.1
Platelets, $\times 10^9$ L	58	1 to 1,069
Bone marrow blasts (%)	62	21 to 95
	Number of patients	(%)
Cytogenetic risk		
Favorable	0	0
Intermediate	67	88
Adverse	9	12
Treatment		
Intensive chemotherapy	51	64
Hypomethylating agent	23	29
Low-dose cytarabine	6	7
<i>IDH1/2</i> mutation		
Single		
p.R132C	13	16.7
p.R132H	5	6.4
p.R132S	3	3.8
p.R132L	2	2.6
p.R132G	1	1.3
p.R140Q	44	56.4
p.R140L	1	1.3
p.R140W	1	1.3
p.R172K	6	7.7
p.R172G	1	1.3
p.R172_H173delinsSA	1	1.3
Double		
p.R132C and p.R140Q	2	NA

between patients in the high and low MAF groups (6.2 and 9.9 months, respectively) was not statistically significant ($P=0.18$). We also assessed different MAF as cutoffs for high versus low mutation burden (5%, 20% and 30%), but correlation with relapse was not observed using any of these cutoffs (*data not shown*).

IDH mutations in remission predict relapse in the context of co-mutations

Other gene mutations were detected in 89% of patients who had *IDH1/2* mutations in a pre-treatment sample.

NPM1 (n=38) was the most frequently co-mutated gene followed by *DNMT3A* R882 (n=25), *FLT3*-ITD (internal tandem duplication) (n=22), and *KRAS/NRAS* (n=12). Few (<5) patients had mutations in *ASXL1*, *BRAF*, *CEBPA*, *JAK2*, *RUNX1*, *TET2* or *TP53*. CR^{IDH+} was significantly more common (39%) than CR^{FLT3+} (n=3, 14%), CR^{NPM1+} (n=4, 11%), and $CR^{KRAS/NRAS+}$ (n=0, 0%) in CR or CRi ($P>0.05$). $CR^{DNMT3A+}$ was present with a similar frequency, being seen in 36% of patients (n=9). We assessed the effect of CR^{IDH+} in the context of co-mutations using the Fine and Gray regression model. By univariate analysis,

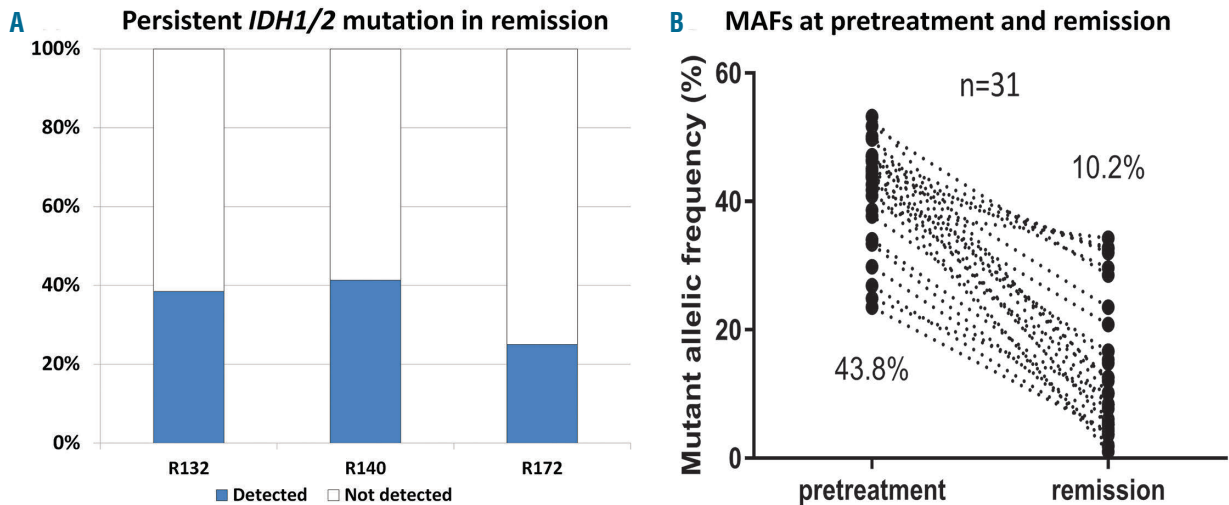


Figure 1. Persistent IDH1/2 mutations in remission and changes in mutant allelic frequencies in pretreatment and remission samples. (A) Percentages of persistent IDH1/2 mutations in patients with acute myeloid leukemia in remission. Persistent mutations occur at similar frequencies for the different mutations. (B) Mutant allelic frequency (MAF) of IDH mutations present in pretreatment samples and remission samples. The median mutant allelic frequency is shown for each occasion.

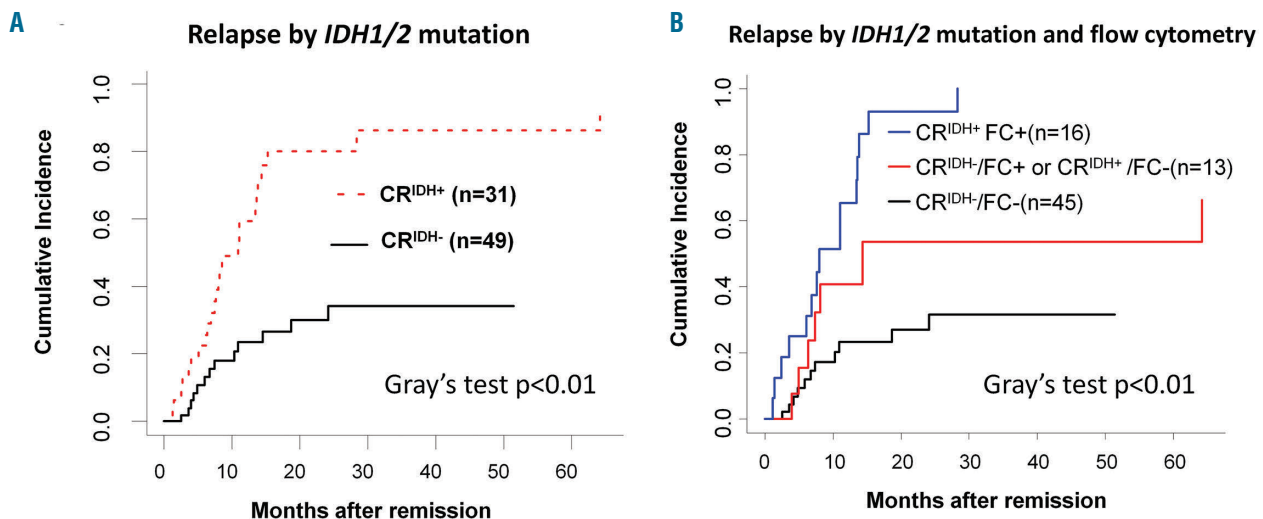


Figure 2. Cumulative incidence rates of relapse in patients with persistent IDH1/2 mutations in remission. (A) Cumulative incidences of relapse in patients with persistent IDH1/2 mutation and patients without detectable IDH1/2 mutation. The cumulative incidence rate is significantly higher in patients with persistent IDH1/2 mutation in remission (CR^{IDH+}) than in patients without detectable IDH1/2 mutation in remission (CR^{IDH-}). (B) Cumulative incidence rate of relapse in patients with respect to mutational status in IDH1/2 and flow cytometrically determined presence of minimal residual disease in remission. The cumulative incidence of relapse was significantly higher in patients who were positive in both molecular and flow cytometry tests compared to patients with a positive result in either of the tests or negative in both. CR^{IDH+} : persistent IDH1/2 mutation in remission, CR^{IDH-} : non-detectable IDH1/2 mutation in remission, FC+: positive for minimal residual disease by flow cytometry, FC-: negative for minimal residual disease by flow cytometry.

Table 2. Risk of relapse according to the presence of persistent mutations in different genes (Fine and Gray regression model).

	HR	Univariate 95% CI	P value	HR	Multivariate 95% CI	P value
Age ≥ 60 years	0.99	0.96 to 1.03	0.9			
Female gender	1.23	0.56 to 2.67	0.61			
CR ^{IDH+}	3.89	1.98 to 7.62	<0.01	4.45	2.15 to 9.19	<0.01
CR ^{FLT3+}	12.6	1.66 to 95.1	0.01	20.2	3.99 to 102	<0.01
CR ^{NPM1+}	1.38	0.25 to 7.75	0.71			
CR ^{DNMT3A+}	2.01	0.92 to 4.39	0.08			

HR; hazard ratio, CI; confidence interval, CR^{IDH+}; persistent *IDH1/2* mutation in CR or CRi, CR^{FLT3+}; persistent *FLT3*-ITD mutation in CR or CRi, CR^{NPM1+}; persistent *NPM1* mutation in CR or CRi, CR^{DNMT3A+}; persistent *DNMT3A* mutation in CR or CRi.

CR^{FLT3+} also demonstrated an increased risk of relapse. By multivariate analysis, CR^{IDH+} and CR^{FLT3+} remained significant for an increased risk of relapse.

We also assessed the dynamic changes of clonal architecture in 25 CR^{IDH+} patients who relapsed. Comparing mutational profiles at CR/CRi and relapse, four patients acquired novel mutations at relapse. These mutations were *ERBB2* p.R784C (MAF: 12.3%), *FLT3* p.D835Y (1.5%), *TP53* p.G245D (3.8%) and *WT1* p.K467fs (12.2%). These mutations showed subclonal fraction patterns compared with the MAF of *IDH1/2* mutation at relapse.

Flow cytometry is comparable to next-generation sequencing in predicting relapse

We compared molecular test results to those of multiparametric flow cytometry (FC) immunophenotyping in CR/CRi bone marrow specimens. Flow cytometric results were available for a total of 79 patients. Minimal residual disease (MRD) determination by FC has been described previously.^{52,53} The sensitivity of the flow cytometry was validated to 0.1% - 0.01% depending on the leukemic cell phenotype. According to FC, 19 (26%) patients had MRD, 55 (76%) were MRD-negative and results were indeterminate in five patients. Among 74 patients in whom both FC and molecular MRD tests were performed, the results were concordant in 61 (82%) patients and discordant in 13 (18%) patients with a statistically significant association ($P < 0.01$). Of the 13 patients with discordant FC and molecular testing MRD results, ten patients were positive by FC only and three patients were positive by molecular testing only. Four of the five patients for whom FC was indeterminate with regards to MRD had a persistent *IDH2* p.R140Q mutation with various MAF values (median 12.5%; range, 1 to 28.5%) and all of them relapsed. Similarly to CR^{IDH+} patients, those who were positive for MRD assessed by flow cytometry (FC⁺) showed an increased risk of relapse compared to patients who were negative for MRD by flow cytometry (FC⁻) after 1 year of follow-up (63% versus 27%; hazard ratio, 4.24; 95% confidence interval: 2.22 to 8.13; $P < 0.01$). Patients with positive results in both methods (CR^{IDH+}/FC⁺) had a significantly higher risk of relapse compared to those with discordant results (CR^{IDH+}/FC⁻ or CR^{IDH-}/FC⁺) or negative results in both methods (CR^{IDH-}/FC⁻) (Figure 2 and Table 3).

Discussion

IDH1 and *IDH2* mutations are not uncommon in AML. In addition, *IDH1/2* mutations can be found in the pre-

Table 3. Risk of relapse with respect to presence or absence of a persistent *IDH1/2* mutation and flow cytometry determined minimal residual disease status (Fine and Gray regression model).

Group	HR	95% CI	P value
CR ^{IDH+} /FC ⁺ (n=16)	Reference		
CR ^{IDH+} /FC ⁻ or CR ^{IDH-} /FC ⁺ (n=13)	0.36	0.15 to 0.87	0.02
CR ^{IDH-} /FC ⁻ (n=45)	0.17	0.08 to 0.34	<0.01

CR^{IDH+}; persistent *IDH1* or *IDH2* mutation in remission, CR^{IDH-}; no detectable *IDH1* or *IDH2* mutation in remission, FC⁺; positive for minimal residual disease by flow cytometry, FC⁻; negative for minimal residual disease by flow cytometry.

leukemic clone in individuals without pathology-proven AML or even in healthy individuals as a sign of age-related clonal hematopoiesis.^{15,34-36} For these reasons, in this study, we only selected AML patients with an *IDH1/2* mutation as a predominant clone (MAF >10%) in pretreatment samples to minimize the effect on our analysis of subclonal *IDH1/2* mutations.

In patients with AML associated with *IDH1/2* mutations, karyotyping is not a preferred method for monitoring persistent aberrancy during remission for at least two reasons: cytogenetic analysis is less sensitive and *IDH1/2* mutations are enriched in AML with a normal karyotype.^{5,8,11,57} In support of this statement, 68% of patients had a diploid karyotype at diagnosis. Out of 24 patients who had cytogenetic abnormalities in pretreatment samples, only two had persistent cytogenetic abnormalities in the remission sample. Therefore, follow-up with more sensitive methods is necessary to monitor for MRD.

In this cohort of patients, *IDH2* mutations (69%) were more common than *IDH1* mutations (31%). In the *IDH2* group, mutations at codon 140 were far more frequent than R172 mutations in an approximately 5.8 to 1 ratio. *IDH1* mutations were present in almost one-third of patients. These frequency data are consistent with those from other studies of AML in the literature.^{5,11,57} The median MAF of an *IDH1/2* mutation was 43.8%, indicating that the *IDH1/2* mutation was the predominant clone in most patients. The median MAF of the *IDH1* R132 mutation (39.2%) was slightly lower than that of *IDH2* mutations (44.1% and 42.5% for *IDH2* R140 and R172 mutations, respectively).

Persistent *IDH1/2* mutations in patients with AML who are in complete remission (CR^{IDH+}) have been reported by others.^{18,19} We observed that approximately 40% of AML patients in remission had persistent *IDH1/2* mutations with decreased MAF regardless of *IDH* mutation subtype (*IDH1* R132, *IDH2* R140 and *IDH2* R172) or treatment

type (intensive chemotherapy *versus* hypomethylating agents). Approximately 50% of patients with CR^{IDH+} had MAF below the assay sensitivity of the Sanger sequencing (<10%). This indicates that a NGS-based approach is necessary to monitor persistent *IDH1/2* mutations. CR^{IDH+} was associated with an increased risk of relapse (hazard ratio, 3.89; 95% confidence interval: 1.98-7.62; $P < 0.01$) compared to patients with CR^{IDH-}. However, CR^{IDH+} was not associated with a shorter time to relapse (median 8.1 months *versus* 6.9 months in patients with CR^{IDH-}; $P = 0.71$). Interestingly, high mutation burden did not correlate with relapse in this study because patients with lower *IDH1/2* mutation burden (MAF <10%) relapsed with a similar frequency as patients with a higher mutation burden (MAF $\geq 10\%$) (77% and 86%, respectively, $P = 0.66$). Accordingly, these data suggest that presence of persistent *IDH1/2* mutation in remission is *per se* associated with relapse in AML patients and that mutation burden does not have an additive predictive effect.

Focusing on a single event (*IDH1/2* mutation) as a predictive marker of relapse in AML is potentially problematic because of frequent co-mutations in other genes including *FLT3*, *NPM1* and *DNMT3A*. Indeed, co-mutations in other genes were detected in the majority of patients (89%) in this study cohort. However, persistent mutation in other genes in remission was rare, except for *DNMT3A*. By univariate analysis, CR^{FLT3+} also showed an increased risk of relapse. This result might not be reliable because only a few patients ($n = 3$) had persistent *FLT3* mutation in remission.

We noticed that a few AML patients acquired novel mutations at relapse, but at a relatively low burden (MAF range: 1.5% – 12.2%). These mutations occurred in genes in the activated signaling (*FLT3* or *KRAS*) and tumor suppressor (*TP53* and *WT1*) classes, apparently providing either proliferative or survival signals to the *IDH*-mutated clone.

FC is a powerful tool for detecting residual leukemic cells and can be used to predict relapse in patients with AML. The concordance rate for detecting MRD between FC and molecular methods was 82%, similar to earlier studies.^{38,39} Positivity for MRD determined by FC was also

associated with an increased risk of relapse in this cohort. Interestingly, patients with positive results according to both methods (CR^{IDH+/FC+}) had a significantly higher risk of relapse compared to those with discordant results (CR^{IDH+/FC-} or CR^{IDH-/FC+}) or negative results by both methods (CR^{IDH-/FC-}). These findings suggest that mutational analysis and FC are complementary methods useful for predicting relapse in AML patients in CR or CRi.

The data we present are in accordance with those of a recent study by Jongen-Lavrencic *et al.*, who investigated 430 patients with AML or refractory anemia with excess blasts treated according to the clinical protocol of either the HOVON or SAKK with achievement of either CR or CRi after two cycles of induction chemotherapy.³⁹ Mutational screening was performed at the time of diagnosis and at CR/CRi using a targeted, 54-gene NGS panel (limit of detection: $\leq 1\%$ of mutant allele). Their study showed that persistent mutation in genes other than *DNMT3A*, *TET2* and *ASXL1* in CR/CRi was an independent risk factor for relapse. *IDH1* and *IDH2* mutations were included in their study and showed a similar frequency of persistent mutation in CR/CRi (28%). However, the authors did not focus on particular genes with respect to the increased risk of relapse.

To the best of our knowledge, our study is the largest cohort ($n = 80$) investigating the impact of persistent *IDH1/2* mutations in CR/CRi in AML patients. However, our study does have some limitations: (i) the time point of *IDH1/2* analysis in remission was not uniform, and (ii) our cohort was not sufficiently large to reliably investigate the effect of co-mutations in remission. Larger-scale studies are necessary to reproduce the results of our study.

In summary, approximately 40% of AML patients with an *IDH1/2* mutation at initial diagnosis will have persistent mutations after therapy in remission bone marrow samples. A persistent *IDH1/2* mutation is associated with an increased risk of relapse. Monitoring *IDH1/2* mutations in AML patients during remission using a highly sensitive NGS-based assay may provide useful information to guide early interventions with the aim of achieving longer remissions and better outcomes.

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