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FLT3-ITD and *CEBPA* Mutations Predict Prognosis in Acute Myelogenous Leukemia Irrespective of Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Cytogenetic and genetic changes have prognostic significance in acute myelogenous leukemia (AML). In our study, we compared the cytogenetic changes and gene mutations (*NPM1*, *CEBPA*, *DNMT3A*, *FLT3*-ITD, *FLT3*-TKD, and *C-KIT*) with clinical outcomes in 1132 patients with AML enrolled at our center over a 10-year period. A total of 977 patients provided gene mutation data. There were subsets of patients who exhibited mutations in *NPM1* (17.9%), *CEBPA* (16.4%), *FLT3*-ITD (18.5%), *FLT3*-TKD (3.9%), *DNMT3A* (8.6%), and *C-KIT* (8.8%). A total of 557 patients (49.2%) underwent hematopoietic stem cell transplantation (HSCT) as consolidation therapy. Multivariate analysis identified an adverse karyotype (hazard ratio [HR], 1.48; $P = .001$), the presence of *FLT3*-ITD (HR, 1.90; $P < .001$), and receipt of nonstandard first-line induction chemotherapy (HR, 1.45; $P = .003$) as significant risk factors for poor overall survival (OS), and the presence of $CEBPA^{mut}$ (HR, .42; $P < .001$) and receipt of HSCT (HR, .35; $P < .001$) as prognostic factors for favorable OS. In addition, the presence of $FLT3\text{-ITD}^{mut}$ (HR, 2.11; $P < .001$) was identified as an independent risk factor for poor disease-free survival (DFS), and receipt of HSCT was correlated with improved DFS (HR, .74; $P = .046$). Compared with chemotherapy as consolidation therapy, HSCT improved the prognosis and overcame the prognostic effect of karyotype from the initial diagnosis; however, the presence of *FLT3*-ITD or *CEBPA* mutation can predict prognosis in AML irrespective of HSCT.

INTRODUCTION

Acute myelogenous leukemia (AML) is a heterogeneous disease with a variable prognosis. Chromosomal aberrations are found in approximately 55% of adult patients with AML and have high prognostic significance in terms of response to therapy, risk of relapse, and overall survival (OS) [1-4]. Three cytogenetic risk groups are currently recognized: favorable, intermediate, and adverse. The adverse cytogenetic risk group is largely homogenous and generally includes abnormalities of 3q, such as inv(3q), t(3;3), 5q/-5, 7q/-7, abn(17p), t(6;9), and 11q23 [except t(9;11)], as well as complex karyotypes [3].

A practical limitation of this cytogenetic risk group classification scheme is that it assigns the majority of patients to the intermediate-risk group. In an analysis of 1612 patients with cytogenetic data in the Medical Research Council's AML 10 trial, >66% met the definition for this category [5]. Additional prognostic factors and more precise prognostic stratification would be particularly useful for the patients in this group.

Recently, numerous genetic mutations associated with AML have been identified and used in attempts to stratify AML prognoses. Among these mutations, internal tandem duplications (ITDs) in the fms-related tyrosine kinase 3 gene (*FLT3*-ITD), as well as mutations in Nucleophosmin 1 (*NPM1*) and CCAAT/enhancer binding protein A (*CEBPA*), *ASXL1*, *RUNX1*, and *TP53*, have been shown to have prognostic significance [6-10]. These prognostic factors have been incorporated into the guidelines of the European LeukemiaNet [11]. Following the development of whole-genome sequencing technology, an array of other potentially predictive gene mutations have been identified. These include mutations in the DNA methyltransferase 3A (*DNMT3A*), *C-KIT*, and *TET2* [12-15], all of which are associated with prognosis in patients with AML.

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Hematopoietic stem cell transplantation (HSCT) is considered the standard consolidation therapy in patients with high-risk AML. Cytogenetic and molecular markers have been adopted to enable prognostic stratification of AML and guide selection of further treatment, either consolidative conventional chemotherapy or HSCT [3]. Therefore, in the present study, we investigated the relevance of cytogenetic changes and gene mutations (*NPM1*, *CEBPA*, *DNMT3A*, *FLT3*-ITD, *FLT3*-TKD, and *C-KIT*) in AML on OS and disease-free survival (DFS). We also evaluated the impact of HSCT on outcomes in patients classified by cytogenetic risk and gene mutations.

METHODS

Patient Characteristics

A total of 1132 patients with newly diagnosed AML were included in our analysis. Patients were enrolled between April 2006 and July 2016 at the First Affiliated Hospital of Soochow University. Informed consent was obtained from all patients before data collection. This study was approved by the Committee for the Ethical Review of Research at First Affiliated Hospital of Soochow University and was conducted in accordance with institutional guidelines and the Declaration of Helsinki.

Treatments

Induction therapies included standard first-line treatment and nonstandard first-line treatment, as determined by each patient's age and organ function. The majority of our patients (n = 770; 68%) received standard first-line treatment with an IA/DA regimen, composed of either idarubicin 8 to 12 mg/m^2 or daunorubicin 60 to 90 mg/m^2 on days 1 to 3 and 100 mg/m^2 cytarabine on days 1 to 7. Older patients and patients with organ dysfunction were treated with combination chemotherapies, including the CAG regimen (containing granulocyte colony-stimulating factor priming the combination of low-dose cytarabine and aclarubicin), the IAG regimen (containing granulocyte colony-stimulating factor priming the combination of low-dose cytarabine and idarubicin), or hypomethylating agents with or without CAG or IAG. After achieving complete remission (CR) following induction chemotherapy, patients subsequently received ≥4 cycles of intermediate/high-dose cytarabine-based combination chemotherapy (1 to 2 g/m^2 on days 1 to 3) or underwent HSCT. Generally, all high-risk patients with cytogenetic and molecular findings were referred for HSCT consultation. The decision to proceed with HSCT was also based on donor availability, patient preference, and treating physician preference.

HSCT Conditioning Regimens

Pretransplantation myeloablative regimens included busulfan (3.2 mg/kg/day on days −7 to −5) and cyclophosphamide (1.8 g/m^2/day on days −4 to −3). Patients at high risk for central nervous system leukemia received total body irradiation (8 Gy, with lung shielding at 6.5 Gy, on days −8 to −6), cytarabine (2 g/m^2/day on day −5), and cyclophosphamide (1.8 g/m^2/day on days −4 to −3). Rabbit antithymocyte globulin (ATG/Thymoglobulin; Imtix Sangstat, Lyon, France) was administered to patients receiving an HLA-matched unrelated donor transplant (10 mg/kg total dose on days −5 to −2) or a haploidentical donor transplant (10 mg/kg total dose on days −6 to −3). Patients of advanced age or with a concurrent medical condition received reduced-intensity conditioning, composed of fludarabine (30 mg/m^2/day on days −10 to −6), cytarabine (1.5 g/m^2/day on days −10 to −6), and busulfan (3.2 mg/kg/day on days −5 to −3). In addition, ATG was administered to patients receiving an HLA-matched unrelated donor transplant (6 mg/kg total dose on days −4 to −1).

Cytogenetic Risk Classification and Mutation Analyses

All cytogenetic samples were taken from bone marrow (BM) cells. Cytogenetic analyses were performed using standard techniques for chromosome banding after 24 hours of unstimulated culture. At least 20 metaphases were analyzed whenever possible. Favorable risk was defined by the presence of t(8, 21) or inv(16)/t(16;16). Karyotypes with neither favorable nor adverse risk markers were classified as intermediate risk. Adverse risk was defined by the presence of -5/5q, -7/7q, 11q23 [except when combined with t(9; 11)], inv(3)/t(3; 3), t(6; 9), or a complex karyotype [3].

For gene mutation analysis, genomic DNA was extracted from BM-derived mononuclear cells using the Purelink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. A variety of AML-related gene mutations were evaluated, including *NPM1*, *CEBPA*, *FLT3*-ITD, *FLT3*-TKD, *DNMT3A*, and *C-KIT*. The gene mutations were detected by PCR amplification of the entire or a portion of the coding region, followed by direct bidirectional DNA sequencing.

Definitions, Study Endpoints, and Statistical Analysis

Definitions of treatment responses in patients with AML, including CR, partial remission (PR), and treatment failure, were based on previously reported criteria [16]. Relapse was defined as the reappearance of leukemic blasts in peripheral blood, ≥5% blasts in BM, or the appearance of extramedullary disease after CR. Study endpoints were OS and DFS. OS was defined as the interval from diagnosis to either death (regardless of cause) or last follow-up. DFS was calculated only for patients who achieved CR and was defined as the interval from achievement of CR to the date of relapse or death from any cause.

The distributional properties of continuous variables are expressed as median and range, and categorical variables are expressed as frequency and percentage. Differences between categorical covariates were assessed using the χ^2 and Fisher exact tests. Differences between continuous covariates were assessed using the Mann-Whitney *U* test. OS and DFS were computed using the Kaplan-Meier method, and the log-rank test was used for univariate comparisons. Prognostic factors with a *P* value <.10 on univariate analysis were entered into a Cox proportional hazards model to evaluate the effects of those factors on survival. Differences between groups were considered statistically significant at $P < .05$ in a 2-tailed test. All analyses were performed using SPSS version XX (SPSS, Chicago, IL).

RESULTS

Patient Characteristics

A total of 1132 consecutive patients with AML who were diagnosed at our center between April 2006 and July 2016 were included in this study. The median patient age was 42 years (range, 9 to 81 years), and 634 patients (56.0%) were male. Of the 1132 patients, 977 (86.3%) had complete data on *NPM1*, *CEBPA*, *FLT3*-ITD, *FLT3*-TKD, *DNMT3A*, and *C-KIT* mutations. Karyotype analyses were performed in 1078 patients (95.2%). Standard first-line induction chemotherapy (using IA or DA) was applied in 770 patients (68%). Treatment response was evaluated after 2 cycles of induction chemotherapy; the results are presented in Table 1. After consolidation therapy, 557 patients (49.2%) underwent HSCT. The median duration of follow-up in survivors was 18.6 months (range, .1 to 120.5 months). Up to the last follow-up, 239 patients (23.1%) had experienced disease relapse and 407 patients (36%) had died. Baseline patient characteristics are presented in Table 1.

Cytogenetic Risk Classification and Mutation Frequency

Cytogenetic data were available for 1078 patients, of whom 477 (44.2%) presented with an abnormal karyotype. Based on

Table 1
Patient Characteristics

Characteristic	Value
Sex, male/female, n	634/498
Age, yr, median (range)	42 (9-81)
Diagnosis (N = 1132), n (%)	
AML with recurrent genetic abnormalities	618 (54.6)
Therapy-related myeloid neoplasms	50 (4.4)
AML not otherwise specified	464 (41.0)
Karyotype (N = 1078), n (%)	
Normal	601 (55.8)
Abnormal	477 (44.2)
Mutation (N = 977), n (%)	
NPM1^{mut}	175 (17.9)
CEBPA^{mut}	160 (16.4)
FLT3-ITD^{mut}	181 (18.5)
FLT3-TKD^{mut}	38 (3.9)
DNMT3A^{mut}	84 (8.6)
C-KIT^{mut}	86 (8.8)
Induction chemotherapy (N = 1132), n (%)	
IA/DA	770 (68.0)
Other*	362 (32.0)
Treatment response (N = 1132), n (%)†	
CR	914 (80.7)
PR	55 (4.9)
Treatment failure	163 (14.4)
Death, n (%)	407 (36.0)
Relapse, n (%)	239 (23.1)
Transplantation, n (%)	557 (49.2)

* Nonstandard first-line treatment, including CAG, IAG, hypomethylating agents with or without CAG or IAG, and other therapies.

† Treatment response was evaluated after 2 cycles of induction chemotherapy.

risk classification, 142 patients (13.2%) were in the favorable risk group, 789 patients (73.2%) were in the intermediate risk group, and 147 patients (13.6%) were in the adverse risk group.

We also analyzed the frequency of mutations in the following genes: *NPM1*, *CEBPA*, *FLT3*-ITD, *FLT3*-TKD, *DNMT3A*, and *C-KIT*. Of the 977 patients with complete gene mutation data, 541 (55.4%) exhibited or more gene mutations, including 355 (36.3%) with mutation in a single gene, 131 (13.4%) with mutations in 2 genes, and 55 (5.6%) with mutations in ≥3 genes. No mutations in the foregoing genes were seen in 436 patients (44.6%). The frequencies of each gene mutation across the entire cohort are shown in Table 1.

Risk Factors Affecting OS and DFS

The results of univariate analyses of risk factors that affected OS are presented in Table 2. An older age, higher WBC count, nonstandard first-line induction chemotherapy, adverse karyotype, and gene mutation status (*FLT3*-ITD^{mut} and *DNMT3A*^{mut}) were poor prognostic factors. *CEBPA*^{mut} and receipt of HSCT as consolidation therapy were associated with favorable OS. When these factors were fitted into a multivariate regression model, an adverse karyotype (hazard ratio [HR], 1.48; $P = .001$), presence of *FLT3*-ITD^{mut} (HR, 1.90; $P < .001$), and receipt of nonstandard first-line induction chemotherapy (HR, 1.45; $P = .003$) remained statistically significant factors for worse OS, and *CEBPA*^{mut} (HR, .42; $P < .001$) and receipt of HSCT (HR, .35; $P < .001$) remained significant prognostic factors for improved OS (Table 3).

Table 2
Univariate Analysis of Risk Factors Affecting OS

Variable	No. of Patients	Median OS	HR	*P* Value
Sex			.95	.618
Male	634	56.3		
Female	498	50.5		
Age, yr			1.03	<**.001**
<60	1028	67.5		
≥60	104	18.3		
WBC, × 10^9/L			1.55	**.001**
<100	969	60.7		
≥100	163	29.0		
Hb, g/L			1.00	.977
≥100	331	56.3		
<100	801	53.5		
PLT, ×10^9/L			.99	.956
<100	964	56.3		
≥100	168	101.4		
Karyotype			1.53	<**.001**
Favorable	142	NA		
Intermediate	789	60.7		
Adverse	147	28.4		
NPM1			.98	.919
mut	175	60.7		
wt	802	56.3		
CEBPA			.44	<**.001**
mut	160	NA		
wt	817	43.8		
FLT3-ITD			1.95	<**.001**
mut	181	20.6		
wt	796	64.0		
FLT3-TKD			.98	.949
mut	38	NA		
wt	939	56.3		
DNMT3A			1.41	**.049**
mut	84	28.1		
wt	893	60.7		
C-KIT			.66	**.065**
mut	86	NA		
wt	891	53.5		
Induction chemotherapy			1.80	<**.001**
IA/DA	770	67.5		
Other*	362	27.0		
Transplantation			.34	<**.001**
Yes	557	NA		
No	575	24.2		

Significant *P* values are in bold type.
Hb indicates hemoglobin; PLT, platelets.

Table 3
Multivariate Analysis of Risk Factors Affecting OS

Variable	Multivariate *P* Value	HR (95% CI)
Age, ≥60 yr versus <60 yr	.317	1.19 (.85-1.65)
WBC, ≥100 versus < 100 ×10^9/L	.578	1.10 (.79-1.52)
Karyotype, adverse versus other	**.001**	1.48 (1.18-1.86)
CEBPA, mut versus wt	<**.001**	.42 (.29-.63)
FLT3-ITD, mut versus wt	<**.001**	1.90 (1.46-2.47)
DNMT3A, mut versus wt	.430	1.15 (.81-1.64)
C-KIT, mut versus wt	.957	.99 (.63-1.55)
Induction chemotherapy, other* versus IA/DA	**.003**	1.45 (1.14-1.85)
Transplantation, yes versus no	<**.001**	.35 (.27-.44)

Significant *P* values are in bold type.
* Nonstandard first-line treatment, including CAG, IAG, hypomethylating agents with or without CAG or IAG, and other therapies.

Table 4
Univariate Analysis of Risk Factors Affecting DFS

	Univariate Analysis of DFS		
Variable	No. of Patients	3-Year DFS, %	*P* Value
Sex			.724
Male	574	66	
Female	460	67	
Age, yr			**.030**
<60	956	69	
≥60	78	35	
WBC, ×10^9/L			.840
<100	898	67	
≥100	136	66	
Hb, g/L			.325
≥100	304	64	
<100	730	67	
PLT, × 10^9/L			.630
<100	871	67	
≥100	163	65	
Karyotype			.702
Favorable	141	67	
Intermediate	728	66	
Adverse	121	63	
NPM1			***.039***
mut	161	60	
wt	738	67	
CEBPA			**.033**
mut	154	75	
wt	745	64	
FLT3-ITD			<**.001**
mut	162	55	
wt	737	69	
FLT3-TKD			.841
mut	34	75	
wt	865	66	
DNMT3A			**.012**
mut	74	54	
wt	825	67	
C-KIT			.859
mut	85	68	
wt	814	66	
Induction chemotherapy			**.054**
IA/DA	731	68	
Other*	303	63	
Transplantation			**.046**
Yes	550	70	
No	484	61	

Significant *P* values are in bold type.

Table 5
Multivariate Analysis of Risk Factors Affecting DFS

Variable	Multivariate *P* Value	HR (95% CI)
Age, ≥60 yr versus <60 yr	.831	1.06 (.64-1.75)
NPM1, mut versus wt	.762	1.06 (.73-1.54)
CEBPA, mut versus wt	.074	.69 (.46-1.04)
FLT3-ITD, mut versus wt	**<.001**	2.11 (1.51-2.94)
DNMT3A, mut versus wt	.096	1.47 (.93-2.30)
Induction chemotherapy, other* versus IA/DA	.092	1.31 (.96-1.79)
Transplantation, yes versus no	**.046**	.74 (.56-.99)

Significant *P* values are in bold type.
* Nonstandard first-line treatment, including CAG, IAG, hypomethylating agents with or without CAG or IAG, and other therapies.

We also evaluated risk factors affecting DFS by univariate and multivariate analyses (Tables 4 and 5). Our results identify *FLT3*-ITD^{mut} as an independent risk factor affecting DFS. The 3-year DFS of patients with *FLT3*-ITD^{mut} was significantly lower than that of patients with *FLT3*-ITD^{wt} (wild type) (55% versus 69%; $P < .001$). Receipt of HSCT remained a prognostic factor for favorable DFS (HR, .74; $P = .046$); however, no significant difference in DFS was observed among patients in the different cytogenetic risk groups ($P = .702$).

Comparison of Clinical Characteristics between Patients Who Received and Did Not Receive HSCT as Consolidation Therapy

A total of 557 patients underwent HSCT as consolidation therapy, and 575 patients received chemotherapy as consolidation treatment. The patients who underwent HSCT demonstrated a significantly better prognosis than those who received chemotherapy, in terms of both OS and DFS (Figure 1). Clinical and molecular characteristics of these 2 groups are compared in Table 6. Patients in the HSCT treatment group were younger, more frequently treated with standard first-line induction chemotherapy, and exhibited a lower frequency of the intermediate karyotype. The 2 groups also exhibited differences in the frequencies of *NPM1*, *FLT3*-TKD, and *C-KIT* mutations. Although *CEBPA* and *FLT3*-ITD mutations were identified as risk factors affecting OS or DFS, no significant differences in the mutation frequency of these 2 genes were observed.

Table 6
Clinical and Molecular Characteristics of Patients with AML Who Received Chemotherapy or HSCT as Consolidation Therapy

Variable	Chemotherapy (N = 575)	HSCT (N = 557)	*P* Value
Sex, n (%)			**.006**
Male	299 (52.0)	335 (60.1)	
Female	276 (48.0)	222 (39.9)	
Age, yr, median (range)	48 (10-81)	36 (9-66)	**<.001**
WBC, $\times 10^9$/L, median (range)	14.5 (.52-444)	18.4 (.15-406)	.108
Hb, g/L, median (range)	85 (11-167)	82 (11-177)	.285
PLT, $\times 10^9$/L, median (range)	37 (.58-999)	38 (1-557)	.676
Karyotype, n (%)			**.035**
Favorable	58 (10.6)	84 (15.8)	
Intermediate	415 (76.0)	374 (70.3)	
Adverse	73 (13.4)	74 (13.9)	
NPM1, n (%)			**.009**
mut	103 (21.1)	72 (14.7)	
wt	384 (78.9)	418 (85.3)	
CEBPA, n (%)			.411
mut	75 (15.4)	85 (17.3)	
wt	412 (84.6)	405 (82.7)	
FLT3-ITD, n (%)			.971
mut	90 (18.5)	91 (18.6)	
wt	397 (81.5)	399 (81.4)	
FLT3-TKD, n (%)			**.045**
mut	25 (5.1)	13 (2.7)	
wt	462 (94.9)	477 (97.3)	
DNMT3A, n (%)			.797
mut	43 (8.8)	41 (8.4)	
wt	444 (91.2)	449 (91.6)	
C-KIT, n (%)			**.002**
mut	29 (6.0)	57 (11.6)	
wt	458 (94.0)	433 (88.4)	
Induction chemotherapy, n (%)			**<.001**
IA/DA	341 (59.3)	429 (77.0)	
Other*	234 (40.7)	128 (23.0)	

Significant *P* values are in bold type.
* Nonstandard first-line treatment, including CAG, IAG, hypomethylating agents with or without CAG or IAG, and other therapies.

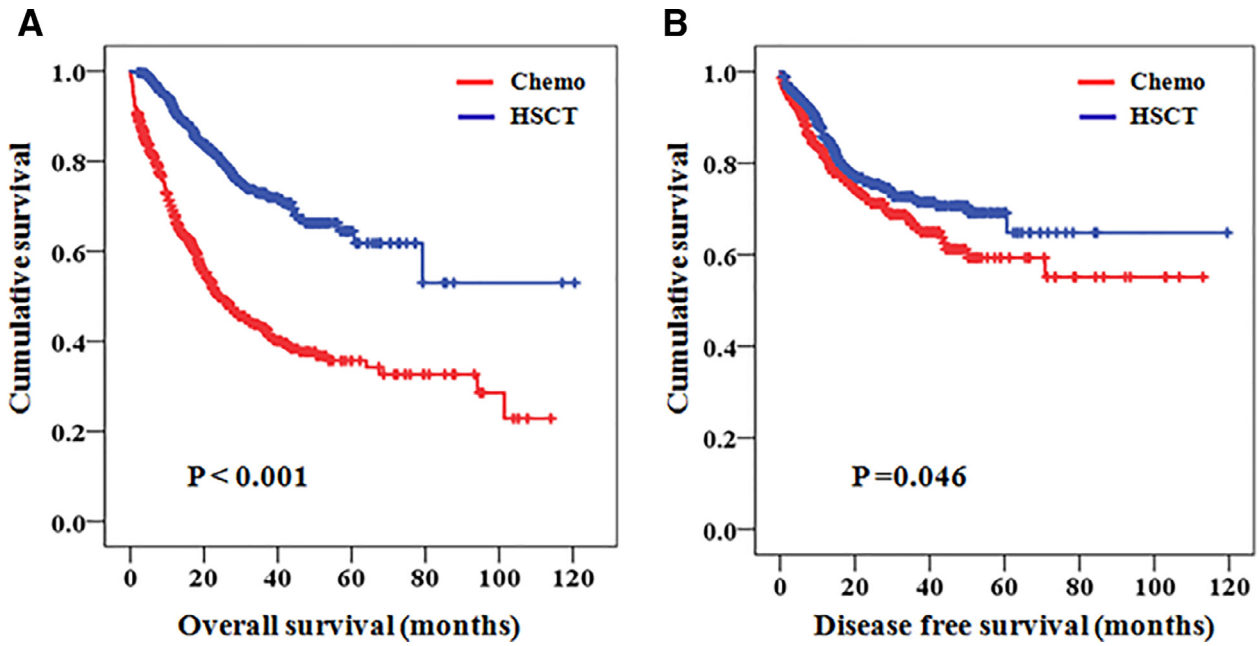

Figure 1. OS (A) and DFS (B) of patients with AML who received various consolidation therapies. Chem, chemotherapy.

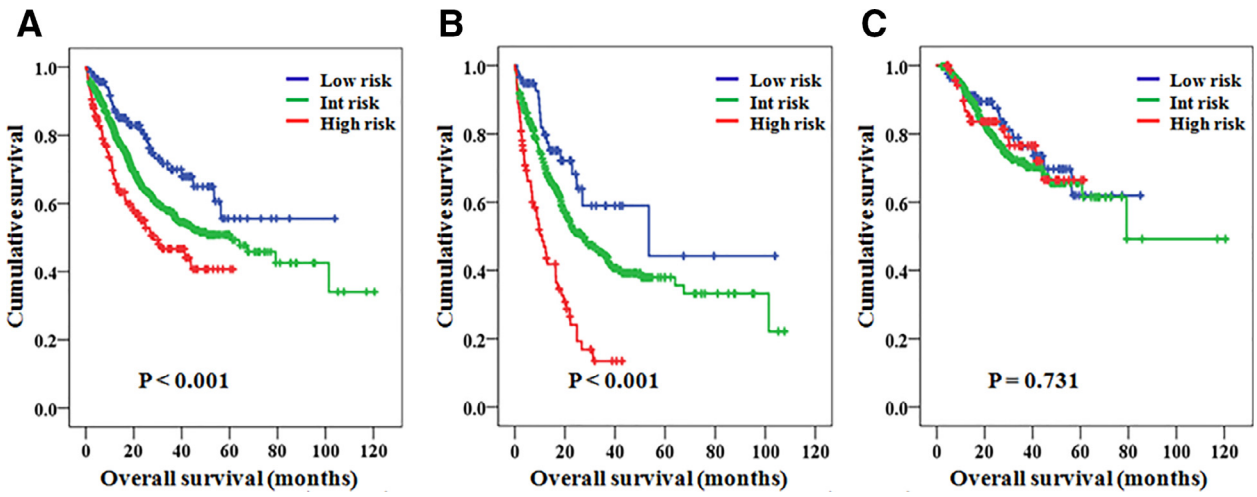

Figure 2. OS of the entire cohort of patients with AML (A), the subgroup of patients who received chemotherapy as consolidation treatment (B), and the subgroup of patients who underwent HSCT as consolidation therapy (C), according to their cytogenetic risk.

Impact of HSCT on Outcome of Patients Classified by Cytogenetic Risk and Gene Mutations

To evaluate the impact of HSCT on outcome in patients classified by cytogenetic risk and the presence of mutations in either *CEBPA* or *FLT3*-ITD, we performed further analyses to stratify patients in relation to their HSCT treatment status. Cytogenetic risk could effectively predict OS (Figure 2A and B), in both the full patient cohort and the subgroup of patients who received chemotherapy as consolidation treatment. However, in the subgroup of patients who received HSCT, cytogenetic risk was not identified as a risk factor that could predict OS (Figure 2C). This suggests that HSCT can significantly improve OS and help overcome the prognostic effect of cytogenetic risk classification (in contrast to the patients who received chemotherapy as consolidation). Figures 3A and 4A show the OS according to mutation status for *CEBPA* and *FLT3*-ITD genes across the entire patient cohort. OS was superior in patients with *CEBPA*^{mut} compared with those with *CEBPA*^{wt} in the entire cohort (Figure 3A; $P < .001$). Similarly, patients with *FLT3*-ITD^{mut} had worse OS (Figure 4A; $P < .001$). A similar trend was seen in patients who received both chemotherapy and HSCT as consolidation therapy (Figures 3B and C and 4B and C). In addition, *FLT3*-ITD^{mut} was identified as an effective predictor of poor DFS, both in the entire patient cohort and in the chemotherapy and HSCT subgroups (Figure 4D-F). These results suggest that HSCT, which significantly improves overall patient prognosis, cannot overcome the prognostic significance driven by the presence of *CEBPA*^{mut} and *FLT3*-ITD^{mut}.

DISCUSSION

In this study, we analyzed the frequency of cytogenetic changes and gene mutations in a large cohort at a single institution between April 2006 and July 2016. Of the 6 gene mutations in our analysis (*NPM1*^{mut}, *CEBPA*^{mut}, *DNMT3A*^{mut}, *FLT3*-ITD^{mut}, *FLT3*-TKD^{mut}, and *C-KIT*^{mut}), *FLT3*-ITD^{mut} was associated with a significantly worse prognosis for OS and DFS in our entire cohort of patients with AML. In addition, multivariate analysis identified *CEBPA*^{mut} as an independent risk factor for favorable OS.

Cytogenetic characterization at diagnosis is recognized as the strongest prognostic factor, both for the response to induction therapy and for long-term survival [17,18]. Younger adult patients are commonly categorized into 3 risk groups: favorable, intermediate, and adverse [1,2,5]. Consistent with this classification system, our analyses demonstrated that an adverse karyotype at diagnosis conferred a poor OS in patients

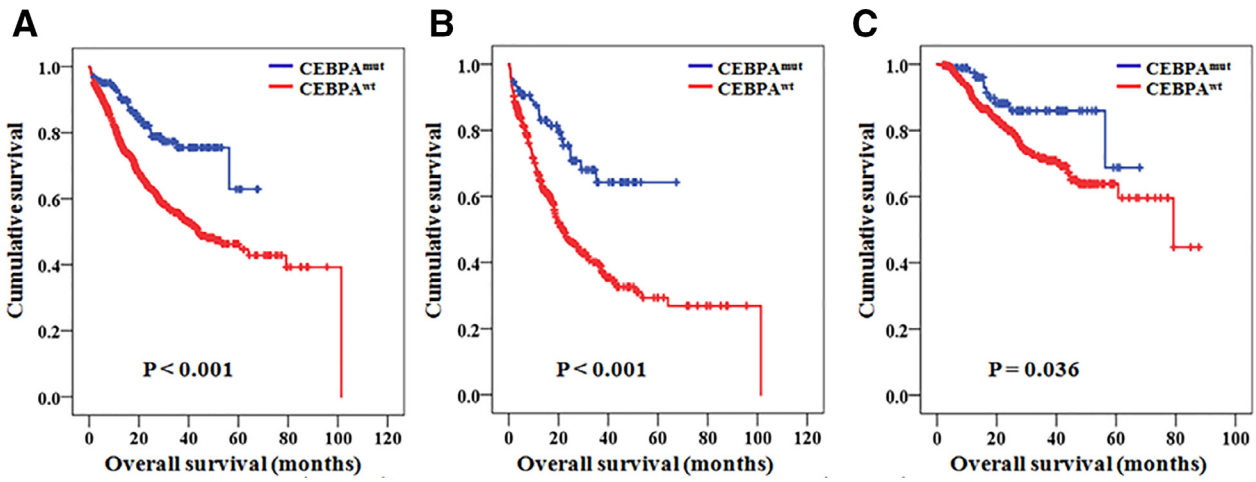

Figure 3. OS of the entire cohort of patients with AML (A), the subgroup of patients who received chemotherapy as consolidation treatment (B), and the subgroup of patients who underwent HSCT as consolidation therapy (C), according to the presence of a *CEBPA* mutation.

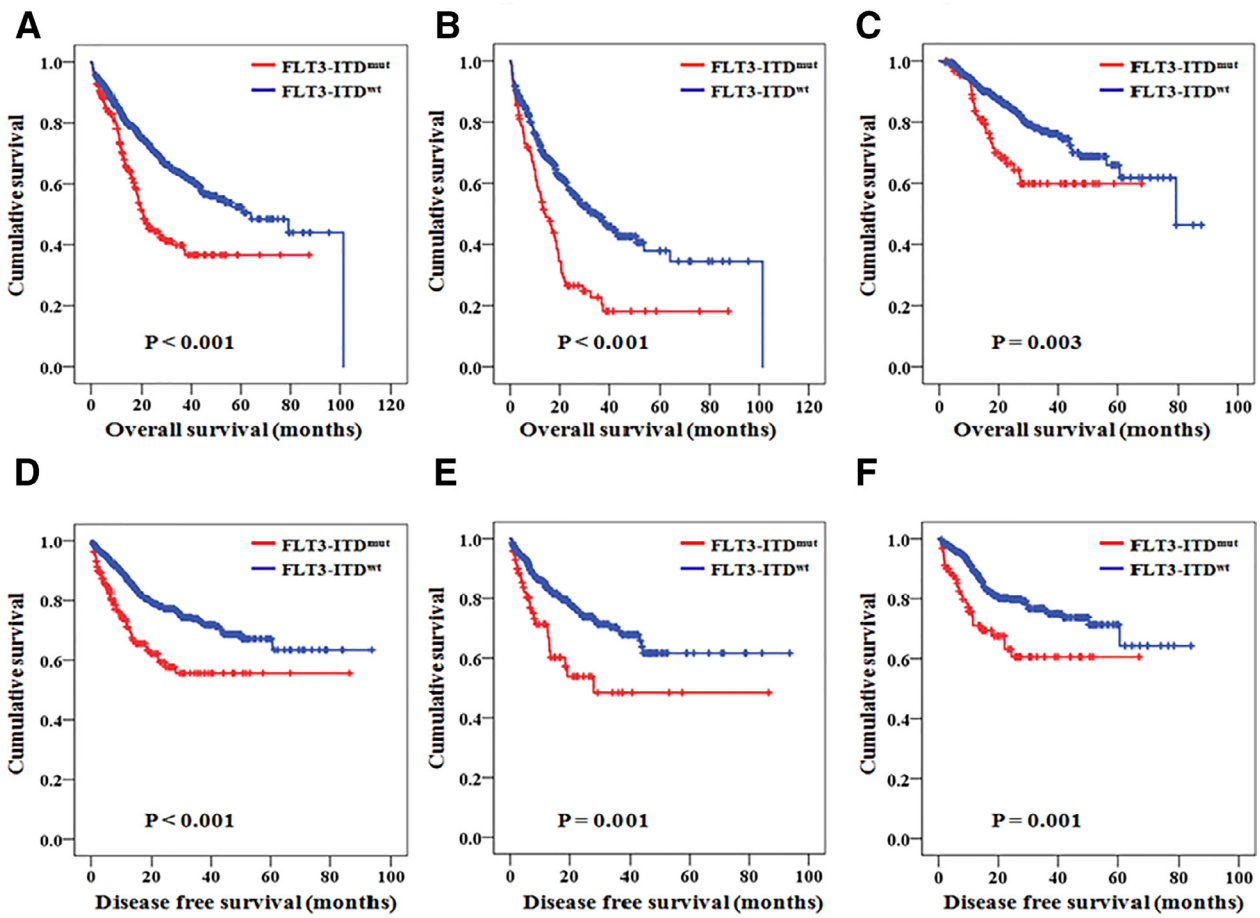

Figure 4. (A-C) OS of the entire cohort of patients with AML (A), the subgroup of patients who received chemotherapy as consolidation treatment (B), and the subgroup of patients who underwent HSCT as consolidation therapy (C), according to the presence of an *FLT3*-ITD mutation. (D-F) DFS of the entire cohort of patients with AML (D), the subgroup of AML patients who received chemotherapy as consolidation treatment (E), and the subgroup of patients who underwent HSCT as consolidation therapy (F), according to the presence of an *FLT3*-ITD mutation.

with AML, especially in patients who received chemotherapy for consolidation. Furthermore, we found that HSCT as consolidation therapy may significantly improve OS in all patients, regardless of karyotype, compared with patients who do not undergo HSCT. This suggests that HSCT can overcome the prognosis conferred by a patient's intrinsic cytogenetic risk.

In recent years, acquired gene mutations, as well as pathogenic deregulation of gene expression, have been identified in patients with AML [19-21]. These acquired mutations have prognostic relevance; *FLT3*-ITD^{mut} is associated with shortened relapse-free survival and OS [6,22-25], whereas more favorable outcomes are associated with cytogenetically normal cases of AML that exhibit mutations in *CEBPA* [26] or *NPM1* (without concomitant *FLT3*-ITD^{mut}) [7,27]. In previous reports, patients with *FLT3*-ITD^{mut} composed approximately 20% to 30% of patients with AML [23,25], similar to the 18.5% prevalence of *FLT3*-ITD^{mut} seen in our cohort. In a previous study, Gale et al [28] found no beneficial effects of transplantation therapy in patients with *FLT3*-ITD^{mut}. Another study of 872 patients with cytogenetically normal AML found that HSCT therapy may improve the prognosis of patients with *FLT3*-ITD^{mut} [29]. Our data support the prognostic importance of *FLT3*-ITD^{mut}, demonstrating worsened OS and DFS. In addition, patients with *FLT3*-ITD^{mut} who did not undergo HSCT consolidation therapy exhibited the worst OS and DFS in our study. Furthermore, in patients who underwent HSCT consolidation therapy, the presence of *FLT3*-ITD^{mut} remained the predictive factor that conferred the worst OS and DFS. This finding suggests that HSCT, although significantly improving patient prognosis, could not overcome the poor prognosis resulting from *FLT3*-ITD^{mut}. New *FLT3* detection techniques, such as next-generation sequencing, might improve detection sensitivity, thereby allowing mutated *FLT3* to serve as a useful prognostic marker [30,31]. Our analysis provides a rationale for testing *FLT3*-ITD as a marker for minimal residual disease; this marker could assist clinicians in identifying high-risk patients and would be particularly useful in guiding preemptive *FLT3*-targeted therapies.

CEBPA is a leucine zipper transcription factor with a pivotal role in myeloid differentiation [32,33]. Mutated *CEBPA* has been associated with relatively favorable outcomes and may serve as a prognostic marker [26,34]. In this study, *CEBPA*^{mut} was found in 16.4% of our patients with AML, similar to the approximately 15% reported in previous studies [35,36]. Taskesen et al [37] studied a cohort of 1182 cytogenetically normal patients with AML, reporting that both single and double mutations in *CEBPA* were associated with more favorable outcomes compared with *CEBPA*^{wt}. Our findings are consistent with that previous report, with *CEBPA*^{mut} serving as an independent factor for prediction of favorable OS. Furthermore, we found that HSCT can improve outcomes in patients with *CEBPA*^{wt}, as has been reported previously [29]. Recent analyses of collaborating mutations in patients with AML and *CEBPA*^{mut} showed that a favorable prognosis may be limited to patients with double *CEBPA* mutations and not those with single *CEBPA*

mutations [8,38]; this benefit appears to be restricted to patients without cytogenetic aberrations or *FLT3*-ITD^{mut} expression [38]. In our comparison of consolidation therapy methods, we found that HSCT can greatly improve prognosis irrespective of *CEBPA* status but cannot entirely overcome the prognostic effect associated with *CEBPA*^{mut}.

In our present analysis, the small number of patients with mutant *FLT3*-TKD^{mut} limited our ability to determine the influence of *FLT3*-TKD on disease outcome. A previous meta-analysis reported that *FLT3*-TKD negatively influences disease outcome [39]; however, in a recent study by the Medical Research Council, *FLT3*-TKD^{mut} was associated with favorable outcomes across the entire cohort, as well as in a subgroup of patients with cytogenetically normal AML [40]. In our cohort, the prevalence of *NPM1*^{mut} was 17.9%, and our findings do not suggest a significant correlation between disease prognosis and the presence of *NPM1* mutations. It has been shown that *NPM1* can serve as an important predictive marker in patients with AML [29,41]; however, most of the data were established in patients with normal karyotype [29,41] or those aged <60 years [7,29,41]. *DNMT3A* mutations have been associated with poor prognosis in patients with AML [12,13]. In our study, although we found the same correlation between *DNMT3A* mutations and OS in univariate analysis, *DNMT3A* mutations were not identified as an independent risk factor on multivariate analysis. These discrepancies may be explained by differences in the criteria used for different study populations. More large multicenter prospective studies are needed to verify the prognostic value of these genes in AML.

In conclusion, our data show that HSCT can improve the prognosis of the overall AML patient population but cannot entirely overcome the prognostic effects associated with mutations in *FLT3*-ITD and *CEBPA*; that is, *FLT3*-ITD^{mut} or *CEBPA*^{mut} can predict prognosis in AML independent of HSCT.

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