MYELOID NEOPLASIA

CME Article

Prognostic significance of concurrent gene mutations in intensively treated patients with *IDH*-mutated AML: an ALFA study

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KEY POINTS

- **The presence of *NPM1* mutation is the primary prognostic factor for OS in *IDH1*- or *IDH2R140*-mutated AML treated by IC.**

- **In nonfavorable European LeukemiaNet 2010 *IDH*-mutated AML, patients achieving transplantation in CR1 had longer OS and DFS.**

In patients with isocitrate dehydrogenase (IDH)–mutated acute myeloid leukemia (AML) treated by intensive chemotherapy (IC), prognostic significance of co-occurring genetic alterations and allogeneic hematopoietic stem cell transplantation (HSCT) are of particular interest with the advent of IDH1/2 mutant inhibitors. We retrospectively analyzed 319 patients with newly diagnosed AML (127 with *IDH1*, 135 with *IDH2R140*, and 57 with *IDH2R172* mutations) treated with IC in 3 Acute Leukemia French Association prospective trials. In each *IDH* subgroup, we analyzed the prognostic impact of clinical and genetic covariates, and the role of HSCT. In patients with *IDH1* mutations, the presence of *NPM1* mutations was the only variable predicting improved overall survival (OS) in multivariate analysis (*P* < .0001). In *IDH2R140*-mutated AML, normal karyotype (*P* = .008) and *NPM1* mutations (*P* = .01) predicted better OS. *NPM1* mutations were associated with better disease-free survival (DFS; *P* = .0009), whereas the presence of *DNMT3A* mutations was associated with shorter DFS (*P* = .0006). In *IDH2R172*-mutated AML, platelet count was the only variable retained in the multivariate model for OS (*P* = .002). Among nonfavorable European LeukemiaNet 2010–eligible patients, 71 (36%) underwent HSCT in first complete remission (CR1) and had longer OS (*P* = .03) and DFS (*P* = .02) than nontransplanted patients. Future clinical trials testing frontline IDH inhibitors combined with IC may consider stratification on *NPM1* mutational status, the primary prognostic factor in *IDH1*- or *IDH2R140*-mutated AML. HSCT improve OS of nonfavorable *IDH1/2*-mutated AML and should be fully integrated into the treatment strategy. (*Blood*. 2021;137(20):2827-2837)

Introduction

Point mutations in isocitrate dehydrogenase (IDH) genes (*IDH1* and *IDH2*) are found in 7% to 14% and in 8% to 19% of adult patients with acute myeloid leukemia (AML), respectively.[1] IDH1/2-mutant enzymes gain neomorphic enzymatic activity, producing an excess of D-2-hydroxyglutarate,[2-4] leading to histone and DNA hypermethylation and cell differentiation blockade.[5-9] Despite a shared oncogenic mechanism, *IDH* mutation subtypes (*IDH1*, *IDH2R140*, and *IDH2R172*) have distinct patterns of co-occurring genetic alterations that may correspond to distinct entities.[10-13] However, risk stratification within each *IDH* mutation subtype remains conflicting in patients with AML treated with intensive chemotherapy (IC).[11,14-22] One large comprehensive series investigated the mutational landscape of patients with AML that have different *IDH* mutation subtypes, but it did not investigate the prognostic impact of co-occurring mutations in each group.[12] Furthermore, some studies addressed the role of allogeneic hematopoietic stem cell transplantation (HSCT) in limited cohorts of *IDH*-mutated AML, but none analyzed its impact in specific *IDH* subgroups.[23,24] Oral targeted inhibitors of mutant IDH1 and IDH2 enzymes (ivosidenib[25] and enasidenib,[26] respectively) were recently approved by the US Food and Drug Administration for the treatment of relapsed/refractory AML as single agents, but also for the treatment of newly diagnosed AML with a susceptible *IDH1* mutation for ivosidenib, in patients who are at least 75 years old or who have comorbidities that preclude the use of intensive induction chemotherapy.[27-29] In the context of clinical trials evaluating the combination of IDH inhibitors plus IC (#NCT02632708,[30] #NCT03839771), it seems important to identify the main prognostic factors for each *IDH* mutation subtype to guide study design (ie, patients stratification) and data interpretation. Here, we report an *IDH* subgroup analysis of the prognostic impact of clinical and genetic covariates and the outcome after HSCT in a large cohort of 319 newly diagnosed patients with *IDH*-mutated AML who were treated with IC in 3 prospective Acute Leukemia French Association (ALFA) clinical trials.

Patients and methods

Patients

We analyzed 319 newly diagnosed patients with *IDH1/2*-mutated AML treated with IC in 3 ALFA prospective clinical trials (supplemental Figure 1, available on the *Blood* Web site). Patients provided written consent, and the study was conducted according to the Declaration of Helsinki and approved by institutional review boards. Eight patients with dual *IDH1* and *IDH2* mutations were excluded. Fifty-eight older patients (50-70 years) were treated between 2008 and 2010 in ALFA-0701[31] (EudraCT 2007-002933-36), 132 younger patients (18-60 years) were treated between 2009 and 2013 in ALFA-0702[32] (#NCT00932412), and 129 older patients (>60 years) were treated between 2012 and 2016 in ALFA-1200[33] (#NCT01966497) trials. All patients received an induction course, including anthracycline and cytarabine. Patients in ALFA-0701 who were randomized to receive gemtuzumab ozogamicin were excluded

Table 1. Characteristics of patients

	All	IDH1[R132]	IDH2[R140]	IDH2[R172]
Patients	319 (100)	127 (40)	135 (42)	57 (18)
Age, median (IQR), y	61 (52-67)	61 (52-67)	61 (50-67)	62 (56-68)
Males	164 (52)	68 (54)	74 (55)	22 (39)
sAML	16 (5)	10 (8)	4 (3)	2 (4)
WBCs, median (IQR), $\times10^9$/L	3.7 (1.6-23.8)	4.5 (1.6-20.7)	8.8 (2.3-31.6)	1.8 (1.2-2.7)
Cytogenetics				
Normal	198 (67)	72 (63)	98 (77)	28 (52)
Complex	11 (4)	9 (8)	2 (2)	0
Trisomy 8/8q	31 (10)	16 (14)	10 (8)	5 (9)
Monosomy 7	12 (3)	10 (9)	2 (2)	0
Trisomy 11/11q	13 (4)	3 (3)	2 (2)	8 (15)
Not available, n	22	12	7	3
ELN 2010 risk groups				
Favorable	86 (29)	38 (33)	48 (37)	0 (0)
Intermediate-1	112 (38)	34 (29)	50 (39)	28 (52)
Intermediate-2	75 (25)	25 (22)	24 (19)	26 (48)
Adverse	24 (8)	18 (16)	6 (5)	0 (0)
Not available, n	22	12	7	2
Trials				
ALFA-0701	58 (18)	26 (20)	21 (16)	11 (19)
ALFA-0702	132 (41)	54 (43)	57 (42)	21 (37)
ALFA-1200	129 (41)	47 (37)	57 (42)	25 (44)
HSCT in CR1				
Eligible patients*	197 (62)	69 (54)	77 (57)	51 (89)
HSCT performed	71 (36)	23 (33)	28 (36)	20 (39)

Unless otherwise noted, data are n (%).

sAML, secondary AML; WBCs, white blood cells.

*Patients with nonfavorable AML and not receiving gemtuzumab ozogamicin in ALFA-0701.

from survival analyses to obtain a homogenous patient cohort treated with IC. ALFA-0701 and ALFA-0702 trials included a salvage course based on high-dose cytarabine. Patients in ALFA-1200 received a second intermediate-dose course of cytarabine (IDAC), regardless of response. Consolidation included daunorubicin plus cytarabine in ALFA-0701, high-dose cytarabine or clofarabine plus IDAC in ALFA-0702, and IDAC in ALFA-1200. Patients with nonfavorable risk according to the 2010 European LeukemiaNet study (ELN 2010)[34] were eligible for HSCT if they had a sibling donor or a fully 10/10 HLA-matched unrelated donor.

Cytogenetic and molecular analyses

Conventional karyotype and fluorescence in situ hybridization were centrally reviewed. Molecular analyses were performed in the ALFA Central Laboratory (Lille University Hospital; C.P.) by high-throughput sequencing on diagnostic peripheral blood or bone marrow samples, as reported previously for the ALFA-0701,[35] ALFA-0702,[36] and ALFA-1200[33] studies. Analyses focused on the 37 genes overlapping in the 3 studies: *IDH1, IDH2, ASXL1, BCOR, BCORL1, CALR, CBL, CEBPA, CSF3R, DNMT4A, ETV6, EZH2, FLT3, GATA2, JAK2, KIT, KRAS, MPL, NIPBL, NPM1, NRAS, PHF6, PTPN11, RAD21, RIT1, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF3, STAG2, TET3, TP53,* *U2AF1, WT1,* and *ZRSR2* (supplemental Table 1). Screening for *NPM1* mutations and *FLT3* internal tandem duplications (*FLT3-ITD*) was also performed by fragment analysis; screening for mutations in *CEBPA* was performed by Sanger sequencing.

Statistical analyses

Continuous variables are reported as median and interquartile range (IQR), and categorical and ordinal variables are reported as number and proportion. Differences in quantitative variables between groups was assessed using the Kruskal-Wallis test, followed by a pairwise Mann-Whitney *U* test, if significant. Correlation between genotype and frequent covariates (present in >5% of the whole *IDH* cohort) was made using point biserial correlation for continuous variables and evaluated with the Φ coefficient and tested with the Fisher test for dichotomic variables. *P* values were corrected for multiple testing using the Benjamini-Hochberg procedure[37] (*q* values). Standard National Cancer Institute criteria were used to define CR and CRp[38] after the first course and salvage therapy in ALFA-0701 and ALFA-0702 and after the first and second courses in ALFA-1200. Patients alive after induction or induction and salvage, but not reaching CR/CRp criteria, were considered to have refractory disease. Relapse was defined as reappearance of circulating
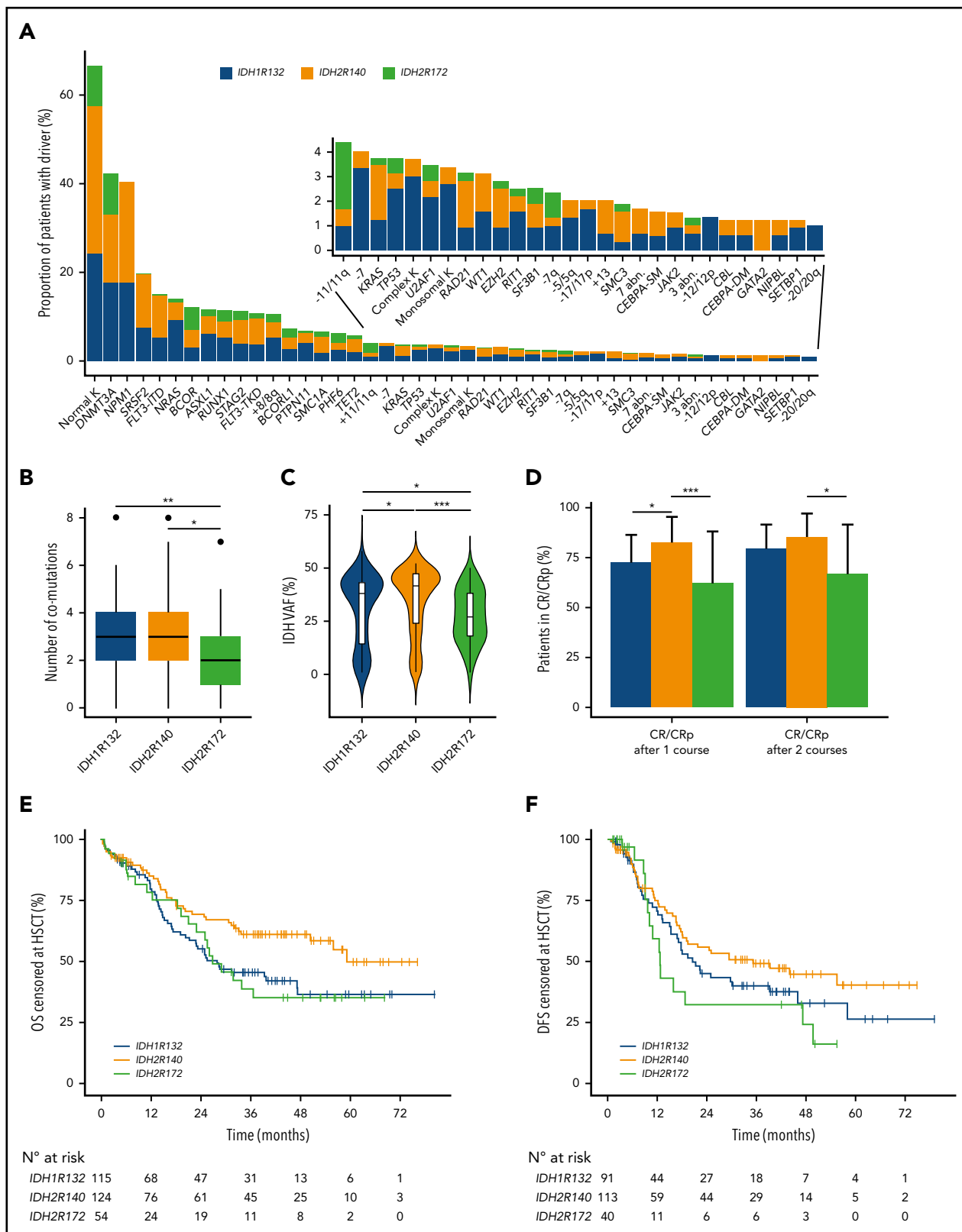
Figure 1. Characteristics of the *IDH1/2*-mutated AML cohort. (A) Molecular and cytogenetic characteristics of the *IDH*-mutated AML cohort according to *IDH* subgroup. (B) Box plots showing the number of co-occurring mutations in each *IDH* subgroup. (C) Violin plots showing the variant allele frequency of the *IDH* variant in each *IDH* subgroup. (D) Bar graph showing the CR/CRp rates after 1 and 2 courses of IC in each *IDH* subgroup. The error bars represent the upper limit of the 95% confidence interval. OS (E) and DFS (F) censored at HSCT according to *IDH* subgroup. *q < 0.05; **q < 0.01; ***q < 0.001.
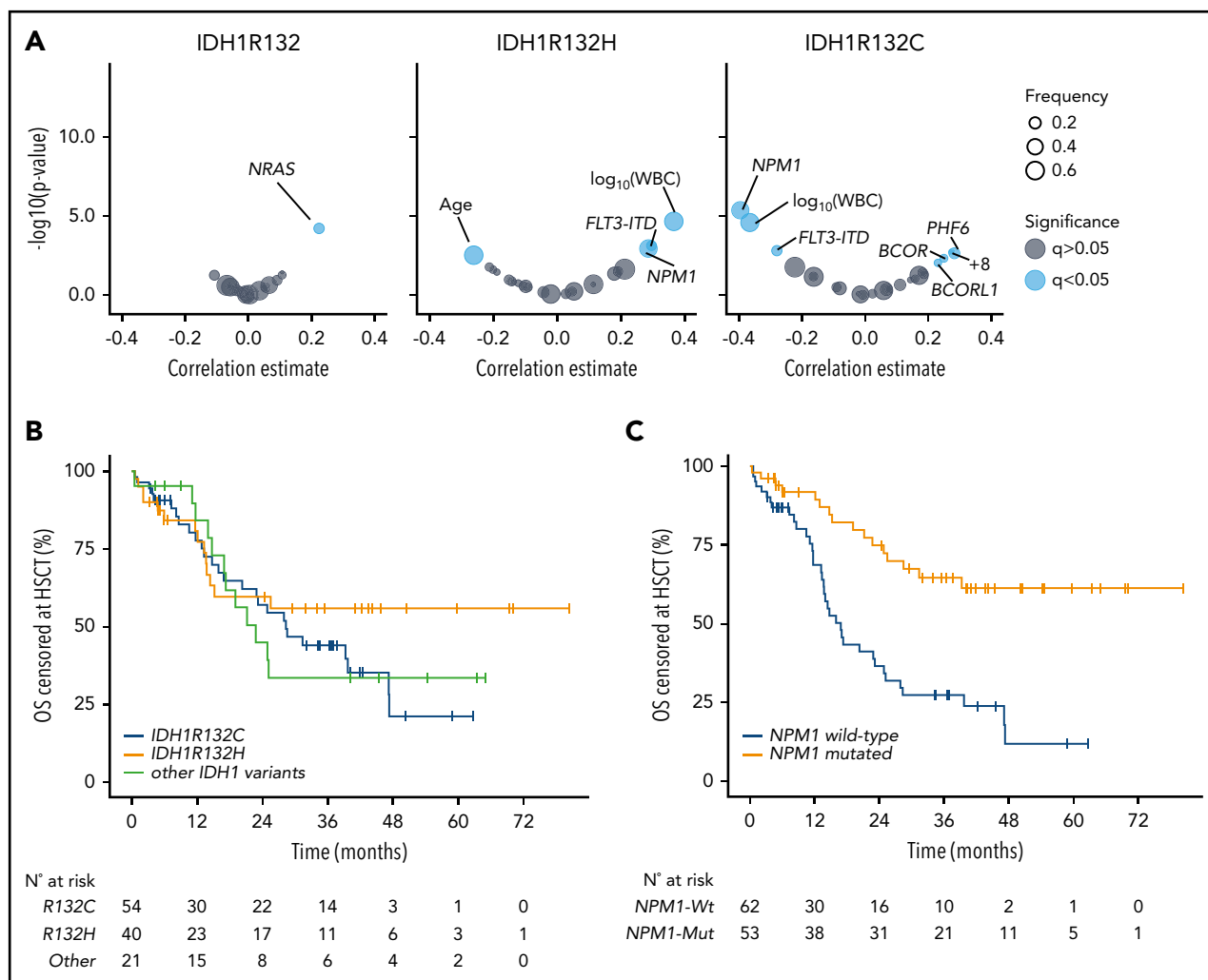
Figure 2. Characteristics of patients with _IDH1_-mutated AML. (A) Volcano plot representing the association between _IDH1_, _IDH1R132C_, and _IDH1R132H_ variants and covariates (estimate of the point-biserial correlation [continuous variables] or Φ [dichotomous variables] on the x-axis) and the significance of the difference. The _P_ value was calculated using the Mann-Whitney _U_ test (for continuous variables) or Fisher's exact (dichotomous) test, expressed on an inverted logarithmic scale on the y-axis. The size of the circle corresponds to the frequency of the variable in the cohort. Only covariates with _q_ < 0.05 are highlighted. (B) OS censored at HSCT according to the type of _IDH1_ variant. (C) OS censored at HSCT according to the mutational status of _NPM1_.

leukemic blasts, recurrence of >5% marrow blasts, and/or appearance of extramedullary leukemia. Bivariate analyses for response were done by logistic regression stratified on the trial. Follow-up duration was calculated with the inverse method. Overall survival (OS) analyses were considered from the date of diagnosis to the date of death or last follow up. Analyses of disease-free survival (DFS) were restricted to patients achieving complete remission (CR)/CR with incomplete platelet recovery (CRp) after 2 courses and were considered from the date of response to the date of death, relapse, or last follow-up. OS and DFS were obtained according to the Kaplan-Meier method and censored at HSCT. For survival analysis, frequent variables (present in >10% of each _IDH_ subgroup) were selected using a LASSO penalized regression with the R package glmnet, using the regularization parameter λ-min determined on 100 cross-validations. Selected variables were included in multivariate Cox models, followed by backward regression. The proportional-hazards assumption was tested on the basis of Schoenfeld residuals. To determine the role of HSCT in first CR, HSCT was considered a time-dependent covariate; survival curves for OS and

DFS were obtained using the Simon-Makuch method and compared using a time-dependent bivariate Cox model. All tests were 2 sided, and statistical significance was defined as a _P_ value or _q_ value <.05; all analyses for response and survival were stratified on the clinical trial. All analyses were performed with R version 3.5.2.

Results

Characteristics of patients

A total of 319 patients with _IDH1/2_-mutated AML from ALFA-0701 (n = 58), ALFA-0702 (n = 132), and ALFA-1200 (n = 129) prospective clinical trials was analyzed in the study, including 127 with _IDH1_-mutated AML (40%), 135 with _IDH2R140_-mutated AML (42%), and 57 with _IDH2R172_-mutated AML (18%) (supplemental Figure 1). Characteristics of patients at diagnosis are listed in Table 1. Overall, 164 were men (52%) with a median age of 61 years (IQR, 52-67). Most patients had a normal karyotype (67%); ELN 2010 risk was favorable, intermediate-1, intermediate-2, and high in 29%, 38%, 25%, and 8% of evaluable patients,

Table 2. Multivariate analyses for survival

	OS censored at HSCT, stratified on the trial			DFS censored at HSCT, stratified on the trial		
	IDH1	*IDH2R140*	*IDH2R172*	*IDH1*	*IDH2R140*	*IDH2R172*
NPM1, mutated	0.29 (0.16-0.54) *P* < .0001	0.41 (0.21-0.81) *P* = .01	—	—	0.33 (0.17-0.63) *P* = .0009	—
DNMT3A, mutated	—	—	—	—	3.05 (1.61-5.78) *P* = .0006	—
Karyotype, normal	—	0.38 (0.19-0.78) *P* = .008	—	—	—	—
Log$_{10}$(platelets)	—	—	0.19 (0.07-0.53) *P* = .002	—	—	—

Data are shown as HR (95% CI).

—, variable not retained in the multivariate analysis.

respectively. Among patients with *IDH1*, 60 had a p.R132C mutation (47%), 44 had a p.R132H mutation (35%), 11 had a p.R132G mutation (9%), 10 had a p.R132S mutation (8%), and 2 had a p.R132L mutation (1%). Among patients with *IDH2R140* mutations, 127 had a p.R140Q mutation (94%), 7 had a p.R140W mutation (5%), and 1 had a p.R140L mutation (1%). The *IDH2R172* variants were p.R172K (n = 56) and p.R172S (n = 1). The majority of patients with *IDH* mutations (>95%) had ≥1 comutation, primarily in *DNMT3A* (42%), *NPM1* (40%), *SRSF2* (20%), *FLT3-ITD* (15%), or *NRAS* (14%) (Figure 1A-B; supplemental Figure 2). Patiens with *IDH2R172* mutations had significantly fewer comutations than did other patients: a median of 2 (IQR, 1-3) for *IDH2R172* vs a median 3 (IQR, 2-4) for *IDH1* and a median of 3 (IQR, 2-4) for *IDH2R140*; q = 0.004 and q = 0.01, respectively (Figure 1B). Patients with *IDH2R172* mutations also had a significantly smaller allele burden (median variant allele frequency of 27% [IQR, 18-38] vs 38% [IQR, 15-43] for *IDH1* and 42% [IQR, 24-47] for *IDH2R140*, q = 0.04 and q < 0.001, respectively; Figure 1C).

After exclusion of the 26 patients who received gemtuzumab ozogamicin, 224 patients (76%) and 244 patients (83%) achieved a CR/CRp after 1 and 2 courses of IC, respectively. Upon bivariate analyses stratified on the trial, *IDH2R140* mutations predicted higher response rates after 1 course of IC (86% vs 74% for *IDH1* mutations and 59% for *IDH2R172* mutations, q = 0.03 and q < 0.001, respectively) and after 2 courses of IC (91% vs 79% for *IDH1* mutations and 74% for *IDH2R172* mutations, q = 0.02 and q = 0.02, respectively; Figure 1D). Of note, concomitant *NPM1* mutations were associated with significantly higher CR/CRp rates for *IDH1* mutations (94% [50/53] vs 66% [41/62] for *NPM1* wild-type; *P* = .0002) and for *IDH2R140* mutations (100% [62/62] vs 82% [51/62] for *NPM1* wild-type; *P* = .0003).

Median follow-up was 46.0 months (IQR, 38.0-58.4). Median OS censored at HSCT was 39.7 months (IQR, 14.7-not reached); in bivariate analyses stratified on the clinical trial, patients with *IDH2R140* mutations had better OS censored at HSCT than did patients with *IDH1* mutations (3-year OS, 61% vs 46%; hazard ratio [HR], 0.60; 95% confidence interval [CI], 0.40-0.93; *P* = .02), but it was not better than for patients with *IDH2R172* mutations

(3-year OS, 61% vs 39%; HR, 0.65; 95% CI, 0.38-1.09; *P* = .10; Figure 1E). Median DFS censored at HSCT was 22.3 months (IQR, 10.8-not reached), and there was no significant difference according to *IDH* subtype (Figure 1F). Of note, the ELN 2010 and the most recent ELN 2017 risk stratification poorly discriminated intermediate-risk and adverse-risk patients with *IDH*-mutated AML (supplemental Figure 3).

IDH1R132-mutated cohort

Only frequent covariates (>5% of the entire *IDH* cohort; supplemental Table 2) were included in the correlation analyses. The only covariate associated with *IDH1* mutations was *NRAS* mutation (24% vs 8% in *IDH1* wild-type; q = 0.003). We investigated the associations of the 2 most frequent *IDH1* variants (ie, p.R132H and p.R132C) within the *IDH1*-mutated cohort. p.R132H variants were present in younger patients (median age, 56 years vs 63 years for other *IDH1* variants; q = 0.02) and were associated with higher rates of *NPM1* mutations (64% vs 34%; q = 0.01) and *FLT3-ITD* (27% vs 6%; q = 0.01), as well as higher white blood cell (WBC) counts (17.9 × 10^9/L vs 2.4 × 10^9/L; q = 0.0006). p.R132C variants were significantly associated with higher rates of trisomy 8 (23% vs 5%; q = 0.01), as well as mutations in *PHF6* (13% vs 0%; q = 0.01), *BCOR* (15% vs 1%; q = 0.03), and *BCORL1* (13% vs 1%, q = 0.048; Figure 2A). Despite these 2 distinct mutational patterns, there was no difference in survival between patients with p.R132C or p.R132H variants (Figure 2B).

To identify the prognostic impact of covariates in *IDH1*-mutated AML, we included all covariates present in ≥10% of *IDH1* patients (list in supplemental Table 3) in a LASSO penalized regression for OS and DFS censored at HSCT. The final multivariate Cox models stratified on the trial are summarized in Table 2. *NPM1* mutational status was the only variable predicting prolonged OS (3-year OS of 65% in mutated *NPM1* vs 28% in wild-type *NPM1*; HR, 0.29; 95% CI, 0.16-0.54; *P* < .0001; Figure 2C). No variable was retained in the multivariate analysis for DFS.

IDH2R140-mutated cohort

Compared with other *IDH* mutations, *IDH2R140* variants were associated with higher rates of *NPM1* mutations (53% vs 30%; q = 0.0005), *FLT3-ITD* (22% vs 10%; q = 0.01), *SRSF2* mutations

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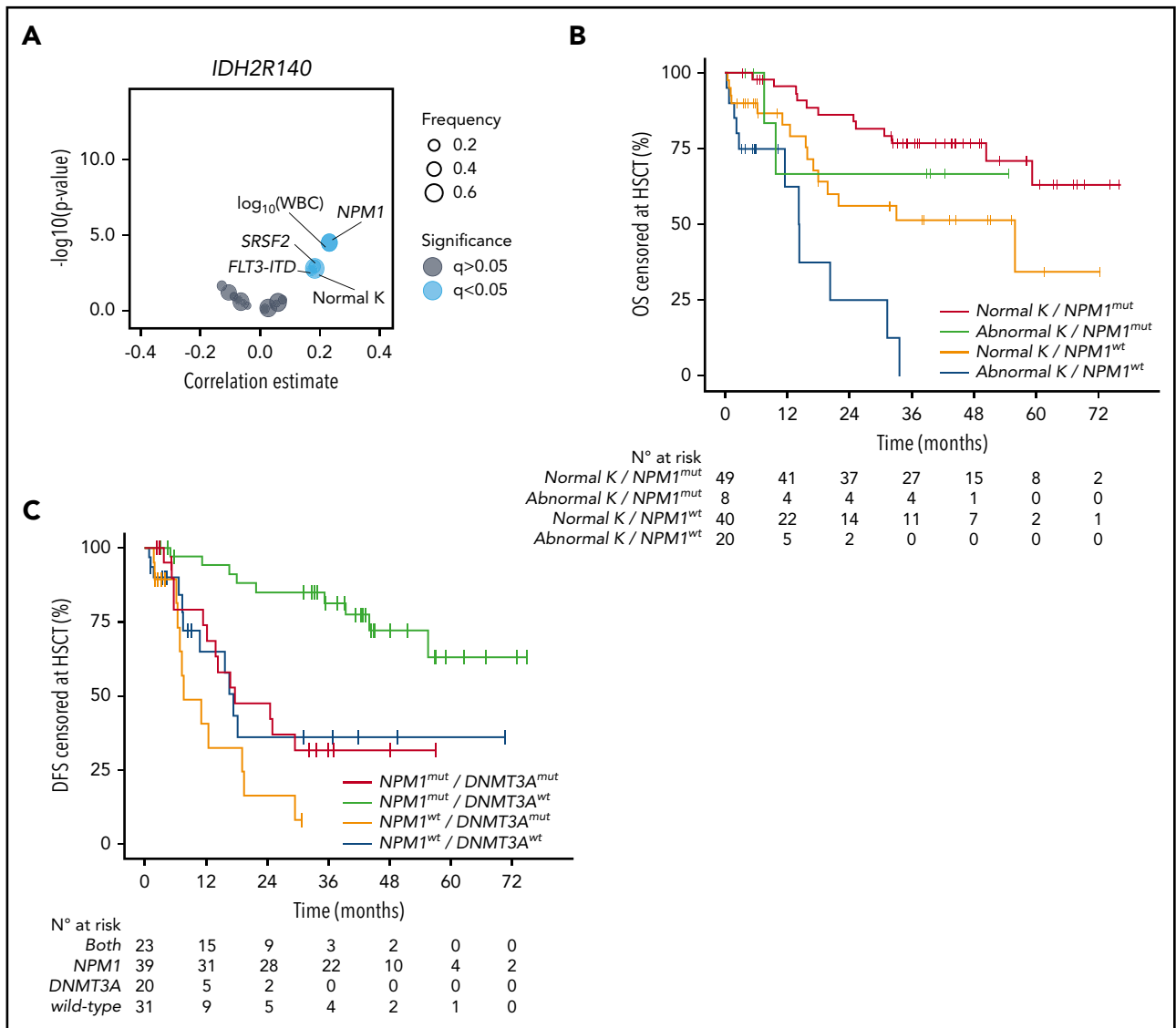
Figure 3. Characteristics of patients with *IDH2R140*-mutated AML. (A) Volcano plot representing the association between *IDH2R140* variants and covariates (estimate of the point-biserial correlation [continuous variables] or Φ [dichotomous variables] on the x-axis) and the significance of the difference. The *P* value was calculated using a Mann-Whitney (continuous variables) *U* test or Fisher's exact (dichotomous) test, expressed on an inverted logarithmic scale on the y-axis. The size of the circle corresponds to the frequency of the variable in the cohort. Only covariates with *q* < 0.05 are highlighted. (B) OS censored at HSCT in *IDH2R140*-mutated patients according to the karyotype and *NPM1* mutational status. (C) DFS censored at HSCT according to the mutational statuses of *NPM1* and *DNMT3A*.

(28% vs 14%; *q* = 0.01), normal karyotypes (77% vs 59%; *q* = 0.01), and higher WBC counts (median, 8.80×10^9/L vs 2.60×10^9/L; *q* = 0.0005; Figure 3A).

Results of the multivariate analyses for OS and DFS censored at HSCT are summarized in Table 2. Normal karyotype (3-year OS of 67% vs 28%; HR, 0.38; 95% CI, 0.19-0.78; *P* = .008) and *NPM1* mutations (3-year OS of 77% vs 40%; HR, 0.41; 95% CI, 0.21-0.81; *P* = .01) predicted prolonged OS (Figure 3B). *NPM1* mutations predicted prolonged DFS (3-year DFS of 63% vs 23%; HR, 0.33; 95% CI, 0.17-0.63; *P* = .0009), whereas *DNMT3A* mutations were detrimental (3-year DFS of 22% vs 67%; HR, 3.05; 95% CI, 1.61-5.78; *P* = .0006; Figure 3C).

IDH2R172-mutated cohort

IDH2R172 variants were associated with higher rates of *BCOR* mutations (30% vs 8%; *q* < 0.001), no *NPM1* mutations (0% vs

49%; *q* < 0.001), fewer *SRSF2* mutations (2% vs 24%; *q* < 0.001) and *FLT3*-ITD (2% vs 18%; *q* < 0.001), and lower WBC count (median, 1.80×10^9/L vs 6.25×10^9/L; *q* < 0.001; Figure 4A).

Results of the multivariate analyses for OS and DFS censored at HSCT are summarized in Table 2. Log-transformed platelet count was the only variable retained in the multivariate model for OS (HR, 0.19; 95% CI, 0.07-0.53; *P* = .002). Using an arbitrary cutoff for platelets of 100×10^9/L, 3-year OS was 17% for patients with thrombocytopenia vs 52% for those without (Figure 4B). Patients with *IDH2R172* mutations that have low platelet counts ($<100 \times 10^9$/L) suffered more early deaths (3/20 vs 0/34 with high platelet counts) and had significantly higher rates of induction failure after 1 course of IC (CR/CRp rates of 25% vs 79%; *P* = .001) or 2 courses of IC (CR/CRp rates of 45% vs 91%; *P* = .001). No variable was retained as an independent prognostic factor for DFS. Of note, the 16 patients with *IDH2R172*
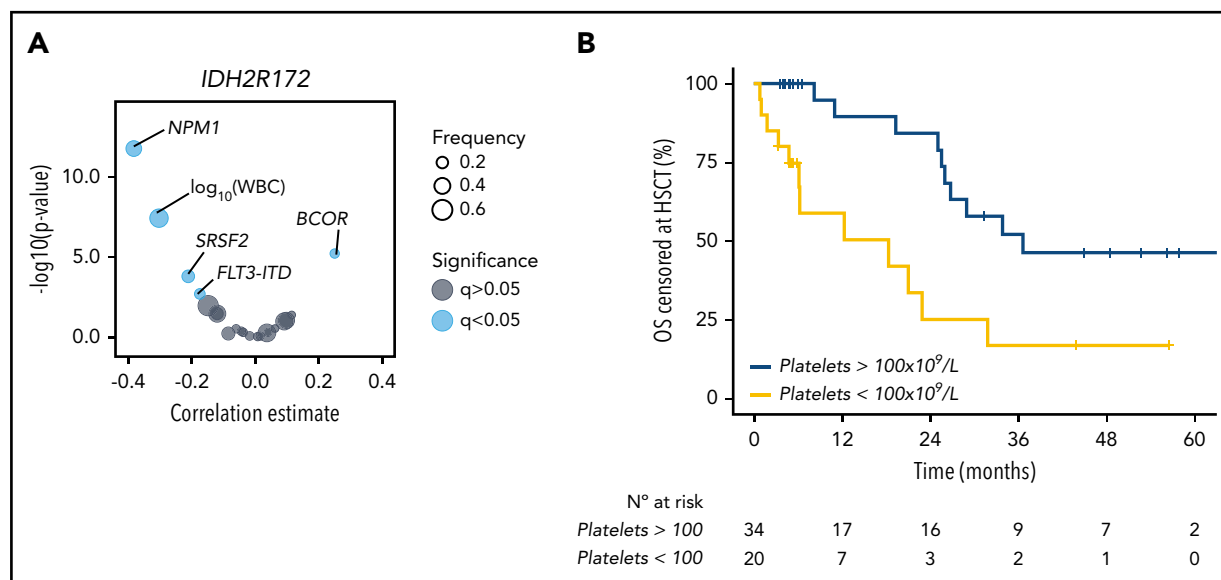
Figure 4. Characteristics of the patients with *IDH2R172*-mutated AML. (A) Volcano plot representing the association between *IDH2R172* variants and covariates (estimate of the point-biserial correlation [continuous variables] or Φ [dichotomous variables] on the x-axis) and the significance of the difference, expressed on an inverted logarithmic scale on the y-axis. The *P* value was calculated using the Mann-Whitney *U* test (continuous variables) or Fisher's exact (dichotomous) test. The size of the circle corresponds to the frequency of the variable in the cohort. Only covariates with *q* < 0.05 are highlighted. (B) OS censored at HSCT in patients with *IDH2R172* mutations, according to the platelet counts at diagnosis (arbitrary cutoff at 100 × 10⁹/L).

mutations without other classifying abnormalities, as defined by Papaemmanuil et al,[11] did not have distinct comutations or prolonged outcomes (supplemental Figure 4).

Impact of HSCT in patients with *IDH* mutations

Finally, we investigated the role of HSCT in first complete remission (CR1) for the 197 eligible patients (ie, patients with nonfavorable AML according to ELN 2010 risk and who did not receive gemtuzumab ozogamicin). Overall, 71 (36%) eventually received an HSCT after a median time from diagnosis of 5.3 months (IQR, 4.5-6.1), including 23 (33%), 28 (36%), and 20 (39%) patients in the *IDH1R132*-mutated, *IDH2R140*-mutated,

and *IDH2R172*-mutated groups, respectively (Table 1). The main characteristics were similar between the transplant and notransplant cohorts; however, most transplanted patients came from the ALFA-0702 trial, resulting in a younger age in this group (supplemental Table 4). When we considered HSCT as a time-dependent covariate in bivariate analyses stratified on the clinical trial, transplantation in CR1 was associated with a prolonged OS (HR, 0.60; 95% CI, 0.37-0.96; *P* = .03; Figure 5A) and a prolonged DFS (HR, 0.55; 95% CI, 0.34-0.89; *P* = .02; Figure 5B). Analyses in *IDH* subtypes revealed a benefit in OS only for patients with *IDH1R132* mutations (HR, 0.48; 95% CI, 0.23-0.99; *P* = .048) and a trend toward better DFS for patients
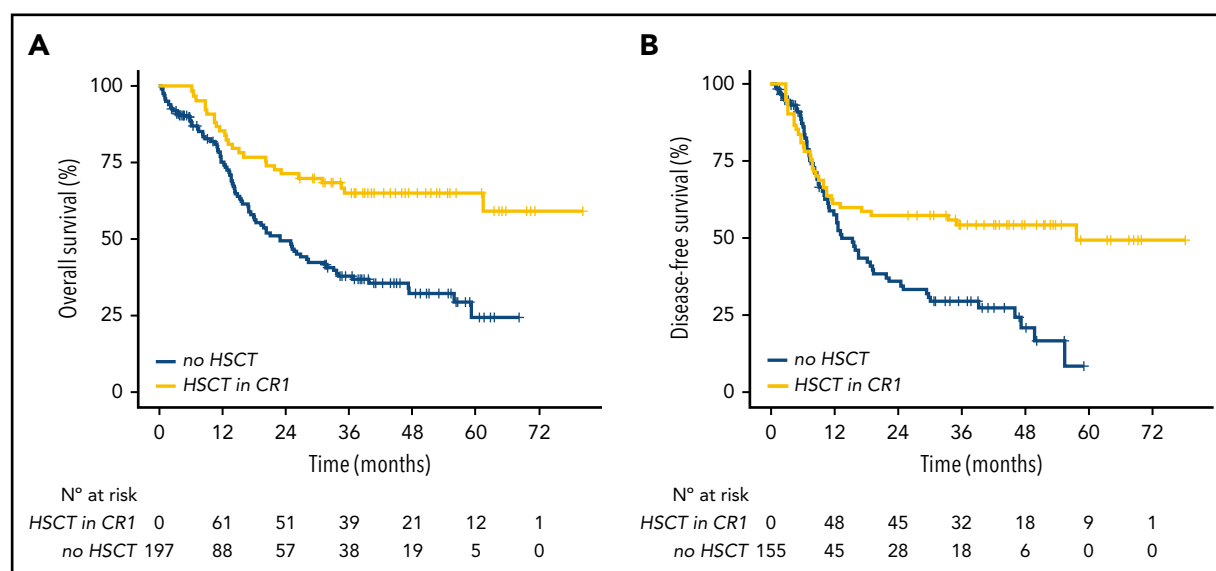
Figure 5. Impact of HSCT in first CR/CRp in patients with *IDH*-mutated AML. Simon-Makuch plot of OS (A) and DFS (B) according to achievement of HSCT. HSCT was considered a time-dependent variable.

with *IDH2R172* mutations (HR, 0.41; 95% CI, 0.14-1.20; *P* = .10; supplemental Figure 5).

Discussion

In recent years, there has been renewed interest in *IDH1/2* mutations in AML with the advent of specific inhibitors. However, the prognostic impact of *IDH1/2* mutations and their comutations remained unclear because, in most studies, *IDH1, IDH2R140*, and *IDH2R172* mutations were analyzed together[12,20,21] and in cohorts treated with different-intensity regimens.[21,39] The present study represents one of the largest series of patients with *IDH1/2*-mutated AML prospectively enrolled in 3 clinical trials with sequencing data on 37 genes; we assessed the prognostic impact of clinical and genetic covariates, as well as the impact of HSCT in CR1, for each *IDH* mutation subtype.

Our cohort included 127 patients with *IDH1* mutations (40%), 135 patients with *IDH2R140* mutations (42%), and 57 (18%) patients with *IDH2R172* mutations; these proportions are in line with previous reports.[12,17,40] Clinical and genetic covariates differed between *IDH* mutation subtypes. Among patients with *IDH1* mutations, we could emphasize that p.R132H variants were more frequently associated with *NPM1* and *FLT3-ITD* mutations than were other *IDH1* subtypes, as reported previously.[13] In line with previous reports,[11,12] patients with *IDH2R140* mutations had significantly higher WBC counts, as well as more frequent normal karyotypes and *NPM1, FLT3-ITD*, and *SRSF2* mutations, whereas patients with *IDH2R172* mutations had lower WBC counts, more frequent *BCOR* mutations, and no *NPM1* mutations. These differences in clinical and genetic covariates suggest that *IDH* mutation subtypes are distinct entities that should be considered separately; which is supported by biological differences.[41,42]

After intensive treatment, CR/CRp rates were significantly higher in patients with *IDH2R140* mutations (86%) than in patients with *IDH1* (74%) and *IDH2R172* (59%) mutations, as reported previously.[40] *NPM1* mutations were associated with remarkably high CR/CRp rates in *IDH1R132*-mutated (94%) and *IDH2R140*-mutated (100%) patients. Considering long-term outcome, patients with *IDH2R140* mutations had a significantly prolonged OS compared with patients who had *IDH1* mutations (*P* = .02), as well as a trend toward prolonged OS compared with patients who had *IDH2R172* mutations (*P* = .1). Higher rates of *NPM1* mutations and normal karyotypes may account for the better outcome in patients with *IDH2R140* mutations. In keeping with studies on patients accrued to the ALFA,[16] MRC,[40] and CALGB[10] clinical trials, patients with *IDH2R172* mutations did not have a prolonged survival compared with other patients who had IDH mutations in our cohort, even in the 16 patients with *IDH2R172* mutations without other classifying abnormalities, as defined by Papaemmanuil et al[11] (supplemental Figure 4). These results conflict with 2 other studies,[11,12] perhaps owing to the greater treatment heterogeneity in them.

Most importantly, our study identified the main prognostic factors for long-term outcome in each *IDH* mutation subtype. *NPM1* was the only mutation predicting OS in multivariate analysis for patients with *IDH1* and *IDH2R140* mutations. Surprisingly, other stratifying mutations, such as *FLT3* mutations[11,43,44] (supplemental Figure 6), did not have any significant prognostic impact in our cohort; however, we acknowledge that the number

of patients with a high allelic ratio of *FLT3-ITD* was low (n = 11). We report for the first time the negative impact of cytogenetic abnormalities in *IDH2R140* on OS, such as in *NPM1*-mutated AML.[45] We also found that platelet count was the only variable retained in the multivariate model for OS in *IDH2R172*. Patients with *IDH2R172* mutations who had low platelet counts (<100 × 10⁹/L) had a more advanced and resistant disease with higher rates of induction failure. Interestingly, no variable was retained as an independent prognostic factor for DFS in patients with *IDH1* or *IDH2R172* mutations. In patients with *IDH2R140* mutations, *NPM1* mutations were associated with prolonged DFS only in patients with wild-type *DNMT3A*. These results are in line with previous results in *NPM1*-mutated AML.[46] In patients with both *IDH* and *DNMT3A* mutations (n = 135), cancer cell fractions of *IDH* and *DNMT3A* mutations were similar across all 3 *IDH* subgroups (supplemental Table 5). However, in patients with *IDH1* and *IDH2R140* mutations, *NPM1* mutations had a lower allelic burden (*P* < .001), suggesting that they are later events. The poorer prognosis related to the association between *IDH2R140* and *DNMT3A* mutations has already been reported.[11] Both mutations are early events and are associated with clonal dominance in single-cell genotyping studies,[47] a feature that is associated with a poorer prognosis in AML.[48] Mouse models suggest an epigenetic cooperation between the 2 alterations, leading to activation of a stem cell–like gene signature.[49] Following previous reports in refractory/relapsing *IDH*-mutated AML,[50,51] it will be interesting to further study the genetic landscape and molecular predictors in patients with relapsed *IDH*-mutated AML, to compare trials conducted in this population.

In the 3 prospective trials, HSCT in CR1 was only recommended for patients with intermediate- or adverse-risk AML, according to the ELN 2010 classification, when a fully 10/10 HLA-matched donor could be identified. ELN 2010 is a valid stratification for allogeneic HSCT, and the more recent ELN 2017 reclassifies <5% of patients considered as favorable.[52] Seventy-one (36%) of the eligible patients aged 70 years or younger underwent transplantation in CR1, and we were able to compare transplant and no-transplant cohorts with similar primary characteristics. As expected, HSCT in CR1 was associated with improved OS and DFS. However, analyses in *IDH*-mutated subtypes were impaired by limited power and only revealed a benefit in OS for patients with *IDH1R132* mutations.

Combining IDH inhibitors with intensive induction and consolidation chemotherapy could be synergistic because primary resistance to IDH inhibitors is related to the number of co-occurring mutations,[28,50] as well as to the expansion of a clone harboring mutations in receptor tyrosine kinase pathway genes (ie, *NRAS, KRAS, PTPN11, KIT*, and *FLT3*), which are associated with significantly lower CR with partial hematologic recovery rates.[28,50] In the present cohorts, mutations in signaling pathway genes, including *FLT3* mutations, did not have any impact on the outcome after IC (supplemental Figure 6). Thus, the combination of IC plus IDH inhibitors might prevent primary resistance and relapses conveyed by signaling mutations. This is supported by a recent report of a phase 1 study evaluating the association between IDH inhibitors and IC in newly diagnosed AML,[30] in which *FLT3* and *RAS* mutations were cleared after induction chemotherapy. It has yet to be proven whether combination therapies might also prevent secondary resistance to IDH

inhibitors related to second site mutations hampering the binding of the drugs or a mutational switch.[50,53,54]

Thus, future clinical trials testing frontline IDH inhibitors with IC should consider stratification on *NPM1* mutational status. HSCT should be fully integrated into the treatment strategy, with important implications for trial design (guidelines for HSCT indications, censoring at HSCT in survival outcomes analyses, post-HSCT maintenance). Future studies should also evaluate alternative strategies (ie, HSCT indications based on minimum residual disease levels, maintenance therapy with IDH inhibitors).

Authorship

Contribution: M.D. and S.d.B. designed the study, analyzed the data, and wrote the manuscript; R.I., H.D., and S.d.B. supervised the study; M.D. performed statistical analyses; N.D. and C. Preudhomme performed genotyping; C.T. performed cytogenetic analyses; J.-B.M., E.R., X.T., J.-P.M., T.B., L.A., S.C., E.L., C.B., J.-V.M., C. Pautas, J.L., N.B., K.C.-L., D.C., P.T., N.V., A.P., C.R., C.G., R.I., H.D., and S.d.B. provided clinical data; and all authors edited the manuscript and approved its final version.

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