developed overt leukemia throughout the median follow-up period of 106 days (WT and INS, n = 12 each). We speculate that this might be partly attributable to the limited engraftment of WT/INS-transduced T-cell progenitors in thymus (data not shown).

INS exacerbates the in vivo oncogenic activity of Notch1

INS-like mutations were reported to occur in 10% of T-ALL patients.[3,4] In contrast, Notch1 mutations were more frequently found in T-ALL patients and were equally distributed between patients with WT and INS.[4] The DND-41 cell line carries both INS and Notch1 mutations.[6,13] Moreover, IL7 signaling coordinates with Notch1 in proper T-cell developmental programming.[14,15] We hypothesized that INS may cooperate with active Notch1 mutants in T-cell leukemogenesis. Therefore, Lin^- cells were transduced with mock or IL7Rα-WT/INS along with an active form of intracellular Notch1 (ICN1), followed by syngeneic transplantation. As reported previously,[16] within 4 to 6 weeks after transplantation, all mice developed T-ALL, characterized by extrathymic expansion of leukemic cells (Figure 2A-B; supplemental Figure 8B). Clonality of INS/ICN1-induced leukemia was confirmed by Southern blot analysis around day 35 (supplemental Figure 5, *right*). Despite similar immunophenotypes ($CD3^+CD4^+CD8^+TCR\text{-}\beta^+$) between WT/ICN1 and INS/ICN1 cells (Figure 2A, *Upper*; supplemental Figures 8A and 9), histological examinations of the liver, SP, and BM in recipient mice revealed that systemic expansion of INS/ICN1-Lin^- cells was much more aggressive than that of WT/$ICN1^-$ and mock/$ICN1^-Lin^-$ cells (Figure 2A, *Lower*, and 2B). Furthermore, the median survival time of INS/ICN1 mice (44 days; n = 54) was significantly shorter than that of mock/ICN1 (60 days; n = 44) and WT/ICN1 (57 days; n = 42) mice ($P < .001$ by log-rank test; Figure 2C). Taken together, INS clearly exaggerated ICN1-induced T-ALL.

Forced expression of INS in B-cell progenitors caused mature B-ALL/lymphoma

Because the IL7Rα gene is transcriptionally active in common lymphoid progenitors (CLPs; $Lin^-c\text{-}kit^{low}Sca1^+$ $IL7R\alpha^+$) and their progenies and not expressed in stem cell compartments,[17] the INS allele could target the same cell populations. Then, CLPs were transduced with INS or WT IL7Rα and cultured on the OP9 stromal layer with a cytokine cocktail for 18 days, followed by transplantation of resulting pro-B cells into syngeneic recipient mice (supplemental Figure 10A). All but 1 of the INS-CLP recipients died of mature B-ALL/lymphoma, whereas no WT-CLP recipients died ($P < .01$; Figure 2D-E). Autopsy specimens revealed massive infiltration of $B220^+$ leukemic blasts into the BM, SP, and lymph nodes (Figure 2F; data not shown). This mature B-cell ALL/lymphoma was transplantable to secondary recipients, resulting in more aggressive mature B-ALL/lymphoma with much shorter survival periods (Figure 2E). INS-induced mature B-ALL/lymphoma was biclonal, as evidenced by Southern blot analysis (supplemental Figure 5, *right*). Under these experimental conditions, INS-CLPs had already committed to the cytokine-independent clonogenic pro-B cells before transplantation (supplemental Figure 10B-C; data not shown).

Finally, we wished to identify the downstream signals involved in INS-induced leukemogenesis. Using microarray analysis (Gene Expression Omnibus accession number GSE51211) of the resultant transformed cells in vitro and in vivo, we performed a comparative analysis of gene expression profiles from WT and INS-transduced hematopoietic stem/progenitor cells, as well as resultant leukemia cells that developed in vivo. As a result, we found a list of candidate genes (n = 6133) that were up- or downregulated by INS in comparison with WT. Among those genes, by reviewing hierarchical clustering analysis, several genes could be candidate mediators downstream of INS in comparison with WT, including hairy and enhancer of split-1 (*HES1*) for MPD, proviral insertion site in Moloney murine leukemia virus 1 (*PIM1*) for B-ALL, and insulin-like growth factor 1 receptor (*IGF1R*) for T-ALL (supplemental Figure 11). Quantitative RT-PCR verified their differential expression in comparison with WT (supplemental Figure 12). Putative involvement of these genes in INS-induced leukemogenesis was supported by previous data reporting the significance of *HES1* overexpression reported in advanced chronic myelogenous leukemia,[18] *PIM1* activation involved in pre-B-cell transformation,[19] *PIM1* overexpression reported in B-ALL,[20] and high-level expression of *IGF1R* in T-ALL.[20,21] In addition, we performed gene set enrichment analysis[22] to find significant overlaps between INS/ICN1 (in comparison with WT/ICN1) gene expression signature and gene sets present in the public database (supplemental Discussion). As a result, we found that in vivo INS/ICN1 was characterized by overexpression of interferon (IFN)-stimulated genes[23] and IGF1-signal-related genes,[24] suggesting constitutive activation of IFN^- as well as the $IGF1^-$ signal pathway (supplemental Figures 13 and 14; supplemental Tables 2 and 3). These are consistent with the previous report that JAK1-mutated T-ALL samples were characterized by the IFN-pathway[23] signature, as well as our findings of a higher *IGF1R* transcript level in INS/ICN1 cells compared with that of WT/ICN1 (supplemental Figures 11 and 12).

In conclusion, we provided evidence that INS has significant in vivo leukemogenic activity and that determination of the lineage of resulting leukemias depends on the developmental stage during which they occur and/or concurrent mutations. In addition, as far as we know, this is the first report in which transformation of CLP leading to in vivo malignancy is shown. This is also of general relevance for the field of lymphoid malignancies. Given that either IL7Rα or Jak1 gain-of-function mutations have been found in approximately 10%[3-5] or 19%[25] of T-ALL patients and that IFN-pathway signatures have been associated with Jak1-mutated T-ALL,[23] it is fairly certain that IFN-pathway signatures induced by aberrant IL7R/Jak1 axis might substantially contribute to the pathogenesis of T-ALL in close association with activating mutations in the Notch pathways.

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Authorship

Contribution: K.Y., N.Y., and K.I. performed experiments; K.Y. wrote the manuscript; A.H., A.K., and K.H. provided vital reagents; and A.T. supervised the research.

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References

1. Barata JT, Cardoso AA, Boussiotis VA. Interleukin-7 in T-cell acute lymphoblastic leukemia: an extrinsic factor supporting leukemogenesis? *Leuk Lymphoma.* 2005;46(4): 483-495.
2. Silva A, Laranjeira AB, Martins LR, et al. IL-7 contributes to the progression of human T-cell acute lymphoblastic leukemias. *Cancer Res.* 2011;71(14):4780-4789.
3. Shochat C, Tal N, Bandapalli OR, et al. Gain-of-function mutations in interleukin-7 receptor-α (IL7R) in childhood acute lymphoblastic leukemias. *J Exp Med.* 2011;208(5):901-908.
4. Zenatti PP, Ribeiro D, Li W, et al. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. *Nat Genet.* 2011; 43(10):932-939.
5. Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature.* 2012;481(7380):157-163.
6. Porcu M, Kleppe M, Gianfelici V, et al. Mutation of the receptor tyrosine phosphatase PTPRC (CD45) in T-cell acute lymphoblastic leukemia. *Blood.* 2012;119(19):4476-4479.
7. Kim K, Khaled AR, Reynolds D, Young HA, Lee CK, Durum SK. Characterization of an interleukin-7-dependent thymic cell line derived from a p53 (-/-) mouse. *J Immunol Methods.* 2003;274(1-2): 177-184.
8. Harvey M, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A, Donehower LA. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. *Nat Genet.* 1993;5(3):225-229.
9. Haines BB, Ryu CJ, Chang S, et al. Block of T cell development in P53-deficient mice accelerates development of lymphomas with characteristic RAG-dependent cytogenetic alterations. *Cancer Cell.* 2006;9(2):109-120.
10. Jiang Q, Li WQ, Aiello FB, Klarmann KD, Keller JR, Durum SK. Retroviral transduction of IL-7Ralpha into IL-7Ralpha-/- bone marrow progenitors: correction of lymphoid deficiency and induction of neutrophilia. *Gene Ther.* 2005;12(24): 1761-1768.
11. Kharas MG, Okabe R, Ganis JJ, et al. Constitutively active AKT depletes hematopoietic stem cells and induces leukemia in mice. *Blood.* 2010;115(7):1406-1415.
12. Kato Y, Iwama A, Tadokoro Y, et al. Selective activation of STAT5 unveils its role in stem cell self-renewal in normal and leukemic hematopoiesis. *J Exp Med.* 2005;202(1):169-179.
13. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science.* 2004; 306(5694):269-271.
14. Magri M, Yatim A, Benne C, et al. Notch ligands potentiate IL-7-driven proliferation and survival of human thymocyte precursors. *Eur J Immunol.* 2009;39(5):1231-1240.
15. García-Peydró M, de Yébenes VG, Toribio ML. Notch1 and IL-7 receptor interplay maintains proliferation of human thymic progenitors while suppressing non-T cell fates. *J Immunol.* 2006; 177(6):3711-3720.
16. Pear WS, Aster JC, Scott ML, et al. Exclusive development of T cell neoplasms in mice transplanted with bone marrow expressing activated Notch alleles. *J Exp Med.* 1996;183(5): 2283-2291.
17. Miller JP, Izon D, DeMuth W, Gerstein R, Bhandoola A, Allman D. The earliest step in B lineage differentiation from common lymphoid progenitors is critically dependent upon interleukin 7. *J Exp Med.* 2002;196(5):705-711.
18. Nakahara F, Sakata-Yanagimoto M, Komeno Y, et al. Hes1 immortalizes committed progenitors and plays a role in blast crisis transition in chronic myelogenous leukemia. *Blood.* 2010;115(14): 2872-2881.
19. Guo G, Qiu X, Wang S, et al. Oncogenic E17K mutation in the pleckstrin homology domain of AKT1 promotes v-Abl-mediated pre-B-cell transformation and survival of Pim-deficient cells. *Oncogene.* 2010;29(26): 3845-3853.
20. Haferlach T, Kohlmann A, Wieczorek L, et al. Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. *J Clin Oncol.* 2010;28(15): 2529-2537.
21. Medyouf H, Gusscott S, Wang H, et al. High-level IGF1R expression is required for leukemia-initiating cell activity in T-ALL and is supported by Notch signaling. *J Exp Med.* 2011;208(9): 1809-1822.
22. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA.* 2005;102(43):15545-15550.
23. Hornakova T, Chiaretti S, Lemaire MM, et al. ALL-associated JAK1 mutations confer hypersensitivity to the antiproliferative effect of type I interferon. *Blood.* 2010;115(16): 3287-3295.
24. Creighton CJ, Casa A, Lazard Z, et al. Insulin-like growth factor-I activates gene transcription programs strongly associated with poor breast cancer prognosis. *J Clin Oncol.* 2008;26(25): 4078-4085.
25. Flex E, Petrangeli V, Stella L, et al. Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia. *J Exp Med.* 2008;205(4): 751-758.

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Brief Articles

Effect of ABO Blood Group Incompatibility on the Outcome of Single-Unit Cord Blood Transplantation after Myeloablative Conditioning

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ABO blood group incompatibility between donor and recipient has been associated with poor transplant outcomes in allogeneic hematopoietic stem cell transplantation. However, its effect on the outcome of cord blood transplantation (CBT) has yet to be clarified. We retrospectively analyzed 191 adult patients who received single-unit CBT after myeloablative conditioning for malignant disease in our institute. Major mismatch showed a significantly lower incidence of platelet engraftment compared with ABO match as a reference (hazard ratio, .57; $P = .01$). Nevertheless, there was no increase in graft-versus-host disease, transplant-related mortality, and overall mortality after ABO-incompatible CBT. These data suggested that donor–recipient ABO incompatibility does not have a significant impact on outcome after myeloablative CBT for hematological malignancies.

INTRODUCTION

In contrast to solid organ transplantation, ABO blood group incompatibility between donor and recipient is reportedly a more common situation after allogeneic hematopoietic stem cell transplantation (allo-HSCT). It is well known that ABO incompatibility of allo-HSCT can cause an increased risk of delayed erythroid reconstitution, pure red cell aplasia, and acute and delayed hemolysis [1,2]. However, the association between ABO incompatibility and transplantation outcomes, such as neutrophil and platelet recovery, graft-versus-host disease (GVHD), and survival, is controversial [1,2]. Moreover, most of these studies analyzed patients receiving allo-HSCT using bone marrow or mobilized peripheral blood as a stem cell source from related and unrelated donors [1-5].

Cord blood transplantation (CBT) from an unrelated donor is increasingly used as an alternative transplant method for adult patients without HLA-compatible related or unrelated donors. Although most patients receive an HLA-mismatched cord blood unit, the lower risk of GVHD without compromising graft-versus-leukemia effects is one of the most attractive advantages of CBT. We previously reported that ABO incompatibility influenced platelet engraftment and transfusion requirement of RBCs and platelets in CBT [6]. However, the effects of ABO incompatibility on GVHD and survival after myeloablative CBT are limited. In the present study, we analyzed the neutrophil and platelet recovery, GVHD, transplant-related mortality (TRM), relapse, and survival in myeloablative CBT in adult patients with malignant disease in our institute.

METHODS

This retrospective study included data from 191 adult patients who underwent unrelated first allogeneic transplantation using single-unit CBT at our institute between August 1998 and February 2013. Donor–recipient ABO compatibility was categorized as follows: ABO match in 55 patients, major mismatch in 47, minor mismatch in 58, and bidirectional mismatch in 31. All patients received 12 Gy total body irradiation (TBI)-based myeloablative conditioning regimens and cyclosporine with or without short-term methotrexate as a GVHD prophylaxis, and cord blood units were selected as reported previously [7,8]. The institutional review board of the Institute of Medical Science, The University of Tokyo approved this study. This study was conducted in accordance with the Declaration of Helsinki.

The primary study endpoint was overall survival (OS), defined as the time from the date of transplantation to the date of death or last contact. Secondary endpoints were relapse, TRM, GVHD, and neutrophil and platelet recovery. Relapse was defined by morphologic evidence of disease in peripheral blood, bone marrow, or extramedullary sites. TRM was defined as death during a remission. Both acute GVHD (aGVHD) and chronic GVHD (cGVHD) were graded according to previously published criteria [9,10]. The incidence of aGVHD was evaluated in all engrafted patients, whereas the incidence of cGVHD was evaluated in engrafted patients surviving more than 100 days. Neutrophil engraftment was defined as being achieved on the first of 3 consecutive days during which the absolute neutrophil count was at least 0.5×10^9/L. Platelet engraftment was defined as being achieved on the first of 3 days when the platelet count was higher than 50×10^9/L without transfusion support.

Baseline patient and transplant characteristics were compared using the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables. The probability of OS was estimated according to the

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Kaplan-Meier method, and groups were compared using the log-rank test. The probabilities of the others were estimated based on a cumulative incidence method to accommodate competing risks. Multivariate analysis was performed with a Cox proportional hazard model adjusted for OS, and a Fine and Gray proportional hazards model for the others.

The following variables for multivariate analysis were considered: age (<45 versus ≥45 years), disease status at CBT (standard risk versus high risk), cord blood nucleated cell count (<2.5 × 10^7 versus ≥2.5 × 10^7/kg), cord blood CD34 + cell count (<1 × 10^5 versus ≥1 × 10^5/kg), HLA disparities based on antigen level HLA-A and -B and allele level HLA-DRB1 (1 versus 2 versus ≥3), sex compatibility between donor and recipient (female donor to male recipient versus other), year of CBT (1998 to 2005 versus 2006 to 2013), and ABO compatibility between donor and recipient (match versus major mismatch versus minor mismatch versus bidirectional mismatch). The ABO match was considered the reference group in the multivariate analyses.

All statistical analyses were performed with EZR, a graphic user interface for R 2.13.0 [11]. $P < .05$ was considered significant. Analysis of data was performed in August 2013. The median follow-up of surviving patients was 92 months (range, 5 to 181) after CBT in the entire cohort.

RESULTS

The characteristics of patients and cord blood units are shown in Table 1. There were no significant differences among the 4 groups, except for HLA disparities. The major mismatch group contained a slightly higher number of HLA disparities as compared with the minor mismatch group ($P = .07$) or the bidirectional mismatch group ($P = .08$), although these were not statistically significant.

The probability of OS at 5 years significantly differed among the 4 groups in univariate analysis ($P = .03$) (Figure 1A). However, multivariate analysis of mortality adjusting for other variables showed no significant difference between ABO match and major (hazard ratio [HR], 1.20; $P = .62$), minor (HR, .72; $P = .41$), or bidirectional (HR, 1.76; $P = .14$) mismatch (Table 2). In univariate analysis, ABO incompatibility was not associated with cumulative incidence of TRM (Figure 1B) or relapse (Table 2). In multivariate analysis, a trend toward a higher incidence of TRM was observed in the major mismatch compared with the match group, but this was not significant ($P = .05$).

In univariate analysis, there was no significant difference in the cumulative incidence of grades II to IV aGVHD among the 4 groups ($P = .91$) (Figure 1C). In multivariate analysis, a higher number (≥3) of HLA disparities (HR, 1.56; 95% confidence interval [CI], 1.05 to 2.32; $P = .02$), a higher cord blood CD34 + cell count (HR, 1.51; 95% CI, 1.05 to 2.18; $P = .02$), and older year of CBT (HR, 1.85; 95% CI, 1.30 to 2.65; $P < .01$) were associated with a higher incidence of grades II to IV aGVHD, but ABO incompatibility was not associated with the incidence of grades II to IV aGVHD (Table 2). The cumulative incidence of grades III to IV aGVHD significantly differed among the 4 groups in univariate analysis ($P = .02$). However, multivariate analysis adjusting for other variables showed no significant difference in the cumulative incidence of grades III to IV aGVHD between ABO match and major (HR, 2.56; $P = .19$), minor (HR, .59; $P = .56$), or bidirectional (HR, 1.46; $P = .67$) mismatch (Table 2). In univariate analysis, there was no significant difference in the cumulative incidence of extensive cGVHD among the 4 groups ($P = .86$) (Figure 1D). In multivariate analysis, older age (HR, 1.85; 95% CI, 1.06 to 3.23;

Table 1
Characteristics of Patients, Cord Blood Units, and Transplantation

	Total	Match	Major Mismatch	Minor Mismatch	Bidirectional Mismatch	P
Number (%)	191	55 (28)	47 (24)	58 (30)	31 (16)	
Age, yr, median (range)	40 (16-55)	40 (16-55)	40 (16-53)	40 (16-53)	41 (18-52)	.94
Disease type, n (%)						.61
AML	101 (52)	30 (54)	24 (51)	30 (51)	17 (54)	
ALL	45 (23)	17 (30)	10 (21)	11 (18)	7 (22)	
MDS	25 (13)	5 (9)	5 (10)	10 (17)	5 (16)	
CML	11 (5)	1 (1)	4 (8)	4 (6)	2 (6)	
NHL	9 (4)	2 (3)	4 (8)	3 (5)	0 (0)	
Disease status at CBT,* n (%)						.09
Standard risk	79 (41)	24 (44)	17 (36)	30 (51)	8 (25)	
High risk	112 (58)	31 (54)	30 (64)	28 (48)	23 (74)	
Conditioning regimen, n (%)						.36
TBI12Gy+Ara-C/G-CSF+CY	131 (68)	34 (61)	33 (70)	40 (68)	24 (77)	
TBI12Gy+Ara-C+CY	31 (16)	9 (16)	11 (23)	9 (15)	2 (6)	
TBI12Gy+CY	16 (8)	6 (10)	1 (2)	5 (8)	4 (12)	
TBI12Gy+others	13 (6)	6 (10)	2 (4)	4 (6)	1 (3)	
GVHD prophylaxis, n (%)						.10
Cyclosporine A + methotrexate	188 (98)	55 (100)	47 (100)	57 (98)	29 (93)	
Cyclosporine A	3 (1)	0 (0)	0 (0)	1 (2)	2 (6)	
Number of nucleated cells, ×10^7/kg, median (range)	2.43 (1.32-5.69)	2.52 (1.32-5.50)	2.47 (1.65-4.92)	2.38 (1.51-5.69)	2.58 (1.65-5.07)	.79
Number of CD34$^+$cells, ×10^5/kg, median (range)	.92 (.17-7.75)	.88 (.28-3.15)	.93 (.17-1.99)	.91 (.28-7.75)	1.14 (.44-2.84)	.20
HLA disparities,† n (%)						.05
1	23 (12)	4 (7)	7 (14)	8 (13)	4 (12)	
2	106 (55)	32 (58)	16 (34)	37 (63)	21 (67)	
3	57 (29)	17 (30)	23 (48)	12 (20)	5 (16)	
4	5 (2)	2 (3)	1 (2)	1 (1)	1 (3)	
Sex compatibility, n (%)						
Female donor to male recipient	58 (30)	19 (34)	13 (27)	17 (29)	9 (29)	.88
Other	133 (69)	36 (65)	34 (72)	41 (70)	22 (70)	
Year of CBT, n (%)						.58
1998-2005	102 (53)	28 (50)	22 (46)	33 (56)	19 (61)	
2006-2013	89 (46)	27 (49)	25 (53)	25 (43)	12 (38)	

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia, MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; NHL, non-Hodgkin lymphoma, Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide.

* For disease status at CBT, patients in complete remission (CR) 1 or CR2 without poor prognostic karyotype for AML and ALL, refractory anemia for MDS, chronic phase for CML, and CR1 or CR2 for NHL were classified as standard risk, whereas patients in all other situations were classified as high risk.

† The number of HLA disparities defined as low resolution for HLA-A and -B and high resolution for HLA-DRB1.

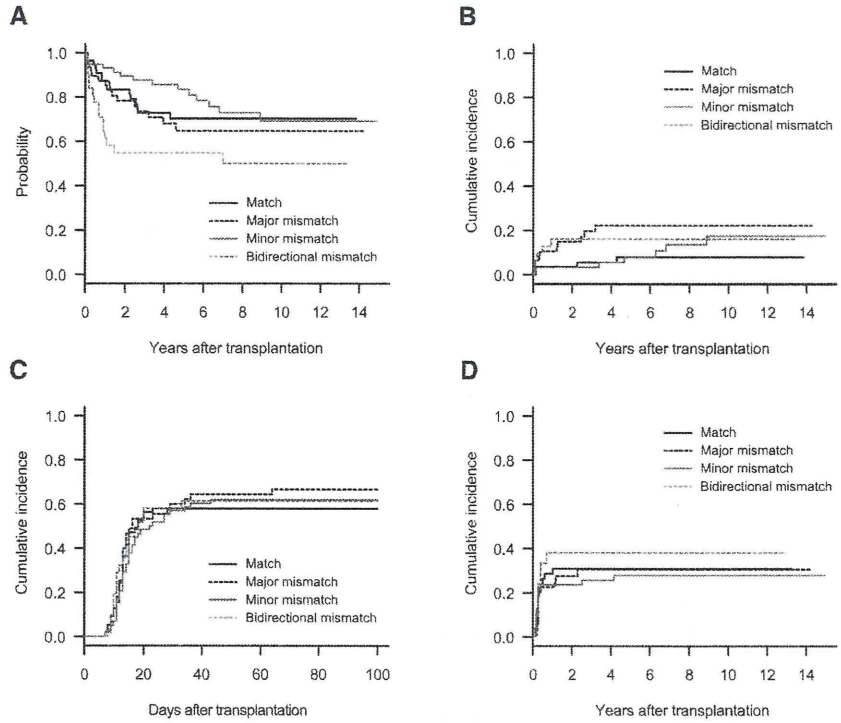

Figure 1. Probability of OS (A), cumulative incidence of TRM (B), grades II to IV aGVHD (C), and extensive cGVHD (D) according to donor–recipient ABO incompatibility after myeloablative CBT.

$P = .03$) and female donor to male recipient (HR, 1.79; 95% CI, 1.02 to 3.15; $P = .04$) were associated with a higher incidence of extensive cGVHD, but ABO incompatibility was not associated with the incidence of extensive cGVHD (Table 2).

ABO incompatibility was not associated with cumulative incidence of neutrophil engraftment among the 4 groups in univariate analysis ($P = .73$). In multivariate analysis, a lower cord blood CD34 + cell count (HR, .51; 95% CI, .37 to .70; $P < .001$), high risk of disease status at CBT (HR, .68; 95% CI, .50 to .93; $P = .01$), and older year of CBT (HR, .71; 95% CI, .53 to .96; $P = .02$) were associated with a lower incidence of neutrophil engraftment, but ABO incompatibility was not associated with neutrophil engraftment (Table 2). The cumulative incidence of platelet recovery was not significantly different among the 4 groups in univariate analysis ($P = .30$). In multivariate analysis, major mismatch (HR, .57; $P = .01$) showed a significantly lower incidence of platelet engraftment when compared with ABO match (Table 2). In addition, a lower cord blood CD34 + cell count (HR, .63; 95% CI, .45 to .88; $P < .01$), lower cord blood nucleated cell count (HR, .70; 95% CI, .52 to .94; $P = .01$), and high risk of disease status at CBT (HR, .65; 95% CI, .45 to .94; $P = .02$) were associated with a lower incidence of platelet engraftment.

We also analyzed the effect of major/bidirectional mismatch group defined as combined group of major and bidirectional mismatch. However, we were unable to find any impact of major/bidirectional mismatch on outcomes in multivariate analysis, except for platelet engraftment (Supplemental Table 1).

DISCUSSION

The ABO blood group antigens consist of oligosaccharide glycoproteins and are expressed not only in erythrocytes but also in neutrophils, platelets, and, vascular endothelial and epithelial cells. The ABO antigens could be immunological targets for ABO-incompatible donor or recipient lymphocytes, affecting GVHD and engraftment. Many previous studies have reported an increased risk of aGVHD after ABO-incompatible allogeneic bone marrow transplantation from related and unrelated donors, particularly in minor and bidirectional mismatch [3-5]. Igarashi et al. [12] reported an association between the anti-host isohemagglutinin produced by donor-derived B lymphocytes and the development of aGVHD after minor and bidirectional mismatched allogeneic bone marrow transplantation and peripheral blood stem cell transplantation from related and unrelated donors. These effects might be associated with ABO-incompatible immune responses against ABO antigens in vascular endothelial and epithelial cells of recipients. However, it has been reported that donor-derived isohemagglutinin was not identified in patients after minor and bidirectional mismatched CBT [12,13]. The higher proportion of naïve B lymphocytes in cord blood grafts might contribute to defective isohemagglutinin production after ABO-incompatible CBT, which might have contributed to the low incidence of severe GVHD even after ABO-incompatible CBT. Therefore, the effect of ABO incompatibility on transplant outcome might differ depending on the kinds of stem cell sources in allo-HSCT.

Several studies have reports on associations between ABO incompatibility and outcomes after CBT [14-19]. Romee et al. [14] reported no impact of ABO incompatibility on aGVHD and cGVHD in 503 CBT recipients. Berglund et al. [15] reported an increased incidence of grades II to IV aGVHD in major mismatch recipients (n = 23) of CBT. Moreover, previous studies demonstrated lower survival for major

Table 2
Univariate and Multivariate Analysis of ABO Compatibility for the Outcomes of CBT

	Univariate Analysis			Multivariate Analysis		
	Number	Percent (95% CI)	*P*	HR	95% CI	*P*
OS*		At 5 yr	.03			
Match	55	70.2 (55.3-81.0)		1.00		Reference
Major mismatch	47	64.8 (48.0-77.3)		1.20	.57-2.50	.62
Minor mismatch	58	83.2 (70.1-90.9)		.72	.33-1.57	.41
Bidirectional mismatch	31	54.6 (35.7-70.1)		1.76	.82-3.77	.14
Relapse†		At 5 yr	.09			
Match	55	26.9 (15.6-39.6)		1.00		Reference
Major mismatch	47	15.8 (6.8-28.2)		.54	.20-1.42	.21
Minor mismatch	58	14.4 (6.6-24.9)		.54	.22-1.32	.18
Bidirectional mismatch	31	32.5 (16.7-49.3)		1.08	.43-2.71	.86
TRM‡		At 5 yr	.19			
Match	55	8.1 (2.5-18.1)		1.00		Reference
Major mismatch	47	22.2 (11.3-35.4)		3.19	.97-10.46	.05
Minor mismatch	58	7.9 (2.5-17.6)		1.34	.34-5.33	.67
Bidirectional mismatch	31	16.1 (5.7-31.2)		1.99	.49-8.03	.33
Grades II-IV aGVHD		At 100 d	.91			
Match	55	58.2 (43.9-70.1)		1.00		Reference
Major mismatch	45	66.7 (50.5-78.6)		1.06	.64-1.73	.81
Minor mismatch	58	62.1 (48.1-73.3)		1.11	.68-1.80	.66
Bidirectional mismatch	31	61.3 (41.4-76.2)		1.28	.73-2.24	.37
Grades III-IV aGVHD§		At 100 d	.02			
Match	55	5.5 (1.4-13.7)		1.00		Reference
Major mismatch	45	20.0 (9.8-32.8)		2.56	.63-10.37	.19
Minor mismatch	58	3.4 (.6-10.7)		.59	.10-3.46	.56
Bidirectional mismatch	31	9.7 (2.4-23.2)		1.46	.25-8.44	.67
Extensive cGVHD		At 5 yr	.86			
Match	49	28.6 (16.7-41.6)		1.00		Reference
Major mismatch	40	30.5 (16.9-45.3)		1.18	.56-2.47	.65
Minor mismatch	55	27.9 (16.5-40.4)		1.24	.57-2.72	.58
Bidirectional mismatch	21	38.1 (17.8-58.3)		1.56	.67-3.63	.30
Neutrophil engraftment		At 60 d	.73			
Match	55	96.4 (83.6-99.2)		1.00		Reference
Major mismatch	47	92.6 (75.2-98.0)		.82	.56-1.20	.33
Minor mismatch	58	94.8 (83.3-98.5)		1.09	.78-1.53	.59
Bidirectional mismatch	31	88.7 (64.1-96.8)		1.06	.66-1.68	.80
Platelet engraftment		At 100 d	.30			
Match	55	88.9 (76.0-95.0)		1.00		Reference
Major mismatch	47	70.0 (53.6-81.6)		.57	.36-.90	.01
Minor mismatch	58	93.1 (81.2-97.6)		.92	.66-1.28	.64
Bidirectional mismatch	31	73.3 (51.5-86.4)		.78	.45-1.34	.37

* HR for overall mortality. In multivariate analysis, there were no significant variables, but there was a trend toward a higher mortality among those with a high risk of disease status at CBT (HR, 1.60; 95% CI, .88-2.89; $P = .11$) and female donor to male recipient (HR, 1.64; 95% CI, .94-2.85; $P = .07$).

† In multivariate analysis, there were no significant variables, but there was a trend toward a higher relapse among those with a high risk of disease status at CBT (HR, 1.71; 95% CI, .85-3.44; $P = .13$).

‡ In multivariate analysis, there were no significant variables, but there was a trend toward a higher TRM among those with female donor to male recipient (HR, 2.05; 95% CI, .87-4.81; $P = .09$).

§ In multivariate analysis, there were no significant variables, but there was a trend toward a higher incidence of grades III-IV aGVHD among those with a lower cord blood CD34 + cell count (HR, 2.75; 95% CI, .84-9.00; $P = .09$) and a high risk of disease status at CBT (HR, 3.98; 95% CI, .80-19.65; $P = .08$).

mismatch recipients of single-unit CBT [16,17], whereas other studies did not [14,18,19]. However, these studies included a relatively heterogeneous group of patients receiving single or double CBT after reduced-intensity or myeloablative conditioning regimen. In most of these studies, 3 groups of ABO mismatch, namely, major, minor, and bidirectional mismatch, were not evaluated separately. Of note, the advantage of our study is the relatively homogeneous adult patient population with hematological malignancies treated with single-unit CBT after 12 Gy TBI-based myeloablative conditioning regimens and a cyclosporine-based GVHD prophylaxis. Moreover, 3 groups of ABO mismatch were evaluated separately. Therefore, we were able to determine the potential effect of ABO incompatibility in CBT.

In conclusion, our data showed that ABO incompatibility affected the incidences of platelet engraftment but did not have a significant effect on the incidence of GVHD, relapse, TRM, and OS after CBT. These results should be interpreted with caution because this retrospective study included a relatively small number of Japanese patients who received single-unit CBT after 12 Gy TBI-based myeloablative conditioning regimens for hematological malignancies. Although these findings should be confirmed in large prospective studies, ABO incompatibility does not appear to have had a significant impact on the outcome after CBT in our study.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2013.12.563.

REFERENCES

1. Booth GS, Gehrie EA, Bolan CD, Savani BN. Clinical guide to ABO-incompatible allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2013;19:1152-1158.
2. Rowley SD, Donato ML, Bhattacharyya P. Red blood cell-incompatible allogeneic hematopoietic progenitor cell transplantation. *Bone Marrow Transplant*. 2011;46:1167-1185.
3. Seebach JD, Stussi G, Passweg JR, et al. ABO blood group barrier in allogeneic bone marrow transplantation revisited. *Biol Blood Marrow Transplant*. 2005;11:1006-1013.
4. Kimura F, Sato K, Kobayashi 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;93:1686-1693.
5. Bacigalupo A, Van Lint MT, Occhini D, et al. ABO compatibility and acute graft-versus-host disease following allogeneic bone marrow transplantation. *Transplantation*. 1988;45:1091-1094.
6. Tomonari A, Takahashi S, Ooi J, et al. Impact of ABO incompatibility on engraftment and transfusion requirement after unrelated cord blood transplantation: a single institute experience in Japan. *Bone Marrow Transplant*. 2007;40:523-528.
7. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood*. 2004;104:3813-3820.
8. Takahashi S, Ooi J, Tomonari A, et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. *Blood*. 2007;109:1322-1330.
9. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
10. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204-217.
11. Kanda Y. Investigation of the freely available easy-to-use software "EZR" for medical statistics. *Bone Marrow Transplant*. 2013;48:452-458.
12. Igarashi A, Kakihana K, Haraguchi K, et al. Anti-host isohemagglutinin production is associated with a higher risk of acute GVHD in ABO-incompatible transplantation. *Bone Marrow Transplant*. 2012;47:1356-1360.
13. Snell M, Chau C, Hendrix D, et al. Lack of isohemagglutinin production following minor ABO incompatible unrelated HLA mismatched umbilical cord blood transplantation. *Bone Marrow Transplant*. 2006;38:135-140.
14. Romee R, Weisdorf DJ, Brunstein C, et al. Impact of ABO-mismatch on risk of GVHD after umbilical cord blood transplantation. *Bone Marrow Transplant*. 2013;48:1046-1049.
15. Berglund S, Le Blanc K, Remberger M, et al. Factors with an impact on chimerism development and long-term survival after umbilical cord blood transplantation. *Transplantation*. 2012;94:1066-1074.
16. Arcese W, Rocha V, Labopin M, et al. Unrelated cord blood transplants in adults with hematologic malignancies. *Haematologica*. 2006;91:223-230.
17. Narimatsu H, Miyakoshi S, Yamaguchi T, et al. Chronic graft-versus-host disease following umbilical cord blood transplantation: retrospective survey involving 1072 patients in Japan. *Blood*. 2008;112:2579-2582.
18. Cohen YC, Scaradavou A, Stevens CE, et al. Factors affecting mortality following myeloablative cord blood transplantation in adults: a pooled analysis of three international registries. *Bone Marrow Transplant*. 2011;46:70-76.
19. Blin N, Traineau R, Houssin S, et al. Impact of donor-recipient major ABO mismatch on allogeneic transplantation outcome according to stem cell source. *Biol Blood Marrow Transplant*. 2010;16:1315-1323.

Unrelated Donor Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Hemoglobinopathies Using a Reduced-Intensity Conditioning Regimen and Third-Party Mesenchymal Stromal Cells

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Allogeneic hematopoietic stem cell transplantation for patients with a hemoglobinopathy can be curative but is limited by donor availability. Although positive results are frequently observed in those with an HLA-matched sibling donor, use of unrelated donors has been complicated by poor engraftment, excessive regimen-related toxicity, and graft-versus-host disease (GVHD). As a potential strategy to address these obstacles, a pilot study was designed that incorporated both a reduced-intensity conditioning and mesenchymal stromal cells (MSCs). Six patients were enrolled, including 4 with high-risk sickle cell disease (SCD) and 2 with transfusion-dependent thalassemia major. Conditioning consisted of fludarabine (150 mg/m^2), melphalan (140 mg/m^2), and alemtuzumab (60 mg for patients weighing > 30 kg and .9 mg/kg for patients weighing <30 kg). Two patients received HLA 7/8 allele matched bone marrow and 4 received 4-5/6 HLA

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Single-Unit Cord Blood Transplantation after Granulocyte Colony-Stimulating Factor–Combined Myeloablative Conditioning for Myeloid Malignancies Not in Remission

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High disease burden in myeloablative allogeneic hematopoietic stem cell transplantation is associated with adverse outcomes in patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS). Quiescent leukemia stem cells could be induced to enter cell cycle by granulocyte colony-stimulating factor (G-CSF) administration and become more susceptible to chemotherapy. We report on the outcome of unrelated cord blood transplantation (CBT) using a conditioning regimen of 12 Gy total body irradiation, G-CSF–combined high-dose cytarabine, and cyclophosphamide in 61 adult patients with AML or advanced MDS not in remission. With a median follow-up of 97 months, the probability of overall survival and cumulative incidence of relapse at 7 years were 61.4% and 30.5%, respectively. In multivariate analysis, poor-risk cytogenetics and high lactate dehydrogenase values at CBT were independently associated with inferior survival. These data demonstrate that CBT after G-CSF–combined myeloablative conditioning is a promising curative option for patients with myeloid malignancies not in remission.

INTRODUCTION

The prognoses of patients with acute myelogenous leukemia (AML) and advanced myelodysplastic syndrome (MDS) who have not achieved remission after chemotherapy have been poor. Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only potentially curative therapy for such patients, high disease burden has been reported to be associated with increased relapse or poor survival rate after allo-HSCT [1-9]. Recently, cord blood (CB) has been considered an acceptable alternative as a source of hematopoietic stem cells in unrelated allo-HSCT for adult patients without HLA-identical related or unrelated donors [9-16]. In comparison with other sources of allo-HSCT, one of the main advantages of using CB for patients with a high disease burden who require urgent transplantation is its rapid and convenient availability. Because it was shown that administration of granulocyte colony-stimulating factor (G-CSF) increased the susceptibility of cell-cycle–specific agent cytarabine in leukemia cells in vitro [17], we administered G-CSF–combined high-dose cytarabine in myeloablative conditioning for allo-HSCT [18,19] and reported that a G-CSF–combined conditioning regimen provided better engraftment and survival results in cord blood transplantation (CBT) for myeloid malignancies [13-16]. The objective of this retrospective study was to confirm the effects of CBT after G-CSF–combined myeloablative conditioning in adult patients with myeloid malignancies not in remission and to identify variables influencing long-term outcomes.

PATIENTS AND METHODS

Patients and Transplantation Procedures

This retrospective study included 61 consecutive adult patients who underwent unrelated transplantation using single-unit CB for AML or advanced MDS not in remission at our institute between 1998 and 2013. Thirty-two patients were included in our previous study [15,16] and extended the follow-up. The diagnoses of AML and MDS were made according to the World Health Organization classification. Advanced MDS was defined as having refractory anemia with excess blasts type 1 or refractory anemia with excess blasts type 2 by World Health Organization classification. Myeloid malignancies not in remission were defined as more than 5% blasts in the bone marrow (BM), or circulating blasts in peripheral blood (PB) or central nervous system. The cytogenetic subgroups were defined according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML [20] and International Prognostic Scoring System criteria for MDS [21]. All patients received 12 Gy total body irradiation (TBI) in 4 divided fractions on days −8 and −7, cytarabine on days −5 and −4 (total dose 12 g/m2, and 3 g/m2 every 12 hours for 2 days) with 5 μg/kg G-CSF (lenograstim) from 12 hours before the first dose of cytarabine to the end of cytarabine dosing, and cyclophosphamide (total dose 120 mg/kg) on days −3 and −2 [15,16]. Fifty-eight patients received cyclosporine (CSP) (3 mg/kg/day) with a short course of methotrexate (15 mg/m2 on day +1 and 10 mg/m2 on days +3 and +6), and 3 patients received CSP only as graft-versus-host disease (GVHD) prophylaxis. CB units were obtained from the Japanese Cord Blood Bank Network. Donor-recipient HLA-matching status was based on antigen level HLA-A and -B and on allele level HLA-DRB1 typing. All patients received similar supportive care and CB units were

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Table 1
Characteristics of Patients, Cord Blood Units, and Transplantation

Characteristic	Value
No. of patients	61
Sex	
Male	36 (59)
Female	25 (41)
Age, median (range), yr	41 (18-55)
CMV serostatus	
Positive	54 (86)
Negative	7 (11)
Disease type	
De novo AML	24 (39)
AML secondary to MDS	24 (39)
Advanced MDS*	13 (21)
Cytogenetics†	
Good	1 (2)
Intermediate	27 (44)
Poor	30 (49)
Unknown	3 (5)
Bone marrow blasts at CBT, median (range), %	17.7 (1.4-86.0)¶
< 25%	39
≥ 25%	22
Peripheral blood blasts at CBT, median (range), %	6.5 (0-68.5)
Absent	12
Present	49
LDH at CBT	
≤ ULN	41 (67)
> ULN	20 (33)
Disease status at CBT‡	
Untreated	31 (51)
Primary refractory	14 (23)
Refractory relapse	16 (26)
Time from diagnosis to CBT, median (range), mo	7 (1-219)
Conditioning regimen	
TBI12Gy+Ara-C/G-CSF+CY	61
GVHD prophylaxis	
CyclosporineA+methotrexate	58 (95)
CyclosporineA	3 (5)
Number of nucleated cells, median (range), $\times 10^7$/kg	2.43 (1.32-5.50)
Number of $CD34^+$cells, median (range), $\times 10^5$/kg	1.03 (.21-2.27)
HLA disparities§	
1	13 (21)
2	32 (52)
3	14 (22)
4	2 (3)

CMV indicates cytomegalovirus; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; CBT, cord blood transplantation; LDH, lactate dehydrogenase; ULN, upper limit of normal; TBI, total body irradiation; Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide; GVHD, graft-versus-host disease; HLA, human leukocyte antigen.
Data presented are n (%) unless otherwise indicated.

* Advanced MDS are defined as having refractory anemia with excess blasts-1 (RAEB-1) or RAEB-2 by WHO criteria.

† The cytogenetic subgroups according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML and International Prognostic Scoring System criteria for MDS.

‡ Untreated was defined as no treatment before conditioning regimen, indicating that the majority of patients with AML secondary to MDS or advanced MDS received CBT as an up-front treatment. Primary refractory was defined as failure to achieve complete remission with induction chemotherapy. Refractory relapse was defined as failure to achieve complete remission with salvage chemotherapy after first or subsequent relapse.

§ The number of HLA disparities, defined as the low resolution for HLA-A and -B and the high resolution for HLA-DRB1.

¶ The 5 patients with less than 5% blasts in the bone marrow included circulating blasts in peripheral blood (n = 3) or central nervous system (n = 2).

selected, as previously reported [15,16]. The institutional review board of the Institute of Medical Science, University of Tokyo approved this study. This study was conducted in accordance with the Declaration of Helsinki.

End Points and Statistical Analysis

The primary study end point was overall survival (OS), defined as time from the date of transplantation to the date of death or last contact. Secondary end points were relapse, including disease progression before engraftment; transplantation-related mortality (TRM); neutrophil and platelet engraftment; acute graft-versus-host disease (aGVHD); and chronic GVHD (cGVHD). Relapse was defined as morphologic evidence of disease in PB, BM, or extramedullary sites. TRM was defined as death during remission. Neutrophil engraftment was defined as the first of 3 consecutive days during which the absolute neutrophil count was at least .5 $\times$ 10^9/L. Platelet engraftment was achieved on the first of 3 days when the platelet count was higher than 50 $\times$ 10^9/L without transfusion support. Both aGVHD and cGVHD were graded according to the previously published criteria [22,23]. The incidence of aGVHD was evaluated in all engrafted patients, whereas the incidence of cGVHD was evaluated in engrafted patients surviving more than 100 days.

The probability of OS was estimated according to the Kaplan-Meier method, and the groups were compared using the log-rank test. The probabilities of relapse, TRM, neutrophil and platelet engraftment, and acute and chronic GVHD were estimated based on a cumulative incidence method to accommodate competing risks [24]. Multivariate analysis was performed with a Cox proportional hazard model adjusted for OS and Fine and Gray proportional hazards model for relapse [25]. The following variables were considered: age (< 45 versus ≥ 45 years), disease type (de novo AML versus AML secondary to MDS versus advanced MDS), cytogenetic risk (other than poor versus poor), proportion of blasts in BM (< 25 versus ≥ 25%), the presence of blasts in PB (absent versus present), lactate dehydrogenase (LDH) at CBT (≤ upper limit of normal versus > upper limit of normal), disease status at CBT (untreated versus primary refractory versus refractory relapse), cord blood nucleated cell count (< 2.5 versus ≥ 2.5 $\times$ 10^7/kg), and HLA disparities based on antigen level HLA-A and -B and allele level

HLA-DRB1 (≤ 2 versus ≥ 3). All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria) [26]. $P < .05$ was considered significant. Analysis of data was performed in August 2013.

RESULTS

Patient and CB unit characteristics are shown in Table 1. The median age was 41 years (range, 18 to 55 years), the median number of nucleated cells was 2.43×10^7/kg (range, 1.32 to 5.50×10^7/kg), and the median number of CD34+ cells was 1.03×10^5/kg (range, .21 to 2.27×10^5/kg). Disease types were de novo AML in 24 patients, AML secondary to MDS in 24, and advanced MDS in 13. The majority of patients with de novo AML with multilineage dysplasia (n = 2), AML secondary to MDS (n = 19), or advanced MDS (n = 10) received CBT as an up-front treatment, which was classified as untreated group (n = 31). Among patients with primary refractory status (n = 14), 3 patients received CBT after the first cycle of induction chemotherapy. The median number of prior chemotherapy treatments before CBT for primary refractory status was 3 (range, 1 to 5). The median time from diagnosis to CBT was 7 months (range, 1 to 219 months), and the median period of follow-up for survivors after CBT was 97 months (range, 5 to 181 months).

The cumulative incidence of neutrophil recovery was 93.4% (95% confidence interval [CI], 81.0% to 97.8%) at 60 days after CBT with a median time to achieve greater than $.5 \times 10^9$/L neutrophils of 22 days (range, 18 to 41 days). Disease progression before engraftment occurred in 2 patients. The cumulative incidence of platelet recovery was 78.7% (95% CI, 65.7% to 87.2%) at 100 days after CBT with a median time to an untransfused platelet count greater than 50×10^9/L of 50 days (range, 30 to 179 days). The cumulative incidences of grade II to IV acute GVHD and extensive chronic GVHD were 62.3% (95% CI, 48.7% to 73.2%) at 100 days and 32.9% (95% CI, 21.4% to 44.9%) at 3 years after CBT, respectively. The probability of OS at 7 years was 61.4% (95% CI, 47.1% to 72.9%). The cumulative incidence of relapse at 7 years was 30.5% (95% CI, 19.2% to 42.6%). The cumulative incidence of TRM at 100 days and at 1 year was 6.6% (95% CI, 2.1% to 14.7%) and 8.2% (95% CI, 3.0% to 16.9%), respectively (Figure 1).

In multivariate analysis, poor-risk cytogenetics (hazard ratio [HR], 7.14; 95% CI, 2.33 to 21.80; $P < .001$) and high LDH value (HR, 4.00; 95% CI, 1.33 to 12.07; $P = .013$) were associated with inferior survival (Figure 2, Table 2). De novo AML (HR, 9.66; 95% CI, 1.06 to 87.75; $P = .044$), primary refractory status at CBT (HR, 6.47; 95% CI, 1.86 to 22.51; $P = .003$), and high LDH value (HR, 3.75; 95% CI, 1.11 to 12.57; $P = .032$) were associated with an increased relapse incidence (Table 3, Supplemental Figure 1). In contrast, the proportion of blasts in BM and the presence of blasts in PB did not show any impact on survival and relapse incidence.

DISCUSSION

Previous reports have suggested that the only potentially curative therapy for patients with myeloid malignancies not in remission is allo-HSCT. However, the incidence of relapse has been reported to be high, and several reports showed long-term survival rates of only 10% to 30% [1-6]. Several factors, including blasts in BM or PB, cytogenetics, and donor availability, have been associated with outcome. In this study, poor-risk cytogenetics and high LDH value were significantly associated with inferior OS. De novo AML, primary refractory status, and high LDH value were associated with increased relapse. However, we found no impact of disease burden on survival and relapse. In fact, several retrospective studies did not show any advantage of induction chemotherapy before allo-HSCT to reduce the disease burden for patients with advanced MDS or AML secondary to MDS [27-29]. Therefore, the majority of patients with advanced MDS or AML secondary to MDS received G-CSF–combined myeloablative conditioning followed by CBT without prior induction chemotherapy in our institute.

After physicians have decided that allo-HSCT is appropriate for patients with myeloid malignancy not in remission, the elective timing of the transplantation is the main advantage of CBT. In fact, CBT timing is decided depending on the patient's conditions, such as control of infection and disease burden. Such elective timing of CBT might have contributed to disease burden not being shown to influence outcome in our study. On the other hand, the use of CB as a source of hematopoietic stem cells could offer the opportunity for patients to receive allo-HSCT without related or unrelated donors. Moreover, the lower incidence of severe GVHD without compromising graft-versus-leukemia effects in CBT may also have contributed to long-term survival in our study.

Relapse is the most important cause of treatment failure after allo-HSCT, particularly in patients with myeloid malignancies not in remission. This is mainly due to the residual leukemic cells that have escaped the cytotoxic effect of conditioning before transplantation. To reduce disease relapse, the role of a more intense conditioning regimen has been analyzed extensively [30]. Since chemosensitization of leukemia cells with G-CSF enhances the cytotoxicity of the cell-cycle–specific agent cytarabine [17], we administered G-CSF–combined high-dose cytarabine in the standard conditioning regimen of TBI/cyclophosphamide. The clinical efficacy of concomitant use of G-CSF with chemotherapy has remained controversial in newly diagnosed or relapsed refractory AML and MDS [31,32]. Recently, Pabst et al. reported

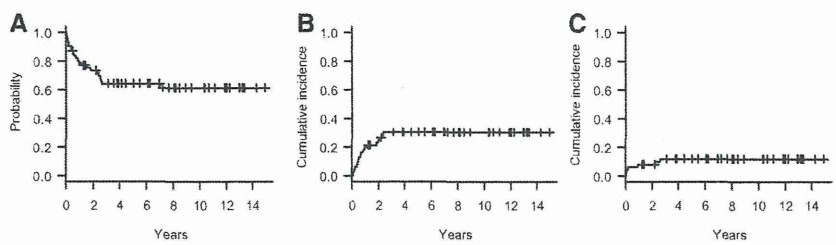

Figure 1. Probability of overall survival and cumulative incidences of relapse and transplant-related mortality after G-CSF–combined myeloablative CBT. Overall survival (A), relapse (B), and transplantation-related mortality (C) in 61 patients with AML or advanced MDS not in remission after G-CSF–combined myeloablative CBT.