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Significance of Persistent Cytogenetic Abnormalities on Myeloablative Allogeneic Stem Cell Transplantation in First Complete Remission

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Risk stratification is important to identify patients with acute myelogenous leukemia (AML) who might benefit from allogeneic hematopoietic stem cell transplantation (allo-HSCT) in first complete remission. We retrospectively studied 150 patients with AML and diagnostic cytogenetic abnormalities who underwent myeloablative allo-HSCT while in first complete remission to evaluate the prognostic impact of persistent cytogenetic abnormalities at allo-HSCT. Three risk groups were identified. Patients with favorable/intermediate cytogenetics at diagnosis (n = 49) and patients with unfavorable cytogenetics at diagnosis but without a persistent abnormal clone at allo-HSCT (n = 83) had a similar 3-year leukemia-free survival of 58%-60% despite the higher 3-year relapse incidence (RI) in the latter group (32.3%, versus 16.8% in the former group). A third group of patients with unfavorable cytogenetics at diagnosis and a persistent abnormal clone at allo-HSCT (n = 15) had the worst prognosis, with a 3-year RI of 57.5% and 3-year leukemia-free survival of only 29.2%. These data suggest that patients with AML and unfavorable cytogenetics at diagnosis and a persistent abnormal clone at allo-HSCT are at high risk for relapse after allo-HSCT. These patients should be considered for clinical trials designed to optimize conditioning regimens and/or to use preemptive strategies in the posttransplantion setting aimed at decreasing RI.

INTRODUCTION

The current recommendations for allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with acute myelogenous leukemia (AML) in first complete remission (CR1) are limited to patients in whom the risk of relapse significantly exceeds the risk of mortality from allo-HSCT, based on cytogenetic stratification into good-risk, intermediate-risk, and poor-risk AML. These recommendations are summarized in the National Comprehensive Cancer Network guidelines [1]. Of course, practice may continue to evolve with increasing knowledge of the pathobiology of AML and identification of new molecular markers. In that context, identifying new independent prognostic features in addition to well-established classical risk factors for high risk of relapse after allo-HSCT will help optimize the decision-making process for patients with AML undergoing allo-HSCT in CR1.

Studies of more sensitive and objective methods that can detect leukemia cells undetectable by morphology (ie, minimal residual disease [MRD]) have provided a rationale for the incorporation of MRD testing in risk assignment strategies [2,3]. The presence of MRD at the time of allo-HSCT has emerged as a valuable prognostic indicator of relapse posttransplantation in patients with chronic myelogenous leukemia [4,5] and pediatric acute lymphoblastic leukemia [6,7]; however, its independent prognostic significance in patients with AML is less clear [8]. Here we report a retrospective analysis assessing the prognostic impact of a persistent abnormal karyotype before allo-HSCT in patients with AML in CR1.

PATIENTS AND METHODS

Patient Population

This study included patients age >18 years with AML with cytogenetic abnormalities at initial diagnosis who were in first morphological complete remission at allo-HSCT, had either a matched sibling donor (MSD) or a matched unrelated donor (MUD), and underwent first allo-HSCT with a myeloablative conditioning regimen between January 1, 2001, and June 1, 2011 at the University of Texas M.D. Anderson Cancer Center. The study group included patients with a previous diagnosis of myelodysplastic syndrome or a myeloproliferative disorder who progressed to AML. Treatment protocols and this retrospective analysis were approved by the University of Texas M.D. Anderson Cancer Center's Institutional Review Board. All patients provided written informed consent for allo-HSCT.

Conditioning Regimen and Graft-versus-Host Disease Prophylaxis

The patients received a busulfan and fludarabine conditioning regimen consisting of i.v. busulfan either at a dose calculated to target an average daily systemic exposure dose, represented by an area under the concentration-versus-time curve of 6000 μMol·min ± 10%, or 130 mg/m^2, along with fludarabine 40 mg/m^2 for 4 days [9]. Six of the 150 patients received cloforabine [10], and 9 received plerifaxor in addition to busulfan and fludarabine. MUD graft recipients received either equine antithymocyte globulin 20 mg/kg/day for 3 days or rabbit antithymocyte globulin 0.5 mg/kg/day for 1 day, followed by 1.5 mg/kg the next day and 2.0 mg/kg the day after that. In 145 patients (96.6%), graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and methotrexate 5 mg/m^2 i.v. on posttransplantation days 1, 3, 6, and 11. Fourteen of these 145 patients also received pentostatin as described previously [11]. The other 5 patients received posttransplantation cyclophosphamide for GVHD prophylaxis.

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Cytogenetic Analysis and Definitions

All samples were analyzed by standard cytogenetic techniques. At least 20 metaphases from bone marrow (BM) were analyzed before a karyotype was defined as normal. For samples with abnormal cytogenetics, analyses with fewer than 20 metaphases were acceptable. An abnormality was considered clonal and thus mentioned in the karyotype when at least 2 metaphases had the same aberration in cases of a structural abnormality or an extra chromosome, in accordance with the International System for Human Cytogenetic Nomenclature [12]. Any monosomy had to be present in at least 3 metaphases. Cytogenetic risk group was assigned based on criteria published by the Southwest Oncology Group and Eastern Cooperative Oncology Group [13]. The unfavorable-risk group included patients with −5/del(5q), −7/del(7q), inv(3q), abn 11q, 20q, or 21q, del(9q), t(6;9), t(9;22), abn 17p, or a complex karyotype (3 or more abnormalities). All other patients and patients with inv(16)/t(16;16) with or without any additional abnormalities and with t(8;21) without del(9q) and not part of a complex karyotype and who retained their favorable characterization were included in the intermediate/favorable-risk group.

Complete remission (CR) was defined as ≤5% blast cells, no Auer rods, and no cluster of blast cells on BM analysis, as well as no evidence of extramedullary leukemia. Patients with an absolute neutrophil count <1000/μL and a platelet count <100,000/μL with transfusion dependence were classified as CR incomplete (CRi). Relapse was defined as ≥5% blasts in the BM or the development of extramedullary leukemia.

Statistical Analyses

The primary study endpoint was cumulative incidence of relapse (CIR). Other study endpoints included probabilities of leukemia-free survival (LFS), overall survival (OS), and transplantation-related mortality (TRM). LFS was defined as survival without disease progression or relapse; patients alive without disease progression or relapse were censored at the time of last follow-up. OS was defined as the time from stem cell infusion to death from any cause. Patients who were alive were censored at the time of last contact.

Patient and transplant characteristics by persistence of an abnormal clone at allo-HSCT were compared using the χ^2 test for categorical data and the Wilcoxon rank sum test for continuous data. LFS and OS were calculated using the Kaplan-Meier method. Univariate comparisons of all endpoints were performed using the log-rank test. Cumulative incidence was used to estimate the endpoints of CIR and TRM. A Cox proportional hazards model [14] or the Fine and Gray method [15] for competing hazards was used for multivariate regression. Variables included in the models were age (≤50 years versus >50 years), sex, secondary AML, cytogenetic risk group at diagnosis (favorable/intermediate versus unfavorable), CR with recovery of peripheral blood stem cell (PBSC) counts, donor type (MSD versus MUD), and presence of persistent abnormal clone at allo-HSCT. Because stem cell source (BM versus PBSCs) was highly correlated with donor type, it not included in the models. All factors were tested for the proportional hazards assumption. The presence of a persistent abnormal clone detected by cytogenetic analysis was included in each model. Analyses were performed using Stata version 11.2 for Windows (StataCorp, College Station, TX).

RESULTS

Patient Characteristics

Among the 251 patients undergoing first allo-HSCT with myeloablative conditioning and had either an MSD or an MUD in CR1, 156 (62.2%) had a cytogenetic abnormality at diagnosis. Eighteen of these 156 patients (11.5%) had a persistent clone with an abnormal karyotype at transplantation (AKAT) that was identical to the clone present at diagnosis, 132 (82.6%) had a normal karyotype at transplantation (NKAT), and 6 (3.9%) did not undergo cytogenetic evaluation before allo-HSCT. Patient and disease characteristics are summarized in Tables 1 and 2. The interval between cytogenetic evaluation and allo-HSCT did not differ between patients with AKAT and those with NKAT (median, 18 days [interquartile range (IQR), 14-29 days] versus 16 days [15-19 days]; $P = .30$). Patients with AKAT were more likely to have AML with unfavorable cytogenetics at diagnosis ($P = .09$), a higher frequency of secondary AML ($P < .001$), and incomplete recovery of peripheral blood (PB) counts (CRi; $P < .001$). Median age at transplantation, donor type (MSD versus MUD), and stem cell source (BM versus PBSCs) were similar in the 2 groups, as well as the median interval from diagnosis of AML to allo-HSCT (median, 5.2 months [range, 1.9-38.4 months] versus 5.2 months [3.3-77.4 months]; $P = .30$).

Table 1
Clinical and Transplantation Characteristics of the Study Population

	NKAT (n = 132)		AKAT (n = 18)		
	n	%	n	%	*P*
Age, years					
Median	49		50.5		
IQR	36-55		39-56		.70
Sex					
Female	63	47.7	6	33.3	
Male	69	52.3	12	66.7	.20
Secondary AML					
No	120	90.9	11	61.1	
Yes	12	9.1	7	38.9	<.001
Cytogenetic risk group					
Favorable/intermediate	49	37.1	3	16.7	
Unfavorable	83	62.9	15	83.3	.09
CR with recovery of PBSC counts					
Yes	122	92.4	11	61.1	
No	10	7.6	7	38.9	<.001
Time from cytogenetic evaluation to HSCT, days					
Median	16		18		
IQR	15-19		14-29		.30
Donor type					
MRD	73	55.3	9	50.0	
MUD	59	44.7	9	50.0	.70
Source of stem cells					
BM	44	33.3	6	33.3	
PBSC	88	66.7	12	66.7	1.0
Time from diagnosis to HSCT, months					
Median	5.1		5.2		
IQR	4-7.6		4-9.1		.30

NKAT indicates normal karyotype at transplantation; AKAT, abnormal karyotype at transplantation; IQR, interquartile range; CR, complete remission; PBSC, peripheral blood stem cells; HSCT, hematopoietic stem cell transplantation; MRD, matched related donor; MUD, matched unrelated donor; BM, bone marrow; PBSC, peripheral blood stem cells.

The stem cell source was PBSCs in 100 patients (66.7%) and BM in 50 patients (33.3%). PBSCs were used more often in MSD transplantations than in MUD transplantations (93.9% versus 33.8%; $P < .001$).

OS, LFS, CIR, and TRM

The median follow-up duration in allo-HSCT survivors was 40.9 months (IQR, 16.6-63.4 months) and was similar in both cytogenetic groups ($P = .80$). Estimated 3-year OS was 52.4% (95% confidence interval [CI], 25.9%-73.4%) for patients with AKAT and 66.7% (95% CI, 57.7%-74.4%; $P = .03$) for patients with NKAT, and estimated 3-year LFS was 36.5% (95% CI, 14.9%-58.6%) for the former and 59.0% (95% CI, 49.8%-67.1%) for the latter. Although median OS was not reached (NR) in both groups, median LFS was shorter in patients with AKAT compared with those with NKAT (11.4 months [IQR, 3 months to NR] versus 71.8 months [IQR, 8.4 months to NR]; $P = .06$).

CIR was higher in patients with AKAT, with a 3-year cumulative incidence of 46.9% (95% CI, 22.4%-68.1%), compared with 26.5% in patients with NKAT (95% CI, 19.2%-34.4%), although the difference did not reach statistical significance ($P = .10$). The 3-year TRM rate was similar in the 2 groups (16.7% [95% CI, 4.1%-36.5%] in AKAT versus 14.4% [95% CI, 8.9%-21.2%] in NKAT; $P = .90$).

A longer interval between cytogenetic assessment and allo-HSCT could theoretically allow time for reappearance of disease, resulting in bias in classification. Nonetheless, a comparison of estimated OS, LFS, CIR, and TRM in 12 NKAT

Table 2
Clinical Characteristics of Patients with Abnormal Karyotype at Transplantation

Patient	Age, Years	Diagnostic Cytogenetic Abnormality	Diagnostic Molecular Abnormality	Therapy-Related AML	Antecedent Hematologic Disorder	CR Status before HSCT	Relapse after HSCT	Time to Relapse after HSCT	Therapy for Relapse after HSCT	Survival after Relapse	Survival after HSCT	Cause of Death
1	52	45, XY, add(5)(q11.2), t(6; 11)(q21; p15), −17, +mar	NPM-1(−); C-KIT(−); RAS(−); FLT-3(−)	Yes	No	CRi	No				2.3 months	MOF
2	45	43-44, XY, del(5)(q13q33), −6, −7, −18, add(19)(q13.1)	FLT-3(−); RAS(−)	No	No	CRi	No				48.6 months	Alive
3	43	47, XY, del(3)(q21q27), +21	NP	No	MDS	CR	No				97.4 months	Alive
4	32	44, XY, −5, del(7)(q22), +11, −17, −20	NP	No	No	CR	Yes	19.6 months	Palliative care	1 month	20.6 months	Relapse
5	43	47, XX, der(3) inv(3)(p12q12) del(3)(q13.3), t(11; 15)(q24; q21), +21	NP	No	No	CR	No				97.4 months	Alive
6	19	46, XY, t(3; 6)(q25; q26), del(5)(q13q33)	NP	No	AA	CRi	Yes	4.9 months	Fludarabine and cytarabine; decitabine	5.8 months	10.8 monhts	Leukemia
7	58	49, XY, +8, +9, +14	FLT-3(−); RAS(−)	No	No	CRi	No				34.7 months	Alive
8	57	47, XY, del(7)(q22q36), +9	NP	No	CMML	CRi	Yes	90 days	Palliative care	1.3 months	4.3 months	Leukemia
9	56	46, XY, del(7)(q11.2q32)	FLT-3(+); NMP1(−); RAS(−)	No	No	CR	Yes	91 days	5-Azacitidine with sorafenib; mitoxantrone with etoposide	309 days	14 months	Alive
10	52	46, XX, t(6; 9)(p23; q34)	NPM-1(−); C-KIT(−); RAS(−); FLT-3(+)	Yes	No	CR	No				12 months	Alive
11	62	45, XY, −7	FLT-3(−); RAS(−)	No	MDS	CRi	NE				1 month	Graft failure
12	35	45, XY, t(3; 12)(q26.2; p13), −7	FLT3(+); RAS(−); C-KIT(−)	No	No	CR	Yes	341 days	Idarubicin, cytarabine, and decitabin	1120 days	48.7 months	Alive
13	51	47-51, XX, −2, −3, −5, −7, −12, −13, +6-9 mar	NP	No	Ph negative MPD	CRi	Yes	62 days	Palliative care	93 days	5.2 months	Leukemia
14	46	46, XX, +i(1)(p10), del(4)(q21q35), add(5)(q11.2), −17	NP	No	No	CR	Yes	134 days	Decitabine and SAHA	77 days	7 months	Leukemia
15	40	46, XX, t(6; 11)(q27; q23)	FLT3(+); NPM1(−); CEBPA(−)	No	No	CRi	No				13 months	Alive
16	55	47, XY, +8	FLT-3(−); RAS(−)	No	MDS	CR	No				69 months	Alive
17	30	46, XX, t(2; 3)(p23; q29)	FLT-3(−); RAS(−)	No	MDS	CR	No				36.1 months	Alive
18	64	46, XY, del(12)(p12p13)	FLT-3(+); RAS(−)	No	CMML	CRi	NE				0.5 month	MOF

AA indicates aplastic anemia; CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; MOF, multiorgan failure; MPD, myeloproliferative disorder; NE, not evaluable; NP, not performed.

Table 3
Univariate Analyses for Disease Outcomes

	OS			LFS			RI			TRM		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age												
<50 years	1.0			1.0			1.0			1.0		
≥50 years	1.7	1.1-2.9	.05	15	0.9-2.4	.10	0.9	0.5-1.6	.60	3.5	1.4-8.7	.008
Sex												
Female	1.0			1.0			1.0			1.0		
Male	2.0	1.1-3.5	.02	2.0	1.2-3.4	.05	1.5	0.8-2.7	.20	2.7	1.1-6.7	.03
Secondary AML												
No	1.0			1.0			1.0			1.0		
Yes	1.8	0.9-36	.10	1.3	0.6-2.6	.50	0.9	0.3-2.7	.90	1.5	0.5-4.5	.50
Cytogenetic risk group												
Favorable/intermediate	1.0			1.0			1.0			1.0		
Unfavorable	1.1	0.6-2.0	.60	1.4	0.8-2.4	.20	2.8	1.3-5.9	.009	0.5	0.2-1.1	.06
CR with recovery of PBSC counts												
Yes	1.0			1.0			1.0			1.0		
No	1.3	0.6-3.0	.40	1.4	0.7-2.7	.30	1.5	0.6-3.7	.40	0.7	0.2-3.3	.60
Cytogenetics at allo-HSCT												
NKAT	1.0			1.0			1.0			1.0		
AKAT	1.5	0.7-3.2	.30	1.8	0.9-3.5	.07	1.8	0.8-3.8	.10	1.1	0.3-4.0	.90
Donor type												
MRD	1.0			1.0			1.0			1.0		
MUD	0.8	0.5-1.4	.40	0.8	0.5-1.2	.20	0.6	0.3-1.0	.06	1.5	0.6-3.2	.30
Stem cell source												
PBSC	1.0			1.0						1.0		
BM	1.0	0.6-1.8	.90	1.0	0.6-1.7	.90	0.5	0.3-1.0	.06	2.9	1.3-6.5	.009

OS indicates overall survival; LFS, leukemia free survival; CIR, cumulative incidence of relapse; TRM, transplant related mortality; HR, hazard ratio; CI, confidence interval; CR, complete remission; PBSC, peripheral blood stem cell; allo-HCT, allogeneic hemetopoietic stem cell transplantation; NKAT, normal karyotype at transplantation; AKAT, abnormal karyotype at transplantation; MRD, matched related donor; MUD, matched unrelated donor; BM, bone marrow.

patients with an interval of >28 days and 120 patients with an interval of ≤28 days showed no significant differences ($P = .50$ for OS, $P = .30$ for LFS, $P = .40$ for CIR, and $P = .50$ for TRM).

Univariate and multivariate regression models were applied to analyze OS, LFS, CIR, and TRM. The unadjusted hazard ratio (HR) of AKAT versus NKAT was 1.5 (95% CI, 0.7-3.2; $P = .30$) for OS, 1.8 (95% CI, 0.9-3.5; $P = .07$) for LFS, 1.8 (95% CI, 0.8-3.8; $P = .10$) for CIR, and 1.1 (95% CI, 0.3-4.0; $P = .90$) for TRM (Table 3). Age ≥50 years and male sex were associated with worse OS, with unadjusted HRs of 1.7 (95% CI, 1.1-2.9; $P = .05$) and 2.0 (95% CI, 1.1-3.5; $P = .02$), respectively. For LFS, male sex was prognostic of shorter LFS (unadjusted HR, 2.0; 95% CI, 1.2-3.4; $P = .05$). The risk of CIR was increased by the presence of unfavorable cytogenetics at the time of diagnosis (unadjusted HR, 2.8; 95% CI, 1.3-5.9; $P = .009$). Undergoing MUD allo-HSCT versus MSD and using BM versus PBSCs as the stem cell source were associated with decreased CIR, with unadjusted HRs of 0.6 (95% CI, 0.3-1.0; $P = .06$) and 0.5 (95% CI, 0.3-1.0; $P = .06$), respectively. For TRM, age ≥50 years (unadjusted HR, 3.5; 95% CI, 1.4-8.7; $P = .008$), male sex (2.7; 95% CI, 1.1-6.7; $P = .03$), and a BM stem cell source (2.9; 95% CI, 1.3-6.7; $P = .009$) were associated with worse outcomes.

After adjustment for other covariates, the adjusted HR of AKAT versus NKAT was 1.5 (95% CI, 0.7-3.2, $P = .30$) for OS and 1.7 (95% CI, 0.9-3.2; $P = .10$) for LFS. For OS, age ≥50 years and male sex were prognostic factors (adjusted HR, 1.7; 95% CI, 1.1-3.0; $P = .04$ and 1.9; 95% CI, 1.1-3.3; $P = .03$, respectively). For LFS, only male sex was a poor prognostic factor (adjusted HR, 2.0; 95% CI, 1.2-.3.3; $P = .007$). In patients with AKAT, the adjusted HR was 1.6 (95% CI, 0.8-3.4; $P = .20$) for CIR and 1.1 (95% CI, 0.3-4.0; $P = .90$) for TRM. Unfavorable cytogenetics at diagnosis was found to increase the CIR (adjusted HR, 2.8; 95% CI, 1.3-5.9; $P = .009$), whereas an MUD allo-HSCT was associated with decreased CIR (adjusted HR, 0.5; 95% CI, 0.3-0.9; $P = .04$). For TRM, age ≥50 years (adjusted HR, 3.6; 95% CI, 1.5-8.8; $P = .005$) and male sex (adjusted HR, 2.9; 95% CI, 1.1-7.3; $P = .03$) were found to be poor prognostic factors.

Regression Models after Combining Diagnostic Cytogenetics and Presence of a Persistent Abnormal Clone

To test our hypothesis that AKAT is associated with increased risk of relapse and subsequent decreased LFS, we categorized the patients into 3 risk groups: favorable/intermediate cytogenetics at diagnosis (n = 49), unfavorable cytogenetics at diagnosis with NKAT (n = 83), and unfavorable cytogenetics at diagnosis with AKAT (n = 15). Only 3 patients with favorable/intermediate cytogenetics at diagnosis had AKAT, and thus they were not included in this analysis.

We found that OS was similar among the 3 risk groups ($P = .50$), but LFS was lower in the patients with unfavorable cytogenetics at diagnosis with AKAT compared with the other 2 groups ($P = .05$) (Table 4 and Figure 1A and B). CIR was also highest in the patients with unfavorable cytogenetics at diagnosis with AKAT (unadjusted HR, 4.2; 95% CI, 1.6-11.0; $P = .003$), followed by patients with unfavorable cytogenetics at diagnosis with NKAT (unadjusted HR, 2.3; 95% CI, 1.1-5.0; $P = .03$). The TRM rate was comparable in the 3 groups ($P = .20$).

Table 4
Summary of Outcomes: Three-Year Estimates of OS, LFS, RI, and TRM

	Favorable/ Intermediate Cytogenetics		Unfavorable Cytogenetics with NKAT		Unfavorable Cytogenetics with AKAT		
		95% CI		95% CI		95% CI	*P*
OS	65.6	49.1-77.9	67.1	55.4-76.4	48.0	18.9-72.4	.50
LFS	60.8	45-73.5	57.8	46.2-67.8	29.2	8.4-54.2	.05
RI	16.8	7.9-28.7	32.3	22.2-42.6	57.5	27.5-78.9	.002
TRM	22.3	11.4-35.4	10.0	4.6-17.6	13.3	6.8-34.6	.20

OS indicates overall survival; LFS, leukemia free survival; CIR, cumulative incidence of relapse; TRM, transplant related mortality; NKAT, normal karyotype at transplantation; AKAT, abnormal karyotype at transplantation; CI, confidence interval.

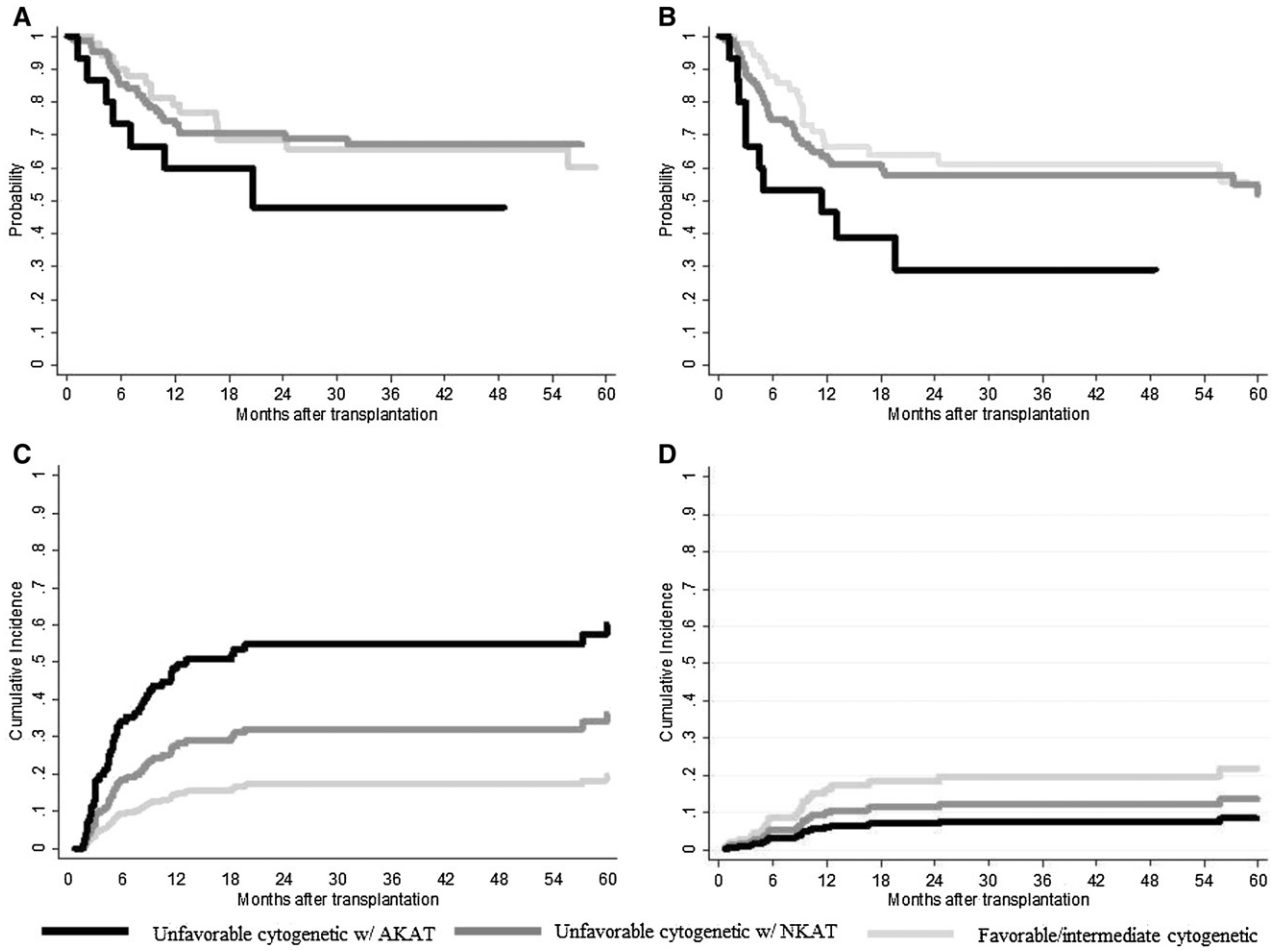

Figure 1. Probabilities of OS (A), LFS (B), CIR (C), and TRM (D) by diagnostic cytogenetics and persistence of an abnormal clone at allo-HSCT.

Multivariate regression analyses with risk groups combining diagnostic cytogenetics and persistence of an abnormal clone at allo-HSCT revealed increased CIR (HR, 4.5; 95% CI, 1.7-11.9; $P = .003$) and decreased LFS (adjusted HR, 2.4; 95% CI, 1.1-5.2; $P = .02$) in the patients with unfavorable cytogenetics with AKAT (Table 5). The patients with unfavorable cytogenetics with NKAT also exhibited increased CIR (adjusted HR, 2.4; 95% CI, 1.1-5.3; $P = .02$). This risk group was not prognostic for OS and TRM, however. Only male sex was identified as a poor prognostic factor for OS (adjusted HR, 1.9; 95% CI, 1.1-3.4; $P = .03$). Undergoing MUD allo-HSCT was associated with decreased CIR (adjusted HR, 0.5; 95% CI, 0.3-0.9; $P = .04$). For TRM, age ≥50 years was the sole poor prognostic factor identified (adjusted HR, 3.4; 95% CI, 1.3-8.9; $P = .01$).

DISCUSSION

In this study involving a relatively large cohort (n = 150) of patients with AML in morphological CR1 treated with allo-HSCT, residual disease, defined as the persistence of

Table 5
Multivariate Regression Models for OS, LFS, RI, and TRM

	OS			LFS			RI			TRM		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age												
<50 years	1.0									1.0		
≥50 years	1.7	0.9-2.9	.06							3.4	1.3-8.9	.01
Sex												
Female	1.0			1.0						1.0		
Male	1.9	1.1-3.4	.03	2.0	1.2-3.4	.07				2.4	0.9-5.9	.06
Cytogenetic and persistent abnormal clone												
Favorable/intermediate cytogenetics	1.0			1.0			1.0			1.0		
Unfavorable cytogenetics with NKAT	1.1	0.6-2.1	.70	1.4	0.8-2.4	.20	2.4	1.1-5.3	.02	0.5	0.2-1.2	.10
Unfavorable cytogenetics with AKAT	1.8	0.7-4.4	.20	2.4	1.2-5.2	.02	4.5	1.7-11.9	.003	0.6	0.1-2.8	.50
Donor type												
MRD							1.0			1.0		
MUD							0.5	0.3-0.9	.04	2.9	0.8-4.4	.1

LFS indicates leukemia free survival; CIR, cumulative incidence relapse; TRM, transplant related mortality; HR, hazard ratio; CI, confidence interval; NKAT, normal karyotype at transplantation; AKAT, abnormal karyotype at transplantation; MRD, matched related donor; MUD, matched unrelated donor.

previously detected cytogenetically abnormal clones at the time of transplantation (AKAT), was associated with poor prognosis, with increased RI and decreased LFS. In our cohort, AKAT was significantly associated with other poor-risk features, including secondary AML, unfavorable cytogenetics at diagnosis, and incomplete recovery of PB counts before allo-HSCT, consistent with the literature [8]. However, given that 15 of 18 patients in the AKAT group had unfavorable cytogenetics at diagnosis, this association was strongest with unfavorable cytogenetics at diagnosis. Ultimately, we combined diagnostic cytogenetic risk groups and the presence of cytogenetically abnormal clones at allo-HSCT to identify 3 risk groups. The patients with favorable/intermediate cytogenetics at diagnosis and those with unfavorable cytogenetics at diagnosis with NKAT exhibited a similar 3-year LFS of approximately 58%-60%. The third group of patients with unfavorable cytogenetics at diagnosis and AKAT had the worst prognosis, with a 3-year LFS of only 29.2%. OS differed among the groups, but the difference between the group with unfavorable cytogenetics and NKAT and the group with unfavorable cytogenetics and AKAT did not reach statistical significance, most likely owing to the relatively small size of the latter group. Despite its limitations, this risk classification allowed us to control for the confounding effect of diagnostic cytogenetics while interpreting the impact of a persistent abnormal clone at the time of allo-HSCT on disease outcomes, and also provided a prognostic tool for predicting CIR and LFS.

Persistent cytogenetic and/or molecular abnormalities after induction chemotherapy are known to be poor independent prognostic factors in AML [16,17]. For patients without baseline cytogenetic or molecular abnormalities, demonstration of MRD by multicolor flow cytometry (MFC) is also correlated with inferior outcomes [3]. Among the techniques for detecting MRD, cytogenetic evaluation has the disadvantages of decreased sensitivity (5%) and the need to prepare metaphase chromosomes, with only 50%-55% of patients with AML exhibiting recurrent cytogenetic abnormalities for AML at diagnosis. Despite the limitations of MRD detection by chromosome banding, the persistence of cytogenetic abnormalities after induction or consolidation therapy has been shown to be predictive of residual disease in patients with AML [18-20]. Recently, Chen et al. [16] reported the independent prognostic value of persistent cytogenetic abnormalities at CR after induction chemotherapy in patients with AML, with only allo-HSCT improving outcomes in this group of patients. Those findings support the idea that the presence of MRD, even when detected by a less-sensitive technique, can help predict outcomes and guide the choice of treatment options.

The impact of MRD at allo-HSCT on disease outcomes and its potential to deliver tailored therapies are not yet well established. Walter et al. [8] reported a negative impact of MRD at allo-HSCT on disease outcomes. In their study of 99 patients, the patients with MRD, indicated by positive MFC results, had a 2-year disease-free survival (DFS) of 9%, compared with 74.8% in those without MRD. Similar to our findings, the authors reported an association of MRD with other adverse risk factors, including unfavorable cytogenetics at diagnosis, suggesting that MRD may be a surrogate marker for high-risk disease rather than an independent poor prognostic factor for disease outcomes after allo-HSCT.

Whether MRD at allo-HSCT should be an indication for further chemotherapy to eradicate the persistent clone, or whether patients with MRD should undergo allo-HSCT with no further delay considering that the persistent clone is more likely chemotherapy-resistant, has not yet been investigated. The available data indicating no benefit from postremission chemotherapy before allo-HSCT in patients with AML in CR1 did not take the presence of MRD into account [21-23]. On the other hand, among patients with MRD at postinduction or postconsolidation therapy, better outcomes have been reported in those who proceed to allo-HSCT compared with those who receive further chemotherapy. Maurillo et al. [3] reported a 5-year relapse-free survival (RFS) of only 11% in patients with MRD detected by MFC after consolidation who did not undergo allo-HSCT, compared with 47% in those who did undergo allo-HSCT. Buccisano et al. [24] reported preliminary results of a risk stratification model in AML CR1 aimed at identifying patients who might benefit from allo-HSCT earlier in their disease course. In their high-risk group, including patients with MRD, they observed a 70% DFS rate in the patients who underwent allo-HSCT, compared with 20% in a historical control group who received further chemotherapy but did not proceed to allo-HSCT. Until a well-designed randomized clinical trial investigating the role of further chemotherapy to eradicate MRD before allo-HSCT is available, it might be better to proceed with allo-HSCT with no further delay considering the dismal outcomes in patients with MRD who do not undergo transplantation.

In conclusion, our results indicate that MRD at allo-HSCT, even when detected by a less-sensitive technique, can identify patients at high risk for relapse with decreased LFS after transplantation. Despite the poor outcomes reported in this population in the transplantation setting, these patients should still be considered for allo-HSCT while in CR1, and future efforts should focus on improving transplantation outcomes in this high-risk group. Recent studies reporting the use of hypomethylating agents given preemptively in the posttransplantation setting [25] and modifications of conditioning regimens [10,26] to decrease the risk of relapse have shown promising results, and prospective multicenter trials to confirm those results in larger patient populations should be prioritized within the transplantation community.

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