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Allogeneic Transplantation in First Remission Improves Outcomes Irrespective of *FLT3*-ITD Allelic Ratio in *FLT3*-ITD–Positive Acute Myelogenous Leukemia

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The adverse prognosis of internal tandem duplication in the FMS-like tyrosine kinase 3 gene(s) (*FLT3*-ITD) in patients with acute myelogenous leukemia (AML) may depend on allelic burden. We compared postremission treatment with chemotherapy and hematopoietic stem cell transplantation (HSCT) in 169 *FLT3*-ITD*mut* intermediate cytogenetic risk AML patients with allelic ratio evaluable at diagnosis who achieved first complete remission (CR1) with induction therapy. To minimize selection bias, the analysis was limited to patients who remained in CR1 for at least 4 months (median time to HSCT) after achieving CR1, and propensity score matching was implemented. Sensitivity analysis including patients who remained in CR1 for at least 3 months was applied as well. HSCT in CR1 was associated with longer relapse-free survival (RFS) and overall survival (OS), with 3-year estimated rates of 18% and 24%, respectively ($P < .001$), for patients receiving chemotherapy and 46% and 54%, respectively ($P < .001$), for those undergoing HSCT. Multivariate regression models showed that HSCT remained statistically significant with improved RFS and OS independent of *FLT3*-ITD allelic ratio and *NPM1* status. Irrespective of postremission therapy, relapse remains the main reason for treatment failure, with a 3-year incidence of 68% in chemotherapy recipients versus 41% in HSCT recipients. Allogeneic HSCT improved disease outcomes compared with chemotherapy after propensity score matching was applied. The improvement observed for RFS (hazard ratio [HR], 0.55; $P = .09$) and OS (HR, 0.58; $P = .10$) with HSCT as postremission therapy in patients who remained in CR1 for at least 4 months did not reach statistical significance; however, the sensitivity analyses including patients who remained in CR1 for at least 3 months showed significant improvement in both RFS (HR, 0.31; $P = .002$) and OS (HR, 0.27; $P = .02$) after propensity score matching. Our results indicate that HSCT in CR1 for AML *FLT3*-ITD*mut* patients is associated with longer RFS and OS. Innovative transplantation strategies to improve relapse incidence are urgently needed.

INTRODUCTION

Acute myelogenous leukemia (AML) is a heterogeneous disease with a variable prognosis. Overall, FMS-like tyrosine kinase 3 (gen)s (*FLT3*-ITD) mutations occur in 23% to 25% of patients with AML at diagnosis [1,2]. *FLT3*-ITD*mut* AML represents a distinct entity in patients with intermediate-risk karyotype, conferring poor prognosis [1-3]. The probability of reaching first complete remission (CR1) is similar in *FLT3*-ITD*mut* AML and in other intermediate-risk forms of AML in both young patients and elderly patients [3-5]; however, the high risk of relapse, frequently occurring in the first months post-transplantation, is the cause of the shorter disease-free survival (DFS) and overall survival (OS) in this group.

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Recent studies have shown that the risk conferred by *FLT3*-ITD*mut* is related to specific characteristics, including allelic burden, length of mutation, and site of insertion [4,6]. Moreover, the coexistence of other poor-risk molecular markers in *FLT3*-ITD*mut* AML can further worsen the prognosis, as has been demonstrated for *WT1* and *DMNT3A* mutations [7]. In contrast, whether certain additional genetic mutations in *FLT3*-ITD*mut* AML make the outcomes relatively favorable, as is the case with *NPM1* mutations, is a matter of debate. Mutations of the *NPM1* gene are found in 50% of cytogenetically normal AML (CN-AML) [8] and lead to favorable survival. Of note, in 20% of patients with *NPM1* mutation, *FLT3*-ITD is also identified, and the presence of *FLT3*-ITD may negate the favorable impact of mutated *NPM1* [9-11].

Hematopoietic stem cell transplantation (HSCT) is usually indicated for patients with *FLT3*-ITD*mut* AML, owing to these patients' short relapse-free survival (RFS) even after achieving CR1 and high resistance to salvage chemotherapies. Despite this common practice, some still argue that the benefit of this high-risk procedure for *FLT3*-ITD*mut* AML in CR1 remains to be proven [12,13]. Several studies have shown better survival with allogeneic HSCT compared with chemotherapy when performed in CR1, but those studies have been criticized for comparing outcomes with historical controls or not including matched unrelated donors as the donor source [11,14-16]. More recently, the importance of taking into account not only the mutational status of *FLT3*-ITD at diagnosis, but also the allele ratio, has been addressed, and improved outcomes after HSCT, even in patients with high allelic ratio HSCT, have been reported [17].

In this study, we compared postremission treatment with chemotherapy and allogeneic HSCT in intermediate-risk AML patients with *FLT3*-ITD*mut* who achieved CR1 after induction chemotherapy. In particular, we aimed to investigate the impact of the *FLT3*-ITD allelic ratio and presence of *NPM1* at diagnosis on outcomes, with the goal of identifying a subgroup of patients who might not need to proceed with HSCT in CR1.

MATERIALS AND METHODS

The study population comprised 227 adult AML patients (age ≥18 years) with *FLT3*-ITD*mut* who were diagnosed and treated at MD Anderson Cancer Center (MDACC) between July 2000 and November 2013. Patients who received antileukemia therapy at an outside institution before referral to MDACC were excluded. Patients were eligible for analysis if they had achieved CR1 with induction chemotherapy and had not undergone previous HSCT for another hematologic malignancy (Figure 1). Patients with poor-risk cytogenetics at diagnosis like del5q/-5 and/or del7q/-7, were excluded; only patients with intermediate-risk cytogenetics were included. Patients who underwent HSCT as consolidation therapy in CR1 using a mismatched donor, including cord blood units, were excluded as well. To minimize time to transplantation selection bias, our analysis was limited to patients who remained in CR1 for at least 4 months (median time to HSCT) after achieving CR1. Finally, a total of 169 patients were included in this analysis.

Analysis of FLT3-ITD and FLT3-ITDwild Allelic Burden and NPM1

All samples were obtained at diagnosis, and detection of *NPM1* and *FLT3*-ITD mutations was performed on genomic DNA from bone marrow aspirates. *FLT3*-ITD and codon 835/836 tyrosine-kinase domain mutational status was determined using DNA from unsorted bone marrow aspirate samples obtained at initial presentation using a semiquantitative DNA-based PCR-capillary electrophoresis assay, as described previously [6], with an analytical sensitivity of 1% to 2% mutation-bearing cells. *FLT3*-ITD-*wild* allelic burden at diagnosis was calculated as the ratio of the area under the curve (AUC) of mutant and wild type alleles (*FLT3*-ITD/*FLT3*-ITD*wild*) [18]. In cases with more than 1 mutation, all *FLT3*-ITD mutations were summed. *NPM1* exon12 mutations were identified by PCR amplification as described previously [19].

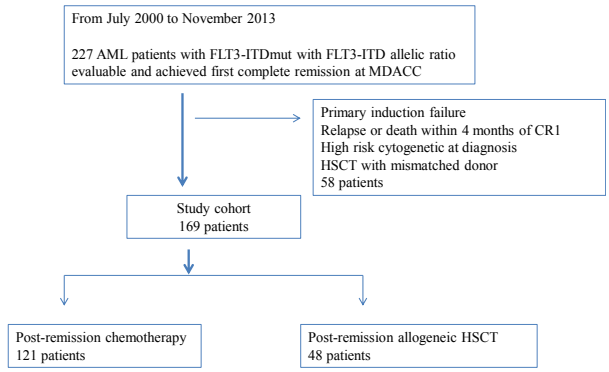

Figure 1. Flow chart of the patient selection strategy. Among all AML patients diagnosed and treated at MDACC between July 2000 and November 2013, only those with *FLT3*-ITD*mut* and FLT3 allelic ratio available and who achieved CR1 with antileukemia treatment were included. The final study cohort comprised 169 patients.

Treatments

Induction therapies were provided according to age-appropriate front-line regimens, and all were administered at MDACC during the study period. At this institution, the preferred induction chemotherapy for AML is combination chemotherapy, including high-dose cytarabine, for patients aged ≤65 years who are medically fit. Older patients are treated with combination chemotherapies based on low-dose cytarabine or hypomethylating agents. Overall, induction chemotherapy was composed predominantly of high-dose cytarabine-based regimens in 119 patients (70%), hypomethylating agents in 28 patients (17%), clofarabine in 9 patients (5%), and cladribine in 6 patients (4%). The majority (90%) of patients aged ≤65 years were treated with a high-dose cytarabine-based regimen. Patients who did not undergo HSCT as postremission therapy received consolidation chemotherapy similar to their induction therapy.

Among the 169 patients in the total cohort, 37 (22%) received an FLT3 inhibitor as a part of their induction/consolidation chemotherapy. In our cohort, patients were enrolled into clinical trials (including high-dose cytarabine-based, hypomethylating agent-based, or low-dose cytarabine-based front-line regimens) based on patient age and organ function; the majority of the patients (n = 134; 79%) were treated on clinical trials.

In general, all high-risk patients with cytogenetic and molecular findings (including *FLT3*-ITD*mut* patients) were referred for HSCT consultation. The decision to proceed with HSCT in CR1 was based on donor availability, patient preference, and treating physician preference. Postremission therapy was consolidation chemotherapy in 121 patients (71.6%; chemo group) and allogeneic HSCT in 48 patients (28.4%; HSCT group). Among the 121 patients in the chemo group, 5 underwent HSCT after disease relapse and 2 underwent HSCT without relapse but later in the course of disease; these 7 patients were analyzed in the chemo group.

Forty-five of the 48 patients who underwent HSCT as postremission consolidation therapy did so at MDACC. The hematopoietic stem cell source was peripheral blood in 30 patients (66.6%) and bone marrow in 15 patients (33.3%). Until July 2005, serologic or low-resolution molecular techniques were used for class I antigens, and high-resolution molecular typing by polymerase chain reaction was used for class II alleles. After July 2005, all donors underwent high-resolution molecular typing of class I and II antigens and were matched with recipients at HLA-A, -B, -C, and -DRB1. More than one-half of the donors were matched unrelated (n = 26; 57.8%), as described by Weisdorf et al. [20], and the remainder were matched related (n = 19; 42.2%).

The conditioning regimen for HSCT was myeloablative in 33 of 45 (73.3%) patients and consisted of fludarabine given as 40 mg/m^2 for 4 days with i.v. busulfan either as a fixed dose of 130 mg/m^2 or as a targeted dose to achieve an AUC of 5000 to 6000 for 4 days. Reduced-intensity conditioning, given to 12 patients, consisted of fludarabine 25 to 40 mg/m^2 for 3 to 4 days with either melphalan 140 mg/m^2 or 100 mg/m^2 or busulfan as either a fixed dose of 100 mg/m^2 or a targeted dose to achieve an AUC of 4000 for 4 days. Tacrolimus (0.015 mg/kg) and methotrexate 5 mg/m^2 i.v. on post-transplantation days +1, +3, +6, and +11 were used for graft-versus-host disease prophylaxis in the majority of the patients (93.3%). Patients with an unrelated donor received rabbit antithymocyte globulin (Thymoglobulin; Genzyme, Cambridge, MA), 0.5 mg/kg on day −3, 1.5 mg/kg on day −2, and 2.0 mg/kg on day −1.

Approval for these studies was obtained from the MDACC Institutional Review Board. Informed consent was provided in accordance with the Declaration of Helsinki.

Statistical Methods

Patient characteristics were tabulated by the status of stem cell transplantation. Differences between categorical covariates were tested using Fisher's exact test, and differences between continuous covariates were compared using Wilcoxon's rank-sum test. OS was defined as the interval between diagnosis date and death date, and was censored at the last follow-up date for patients who were alive. RFS was defined as the interval between remission dates and relapse date or death date, whichever came first, and was censored at last follow-up date for patients who were alive without relapse. Survival curves were estimated using the Kaplan-Meier method [21]. Univariate and multivariate Cox proportional hazards regression models [22] were used to assess the association between patient characteristics and OS or RFS. Patient characteristics that were significant in the univariate models at the 0.10 level were included in the multivariate model. Backward elimination was implemented until all remaining predictors had a P value $< .05$. Predictive variables were transformed as appropriate.

Because in this study the decision regarding postremission therapy was not randomized, we used propensity score matching to reduce the possibility of selection bias [23,24]. The Greedy 8→1 digit match algorithm was applied in propensity score matching. Age at diagnosis, *FLT3*-ITD allelic ratio, presence of *NPM1* mutation, receipt of intermediate/high-dose cytarabine as part of induction and/or consolidation chemotherapy, and year of diagnosis (before 2008 versus after 2008) were the criteria used to estimate the propensity scores. The year 2008 was used as a cutoff for matching, because since that time FLT3 inhibitors have been the most frequently used agent in front-line and salvage settings. HSCT patients were 1:1 matched to chemotherapy patients. For the matched data, differences in patient characteristics were evaluated using McNemar's test for categorical covariates with 2 levels, generalized estimating equation methods for categorical covariates with 3 levels, and Wilcoxon's signed-rank test for continuous covariates. The stratified log-rank test was applied to assess the difference in OS and RFS between the 2 matched groups (ie, HSCT versus chemotherapy). Univariate and multivariate Cox proportional hazards regression models stratifying on the matched pairs were used to assess the associations between patient characteristics and OS or RFS. Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC), and graphics were created using Stata (StataCorp, College Station, TX).

RESULTS

Table 1 presents the disease and patient characteristics by postremission therapy patients received in CR1. Treatment groups were comparable except that patients in the HSCT group were younger (median age, 55 years versus 62 years; $P = .002$) and more often received high-dose cytarabine as a part of their induction/consolidation chemotherapy (87.5% versus 58.7%; $P < .001$) compared with those in the chemotherapy group. ECOG performance status (PS) [25] at diagnosis was available in 155 of 169 patients and more than three-quarters of the patients in both treatment groups had an ECOG PS of 0 to 1 (83.6 % in the chemo group versus 93.3% in the HSCT group).

The median follow-up from the date of achieving CR1 was 29.4 months for the HSCT group and 32.8 months for the chemo group ($P = .71$). The median time from diagnosis to transplantation among the 48 HSCT patients was 4.1 months (range, 2.5 to 8.9 months).

RFS

Of 169 patients, 121 (72%) had disease relapse or died and 48 (28%) were alive without disease relapse at last follow-up. The median RFS was 8.5 months (95% confidence interval [CI], 7.0 to 10.9 months) and the 3-year RFS rate was 26% (95% CI, 20% to 34%). Figure 2A shows the Kaplan-Meier estimates

Table 1
Patient Characteristics before and after Propensity Score Matching

Characteristic	Without Propensity Score Matching				With Propensity Score Matching		
	All Patients (n = 169)	Chemo Group (n = 121)	HSCT Group (n = 48)	*P*	Chemo Group (n = 41)	HSCT Group (n = 41)	*P*
Age at diagnosis, yr, median (IQR)	59 (50-68)	62 (52-70)	55 (47-62)	.002	56 (49-65)	56 (52-62)	.48
WBC at diagnosis, median (IQR)	11.6 (3.8-29.7)	12 (4-28.2)	9 (3.1-36.75)	.99	9.8 (4.9-19.4)	11.3 (4.2-51.4)	.24
Platelet count at diagnosis, median (IQR)	45 (25-74)	45 (25-71)	45 (22.5-83)	.99	43 (30-79)	52 (31-91)	.73
BM blasts at diagnosis, median (IQR)	43 (12-74)	46 (13-75)	32.5 (6.5-63.5)	.18	58 (28-82)	32 (7-64)	.07
Cytogenetics at diagnosis, n/N	164/169	119/121	45/48				
Diploid, n (%)	128 (78)	95 (79.8)	33 (73.3)		34 (85)	29 (74.4)	
Other, n (%)	36 (22)	24 (20.2)	12 (26.7)	.40	6 (15)	10 (25.6)	.13
FLT3-ITD mutations at diagnosis, n/N	163/169	116/121	47/48				
1, n (%)	130 (79.8)	95 (81.9)	35 (74.5)		33 (80.5)	31 (75.6)	
>1, n (%)	33 (20.2)	21 (18.1)	12 (25.5)	.29	8 (19.5)	10 (24.4)	.56
FLT3-ITD AR at diagnosis, median (IQR)	0.34 (0.11-0.48)	0.35 (0.12-0.49)	0.3 (0.04-0.48)	.29	0.18 (0.04-0.45)	0.32 (0.05-0.48)	.46
FLT3-ITD AR ≥0.3 at diagnosis, n/N	163/169	116/121	47/48				
Yes, n (%)	88 (54)	65 (56)	23 (48.9)		19 (46.3)	22 (53.7)	
No, n (%)	75 (46)	51 (44)	24 (51.1)	.49	22 (53.7)	19 (46.3)	.44
FLT3-ITD AR ≥0.5 at diagnosis, n/N	163/169	116/121	47/48				
Yes, n (%)	37 (22.7)	29 (25)	8 (17)		9 (22)	7 (17.1)	
No, n (%)	126 (77.3)	87 (75)	39 (83)	.31	32 (78)	34 (82.9)	.62
Presence of *NPM1* mutation, n (%)							
Yes	56 (33.1)	37 (30.6)	19 (39.6)		18 (43.9)	17 (41.5)	
No	57 (33.7)	42 (34.7)	15 (31.3)		12 (29.3)	14 (34.1)	
Unknown	56 (33.1)	42 (34.7)	14 (29.2)	.55	11 (26.8)	10 (24.4)	.85
Use of FLT3 inhibitor in induction/consolidation, n (%)							
Yes	37 (21.9)	27 (22.3)	10 (20.8)		10 (24.4)	8 (19.5)	
No	132 (78.2)	94 (77.7)	38 (79.2)	1.0	31 (75.6)	33 (80.5)	.53
Year of diagnosis after 2008, n (%)							
Yes	106 (62.7)	68 (56.2)	38 (79.2)		31 (75.6)	31 (75.6)	
No	63 (37.3)	53 (43.8)	10 (20.8)	.01	10 (24.4)	10 (24.4)	1.00
Use of high-dose cytarabine, n (%)							
Yes	113 (66.9)	71 (58.7)	42 (87.5)		34 (82.9)	35 (85.4)	
No	56 (33.1)	50 (41.3)	6 (12.5)	<.001	7 (17.1)	6 (14.6)	.65
ECOG performance status at diagnosis, n/N	155/169	110/121	45/48		36/41	38/41	
0-1, n (%)	134 (86.5)	92 (83.6)	42 (93.3)		31 (86.1)	36 (94.7)	
2-3, n (%)	55 (13.5)	18 (16.4)	3 (6.7)	.10	5 (13.9)	2 (5.3)	.20

IQR indicates interquartile range; BM, bone marrow; AR, allelic ratio; ECOG, Eastern Cooperative Oncology Group.

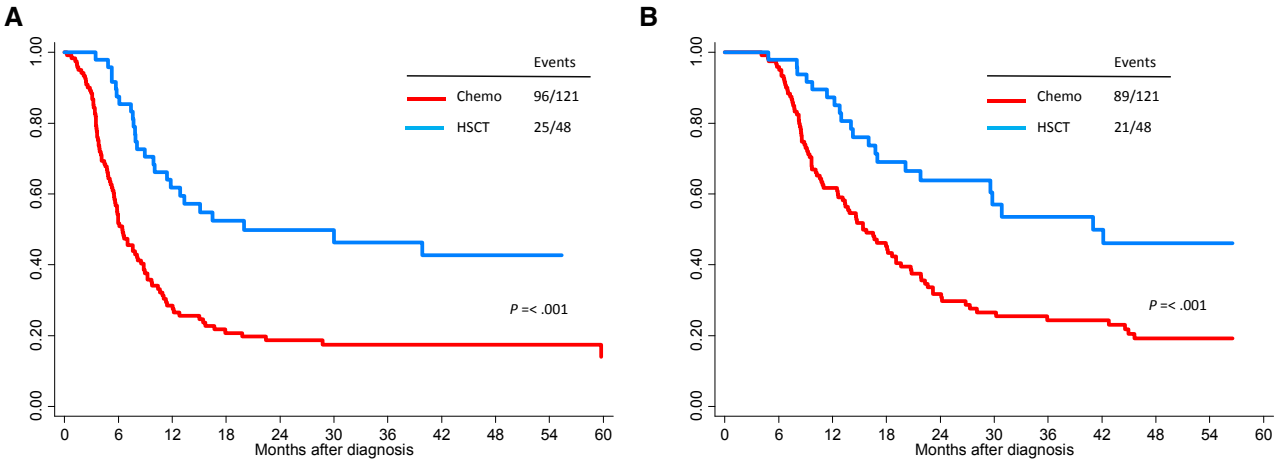

Figure 2. RFS (A) and OS (B) by postremission therapy in CR1. RFS at 3 years was 18% in the chemo group and 46% in the HSCT group. OS was 24% in the chemo group and 54% in the HSCT group.

for RFS by postremission therapy. Among the 48 patients in the HSCT group, 25 patients relapsed or died. The median RFS was 20 months (95% CI, 11.8 months to not estimable), and the 3-year RFS rate was 46% (95% CI, 33% to 64%). Among the 121 patients in the chemo group, 96 relapsed or died. The median RFS was 6.4 months (95% CI, 5.6 to 8.8 months) and the 3-year RFS rate was 18% (95% CI, 12% to 26%). There was a significant difference in RFS between the HSCT and chemo groups ($P < .001$, log-rank test).

Table 2 presents the results of univariate Cox proportional hazards model for RFS. Whereas log(WBC at diagnosis) (HR, 1.16; 95% CI, 1.01 to 1.33; $P = .03$) and log(FLT3-ITD allelic ratio at diagnosis) (HR, 1.16; 95% CI, 1.0 to 1.35; $P = .05$) were poor prognostic factors, receipt of high-dose cytarabine as part of induction/consolidation chemotherapy (HR, 0.61; 95% CI, 0.42 to 0.89; $P = .001$), receipt of allogeneic HSCT as postremission therapy (HR, 0.39; 95% CI, 0.25 to 0.61; $P < .001$), and presence of an *NPM1* mutation (HR, 0.56; 95% CI, 0.36 to 0.88; $P = .01$) were associated with improved RFS. When these prognostic variables were fitted into a multivariate regression model, allogeneic HSCT as postremission therapy remained statistically significant factor in improved RFS (HR, 0.42; 95% CI, 0.27 to 0.66; $P < .001$), and log(WBC at diagnosis) (HR, 1.18; 95% CI, 1.03 to 1.35; $P = .02$) remained a poor prognostic factor for RFS (Table 3).

Table 2
Univariate Analysis for RFS and OS for the Entire Study Cohort

Variable	RFS			OS		
	HR	95% CI	*P*	HR	95% CI	*P*
Age	1.01	0.99-1.02	.32	1.01	1-1.03	.05
Log(WBC) at diagnosis	1.16	1.01-1.33	.03	1.19	1.03-1.37	.02
Log(platelet) count at diagnosis	1.13	0.91-1.4	.26	1.12	0.90-1.4	.30
BM blasts at diagnosis	1	1-1.01	.63	1	0.99-1.01	.79
Cytogenetics at diagnosis (diploid versus other)	1.04	0.67-1.61	.87	1.45	0.89-2.36	.13
No of FLT3-ITD mutations at diagnosis (>1 versus 1)	0.79	0.50-1.26	.32	0.78	0.48-1.26	.31
Log (FLT3-ITD allelic ratio) at diagnosis	1.16	1-1.35	.05	1.18	1-1.38	.05
FLT3-ITD allelic ratio ≥0.3 at diagnosis (yes versus no)	1.3	0.9-1.87	.16	1.29	0.87-1.9	.20
FLT3-ITD allelic ratio ≥0.5 at diagnosis (yes versus no)	0.94	0.6-1.46	.77	1.07	0.68-1.68	.77
Presence of *NPM1* mutation						
Yes versus no	0.56	0.36-0.88	.01	0.64	0.4-1.04	.07
Unknown versus no	0.8	0.53-1.22	.30	0.91	0.58-1.42	.68
Use of FLT3 inhibitor as induction/consolidation (yes versus no)	1.1	0.71-1.71	.67	1.18	0.75-1.84	.47
Year of diagnosis after 2008 (yes versus no)	0.82	0.57-1.17	.27	0.82	0.56-1.2	.30
Allogeneic HSCT (yes versus no)	0.39	0.25-0.61	<.001	0.43	0.27-0.69	<.001
Use of high-dose cytarabine (yes versus no)	0.61	0.42-0.89	.01	0.68	0.46-1.01	.06

OS

Of the 169 patients, 110 (65%) died and 59 (35%) were alive at last follow-up. The median OS among the 59 survivors was 33.6 months (range, 5.0 to 159.4 months). Figure 2B shows the Kaplan-Meier estimates for OS by postremission therapy. Among the 48 patients in the HSCT group, 21 died. The median OS was 41 months (95% CI, 29.6 months to not estimable), and the 3-year OS rate was 54% (95% CI, 40% to 72%). Among the 121 patients in the chemo group, 89 died. The median OS was 15.4 months (95% CI, 13.1 to 19.6 months), and the 3-year OS rate was 24% (95% CI, 17% to 34%). OS was significantly better in the HSCT group compared with the chemo group ($P < .001$).

Table 2 presents the results of univariate Cox proportional hazards models for OS. Whereas older age (HR, 1.01; 95% CI, 1 to 1.03; $P = .05$), log(WBC at diagnosis) (HR, 1.19; 95% CI, 1.03 to 1.37; $P = .02$), and log(FLT3-ITD allelic ratio at diagnosis) (HR, 1.18; 95% CI, 1 to 1.38; $P = .05$) were poor risk factors for

Table 3
Multivariate Regression for RFS and OS for the Entire Study Cohort*

Variable	RFS			OS		
	HR	95% CI	*P*	HR	95%CI	*P*
Log(WBC) at diagnosis	1.18	1.03-1.35	.02	1.20	1.04-1.39	.01
Allogeneic HSCT (yes versus no)	0.42	0.27-0.66	<.001	0.47	0.29-0.76	.002

* The regression model included log(*FLT3*-ITD allelic ratio) at diagnosis, presence of *NPM1* mutation, and the use of intermediate/high cytarabine doses as induction/consolidation chemotherapy.

OS, receipt of high-dose cytarabine as part of induction/consolidation chemotherapy (HR, 0.68; 95% CI, 0.46 to 1.01; $P = .06$), and undergoing HSCT as postremission therapy were associated with improved OS (HR, 0.43; 95% CI, 0.27 to 0.69; $P < .001$). On multivariate analysis, HSCT was associated with improved OS (HR, 0.47; 95% CI, 0.29 to 0.76; $P = .002$), and log(WBC at diagnosis) (HR, 1.2; 95% CI, 1.04 to 1.39; $P = .01$) was a poor prognostic factor for OS (Table 3).

Interactions Among HSCT and FLT3-ITD Allelic Ratio at Diagnosis, NPM1, and Cytarabine-Containing Chemotherapy Regimens

The impact of HSCT and FLT3-ITD allelic ratio at diagnosis on RFS and OS was further investigated by adding the interaction term between HSCT and FLT3-ITD allelic ratio ($\geq$0.3 versus <0.3) in the Cox proportional regression model. The effects of the interaction on RFS and OS were not significant ($P = .91$ and .18, respectively), suggesting that the effect of HSCT on RFS and OS was not influenced by FLT3-ITD allelic ratio at diagnosis. Similar interaction effects were examined for the presence of NPM1 and the effect of HSCT. The effects of the interaction of the presence of NPM1 and HSCT was not significant for RFS ($P = .42$) or OS ($P = .43$). Similarly, the use of high-dose cytarabine as part of induction/consolidation chemotherapy did not influence the effect of HSCT on RFS and OS, with nonsignificant interactions between the use of high-dose cytarabine and HSCT ($P = .11$ and .24, respectively).

Relapse and NRM Incidence

The 3-year cumulative incidence rate of relapse was 41% (95% CI, 26% to 55%) in the HSCT group and 68% (95% CI, 58% to 76%) in the chemo group, when NRM was the competing event (Figure 3A). The 3-year cumulative incidence rate of NRM was 13% (95% CI, 5% to 25%) in the HSCT group and 15% (95% CI, 9% to 22%) in the chemo group (Figure 3B).

Calculation of Propensity Score and Propensity Score Matching

We used a propensity score–based approach for the comparison of outcomes between patients in the chemo and HSCT groups as described above, because postremission therapy with chemotherapy or allogeneic HSCT was not allocated through randomization. From among the 169 patients, we selected 82 propensity score–matched chemotherapy and allogeneic HSCT recipients for comparison.

Table 1 compares patient characteristics by type of postremission therapy before and after propensity score matching. In the original population, the 2 groups were significantly different in terms of age at diagnosis, year of diagnosis, and the use of high-dose cytarabine as a part of induction or consolidation chemotherapy. After propensity score matching, all patient and disease characteristics were similar in the 2 groups (Table 1). The median age at diagnosis was 56 years in both groups ($P = .48$), and high-dose cytarabine was administered to 34 of 41 patients in the chemo group and to 35 of 41 patients in the HSCT group ($P = .65$).

RFS and OS

Among 82 patients, 47 (57%) had disease relapse or died and 35 (43%) were alive without disease relapse at last follow-up. The median follow-up of survivors from the date of achieving CR1 was 44.6 months in the chemo group and 30.7 months in the HSCT group. Figure 4A shows the Kaplan-Meier estimates for RFS by postremission therapy status. Among the 41 patients in the HSCT group, the median RFS was 30 months (95% CI, 11.4 months to not estimable), and among the 41 patients in the chemo group, the median RFS was 8.0 months (95% CI, 5.9 months to not estimable). This difference in RFS observed between the HSCT and chemo groups did not reach statistical significance ($P = .09$, stratified log-rank test). RFS at 3 years was 47% (95% CI, 30% to 62%) for the HSCT group versus 34% (95% CI, 20% to 49%) for the chemo group.

Figure 4B shows the Kaplan-Meier estimates for OS by HSCT. The median OS was 42.2 months (95% CI, 21.8 months to not estimable) in the HSCT group versus 16.5 months (95% CI, 12.6 months to not estimable) in the chemo group. Similar to RFS, the difference in OS between the HSCT and chemo groups did not reach statistical significance ($P = .14$, stratified log-rank test). The OS at 3 years was 54% (95% CI, 36% to 69%) for the HSCT group versus 39% (95% CI, 24% to 55%) for the chemo group.

Table 4 presents the results of stratified univariate Cox proportional hazards models for RFS and OS. Among the variables tested, allogeneic HSCT as postremission therapy improved RFS (HR, 0.55; 95% CI, 0.27 to 1.1; $P = .09$) and OS

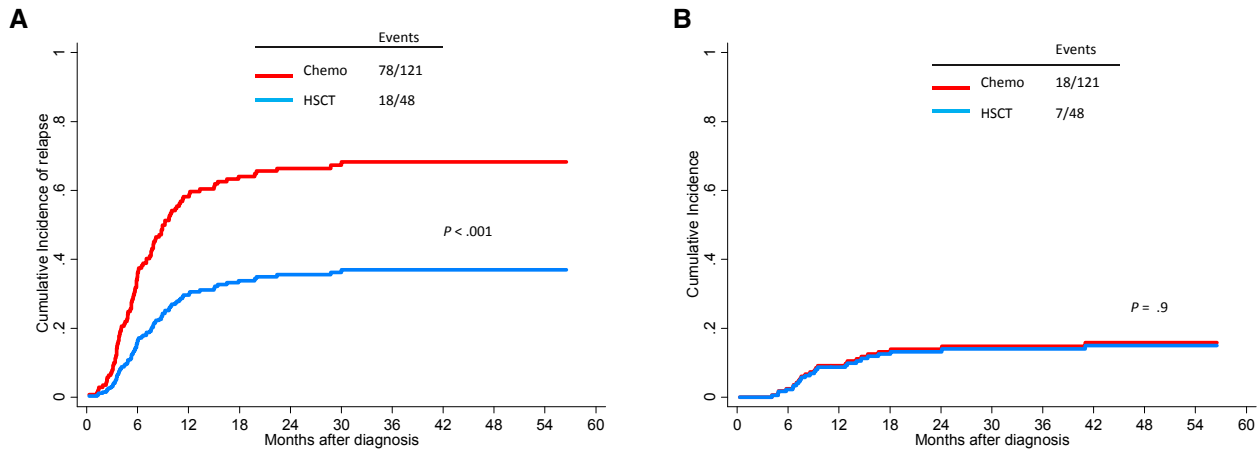

Figure 3. Cumulative incidence of relapse with NRM as the competing event (A) and NRM with relapse as the competing event (B) by the type of postremission therapy in CR1. The 3-year cumulative incidence of relapse was 68% in the chemo group and 41% in the HSCT group. The 3-year cumulative incidence of NRM was 15% in the chemo group and 13% in the HSCT group.

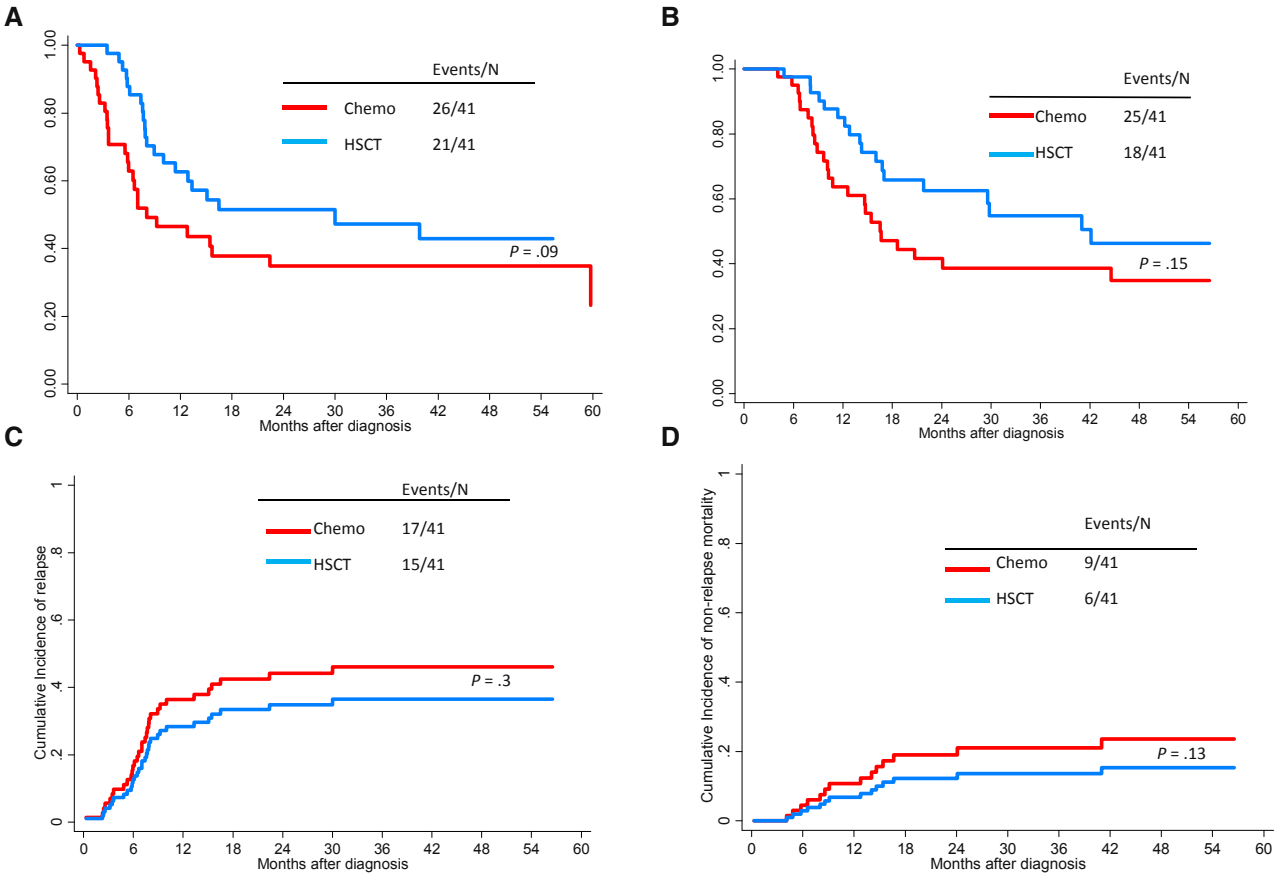

Figure 4. Estimates of RFS (A), OS (B), cumulative incidence of relapse (C), and NRM (D) by postremission therapy in 82 patients in CR1 for at least 4 months after propensity score matching. RFS at 3 years was 34% in the chemo group and 47% in the HSCT group. OS was 39% in the chemo group versus 54% in the HSCT group. The 3-year cumulative incidence of relapse was 45% in the chemo group versus 40% in the HSCT group, and NRM was 21% in the former versus 13% in the latter.

(HR, 0.58; 95% CI, 0.28 to 1.22; $P = .10$), but the difference did not reach statistical significance. No other variable was found to be a significant prognostic factor for RFS or OS.

Table 4
Univariate Analyses for RFS and OS After Propensity Score Matching*

Variable	RFS			OS		
	HR	95% CI	*P*	HR	95% CI	*P*
Age	0.99	0.96-1.02	.54	1.01	0.97-1.05	.66
Log(WBC) at diagnosis	0.97	0.65-1.47	.90	1.05	0.69-1.60	.80
Log(platelet) count at diagnosis	1.17	0.62-2.19	.63	1.00	0.52-1.92	.99
BM blasts at diagnosis	1.00	0.98-1.02	.97	1.00	0.98-1.02	.96
Cytogenetics at diagnosis (diploid versus other)	4.00	0.85-18.84	.08	4.00	0.85-18.84	.08
Log (*FLT3*-ITD allelic ratio) at diagnosis	0.93	0.62-1.37	.70	0.92	0.61-1.38	.68
FLT3-ITD allelic ratio ≥0.3 at diagnosis (yes versus no)	1.40	0.44-4.41	.57	1.40	0.44-4.41	.57
Presence of *NPM1* mutation						
Yes versus no	0.77	0.26-2.31	.64	0.79	0.24-2.64	.70
Unknown versus no	0.67	0.15-3.05	.61	0.52	0.10-2.63	.43
Year of diagnosis after 2008 (yes versus no)	1.50	0.25-8.98	.66	4.00	0.45-35.79	.22
Allogeneic HSCT (yes versus no)	0.55	0.27-1.10	.09	0.58	0.28-1.22	.15
Use of high-dose cytarabine (yes versus no)	0.33	0.03-3.20	.34	0.33	0.03-3.20	.34

* Number of *FLT3*-ITD mutations and the use of FLT3 inhibitors were not analyzed for their impact on RFS and OS owing to sample size <10 in groups.

The cumulative incidence of relapse at 3 years was 40% (95% CI, 24% to 56%) for the HSCT group and 45% (95% CI, 28% to 60%) for the chemo group (Figure 4C). There was no difference in NRM between the 2 groups (Figure 4D).

Sensitivity Analyses for RFS and OS

We repeated our analyses with another "minimum time" to be in CR1 and alive, to address lead time bias and confirm our results with a sensitivity analysis. In the repeat analyses, we included patients who had remained in CR1 for at least 3 months. This increased the size of our study cohort to 184 patients, 136 in the chemo group and 48 in the HSCT group. Similar to the cohort of patients who remained in CR1 for at least 4 months, in this expanded cohort the patients in the HSCT group were younger (median age, 55 years versus 61.5 years; $P = .003$), more often diagnosed after 2008 (79.2% versus 55.9%; $P = .01$), and more often received high-dose cytarabine as part of induction/consolidation therapy (87.5% versus 61%; $P = .001$) (Supplementary Table 1). The results of univariate and multivariate regression analyses were very similar (Supplementary Table 2 and Table 3) to those for the study cohort with a lead time bias of 4 months. Multivariate regression showed that HSCT as postremission therapy improved RFS (HR, 0.39; 95% CI, 0.25 to 0.6; $P < .001$) and OS (HR, 0.43; 95% CI, 0.26 to 0.68; $P < .001$), whereas log(WBC) was a poor prognostic factor for both outcomes.

When propensity score matching was applied to the cohort that remained in CR1 for at least 3 months, we had 44

patients in the HSCT group and 44 in the chemo group. Age at diagnosis, log(*FLT3*-ITD allelic ratio), diagnosis after 2008, presence of *NPM1* mutation, and the use of high-dose cytarabine in induction/consolidation were the criteria used to estimate the propensity scores.

RFS and OS at 3 years were significantly improved with HSCT as postremission therapy, as shown in Figure 5A and B. RFS at 3 years was 45.5% (95% CI, 29% to 60.7%) for the HSCT group versus 21.8% (95% CI, 10.3% to 38.4%) for the chemo group. Univariate regression in this group identified the use of HSCT instead of chemotherapy as postremission therapy as the sole prognostic factor for RFS (HR, 0.31; $P = .002$) and OS (HR, 0.27; $P = .02$) (Supplementary Table 4).

The cumulative incidence of relapse was also lower in the HSCT group (Figure 5C). The incidence of relapse at 3 years was 40% (95% CI, 24% to 55%) in the HSCT group versus 55% (95% CI, 37% to 69%) in the chemo groups. NRM was similar in the 2 groups (Figure 5D).

DISCUSSION

As the use of molecular data for assigning prognosis in AML has become mainstream, it is increasingly important to define the role of HSCT in molecularly defined prognostic groups. In the present study, we evaluated the impact of allogeneic HSCT compared with chemotherapy as postremission therapy on clinical outcomes in 169 patients with AML and an *FLT3*-ITD mutation in CR1 after taking the *FLT3*-ITD allelic ratio and *NPM1* mutation into consideration. Our results indicate that allogeneic HSCT in CR1 is associated with prolonged RFS and OS independent of the *FLT3*-ITD allelic ratio and NPM1 mutation status in *FLT3*-ITD*mut* patients. Given that slightly less than one-quarter of our patients received an FLT3 inhibitor as part of induction therapy, our study is not adequately powered to analyze its impact on outcomes.

The role of allogeneic HSCT in treating *FLT3*-ITD*mut* AML has been a matter of debate ever since Gale et al. [12] published their experience with *FLT3*-ITD*mut* AML patients in CR1. In that study, patients were grouped according to the availability of a matched related donor into donor and no donor groups, and the donor group was found to have a lower (albeit nonsignificantly so) relapse incidence with no impact on OS. More recently reported data indicate a clinical benefit in *FLT3*-ITD*mut* AML after allogeneic HSCT, with significant improvements in both RFS and OS [11,16,26]. Our results also support the notion that allogeneic HSCT provides improvement in RFS and OS in *FLT3*-ITD*mut* AML patients when performed in CR1 with a matched donor.

Given the retrospective nature of our study, we used propensity score adjustment to accurately identify the impact of chemotherapy and allogeneic HSCT as postremission therapy on outcomes by balancing the covariates in the 2 groups and reducing selection bias when treatment assignment was not random [24]. However, this might have led to a large reduction in sample size while accounting for the selection bias associated with observed confounding variables but not observed latent confounding variables. We also included only patients who did not die or relapse within 4 months after achieving CR1, which was the median time to

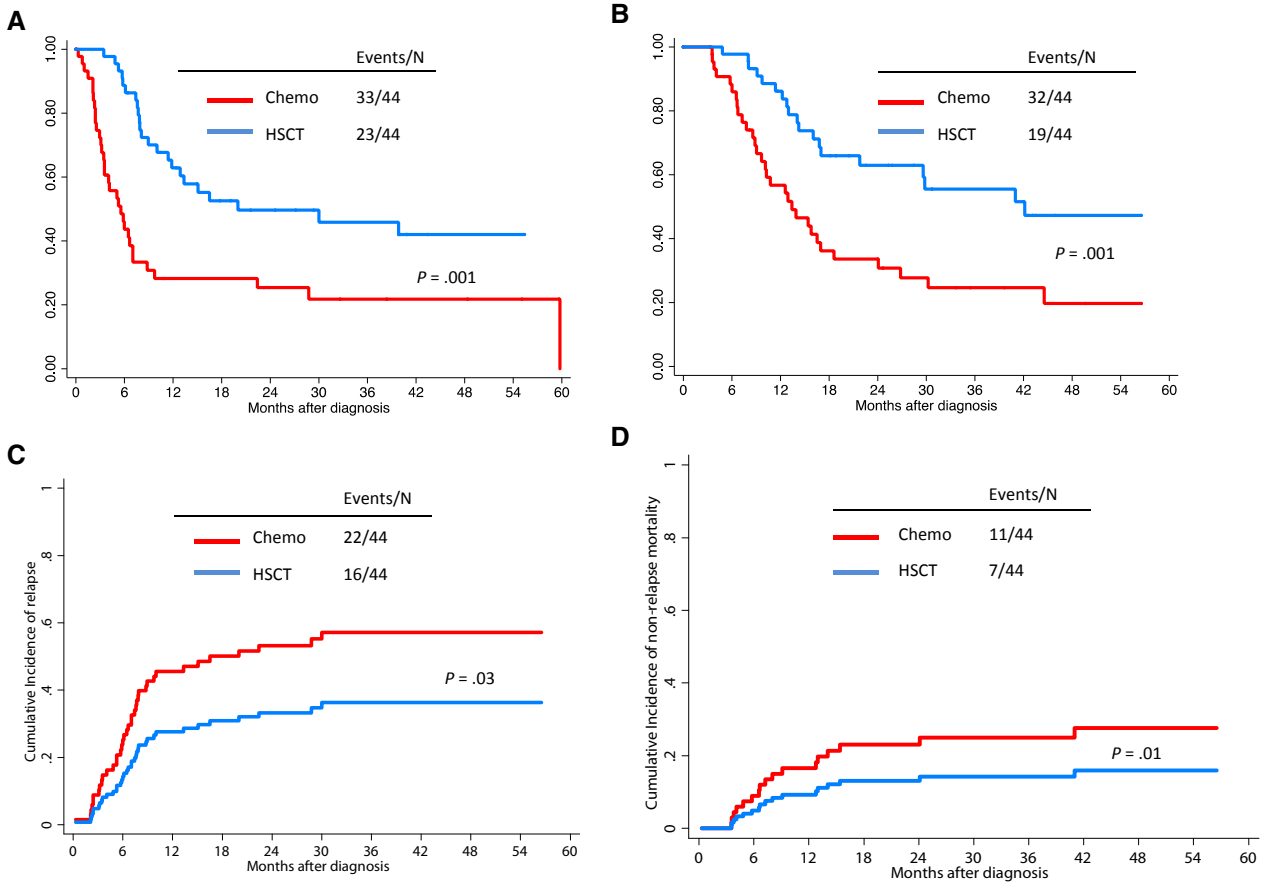

Figure 5. Estimates of RFS (A), OS (B), cumulative incidence of relapse (C), and NRM (D) by postremission therapy in 88 patients in CR1 for at least 3 months after propensity score matching. RFS and OS at 3 years were 46% and 55.5%, respectively, in the HSCT group versus 22% and 24.6% in the chemo group. The cumulative incidence of relapse was 40% in the HSCT group and 55% in the chemo group.

HSCT in AML patients at our institution, to reduce the lead time bias and make the study population as homogeneous as possible. We selected another minimum time to be alive and in CR1 and repeated our analyses to confirm our findings with a sensitivity analysis. Mismatched donor recipients were also excluded, to increase the homogeneity of the study population. Despite all of these efforts, however, the limitations of a nonprospective and nonrandomized study remain, as is true for other available data so far. Because our patients did not receive FLT3 inhibitors uniformly postrelapse, this study could not address the issue of any potential favorable impact of FLT3 inhibitors as salvage therapy on OS; therefore, our results should be interpreted cautiously until well-designed prospective clinical trials can confirm the findings.

AML is a polyclonal disease, and the allelic ratio is to some degree a reflection of the clonal burden of the *FLT3*-ITD*mut* cells within the leukemia cell population. Despite several studies showing that a higher mutant to wild-type allelic ratio is predictive of worse outcomes, the importance of taking into account not only the mutational status of *FLT3*-ITD at diagnosis, but also the allelic ratio for postremission therapy was not addressed until recently. Studies by German-Austrian AML Study Group [27] and the Spanish cooperative group CETLAM [9] showed that the benefit of allogeneic HSCT performed in CR1 may be restricted to patients with an allelic ratio of $\geq$0.51, and that allogeneic HSCT did not improve outcomes in patients with a low allelic ratio, suggesting that in these patients, the risk associated with allogeneic HSCT was not outweighed by its benefit. Our results, however, show improved RFS and OS after allogeneic HSCT in CR1 independent of the FLT3 allelic ratio. Our study differed from the previously published series in that the *FLT3*-ITD allelic ratio at diagnosis was lower, with a median of 0.3. After propensity score matching, the limited study group had an even lower allelic ratio at diagnosis (median, 0.18). The number of patients with an allelic ratio of $\geq$0.5, the generally accepted cutoff for high allelic ratio, was limited in our study cohort. Despite the lower allelic ratios at diagnosis compared with previously published studies, relapse incidence was the major reason for failure in both the chemo and HSCT groups, and the relapse incidence was similar with reports of higher allelic ratios. These results suggest that chemoresistance can be observed with any subclone of *FLT3*-ITD mutated cells during chemotherapy and may be the primary reason for treatment failure.

Similar to the clinical impact of the *FLT3*-ITD allelic ratio, the impact of *NPM1* mutation in *FLT3*-ITD*mut* patients is unclear [9,10,28,29]. The interaction of *NPM1* status and *FLT3*-ITD mutant level is important, particularly in post-remission therapy decisions. In our study, two-thirds of the patients had *NPM1* status evaluable and one-third of the patients had *NPM1* mutation. Although the presence of *NPM1* mutation was associated with favorable RFS and OS, this effect lost its significance on multivariate regression, suggesting that *FLT3*-ITD*mut* trumps the favorable prognosis of *NPM1* mutations and that those patients should be considered high risk. We believe that patients with *FLT3*-ITD and *NPM1* mutations will benefit from aggressive consolidation therapy with HSCT. The reported discrepancies in the literature may be attributed to the small size of the minor subgroups in some of the studies, the use of different thresholds for *FLT3*-ITD levels, and because *FLT3*-ITD levels might be underestimated in samples with low leukemic cell purity. However, a larger cohort of intermediate-risk patients treated through Medical Research Council showed similar outcomes in patients with an *NPM1* mutation when adjusted by high or low *FLT3*-ITD allelic ratio, suggesting that patients with an *NPM1* mutation and a low *FLT3*-ITD allelic ratio should not be considered different from those with a higher allelic ratio [29].

Despite improved outcomes compared with postremission chemotherapy, relapse remains the major reason for failure after allogeneic HSCT in AML. A recent European Group for Blood and Marrow Transplantation analysis showed that *FLT3*-ITD*mut* patients had a 2-year cumulative incidence of relapse in the range of 30% after allogeneic HSCT, double that seen in the *FLT3*-ITD*wild* group [15]. A recent study investigating transplantation outcomes in poor-risk patients by cytogenetics and the presence of *FLT3*-ITD*mut* showed a 3-year relapse incidence of 28% to 36% in normal karyotype AML patients with *FLT3*-ITD*mut* [26]. These results argue for innovative strategies to reduce relapse incidence and improve leukemia-free survival in *FLT3*-ITD*mut* AML [30,31].

FLT3 kinase inhibitors with promising evidence of clinical efficacy have been investigated alone and in combination with chemotherapy not only in front-line and salvage AML therapy [32-36], but also in the post-transplantation setting to prevent relapse. Most recently, Stone et al. [37] reported improved survival with the addition of FLT3 kinase inhibitor to standard chemotherapy compared with standard chemotherapy in a multicenter phase III trial. Similarly, the safety and efficacy of using FLT3 kinase inhibitors as maintenance therapy in the post-transplantation setting have been reported. Chen et al. [38] found a 2-year progression-free survival of 86% when sorafenib was given as maintenance therapy in *FLT3*-ITD*mut* AML patients who underwent transplantation in CR1 or CR2. It is also plausible that the addition of FLT3 inhibitors to induction and/or consolidation therapy before HSCT will yield a potential benefit of reducing early relapse and increasing the likelihood of proceeding with HSCT. Recent studies demonstrating improved CR rates and prolonged CR duration when FLT3 inhibitors are used in combination with hypomethylating agents [34] or chemotherapy [39-41] show promise that more patients can undergo allogeneic HSCT without early relapse. We believe that it is worth investigating the feasibility of using new-generation FLT3 inhibitors incorporated into leukemia treatment before HSCT, into conditioning regimens before HSCT, and then in post-transplantation maintenance after HSCT in *FLT3*-ITD*mut* AML patients. It is plausible that using such an integrated approach throughout different stages of leukemia treatment may lead to prolonged leukemia-free survival with low relapse rates and change the prognosis in *FLT*-ITD*mut* AML patients.

In summary, our analyses show that allogeneic HSCT with a matched related or unrelated donor provides favorable outcomes compared with consolidation chemotherapy in *FLT3*-ITD*mut* AML CR1 patients independent of their allelic ratio and *NMP1* mutation status. With the introduction of kinase inhibitors at different stages of disease treatment, transplantation outcomes may continue to improve. Well-designed prospective studies are needed to define what these promising drugs can offer when integrated with current treatment approaches.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2016.03.027.

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