Acute megakaryoblastic leukemia, unlike acute erythroid leukemia, predicts an unfavorable outcome after allogeneic HSCT

Acute megakaryoblastic leukemia, unlike acute erythroid leukemia, predicts an unfavorable outcome after allogeneic HSCT

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Abstract

Acute erythroid leukemia (FAB-M6) and acute megakaryoblastic leukemia (FAB-M7) exhibit closely related properties in cells regarding morphology and the gene expression profile. Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered the mainstay of the treatment for both subtypes of leukemia due to their refractoriness to chemotherapy and high rates of relapse, it remains unclear whether allo-HSCT is curative in such cases due to their scarcity. We retrospectively examined the impact of allo-HSCT in 382 patients with M6 and 108 patients with M7 using nationwide HSCT data and found the overall survival (OS) and relapse rates of the M6 patients to be significantly better than those of the M7 patients after adjusting for confounding factors and statistically comparable with those of the patients with M0/M1/M2/M4/M5 disease. Consequently, the factors of age, gender, performance status, karyotype, disease status at HSCT and development of graft-versus-host disease predicted the OS for the M6 patients, while the performance status and disease status at HSCT were predictive of the OS for the M7 patients. These findings substantiate the importance of distinguishing between M6 and M7 in the HSCT setting and

suggest that unknown mechanisms influence the HSCT outcomes of these closely related subtypes of leukemia.

Keywords: acute erythroid leukemia, acute megakaryoblastic leukemia, allogeneic hematopoietic stem cell transplantation.

1 Introduction

All blood cell lineages are derived from a common hematopoietic stem cell[1]. A current dendrogram describing the process of blood cell fate determination postulates megakaryocyte and erythroid series to arise from common megakaryocyte-erythroid progenitors[2-6], and similarity between the erythroid and megakaryocytic lineages has been observed in terms of differentiation, regulation by growth factors and epigenetics[7-10]. In an analogous fashion, two rare subtypes of acute myeloid leukemia (AML), acute erythroid leukemia (M6 according to the FAB classification) and acute megakaryoblastic leukemia (M7 according to the FAB classification), are considered to be closely related in origin due to their morphologic similarity[3] as well as common patterns of the gene expression[11]. Both M6 and M7 are considered indications for allogeneic hematopoietic stem cell transplantation (allo-HSCT) in view of the poor prognosis of patients not treated with this procedure[12-15]. However, it remains uncertain whether the use of allo-HSCT for M6 or M7 of AML definitively improves the prognosis because the data are limited based on the fact that M6 and M7 comprise less than 5% of all AML cases. If M6 and M7 are innately identical, there may be similarities in

allo-HSCT outcomes between these two diseases. We therefore conducted a retrospective study to examine the outcomes of allo-HSCT in patients diagnosed with AML M6 or M7 using data obtained from a nationwide Japanese survey.

2 Methods

2.1 Study population

The data for *de novo* AML patients 16 years of age or older who underwent initial allo-HSCT between January 1996 and December 2010 were obtained from the Transplant Registry Unified Management Program (TRUMP) in Japan[16]. The clinical features and outcomes of these patients were investigated. The subtypes of M6 according to the FAB classification, M6a and M6b, were not distinguished in the database. The diagnosis which was made according to the results of a FACS analysis and the data and the risk status based on the cytogenetic subgroup was categorized at each institution, instead of a central review, according to the Southwestern Oncology Group criteria for favorable and unfavorable risks[17]; all others were included in the intermediate-risk category[18]. In addition, clinical data were collected from the databases of the

Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Cord Blood Bank Network using a standardized report form. This study was approved as an adult AML working group study by the Committee for Nationwide Survey Data Management of the JSHCT (study #2-12).

2.2 Statistical analysis

The characteristics of the M6 and M7 patients were compared using Fisher's exact test for categorical variables and the t-test for continuous variables. Overall survival (OS) was defined as the number of days from HSCT until death from any cause. Relapse-free survival (RFS) was defined as the number of days from HSCT to relapse of the underlying disease. Non-relapse mortality (NRM) was defined as death without relapse. Any patient who remained alive on the last date of follow-up was censored. The OS rates were calculated using the Kaplan-Meier method and compared using the log-rank test. The cumulative incidences of NRM (CI-NRM) and relapse were calculated considering each other type of event as a competing risk and compared using the stratified Grey test. Multivariate Cox models were used to evaluate the hazard ratios associated with the prognosis. The following variables related to survival were compared in a univariate analysis:

recipient characteristics (age: younger than 50 years *vs.* older than 50 years, blood type, gender, performance status at diagnosis: 0 to 2 *vs.* 3 or 4, FAB classification: M6 *vs.* M7 and cytogenetic subgroup), donor characteristics (blood type, blood type compatibility, gender, gender compatibility, relationship: related *vs.* unrelated and serological HLA compatibility), transplant characteristics (days from diagnosis to HSCT: less than 90 days, 90 days to 180 days, longer than 180 days, disease status at allo-HSCT: complete remission (CR) *vs.* non-CR, intensity of the preconditioning regimen (myeloablative *vs.* reduced intensity), use of total body irradiation as a preconditioning regimen, source of the graft: bone marrow (BM), peripheral blood stem cells (PBSCs) or cord blood (CB), the year of HSCT (before 2005 or after 2006) and transplant outcomes (development of acute graft-versus-host disease (GVHD): 0 or 1 *vs.* 2 to 4, development of chronic GVHD and relapse). The development of GVHD was treated as a time-dependent covariate. Covariates found to be significant in the univariate analyses ($P \leq 0.10$) were included in the Cox's proportional hazards models and Fine and Gray's proportional hazards models. For both the univariate and multivariate analyses, P values were two-sided and

outcomes were considered to be significant for $P \leq 0.05$. Matched-pair analysis was performed matching for the recipient' age, cytogenetic subgroup, disease status at HSCT, conditioning regimen, donor selection and graft source. All statistical analyses were performed using the EZR program (Saitama Medical Center, Jichi Medical University)[19], a graphical user interface for R (The R Foundation for Statistical Computing; http://www.r-project.org, version 2.14.1).

3 Results

3.1 Characteristics of the patients

The number of AML patients with M6 and M7 was 382 and 108, respectively (Table 1). No favorable cytogenetic risk patients were included in this study. There were no significant difference in age, WBC, the proportion of patients with CR1 at allo-HSCT or the cytogenetic subgroup between the two groups; however, the proportion of patients with any CR at allo-HSCT was lower in the M7 group than in the M6 group (34% *vs.* 46%, $p<0.04$) and the degree of HLA disparity was more significant in the M7 group than in the M6 group (proportion of HLA match HSCT: 64% in M6 *vs.* 57% in M7, $p<0.02$). These

findings were consistent with the low remission rates in M7.

3.2 Outcomes after allo-HSCT

The OS and relapse-free survival (RFS) rates were significantly inferior in the M7 patients than in the M6 patients (Figure 1, 5-year OS of the M6 patients and M7 patients: 35% and 17%, respectively ($P<0.0003$); 5-year RFS of the M6 patients and M7 patients: 34% and 14%, respectively ($P<0.0002$)). The CI-NRM was not significantly different between these two groups (Figure 2(a), 3-year CI-NRM: 22% in the M6 patients and 27% in the M7 patients, $P=0.29$); however, the CI-relapse rate was significantly worse in the M7 patients than in the M6 patients (Figure 2(b), 3-year CI-relapse for the M6 patients and M7 patients: 30% and 46%, respectively ($P<0.02$)). The CI-relapse rates among the patients with CR at HSCT were significantly worse in the M7 group than in the M6 group, whereas those for the patients without CR at HSCT were comparable between these two groups (Figure 2(c) and 2(d), 3-year CI-relapse for the M6 patients with and without CR and the M7 patients with and without CR: 19% and 43% ($P<0.004$) and 42% and 48% ($P=0.59$), respectively). Therefore, we speculate that the primary factor of a worse OS in the M7 patients than in the M6 patients was caused by the

relatively higher rate of relapse in the M7 patients with CR.

When the outcomes after allo-HSCT were compared between the M6 and M7 patients and the M0-M5 (except M3) patients using a matched-pair analysis (Table 2), the M7 patients showed significantly worse OS, RFS and CI-relapse rates than the M0-M5 patients, while the M6 patients demonstrated comparable outcomes with the M0-M5 patients (Figure 3, 5-year OS, 5-year RFS, 3-year CI-relapse and 3-year CI-NRM for the M7 patients and paired M0-M5 patients: 12% and 34% ($P<0.001$), 17% and 33% ($P<0.01$), 47% and 33% ($P<0.05$) and 36% and 35%, respectively). The current results may therefore suggest that only M7 is a poor prognostic factor in HSCT for AML patients.

3.3 Prognostic factors affecting the OS in the M6 patients and M7 patients

The univariate analysis of the M6 and M7 patients showed that age, gender, performance status at HSCT, FAB classification, cytogenetic subgroup, disease status at HSCT, graft source, HLA disparities, HSCT year and the development of GVHD were associated with the OS (Table 3). Furthermore, age, gender, performance status at HSCT, FAB classification, cytogenetic

subgroup, disease status at HSCT and the development of chronic GVHD remained significant factors in the multivariate analysis using Cox's proportional hazards model. The competing risks of relapse and non-relapse mortality were affected by age, performance status at HSCT, cytogenetic subgroup, disease status at HSCT, major ABO mismatch, graft source and the development of chronic GVHD for relapse mortality and HLA disparities for non-relapse mortality using a fine-gray analysis. When the patients with M6 and patients with M7 were analyzed separately, age, gender, performance status at HSCT, cytogenetic subgroup, disease status at HSCT and the development of GVHD where found to predict the OS rate in the M6 patients, while the performance status and disease status at HSCT predicted the OS in the M7 patients (Table 4).

4 Discussion

Allo-HSCT is expected to provide curability for patients with AML by eliminating leukemic stem cells with allo-reactive donor T-cells[20-23]. We hypothesized that two infrequent subtypes of AML, M6 and M7, comprise leukemic stem cells with the same properties in the context of the

graft-versus-leukemia effect, thus showing similar transplant outcomes, since M6 and M7 are considered to originate from a common megakaryocyte-erythroid progenitor. The current study revealed that patients with M7 show inferior survival rates after allo-HSCT to those with M6, primarily due to the higher relapse rate observed in patients with M7. One plausible explanation for this difference in outcome is that the M7 subtype is more prone to internal tandem duplications of FLT3 (FLT3-ITD), the most common mutations associated with an adverse disease outcome, than the M6 subtype[24]. Another possibility is that myelofibrotic changes may occur frequently in M7 patients[15], and the post-transplant outcomes of patients with M7 associated with myelofibrosis, especially in severe cases, is dismal[25, 26]. In contrast, of the detection of myelofibrotic changes is rare in patients with M6 disease, as supported by the findings of a previous study[27]. Unfortunately, the present registry-based data did not include information regarding genetic abnormalities or fibrotic changes, and an examination of these parameters was outside of the scope of the present study. Therefore, further studies are warranted.

It is well known that M7 is associated with Down syndrome. There were no

M7 cases complicated with Down syndrome; however, 1 patient had a sole trisomy 21 abnormality. One patient had trisomy 21 and trisomy 8, and 4 patients had a complex cytogenetic abnormality containing trisomy 21 in our cohort. In contrast, 9 pediatric patients with M7 had sole trisomy 21 and received allo-HSCT. According to the current data, adult M7 patients with trisomy 21 did not receive allo-HSCT for some reason.

The current findings demonstrated a poor prognosis among the M7 patients after allo-HSCT. However, the outcome analysis showed a better performance status and CR at the time of allo-HSCT to be favorable prognostic factors. Although the transplantation of cord blood is superior to other graft sources in terms of competing risks of relapse, no superiority of cord blood with respect to overall survival was observed in the multivariate analysis. One possible reason for this finding is that the benefit of a lower risk of relapse induced by cord blood transplantation is offset by a higher risk of non-relapse mortality associated with HLA disparities resulting from cord blood transplantation. New treatment strategies are thus needed to improve the outcomes of M7 patients who do not achieve CR with remission induction therapy; unfortunately however, no promising reports have been

published regarding specific gene abnormalities for M7, and molecular-targeted therapy is not expected to achieve a significant improvement in treatment outcomes. As it stands, therefore, it is necessary to reconsider which treatment strategy will obtain the best outcome using currently available tools and techniques.

5 Conclusions

In the present study, the allo-SCT outcomes of the M7 patients were significantly inferior to those of the M6 patients, suggesting that M7 differs clinically from M6 as a disease entity. Employing a centralized database enables researchers to analyze rare disease entities, such as M6 and M7. Nevertheless, further prospective validation studies including genetic analyses are needed to verify the current results.

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Figure legends

Figure 1. Survival of the M6 and the M7 patients.

(a) Rates of overall survival (OS). (b) Rates of relapse-free survival (RFS).

Solid line, M6 patients; dashed line, M7 patients.

Figure 2. Cumulative incidence (CI) of events after allo-HSCT.

(a) CI of non-relapse mortality (NRM).

(b)-(d) CI of relapse. (b) All patients; (c) patients in CR at HSCT; (d) patients in non-CR at HSCT.

Solid line, M6 patients; dashed line, M7 patients.

Figure 3. Survival and the CI of events after allo-HSCT of the M6 and the M7 patients compared with matched M0-M5 patients.

(a) Rates of OS. (b) Rates of RFS. (c) CI of NRM. (d) CI of relapse.

Solid line, M6 patients; dashed line, M7 patients; dotted line, M0-M5 patients (except M3 patients).

Table captions

Table 1. Characteristics of patients.

*: one patient transplanted BM+PBSC are not included.

Abbreviations: BM; bone marrow, CB; cord blood, CR; complete remission, HSCT; hematopoietic stem cell transplantation, PBSC; peripheral blood stem cell.

Table 2. Characteristics of patients in matched-pair analysis.

*: one patient transplanted BM+PBSC are not included.

Abbreviations: BM; bone marrow, CB; cord blood, CR; complete remission, HSCT; hematopoietic stem cell transplantation, PBSC; peripheral blood stem cell.

Table 3. Prognostic factors affecting clinical outcomes.

a. overall survival.

Abbreviations: BM; bone marrow, CB; cord blood, CR; complete remission, GVHD; graft-versus-host disease, HSCT; hematopoietic stem cell transplantation, PBSC; peripheral blood stem cell.

b. competing risk, relapse.

Abbreviations: GVHD; graft-versus-host disease, HSCT; hematopoietic stem cell transplantation.

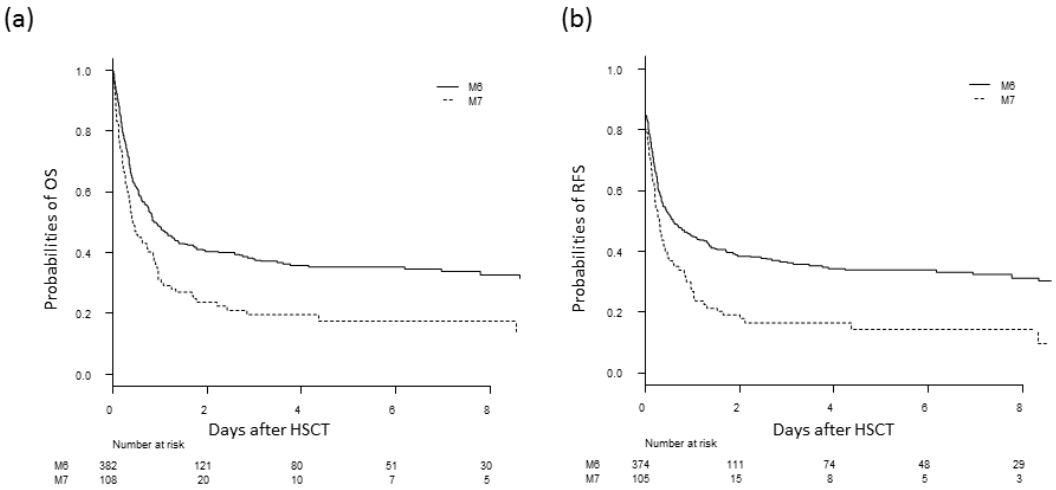
c. competing risk, non-relapse death.

Table 4. Prognostic factors affecting clinical outcomes, distinctively from M6 to M7 patients.

a. M6 patients.

b. M7 patients.

Abbreviations: GVHD; graft-versus-host disease, HSCT; hematopoietic stem cell transplantation.



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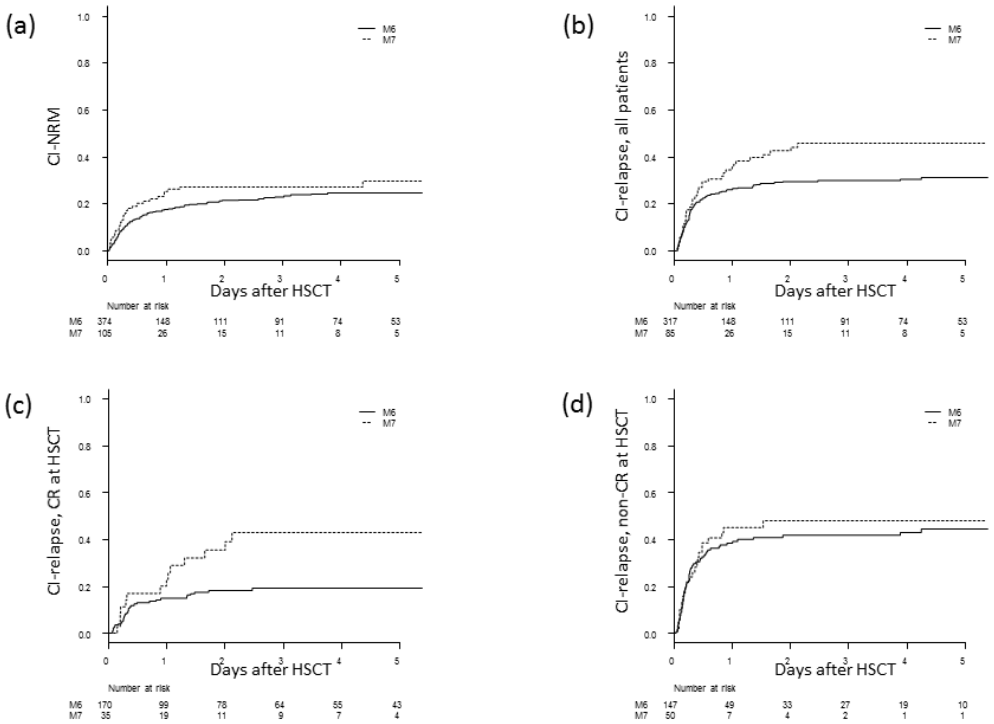
(a) The probabilities of overall survival (OS). (b) The probabilities of relapse-free survival (RFS).

Solid line, M6 patients; dashed line, M7 patients.



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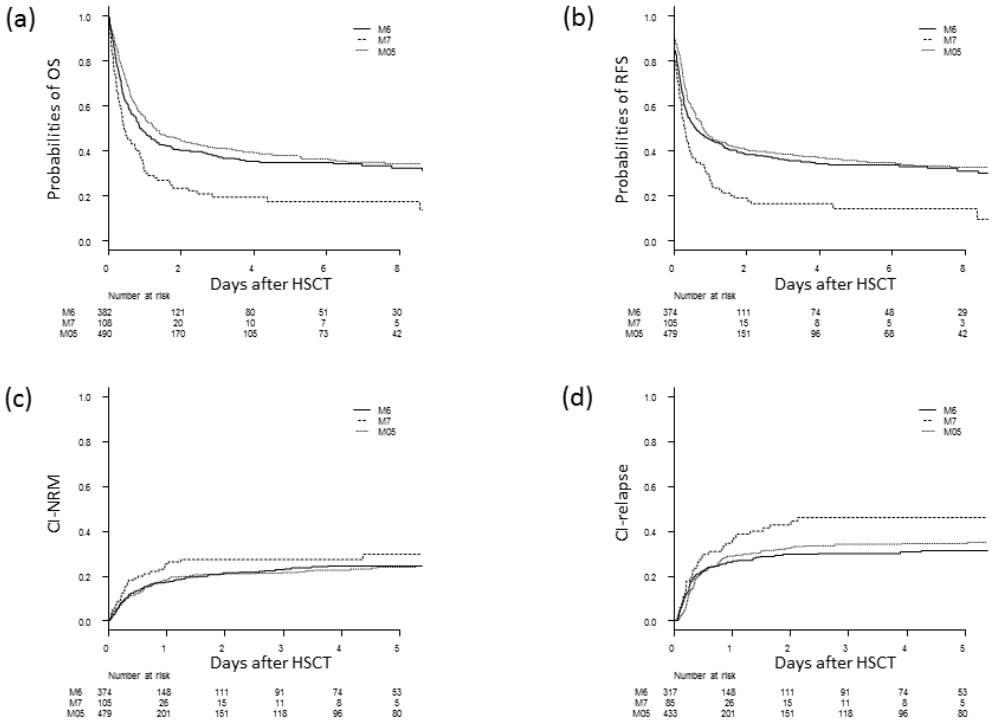
Solid line, M6 patients; dashed line, M7 patients.



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Solid line, M6 patients; dashed line, M7 patients; dotted line, M0-M5 patients (except M3).

Table 1. Characteristics of patients

	FAB M6 (n=382)	FAB M7 (n=108)	
Age, mean (range)	46.4 (16-73)	45.3 (16-70)	p=0.54
Gender, male/female	268 / 114	78 / 30	p=0.72
WBC count at diagnosis, mean (range)	6625 (0-207000)	5024 (500-63500)	p=0.39
Cytogenetic subgroup, intermediate / poor	260 / 122	63 / 45	p=0.07
Performance status, 0-1 / 2-4	264 / 48	68 / 19	p=0.19
HSCT Year, -2000 / 2001-2005 / 2006-	67 / 95 / 220	20 / 26 / 62	p=0.98
Diagnosis to HSCT, <=90 / 90<SCT<=180 / 180<	45 / 144 /190	12 / 43 / 51	p=0.89
Disease status at HSCT, CR/non-CR	175 / 207	37 / 71	***p<0.04***
Conditioning regimen, Myeloablative / Reduced intensity	233 / 149	60 / 48	p=0.32
Donor selection, Related / Unrelated	148 / 234	49 / 59	p=0.22
Graft source, BM / PBSC / CB	220* / 67 / 94	70* / 20 / 17	p=0.16
HLA disparities, 0 / 1 / 2 / 3	223 / 50 / 73 / 4	56 / 24 / 14 / 4	***p<0.02***

*: one patient transplanted BM+PBSC are not included.

Abbreviations: BM; bone marrow, CB; cord blood, CR; complete remission, HSCT; hematopoietic stem cell transplantation, PBSC; peripheral blood stem cell.

Table 2. Characteristics of patients in matched-pair analysis

	FAB M6 (n=382)	FAB M0-M5, matched for M6 (n=382)		FAB M7 (n=108)	FAB M0-M5, matched for M7 (n=108)	
Age, mean (range)	46.4 (16-73)	46.0 (16-70)	p=0.99	45.3 (16-70)	45.2 (16-68)	p=0.96
Gender, male/female	268 / 114	226 / 156	***p<0.002***	78 / 30	42 / 66	***p<0.0001***
Cytogenetic subgroup, intermediate / poor	260 / 122	260 / 122	p=1.00	63 / 45	64 / 44	p=1.00
Performance status, 0-1 / 2-4	264 / 48	263 / 49	p=1.00	68 / 19	70 / 11	p=0.23
HSCT Year, -2000 / 2001-2005 / 2006-	67 / 95 / 220	67 / 106 / 209	p=0.66	20 / 26 / 62	28 / 21 / 59	p=0.22
Diagnosis to HSCT, <=90 / 90<SCT<=180 / 180<	45 / 144 / 190	25 / 100 / 255	***p<0.0001***	12 / 43 / 51	56 / 92 / 12	p=0.38
Disease status at HSCT, CR/non-CR	175 / 207	175 / 207	p=1.00	37 / 71	37 / 71	p=1.00
Conditioning regimen, Myeloablative / Reduced intensity	233 / 149	233 / 149	p=1.00	60 / 48	61 / 47	p=1.00
Donor selection, Related / Unrelated	148 / 234	148 / 234	p=1.00	49 / 59	49 / 59	p=1.00
Graft source, BM / PBSC / CB	220* / 67 / 94	221 / 67 / 94	p=1.00	70* / 20 / 17	71 / 20 / 17	p=1.00

*: one patient transplanted BM+PBSC are not included.

Abbreviations: BM; bone marrow, CB; cord blood, CR; complete remission, HSCT; hematopoietic stem cell transplantation, PBSC; peripheral blood stem cell.

Table 3. Prognostic factors affecting clinical outcomes

a. overall survival

Variables	Risk factors	univariate	mulivariate		
			HR	95% CI	*P*
Age	16-49		1		
	≥50	***<0.0001***	1.39	1.07-1.81	***<0.02***
Gender, receipient	female		1		
	male	***<0.0001***	1.57	1.16-2.11	***<0.004***
Performance status at HSCT	0, 1		1		
	≥2	***<0.0001***	2.50	1.82-3.43	***<0.0001***
FAB classification	M6		1		
	M7	***<0.0003***	1.60	1.20-2.13	***<0.002***
Cytogenetic subgroup	intermediate		1		
	poor	***<0.0001***	2.09	1.59-2.74	***<0.0001***
Disease Status at HSCT	CR		1		
	non-CR	***<0.0001***	1.93	1.43-2.59	***<0.0001***
Graft source	BM				
	PBSC	***<0.02***		NA	
	CB	***<0.0001***			
HLA disparities	0			NA	
	≥1	***<0.0001***			
HSCT year	-2005			NA	
	2006-	***<0.03***			
acute GVHD	0, 1			NA	
	≥2	***<0.006***			
chronic GVHD	no		1		
	yes	***<0.0003***	0.36	0.25-0.50	***<0.0001***

Abbreviations: BM; bone marrow, CB; cord blood, CR; complete remission, GVHD; graft-versus-host disease, HSCT; hematopoietic stem cell transplantation, PBSC; peripheral blood stem cell.

b. competing risk, relapse

Variables	Risk factors	mulivariate		
		HR	95% CI	*P*
Age	16-49	1		
	≥50	1.45	1.01-2.08	***<0.05***
Performance status at HSCT	0, 1	1		
	≥2	2.33	1.47-3.67	***<0.0003***
Cytogenetic subgroup	intermediate	1		
	poor	2.46	1.65-3.65	***<0.0001***
Disease Status at HSCT	CR	1		
	non-CR	2.07	1.38-3.10	***<0.0005***
ABO Major mismatch	no	1		
	yes	1.46	1.01-2.11	***<0.05***
Graft source	BM	1		
	PB	1.23	0.81-1.85	0.33
	CB	0.46	0.26-0.81	***<0.008***
chronic GVHD	no	1		
	yes	0.40	0.26-0.62	***<0.0001***

Abbreviations: GVHD; graft-versus-host disease, HSCT; hematopoietic stem cell transplantation.

c. competing risk, non-relapse death

Variables	Risk factors	mulivariate		
		HR	95% CI	*P*
HLA disparities	0	1		
	1	2.12	1.17-3.85	***<0.02***
	2	1.72	0.90-3.26	0.1
	3	4.38	1.16-16.5	***<0.03***

Table 4. Prognostic factors affecting clinical outcomes, distinctively from M6 to M7 patients.

(a) M6 patients

Variables	Risk factors	mulivariate		
		HR	95% CI	*P*
Age	16-49	1		
	≥50	1.62	1.19-2.21	***<0.003***
Gender	female	1		
	male	1.79	1.25-2.58	***<0.002***
Performance status at HSCT	0, 1	1		
	≥2	2.06	1.40-3.03	***<0.0003***
Cytogenetic subgroup	intermediate	1		
	poor	2.48	1.79-3.44	***<0.0001***
Disease Status at HSCT	CR	1		
	non-CR	1.84	1.31-2.57	***<0.0001***
acute GVHD	0, 1	1		
	≥2	0.71	0.52-0.97	***<0.04***
chronic GVHD	no	1		
	yes	0.37	0.25-0.55	***<0.0001***

(b) M7 patients

Variables	Risk factors	mulivariate		
		HR	95% CI	*P*
Performance status at HSCT	0, 1	1		
	≥2	3.17	1.80-5.60	***<0.0001***
Disease Status at HSCT	CR	1		
	non-CR	3.55	1.87-6.75	***<0.0002***

Abbreviations: GVHD; graft-versus-host disease, HSCT; hematopoietic stem cell transplantation.