Clinical relevance of *IDH1/2* mutant allele burden during follow-up in acute myeloid leukemia. A study by the French ALFA group

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ABSTRACT

Assessment of minimal residual disease has emerged as a powerful prognostic factor in acute myeloid leukemia. In this study, we investigated the potential of *IDH1/2* mutations as targets for minimal residual disease assessment in acute myeloid leukemia, since these mutations collectively occur in 15-20% of cases of acute myeloid leukemia and now represent druggable targets. We employed droplet digital polymerase chain reaction assays to quantify *IDH1R132*, *IDH2R140*, and *IDH2R172* mutations on genomic DNA in 322 samples from 103 adult patients with primary *IDH1/2* mutant acute myeloid leukemia and enrolled on Acute Leukemia French Association -0701 or -0702 clinical trials. The median *IDH1/2* mutant allele fraction in bone marrow samples was 42.3% (range, 8.2 - 49.9%) at diagnosis of acute myeloid leukemia, and below the detection limit of 0.2% (range, <0.2 - 39.3%) in complete remission after induction therapy. In univariate analysis, the presence of a normal karyotype, a *NPM1* mutation, and an *IDH1/2* mutant allele fraction <0.2% in bone marrow after induction therapy were statistically significant predictors of longer disease-free survival. In multivariate analysis, these three variables remained significantly predictive of disease-free survival. In 7/103 (7%) patients, *IDH1/2* mutations persisted at high levels in complete remission, consistent with the presence of an *IDH1/2* mutation in pre-leukemic hematopoietic stem cells. Five out of these seven patients subsequently relapsed or progressed toward myelodysplastic syndrome, suggesting that patients carrying the *IDH1/2* mutation in a pre-leukemic clone may be at high risk of hematologic evolution.

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Introduction

Assessment of minimal residual disease (MRD) has emerged as a powerful prognostic factor in acute myeloid leukemia (AML).[1-6] Many studies have shown that MRD detection using multiparameter flow cytometry or real-time quantitative polymerase chain reaction (qPCR) provides powerful independent prognostic information in AML.[7,8] Chimeric fusion genes, such as *PML-RARA*, *CBFB-MYH11* or *RUNX1-RUNX1T1*, *NPM1* mutations, as well as *WT1* expression are well-established molecular markers for MRD monitoring in AML. However, sensitive and leukemia-specific MRD markers are lacking in approximately 40% of AML patients. This prompted us to investigate the potential of other recurrent molecular

abnormalities as targets for MRD assessment in AML, such as mutations in isocitrate dehydrogenase (*IDH*) 1 and 2.

IDH1/2 mutations affecting *IDH1R132*, *IDH2R140*, and *IDH2R172* residues are single-nucleotide mutations that collectively occur in 15-20% of AML and represent driver mutations in leukemogenesis.[9] Mutant IDH1/2 enzymes have neomorphic activity and catalyze the reduction of α-ketoglutarate to an oncometabolite, the R-enantiomer of 2-hydroxyglutarate (2-HG), which promotes DNA and histone hypermethylation, altered gene expression, and impaired hematopoietic differentiation.[10,11] Quantification of single-nucleotide mutations by qPCR can be challenging because of problems with background amplification from the wild-type allele. Recently, the development of digital PCR has enabled absolute quantification of various genomic targets with high precision and sensitivity and has, therefore, turned out to be a promising technique for MRD monitoring, especially for gene mutations.[7,12]

The clinical significance of residual *IDH1/2* mutations in bone marrow in complete remission after chemotherapy is currently unknown. In this study, we employed digital PCR assays to quantify *IDH1/2* mutant allele fraction at AML diagnosis and during follow-up in a large cohort of AML patients intensively treated in the Acute French Leukemia Association (ALFA) trials to investigate whether *IDH1/2* mutations are suitable MRD markers that could predict clinical outcome in AML patients and provide further information for risk-adapted therapy.

Methods

Patients and treatment

This study was performed in 103 adult patients (18-70 years) with previously untreated primary *IDH1/2* mutated AML and enrolled on the prospective ALFA-0701 (Eudra-CT 2007-002933-36; ClinicalTrials.gov NCT00927498 or ALFA-0702 (Eudra-CT 2008-000668-18; ClinicalTrials.gov NCT00932412) trials. Treatment schemes have been previously reported for both trials.[13,14] These studies were approved by the ethics committee of Saint-Germain en Laye and Sud Est IV, France, respectively, and the institutional review board of the French Regulatory Agency. Bone marrow or peripheral blood samples collected at the time of diagnosis of AML and during follow-up were obtained from the tissue bank *Tumorothèque du Centre de Référence Régional en Cancérologie de Lille* (CRRC)" and approval for this study was obtained from the institutional review board of CHRU of Lille (CSTMT089). All patients provided written informed consent to both treatment and genetic analysis before inclusion in the study, in accordance with the declaration of Helsinki. Among all patients included in the ALFA-0701 (n=278) or ALFA-0702 (n=704) trials, we selected patients meeting the following criteria: (i) the presence of an *IDH1*R132 or an *IDH2*R140/R172 mutation at AML diagnosis (n=160), (ii) achievement of complete remission after induction therapy (n=130), and (iii) one or more bone marrow follow-up sample available for *IDH1/2* variant allele fraction (*IDH1/2*-VAF) assessment (n=103) (Figure 1).

Molecular analysis

Droplet Digital™ PCR (ddPCR) assays were used to quantify the *IDH1/2* mutant allele and its wild-type counterpart in diagnostic and follow-up samples. During complete remission, only bone marrow samples were analyzed for *IDH1/2*-VAF assessment. *IDH1/2*-VAF was quantified on genomic DNA using Bio-Rad™ reagents, primers and probes (HEX-labeled wild-type allele; FAM-labeled mutant alleles). All samples were tested in duplicate wells, using 90 ng of DNA per well. The PCR product from each well was then subjected to the QX100 droplet reader (Bio-Rad™), which measures the fluorescence of each droplet individually using a two-color detection system. Raw data were analyzed using QuantaSoft software, version 1.7.4.0917 (Bio-Rad™). Representative two-dimensional plots of droplet fluorescence for
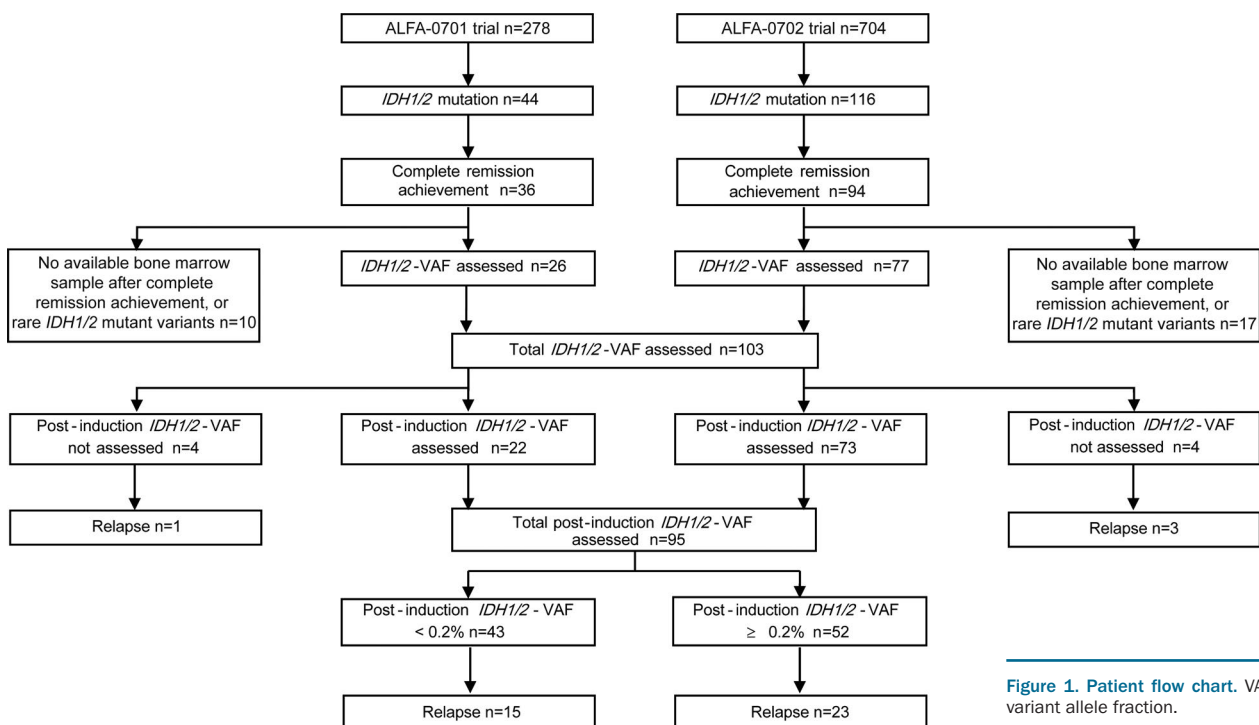

Figure 1. Patient flow chart. VAF, variant allele fraction.

IDH1/2 wild-type controls and *IDH1/2* mutant samples are shown in *Online Supplementary Figure S1*. The mutant allele frequency was then estimated using a Poisson distribution model as the fraction of positive droplets divided by total droplets containing a target. The limit of detection was defined for each mutation as the mean value of *IDH1/2* wild-type controls plus three standard deviations (*Online Supplementary Table S1*). The upper detection limit of these ddPCR assays (rounded to 0.2% of mutant allele frequency) was further considered as the threshold for statistical analysis. An *IDH1/2*-VAF level below 0.2% was hereafter considered as negative MRD. Gene mutation analysis and next-generation sequencing assays are described in the *Online Supplementary Methods* and *Online Supplementary Tables S2-S4*.

Statistical analysis

Group comparison for categorical and continuous variables was performed with the Fisher exact and Mann-Whitney test, respectively. Overall survival was calculated from the date of AML diagnosis to the last follow-up date by censoring patients alive at that date. Disease-free survival was calculated from the date of complete remission to the date of relapse or death, censoring patients alive without an event at the last follow-up date. In some analyses, data were censored at the time of allogeneic stem cell transplantation. Univariate and multivariate analyses assessing the impact of categorical and continuous variables were performed with a Cox model.[15] The proportional-hazards assumption was checked before conducting multivariate analyses.[16] Covariates with a *P*-value <0.1 in univariate analysis were included in the multivariable models. Statistical analyses were performed with STATA software (STATA 12.0 Corporation, College Station, TX, USA). *P*-values were two-sided, with *P*<0.05 denoting statistical significance.

Results

Baseline characteristics of the patients and acute myeloid leukemias

The patients' median age was 54 years (range, 22-70). The median follow-up was 2.7 years (95% CI: 2.3-3.0). Results of conventional cytogenetic studies were available for 98/103 (95%) patients, of whom 72% had normal karyotype AML. A concomitant *NPM1* mutation was found in 50/103 (48%) patients. Only 4/103 (4%) patients harbored a concomitant *TET2* mutation (Table 1), in accordance with the fact that *IDH1/2* and *TET2* mutations tend to be mutually exclusive.[10] As opposed to *IDH1*R132 and *IDH2*R140 mutations, *IDH2*R172 mutations are less likely to be accompanied by additional frequently recurring

mutations in AML.[9,17] In our cohort, *IDH2*R172K mutations were mutually exclusive with *NPM1* and *FLT3* mutations, but co-occurred with *DNMT3A* mutations. An isolated trisomy 11 was identified in 5/21 (24%) patients with the *IDH2*R172K mutation, while this cytogenetic abnormality was not found in any patient with other types of *IDH1/2* mutations (24% *versus* 0%; *P*<0.001) (Figure 2). In the subgroup of *IDH2*R172K mutant AML (n=21), single-nucleotide polymorphism array analysis revealed an additional genomic lesion involving chromosome 11, consisting of a 11p11.2-q12.1 uniparental disomy, in one patient with normal karyotype AML. No *MLL* partial tandem duplication, known to be strongly associated with trisomy 11,[18] was found by reverse transcriptase

Table 1. Baseline characteristics of the patients and acute myeloid leukemias.

| | Number of patients (%) | | |
	ALFA-0701	ALFA-0702	Total
Gender			
Male	10	38	48 (47)
Female	16	39	55 (53)
Median age (range), years	62 (51-70)	50 (22-60)	54 (22-70)
Median white blood cell count (range), x 10⁹/L	18 (1-157)	5 (1-377)	7 (1-377)
Cytogenetics			
Normal	21	50	71 (69)
Abnormal	4	23	27 (26)
Failure	1	4	5 (5)
IDH1/2 mutation			
IDH1 p.R132H/C/G	10	26	36 (35)
IDH2 p.R140Q	10	36	46 (45)
IDH2 p.R172K	6	15	21 (20)
Other gene mutations			
NPM1 mutation	16	34	50 (48)
FLT3 internal tandem duplication	3	16	19 (19)
FLT3-tyrosine kinase domain mutation	2	7	9 (9)
DNMT3A mutation	6	23	29 (35)
TET2 mutation	2	2	4 (4)
CEBPA mutation	1 (1 sm)	3 (2 sm, 1 dm)	4 (4)
European LeukemiaNet 2008 risk-group			
Favorable	13	20	33 (32)
Non-favorable	12	52	64 (62)
Not defined	1	5	6 (6)

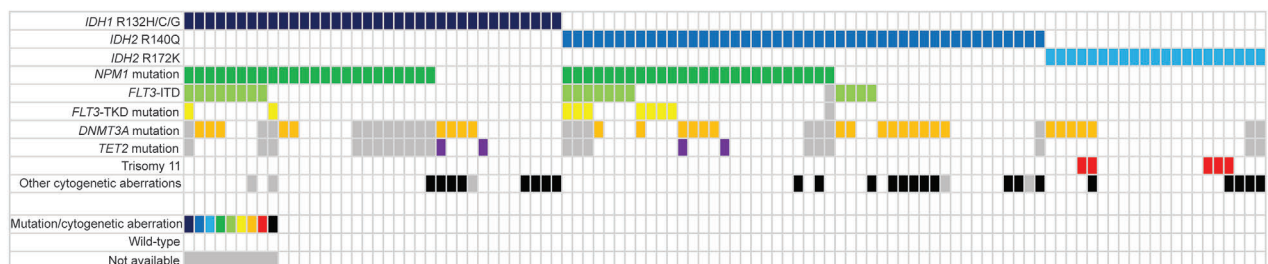sm: single mutation; dm: double mutation.

Figure 2. Barcoding representing the co-occurrence of gene mutations and cytogenetic alterations in our cohort of 103 patients with *IDH1/2* mutant acute myeloid leukemia. ITD: internal tandem duplication; TKD: tyrosine kinase domain.

PCR in this subgroup (*data not shown*). The association between *IDH2*R172 mutation and trisomy 11 observed in our cohort is consistent with results from a previous study,[19] and suggests a potential cooperation between these two genetic alterations in leukemogenesis.

IDH1/2 mutation level at diagnosis of acute myeloid leukemia and during follow-up

At AML diagnosis, *IDH1/2*-VAF could be assessed by next-generation sequencing in 80/103 patients (*Online Supplementary Table S5*). The median *IDH1/2*-VAF value was 41% (range, 16-53%) in bone marrow and 39.5% (range, 6-50%) in peripheral blood samples. In the subset of *NPM1*-mutated AML, *IDH1/2*-VAF was systematically higher than *NPM1*-VAF, except in one patient with similar VAF for both mutations [n=34 comparisons; median difference *IDH1/2*-VAF - *NPM1*-VAF, 10.5% (range, 0-25%); *P*<0.001] (*Online Supplementary Figure S2*). This finding supports the notion that *IDH1/2* mutations were present in pre-existing clones that subsequently acquired *NPM1* mutations.

We also performed ddPCR assays in diagnostic and follow-up samples to quantify the *IDH1/2*-VAF. A total of 322 samples from 103 patients with *IDH1/2* mutations were analyzed by ddPCR at diagnosis (n=97, of which 69 were bone marrow and 28 peripheral blood samples), during hematologic remission (n=211 bone marrow samples), and at relapse (n=14 bone marrow samples). At AML diagnosis, the median *IDH1/2*-VAF assessed by ddPCR was 42.3% (range, 8.2-49.9%) in bone marrow and 40.6% (range, 5.5-53%) in peripheral blood samples, consistent with our next-generation sequencing data. After induction therapy, the *IDH1/2* mutant allele fraction in bone marrow samples decreased significantly compared to the pretreatment levels (*P*<0.001) with a median value below 0.2% (range, <0.2-39.3%). At AML relapse, the median *IDH1/2*-VAF was 21.3% (range, 0.2-38.5%). Among the 14 patients for whom a bone marrow sample was available for molecular analysis at AML relapse, only one lost the mutation during disease evolution (Figure 3A).

Persistent clonal hematopoiesis with *IDH1/2* mutations

IDH1/2 mutations persisted at high levels during hematologic remission in 7/103 (7%) patients, including four with an *IDH2*R140 mutation and three with an *IDH1*R132 mutation, but none with an *IDH2*R172 mutation. The main characteristics of these seven patients are summarized in Table 2 and their *IDH1/2*-VAF profiles are shown in Figure 3B. The only common characteristic identified in these patients was age over 50 years. In this subgroup, the median *IDH1/2*-VAF was 8% (range, 0.8-28.5%) after induction and 40% (range, 26-43.5%) after consolidation therapy. Of these seven patients, only one is still alive in first complete remission, one died from transplant-related mortality, three relapsed, and two developed overt myelodysplastic syndrome. Altogether, 5/7 (71%) patients with persistent clonal hematopoiesis with *IDH1/2* mutations relapsed or progressed toward myelodysplastic syndrome within 1 to 4 years after AML diagnosis.

Univariate and multivariate prognostic analyses

The prognostic impact of *IDH1/2* mutations in AML remains controversial.[9] In the present cohort composed exclusively of *IDH1/2*-mutated AML, the presence of an *IDH2*R172 mutation was associated with a shorter disease-free survival compared to other *IDH1/2* mutation types, but without the difference reaching statistical significance (*P*=0.088). No difference according to the type of *IDH1/2* mutation was observed regarding overall survival (Table 3; Figure 4).

The prognostic impact of *IDH1/2*-VAF was evaluated in complete remission after induction therapy in a subset of 95 patients for whom a post-induction bone marrow sample was available for *IDH1/2*-VAF assessment (Figure 1). We were not able to perform statistical analysis at later follow-up time-points, such as post-consolidation, because of the lack of available DNA samples for many patients. Variables considered for univariate and multivariate analyses were age, white blood cell count, cytogenetics, mutational status of five genes, and *IDH1/2*-VAF after induction therapy. In univariate analysis for disease-free survival, the presence of a normal karyotype, a *NPM1* mutation, and a *IDH1/2*-VAF <0.2% were significantly associated with a longer disease-free survival. In multivariate analysis, these three variables remained significantly predictive of disease-free survival. Factors significantly associated with overall survival were age, the presence of a normal karyotype, the presence of a *NPM1* mutation or a *TET2* mutation. Other molecular abnormalities studied,
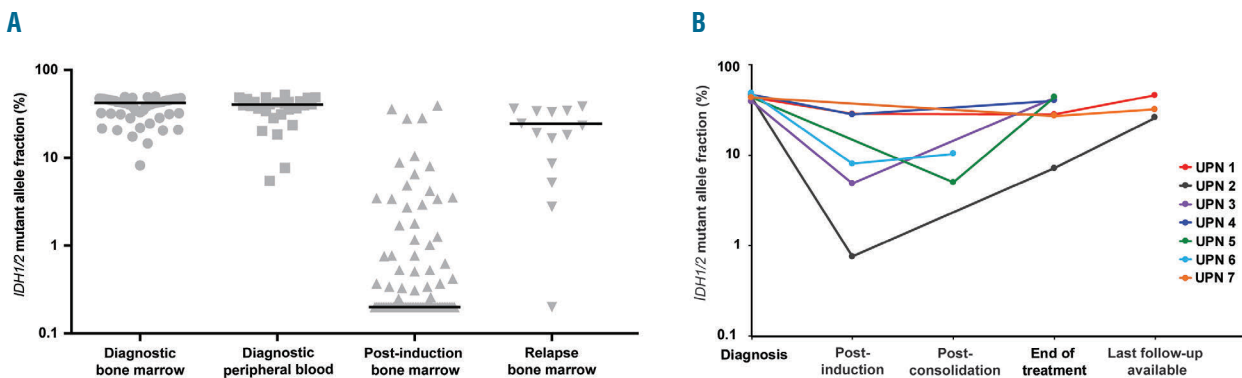

Figure 3. *IDH1/2* mutant allele fraction assessed by droplet digital polymerase chain reaction at diagnosis of acute myeloid leukemia and during follow-up (A) in the whole cohort and (B) for the seven patients with persistent clonal hematopoiesis with *IDH1/2* mutations. The plain lines in the dot plot indicate the median values.

as well as *IDH1/2*-VAF, had no impact on overall survival (Table 3; Figure 5).

Discussion

In this study including 103 adult patients with primary *IDH1/2* mutant AML who were intensively treated, we showed the feasibility of *IDH1/2*-VAF monitoring using ddPCR and its prognostic relevance after induction therapy, independently of pretreatment risk factors. Our findings also suggest that patients with persistent *IDH1/2*-mutated clonal hematopoiesis may be at high risk of dismal hematologic evolution.

The prognostic value of *IDH1/2* mutations is still a matter of debate[9] and may be influenced by the type of mutations, as we previously reported,[20,21] or the profile of concomitant mutations, such as *NPM1* or *DNMT3A* mutations.[17,22] The present study, which only included patients with *IDH1/2* mutations, was not designed to explore the prognostic significance of *IDH1/2* mutations.

The role of MRD in the management of AML patients is growing. Because of the marked heterogeneity of AML, no single MRD marker can be applied to all patients. Additionally, the optimal method for measuring clearance of leukemia cells after chemotherapy remains to be determined. Here, we focused on *IDH1/2* mutations because they are recurrent genetic events in AML, mostly in normal karyotype AML, and now represent druggable targets. The digital PCR technique had been previously shown to allow absolute quantification of a nucleic acid target with high precision and sensitivity.[12] Our data provide evidence that measurement of *IDH1/2*-VAF by ddPCR is feasible.

Table 2. Clinical and biological characteristics of the seven patients with persistent clonal hematopoiesis with *IDH1/2* mutations.

	UPN 1	UPN 2	UPN 3	UPN 4	UPN 5	UPN 6	UPN 7
Age (years)	50	55	55	50	68	60	63
Gender	F	M	M	M	F	F	F
WBC count, x 10⁹/L	28	2.4	4.7	43	34	100	3.2
Cytogenetics	Normal	Trisomy 8	Normal	Normal	Failure	Normal	Normal
NPM1 mutation	Pos.	Neg.	Pos.	Neg.	Pos.	Pos.	Neg.
FLT3-ITD	Pos.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.
FLT3-TKD mutation	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
CEBPA mutation	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
DNMT3A mutation	NA	p.R882H (VAF 26%)	NA	p.R882H (VAF 48%)	Neg.	Neg.	Neg.
TET2 mutation	NA	Neg.	NA	Neg.	Neg.	Neg.	Neg.
IDH1/2 mutation (VAF at diagnosis)	*IDH2* p.R140Q (44%)	*IDH2* p.R140Q (43%)	*IDH2* p.R140Q (39%)	*IDH2* p.R140Q (47%)	*IDH1* p.R132G (44%)	*IDH1* p.R132C (48%)	*IDH1* p.R132C (43%)
IDH1/2-VAF in CR after induction	28.5%	0.76%	4.87%	28.1%	NA	8.2%	NA
IDH1/2-VAF in CR after consolidation	28.1%	7.2%	42.9%	39.9%	43.5%	NA	27.1%
Clinical outcome	Alive in CR1 2 years after AML diagnosis	Relapse 4 years after AML diagnosis	MDS 1 year after AML diagnosis	Relapse 1.5 year after AML diagnosis	Death after allo-SCT	Relapse 1.5 year after AML diagnosis	MDS 2.5 years after AML diagnosis

UPN: unique patient number; F: female; M: male; WBC: white blood cell; Pos.: positive; Neg.: negative; ITD: internal tandem duplication; TKD: tyrosine kinase domain; NA: not available; VAF: variant allele fraction; CR: complete remission; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; allo-SCT: allogeneic stem cell transplantation.

Table 3. Prognostic analysis for disease-free survival and overall survival.

	Disease-free survival						Overall survival		
	Univariate			Multivariate			Univariate		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age*	1.04	0.98 1.10	0.202	-	- -	-	1.13	1.00 1.27	0.047
Log₁₀ (white blood cell count)*	1.00	0.99 1.01	0.537	-	- -	-	1.00	0.99 1.01	0.698
NPM1 mutation	0.23	0.11 0.50	<0.001	0.32	0.12 0.88	0.027	0.19	0.05 0.72	0.014
Normal karyotype	0.26	0.12 0.59	0.001	0.41	0.17 0.99	0.046	0.24	0.07 0.76	0.016
FLT3 internal tandem duplication	1.11	0.33 3.70	0.865	-	- -	-	1.00	0.12 8.08	1.000
FLT3 tyrosine kinase domain mutation	0.20	0.03 1.48	0.115	-	- -	-	-	- -	0.078
DNMT3A mutation	1.42	0.61 3.31	0.413	-	- -	-	2.42	0.64 9.15	0.192
TET2 mutation	2.66	0.58 12.30	0.209	-	- -	-	12.59	1.68 94.62	0.014
IDH2 p.R172K mutation	2.04	0.90 4.61	0.088	0.85	0.31 2.32	0.751	1.44	0.39 5.34	0.586
IDH1/2-VAF after induction <0.2%	0.32	0.15 0.69	0.004	0.40	0.18 0.90	0.026	0.46	0.14 1.54	0.208

HR: hazard ratio; CI: confidence interval; VAF: variant allele fraction; *: continuous variable.

However, despite technical optimizations, we were not able to reach the 0.01% or even 0.1% threshold that we would expect as the quantitative detection limit with the specific ddPCR assays. This problem was due to a relatively high background observed in negative controls, which always consisted of double-positive (actually false-positive) droplets. Polymerase errors occurring during the PCR amplification step seem to be responsible for the generation of these false-positive signals.

The present study is the first to quantify *IDH1/2* mutation levels in a large cohort of AML patients. Previous studies using Sanger sequencing,[23] qPCR,[24] or next-generation sequencing technology[25] suggested that the presence or the level of *IDH1/2* mutations was correlated to disease status in most patients with AML, but the small number of *IDH1/2*-mutated patients included in these studies precluded statistical analysis. Our study revealed that a positive *IDH1/2*-VAF after induction chemotherapy was associated with a shorter disease-free survival. Whether patients with residual *IDH1/2* mutations in complete remission may benefit from allogeneic stem cell transplantation remains to be addressed by future studies. In clinical practice, *IDH1/2*-VAF assessment during and after treatment could be especially valuable in AML patients without recurrent fusion genes or *NPM1* mutations, which are both leukemia-specific and more sensitive MRD markers. Keeping in mind the caveat that *IDH1/2* mutations can be present in the pre-leukemic clone in some cases, one could argue that these mutations could be good MRD markers for those patients in whom MRD becomes undetectable after induction or at early follow-up time-points. However, sequential monitoring of *IDH1/2*-VAF after consolidation therapy or allogeneic stem cell transplantation could still help to detect disease persistence and guide pre-emptive therapy to prevent hematologic relapse, as suggested in a recent study.[26] An alternative approach to MRD monitoring in *IDH1/2*-mutated patients is to quantify the oncometabolite 2-HG.[27,28] A previous study from the ALFA group showed that total 2-HG serum levels <2 μmol/L after induction were associated with better disease-free survival and overall survival.[29] We were not able to correlate *IDH1/2*-VAF and 2-HG levels in this study because of the lack of serum samples.
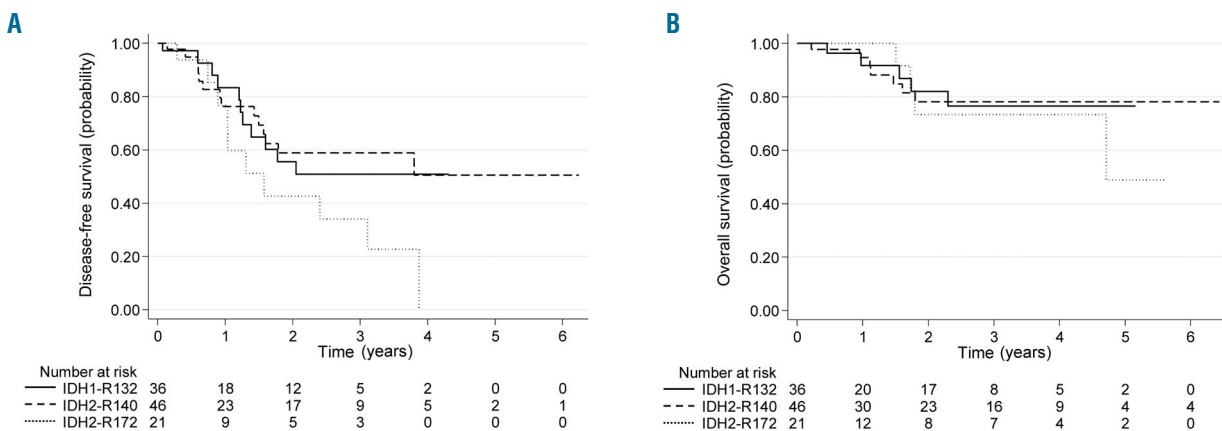
We found that 7/103 (7%) patients had an *IDH1/2*

Figure 4. Kaplan-Meier estimates of (A) disease-free survival and (B) overall survival according to the type of *IDH1/2* mutation.
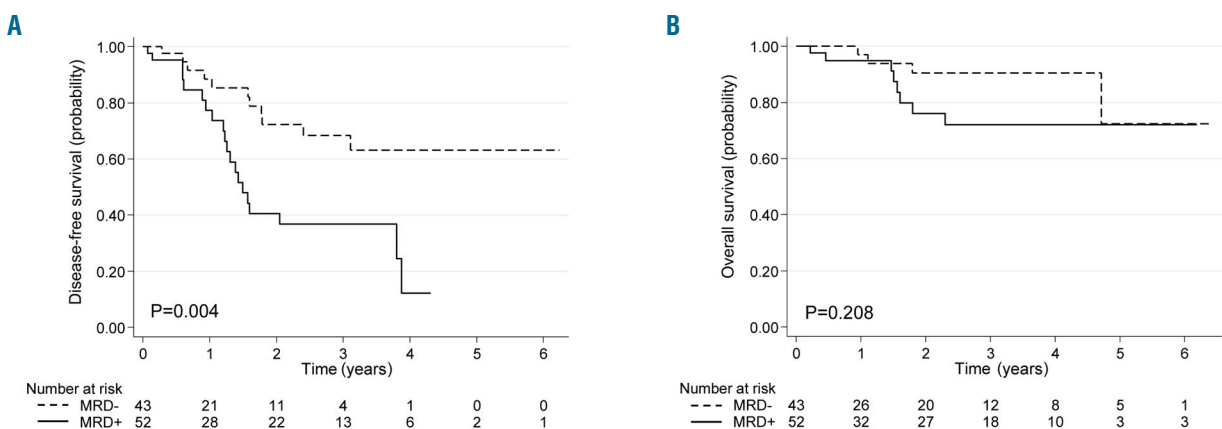

Figure 5. Prognostic analysis according to post-induction *IDH1/2* mutant allele fraction. Kaplan-Meier estimates of (A) disease-free survival and (B) overall survival according to *IDH1/2* variant allele fraction (*IDH1/2*-VAF). MRD+ denotes I*DH1/2*-VAF ≥0.2% and MRD- denotes *IDH1/2*-VAF <0.2%.

mutation that persisted at high levels in hematologic remission, consistent with the presence of this mutation in pre-leukemic hematopoietic stem cells. Unlike AML blasts, these hematopoietic stem cells survive chemotherapy and persist in remission bone marrow, providing a potential reservoir for leukemic progression.[30] In our study, 5/7 (71%) patients with persistent clonal hematopoiesis with *IDH1/2* mutations relapsed or progressed toward myelodysplastic syndrome, suggesting that these patients may be at high risk of hematologic evolution and should probably be monitored more closely. Klco *et al.* showed that initiating mutations, such as *DNMT3A, TET2*, and *IDH1/2* mutations, are less likely to be cleared after chemotherapy than cooperating mutations,[31] in accordance with our own and previous data.[25,32] Furthermore, the prognostic value of persisting somatic mutations in complete remission appears to vary depending on the gene involved. Recent studies suggested that the presence of persistent mutations in *DNMT3A, TET2* or *ASXL1* lacks prognostic impact in terms of AML relapse or survival,[33,34] in contrast with what we observed for *IDH1/2* mutations.

Patients with *IDH1/2* mutations are candidates for targeted therapies. Small-molecule inhibitors of mutant IDH1 such as ivosidenib or IDH2 such as the recently approved enasidenib are currently under clinical investigation and, when used as single agents, have shown promising results in patients with AML or myelodysplastic syndrome as a first-line treatment or in relapsed or refractory diseases.[9] These molecules have been shown to induce differentiation of primary leukemic cells *in vitro*[35,36] and *in vivo*[37] to promote clinical responses. Future studies should determine whether patients with high levels of *IDH1/2*-VAF after induction therapy could benefit from a consolidation or maintenance therapy including *IDH1/2* inhibitors. Ultimately, one could imagine that the use of these small

molecules might also be considered in patients with persistence of clonal hematopoiesis with *IDH1/2* mutations, although clearance of the clone carrying the drug targets seems to occur only in a small subset of treated patients, even with the most potent inhibitors.[38] Additionally, preclinical and clinical data indicate that *IDH1/2* mutations may identify patients likely to respond to pharmacological BCL-2 inhibition.[39,40] The use of *IDH1/2*-VAF monitoring in patients treated with an *IDH1/2* or BCL-2 inhibitor, such as venetoclax, could therefore contribute to the evaluation of treatment efficacy.

In conclusion, our study is the first to show that *IDH1/2* mutant allele fraction in complete remission after induction therapy significantly correlates with disease-free survival, independently of pretreatment prognostic factors. However, this difference did not translate into distinct overall survival rates in our cohort. Our data provide evidence that *IDH1/2* mutant allele fraction has the potential to become a useful tool for the management of AML patients as a biomarker of treatment response, in addition to being a molecular predictor of response to targeted therapies. Further studies based on larger cohorts of patients are required to confirm and extend our findings, and to address the question of whether the residual level of *IDH1/2* mutation may help to refine the assignment into distinct risk groups and guide the decision of whether to perform allogeneic stem cell transplantation or give targeted therapies.

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