

Figure 1. Skin biopsy specimens, infiltrating cells, and overall survival. Immunohistochemical analysis of representative skin biopsy specimen (A), the cell count distribution of CD8⁺, CD163⁺, and CD1a⁺ cells (B), and the impact of MM on OS (C-D). (A) Tissue sections of skin biopsy were stained with hematoxylin and eosin (ai,bi,ci), or antibodies to CD8⁺ (aii, bii, cii), or CD163⁺ (aiii, biii, ciii) as detailed in "Methods." Shown are representative specimens of an MT/FM patient (ai-iii), a MT/MM patient (bi-iii), and an FT/MM patient (ci-iii). Original magnifications ×200. (B) Distribution of infiltrating cell counts (Bi, CD8⁺; Bii, CD163⁺; and Biii, CD1a⁺). (C) OS according to CD163⁺ cell counts (≤ 200 [FM] vs > 200 [MM]) in all patients. OS of patients with MM was significantly lower than that of those with FM (FM: 66.2% ± 10.6% at 1 year and 58.3% ± 11.4% at 3 years; MM: 37.8% ± 21.0% at 1 year and 37.8% ± 21.0% at 3 years, respectively; *P* = .005). (D) OS according to CD163⁺ cell counts (≤ 200 vs > 200) in 46 patients undergoing steroid treatments. OS of patients with MM was significantly lower than that of those with FM (FM: 57.7% ± 17.1% at 1 year and 50.7% ± 17.4% at 3 years; MM: 18.2% ± 22.7% at 1 year and 18.2% ± 22.7% at 3 years, respectively; *P* = .002).

the fact that skin biopsies can be performed safely without any critical complications, our results support the importance of conducting skin biopsies of posttransplantation skin lesions. In cases where macrophage-dominant infiltration is observed in a skin biopsy specimen,

macrophage-targeted therapies¹⁸⁻²³ could provide a clue to refractory GVHD.

In conclusion, macrophage infiltration of skin lesions after allo-SCT was shown to be a significant predictive factor for

Table 3. Analyses of risk factors for OS in all 104 patients

Parameter	Univariate		Multivariate	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Older than 50 y	2.27 (1.23-4.20)	.009	—	—
High disease risk	1.87 (1.03-3.40)	.04	1.92 (1.06-3.50)	.03
Graft PB (vs BM)	2.04 (0.91-4.61)	.08	—	—
Unrelated donor	0.71 (0.40-1.29)	.26	—	—
HLA mismatch	1.13 (0.61-2.10)	.70	—	—
Conventional regimens	0.74 (0.39-1.39)	.35	—	—
Skin stage 3 or 4 at biopsy	1.11 (0.57-2.15)	.76	—	—
MT (> 100 CD8 ⁺ cells)	1.10 (0.59-2.08)	.76	—	—
MM (> 200 CD163 ⁺ cells)	2.38 (1.27-4.49)	.006	2.45 (1.30-4.61)	.006

— indicates not applicable.

refractory GVHD, as well as being a negative prognostic factor for OS. Our results indicate the importance of skin biopsies after allo-SCT and suggest the possibility of developing infiltrating cell-based strategies.

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References

- Gordon S. The role of the macrophage in immune regulation. *Res Immunol*. 1998;149(7):685-688.
- Stoy N. Macrophage biology and pathobiology in the evolution of immune responses: a functional analysis. *Pathobiology*. 2001;69(4):179-211.
- Haniffa M, Ginhoux F, Wang XN, et al. Differential rates of replacement of human dermal dendritic cells and macrophages during hematopoietic stem cell transplantation. *J Exp Med*. 2009; 206(2):371-385.
- Kristiansen M, Graversen JH, Jacobsen C, et al. Identification of the haemoglobin scavenger receptor. *Nature*. 2001;409(6817):198-201.
- Fabrick BO, Dijkstra CD, van den Berg TK. The macrophage scavenger receptor CD163. *Immunobiology*. 2005;210(2-4):153-160.
- Moestrup SK, Moller HJ. CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. *Ann Med*. 2004; 36(5):347-354.
- Zaba LC, Fuentes-Duculan J, Steinman RM, Krueger JG, Lowes MA. Normal human dermis contains distinct populations of CD11c⁺BDCA-1⁺ dendritic cells and CD163⁺FXIIIa⁺ macrophages. *J Clin Invest*. 2007;117(9):2517-2525.
- Przeplorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15(6):825-828.
- Inamoto Y, Ito M, Suzuki R, et al. Clinicopathological manifestations and treatment of intestinal transplant-associated microangiopathy. *Bone Marrow Transplant*. 2009;44(1):43-49.
- Nishida T, Hamaguchi M, Hirabayashi N, et al. Intestinal thrombotic microangiopathy after allogeneic bone marrow transplantation: a clinical imitator of acute enteric graft-versus-host disease. *Bone Marrow Transplant*. 2004;33(11): 1143-1150.
- Kennedy MS, Deeg HJ, Storb R, et al. Treatment of acute graft-versus-host disease after allogeneic marrow transplantation: randomized study comparing corticosteroids and cyclosporine. *Am J Med*. 1985;78(6):978-983.
- Ratanatharathorn V, Nash RA, Przeplorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood*. 1998;92(7):2303-2314.
- Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med*. 1986;314(12):729-735.
- Lin MT, Storer B, Martin PJ, et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med*. 2003;349(23):2201-2210.
- Sugimoto K, Murata M, Onizuka M, et al. Decreased risk of acute graft-versus-host disease following allogeneic hematopoietic stem cell transplantation in patients with the 5,10-methylenetetrahydrofolate reductase 677TT genotype. *Int J Hematol*. 2008;87(5):451-458.
- Remberger M, Mattsson J, Hassan Z, et al. Risk factors for acute graft-versus-host disease grades II-IV after reduced intensity conditioning allogeneic stem cell transplantation with unrelated donors: a single centre study. *Bone Marrow Transplant*. 2008;41(4):399-405.
- Lee KH, Choi SJ, Lee JH, et al. Prognostic factors identifiable at the time of onset of acute graft-versus-host disease after allogeneic hematopoietic cell transplantation. *Haematologica*. 2005; 90(7):939-948.
- Patriarca F, Sperotto A, Damiani D, et al. Infliximab treatment for steroid-refractory acute graft-versus-host disease. *Haematologica*. 2004; 89(11):1352-1359.
- Couriel D, Saliba R, Hicks K, et al. Tumor necrosis factor-alpha blockade for the treatment of acute GVHD. *Blood*. 2004;104(3):649-654.
- Oussoren C, Storm G. Role of macrophages in the localisation of liposomes in lymph nodes after subcutaneous administration. *Int J Pharm*. 1999; 183(1):37-41.
- Van Rooijen N, Sanders A. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. *J Immunol Methods*. 1994;174(1):83-93.
- Liu Q, Hamblin MR. Macrophage-targeted photodynamic therapy: scavenger receptor expression and activation state. *Int J Immunopathol Pharmacol*. 2005;18(3):391-402.
- Demidova TN, Hamblin MR. Macrophage-targeted photodynamic therapy. *Int J Immunopathol Pharmacol*. 2004;17(2):117-126.

Authorship

Contribution: S.N., S.T., M.I., and K.M. designed the research; S.N., S.T., and M.I. analyzed and interpreted the data; S.N. and S.T. performed statistical analysis; and S.N., T.G., A.S., K.W., M.Y., N.I., S.T., M.S., Y.O., M.I., and K.M. collected clinical data and wrote the paper.

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Correspondence: Masafumi Ito, Department of Hematology, Japanese Red Cross Nagoya First Hospital, 3-35 Michishita-cho, Nakamura-ku, Nagoya 453-8511, Japan; e-mail: itom@nagoya-1st.jrc.or.jp.

Hematopoietic stem cell transplantation for core binding factor acute myeloid leukemia: t(8;21) and inv(16) represent different clinical outcomes

Yachiyo Kuwatsuka,¹ Koichi Miyamura,¹ Ritsuro Suzuki,² Masaharu Kasai,³ Atsuo Maruta,⁴ Hiroyasu Ogawa,⁵ Ryuji Tanosaki,⁶ Satoshi Takahashi,⁷ Kyuhei Koda,⁸ Kazuhiro Yago,⁹ Yoshiko Atsuta,² Takashi Yoshida,¹⁰ Hisashi Sakamaki,¹¹ and Yoshihisa Kodera¹

¹Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya; ²Department of HSCT Data Management, Nagoya University School of Medicine, Nagoya; ³Department of Hematology, Sapporo Hokuyu Hospital, Sapporo; ⁴Department of Hematology, Kanagawa Cancer Center, Yokohama; ⁵Department of Molecular Medicine, Osaka University Graduate School of Medicine, Osaka; ⁶Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo; ⁷Department of Hematology, Institute of Medical Science, The University of Tokyo, Tokyo; ⁸Department of Hematology, Asahikawa Red Cross Hospital, Asahikawa; ⁹Department of Hematology, Shizuoka General Hospital, Shizuoka; ¹⁰Hematology Department, Toyama Prefectural Hospital, Toyama; and ¹¹Department of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

We analyzed 338 adult patients with acute myeloid leukemia (AML) with t(8;21) and inv(16) undergoing stem cell transplantation (SCT) who were registered in the Japan Society for Hematopoietic Cell Transplantation database. At 3 years, overall survival (OS) of patients with t(8;21) and inv(16) was 50% and 72%, respectively ($P = .002$). Although no difference was observed when restricted to

allogeneic SCT in first complete remission (CR; 84% and 74%), OS of patients with t(8;21) and inv(16) undergoing allogeneic SCT in second or third CR (45% and 86% at 3 years; $P = .008$) was different. OS was not different between patients in first CR who received allogeneic SCT and those who received autologous SCT for both t(8;21) AML (84% vs 77%; $P = .49$) and inv(16) AML (74% vs 59%; $P = .86$). Patients with inv(16) not in CR did better after allogeneic SCT than those with

t(8;21) (70% and 18%; $P = .03$). Patients with t(8;21) and inv(16) should be managed differently as to the application of SCT. SCT in first CR is not necessarily recommended for inv(16). For t(8;21) patients in first CR, a prospective trial is needed to clarify the significance of autologous SCT and allogeneic SCT over chemotherapy. (Blood. 2009;113:2096-2103)

Introduction

Core binding factor (CBF) acute myeloid leukemia (AML) including t(8;21)(q22;q22) and inv(16)(p13q22)/t(16;16)(p13;q22) [t(8;21) and inv(16)] is considered to be a favorable cytogenetic subgroup in clinical studies.¹⁻⁴ Patients with t(8;21) and inv(16) have shown a markedly improved outcome with repetitive use of high-dose cytarabine.⁵⁻¹³ However, the major treatment failure is disease recurrence.¹⁴⁻¹⁶ These patients frequently become stem cell transplantation (SCT) candidates.

Both t(8;21) and inv(16) AMLs are associated with disruption of genes encoding subunits of the CBF, a heterodimeric transcriptional factor involved in the regulation of hematopoiesis.^{17,18} Although these 2 different cytogenetics also share common clinical characteristics, they are associated with different clinical features such as morphologic presentation and immunophenotypic marker expression.¹⁹

Several reports demonstrated inferior outcome of t(8;21) compared with inv(16), but the number of patients who underwent transplantation was limited.^{14,15,20} A recent study from the Dana-Farber Cancer Institute reported that both patients with t(8;21) and inv(16) de novo AML who underwent allogeneic transplantation performed favorably compared with other karyotypes.²¹ To identify the survival data and prognostic factors among the CBF leukemia population who received SCT, we conducted a retrospective analysis using a Japanese multi-institution database with a large number of patients.

Methods

Study population

A total of 2802 adult patients who underwent autologous or allogeneic SCT from 1996 and 2004 for AML were registered in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) database. Patients who underwent SCT from unrelated donors were registered in the different registry in the study period, but not all of the patients undergoing unrelated SCT were registered in the JSHCT database. Demographic, diagnostic, clinical, cytogenetics, induction, and outcome information were collected for each patient, and were sent to a central registration center. Cytogenetic studies were performed in each center, but a central review of cytogenetic analysis was not performed.

Patients with de novo AML aged 16 to 70 years who received hematopoietic SCT as the first transplant were included in the study. No patients with prior history of autologous or allogeneic SCT were included in the study. Of the remaining 2164 patients, 178 patients with t(15;17) or PML/RAR α were excluded from the analysis below (Table 1). Finally, of the 1986 patients included in the analysis, 255 were reported to have t(8;21) abnormality, and 83 to have inv(16). A total of 194 patients had no available cytogenetic data. The remaining 1454 patients with normal karyotype and other cytogenetic abnormalities were further coded and analyzed according to published Southwest Oncology Group (SWOG) criteria.³ The intermediate risk category included patients characterized by +8, -Y, +6, del(12p), or normal karyotype. The unfavorable risk category was defined by the presence of one or more of -5/del(5q), -7/del(7q), abn 3q, 11q, 20q, or

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Table 1. Cytogenetic risk groups of patients with AML who received autologous SCT and allogeneic SCT

Cytogenetic risk groups	No. patients		
	Auto-SCT	Allo-SCT	Total
t(8;21)	61	194	255
inv(16)	17	66	83
t(15;17)*	65	113	178
Intermediate	140	749	889
Unfavorable	35	325	360
Unknown			
Unknown cytogenetic risk	27	178	205
No available cytogenetic data	44	150	194
Total	389	1775	2164

Auto-SCT indicates autologous stem cell transplantation; Allo-SCT, allogeneic stem cell transplantation.

*Patients with t(15;17) were excluded from the analysis.

21q, del(9q), t(6;9), t(9;22), abn 17p, and complex karyotypes defined as 3 or more abnormalities. Patients with other cytogenetic aberrations were considered an unknown risk group, and were analyzed together with 194 patients with no cytogenetic data.

This study was approved by the Committee for Nationwide Survey Data Management of the JSHCT. Informed consent was obtained in accordance with the Declaration of Helsinki.

Transplantation

A total of 1662 patients underwent allogeneic SCT, and 324 underwent autologous SCT. Patients were treated with various conditioning regimens, but most of those who underwent autologous transplantation received non-total body irradiation (TBI) regimens (97%), including busulfan (BU), cytarabine (CA), and etoposide. The most frequently used conditioning regimens before allogeneic SCT were cyclophosphamide (Cy) plus TBI (n = 327 patients), and BU plus Cy (n = 267). Conditioning regimens before allogeneic SCT also included more intensified regimens such as CA plus Cy plus TBI (n = 262) and BU plus Cy plus TBI (n = 146), or reduced-intensity conditioning regimens with fludarabine (n = 241) or cladribine (n = 19).

Stem cell sources for allogeneic SCT were bone marrow in 871 patients, peripheral blood stem cell in 570 patients, bone marrow plus peripheral blood stem cell in 23 patients, and cord blood in 190 patients. A total of 1242 patients underwent allogeneic SCT from a related donor, and 404 patients underwent SCT from an unrelated donor.

Of the 1637 patients who had available data, 74% received transplants from human leukocyte antigen (HLA)-matched donors. Among patients who received unrelated bone marrow transplants, 156 patients were HLA genotypically matched and 51 were HLA mismatched. HLA data for 39 mismatched unrelated bone marrow transplantation patients were available. A total of 32 patients were one locus mismatched, and 7 patients were 2 loci mismatched. Among patients receiving unrelated cord blood transplants, 19 patients were serologically HLA matched and 170 patients were mismatched. HLA incompatibility was 5 of 6 HLA matched in 57 patients, 4 of 6 HLA matched in 99 patients, 3 of 6 HLA matched in 7 patients, and 1 of 6 HLA matched in 1 patient.

Graft-versus-host disease (GVHD) prophylaxis mostly consisted of methotrexate and a calcineurin inhibitor, either cyclosporin A or tacrolimus. Several other prophylaxes include mycophenolate mofetil, antithymocyte globulin, and CD34⁺ selection. The incidence of acute GVHD was evaluated in 1488 patients who survived more than 28 days, and chronic GVHD was evaluated in 1302 patients who survived more than 100 days after allogeneic SCT. GVHD was evaluated in each center.

Statistical analysis

Correlation between the 2 groups was examined with the chi-square test, Fisher exact test, and the Mann-Whitney *U* test. Disease-free survival (DFS) was calculated from the date of transplantation until the date of

relapse or the date of death in CR. Patient survival data were analyzed with the method of Kaplan and Meier and compared by the log-rank test.

Univariate and multivariate analyses for OS were performed with the aid of the Cox proportional hazard regression model, and variables were selected with the stepwise method. The following variables were evaluated: age, sex, and disease status at transplantation; CR versus not in CR; the number of induction courses to achieve CR; one course versus more than one course and failure; type of transplantation (allogeneic SCT vs autologous SCT); conditioning regimen (reduced intensity vs myeloablative); TBI regimen or not; and the existence of additional karyotype abnormalities or not. For those who received allogeneic SCT, in addition to these variables, the following were also evaluated: type of GVHD prophylaxis; short-course methotrexate plus cyclosporin A or short methotrexate plus FK506; acute GVHD, grade II to IV or grade III to IV; chronic GVHD; HLA mismatch; donor; and donor source. The doses of methotrexate were not surveyed. Each factor was considered to be prognostic if the *P* value was less than .05. Data were analyzed with the Stata 9.2 statistical software (College Station, TX).

Results

Initial characteristics of patients

The median age of all patients with AML in total was 41 years old (range, 16-70 years old). Median follow-up period of living patients was 37.3 months (range, 0.4-108 months). Patients were categorized into 5 cytogenetic subgroups: with t(8;21), with inv(16), intermediate risk cytogenetics, unfavorable cytogenetics, and an unknown risk group. Table 1 shows the number of patients in each cytogenetic subgroup and patients with t(15;17), who were excluded from the analysis.

Characteristics of the patients with CBF who underwent allogeneic SCT or autologous SCT are shown in Table 2. No significant difference was observed between characteristic of 2 groups of patients with CBF who received autologous SCT, except for the initial white blood cell count.

Of the 259 patients with CBF who received allogeneic SCT, significantly more patients with t(8;21) had failed to achieve CR with a single course of induction chemotherapy at diagnosis (*P* = .002), and were not in CR at the time of transplantation (*P* < .001). Among patients in CR at transplantation, the ratio of those in first, second, or third CR was not different between t(8;21) and inv(16) subgroups. Significantly more patients with inv(16) received transplants from an unrelated donor (*P* = .004). Table 3 and Table S1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article) summarize the transplantation data of those undergoing allogeneic SCT. More patients with inv(16) received unrelated transplants compared with t(8;21) patients (*P* = .004).

Overall survival

The OS of 1986 patients with AML at 3 years was 48%, and those with t(8;21), inv(16), intermediate, unfavorable, and unknown cytogenetic risks showed OS of 50%, 72%, 52%, 35%, and 45%, respectively (*P* < .001). Figure 1 shows survival curves of patients with AML patients who underwent allogeneic SCT in first CR (Figure 1A), in second or third CR (Figure 1B), or not in CR (Figure 1C), categorized by the cytogenetic abnormalities. Survival data are listed in Table 4. The OS of patients with t(8;21), inv(16), and intermediate, unfavorable, and unknown risk undergoing allogeneic SCT in first CR was 84%, 74%, 69%, 53%, and 52%, respectively (*P* < .001), and that of patients undergoing allogeneic-SCT

Table 2. Characteristics of patients with CBF AML

	Auto-SCT			Allo-SCT		
	t(8;21) (n = 61), no.	inv(16) (n = 17), no.	P	t(8;21) (n = 194), no.	inv(16) (n = 66), no.	P
Median age, y (range)	44 (17-68)	37 (19-61)	.59	39 (16-70)	34 (16-64)	.054
Median WBC, g/L (range)	8.8 (0.2-94)	33 (2.1-199)	.02	11 (.6-366)	53 (1.8-284)	< .001
Sex						
Male	41	12	.79	117	40	.93
Female	20	5		74	26	
No. of induction chemotherapy at diagnosis of AML						
1 course	48	15	.72	125	55	.002
> 1 or failure*	11	2		56	7	
Additional cytogenetic abnormalities						
None	53	15	> .999	153	54	.61
Positive	8	2		41	12	
Disease status at SCT						
CR	55	16	> .999	108	52	< .001
Not in CR	6	1		85	11	
CR1	43	13	.98	49	21	.29
CR2	7	1		45	26	
CR3	0	1		5	4	
Conditioning regimen						
TBI	0	1	.22	118	47	.078
Not TBI	61	16		71	16	

Correlation between the two groups was examined.

WBC indicates white blood cell count; g/L, 10⁹/L; CR1, first complete remission; and CR2 or 3, second or third CR.

*More than 1 or failure includes patients who did not achieve complete remission after first course of induction chemotherapy, and those who were resistant to induction chemotherapy.

in second or third CR was 45%, 86%, 57%, 44%, and 64%, respectively ($P = .09$). OS of patients undergoing allogeneic SCT not in CR was 18%, 70%, 25%, 15%, and 18%, respectively ($P = .003$).

Table 3. Summary of allogeneic SCT

	t(8;21) (n = 194), no.	inv(16), (n = 66), no.	P
Conditioning regimen			
RIST	31	9	.66
Myeloablative	161	56	
GVHD prophylaxis*			
sMTX+CyA	136	48	.78
sMTX+FK	20	8	
HLA			
Match	146	47	.5
Mismatch	45	18	
Donor			
Related	161	44	.004
Unrelated	32	22	
Stem cell source			
BM	101	40	.27
PB	72	17	
CB	18	7	
aGVHD grade			
0-I	117	37	.54
II-IV	60	22	
cGVHD type			
None	64	28	.28
Lmt/Ext	67	20	

Correlation between the two groups was examined. Some of the missing data was not available, and total numbers do not add up to the number of the patients in each group.

RIST indicates reduced intensity stem cell transplantation; sMTX, short-course methotrexate; CyA, cyclosporin A; FK, tacrolimus; BM, bone marrow; PB, peripheral blood; CB, cord blood; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; Lmt, limited; and Ext, extensive.

*Dose of methotrexate was not surveyed in the study. Detail of other GVHD prophylaxis regimens are in Table S1.

When patients undergoing allogeneic SCT in first CR were analyzed, 3-year OS was not significantly different between patients with t(8;21) and inv(16) (84% and 74%, respectively; $P = .28$), between inv(16) and intermediate risk groups (74% and 69%, respectively; $P = .84$), or between t(8;21) and intermediate risk groups (84% and 69%, respectively; $P = .06$). However, when patients undergoing allogeneic SCT in second or third CR were analyzed, the 3-year OS of patients with inv(16) was significantly better than patients with t(8;21) (86% and 45%, respectively; $P = .008$), and better than intermediate risk patients (86% and 57%, respectively; $P = .03$). Difference was not significant between patients in the intermediate risk group and t(8;21) undergoing allogeneic SCT in second or third CR ($P = .36$). The OS of inv(16) patients undergoing allogeneic SCT not in CR was 70% at 3 years, which was also significantly better than that of t(8;21) (18%; $P = .03$) and the intermediate risk group (25%; $P = .045$).

In addition, the OS of t(8;21) undergoing allogeneic SCT in first CR was significantly better than that of the unfavorable risk group (84% and 53%, respectively; $P < .001$), but the difference between the 2 groups was not significant among patients undergoing allogeneic SCT in second or third CR. In contrast, OS was not different between inv(16) and unfavorable groups undergoing allogeneic SCT in first CR, but it was significantly different when they underwent allogeneic SCT in second or third CR (86% and 44%, for inv(16) and unfavorable groups, respectively; $P = .01$) or allogeneic SCT in non-CR (70% and 15%, respectively; $P = .006$).

Survival curves of patients who underwent autologous SCT in first CR, second or third CR, and not in CR are shown in Figure 2A, 2B, and 2C, respectively. The overall survival of patients with t(8;21), inv(16), and intermediate, unfavorable, and unknown cytogenetic risks in first CR was 77%, 59%, 74%, 38%, and 71%, respectively ($P = .049$), while that of patients undergoing autologous SCT in second or third CR was 43%, 50%, 59%, 44%, and 42%, respectively ($P = .8$). The OS of patients undergoing autologous SCT not in CR with t(8;21), inv(16), intermediate, and

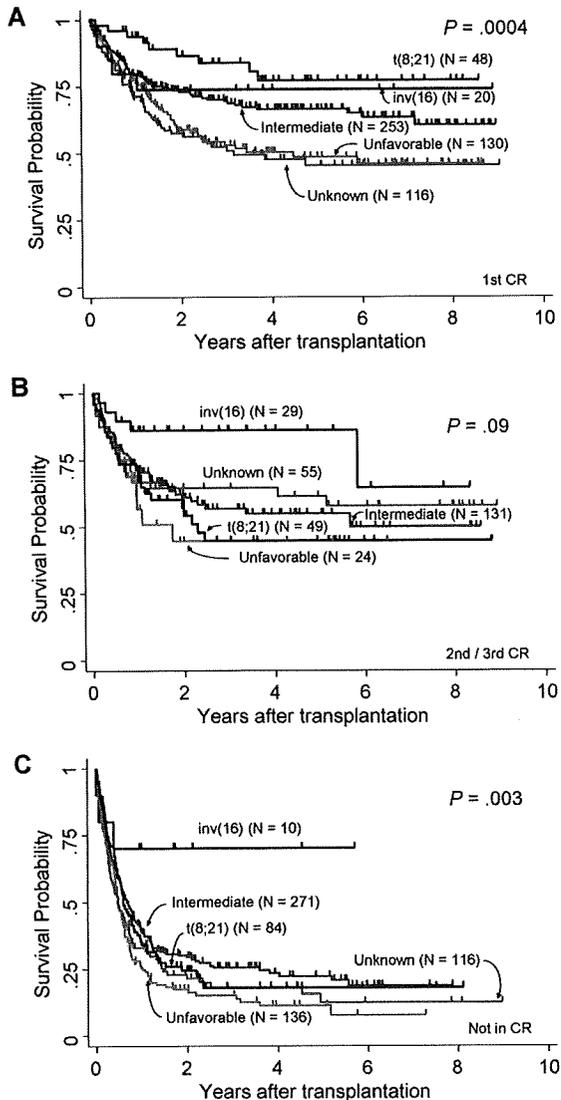


Figure 1. OS difference of patients undergoing allogeneic SCT between cytogenetic subgroups. (A) Survival curves of patients undergoing allogeneic SCT in first CR. (B) Survival curve of patients undergoing allogeneic SCT in second or third CR. (C) Survival curves of patients undergoing allogeneic SCT not in CR. Each are categorized by cytogenetic risk groups, respectively.

unknown risks was 17%, 100%, 25%, and 13%, respectively, and the survival curve of patients in the unfavorable risk group did not reach 3 years ($P = .35$).

Figure 3A and B focus on t(8;21) and inv(16) patients, stratified according to the type of (allogeneic or autologous) and disease status at the time of transplantation (first CR, second or third CR, and not in CR). The 3-year overall survival of t(8;21) patients in first CR was not different between allogeneic and autologous transplantation (84% and 77%, respectively), as well as that of patients in second or third CR (45% and 43%, respectively) and patients not in CR (18% and 17%, respectively). Similarly, the 3-year OS of inv(16) patients was not different between allogeneic and autologous transplantation when they underwent transplantation in first CR (74% and 59%). A significant difference was observed

among the 3 disease status groups of t(8;21) patients ($P < .001$; Figure 3A), but not inv(16) patients ($P = .75$; Figure 3B).

The OS of allogeneic SCT, excluding cord blood transplantation, was not different from the analysis presented here, including bone marrow, peripheral blood, and cord blood transplantation (Table S2; Figures S1,S2).

DFS after SCT was also different among cytogenetic risk groups ($P < .001$). DFS of patients with inv(16) (69% at 3 years) was better compared with t(8;21) (49%), intermediate (46%), unfavorable (31%), and unknown (41%) risk groups. Among patients undergoing allogeneic SCT in first CR, DFS was also different among cytogenetic subgroups ($P < .001$). When t(8;21), inv(16), and intermediate cytogenetic subgroups undergoing allogeneic SCT in first CR were compared, the difference was not statistically significant between t(8;21) and inv(16) (78% and 73% at 3 years; $P = .58$), between t(8;21) and intermediate risk group (78% and 63%; $P = .1$), nor between inv(16) and intermediate risk group (73% and 63%; $P = .65$). DFS of patients with t(8;21) undergoing allogeneic SCT in first CR was better than that of the unfavorable risk group (78% and 47%, respectively; $P < .001$), but the difference was not significant between inv(16) and unfavorable risk groups (73% and 47%, respectively; $P = .16$).

DFS was not significantly different when 5 cytogenetic subgroups among patients undergoing allogeneic SCT in second or third CR were compared ($P = .32$). The DFS of patients undergoing allogeneic SCT in second or third CR was not significantly different between t(8;21) and inv(16) (43% and 71% at 3 years; $P = .053$), t(8;21) and the intermediate group (43% and 47%; $P = .76$), or inv(16) and the intermediate group (71% and 47%; $P = .06$). The difference was also not significant between t(8;21) and unfavorable risk groups (43% and 42%; $P = .7$), nor between inv(16) and unfavorable risk groups (71% and 42%; $P = .06$). The DFS of patients undergoing allogeneic SCT who were not in CR was significantly different among the 5 cytogenetic subgroups ($P = .005$), and that of inv(16) (75% at 3 years) was significantly better than t(8;21) (18%; $P = .02$), the intermediate risk group (22%; $P = .03$) and the unfavorable risk group (10%; $P = .003$).

Relapse and TRM

The relapse rate (RR) after SCT also differed among cytogenetic subgroups ($P < .001$). The RR of patients with inv(16) (18% at 3 years) was lower than t(8;21) (38%), intermediate (38%), and unfavorable (56%) risk groups. The RR of t(8;21) and inv(16) after allogeneic SCT was not statistically different in either first CR (16% and 6%; $P = .45$) or second or third CR (34% and 16%, respectively; $P = .09$).

Transplantation-related mortality (TRM) of all patients with AML was 22% at 3 years. The TRM of t(8;21) (18%), inv(16) (11%), and intermediate (21%), unfavorable (24%), and unknown risk groups (27%) was significantly different among cytogenetic risk groups ($P = .02$).

Evaluation of prognostic variables in CBF

Univariate analyses of t(8;21) showed that age ($P = .004$), not in CR at transplantation ($P < .001$), allogeneic SCT ($P = .01$), and TBI regimen ($P = .006$) were significant prognostic factors indicating poor OS (Table 5). Multivariate analysis for OS revealed older age ($P = .01$) and not in CR at transplantation ($P < .001$) as the independent prognostic variables. Univariate analyses of t(8;21) patients who received allogeneic SCT in CR showed that age ($P = .02$), TBI regimen ($P = .01$), and second and third CR at

Table 4. Outcome of the AML patient population by cytogenetic risk groups

	t(8;21)		inv(16)		Intermediate		Unfavorable		Unknown		P
	%	N	%	N	%	N	%	N	%	N	
OS											
Allogeneic SCT											
CR1	84	48	74	20	69	253	53	130	52	116	< .001
CR2/CR3	45	49	86	29	57	131	44	24	64	55	.09
Non-CR	18	84	70	10	25	271	15	136	18	116	.003
Autologous SCT											
CR1	77	42	59	13	74	89	38	15	71	39	.05
CR2/CR3	43	7	50	2	59	15	44	6	42	18	.8
Non-CR	17	6	100	1	25	16	0	10	13	8	.35
DFS											
Allogeneic SCT											
CR1	78	48	73	19	63	249	47	129	48	113	< .001
CR2/CR3	43	48	71	27	47	129	42	22	57	54	.32
Non-CR	18	81	75	8	22	255	10	128	16	107	.005
Autologous SCT											
CR1	73	41	62	13	64	81	33	15	61	36	.09
CR2/CR3	43	7	50	2	36	14	50	6	39	18	.89
Non-CR	17	6	100	1	25	16	0	10	17	6	.45

transplantation ($P < .001$) were also significantly prognostic for poor OS. These variables remained significant after multivariate analysis. Univariate analyses for inv(16) patients showed only age ($P = .009$) to be a significant prognostic factor (Table 5). The univariate analysis of inv(16) patients who underwent allogeneic SCT in CR showed only additional karyotype abnormalities to be an unfavorable prognostic variable ($P = .009$).

Additional cytogenetic abnormalities to CBF

A total of 49 patients with t(8;21) and 14 with inv(16) had additional cytogenetic abnormalities. Data for additional cytogenetic abnormalities were obtained in 42 patients with t(8;21) and 13 patients with inv(16) (Table 6). Additional abnormalities were selected that have been reported to be prognostic by others, including loss of sex chromosome (X or Y), trisomy 8, trisomy 4, del(7q), and del(9q) for the t(8;21) group, and trisomy 22, trisomy 8, trisomy 21, del(7q), and del(9q) for the inv(16) group.^{14,15,20,22,23} There were no patients with trisomy 21 in the data of patients with CBF. Patients with t(8;21) and patients with inv(16) were analyzed separately. Among t(8;21) patients undergoing allogeneic SCT, survival was not different between patients with and without additional karyotype abnormalities. When patients with inv(16) were analyzed, the survival was not different between patients with ($n = 13$) and without ($n = 67$) additional abnormalities (61% and 74%, respectively; $P = .07$). The survival of patients undergoing allogeneic SCT without additional abnormality ($n = 52$) was significantly better than that with additional abnormality ($n = 11$), (85% and 53%, respectively; $P = .004$). When analysis was restricted to patients in CR with inv(16) undergoing allogeneic SCT, a similar difference was observed (86% without additional abnormality [$n = 42$], and 60% with additional abnormality [$n = 8$], respectively; $P = .03$). Difference in OS was observed among non-CR patients with ($n = 9$) and without ($n = 1$) additional abnormality, but this difference may not be relevant with too few patients in the analysis. We further analyzed subgroups of additional abnormalities of the patients with inv(16). Although the number of patients were limited, significant difference was found among 3 groups of patients; trisomy 8 or trisomy 22 as a sole abnormality ($n = 4$), without additional abnormality ($n = 69$), and other additional abnormality to inv(16) ($n = 10$). The OS at 3 years were 100%, 74%, and 42%, respectively ($P = .002$). The OS of

patients undergoing allogeneic SCT was also different among these 3 groups (100%, $n = 3$; 85%, $n = 52$; and 33%, respectively; $P < .001$).

Discussion

We analyzed the outcome of a large group of patients with adult CBF AML in Japan who were treated with SCT. The current study focused on the different outcome of the 2 different cytogenetic subgroups of patients with CBF AML undergoing SCT. Our study demonstrated a comparable outcome between patients with t(8;21) and inv(16) undergoing SCT in first CR, but the prognosis between these 2 cytogenetic subgroups was different beyond first CR.

In the literature, there have been several reports showing inferior survival of patients with t(8;21) compared with inv(16) patients undergoing induction chemotherapy and SCT.^{14,15,20} Other studies categorized both patients with t(8;21) and inv(16) undergoing allogeneic SCT together as good-risk CBF AML,^{1,21} with a relatively comparable prognosis. In our study, OS of patients with t(8;21) undergoing allogeneic SCT in first CR was not statistically different from intermediate cytogenetic subgroup (84% and 79% at 3 years, respectively; $P = .058$). Moreover, the survival of inv(16) (74% at 3 years) and intermediate cytogenetic subgroups showed no statistically significant difference.

In contrast, we have here demonstrated that the prognosis of patients with t(8;21) undergoing allogeneic SCT with second or third CR disease was significantly poor compared with those with inv(16). This finding is consistent with those of other studies reporting differences between the 2 types of CBF AML.^{14,15} In the present study, non-CR disease with t(8;21) was also significantly poor compared with patients with inv(16). The Acute Leukemia French Association reported that allogeneic donor availability among patients with CBF AML who were in second CR was a prognostic factor for better survival.¹⁶ We believe that different treatment strategies should be applied for patients with t(8;21) and those with inv(16) other than first CR.

Patients with t(8;21) undergoing allogeneic SCT and autologous SCT had a similar survival rate when they underwent transplantation in first CR, and in further CR. No survival difference between allogeneic SCT and autologous SCT was also

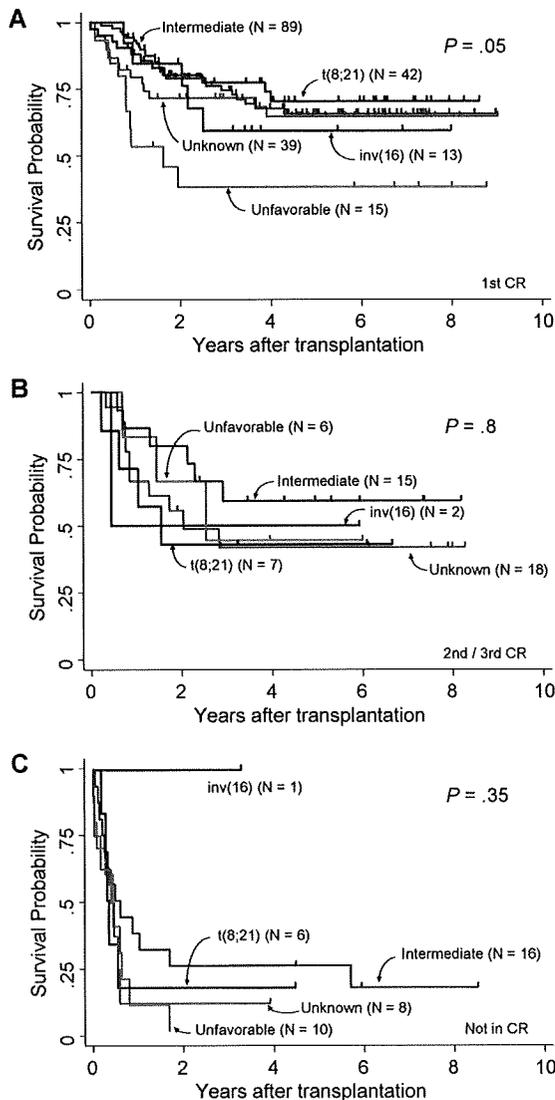


Figure 2. OS difference of patients undergoing autologous SCT between cytogenetic subgroups. (A) Survival curves of patients undergoing autologous SCT in first CR. (B) Survival curves of patients undergoing autologous SCT in second or third CR. (C) Survival curves of patients undergoing autologous SCT not in CR. Each are categorized by cytogenetic risk groups, respectively.

observed among *inv(16)* patients receiving SCT in first CR (74% and 59%, respectively). The University of California, San Francisco (UCSF) group described the good results of patients with advanced AML undergoing autologous SCT in second or third remission, including patients with CBF.²⁴ As in our study, the European Group for Blood and Marrow Transplantation (EBMT) reported that the survival rate of *t(8;21)* patients who received allogeneic bone marrow transplantation was not significantly different from that of patients who received autologous SCT.¹ Results by others showed that allogeneic SCT in first CR did not benefit good-risk cytogenetic subgroups.^{3,25,26} Schlenk et al also demonstrated that *t(8;21)* patients receiving allogeneic SCT or chemotherapy showed no difference in outcome.²³ These results suggest that autologous SCT can be considered as postremission therapy for patients with CBF AML, but it remains unclear whether

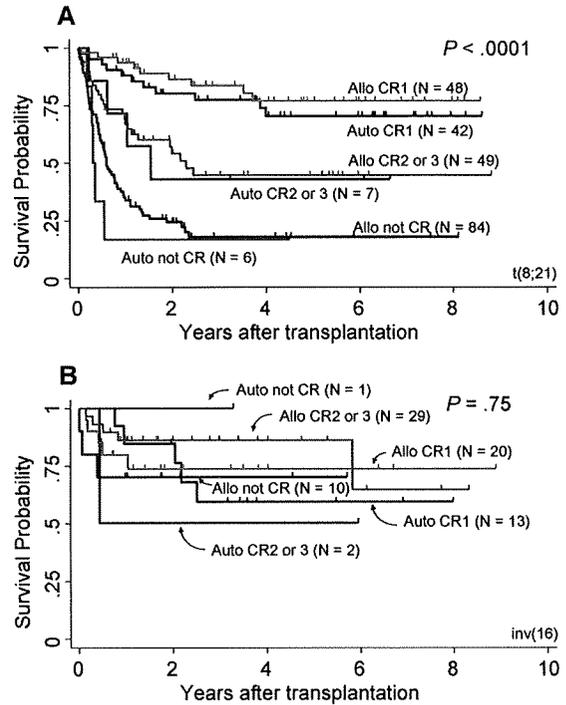


Figure 3. OS of patients with CBF. Survival curves of patients with *t(8;21)* (A) and with *inv(16)* (B). Both are stratified according to the type of transplantation (allogeneic or autologous) and disease status at the time of transplantation (first CR, second or third CR, and not in CR).

SCT is more beneficial for patients with CBF than high-dose cytarabine. Survival of patients with *inv(16)* was favorable beyond first CR. Patients with *inv(16)* in second or third CR, or even non-CR patients, are good candidates for allogeneic SCT. There are long-term survivors after allogeneic SCT in non-CR disease, so *t(8;21)* patients with no other choice of treatment, such as those in further CR or non-CR, can proceed to allogeneic SCT. In order to confirm the appropriate treatment for *t(8;21)* patients in first CR, a prospective trial is needed to compare the results of autologous SCT for *t(8;21)* in first CR with standard chemotherapy. *t(8;21)* patients with suitable related or well-matched donors should be recommended to participate in a risk-adopted prospective trial when they receive allogeneic SCT in first CR.

There were differences between the 2 types of CBF AML with respect to prognostic valuables. Age was a significant and independent prognostic variable in both *t(8;21)* and *inv(16)* patients, a finding in agreement with reports from some,^{14,27} but not all,

Table 5. Prognostic factors affecting overall survival of patients with *t(8;21)*

Variables	Unfavorable factors	Hazard ratio	95% CI	P
t(8;21)				
Age		1.02	1.01-1.04	.004
Disease status at SCT	Not in CR	4.4	3.1-6.5	<.001
Transplantation	Allo-SCT	1.9	1.2-3.0	.01
Conditioning regimen	TBI	1.7	1.2-2.5	.005
inv(16)				
Age		1.1	1.0-1.1	.009

CI indicates confidence interval.

Table 6. Additional cytogenetic abnormalities among patients with CBF

Additional cytogenetic abnormalities	t(8;21), no.	inv(16), no.
None	206	69
With additional abnormalities	49	14*
–Y	10	0
–X	5	0
Trisomy 22	0	3†
Trisomy 8	0	2†
Trisomy 4	2*	0
Complex	7	4
del(7q)	1†	2
del(9q)	6	0
Other abnormalities	27	9‡
Unknown	7	1

*Patients with additional change to inv(16) and trisomy 4 with t(8;21) tended to show poor survival tendency, with $P < .1$.

†All patients with trisomy 22, trisomy 8 with inv(16), and del(7q) with t(8;21) were alive and censored at survival analysis.

‡Other abnormalities with inv(16) was poorly prognostic, with $P < .001$.

investigators.²⁸ Transplantation in CR was a significant and independent prognostic factor for patients with t(8;21), but not for those with inv(16). The Cancer and Leukemia Group B (CALGB) also reported differences between t(8;21) and inv(16) in prognostic factors, in terms of race, sex, and secondary cytogenetic abnormalities.¹⁴ Among patients with CBF AML, t(8;21) and inv(16) patients undergoing SCT should be considered 2 separate clinical entities in future clinical studies.

Several specific additional karyotype abnormalities have been reported to be prognostic in patients with CBF AML. Among t(8;21) patients, no specific additional karyotype abnormality was prognostic for overall survival. The poor prognosis of t(8;21) patients with trisomy 4 has been reported by others,²² but the survival difference was not statistically significant ($P = .085$) in our case series. Since there were limited numbers of patients with additional abnormalities, the real significance of each additional abnormality should be investigated in large numbers of patients.

The reason for the different survival results between patients with t(8;21) and inv(16) undergoing allogeneic SCT in our study remains unclear. The impact of additional mutational events such as c-Kit, FLT3, RAS, and gene-expression profiles was reported to

be associated with the clinical outcome of patients with CBF AML.²⁹⁻³⁴ The effects of these additional mutational events and gene-expression profiles on the clinical outcome of autologous and allogeneic SCT have not yet been studied. Which proportion of the patients with CBF AML benefited from earlier SCT remains to be identified in future clinical studies. Recent studies by others also suggested that prognosis of CBF AML could differ among different ethnic groups or races.^{14,35-37} The background molecular basis among the Japanese population must also be taken into account in future studies.

In conclusion, the survival outcome of patients with CBF AML was similar when they received allogeneic or autologous SCT in first CR. However, the outcomes were significantly different between t(8;21) and inv(16) when they received allogeneic SCT beyond first CR. Therefore, these 2 kinds of CBF AML should be managed differently when applying SCT.

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Authorship

Contribution: Y. Kuwatsuka, K.M., and R.S. contributed to data collection, designed and performed the study, analyzed the data, and wrote the manuscript; M.K., A.M., H.O., R.T., S.T., K.K., K.Y., Y.A., T.Y., and H.S. contributed to data collection and analysis and writing of the paper; and Y. Kodaera contributed to data collection and writing of the paper, conceived the study, and provided intellectual input.

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Correspondence: Yachiyo Kuwatsuka, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan; e-mail: kuwatsuka-ny@umin.ac.jp.

References

- Ferrant A, Labopin M, Frasson F, et al. Karyotype in acute myeloblastic leukemia: prognostic significance for bone marrow transplantation in first remission: a European Group for Blood and Marrow Transplantation study. *Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT)*. *Blood*. 1997;90:2931-2938.
- Grimwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood*. 1998;92:2322-2333.
- Slovak ML, Kopecky KJ, Cassileth PA, et al. Karyotypic analysis predicts outcome of pre-remission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. *Blood*. 2000;96:4075-4083.
- Byrd JC, Mrozek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. 2002;100:4325-4336.
- Wolff SN, Herzig RH, Fay JW, et al. High-dose cytarabine and daunorubicin as consolidation therapy for acute myeloid leukemia in first remission: long-term follow-up and results. *J Clin Oncol*. 1989;7:1260-1267.
- Byrd JC, Dodge RK, Carroll A, et al. Patients with t(8;21)(q22;q22) and acute myeloid leukemia have superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered. *J Clin Oncol*. 1999;17:3767-3775.
- Bloomfield CD, Lawrence D, Byrd JC, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. *Cancer Res*. 1998;58:4173-4179.
- Bishop JF, Matthews JP, Young GA, et al. A randomized study of high-dose cytarabine in induction in acute myeloid leukemia. *Blood*. 1996;87:1710-1717.
- Weick JK, Kopecky KJ, Appelbaum FR, et al. A randomized investigation of high-dose versus standard-dose cytosine arabinoside with daunorubicin in patients with previously untreated acute myeloid leukemia: a Southwest Oncology Group study. *Blood*. 1996;88:2841-2851.
- Kern W, Schoch C, Haferlach T, et al. Multivariate analysis of prognostic factors in patients with refractory and relapsed acute myeloid leukemia undergoing sequential high-dose cytosine arabinoside and mitoxantrone (S-HAM) salvage therapy: relevance of cytogenetic abnormalities. *Leukemia*. 2000;14:226-231.
- Buchner T, Hiddemann W, Wormann B, et al. Double induction strategy for acute myeloid leukemia: the effect of high-dose cytarabine with mitoxantrone instead of standard-dose cytarabine with daunorubicin and 6-thioguanine: a randomized trial by the German AML Cooperative Group. *Blood*. 1999;93:4116-4124.
- Brunet S, Esteve J, Berlanga J, et al. Treatment of primary acute myeloid leukemia: results of a prospective multicenter trial including high-dose cytarabine or stem cell transplantation as post-remission strategy. *Haematologica*. 2004;89:940-949.
- Byrd JC, Ruppert AS, Mrozek K, et al. Repetitive cycles of high-dose cytarabine benefit patients

- with acute myeloid leukemia and inv(16)(p13q22) or t(16;16)(p13;q22): results from CALGB 8461. *J Clin Oncol*. 2004;22:1087-1094.
14. Marcucci G, Mrozek K, Ruppert AS, et al. Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21) differ from those of patients with inv(16): a Cancer and Leukemia Group B study. *J Clin Oncol*. 2005;23:5705-5717.
 15. Schlenk RF, Benner A, Krauter J, et al. Individual patient data-based meta-analysis of patients aged 16 to 60 years with core binding factor acute myeloid leukemia: a survey of the German Acute Myeloid Leukemia Intergroup. *J Clin Oncol*. 2004;22:3741-3750.
 16. de Labarthe A, Pautas C, Thomas X, et al. Allogeneic stem cell transplantation in second rather than first complete remission in selected patients with good-risk acute myeloid leukemia. *Bone Marrow Transplant*. 2005;35:767-773.
 17. Hart SM, Foroni L. Core binding factor genes and human leukemia. *Haematologica*. 2002;87:1307-1323.
 18. de Bruijn MF, Speck NA. Core-binding factors in hematopoiesis and immune function. *Oncogene*. 2004;23:4238-4248.
 19. Ferrara F, Del Vecchio L. Acute myeloid leukemia with t(8;21)/AML1/ETO: a distinct biological and clinical entity. *Haematologica*. 2002;87:306-319.
 20. Appelbaum FR, Kopecky KJ, Tallman MS, et al. The clinical spectrum of adult acute myeloid leukaemia associated with core binding factor translocations. *Br J Haematol*. 2006;135:165-173.
 21. Armand P, Kim HT, DeAngelo DJ, et al. Impact of cytogenetics on outcome of de novo and therapy-related AML and MDS after allogeneic transplantation. *Biol Blood Marrow Transplant*. 2007;13:655-664.
 22. Nishii K, Usui E, Katayama N, et al. Characteristics of t(8;21) acute myeloid leukemia (AML) with additional chromosomal abnormality: concomitant trisomy 4 may constitute a distinctive subtype of t(8;21) AML. *Leukemia*. 2003;17:731-737.
 23. Schlenk RF, Pasquini MC, Perez WS, et al. HLA-identical sibling allogeneic transplants versus chemotherapy in acute myelogenous leukemia with t(8;21) in first complete remission: collaborative study between the German AML Intergroup and CIBMTR. *Biol Blood Marrow Transplant*. 2008;14:187-196.
 24. Linker CA, Damon LE, Ries CA, et al. Autologous stem cell transplantation for advanced acute myeloid leukemia. *Bone Marrow Transplant*. 2002;29:297-301.
 25. Burnett AK, Wheatley K, Goldstone AH, et al. The value of allogeneic bone marrow transplant in patients with acute myeloid leukaemia at differing risk of relapse: results of the UK MRC AML 10 trial. *Br J Haematol*. 2002;118:385-400.
 26. Suciu S, Mandelli F, de Witte T, et al. Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): an intention-to-treat analysis of the EORTC/GIMEMAAML-10 trial. *Blood*. 2003;102:1232-1240.
 27. Delaunay J, Vey N, Leblanc T, et al. Prognosis of inv(16)/t(16;16) acute myeloid leukemia (AML): a survey of 110 cases from the French AML Intergroup. *Blood*. 2003;102:462-469.
 28. Nguyen S, Leblanc T, Fenaux P, et al. A white blood cell index as the main prognostic factor in t(8;21) acute myeloid leukemia (AML): a survey of 161 cases from the French AML Intergroup. *Blood*. 2002;99:3517-3523.
 29. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. *J Clin Oncol*. 2006;24:3904-3911.
 30. Bullinger L, Rucker FG, Kurz S, et al. Gene-expression profiling identifies distinct subclasses of core binding factor acute myeloid leukemia. *Blood*. 2007;110:1291-1300.
 31. Peterson LF, Boyapati A, Ahn EY, et al. Acute myeloid leukemia with the 8q22:21q22 translocation: secondary mutational events and alternative t(8;21) transcripts. *Blood*. 2007;110:799-805.
 32. Nanri T, Matsuno N, Kawakita T, et al. Mutations in the receptor tyrosine kinase pathway are associated with clinical outcome in patients with acute myeloblastic leukemia harboring t(8;21)(q22;q22). *Leukemia*. 2005;19:1361-1366.
 33. Boissel N, Leroy H, Brethon B, et al. Incidence and prognostic impact of c-KIT, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). *Leukemia*. 2006;20:965-970.
 34. Lasa A, Carricondo MT, Carnicer MJ, et al. A new D816 c-KIT gene mutation in refractory AML1-ETO leukemia. *Haematologica*. 2006;91:1283-1284.
 35. Sekeres MA, Peterson B, Dodge RK, et al. Differences in prognostic factors and outcomes in African Americans and whites with acute myeloid leukemia. *Blood*. 2004;103:4036-4042.
 36. Nakase K, Bradstock K, Sartor M, et al. Geographic heterogeneity of cellular characteristics of acute myeloid leukemia: a comparative study of Australian and Japanese adult cases. *Leukemia*. 2000;14:163-168.
 37. Narimatsu H, Yokozawa T, Iida H, et al. Clinical characteristics and outcomes in patients with t(8;21) acute myeloid leukemia in Japan. *Leukemia*. 2008;22:428-432.

NKG2D gene polymorphism has a significant impact on transplant outcomes after HLA-fully-matched unrelated bone marrow transplantation for standard risk hematologic malignancies

J. Luis Espinoza,¹ Akiyoshi Takami,¹ Makoto Onizuka,² Hiroshi Sao,³ Hideki Akiyama,⁴ Koichi Miyamura,⁵ Shinichiro Okamoto,⁶ Masami Inoue,⁷ Yoshinobu Kanda,⁸ Shigeki Ohtake,¹ Takahiro Fukuda,⁹ Yasuo Morishima,¹⁰ Yoshihisa Kodera,¹¹ and Shinji Nakao,¹ for the Japan Marrow Donor Program

¹Department of Hematology and Oncology, Kanazawa University Hospital, Kanazawa; ²Department of Hematology and Oncology, Tokai University School of Medicine, Isehara; ³Department of Hematology, Meitetsu Hospital, Nagoya; ⁴Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo; ⁵Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya; ⁶Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo; ⁷Department of Hematology and Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka; ⁸Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama; ⁹Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo; ¹⁰Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya; ¹¹Department of Promotion for Blood and Marrow Transplantation, Aichi Medical University, Nagoya, Japan

ABSTRACT

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Correspondence:
Akiyoshi Takami,
M.D., Ph.D., Department
of Hematology & Oncology,
Kanazawa University Hospital,
13-1 Takaramachi, Kanazawa,
920-8641, Japan.
E-mail:
takami@med3.m.kanazawa-
u.ac.jp

Background

NKG2D, an activating and co-stimulatory receptor expressed on natural killer cells and T cells, plays pivotal roles in immunity to microbial infections as well as in cancer immunosurveillance. This study examined the impact of donor and recipient polymorphisms in the *NKG2D* gene on the clinical outcomes of patients undergoing allogeneic T-cell-replete myeloablative bone marrow transplantation using an HLA-matched unrelated donor.

Design and Methods

The *NKG2D* polymorphism was retrospectively analyzed in a total 145 recipients with hematologic malignancies and their unrelated donors. The patients underwent transplantation following myeloablative conditioning; the recipients and donors were matched through the Japan Marrow Donor Program.

Results

In patients with standard-risk disease, the donor *NKG2D-HNK1* haplotype, a haplotype expected to induce greater natural killer cell activity, was associated with significantly improved overall survival (adjusted hazard ratio, 0.44; 95% confidence interval, 0.23 to 0.85; $p=0.01$) as well as transplant related mortality (adjusted hazard ratio, 0.42; 95% confidence interval, 0.21 to 0.86; $p=0.02$), but had no impact on disease relapse or the development of grade II-IV acute graft-versus-host disease or chronic graft-versus-host disease. The *NKG2D* polymorphism did not significantly influence the transplant outcomes in patients with high-risk disease.

Conclusions

These data suggest an association between the donor *HNK1* haplotype and better clinical outcome among recipients, with standard-risk disease, of bone marrow transplants from HLA-matched unrelated donors.

Key words: *NKG2D*, *HNK1*, *LNK1*, unrelated donor; bone marrow transplantation, single nucleotide polymorphism.

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Introduction

Hematopoietic stem cell transplantation (SCT) is a potentially curative treatment for a range of hematologic malignancies. Although the use of an HLA-matched unrelated donor is well accepted when an HLA-identical sibling donor is unavailable, the risk of transplantation-related complications may be increased.¹ Despite improvements in clinical and supportive care, transplant-related life-threatening complications, including graft-versus-host disease (GVHD), infections and disease relapse, remain an enormous obstacle to overcome.² Although HLA matching is the major genetic determinant of clinical outcome after allogeneic SCT, recent evidence suggests that non-HLA immune-associated genes are also implicated.³ Previous investigations have revealed that several single nucleotide polymorphisms (SNP) which affect individual immune response to infections and inflammatory reactions are associated with the risk of GVHD and transplant outcomes.⁴⁻¹⁵

NKG2D is an activating and co-stimulatory receptor belonging to the C-type lectin-like family of transmembrane proteins and is expressed as a homodimer on natural killer (NK) cells, CD8⁺ $\alpha\beta$ ⁺ T cells, $\gamma\delta$ ⁺ T cells and activated macrophages.¹⁶⁻¹⁸ The ligands for NKG2D, such as MHC class I-chain related proteins (MICA and MICB), UL16 binding proteins are usually absent or expressed at very low levels in normal cells but are up-regulated by cellular stress including heat shock and microbial infections and are frequently expressed in epithelial tumor cells.¹⁹ Ligand engagement of NKG2D triggers cell-mediated cytotoxicity and co-stimulates cytokine production through a DAP10-phosphoinositol 3-kinase dependent pathway and plays an important role in the elimination of tumors and infected cells.^{16-18,20}

Recently, SNP were identified between *LNK1* and *HNK1* haplotypes of the *NKG2D* gene.²¹ In Japanese individuals, the *HNK1* haplotype is associated with greater activity of NK cells in the peripheral blood^{21,22} and a lower prevalence of cancers originating from epithelial cells.^{21,23,24} The present study investigates the impact of donor and recipient polymorphisms in the *NKG2D* gene on the clinical outcomes of patients undergoing allogeneic myeloablative bone marrow transplantation using an HLA allele-matched unrelated donor.

Design and Methods

Patients

NKG2D genotyping was performed on a total 145 recipients with hematologic malignancies and their unrelated donors who were part of the Japan Marrow Donor Program (JMDP). The recipients underwent transplantation, following myeloablative conditioning, with T-cell-replete marrow from an HLA-A, -B, -C, -DRB1 allele-matched donor between November 1995 and March 2000. HLA genotypes of the HLA-A, -B, -C, and -DRB1 alleles of the patients and donors were determined by the Luminex microbead method described previously. (Luminex 100 System; Luminex, Austin, TX, USA).^{25,26} No

patient had a history of prior transplantation. The final clinical survey of these patients was completed by November 1, 2007. Diagnoses were acute myeloid leukemia (n=49; 34%), acute lymphoblastic leukemia (n=37; 26%), chronic myeloid leukemia (n=41; 28%), myelodysplastic syndrome (n=11; 8%) and malignant lymphoma (n=7; 5%), (Table 1). The recipients were defined as having standard risk disease if they had acute myeloid or lymphoblastic leukemia in first complete remission, malignant lymphoma in complete remission, chronic myeloid leukemia in any chronic phase or myelodysplastic syndrome. All other patients were designated as having high-risk disease. Myeloid malignancies included acute myeloid leukemia, chronic myeloid leukemia and myelodysplastic syndrome, whereas lymphoid malignancies included acute lymphoblastic leukemia and malignant lymphomas. Cyclosporine or tacrolimus-based regimens were used in all patients for GVHD prophylaxis whereas anti-T-cell therapy, such as anti-thymocyte globulin and *ex vivo* T-cell depletion, was not. All patients and donors gave their written informed consent to molecular studies, according to the declaration of Helsinki, at the time of transplantation. The project was approved by the Institutional Review Board of Kanazawa University Graduate School of Medicine and the JMDP.

NKG2D genotyping

NKG2D was genotyped using the TaqMan-Allelic discrimination method²⁷ with a 9700-HT real time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA, USA) and results were analyzed using allelic discrimination software (Applied Biosystems). The genotyping assay was conducted in 96-well PCR plates. The amplification reaction contained template DNA, TaqMan universal master mix and a specific probe (product No. C_9345347_10; Applied Biosystems) for rs1049174, a single locus featuring a G-C substitution to distinguish between the *HNK1* (G) and *LNK1* (C) haplotypes of the *NKG2D* gene.^{21,23,24}

Data management and statistical analysis

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year and annually after transplantation. Pre-transplant cytomegalovirus serostatus was routinely tested only in patients but not in their donors. Engraftment was confirmed by an absolute neutrophil count of more than $0.5 \times 10^9/L$ for at least 3 consecutive days. Acute and chronic GVHD were diagnosed and graded using established criteria.^{28,29} Overall survival was defined as the number of days from transplantation to death from any cause. Disease relapse was defined as the number of days from transplantation to disease relapse. Transplant-related mortality was defined as death without relapse. Any patients who were alive at the last-follow-up date were censored. When collecting data, only the main cause of death was recorded if two or more causes were combined. Data on etiological agents of infections, post-mortem changes and supportive care (including prophylaxis of infections and therapy of GVHD, which were given on an institutional basis), were not available for this

cohort of patients. The analysis was performed using Excel 2007 (Microsoft Corp, Redmond, WA, USA), OriginPro version 8.0J (Lightstone Inc, Tokyo, Japan), and R (The R Foundation for Statistical Computing, Perugia, Italy).³⁰ The probability of overall survival was calculated using the Kaplan-Meier method and compared using the log-rank test. The probabilities of transplant-related mortality, disease relapse, acute GVHD, chronic GVHD, and each cause of death were compared using the Grey test³¹

and analyzed using cumulative incidence analysis,³⁰ considering relapse, death without disease relapse, death without acute GVHD, death without chronic GVHD, and death without each cause as respective competing risks. The analysis was stratified for patients with standard-risk disease and high-risk disease to take into account the already recognized prognostic differences. The variables considered were recipient age at time of transplantation, sex, recipient cytomegalovirus serosta-

Table 1. Characteristics of the donors and recipients.

Variable	Standard-risk disease (n=93, 64%) Donor NKG2D haplotype				p	High-risk disease (n=52, 36%) Donor NKG2D haplotype				p
	HNK1 negative n=55, 59%		HNK1 positive n=38, 41%			HNK1 positive n=28, 54%		HNK1 negative n=24, 46%		
	N.	Ratio	N.	Ratio		N.	Ratio	N.	Ratio	
Age, years										
Recipient					0.08					0.39
Median	31		23			23		22		
Range	1-50		1-50			7-46		2-48		
Donor					0.54					0.02
Median	33		28			34		29		
Range	22-49		21-50			21-47		21-50		
Recipient NKG2D haplotype					0.17					0.48
HNK1 positive	33	60%	28	74%		19	68%	14	58%	
HNK1 negative	22	40%	10	26%		9	32%	10	42%	
Sex, male										
Recipient	30	55%	23	61%	0.37	19	68%	15	63%	0.77
Donor	42	76%	23	61%		19	68%	13	54%	
Recipient/donor sex										
Sex matched	31	56%	20	53%	0.23	18	64%	16	67%	0.86
Male/female	6	11%	9	24%		5	18%	5	21%	
Female/male	18	33%	9	24%		5	18%	3	13%	
Disease										
Acute myeloid leukemia	14	25%	9	24%	0.86	14	50%	12	50%	0.99
Acute lymphoblastic leukemia	10	18%	8	21%		10	36%	9	38%	
Myelodysplastic syndrome	6	11%	5	13%		0	0%	0	0%	
Malignant lymphoma	2	4%	3	8%		1	4%	1	4%	
Chronic myeloid leukemia	23	42%	13	34%		3	11%	2	8%	
ABO matching										
Matched	35	64%	19	50%	0.37	14	50%	17	71%	0.18
Major mismatch	11	20%	10	26%		6	21%	5	21%	
Minor mismatch	9	16%	9	24%		8	29%	2	8%	
Bi-directional	0	0%	1	3%		0	0%	1	4%	
Conditioning regimen										
With total body irradiation	43	78%	30	79%	0.93	26	93%	21	88%	0.51
Without total body irradiation	12	22%	8	21%		2	7%	3	13%	
Pretransplant CMV serostatus										
CMV-negative recipient	14	25%	5	13%	0.30	6	27%	5	21%	0.99
Missing data	4	7%	2	5%		5	18%	4	17%	
GVHD prophylaxis										
With cyclosporine	51	93%	34	89%	0.58	27	96%	20	83%	0.11
With tacrolimus	4	7%	4	11%		1	4%	4	17%	
TNC, ×10 ⁶ /kg										
Median	5.4		5.8		0.40	5.8		8.2		0.04
Range	2.3-14.6		2.3-57.6			2.9-20.0		2.4-42.8		
Engraftment	53	96%	38	100%	0.23	28	100%	23	96%	0.28

CMV, cytomegalovirus; TNC, total nucleated cell count harvested.

tus before transplantation, disease characteristics (disease type and disease lineage), donor characteristics (age, sex, sex compatibility, and ABO compatibility), transplant characteristics (total body irradiation-containing regimen, tacrolimus versus cyclosporine, and total nucleated cell count harvested per recipient weight). The median was used as the cut-off point for continuous variables. The χ^2 test and Mann-Whitney test were used to compare results of two groups. The Hardy-Weinberg equilibrium for the *NKG2D* gene polymorphism was tested using the Haploview program.³² Multivariate Cox models were used to evaluate the hazard ratio associated with the *NKG2D* polymorphism. Co-variables found to be statistically significant in univariate analyses ($p \leq 0.10$) were included in the models. For both the univariate and multivariate analyses, p values were two-sided and outcomes were considered to be statistically significant with $p \leq 0.05$.

Results

Frequencies of *NKG2D* haplotype

The *NKG2D* gene polymorphism was analyzed in 145 pairs of unrelated donors-recipients of bone marrow following myeloablative conditioning (Table 1). The haplotype frequencies of *LNK1/LNK1*, *HNK1/LNK1* and *HNK1/HNK1* were 43%, 42% and 15%, respectively in donors and 35%, 45% and 20%, respectively in recipients. These frequencies were similar to those reported in previous studies in Japanese populations^{21,24} and were in accordance with the Hardy-Weinberg equilibrium ($p=0.80$).

Transplant outcomes according to *NKG2D* haplotype

With a median follow-up of 115 months among survivors (range, 74 to 140 months), 30 recipients (21%) had relapsed or progressed and 62 (47%) had died. Three patients (2%) died before engraftment. The analysis of the influence of the *NKG2D* genotype on clinical out-

comes after transplantation was stratified according to whether the recipients had standard-risk disease or high-risk disease to account for the already recognized prognostic difference. The overall survival at 5 years in patients with standard-risk disease was 63% while that of patients with high-risk disease was 44% ($p=0.06$). The 5-year cumulative incidences of transplant-related mortality were 32% and 27%, respectively ($p=0.33$) and those of disease relapse were 10% and 31%, respectively ($p=0.0006$).

The transplant outcomes according to *NKG2D* genotype are summarized in Table 2. Patients with standard-risk disease receiving transplants from donors with the *HNK1* haplotype had a significantly better 5-year overall survival (73% vs. 49%, $p=0.01$; Figure 1A) and lower transplant-related mortality rate (22% vs. 45%, $p=0.02$; Figure 1B) than those receiving transplants from donors without the *HNK1* haplotype. No difference was noted in disease relapse in relation to the donors' polymorphism (9% vs. 11%, $p=0.81$; Figure 1C) or in the development of grades II to IV acute GVHD (28% vs. 41%, $p=0.25$) or chronic GVHD (37% vs. 41%, $p=0.83$). When patients with acute myeloid leukemia or myelodysplastic syndrome were separately analyzed, there was still no difference in disease relapse in relation to *NKG2D* polymorphisms (*data not shown*). In patients with high-risk disease, the donor *HNK1* haplotype had no significant effects on transplant outcomes (Table 2).

Multivariate analysis

Any factors found to be significant in univariate analyses were included in the multivariate analysis. When patients with standard-risk disease were analyzed, the *HNK1* haplotype in donors remained statistically significant in multivariate analyses for both overall survival and transplant-related mortality (Table 3). The presence of the *HNK1* haplotype in the donor resulted in better overall survival (hazard ratio, 0.44; 95% confidence interval, 0.23 to 0.85; $p=0.01$) and transplant-related mortality (hazard ratio, 0.42; 95% confidence interval, 0.21 to 0.86; $p=0.02$).

Table 2. Univariate analysis of the association of *NKG2D* polymorphisms with clinical outcomes after transplantation.

	N.	5-year OS	p	5-year TRM	p	5-year relapse	p	Grade II-IV acute GVHD	p	Chronic GVHD	p
Standard-risk disease											
Donor <i>NKG2D</i> haplotype			0.01		0.02		0.81		0.25		0.83
<i>HNK1</i> -positive	55	73%		22%		9%		28%		37%	
<i>HNK1</i> -negative	38	49%		45%		11%		41%		41%	
Recipient <i>NKG2D</i> haplotype			0.39		0.31		0.93		0.48		0.98
<i>HNK1</i> -positive	61	62%		33%		10%		37%		39%	
<i>HNK1</i> -negative	32	66%		28%		9%		25%		38%	
High-risk disease											
Donor <i>NKG2D</i> haplotype			0.91		0.77		0.93		0.08		0.47
<i>HNK1</i> -positive	28	43%		26%		33%		54%		44%	
<i>HNK1</i> -negative	24	46%		29%		29%		30%		35%	
Recipient <i>NKG2D</i> haplotype			0.41		0.43		0.10		0.40		0.68
<i>HNK1</i> -positive	33	42%		23%		39%		39%		37%	
<i>HNK1</i> -negative	19	47%		35%		18%		50%		47%	

OS: overall survival; TRM: transplant-related mortality.

The donor and recipient *HNK1* haplotype did not significantly influence the transplant outcomes in patients with high-risk disease.

Main causes of death

The main causes of death according to the *HNK1* haplotype of the donors and recipients are illustrated in Figure 2A for patients with standard-risk disease, and in Figure 2B for those with high-risk disease. In patients with standard-risk disease receiving transplants from *HNK1*-negative donors, the most frequent cause of death was acute GVHD, followed by interstitial pneumonia. Transplants from *HNK1*-positive donors resulted in a statistically significantly reduced incidence of death attributed to acute GVHD (Figure 3A; *p*=0.006) as well as a trend toward a lower incidence of death attributed to interstitial pneumonia (Figure 3B; *p*=0.09). Other causes of death did not differ according to the *HNK1* haplotype.

Discussion

The current study showed an association between the *NKG2D-HNK1* haplotype in unrelated donors of HLA-matched myeloablative bone marrow transplants (haplotype frequency, 61%) and a significantly reduced transplant-related mortality and better overall survival for their recipients with standard-risk disease. The polymorphism of the donor *NKG2D* gene did not influence disease relapse or the development of grades II to IV acute GVHD or chronic GVHD in the patients. One possible explanation for the absence of the beneficial effects of the *HNK1* haplotype in patients with high-risk disease may be that the number of cases in the study was insufficient for a meaningful assessment of the effect. Alternatively, disease progression may precede the emergence of the potential advantageous effects of the *HNK1* donor haplotype that could protect the recipient from severe transplant-related complications. There was a larger difference in disease relapse between patients with

standard-risk disease and those with high-risk disease: 10% and 31% at 3 years after transplantation, respectively.

NKG2D plays important roles in immunity to microbial infections and is especially prominent in controlling viral and bacterial infections.¹⁶ Therefore, the reduced transplant-related mortality in patients with standard-risk disease receiving grafts from donors with the *HNK1* haplotype in this study might be a consequence of increased resistance to infections in the recipients. However, the hypothesis is too speculative because of the unavailability of data on causes of infections in this cohort. Further studies will be needed to clarify whether the *HNK1* haplotype in donors can effectively protect patients against infections.

Several studies have shown that NK cell activity has an important role in the outcomes of patients undergoing allogeneic transplantation.^{33,34} Alloreactive NK cells reduced the risk of relapse of acute myeloid leukemia without increasing the incidence of GVHD, resulting in a marked improvement of event-free survival in a series of haploidentical transplant recipients.^{35,36} In HLA-identical sibling transplants, the absence of HLA-C and HLA-B ligand for donor-inhibitory killer immunoglobulin-like receptors (KIR) provided benefits in terms of survival and relapse of patients with acute myeloid leukemia and myelodysplastic syndrome in recipients of T-cell-depleted SCT.³⁷ On the other hand, the JMDF found that KIR ligand mismatch was unfavorably correlated with relapse of leukemia and survival in patients undergoing T-cell-replete unrelated bone marrow transplants.³⁸ All patients in the present study received grafts from an HLA-A, -B, and -C allele-matched donor, implying KIR ligand match between each patient and donor. It is an open question whether the *NKG2D* polymorphism could affect the outcomes of patients undergoing transplantation with KIR-mismatched grafts.

In this study, major and minor ABO incompatibilities between the donor and recipient tended to be associated with poorer transplant outcomes, regardless of the risk

Table 3. Multivariate analysis of the association of *NKG2D* polymorphisms with clinical outcomes after transplantation.

Variable	Overall survival			Transplant-related mortality			Relapse			Grades II-IV acute GVHD			Chronic GVHD		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Standard-risk disease															
<i>HNK1</i> -positive donor	0.44	0.23-0.85	0.01	0.42	0.21-0.86	0.02	0.71	0.19-2.67	0.61	0.83	0.39-1.75	0.63	0.83	0.39-1.75	0.62
<i>HNK1</i> -positive recipient	1.22	0.60-2.50	0.58	1.32	0.61-2.87	0.48	1.11	0.28-4.48	0.88	1.54	0.66-3.57	0.32	1.06	0.49-2.31	0.88
Donor age, >31 years	-	-	-	-	-	-	-	-	-	2.17	0.95-4.96	0.07	-	-	-
Major ABO incompatibility	-	-	-	-	-	-	-	-	-	3.12	1.49-6.56	0.003	0.50	0.17-1.45	0.20
Minor ABO incompatibility	2.42	1.17-5.03	0.02	-	-	-	-	-	-	-	-	-	0.29	0.07-1.24	0.10
High-risk disease															
<i>HNK1</i> -positive donor	0.68	0.30-1.51	0.34	0.62	0.20-1.91	0.40	1.25	0.41-3.80	0.69	1.87	0.69-5.07	0.22	1.55	0.60-4.01	0.37
<i>HNK1</i> -positive recipient	1.41	0.65-3.07	0.39	0.76	0.25-2.29	0.63	2.35	0.66-8.44	0.19	0.47	0.18-1.22	0.12	0.92	0.35-2.38	0.86
Age, >26 years	1.95	0.93-4.09	0.08	6.30	1.86-21.32	0.003	-	-	-	-	-	-	-	-	-
Donor age, >31 years	-	-	-	-	-	-	0.53	0.17-1.65	0.27	-	-	-	-	-	-
Minor ABO incompatibility	2.94	1.19-7.25	0.02	-	-	-	-	-	-	5.10	2.08-12.52	0.004	-	-	-

category of the disease. These findings are compatible with those of a previous study by the JMDP,³⁹ although the impact of ABO incompatibilities on SCT outcomes is controversial.

This study also identified age as a significant predictive factor for transplant-related mortality in the patients with

standard-risk disease. This is consistent with the results of a previous study⁴⁰ showing that age over 35 years increased the risk of transplant-related mortality after allogeneic myeloablative SCT in high-risk patients.

A possible limitation of this study is the fact that no direct evidence is yet available regarding the ability of *NKG2D* polymorphisms to protect against microbial infections. The association observed between the *NKG2D* haplotype and transplant outcome might be due to another genetic polymorphism in linkage disequilibrium responsible for a better transplant outcome. One candidate gene is *NKG2F* (*KLRC4*), which is located in the NK complex region adjacent to the *NKG2D* gene, because an intrinsic SNP (rs2617171) in the gene has been reported to be in complete linkage with the *NKG2D* genotype.²⁴ Alternatively, polymorphisms may not be directly associated with controlling infection, but rather may be associated with other factors, such as sensitivity to treatment against GVHD or protection against organ toxicities related to transplants, which also influence the transplant outcome. These hypotheses have yet to be verified give the insufficient evidence.

Polymorphisms in genes encoding for nucleotide-binding oligomerization domain 2 (NOD2)/caspase recruitment domain 15 (CARD15),⁹ heme oxygenase-1 (HO-1) promoter,⁶ the Toll-like receptor 4,⁴ CC chemokine ligand (CCL) 5 promoter,³² transforming growth factor (TGF) β 1,¹¹ interleukin (IL) 12, tumor necrosis factor (TNF) α ,¹⁵ IL-23,⁵ mannose-binding lectin (MBL),¹⁰ Fc γ receptor IIa (Fc γ RIIa), myeloperoxidase (MPO), Fc γ RIIIb, IL-1Ra, IL-10,¹² Fc receptor-like 3 (FCRL3), peptidylarginine deimi-

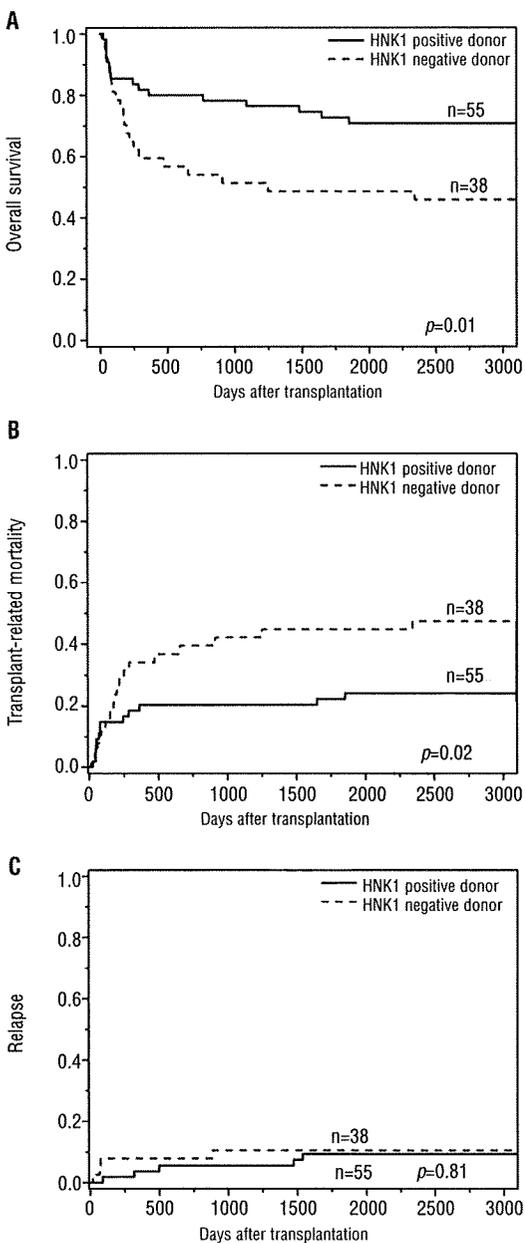


Figure 1. Kaplan-Meier analysis of (A) overall survival, (B) cumulative incidence of transplant-related mortality and (C) disease relapse after transplantation according to the donor *NKG2D* polymorphism in patients with standard-risk disease. Patients with donors with the *HNK1* haplotype had better overall survival and lower transplant-related mortality. Donor haplotype had no significant impact on disease relapse.

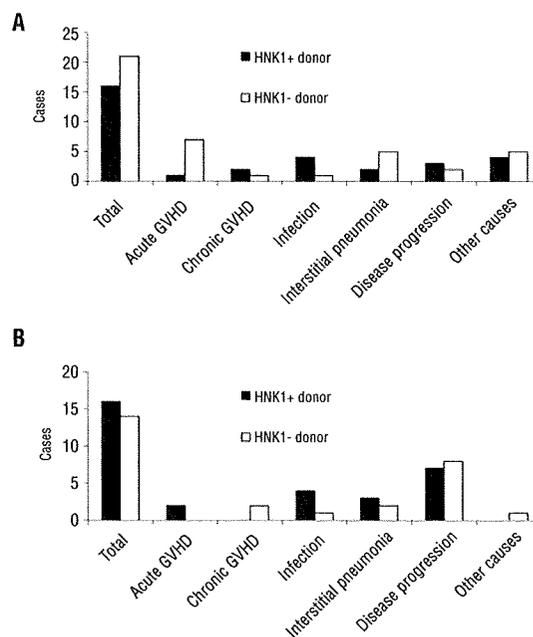


Figure 2. Main causes of death after transplantation according to the *NKG2D* polymorphism in patients with (A) standard-risk disease (B) high-risk disease.

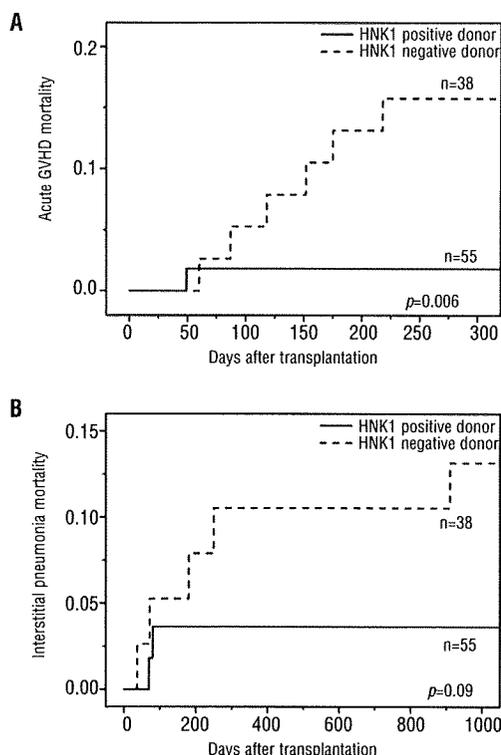


Figure 3. Cumulative incidence of deaths due to (A) acute GVHD and (B) interstitial pneumonia after transplantation in patients with standard-risk disease. The *HNK1* haplotype in donors was associated with a significantly lower incidence of deaths due to acute GVHD ($p=0.006$) as well as a trend toward a lower incidence of deaths due to interstitial pneumonia ($p=0.09$).

ciated with overall survival in the present study. This may prompt the determination of the donor *NKG2D* polymorphism prior to SCT in order to choose the best donor, expected to minimize transplant-related mortality after SCT, when multiple donors for a patient are available. Otherwise, prior information on the donor *NKG2D* polymorphism may be helpful in selecting risk-specific appropriate precautions following transplantation.

In conclusion, the present data suggest that the *NKG2D* polymorphism, in addition to HLA disparity between recipients and donors, affects prognosis after a bone marrow transplant from an unrelated donor. However, care should be made in drawing conclusions because the number of patients in the present study was small. The finding of a gene polymorphism may not be equivalent to differences in gene expression, which may be influenced by multiple factors because the *NKG2D* receptor is found on many tissues and cells.⁴¹ Experimental evidence is required to substantiate the effect of the *NKG2D* polymorphism on immune function. We next plan to conduct a prospective study to confirm these results and to extend this investigation to other transplantation settings, such as related donor SCT, reduced-intensity SCT, HLA-mismatched SCT and SCT for patients with non-hematologic malignancies.

Authorship and Disclosures

JLE and AT designed and performed the research, and contributed to the same aspects of the work; AT, JLE and SN wrote the paper; AT, YKa, and SOH performed the statistical analyses; MO, HS, HA, KM, SOK, MI, TE, YM, and YKo contributed to data collection.

The authors reported no potential conflicts of interest.

nase citullinating enzymes 4 (*PADI4*)¹³ and methylenetetrahydrofolate reductase (*MTHFR*)¹⁴ have been shown to influence the outcome after allogeneic SCT. Most of them are associated with the development of GVHD. Only the *NOD2/CARD15* and *HO-1* promoter polymorphisms have a significant impact on overall survival after SCT. Furthermore, the impact of the *HO-1* promoter polymorphisms depends on donor cells but not on recipient cells, as observed with the *NKG2D* polymorphism which, in the donor, was shown to be significantly asso-

References

- Weisdorf DJ, Anasetti C, Antin JH, Kernan NA, Kollman C, Snyder D, et al. Allogeneic bone marrow transplantation for chronic myelogenous leukemia: comparative analysis of unrelated versus matched sibling donor transplantation. *Blood* 2002; 99:1971-7.
- Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P, et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. *Bone Marrow Transplant* 2005;36:757-69.
- Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E. Genetic polymorphisms predicting the outcome of bone marrow transplants. *Br J Haematol* 2004; 127:479-90.
- Bochud PY, Chien JW, Marr KA, Leisenring WM, Upton A, Janer M, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N Engl J Med* 2008; 359:1766-77.
- Elmaagacli AH, Koldehoff M, Landt O, Beelen DW. Relation of an interleukin-23 receptor gene polymorphism to graft-versus-host disease after hematopoietic-cell transplantation. *Bone Marrow Transplant* 2008; 41:821-6.
- Gerbitz A, Hillemanns P, Schmid C, Wilke A, Jayaraman R, Kolb HJ, et al. Influence of polymorphism within the heme oxygenase-1 promoter on overall survival and transplantation-related mortality after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 2008;14:1180-9.
- Holler E, Rogler G, Brenmoehl J, Hahn J, Herfarth H, Greinix H, et al. Prognostic significance of *NOD2/CARD15* variants in HLA-identical sibling hematopoietic stem cell transplantation: effect on long-term outcome is confirmed in 2 independent cohorts and may be modulated by the type of gastrointestinal decontamination. *Blood* 2006;107:4189-93.
- Holler E, Rogler G, Herfarth H, Brenmoehl J, Wild PJ, Hahn J, et al. Both donor and recipient

- NOD2/CARD15 mutations associate with transplant-related mortality and GvHD following allogeneic stem cell transplantation. *Blood* 2004;104:889-94.
9. Mayor NP, Shaw BE, Hughes DA, Maldonado-Torres H, Madrigal JA, Keshav S, et al. Single nucleotide polymorphisms in the NOD2/CARD15 gene are associated with an increased risk of relapse and death for patients with acute leukemia after hematopoietic stem-cell transplantation with unrelated donors. *J Clin Oncol* 2007;25:4262-9.
 10. Mullighan CG, Heatley S, Doherty K, Szabo F, Grigg A, Hughes TP, et al. Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hematopoietic stem cell transplantation. *Blood* 2002;99:3524-9.
 11. Noori-Daloui MR, Rashidi-Nezhad A, Izadi P, Hossein-Nezhad A, Sobhani M, Derakhshandeh-Peykar P, et al. Transforming growth factor- β 1 codon 10 polymorphism is associated with acute GVHD after allogeneic BMT in Iranian population. *Ann Transplant* 2007;12:5-10.
 12. Rocha V, Franco RF, Porcher R, Bittencourt H, Silva WA Jr, Latouche A, et al. Host defense and inflammatory gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 2002;100:3908-18.
 13. Shimada M, Onizuka M, Machida S, Suzuki R, Kojima M, Miyamura K, et al. Association of autoimmune disease-related gene polymorphisms with chronic graft-versus-host disease. *Br J Haematol* 2007;139:458-63.
 14. Soydan E, Topcuoglu P, Dalva K, Arat M. The impact of methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism on transplant-related variables after allogeneic hematopoietic cell transplantation in patients receiving MTX as GVHD prophylaxis. *Bone Marrow Transplant* 2008;42:429-30.
 15. Viel DO, Tsuneto IT, Sossai CR, Lieber SR, Marques SB, Vigorito AC, et al. IL2 and TNFA gene polymorphisms and the risk of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Scand J Immunol* 2007;66: 703-10.
 16. Burgess SJ, Maasho K, Masilamani M, Narayanan S, Borrego F, Coligan JE. The NKG2D receptor: immunobiology and clinical implications. *Immunol Res* 2008;40:18-34.
 17. Hyka-Nouspikel N, Phillips JH. Physiological roles of murine DAP10 adapter protein in tumor immunity and autoimmunity. *Immunol Rev* 2006;214:106-17.
 18. Jamieson AM, Diefenbach A, McMahon CW, Xiong N, Carlyle JR, Raulat DH. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. *Immunity* 2002;17:19-29.
 19. Raulat DH. Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol* 2003;3:781-90.
 20. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* 2002;419: 734-8.
 21. Hayashi T, Imai K, Morishita Y, Hayashi I, Kusunoki Y, Nakachi K. Identification of the NKG2D haplotypes associated with natural cytotoxic activity of peripheral blood lymphocytes and cancer immunosurveillance. *Cancer Res* 2006;66: 563-70.
 22. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet* 2000;356:1795-9.
 23. Furue H, Kumimoto H, Matsuo K, Suzuki T, Hasegawa Y, Shinoda M, et al. Opposite impact of NKG2D genotype by lifestyle exposure to risk of aerodigestive tract cancer among Japanese. *Int J Cancer* 2008; 123:181-6.
 24. Furue H, Matsuo K, Kumimoto H, Hiraki A, Suzuki T, Yatabe Y, et al. Decreased risk of colorectal cancer with the high natural killer cell activity NKG2D genotype in Japanese. *Carcinogenesis* 2008; 29:316-20.
 25. Kawase T, Morishima Y, Matsuo K, Kashiwase K, Inoko H, Saji H, et al. High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism. *Blood* 2007;110:2235-41.
 26. Sasazuki T, Juji T, Morishima Y, Kinukawa N, Kashiwabara H, Inoko H, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. *Japan Marrow Donor Program. N Engl J Med* 1998;339:1177-85.
 27. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal* 1999;14:143-9.
 28. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995;15:825-8.
 29. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 1980;69:204-17.
 30. Scrucca L, Santucci A, Aversa F. Competing risk analysis using R: an easy guide for clinicians. *Bone Marrow Transplant* 2007;40:381-7.
 31. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med* 1999;18: 695-706.
 32. Kim DH, Jung HD, Lee NY, Sohn SK. Single nucleotide polymorphism of CC chemokine ligand 5 promoter gene in recipients may predict the risk of chronic graft-versus-host disease and its severity after allogeneic transplantation. *Transplantation* 2007;84:917-25.
 33. Dulphy N, Haas F, Busson M, Belhadj S, Peffault de Latour R, Robin M, et al. An unusual CD56(bright) CD16(low) NK cell subset dominates the early posttransplant period following HLA-matched hematopoietic stem cell transplantation. *J Immunol* 2008; 181:2227-37.
 34. Hamby K, Trexler A, Pearson T, Larsen C, Rigby M, Kean L. NK cells rapidly reject allogeneic bone marrow in the spleen through a perforin- and Ly49D-dependent, but NKG2D-independent mechanism. *Am J Transplant* 2007;7:1884-96.
 35. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik W, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002;295:2097-100.
 36. Ruggeri L, Mancusi A, Burchielli E, Capanni M, Carotti A, Aloisi T, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. *Blood Cells Mol Dis* 2008;40: 84-90.
 37. Hsu K, Keever-Taylor C, Wilton A, Pinto C, Heller G, Arkun K, et al. Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. *Blood* 2005;105: 4878-84.
 38. Morishima Y, Yabe T, Matsuo K, Kashiwase K, Inoko H, Saji H, et al. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. *Biol Blood Marrow Transplant* 2007;13: 315-28.
 39. Kimura F, Sato K, Kobayashi S, Ikeda T, Sao H, Okamoto S, et al. Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. *Haematologica* 2008;93: 1686-93.
 40. Goldstone AH, Richards SM, Lazarus HM, Tallman MS, Buck G, Fielding AK, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). *Blood* 2008;111: 1827-33.
 41. Collins RW. Human-MHC class I chain related (MIC) genes: their biological function and relevance to disease and transplantation. *Eur J Immunogenet* 2004;31:105-14.

Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia

Yoshiko Atsuta,¹ Ritsuro Suzuki,¹ Tokiko Nagamura-Inoue,² Shuichi Taniguchi,³ Satoshi Takahashi,⁴ Shunro Kai,⁵ Hisashi Sakamaki,⁶ Yasushi Kouzai,⁷ Masaharu Kasaj,⁸ Takahiro Fukuda,⁹ Hiroshi Azuma,¹⁰ Minoko Takanashi,¹¹ Shinichiro Okamoto,¹² Masahiro Tsuchida,¹³ Keisei Kawa,¹⁴ Yasuo Morishima,¹⁵ Yoshihisa Kodera,¹⁶ and Shunichi Kato,¹⁷ for the Japan Marrow Donor Program and the Japan Cord Blood Bank Network

¹Department of Hematopoietic Stem Cell Transplantation Data Management, Nagoya University School of Medicine, Nagoya; ²Department of Cell Processing & Transfusion, Research Hospital, Institute of Medical Science, University of Tokyo, and Tokyo Cord Blood Bank, Tokyo; ³Department of Hematology, Toranomon Hospital, Tokyo; ⁴Department of Molecular Therapy, Institute of Medical Science, University of Tokyo, Tokyo; ⁵Department of Transfusion Medicine, Hyogo College of Medicine, Nishinomiya; ⁶Division of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo; ⁷Department of Transfusion Medicine, Tokyo Metropolitan Fuchu Hospital, Tokyo; ⁸Department of Hematology, Sapporo Hokuyu Hospital, Sapporo; ⁹Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo; ¹⁰Hokkaido Red Cross Blood Center, Sapporo; ¹¹Japanese Red Cross Tokyo Blood Center, Tokyo; ¹²Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo; ¹³Ibaraki Children's Hospital, Mito; ¹⁴Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi; ¹⁵Aichi Cancer Center Hospital, Nagoya; ¹⁶BMT Center, Japanese Red Cross Nagoya First Hospital, Nagoya; and ¹⁷Department of Cell Transplantation & Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan

We made a disease-specific comparison of unrelated cord blood (CB) recipients and human leukocyte antigen allele-matched unrelated bone marrow (BM) recipients among 484 patients with acute myeloid leukemia (AML; 173 CB and 311 BM) and 336 patients with acute lymphoblastic leukemia (ALL; 114 CB and 222 BM) who received myeloablative transplantations. In multivariate analyses, among AML cases, lower overall survival (hazard ratio [HR] = 1.5; 95% confidence interval [CI], 1.0-2.0, $P = .028$) and

leukemia-free survival (HR = 1.5; 95% CI, 1.1-2.0, $P = .012$) were observed in CB recipients. The relapse rate did not differ between the 2 groups of AML (HR = 1.2; 95% CI, 0.8-1.9, $P = .38$); however, the treatment-related mortality rate showed higher trend in CB recipients (HR = 1.5; 95% CI, 1.0-2.3, $P = .085$). In ALL, there was no significant difference between the groups for relapse (HR = 1.4, 95% CI, 0.8-2.4, $P = .19$) and treatment-related mortality (HR = 1.0; 95% CI, 0.6-1.7, $P = .98$), which contributed to similar

overall survival (HR = 1.1; 95% CI, 0.7-1.6, $P = .78$) and leukemia-free survival (HR = 1.2; 95% CI, 0.9-1.8, $P = .28$). Matched or mismatched single-unit CB is a favorable alternative stem cell source for patients without a human leukocyte antigen-matched related or unrelated donor. For patients with AML, decreasing mortality, especially in the early phase of transplantation, is required to improve the outcome for CB recipients. (Blood. 2009;113:1631-1638)

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) with bone marrow (BM) or peripheral blood, the curative treatment of choice for acute leukemia, is limited by the inadequate supply of human leukocyte antigen (HLA)-identical related donors. Bone marrow from HLA-matched unrelated donors has been a major alternative graft source.¹⁻³ Umbilical cord blood (CB), an alternative stem cell source to BM or peripheral blood stem cells, has been used primarily in children,⁴⁻¹⁰ but its use in adults is increasing.^{11,12}

Clinical comparison studies of cord blood transplantation (CBT) and bone marrow transplantation (BMT) for leukemia from unrelated donors in adult recipients showed comparable outcomes.¹¹⁻¹³ Recipients of CBT showed delayed neutrophil recovery and lower incidence of acute graft-versus-host disease (GVHD).¹¹⁻¹³ Overall treatment-related mortality (TRM) was reported to be similar¹² or higher¹¹ compared with HLA-matched BM. Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are different disease entities that require different chemotherapy regimens for treatment. However, previous comparison

studies have included both diseases because of limitation in the number of CBTs given to adults.

In addition, the study periods of previous studies encompass the pioneering period of CBT, when the general practice was to use these grafts in patients in whom there were no other curative options and when the relevance of cell dose and HLA matching had not yet been recognized.^{6,7,14}

Accumulation of a larger number of CBT results enabled us to make a controlled comparison with unrelated BMTs. To avoid the inclusion of the pioneering period of CBT, the subjects were limited to those who received transplantations in and after 2000.

Methods

Collection of data and data source

The recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN) and the Japan Marrow Donor Program (JMDP).¹⁵

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Peripheral blood stem cell donation from unrelated donors is not permitted in Japan. All 11 CB banks in Japan are affiliated to JCBBN. Both JCBBN and JMDP collect recipients' clinical information at 100 days after transplantation. Patients' information on survival, disease status, and long-term complications, including chronic GVHD and second malignancies, are renewed annually by follow-up forms. This study was approved by the data management committees of JMDP and JCBBN.

Patients

Between January 2000 and December 2005, a total of 1690 adult patients at least 16 years of age with acute leukemia (999 AML, 261 CB and 738 BM; and 691 ALL, 178 CB and 513 BM) received first HSCT with myeloablative conditioning either CB or BM from unrelated donors. Of these, patients who received a single CB unit with 0 to 2 HLA mismatches, or HLA-A, -B, -C, and DRB1 allele-matched BM from unrelated donors were analyzed. HLA matching of CB was performed using low-resolution molecular typing methods for HLA-A and -B, and high-resolution molecular typing for HLA-DRB1. Of 1023 BM recipients with complete HLA high-resolution data, the following recipients with HLA HLA-A, -B, -C, and DRB1 allele mismatches were excluded: 306 recipients with 1 of 8 mismatches (39 for HLA-A, 6 for HLA-B, 137 for HLA-C, and 124 for HLA-DRB1), 150 recipients with 2 of 8 mismatches (36 for 2 class I antigens, and 114 for class I and class II antigens), 33 recipients with 3 of 8 mismatches, and 1 recipient with 4 of 8 mismatches. Of 390 recipients of CB with complete HLA data, 95 recipients with 3 mismatches and 8 patients with 4 mismatches were excluded. A total of 484 patients with AML (173 CBTs and 311 BMTs) and 336 patients with ALL (114 CBTs and 222 BMTs) were the subjects for the analyses. Eighty-five centers performed 287 CBTs analyzed in this study, and 114 centers performed 533 BMTs.

Definitions

Neutrophil recovery was defined by an absolute neutrophil count of at least 500 cells/mm³ for 3 consecutive points; platelet recovery was defined by a count of at least 50 000 platelets/mm³ without transfusion support. Diagnosis and clinical grading of acute GVHD were performed according to the established criteria.¹⁶ Relapse was defined as a recurrence of underlying hematologic malignant diseases. Treatment-related death was defined as death during a continuous remission. Leukemia-free survival (LFS) was defined as survival in a state of continuous remission.

Statistical analysis

Separate analyses were performed for AML and ALL. Descriptive statistical analysis was performed to assess patient baseline characteristics, diagnosis, disease classification, disease status at conditioning, donor-patient ABO mismatches, preparative regimen, and GVHD prophylaxis. The 2-sided χ^2 test was used for categorical variables, and the 2-sided Wilcoxon rank sum test was used for continuous variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of neutrophil and platelet recovery, acute and chronic GVHD, relapse, and TRM.¹⁷ For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event; for GVHD, death without GVHD and relapse were the competing events; for relapse, death without relapse was the competing event; and, for TRM, relapse was the competing event. Gray test was used for group comparison of cumulative incidence.¹⁸ Overall survival (OS) and LFS were calculated using the Kaplan-Meier method. The log-rank test was used for group comparisons. Adjusted comparison of the stem cell source on OS and LFS was performed with the use of the Cox proportional-hazards regression model. For other outcomes, the Fine and Gray proportional-hazards model for subdistribution of a competing risk was used.¹⁹ Adjusted probabilities of OS and DFS were estimated using the Cox proportional-hazards regression model, with consideration of other significant clinical variables in the final multivariate models. The variables considered were the patient's age at transplantation, patient's sex, donor-patient sex mismatch, donor-patient ABO mismatch, disease status at conditioning, and (9;22) chromosome abnormality or others for ALL, cytogenetic information and French-American-British (FAB) classification

of M5/M6/M7 or others for AML, the conditioning regimen, and the type of prophylaxis against GVHD. Factors differing in distribution between CB and BM recipients ($P < .10$) and factors known to influence outcomes (such as patient age at transplantation and chromosome abnormalities and FAB classification of leukemia) were included in the final models. Variables with more than 2 categories were dichotomized for the final multivariate model. The cutoff points of the variables were chosen to make optimal use of the information, with the proviso that smaller groups contain at least 20% of the patients. Variables were dichotomized as follows: patient age greater or younger than 45 years at transplantation, female donor to male recipient donor-recipient sex mismatch versus others for donor-recipient sex matching, donor-recipient ABO major mismatch versus others for ABO matching, M5/M6/M7 FAB classification versus others for classification of AML, chromosome abnormality other than favorable abnormalities for cytogenetics of AML, cyclophosphamide and total body irradiation (TBI) or busulfan and cyclophosphamide or others for conditioning regimen of AML, cyclophosphamide and TBI, or others for conditioning regimen of ALL, and cyclosporine-based versus tacrolimus-based prophylaxis against GVHD. Disease status at transplantation was categorized as first complete remission (1CR), second or later complete remission (2CR), or more advanced disease; which was included in the final model using dichotomized dummy variables. All P values were 2-sided.

The statistical power to detect hazard ratios (HRs) of 2.0 and 1.5 (a regression coefficient equal to 0.6931 and 0.4055, respectively) on Cox regression of the log hazard ratio at a .05 significance level adjusted for event rate were 99% and 78%, respectively, for 484 patients with AML and 97% and 60%, respectively, for 336 patients with ALL. The levels of statistical power for subgroup analyses were as follows: 54% and 22% for 1CR, 51% and 21% for 2CR, 96% and 58% for more advanced in AML patients, 62% and 26% for 1CR, 47% and 20% for 2CR, and 67% and 29% for more advanced in ALL patients.²⁰

Results

Patient characteristics

The characteristics of the patients are shown in Table 1. There was no significant difference in recipients' age at transplantation in AML (median age, CB vs BM = 38 vs 38 years, $P = .61$) and in ALL (median age, CB vs BM = 34 vs 32 years, $P = .29$). The female/male ratio was higher (CB vs BM = 54% vs 38% in AML patients, and CB vs BM = 54% vs 38% in ALL patients, $P < .001$ and $P = .005$, respectively) in CB recipients, resulting in the lower donor-patient sex match rate (CB vs BM = 48% vs 69% in AML patients, and CB vs BM = 46% vs 65% in ALL patients, $P < .001$ and $P = .002$, respectively) in CB recipients. The proportion of ALL patients with Philadelphia chromosome abnormality was higher (CB vs BM = 38% vs 23%) in CB recipients. CB recipients were likely to have more advanced disease status at transplantation (relapse or induction failure, CB vs BM = 47% vs 31% in AML patients, and CB vs BM = 26% vs 19% in ALL patients), and the difference was significant in AML ($P = .003$). HLA-A, -B (low-resolution typing), and -DRB1 (high-resolution typing) was mismatched in 93% of both AML and ALL among CB recipients, whereas HLA -A, -B, -C, and -DRB1 were all genotypically matched for BM recipients. The ABO-matched donor-patient pair proportion was consistently lower for CB (CB vs BM = 34% vs 59% in AML patients and CB vs BM = 32% vs 58% in ALL patients).

A preparative regimen with TBI and cyclophosphamide was used in almost all patients, and cytosine arabinoside was supplemented for CB recipients with AML (36%) in addition to TBI and cyclophosphamide. For GVHD prophylaxis, tacrolimus (CB vs BM = 29% vs 56% in AML patients, and CB vs BM = 37% vs 53% in ALL patients) and