**Table 3**
Univariate and Multivariate Analysis of Prognostic Factors for Relapse

| Variable | Univariate Analysis | | | Multivariate Analysis | | |
|---|---|---|---|---|---|---|
| | Number | 7-year Relapse (95% CI) | *P* | Hazard Ratio | 95% CI | *P* |
| Age | | | | | | |
| < 45 | 36 | 29.3 (15.0-45.2) | | 1 | | |
| ≥ 45 | 25 | 32.0 (14.9-50.6) | .567 | 1.62 | .50-5.17 | .420 |
| Disease type | | | | | | |
| Advanced MDS | 13 | 7.7 (.4-30.5) | | 1 | | |
| AML secondary to MDS | 24 | 29.8 (12.9-49.0) | | 4.37 | .38-49.80 | .230 |
| De novo AML | 24 | 43.4 (22.4-62.7) | .096 | 9.66 | 1.06-87.75 | .044 |
| Cytogenetics* | | | | | | |
| Other than poor | 31 | 23.0 (9.9-39.2) | | 1 | | |
| Poor | 30 | 38.2 (20.5-55.7) | .163 | 2.33 | .90-5.97 | .078 |
| Bone marrow blasts at CBT, % | | | | | | |
| < 25 | 39 | 26.0 (13.3-40.6) | | 1 | | |
| ≥ 25 | 22 | 39.2 (18.0-59.9) | .397 | 1.72 | .57-5.16 | .330 |
| Peripheral blood blasts at CBT | | | | | | |
| Absent | 12 | 16.7 (2.3-42.8) | | 1 | | |
| Present | 49 | 33.8 (20.6-47.4) | .309 | 3.08 | .40-23.70 | .280 |
| LDH value at CBT | | | | | | |
| ≤ ULN | 41 | 25.6 (13.1-40.1) | | 1 | | |
| > ULN | 20 | 40.0 (18.5-60.8) | .240 | 3.75 | 1.11-12.57 | .032 |
| Disease status at CBT | | | | | | |
| Untreated | 31 | 17.8 (6.3-34.1) | | 1 | | |
| Primary refractory | 14 | 50.0 (21.4-73.3) | | 6.47 | 1.86-22.51 | .003 |
| Refractory relapse | 16 | 37.5 (14.5-60.7) | .043 | 1.36 | .26-7.05 | .71 |
| Number of nucleated cells, $\times 10^7$/kg | | | | | | |
| ≥ 2.5 | 29 | 35.5 (18.3-53.1) | | 1 | | |
| < 2.5 | 32 | 25.3 (11.7-41.5) | .525 | .54 | .14-2.12 | .380 |
| HLA disparities† | | | | | | |
| ≤ 2 | 45 | 34.0 (20.4-48.1) | | 1 | | |
| ≥ 3 | 16 | 20.3 (4.5-43.9) | .306 | .53 | .11-2.49 | .420 |

MDS indicates myelodysplastic syndrome; AML, acute myelogenous leukemia; CBT, cord blood transplantation; LDH, lactate dehydrogenase; ULN, upper limit of normal; HLA, human leukocyte antigen; CI, confidence interval.

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

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

significantly improved survival with concomitant use of G-CSF with escalated-dose, but not with conventional-dose cytarabine [31]. In the setting of allo-HSCT, the conditioning regimen consisting of G-CSF–combined high-dose cytarabine and TBI 12 Gy was feasible and might reduce post-transplantation relapse in patients with AML [18,19]. The presence of quiescent leukemia stem cells (LSCs), which are thought to be resistant to chemotherapy, might contribute to relapse after treatment. Recently, a xenograft model demonstrated that cytarabine with G-CSF recruited quiescent LSCs into a phase of the cell cycle, leading to enhanced elimination of LSCs within the niche [33]. This effect might have contributed to reduced relapse in our study. Although these findings should be confirmed in prospective studies, the combination of G-CSF–combined myeloablative conditioning with CBT offered a promising curative option for patients with myeloid malignancies not in remission.

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## SUPPLEMENTARY DATA

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LETTER TO THE EDITOR

# Pretransplant hyperferritinemia has no effect on the outcome of myeloablative cord blood transplantation for acute leukemia and myelodysplastic syndrome

**Takaaki Konuma · Seiko Kato · Maki Oiwa-Monna · Arinobu Tojo · Satoshi Takahashi**

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Dear Editor,

Pretransplant hyperferritinemia has been associated with an increased incidence of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (allo-HSCT) after myeloablative or reduced-intensity conditioning [1–3]. Its effect on the outcome of myeloablative cord blood transplantation (CBT) has yet to be clarified. In this study, we retrospectively analyzed whether hyperferritinemia affected the outcome of myeloablative CBT.

Pretransplant serum ferritin level measured within 100 days preceding single-unit CBT for adult patients with acute leukemia or myelodysplastic syndrome (MDS) in our institute between the year 2001 and 2013 was available for 133 patients in this study. All patients received 12 Gy total body irradiation-based myeloablative conditioning regimens and cyclosporine and short-term methotrexate as a graft versus host disease (GVHD) prophylaxis. Cord blood units were selected as reported previously [4, 5]. In multivariate analysis, the following variables were considered: age (<45 vs. ≥45 years), cytomegalovirus serostatus (negative vs. positive), disease status at CBT (standard risk vs. high risk), cord blood nucleated cell count (<2.5 vs. $\geq 2.5 \times 10^7$/kg), cord blood CD34+ cells (<1 vs. $\geq 1 \times 10^5$/kg), HLA disparities based on antigen level HLA-A and -B and allele level HLA-DRB1 (≤2 vs. ≥3), and pretransplant ferritin level (<1,000 vs. ≥1,000 ng/ml). All statistical analyses were performed with EZR, a graphical user interface for R 2.13.0 [6]. $P<0.05$ was considered significant. Analysis of data was performed on August 2013.

The median pretransplant serum ferritin level was 751(range, 58–6,285) ng/ml, the median age was 40(range, 16–55) years, the median number of nucleated cells was 2.52(range, 1.32–5.69)$\times 10^7$/kg, and the median number of CD34+ cells was 0.91(range, 0.28–7.75)$\times 10^5$/kg. Disease types were acute myelogenous leukemia in 74 patients, acute lymphoblastic leukemia in 45, and MDS in 25. The median follow-up of surviving patients was 42(range, 3–103) months after CBT. In univariate and multivariate analysis, we found no impact of hyperferritinemia on overall survival (OS), relapse, transplant-related mortality (TRM), grades II–IV acute GVHD, extensive chronic GVHD, and neutrophil engraftment (Table 1). We also analyzed the effect of hyperferritinemia using different ferritin threshold (500, 1, 500, 2,000 ng/ml). But, we were unable to find any impact of hyperferritinemia on outcomes (data not shown).

The effect of hyperferritinemia on outcome might differ depending on the kinds of stem cell sources in allo-HSCT. It has been reported that hyperferritinemia was associated with inferior OS in CBT recipients [7]. However, the cumulative incidence of TRM was very low in our entire cohort, which might have contributed to hyperferritinemia not being shown to influence outcome in our study.

Ferritin is not only a marker for iron overload but also an inflammatory indicator. Moreover, iron overload correlates better with magnetic resonance imaging-measured liver iron content (LIC) than hyperferritinemia. Recently, prospective studies demonstrated that LIC did not affect outcomes of allo-HSCT [8, 9], suggesting that it is unclear whether iron overload

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**Table 1** Univariate and multivariate analysis of pretransplant ferritin levels on outcomes of myeloablative CBT

| | | Univariate analysis[b] | | Multivariate analysis[c] | |
|---|---|---|---|---|---|
| | Ferritin[a] | % (95 % CI) | *P* | HR (95 % CI) | *P* |
| Overall survival | | | | | |
| | <1,000 ng/ml | 77 (66–85) at 3 years | 0.71 | 1 | Reference |
| | ≥1,000 ng/ml | 78 (62–87) at 3 years | | 0.77 (0.37–1.60) | 0.49 |
| Relapse | | | | | |
| | <1,000 ng/ml | 20 (12–30) at 3 years | 0.67 | 1 | Reference |
| | ≥1,000 ng/ml | 17 (8–29) at 3 years | | 0.76 (0.33–1.75) | 0.52 |
| Transplant-related mortality | | | | | |
| | <1,000 ng/ml | 8 (3–15) at 3 years | 0.70 | 1 | Reference |
| | ≥1,000 ng/ml | 8 (2–19) at 3 years | | 0.68 (0.23–2.04) | 0.50 |
| Grades II–IV acute GVHD | | | | | |
| | <1,000 ng/ml | 70 (59–78) at 100 days | 0.34 | 1 | Reference |
| | ≥1,000 ng/ml | 63 (47–75) at 100 days | | 0.79 (0.48–1.29) | 0.36 |
| Extensive chronic GVHD | | | | | |
| | <1,000 ng/ml | 20 (12–29) at 3 years | 0.42 | 1 | Reference |
| | ≥1,000 ng/ml | 28 (16–42) at 3 years | | 1.07 (0.54–2.14) | 0.83 |
| Neutrophil engraftment | | | | | |
| | <1,000 ng/ml | 92 (82–96) at 60 days | 0.28 | 1 | Reference |
| | ≥1,000 ng/ml | 95 (81–99) at 60 days | | 1.13 (0.79–1.62) | 0.47 |

*GVHD* graft-versus-host disease, *CI* confidence interval, *HR* hazard ratio

[a] Among the 133 patients, pretransplant serum ferritin level was categorized as ≥1,000 ng/ml ($n=49$) or <1,000 ng/ml ($n=84$)

[b] The probability of overall survival was estimated according to the Kaplan–Meier method, and the 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

[c] Multivariate analysis was performed with a Cox proportional hazard model adjusted for overall survival, and Fine and Gray proportional hazards model for the others

affects outcomes of allo-HSCT. Although hyperferritinemia was not associated with adverse outcomes after CBT in this study, further studies are warranted to evaluate the effect of iron overload on the outcome of CBT for acute leukemia and MDS.

**Conflict of interest** The authors have no conflicts of interest.

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ORIGINAL ARTICLE

# Effects of KIR ligand incompatibility on clinical outcomes of umbilical cord blood transplantation without ATG for acute leukemia in complete remission

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To clarify the effect of killer cell immunoglobulin-like receptor (KIR) ligand incompatibility on outcomes of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) patients in complete remission after single cord blood transplantation (CBT), we assessed the outcomes of CBT registered in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) database. A total of 643 acute leukemia (357 AML and 286 ALL) patient and donor pairs were categorized according to their KIR ligand incompatibility by determining whether or not they expressed HLA-C, Bw4 or A3/A11 by DNA typing. A total of 128 patient–donor pairs were KIR ligand-incompatible in the graft-versus-host (GVH) direction and 139 patient–donor pairs were incompatible in the host-versus-graft (HVG) direction. Univariate and multivariate analyses showed no significant differences between the KIR ligand-incompatible and compatible groups in the GVH direction for both AML and ALL patients of overall survival, disease-free survival, relapse incidence, non-relapse mortality and acute GVH disease. However, KIR incompatibility in the HVG direction ameliorated engraftment in ALL patients (hazard ratio 0.66, 95% confidence interval 0.47–0.91, $P = 0.013$). Therefore, there were no effects of KIR ligand incompatibility in the GVH direction on single CBT outcomes for acute leukemia patients without anti-thymocyte globulin use. However, it is necessary to pay attention to KIR incompatibility in the HVG direction for engraftment.

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**Keywords:** CBT; GVHD; HVG; KIR; NK cell

## INTRODUCTION

Killer cell immunoglobulin-like receptor (KIR) ligand incompatibility may have some important roles in transplantation outcomes such as leukemia relapse and leukemia-free survival.[1–4] Ruggeri *et al.*[5,6] reported surprisingly good clinical results that indicated no relapse, no rejection and no acute graft-versus-host disease (GVHD) after human leukocyte antigen (HLA) haplotype-mismatched transplantations with KIR ligand incompatibility in the GVH direction for acute myeloid leukemia (AML) patients. They also reported that donor allogeneic natural killer (NK) cells attacked host antigen-presenting cells (APCs), resulting in the suppression of GVHD. However, results of studies regarding the clinical advantage of KIR ligand incompatibility in allogeneic stem cell transplantation (allo SCT) from an unrelated donor are discrepant. Davies *et al.*[7] reported that there was no effect of KIR ligand incompatibility on outcomes of unrelated bone marrow transplantation without using anti-thymocyte globulin (ATG), whereas Giebel *et al.*[8] reported a good effect of KIR ligand incompatibility on the outcomes of unrelated bone marrow transplantation using ATG as part of GVHD prophylaxis. Morishima *et al.*[9] reported that KIR ligand mismatching induced adverse effects on acute GVHD and rejection in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor in Japan. It was reported that cord blood transplantation (CBT) for acute leukemia patients in complete remission (CR) from KIR ligand-incompatible donors in the GVH direction was associated with decreased relapse and improved survival.[10] In another study, it was shown that KIR ligand mismatch was associated with development of severe acute GVHD and risk of death after double CBT with reduced-intensity conditioning (RIC) regimen.[11] Therefore, the role of KIR ligand incompatibility in allo SCT remains controversial. To clarify the effect of KIR ligand incompatibility on the outcomes of AML and acute lymphoblastic leukemia (ALL) patients in CR after single CBT, we assessed the outcomes of CBT registered in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) database between 2001 and 2010 (A Study from the HLA Working Group of the JSHCT).

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## MATERIALS AND METHODS

### Study design and data collection

This study was a retrospective analysis of data from a Japanese nationwide multicenter survey. Data were provided by the HLA Working Group of the JSHCT. Outcomes of 643 acute leukemia (357 AML nd 286 ALL) patients in CR were analyzed. Informed consent was obtained from patients and donors according to the Declaration of Helsinki, and approval was obtained from the Institutional Review Board of Hokkaido University Hospital.

### Patient population

This study included AML and ALL patients who received single CBT in CR and (1) patients and donors whose HLA-A, B, C and DR alleles were determined by DNA typing as described previously,[9] (2) underwent transplantation between 2001 and 2010, (3) received a myeloablative conditioning (MAC) regimen ($n=456$) as high-dose radiation and chemotherapy usually in combination with cyclophosphamide or an RIC regimen ($n=187$) defined basically as the use of fludarabine plus low-dose busulfan or melphalan with or without low-dose total body irradiation[12] and (4) did not receive ATG as a preparative regimen.

### Inhibitory KIR ligand assessment

Patients and donors were categorized according to their KIR ligand incompatibility by determining whether or not they expressed HLA-C group 1 or 2, Bw4 or A3/A11 as initially described by Ruggeri *et al.*[5] and Leung.[13] KIR ligand mismatch in the GVH direction was scored when the donor's KIR ligand was not shared by the patient. KIR ligand mismatch in the HVG direction was scored when the patient's KIR ligand was not shared by the donor.

### Transplant procedures

Differences among patients, disease and transplantation-related factors according to conditioning regimens, and GVHD prophylaxis are shown in Tables 1a and b.

### Endpoints

Primary endpoints included overall survival (OS), disease-free survival (DFS), relapse (cumulative incidence of relapse, CIR), non-relapse mortality (NRM) and engraftment. Relapse was defined as clinical and hematological leukemia recurrence. NRM was defined as death during continuous CR after transplantation. Engraftment was defined as a peripheral granulocyte count of $>500/\mu l$ for three consecutive days after transplantation.

### Statistical analysis

Characteristics of patients who received KIR ligand-incompatible CBT in the GVH direction and the compatible group were compared using the $\chi^2$-test for categorical variables and the Wilcoxon two-sample test for continuous variables. To compare the prognosis of the incompatible group with that of the compatible group, univariate survival analyses were conducted for OS, DFS, CIR, NRM, engraftment and acute GVHD (grades II–IV). Survival curves of OS and DFS for each group were depicted using the Kaplan–Meier method and compared using the log-rank test. In the analysis of CIR, NRM, engraftment and acute GVHD, cumulative probabilities were estimated on the basis of cumulative incidence curves to accommodate the following competing events: death for relapse, relapse for transplantation-related mortality, death without GVHD for acute GVHD and death without engraftment for neutrophil engraftment. Groups were compared using the Gray test.[14] To adjust for potential confounders, multivariate analyses were conducted using the Cox proportional hazards model for OS and DFS, and using the Fine–Gray proportional hazards model for CIR and NRM.[15] The variables considered in the multivariate analysis were age at

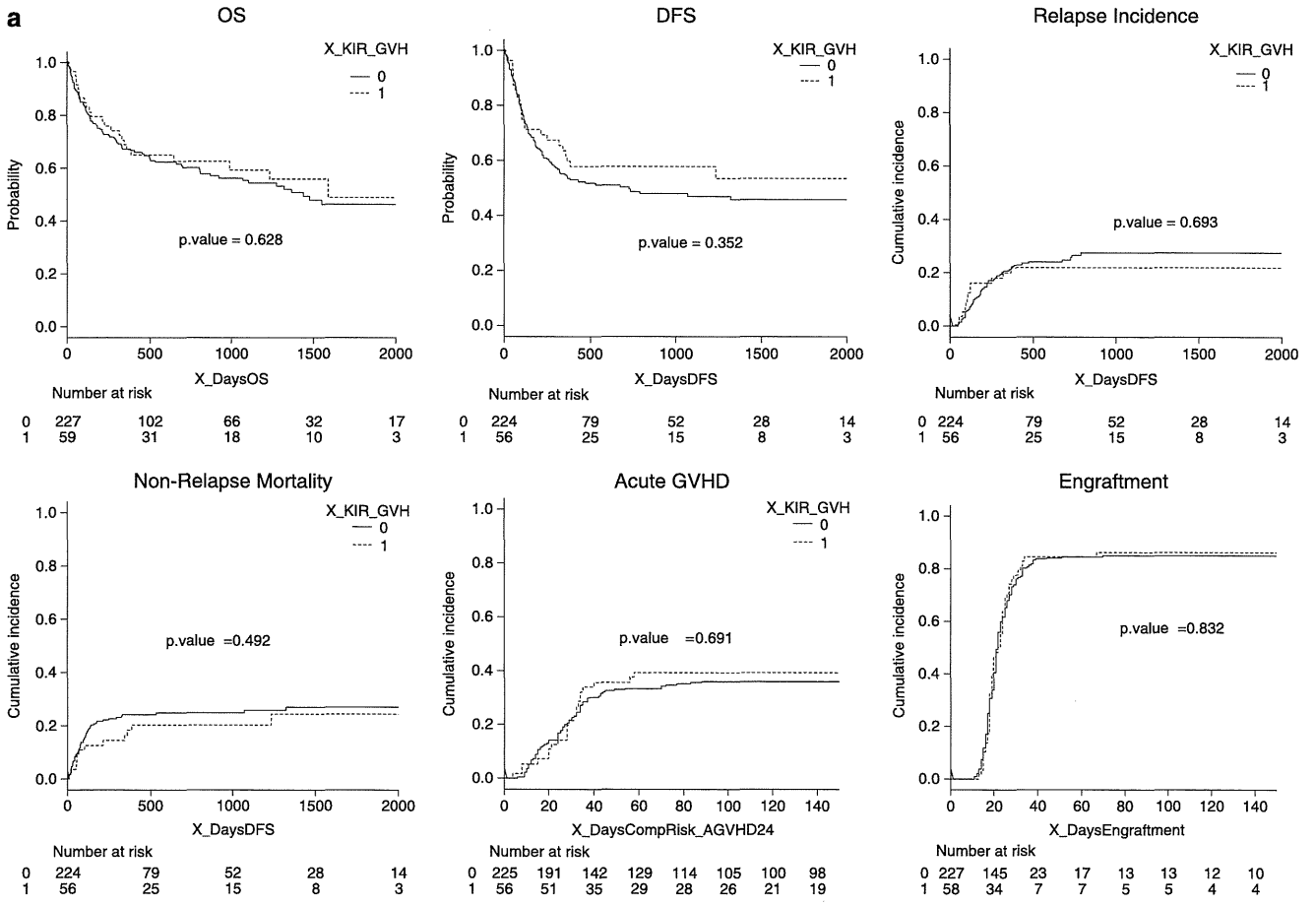


**Figure 1.** Continued

transplantation (40 years or more, 16–39 years and <15 years), performance status before transplantation (2–4 and 0–1), year of transplantation (2006–2009 and 2001–2005), sex (female and male), disease status (CR2 and CR1), conditioning regimens (RIC and MAC), HLA matching and infused cells ($>2.5 \times 10^7$/kg and $<2.5 \times 10^7$/kg) as a clinically important prognostic factor. All statistical analyses were conducted using SAS ver 9.2 (SAS Institute Inc., Cary, NC, USA) and R (www.r-project.org, last accessed 5 April 2012).

## RESULTS

### *Patients and clinical characteristics*

Tables 1a and 1b show clinical and biological characteristics of the 286 ALL and 357 AML patients who received single CBT. One hundred and twenty-eight patient–donor pairs (ALL $n=59$, AML $n=69$) were KIR ligand-incompatible in the GVH direction and 139 patient–donor pairs (ALL $n=65$, AML $n=74$) were incompatible in the HVG direction. Regarding KIR ligand incompatibility in the GVH direction, 59 ALL patients were transplanted with HLA-A, B or C KIR ligand-incompatible cord blood (A3/A11 $n=9$, Bw4 $n=16$, C $n=24$, A+C $n=3$, B+C $n=7$) and 69 AML patients were transplanted with HLA-A, B or C KIR ligand-incompatible cord blood (A3/A11 $n=11$, Bw4 $n=31$, C $n=24$, A+C $n=2$, B+C $n=1$). Regarding KIR ligand incompatibility in the HVG direction, 65 ALL patients were transplanted with HLA-A, B or C KIR ligand-incompatible cord blood (A3/A11 $n=17$, Bw4 $n=13$, C $n=35$, A+B $n=1$, A+C $n=5$) and 74 AML patients were transplanted with HLA-A, B or C KIR ligand-incompatible cord blood (A3/A11 $n=14$, Bw4 $n=14$, C $n=42$, A+C $n=4$). The number of patients mismatched in both the GVH and HVG directions is quite few (15 ALL patients and 18 AML patients). RIC regimens were used in 187 patients (ALL $n=58$ and AML $n=129$). There were no significant differences in other prognostic factors without HLA matching.

### *Impact of KIR ligand mismatch in the GVH direction on transplantation outcomes*

Univariate analysis showed no significant differences between KIR ligand-incompatible and compatible groups in the GVH direction for both AML and ALL patients in OS, DFS, relapse incidence, NRM, acute GVHD and engraftment ($P=0.628$, $P=0.352$, $P=0.693$, $P=0.492$, $P=0.691$, $P=0.832$ for ALL patients and $P=0.674$, $P=0.688$, $P=0.353$, $P=0.766$, $P=0.569$, $P=0474$ for AML patients, respectively; Figures1a and b).

Causes of death are shown in Table 2a. Rates of mortality due to original disease and infections were almost the same in the KIR ligand-compatible and incompatible donor groups.

There were no significant differences in OS, DFS, relapse incidence, NRM, engraftment and acute GVHD between the KIR ligand-incompatible and compatible groups in the GVH direction for both AML and ALL patients by multivariate analysis (hazard ratio (HR) 0.87, $P=0.557$; HR 0.79, $P=0.352$; HR 0.95, $P=0.91$; HR 0.71, $P=0.32$; HR 1.08, $P=0.63$; HR 1.06, $P=0.83$ for ALL patients and HR 0.93, $P=0.752$; HR 1.02, $P=0.945$; HR 0.59, $P=0.12$; HR 0.95, $P=0.86$; HR 0.97, $P=0.89$; HR 0.84, $P=0.51$ for AML patients, respectively; Tables 3a and b). The conditioning regimens (RIC and MAC) did not affect these results.

For ALL patients, age >40 years and CR2 were associated with poor OS (HR 4.25, $P<0.001$ and HR 2.09, $P<0.001$, respectively)

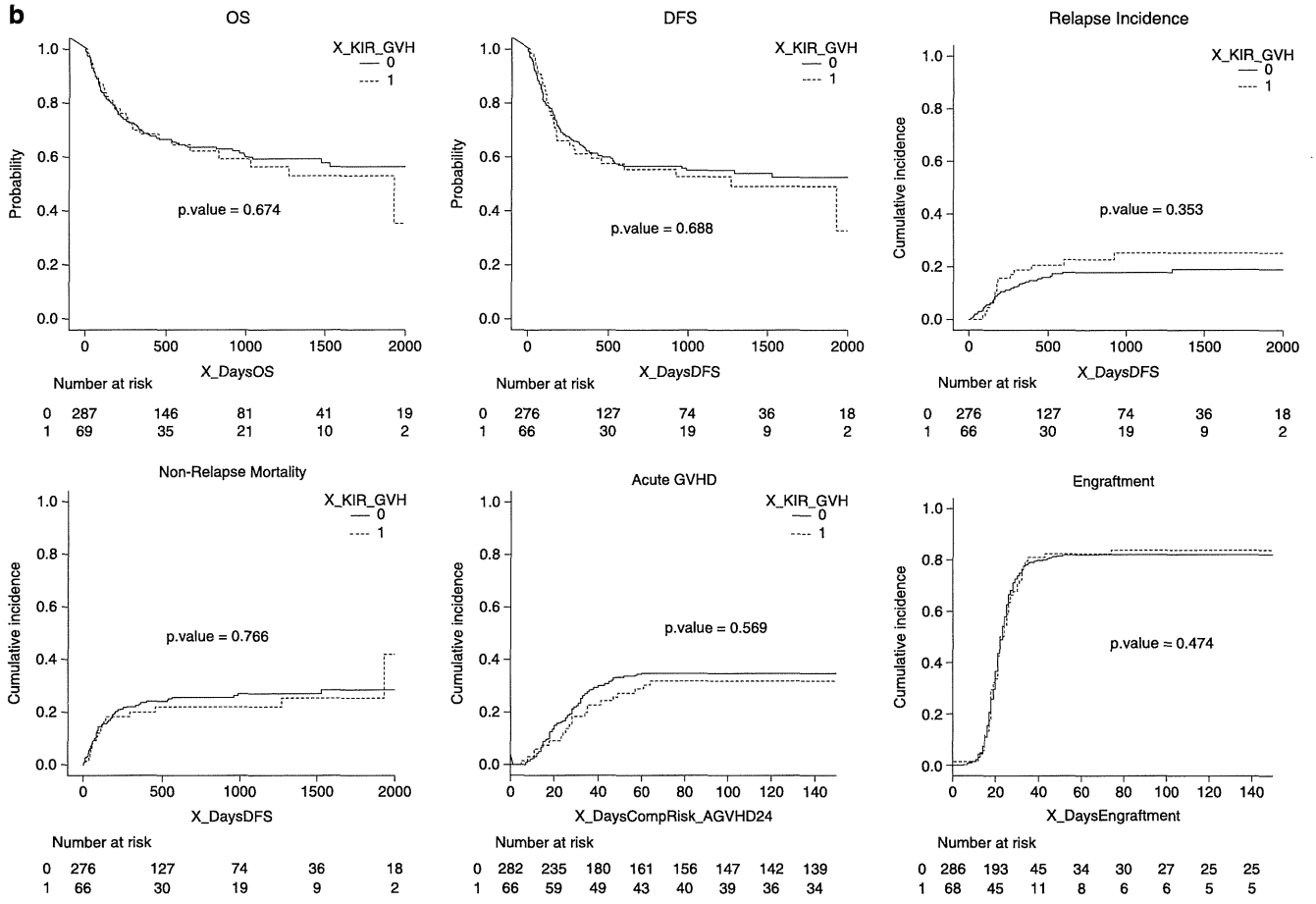


**Figure 1.** *Continued*

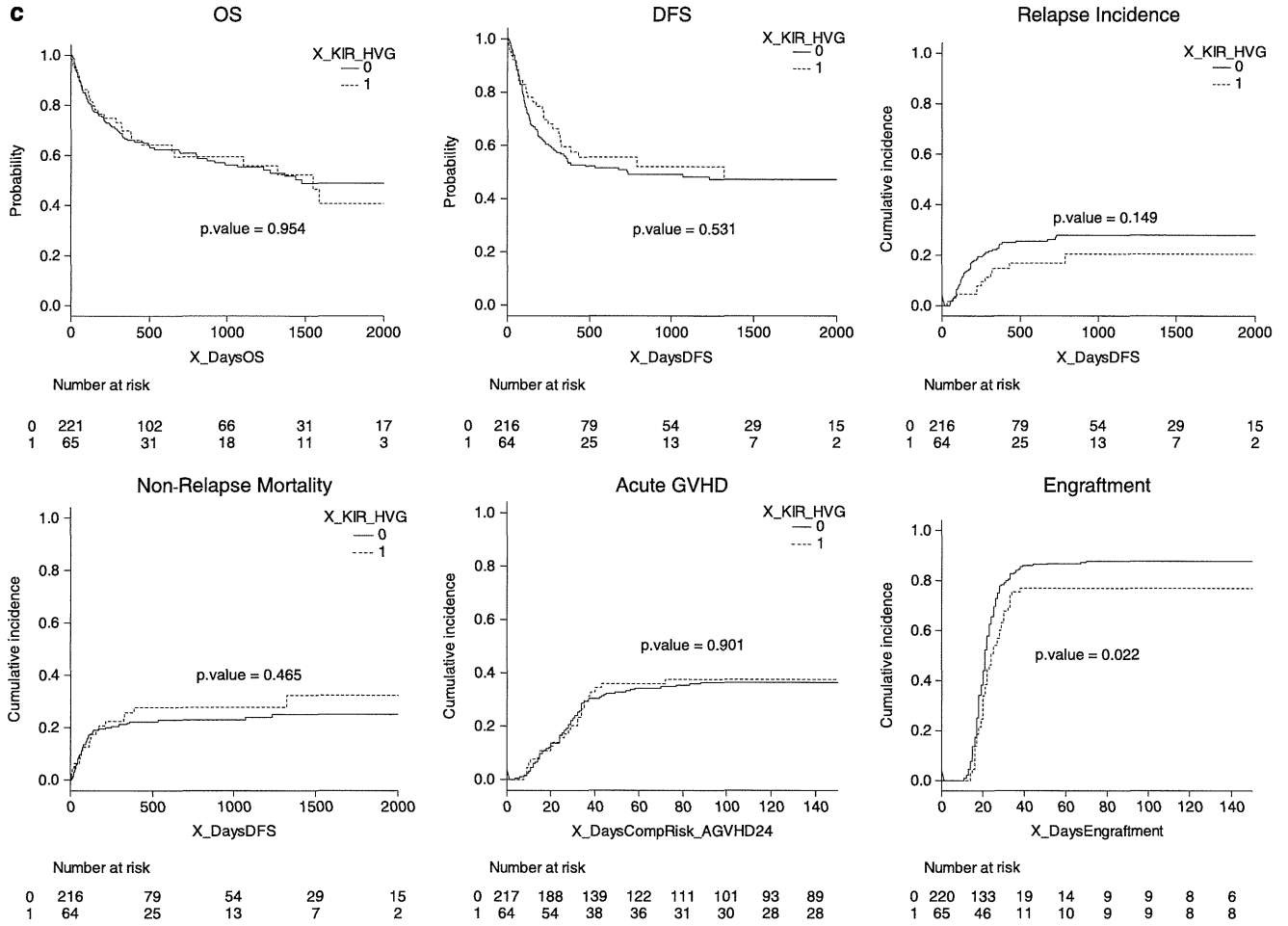


**Figure 1.** Continued

and also with poor DFS (HR 2.41, $P=0.002$ and HR 1.67, $P=0.011$, respectively). Also, age >40 years was associated with higher NRM and lower engraftment rate (HR 6.96, $P<0.001$ and HR 0.55, $P<0.001$, respectively). For AML patients, age >40 years and male gender were associated with poor OS (HR 1.93, $P=0.057$ and HR 1.78, $P=0.003$, respectively) and also with higher NRM (HR 2.59, $P=0.052$ and HR 1.71, $P=0.031$, respectively). Also, male gender was associated with poor DFS (HR 1.48, $P=0.033$). Infused cell number of $>2.5\times10^7$/kg was associated with higher engraftment rate and MAC regimen was associated with lower engraftment rate (HR 1.369, $P=0.018$ and HR 0.686, $P=0.007$, respectively). Age >40 years was associated with lower incidence of GVHD (HR 0.50, $P=0.031$) and HLA mismatch was associated with higher incidence of GVHD (HR 1.58, $P=0.058$).

### Impact of KIR ligand mismatch in the HVG direction on transplantation outcomes

Univariate analysis showed no significant differences between the KIR ligand-incompatible and compatible groups in the HVG direction for both AML and ALL patients in OS, DFS, relapse incidence, NRM and acute GVHD ($P=0.954$, $P=0.531$, $P=0.149$, $P=0.465$, $P=0.901$ for ALL patients and $P=0.264$, $P=0.383$, $P=0.654$, $P=0.598$, $P=0.628$ for AML patients, respectively; Figures1c and d). However, there was a significant difference in engraftment between the KIR ligand-incompatible and compatible groups in the HVG direction for ALL patients ($P=0.022$ for ALL patients and $P=0.151$ for AML patients).

Causes of death are shown in Table 2b. Rates of mortality owing to original disease were almost the same in the KIR ligand-compatible and incompatible donor groups. Rate of mortality owing to infection was higher in the KIR ligand-incompatible donor group with ALL.

Also, there were no significant differences in OS, DFS, relapse incidence, NRM and acute GVHD between the KIR ligand-incompatible and compatible groups in the HVG direction for both AML and ALL patients by multivariate analysis (HR 0.84, $P=0.457$; HR 0.76, $P=0.225$; HR 1.12, $P=0.76$; HR 1.06, $P=0.85$; HR 1.08, $P=0.75$ for ALL patients and HR 0.73, $P=0.197$; HR 0.83, $P=0.414$; HR 0.86, $P=0.68$; HR 0.88, $P=0.66$; HR 1.20, $P=0.42$ for AML patients, respectively; Tables 3c and d). However, there was a significant difference in engraftment between the KIR ligand-incompatible and compatible groups in the HVG direction for ALL patients (HR 0.66, $P=0.013$). The conditioning regimens (RIC and MAC) did not affect these results.

For ALL patients, age >40 years and CR2 were associated with poor OS (HR 4.33, $P<0.001$ and HR 2.11, $P<0.001$, respectively) and also with poor DFS (HR 2.49, $P=0.001$ and HR 1.70, $P=0.009$, respectively). Also, age >40 years was associated with higher NRM and lower engraftment rate (HR 6.87, $P<0.001$ and HR 0.56, $P<0.001$, respectively). For AML patients, age >40 years and male gender were associated with poor OS (HR 2.00, $P=0.045$ and HR 1.76, $P=0.003$, respectively) and also with higher NRM (HR 2.62, $P=0.051$ and HR 1.69, $P=0.032$, respectively). Also, male gender was associated with poor DFS (HR 1.48, $P=0.032$). Infused cell number of $>2.5\times10^7$/kg was

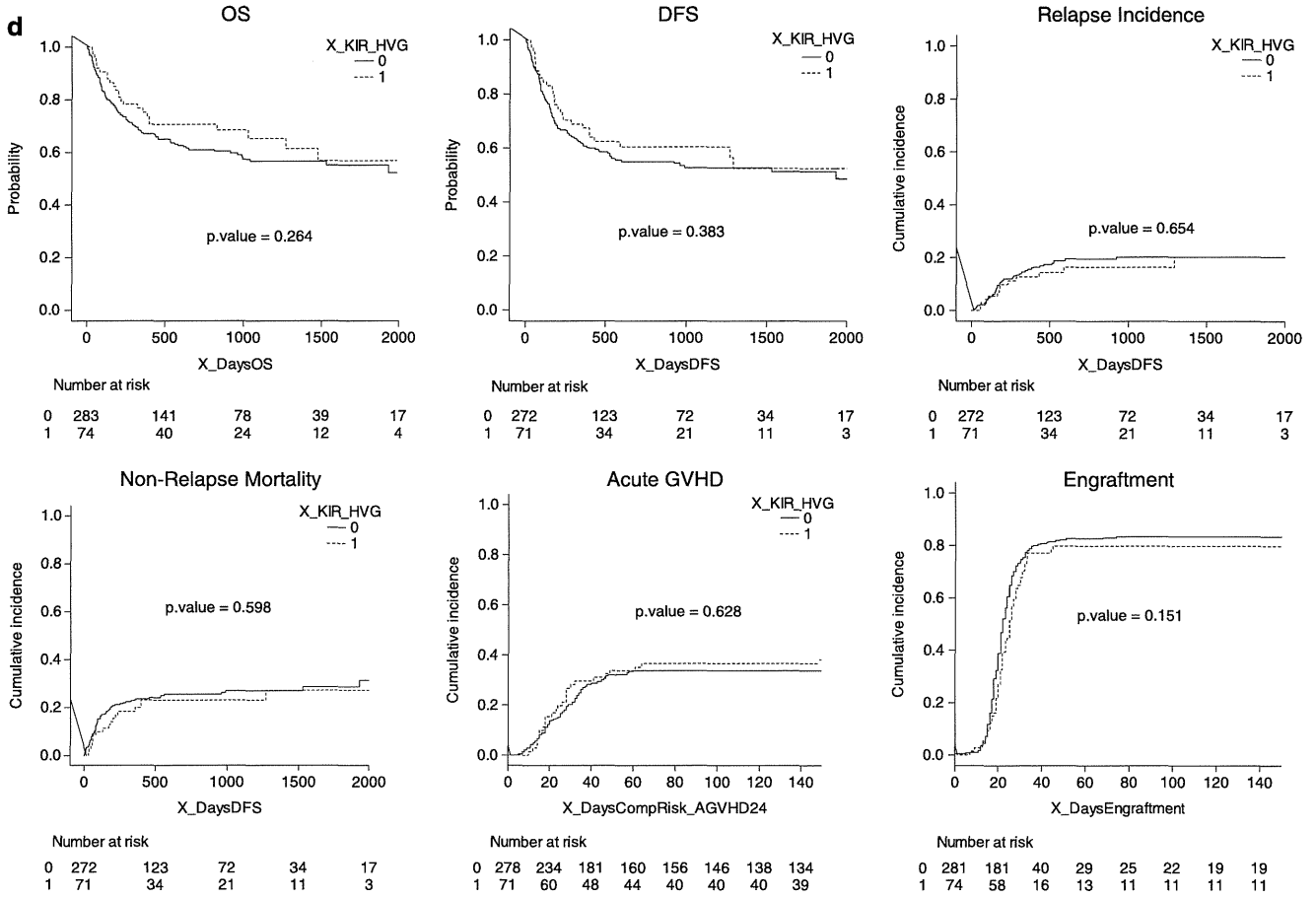


**Figure 1.** Kaplan–Meier curves forOS, DFS, CIR, NRM, acute GVHD and engraftment in (**a**) ALL and (**b**) AML patients transplanted from KIR-compatible and incompatible donors in the GVH direction and in (**c**) ALL and (**d**) AML patients transplanted from KIR-compatible and incompatible donors in the HVG direction.

associated with higher engraftment rate and MAC regimen was associated with lower engraftment rate (HR 1.387, $P=0.014$ and HR 0.694, $P=0.009$, respectively). Age >40 years was associated with lower incidence of GVHD (HR 0.51, $P=0.035$) and HLA mismatch was associated with higher incidence of GVHD (HR 1.49, $P=0.086$).

## DISCUSSION

The role of KIR ligand incompatibility in allo SCT is controversial with various diseases and conditionings.[16,17] It has been suggested that NK cell alloreactivity is associated with better outcome after allo SCT when a high stem cell dose, extensive T-cell depletion and ATG are used.[18,19] NK cell engraftment is earlier and more robust and T-cell engraftment is delayed after CBT.[20,21] Therefore, CBT may represent a setting in which KIR ligand incompatibility is associated with protection from leukemia relapse. Willemze *et al.*[22] reported transplantation outcomes after single-unit CBT for AML patients ($n=94$) and ALL patients ($n=124$). Among those patients, KIR ligand incompatibility was associated with reduced relapse of AML and increased OS. In their study, >80% of the patients were administered ATG or antilymphocyte globulin under MAC. Brunstein *et al.*[23] reported results for 257 patients with single-unit CBT ($n=91$) and double-unit CBT ($n=166$) after myeloablative ($n=155$) and reduced intensity ($n=102$) conditioning. KIR ligand incompatibility was associated with higher rate of acute GVHD and decreased OS under RIC. In their study, only 30% of the patients were administered ATG. Garfall *et al.*[24] reported outcomes of double-unit CBT for 80 patients with various hematological malignancies including 31 AML patients. Among those patients, KIR ligand incompatibility was not associated with relapse reduction. In their study, >70% of the patients were administered ATG with RIC (Flu/Mel/ATG). Those studies that included different transplantation protocols with different disease distributions after single-unit and double-unit CBT showed conflicting results.[25,26]

Lowe *et al.*[27] investigated the relative significance of NK cell and T-cell alloreactivity in 105 pediatric patients who received minimally T-cell-depleted HLA-non-identical bone marrow transplantation. They showed that donor NK cell incompatibility did not improve patient outcome. In contrast, donor T-cell incompatibility was a risk factor for acute GVHD, chronic GVHD and death. Thus, T-cell alloreactivity dominated that of NK cells in minimally T-cell-depleted grafts. It was reported that KIR ligand mismatching induced adverse effects on acute GVHD and rejection and brought no survival benefits to leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor in Japan.[9] Also, Yabe *et al.*[28] reported that KIR ligand incompatibility had potent adverse effects with a higher incidence of acute GVHD and lower OS without ATG, whereas ATG administration ameliorated most of the adverse effects. Therefore, administration of ATG extensively depletes patient's and donor's T cells and becomes a critical factor in attenuating the adverse effects of KIR ligand-incompatible transplantation predominating alloreactive NK cells to induce an antileukemic effect. NK cell cytotoxicity toward a particular target cell is regulated by a

**Table 1a.** Patients characteristics with or without KIR incompatibility in the GVH direction

| *Factor* | *ALL, n (%)* | | | *AML, n (%)* | | |
|---|---|---|---|---|---|---|
| | *KIR compatible* | *KIR incompatible* | P | *KIR compatible* | *KIR incompatible* | P |
| Number of patients | 227 | 59 | | 288 | 69 | |
| Year of transplant | | | 0.621 | | | 0.639 |
| 2001–2005 (%) | 49 (22) | 11 (19) | | 44 (15) | 9 (13) | |
| 2006– | 178 (78) | 48 (81) | | 244 (85) | 60 (87) | |
| Median age (years) | 27 | 33 | 0.895 | 47 | 50 | 0.195 |
| 0–15 | 83 (37) | 16 (27) | 0.355 | 41 (14) | 9 (13) | 0.926 |
| 16–39 | 58 (26) | 19 (32) | | 79 (27) | 18 (26) | |
| >40 | 86 (38) | 24 (41) | | 168 (59) | 42 (61) | |
| Male | 108 (48) | 38 (64) | 0.021 | 145 (50) | 44 (64) | 0.045 |
| Disease status | | | 0.741 | | | 0.077 |
| CR1 | 153 (68) | 43 (73) | | 182 (63) | 37 (54) | |
| CR2 | 69 (30) | 15 (25) | | 95 (33) | 25 (36) | |
| >CR2 | 4 (2) | 1 (2) | | 9 (3) | 6 (9) | |
| TNC infused $\times 10^7$/kg | 3.04 (1.61–24.77) | 2.81 (1.45–24.91) | 0.461 | 2.70 (1.46–38.70) | 2.60 (1.59–10.84) | 0.103 |
| *Conditioning* | | | | | | |
| RIC | 47 (21) | 11 (19) | 0.703 | 101 (35) | 28 (41) | 0.392 |
| TBI | 187 (82) | 52 (86) | 0.457 | 237 (82) | 60 (87) | 0.38 |
| ATG | 0 | 0 | | 0 | 0 | |
| HLA allele matching | | | <0.001 | | | 0.013 |
| 0 miss | 16 (7) | 1 (2) | | 14 (5) | 0 | |
| 1 miss | 25 (11) | 2 (3) | | 19 (7) | 3 (4) | |
| 2 miss | 37 (16) | 3 (5) | | 36 (13) | 3 (4) | |
| 3 miss | 75 (33) | 12 (20) | | 92 (32) | 22 (32) | |
| 4 miss | 46 (20) | 23 (39) | | 73 (25) | 18 (26) | |
| >4 miss | 28 (12) | 18 (31) | | 54 (19) | 23 (33) | |
| GVHD prophylaxis | | | 0.202 | | | 0.687 |
| CsA ± MTX | 96 (42) | 31 (53) | | 133 (46) | 30 (44) | |
| FK ± MTX | 126 (56) | 28 (47) | | 151 (53) | 38 (55) | |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; CR, complete remission; CsA, cyclosporine; FK, tacrolimus; GVH, graft-versus-host; GVHD, GVH disease; HLA, human leukocyte antigen; KIR, killer cell immunoglobulin-like receptor; MTX, methotrexate; RIC, reduced-intensity conditioning; TBI, total body irradiation; TNC, total nucleated cells.

**Table 1b.** Patients characteristics with or without KIR incompatibility in the HVG direction

| *Factor* | *ALL, n (%)* | | | *AML, n (%)* | | |
|---|---|---|---|---|---|---|
| | *KIR compatible* | *KIR incompatible* | P | *KIR compatible* | *KIR incompatible* | P |
| Number of patients | 221 | 65 | | 283 | 74 | |
| Year of transplant | | | 0.413 | | | 0.717 |
| 2001–2005 | 44 (20) | 16 (25) | | 43 (15) | 10 (14) | |
| 2006– | 177 (80) | 49 (75) | | 240 (85) | 64 (86) | |
| Median age (years) | 24 | 35 | 0.134 | 48 | 47 | 0.976 |
| 0–15 | 83 (38) | 16 (25) | 0.149 | 45 (16) | 5 (7) | 0.038 |
| 16–39 | 56 (25) | 21 (32) | | 70 (25) | 27 (36) | |
| >40 | 82 (37) | 28 (43) | | 168 (59) | 42 (57) | |
| Male | 112 (51) | 34 (52) | 0.817 | 152 (54) | 37 (50) | 0.569 |
| Disease status | | | 0.435 | | | 0.372 |
| CR1 | 149 (67) | 47 (72) | | 171 (60) | 48 (65) | |
| CR2 | 68 (31) | 16 (25) | | 95 (34) | 25 (34) | |
| >CR2 | 3 (1) | 2 (3) | | 14 (5) | 1 (1) | |
| TNC infused $\times 10^7$/kg | 3.06 (1.50–24.91) | 2.89 (1.45–17.25) | 0.133 | 2.71 (1.46–18.17) | 2.58 (1.77–38.7) | 0.065 |
| *Conditioning* | | | | | | |
| RIC | 46 (21) | 12 (18) | 0.655 | 107 (38) | 22 (30) | 0.198 |
| TBI | 179 (81) | 59 (91) | 0.064 | 231 (82) | 66 (89) | 0.134 |
| ATG | 0 | 0 | | | | |
| HLA allele matching | | | <0.001 | | | 0.017 |
| 0 miss | 17 (8) | 0 | | 14 (5) | 0 | |
| 1 miss | 26 (12) | 1 (2) | | 21 (7) | 1 (1) | |
| 2 miss | 33 (15) | 7 (11) | | 31 (11) | 8 (11) | |
| 3 miss | 67 (30) | 20 (31) | | 96 (34) | 18 (24) | |
| 4 miss | 50 (23) | 19 (29) | | 69 (24) | 22 (30) | |
| >4 miss | 28 (12) | 18 (27) | | 52 (19) | 25 (34) | |
| GVHD prophylaxis | | | 0.645 | | | 0.171 |
| CsA ± MTX | 96 (43) | 31 (48) | | 124 (44) | 39 (53) | |
| FK ± MTX | 120 (54) | 34 (52) | | 155 (56) | 34 (47) | |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; CR, complete remission; CsA, cyclosporine; FK, tacrolimus; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HVG, host-versus-graft; KIR, killer cell immunoglobulin-like receptor; MTX, methotrexate; RIC, reduced-intensity conditioning; TBI, total body irradiation; TNC, total nucleated cells.

**Table 2a.** Cause of death for patients after single CBT with KIR incompatibility in the GVH direction

| | *ALL, n (%)* | | *AML, n (%)* | |
|---|---|---|---|---|
| | *KIR compatible* | *KIR incompatible* | *KIR compatible* | *KIR incompatible* |
| Original disease | 29 (30) | 11 (46) | 29 (27) | 8 (30) |
| Acute GVHD | 3 (3) | 0 (0) | 5 (5) | 0 (0) |
| Chronic GVHD | 0 (0) | 0 (0) | 1 (1) | 0 (0) |
| Graft failure | 7 (7) | 1 (4) | 4 (4) | 4 (15) |
| Infection | 16 (16) | 5 (21) | 22 (20) | 6 (22) |
| Hemorrhage | 6 (6) | 0 (0) | 2 (2) | 4 (15) |
| Interstitial pneumonitis | 10 (10) | 1 (4) | 9 (8) | 2 (7) |
| ARDS | 4 (4) | 0 (0) | 4 (4) | 0 (0) |
| Organ failure | 7 (7) | 3 (13) | 14 (13) | 2 (7) |
| Others | 15 (15) | 3 (13) | 18 (17) | 1 (4) |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CBT, cord blood transplantation; GVH, graft-versus-host; GVHD, GVH disease; KIR, killer cell immunoglobulin-like receptor; ARDS, acute respiratory distress syndrome.

**Table 2b.** Cause of death for patients after single CBT with KIR incompatibility in the HVG direction

| | *ALL, n (%)* | | *AML, n (%)* | |
|---|---|---|---|---|
| | *KIR compatible* | *KIR incompatible* | *KIR compatible* | *KIR incompatible* |
| Original disease | 32 (34) | 8 (29) | 31 (28) | 6 (25) |
| Acute GVHD | 2 (2) | 1 (4) | 4 (4) | 1 (4) |
| Chronic GVHD | 0 (0) | 0 (0) | 1 (1) | 0 (0) |
| Graft failure | 7 (8) | 1 (4) | 7 (6) | 1 (4) |
| Infection | 13 (14) | 8 (29) | 24 (21) | 4 (17) |
| Hemorrhage | 6 (6) | 0 (0) | 4 (4) | 2 (8) |
| Interstitial pneumonitis | 8 (9) | 3 (11) | 9 (8) | 2 (8) |
| ARDS | 3 (3) | 1 (4) | 1 (1) | 3 (13) |
| Organ failure | 10 (11) | 0 (0) | 15 (13) | 1 (4) |
| Others | 12 (13) | 6 (21) | 16 (14) | 4 (17) |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CBT, cord blood transplantation; GVHD, graft-versus-host disease; HVG, host-versus-graft; KIR, killer cell immunoglobulin-like receptor; ARDS, acute respiratory distress syndrome.

**Table 3a.** Multivariate analysis for each event KIR ligand incompatibility in the GVH direction with ALL patients

| *Variables* | *Reference* | *HR* | *95% CI* | | *P-value* |
|---|---|---|---|---|---|
| *Overall survival* | | | | | |
| KIR incompatible | Compatible | 0.87 | 0.53 | 1.40 | 0.557 |
| Age >40 | Age 0–15 | 4.25 | 2.31 | 7.83 | <0.001 |
| Male | Female | 1.08 | 0.72 | 1.62 | 0.718 |
| CR2– | CR1 | 2.09 | 1.39 | 3.16 | <0.001 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.93 | 0.59 | 1.45 | 0.739 |
| *Disease-free survival* | | | | | |
| KIR incompatible | Compatible | 0.79 | 0.49 | 1.29 | 0.352 |
| Age >40 | Age 0–15 | 2.41 | 1.39 | 4.18 | 0.002 |
| Male | Female | 1.00 | 0.68 | 1.47 | 0.995 |
| CR2– | CR1 | 1.67 | 1.12 | 2.47 | 0.011 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.85 | 0.56 | 1.30 | 0.465 |
| *Relapse incidence* | | | | | |
| KIR incompatible | Compatible | 0.95 | 0.43 | 2.10 | 0.91 |
| Age >40 | Age 0–15 | 0.59 | 0.26 | 1.32 | 0.2 |
| Male | Female | 0.65 | 0.39 | 1.10 | 0.11 |
| CR2– | CR1 | 1.37 | 0.80 | 2.35 | 0.250 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.69 | 0.35 | 1.35 | 0.280 |
| *Non-relapse mortality* | | | | | |
| KIR incompatible | Compatible | 0.71 | 0.37 | 1.39 | 0.32 |
| Age >40 | Age 0–15 | 6.96 | 2.93 | 16.57 | <0.001 |
| Male | Female | 1.44 | 0.79 | 2.64 | 0.24 |
| CR2– | CR1 | 1.62 | 0.90 | 2.92 | 0.100 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.13 | 0.61 | 2.10 | 0.700 |
| *Engraftment* | | | | | |
| KIR incompatible | Compatible | 1.08 | 0.78 | 1.50 | 0.63 |
| Age >40 | Age 0–15 | 0.55 | 0.39 | 0.78 | <0.001 |
| Male | Female | 0.77 | 0.58 | 1.02 | 0.066 |
| CR2– | CR1 | 0.76 | 0.56 | 1.02 | 0.067 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.08 | 0.82 | 1.43 | 0.590 |
| Infused cell $>2.5 \times 10^7$/kg | ≦2.5 | 1.02 | 0.76 | 1.36 | 0.910 |
| MAC | RIC | 0.79 | 0.58 | 1.09 | 0.15 |
| *Acute GVHD* | | | | | |
| KIR-incompatible | Compatible | 1.06 | 0.64 | 1.74 | 0.83 |
| Age >40 | Age 0–15 | 0.95 | 0.53 | 1.71 | 0.87 |
| Male | Female | 1.16 | 0.75 | 1.79 | 0.52 |
| CR2– | CR1 | 1.34 | 0.89 | 2.02 | 0.170 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.40 | 0.86 | 2.28 | 0.180 |

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; CR, complete remission; GVH, graft-versus-host; GVHD, GVH disease; HLA, human leukocyte antigen; HR, hazard ratio; KIR, killer cell immunoglobulin-like receptor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

balance of activating and inhibitory cell–cell contacts. The absence of HLA class I on a target cell allows other activating signals to dominate.[29,30] Inhibitory NK receptors protect self-HLA-expressing normal tissue from NK cells. The second property of an inhibitory NK receptor is to educate or license NK cells to acquire function. NK cells acquire function following engagement of inhibitory receptors with self-ligands after their differentiation from hematopoietic progenitors. Therefore, allo SCT provides a unique environment for NK cell education and NK cell development from hematopoietic stem cells in a short period.[31]

We analyzed the effects of KIR ligand incompatibility in both GVH and HVG directions on single CBT outcomes in 643 acute leukemia patients in CR (ALL $n=286$ and AML $n=357$) without ATG in Japan. In contrast to the results of previous studies indicating that KIR ligand mismatching induced adverse effects on GVHD and survival in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor in Japan,[27,28] our study did not show any positive or negative effects of KIR ligand incompatibility in either the GHV or HVG direction on OS, DFS, CIR, NRM and acute GVHD after single CBT without ATG. CBT may be tolerable to KIR ligand incompatibility in terms of transplantation outcomes such as GVHD, OS and DFS. Therefore, the source of stem cell may also be important to determine the clinical advantage of NK cell alloreactivity after unrelated SCT. We also analyzed transplantation outcomes for only patients with engraftment; however, there were no differences in OS and DFS between patients who received KIR ligand-compatible and incompatible transplantations (data not shown). There was also no difference in outcomes of KIR ligand-compatible and incompatible transplantations in acute leukemia patients combined with ALL and AML in CR. However, multivariate analysis showed a significantly lower rate of engraftment in ALL patients who were KIR ligand incompatible in the HVG direction than compatible patients (HR 0.66, 95% confidence interval 0.47–0.91, $P=0.013$). Also, AML patients who were KIR ligand incompatible in the HVG direction tended to have a lower rate of engraftment (HR 0.799, 95% confidence interval 0.59–1.084, $P=0.15$). It has been reported that NK epitope mismatching in

**Table 3b.** Multivariate analysis for each event KIR ligand incompatibility in the GVH direction with AML patients

| *Variables* | *Reference* | *HR* | *95% CI* | | *P-value* |
|---|---|---|---|---|---|
| *Overall survival* | | | | | |
| KIR incompatible | Compatible | 0.93 | 0.58 | 1.49 | 0.752 |
| Age >40 | Age 0–15 | 1.93 | 0.98 | 3.79 | 0.057 |
| Male | Female | 1.78 | 1.21 | 2.60 | 0.003 |
| CR2– | CR1 | 0.76 | 0.52 | 1.11 | 0.160 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.08 | 0.71 | 1.65 | 0.725 |
| *Disease-free survival* | | | | | |
| KIR incompatible | Compatible | 1.02 | 0.65 | 1.59 | 0.945 |
| Age >40 | Age 0–15 | 1.31 | 0.71 | 2.42 | 0.380 |
| Male | Female | 1.48 | 1.03 | 2.12 | 0.033 |
| CR2– | CR1 | 0.77 | 0.54 | 1.10 | 0.152 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.01 | 0.68 | 1.50 | 0.959 |
| *Relapse incidence* | | | | | |
| KIR incompatible | Compatible | 0.59 | 0.31 | 1.14 | 0.12 |
| Age >40 | Age 0–15 | 0.61 | 0.27 | 1.38 | 0.24 |
| Male | Female | 0.65 | 0.39 | 1.09 | 0.1 |
| CR2– | CR1 | 1.39 | 0.82 | 2.34 | 0.220 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.71 | 0.36 | 1.38 | 0.310 |
| *Non-relapse mortality* | | | | | |
| KIR incompatible | Compatible | 0.95 | 0.52 | 1.72 | 0.86 |
| Age >40 | Age 0–15 | 2.59 | 0.99 | 6.76 | 0.052 |
| Male | Female | 1.71 | 1.05 | 2.77 | 0.031 |
| CR2– | CR1 | 0.85 | 0.54 | 1.36 | 0.510 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.08 | 0.63 | 1.84 | 0.780 |
| *Engraftment* | | | | | |
| KIR incompatible | Compatible | 0.97 | 0.71 | 1.339 | 0.89 |
| Age >40 | Age 0–15 | 0.94 | 0.67 | 1.332 | 0.74 |
| Male | Female | 0.92 | 0.73 | 1.181 | 0.53 |
| CR2– | CR1 | 1.00 | 0.79 | 1.287 | 0.96 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.97 | 0.75 | 1.27 | 0.840 |
| Infused cell $>2.5 \times 10^7$/kg | ≦2.5 | 1.36 | 1.06 | 1.776 | 0.018 |
| MAC | RIC | 0.68 | 0.52 | 0.904 | 0.007 |
| *Acute GVHD* | | | | | |
| KIR incompatible | Compatible | 0.84 | 0.51 | 1.40 | 0.51 |
| Age >40 | Age 0–15 | 0.50 | 0.27 | 0.94 | 0.031 |
| Male | Female | 1.10 | 0.75 | 1.61 | 0.62 |
| CR2– | CR1 | 0.98 | 0.66 | 1.44 | 0.900 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.58 | 0.98 | 2.54 | 0.058 |

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; CR, complete remission; GVH, graft-versus-host; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; KIR, killer cell immunoglobulin-like receptor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

**Table 3c.** Multivariate analysis for each event KIR ligand incompatibility in the HVG direction with ALL patients

| *Variables* | *Reference* | *HR* | *95% CI* | | *P-value* |
|---|---|---|---|---|---|
| *Overall survival* | | | | | |
| KIR incompatible | Compatible | 0.84 | 0.54 | 1.33 | 0.457 |
| Age >40 | Age 0–15 | 4.33 | 2.35 | 7.97 | <0.001 |
| Male | Female | 1.08 | 0.72 | 1.62 | 0.718 |
| CR2– | CR1 | 2.11 | 1.40 | 3.18 | <0.001 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.91 | 0.59 | 1.41 | 0.671 |
| *Disease-free survival* | | | | | |
| KIR incompatible | Compatible | 0.76 | 0.49 | 1.18 | 0.225 |
| Age >40 | Age 0–15 | 2.49 | 1.44 | 4.32 | 0.001 |
| Male | Female | 1.00 | 0.68 | 1.47 | 0.999 |
| CR2– | CR1 | 1.70 | 1.14 | 2.51 | 0.009 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.84 | 0.55 | 1.26 | 0.394 |
| *Relapse incidence* | | | | | |
| KIR incompatible | Compatible | 1.12 | 0.55 | 2.28 | 0.76 |
| Age >40 | Age 0–15 | 0.67 | 0.29 | 1.55 | 0.35 |
| Male | Female | 1.09 | 0.62 | 1.91 | 0.76 |
| CR2– | CR1 | 0.75 | 0.42 | 1.34 | 0.330 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.95 | 0.52 | 1.74 | 0.870 |
| *Non-relapse mortality* | | | | | |
| KIR incompatible | Compatible | 1.06 | 0.59 | 1.89 | 0.85 |
| Age >40 | Age 0–15 | 6.87 | 2.87 | 16.42 | <0.001 |
| Male | Female | 1.43 | 0.77 | 2.64 | 0.26 |
| CR2– | CR1 | 1.62 | 0.90 | 2.90 | 0.110 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.08 | 0.58 | 2.00 | 0.800 |
| *Engraftment* | | | | | |
| KIR incompatible | Compatible | 0.66 | 0.47 | 0.91 | 0.013 |
| Age >40 | Age 0–15 | 0.56 | 0.4 | 0.78 | <0.001 |
| Male | Female | 0.78 | 0.59 | 1.02 | 0.065 |
| CR2– | CR1 | 0.71 | 0.52 | 0.96 | 0.026 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.14 | 0.86 | 1.5 | 0.370 |
| Infused cell $>2.5 \times 10^7$/kg | ≦2.5 | 1.04 | 0.78 | 1.39 | 0.800 |
| MAC | RIC | 0.80 | 0.58 | 1.09 | 0.160 |
| *Acute GVHD* | | | | | |
| KIR incompatible | Compatible | 1.08 | 0.67 | 1.76 | 0.75 |
| Age >40 | Age 0–15 | 0.95 | 0.52 | 1.71 | 0.85 |
| Male | Female | 1.16 | 0.75 | 1.79 | 0.49 |
| CR2– | CR1 | 1.35 | 0.88 | 2.07 | 0.170 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.41 | 0.87 | 2.29 | 0.160 |

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; CR, complete remission; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; HVG, host-versus-graft; KIR, killer cell immunoglobulin-like receptor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

the rejection direction was associated with an increased probability of rejection after unrelated bone marrow transplantation.[9,32] Signaling lymphocytic activation molecule (SLAM)-associated protein-related adaptors and SLAM family receptors were reported to act together in a mechanism that was essential for the elimination of hematopoietic cells but not non-hematopoietic cells by NK cells.[33] Therefore, alloreactive NK cells induced by KIR ligand incompatibility in the HVG direction may attack donor hematopoietic cells to ameliorate donor cell engraftment after CBT with blood containing a relatively small number of hematopoietic stem cells. Administration of ATG as a preparative regimen may be important to obtain some positive effects of KIR ligand incompatibility in the GVH direction on CBT outcomes such as survival and relapse. The present study suggests that it is not necessary to consider KIR ligand compatibility in the GVH direction at CBT without ATG for transplantation outcomes. Also, there is the possibility that KIR ligand incompatibility in the GVH direction induces a graft-versus-leukemia effect for acute leukemia if patients receive ATG as a preparative regimen. On the other hand, it may be necessary to pay attention to KIR ligand compatibility in the HVG direction for engraftment after CBT.

We did not perform KIR genotyping in our cohort study; however, recent data have suggested an important role of KIR polymorphisms and KIR genotype in transplantation outcomes of allo SCT.[34,35] NK cell alloreactivity is regulated by a balance of activating and inhibitory cell–cell contacts. Although phenotypes of the KIR repertoire are personalized by various conditions,[36] however, not only simple algorithm on ligands for inhibitory KIR but also KIR genotypes may be useful for predicting clinically relevant NK cell alloreactivity in a future study.

**Table 3d.** Multivariate analysis for each event KIR ligand incompatibility in the HVG direction with AML patients

| *Variables* | *Reference* | *HR* | *95% CI* | | *P-value* |
|---|---|---|---|---|---|
| *Overall survival* | | | | | |
| KIR incompatible | Compatible | 0.73 | 0.46 | 1.18 | 0.197 |
| Age >40 | Age 0–15 | 2.00 | 1.02 | 3.93 | 0.045 |
| Male | Female | 1.76 | 1.21 | 2.58 | 0.003 |
| CR2– | CR1 | 0.74 | 0.50 | 1.08 | 0.120 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.09 | 0.72 | 1.65 | 0.681 |
| *Disease-free survival* | | | | | |
| KIR incompatible | Compatible | 0.83 | 0.53 | 1.30 | 0.414 |
| Age >40 | Age 0–15 | 1.33 | 0.72 | 2.45 | 0.357 |
| Male | Female | 1.48 | 1.03 | 2.11 | 0.032 |
| CR2– | CR1 | 0.76 | 0.53 | 1.09 | 0.131 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.03 | 0.70 | 1.51 | 0.893 |
| *Relapse incidence* | | | | | |
| KIR incompatible | Compatible | 0.86 | 0.42 | 1.75 | 0.68 |
| Age >40 | Age 0–15 | 0.67 | 0.29 | 1.58 | 0.36 |
| Male | Female | 1.09 | 0.62 | 1.91 | 0.76 |
| CR2– | CR1 | 0.75 | 0.42 | 1.34 | 0.330 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.98 | 0.55 | 1.76 | 0.950 |
| *Non-relapse mortality* | | | | | |
| KIR incompatible | Compatible | 0.88 | 0.49 | 1.57 | 0.66 |
| Age >40 | Age 0–15 | 2.62 | 1 | 6.88 | 0.051 |
| Male | Female | 1.69 | 1.05 | 2.74 | 0.032 |
| CR2– | CR1 | 0.84 | 0.53 | 1.35 | 0.480 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.08 | 0.64 | 1.83 | 0.770 |
| *Engraftment* | | | | | |
| KIR-incompatible | Compatible | 0.799 | 0.59 | 1.084 | 0.15 |
| Age >40 | Age 0–15 | 0.958 | 0.68 | 1.352 | 0.81 |
| Male | Female | 0.918 | 0.72 | 1.17 | 0.49 |
| CR2– | CR1 | 0.994 | 0.78 | 1.264 | 0.96 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 0.997 | 0.77 | 1.291 | 0.98 |
| Infused cell $>2.5 \times 10^7$/kg | ≦2.5 | 1.387 | 1.07 | 1.8 | 0.014 |
| MAC | RIC | 0.694 | 0.53 | 0.914 | 0.009 |
| *Acute GVHD* | | | | | |
| KIR-incompatible | Compatible | 1.20 | 0.76 | 1.90 | 0.42 |
| Age >40 | Age 0–15 | 0.51 | 0.28 | 0.96 | 0.035 |
| Male | Female | 1.09 | 0.75 | 1.59 | 0.64 |
| CR2– | CR1 | 0.98 | 0.66 | 1.45 | 0.910 |
| HLA mismatching (>5/6) | HLA mismatching (6/6, 5/6) | 1.49 | 0.95 | 2.34 | 0.086 |

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; CR, complete remission; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; HVG, host-versus-graft; KIR, killer cell immunoglobulin-like receptor; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Acute kidney injury after myeloablative cord blood transplantation in adults: the efficacy of strict monitoring of vancomycin serum trough concentrations

H. Mae, J. Ooi, S. Takahashi, S. Kato, T. Kawakita, Y. Ebihara, K. Tsuji, F. Nagamura, H. Echizen, A. Tojo. Acute kidney injury after myeloablative cord blood transplantation in adults: the efficacy of strict monitoring of vancomycin serum trough concentrations. Transpl Infect Dis 2013: **15**: 181–186. All rights reserved

**Abstract:** *Background.* Acute kidney injury (AKI) is a common medical complication after myeloablative allogeneic stem cell transplantation (SCT). We have previously performed a retrospective analysis of AKI after cord blood transplantation (CBT) in adults, and found that the maximum of vancomycin (VCM) trough levels were significantly higher in patients with AKI. Following these results, we have monitored VCM serum trough concentrations more strictly, to not exceed 10.0 mg/L, since 2008.
*Methods.* In this report, we performed an analysis of AKI in a new group of 38 adult patients with hematological malignancies treated with unrelated CBT after myeloablative conditioning between January 2008 and July 2011.
*Results.* Cumulative incidence of AKI at day 100 after CBT was 34% (95% confidence interval 19–50). The median of the maximum value of VCM trough was 8.8 (4.5–12.2) mg/L. In multivariate analysis, no factor was associated with the incidence of AKI. No transplant-related mortality was observed. The probability of disease-free survival at 2 years was 83%.
*Conclusion.* These findings suggest that strict monitoring of VCM serum trough concentrations has a beneficial effect on outcomes of CBT.

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Key words: vancomycin; myeloablative conditioning; cord blood transplantation; acute kidney injury

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Acute kidney injury (AKI) is a common medical complication early after myeloablative allogeneic stem cell transplantation (SCT). The incidence of AKI, defined as a 2-fold rise in serum creatinine (sCr) concentration from baseline, has been reported ranging from 36% to 72% in SCT in a myeloablative setting (1–7), and about 20% required hemodialysis. We have previously reported a retrospective analysis of AKI in a group of 54 adult patients with hematological malignancies who received unrelated cord blood transplantation (CBT) after myeloablative conditioning between 2004 and 2007 (8). A statistically significant decrement of renal function from baseline was observed between days 11 and 20. Among the 54 patients, AKI occurred in 27.8% and was associated with a high mortality rate. Although no difference was seen in maximum cyclosporine (CYA) trough levels, the maximum vancomycin (VCM) trough levels were significantly higher in patients with AKI (8). Following these results, we have monitored VCM serum trough concentrations more strictly. In this report, we performed an analysis of AKI in a new group of 38 adult patients with hematological malignancies treated with unrelated CBT after myeloablative conditioning between January 2008 and

July 2011. The main purpose of this retrospective single-center study was to confirm the efficacy of strict monitoring of VCM serum trough concentrations, as well as to identify factors related to the incidence of AKI.

## Patients and methods

### Patients

This was a retrospective single-center analysis. Between January 2008 and July 2011, 39 consecutive adult patients with hematological malignancies were treated with unrelated CBT at The Institute of Medical Science, University of Tokyo. We excluded 1 patient who experienced primary engraftment failure. A total of 38 patients were analyzed. Patients qualified as standard risk if they were in first or second complete remission, had chronic-phase chronic myelogenous leukemia or refractory anemia of myelodysplastic syndrome, or had no high-risk cytogenetics. Patients in third complete remission, in relapse, or in refractory disease, with chronic myelogenous leukemia beyond chronic phase, or with high-risk cytogenetics were classified as high risk. Analyses of data were performed in December 2011. Written informed consent for treatment was obtained from all patients.

### Conditioning

All patients received 4 fractionated 12 Gy total body irradiation on days −8 and −7, in addition to cytosine arabinoside (Ara-C) and cyclophosphamide. Ara-C was administered intravenously (IV) over 2 h at a dose of 3 g/m$^2$ every 12 h on day −5 and −4 (total dose 12 g/m$^2$). In patients with myeloid malignancies, recombinant human granulocyte colony-stimulating factor (G-CSF) was combined with Ara-C. G-CSF was administered by continuous infusion at a dose of 5 μg/kg/day. Infusion of G-CSF was started 12 h before the first dose of Ara-C and stopped at the completion of the last dose. Cyclophosphamide was administered IV over 2 h at a dose of 60 mg/kg once daily on days −3 and −2 (total dose 120 mg/kg). Two days after the completion of conditioning, patients received a CBT.

### Graft-versus-host disease (GVHD) prophylaxis

All patients received standard CYA and methotrexate as GVHD prophylaxis. CYA was given IV every day starting on day −1 at a dose of 3 mg/kg/day. Methotrexate (15 mg/m$^2$ IV) was given on day 1, and 10 mg/m$^2$ on day 3 and 6. Once oral intake could be tolerated, patients were administered oral CYA at a dose of 1:2, in 2 divided doses per day, based on the last intravenous dose. CYA was reduced when sCr levels rose above 1.5 times baseline, or other serious agent-associated toxicities occurred. Physicians could freely modify the CYA dose for patients experiencing severe acute GVHD (aGVHD) or risk of disease relapse. Corticosteroid-based treatment was considered when grade II or higher severe aGVHD occurred (0.5–2 mg/kg).

### Supportive care

All patients received G-CSF by intravenous infusion starting on day 1 until durable granulocyte recovery was achieved. The supportive care regimen, including prophylaxis for infection was the same as previously reported (8, 9).

### Monitoring

All patients were monitored retrospectively 10 days before, and after the first 100 days, of CBT. Daily laboratory data collecting and the detecting method of VCM and CYA trough concentration were the same as previously reported (8). Therapeutic drug monitoring for VCM by assessing serum trough concentration was done twice in weekly, and modified to not exceed 10.0 mg/L.

### End-points and definitions

AKI was defined as 2-fold rise in sCr concentration on daily laboratory results from the baseline (the average of days −10 to 0). Myeloid engraftment was defined as the first of 3 consecutive days, during which the absolute neutrophil count was at least $0.5 \times 10^9$/L. Platelet recovery time was achieved on the first of 3 days when the platelet count was higher than $50 \times 10^9$/L without transfusion support. The aGVHD was graded according to previously published criteria (10). Transplant-related mortality was defined as death from any cause except relapse. Relapse was defined by morphologic evidence of disease in peripheral blood, bone marrow, or extramedullary sites. Disease-free survival was defined as the time from CBT to relapse, death, or the last observation.

### Statistical analysis

Continuous variables are expressed as median and their range. For dichotomous variables, the frequencies of positive occurrence are given along with their corresponding percentages. Continuous variables were divided into high or low with their median values, and a single VCM trough concentration of 10.0 mg/L was defined as a threshold level for analysis. Cumulative incidence of AKI was estimated with competing risk setting, of which death and relapse were defined as competing risk events. Variables considered in univariate analysis were body weight, age, recipient gender, recipient cytomegalovirus serology, disease status at transplant (standard or high risk), total nucleated cell dose, CD34+ cell dose, baseline sCr levels, VCM use, VCM trough levels, CYA trough levels, foscarnet use, aminoglycosides use, days of neutrophil engraftment, aGVHD grade 3–4, and positive blood culture result. Variables with a *P*-value <0.1 for cumulative incidence of AKI were tested in multivariate analysis using Cox proportional hazards models, and *P*-values <0.05 were considered to be statistically significant. The probability of disease-free survival was estimated from the time of CBT according to the Kaplan–Meier method. Endpoints were calculated at the last contact, the date of the last follow-up being December 1, 2011. Statistical software R, version 2.12.2, was used for analysis.

## Results

### Characteristics of patients and cord blood units

The characteristics of 38 patients and cord blood units are shown in Table 1. Among the patients, the median age was 41.5 years (range, 18–52 years), the median weight was 59.5 kg (range, 39–76 kg), the median number of cryopreserved nucleated cells was $2.8 \times 10^7$/kg (range, $1.7–5.7 \times 10^7$/kg), and the median number of cryopreserved CD34+ cells was $0.9 \times 10^5$/kg (range, $0.4–2.6 \times 10^5$/kg). All patients received a single and human leukocyte antigen-mismatched cord blood unit.

### Time courses of changing renal function

No patient had confirmed renal dysfunction before transplantation. The changes of renal function as variations (%) of sCr from baseline levels observed on days 11–20 were greatest and significant (+15.8%,

**Characteristics and clinical course**

| Characteristics | |
|---|---|
| Patients, *n* | 38 |
| Male/Female, *n* | 25/13 |
| Median age, years (range) | 41.5 (18–52) |
| Median weight, kg (range) | 59.5 (39–76) |
| Median number of cryopreserved nucleated cells, $\times 10^7$/kg (range) | 2.8 (1.7–5.7) |
| Median number of cryopreserved CD34+ cells, $\times 10^5$/kg (range) | 0.9 (0.4–2.6) |
| Recipient CMV status, Positive/Negative, *n* | 32/6 |
| Diagnosis | |
| AML, *n* | 12 |
| MDS-related secondary AML, *n* | 6 |
| RAEB, *n* | 3 |
| RA, *n* | 2 |
| CML, *n* | 3 |
| ALL, *n* | 11 |
| NHL, *n* | 1 |
| Disease status at transplant | |
| Standard risk, *n* | 10 |
| High risk, *n* | 28 |
| Conditioning regimen | |
| TBI + Ara-C/G-CSF + CY, *n* | 26 |
| TBI + Ara-C + CY, *n* | 12 |
| GVHD prophylaxis | |
| CYA + MTX, *n* | 38 |
| Baseline sCr, mg/dL (range) | 0.62 (0.33–0.87) |
| Neutrophil >0.5 $\times 10^9$/L, days (range) | 21 (17–30) |
| Patients with positive blood culture, *n* (%) | 6 (16) |
| Patients taking aminoglycosides, *n* (%) | 32 (84) |
| Patients taking foscarnet, *n* (%) | 10 (26) |
| Patients taking liposomal amphotericin, *n* (%) | 16 (42) |
| Maximum CYA trough value, μg/L (range) | 258.5 (40–453) |
| Patients taking VCM, *n* (%) | 32 (84) |
| Duration of VCM therapy, days (range) | 54 (6–100) |
| Maximum VCM trough value, mg/L (range) | 8.8 (5.2–12.2) |
| Patients with maximum VCM trough value, >10.0 mg/L, *n* (%) | 9 (24) |
| Patient requiring hemodialysis, *n* (%) | 0 (0) |

CMV, cytomegalovirus; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess blasts; RA, refractory anemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; TBI, total body irradiation; Ara-C, cytosine arabinoside; G-CSF, recombinant human granulocyte colony-stimulating factor; CY, cyclophosphamide; GVHD, graft-versus-host disease; CYA, cyclosporine; MTX, methotrexate; sCr, serum creatinine; VCM, vancomycin.

*Table 1*

0.57 ± 0.18 mg/dL to 0.71 ± 0.24 mg/dL, $P < 0.001$). No obvious recovery occurred of declined renal function, which remained until day 100.

### Incidence and risk factors of AKI

Cumulative incidence of AKI at day 100 after CBT was 34% (95% CI 19–50) (Fig. 1). The median of the maximum value of VCM trough was 8.8 (4.5–12.2) mg/L. In univariate analysis, baseline sCr levels and foscarnet use were associated with the incidence of AKI (Table 2). In multivariate analysis, no factor was associated with the incidence of AKI (Table 2).

### Transplant outcomes

All patients had myeloid reconstitution, and the median time to >0.5 × $10^9$/L absolute neutrophil count was 21 days (range, 17–30 days). A self-sustained platelet count >50 × $10^9$/L was achieved in 37 patients at a median time of 45.5 days (range, 34–127 days). In 37 of 38 evaluable patients, aGVHD occurred. The grading of aGVHD was grade I in 7 patients, grade II in 25, grade III in 4, and grade IV in 1. No one experienced hepatic veno-occlusive disease. Six of 38 patients (16%) had positive blood culture; however, no one had confirmed hypotension, indicated with decrease in systolic blood pressure >10 mmHg to <90 mmHg. Of 6 patients with positive blood cultures, 4 patients were not administered VCM. The total number of positive blood cultures was 13 of 998 specimens. Ten of 13 bacterial pathogens from blood cultures were gram-positive cocci (Table 3). Vancomycin-resistant *Enterococci* were detected in 1 patient from blood culture, however, this had been continuously detected from stool specimens since admission. No patients required hemodialysis. Among the 38 patients, no patient died of transplant-related causes (transplant-related mortality 0%). Six patients relapsed. Of these 6 patients, 5 patients died of relapse. A total of 32 of 38 patients are alive and free of disease at between 139 and 1400 days (median: 634 days) after CBT. The probability of disease-free survival at 2 years was 83% and 77% at 3 years (Fig. 2).

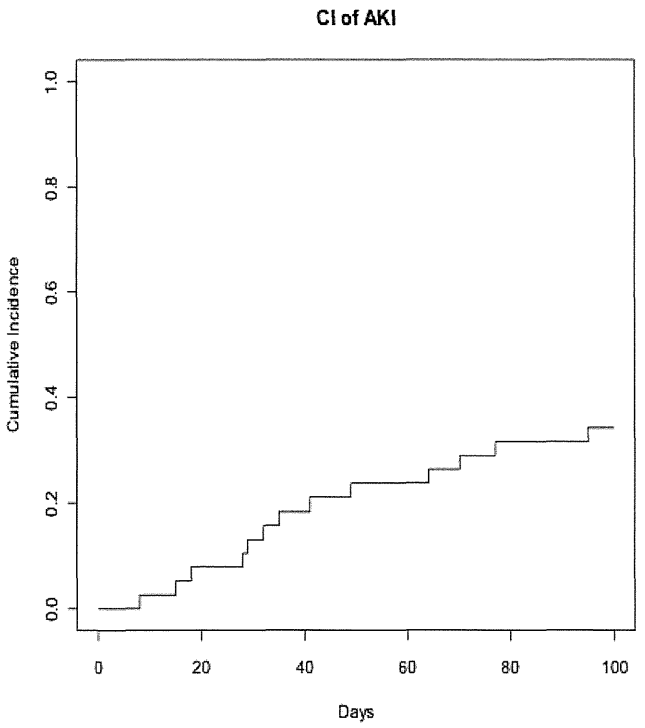


*Fig. 1.* Cumulative incidence (CI) of acute kidney injury (AKI).

## Discussion

In this study, similar trends were observed in the time course of renal function changes as previously reported (8). However, the elevation in sCr was lower in this study, especially in days 11–20 (from 35.0% [8] to 15.8% in this study). Cumulative incidence of AKI was 34%; however, this was not assessed in our previous study (8). When we assessed the incidence of AKI with an identical definition to the previous

**Univariate and multivariate analysis of factors associated with acute kidney injury**

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Hazard ratio (95% CI) | *P* | Hazard ratio (95% CI) | *P* |
| Baseline sCr, mg/dL | | | | |
| >0.62 | 0.27 (0.08–0.98) | 0.047 | 0.33 (0.08–1.32) | 0.12 |
| <0.62 | 1 | | 1 | |
| Foscarnet | | | | |
| (+) | 3.11 (1.07–9.05) | 0.037 | 2.45 (0.71–8.42) | 0.15 |
| (−) | 1 | | 1 | |
| VCM trough, >10.0 mg/L | | | | |
| (+) | 2.68 (0.89–8.09) | 0.081 | 2.64 (0.76–9.19) | 0.13 |
| (−) | 1 | | 1 | |

CI, confidence interval; sCr, serum creatinine; VCM, vancomycin.

*Table 2*

**Isolated bacterial pathogens from blood cultures**

| Pathogens | *n* |
|---|---|
| *Enterococcus faecalis* | 3 |
| Vancomycin-resistant *Enterococcus faecium* | 3 |
| Methicillin-resistant *Staphylococcus* species | 1 |
| Methicillin-resistant *Staphylococcus epidermidis* | 3 |
| *Stenotrophomonas maltophilia* | 1 |
| *Bacillus* species | 1 |
| *Bacillus cereus* | 1 |

*Table 3*

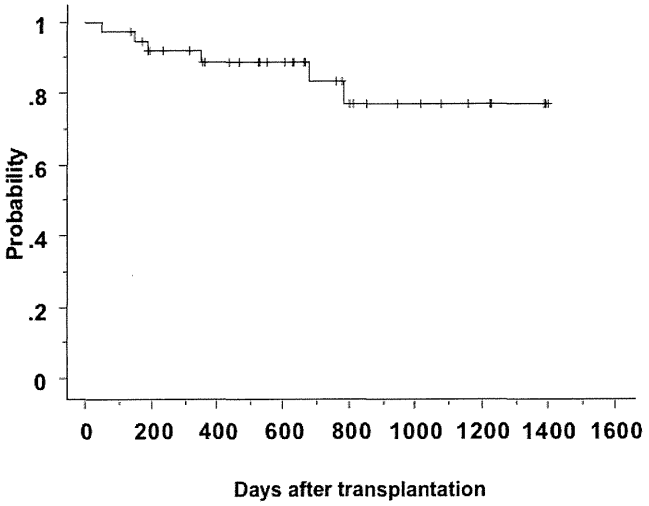


*Fig. 2.* Probability of disease-free survival after cord blood transplantation.

study, defined as just a 2-fold rise in sCr of 10 days average before and after transplantation, the incidence of AKI decreased to 11% in this study. In our previous study, the maximum VCM trough levels were significantly higher in patients with AKI (8); therefore, we have monitored VCM serum trough concentrations more strictly to not exceed 10.0 mg/L since 2008 in this study period. The average maximum value of VCM trough levels was lowered to 8.7 ± 2.1 mg/L from 12.2 ± 4.6 mg/L in the previous study, and proportion of patients with trough levels >10.0 mg/L was also decreased from 57% to 24%. Although baseline sCr levels and foscarnet use were associated with the incidence of AKI, VCM trough levels were not associated with AKI in univariate analysis. No factor was associated with AKI in multivariate analysis. Parikh et al. (11) reported AKI significantly affects survival after myeloablative allogeneic SCT in their meta-analysis, and more recently, Kagoya et al. (7) as well as Gooley et al. (12) reported the association of severity of AKI classification and non-relapse mortality within 100 days after transplantation. Although cumulative incidence of AKI was 34% in this study, no patients required hemodialysis or died of transplant-related causes. Recently, Yazaki et al. (13) reported the association of overall mortality and early bacterial infection of CBT in adults. They reported that cumulative incidence of early bacterial infection at day 100 was 21%, early bacterial infection had a negative effect on survival for adults, and the median day of development was 10 days after transplant, suggesting that prevention of bacterial infection in the very early post-CBT phase is important. Recently, a shift has occurred in the type of infecting organisms that cause bacteremia from predominantly gram-negative organisms to gram-positive cocci. The same trend is confirmed in the CBT (13, 14). VCM has an important role for infection control of gram-positive bacteremia, and was given to almost all the patients in this study. The reduced susceptibility of staphylococci for VCM has been reported since the mid 1990s, and prolonged exposure to lower VCM concentration has been associated with resistance (15). Although very few studies about pharmacokinetics and pharmacodynamics of VCM are available, several studies revealed area under the curve/minimum inhibitory concentration (AUC/MIC) as a preferred parameter, and AUC/MIC >400 associated with successful outcome and prevention of resistance (15, 16). Because of the difficulty of determining multiple concentrations for calculating AUC in the clinical setting, VCM trough concentrations have been recommended as the best surrogate marker for AUC/MIC, and concentrations of 15–20 mg/L – higher than the 5–15 mg/L previously recommended – is recommended as the target range (16). However, because an increased risk of nephrotoxicity with elevated VCM trough concentrations has been reported, and no appropriate pharmacokinetic/pharmacodynamic parameters for VCM have been determined (15, 17, 18), careful assessments are needed for using VCM at high target concentrations. Although we controlled VCM levels to not exceed 10.0 mg/L in this study, no patient died of bacterial infections. Further studies are required to determine the optimal VCM trough concentrations. Few reports are available about monitoring VCM trough concentrations for preventing AKI in allogeneic SCT in adults. Despite the limitations associated with this retrospective review of a small number of patients, our results suggest that strict monitoring of VCM serum trough concentrations has a beneficial effect on outcomes of CBT.

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