Table 2
Multivariate Analysis of Transplantation Outcomes

Outcome		HR (95% CI)	P Value
Overall survival*	Overall		<.001
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM		1.47 (1.24-1.74)	<.001
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM		1.03 (.86-1.24)	.75
Relapse†	Overall		.02
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM		1.35 (1.05-1.74)	.02
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM		1.18 (.89-1.56)	.26
NRM‡	Overall		.013
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM		1.32 (1.06-1.64)	.013
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM		.98 (.77-1.25)	.88
Neutrophil recovery§	Overall		<.001
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM		.42 (.37-.48)	<.001
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM		.47 (.40-.55)	<.001
Platelet recovery‖	Overall		<.001
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM		.36 (.30-.42)	<.001
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM		.44 (.37-.53)	<.001
Grade II-IV acute GVHD¶	Overall		.36
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM		1.10 (.89-1.36)	.38
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM		.69 (.56-.87)	.001
Extensive chronic GVHD#	Overall		.022
4/6-6/6–Matched UCB versus 8/8 HLA–matched UBM		.65 (.46-.92)	.015
4/6-6/6–Matched UCB versus 7/8 HLA–matched UBM		.56 (.38-.82)	.003

UCB indicates umbilical cord blood; UBM, unrelated bone marrow.

* For overall survival, hazard ratio is adjusted with recipient age, sex, primary disease, disease status at transplantation, and year of transplantation.

† For relapse, hazard ratio is adjusted with primary disease, the use of TBI, the use of antithymocyte globulin, and disease status at transplantation.

‡ For NRM, hazard ratio is adjusted with recipient sex, the use of TBI, and year of transplantation.

§ For neutrophil recovery, hazard ratio is adjusted with disease status at transplantation, conditioning regimen, the use of TBI, and GVHD prophylaxis.

‖ For platelet recovery, hazard ratio is adjusted with recipient sex, disease status at transplantation, the use of TBI, year of transplantation, and GVHD prophylaxis.

¶ For grade II to IV acute GVHD, hazard ratio is adjusted with age, disease status at transplantation, and the use of TBI.

For extensive chronic GVHD, hazard ratio is adjusted with recipient sex.

UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT were 29 days (range, 1 to 228 days), 32 days (range, 1 to 323 days), and 66 days (range, 8 to 230 days), respectively. Platelet recovery was also faster in recipients with early phase disease or intermediate phase disease than in those with advanced phase disease in ($P < .001$). A 4/6 to 6/6 HLA–matched UCBT was a strong independent negative predictor for platelet engraftment within the multivariate analysis (versus 8/8 HLA–matched UBMT, HR, .36 [95% CI, .30 to .42]; $P < .001$, versus 7/8 HLA–matched UBMT, HR, .44 [95% CI, .37 to .53]; $P < .001$, respectively) (Table 2). MAC was not a negative predictor for platelet engraftment.

GVHD

The cumulative incidence of grade II to IV acute GVHD by 100 days after transplantation was lower in recipients of an

8/8 HLA–matched UBMT (34% [95% CI, 30% to 39%]) than in recipients of a 7/8 HLA–matched UBMT (50% [95% CI, 44% to 56%]) or a 4/6 to 6/6 HLA–matched UCBT (41% [95% CI, 36% to 45%]). More recipients who received a TBI-containing regimen experienced grade II to IV acute GVHD by day 100 than did those who received a non-TBI regimen (43% [95% CI, 40% to 46%] versus 34% [95% CI, 29% to 39%], $P = .001$). The 4/6 to 6/6 HLA–matched UCBT recipients had a similar risk of grade II to IV acute GVHD to the 8/8 HLA–matched UBMT recipients within the multivariate analysis (HR, 1.10 [95% CI, .89 to 1.36]; $P = .38$) (Table 2). However, the 4/6 to 6/6 HLA–matched UCBT recipients had a significantly lower risk of grade II to IV acute GVHD than did the 7/8 HLA–matched UBMT recipients (HR, .69 [95% CI, .56 to .87]; $P = .001$) (Table 2).

The cumulative incidence of the extensive type of chronic GVHD by 2 years after transplantation was lower in recipients of the 4/6 to 6/6 HLA–matched UCB (15% [95% CI, 11% to 19%]) than in those who received the 8/8 HLA–matched UBMT or 7/8 HLA–matched UBMT (23% [95% CI, 19% to 27%] and 25% [95% CI, 20% to 32%], respectively). The same relationship was observed when performing the multivariate analysis (versus 8/8 HLA–matched UBMT, HR, .65 [95% CI, .46 to .92]; $P = .015$, versus 7/8 HLA–matched UBMT, HR, .56 [95% CI, .38 to .82]; $P = .003$, respectively) (Table 2).

Relapse

The cumulative incidence of relapse by 2 years was significantly higher in patients receiving the 4/6 to 6/6 HLA–matched UCBT (26% [95% CI, 22% to 30%]) than in those who received the 8/8 HLA–matched UBMT (18% [95% CI, 15% to 22%]) or those who received the 7/8 HLA–matched UBMT (21% [95% CI, 16% to 26%]). However, according to disease status at transplantation, the relapse rate by 2 years after the 8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT were not statistically different regardless of disease status at transplantation (8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT; early phase disease, 11% [95% CI, 7% to 16%], 15% [95% CI, 8% to 23%], and 19% [95% CI, 12% to 26%]; intermediate phase disease, 22% [95% CI, 13% to 32%], 26% [95% CI, 15% to 39%], and 17% [95% CI, 10% to 27%]; advanced phase disease, 35% [95% CI, 28% to 42%], 36% [95% CI, 28% to 44%], and 43% [95% CI, 38% to 49%], respectively) (Figure 1A–C). On multivariate analysis, the 4/6 to 6/6 HLA–matched UCBT recipients had a significantly higher risk of relapse than did the recipients of the 8/8 HLA–matched UCBT (HR, 1.35 [95% CI, 1.05 to 1.74]; $P = .02$) and had a similar risk to that of the 7/8 HLA–matched UBMT recipients (HR, 1.18 [95% CI, .89 to 1.56]; $P = .26$) (Table 2).

According to primary disease, the cumulative incidence of relapse after the 4/6 to 6/6 HLA–matched UCBT was higher than that after the 8/8 HLA–matched UBMT only in MDS patients and was similar both in AML patients and in ALL patients (Supplemental Table 4).

According to conditioning regimen, the cumulative incidence of relapse after the 4/6 to 6/6 HLA–matched UCBT was higher than that after the 8/8 HLA–matched UBMT only in recipients of MAC (Supplemental Table 5). Among the patients who received RIC, the cumulative incidence of relapse after the 4/6 to 6/6 HLA–matched UCBT was significantly higher than that after the UBMT in recipients without extensive chronic GVHD. However, the cumulative incidence of relapse after the 4/6 to 6/6
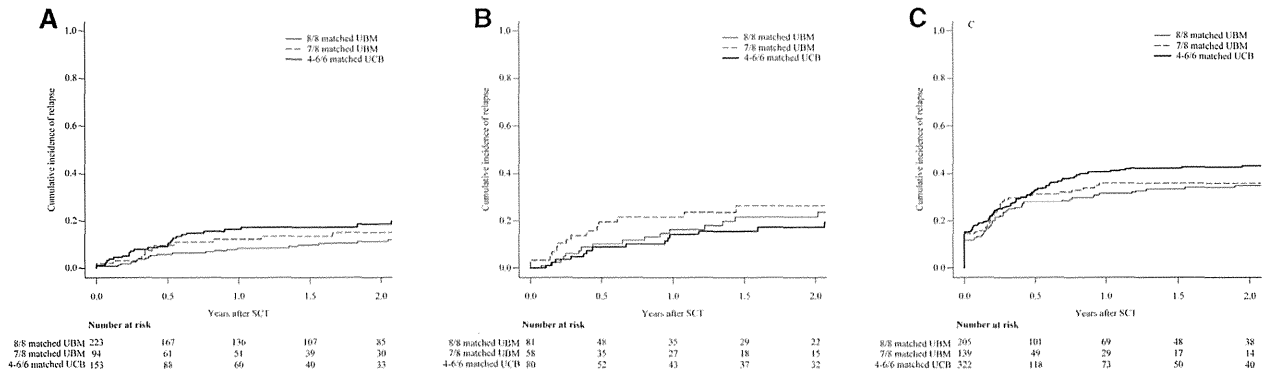
Figure 1. Cumulative incidence of relapse in patients with early phase disease, in those with intermediate phase disease, and in those with high-risk disease according to hematopoietic stem cell source and donor-recipient HLA match. (A) The cumulative incidences of relapse in patients with early phase disease by 2 years after an 8/8 HLA–matched unrelated bone marrow transplantation (UBMT), a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched umbilical cord blood transplantation (UCBT) were 11% (95% CI, 7% to 16%), 15% (95% CI, 8% to 23%), and 19% (95% CI, 12% to 26%), respectively. (B) The cumulative incidences of relapse in patients with intermediate phase disease by 2 years after an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 22% (95% CI, 13% to 32%), 26% (95% CI, 15% to 39%), and 17% (95% CI, 10% to 27%), respectively. (C) The cumulative incidences of relapse in patients with intermediate phase disease by 2 years after an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 35% (95% CI, 28% to 42%), 36% (95% CI, 28% to 44%), and 43% (95% CI, 38% to 49%), respectively.

HLA–matched UCBT was not statistically different from that after UBMT among the recipients of MAC (Supplemental Figure 1).

NRM

The 2-year cumulative incidences of NRM after the 8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT were 32% (95% CI, 27% to 36%), 40% (95% CI, 33% to 46%), and 38% (95% CI, 34% to 43%), respectively. Among patients with early phase disease, the cumulative incidence of NRM at 2 years after the 8/8 HLA–matched UBMT was significantly lower than that after the 7/8 HLA–matched UBMT or 4/6 to 6/6 HLA–matched UCBT (25% [95% CI, 19% to 32%], 35% [95% CI, 25% to 45%], and 37% [95% CI, 29% to 46%]) (Figure 2A). Among patients with intermediate phase disease or advanced phase disease, NRM by 2 years was not statistically different among 3 groups (8/8 HLA–matched UBMT, 7/8 HLA–matched UBMT, and 4/6 to 6/6 HLA–matched UCBT; intermediate phase disease; 32% [95%

CI, 31% to 43%], 27% [95% CI, 16% to 40%], and 28% [95% CI, 18% to 38%]; advanced phase disease, 34% [295% CI, 7% to 41%], 41% [95% CI, 32% to 50%], and 36% [95% CI, 30% to 41%], respectively) (Figure 2B,C). On multivariate analysis, the 4/6 to 6/6 HLA–matched UCBT recipients had a higher risk of NRM than the 8/8 HLA–matched UBMT recipients (HR, 1.32 [95% CI, 1.06 to 1.64]; $P = .013$); however, they had a similar risk to the 7/8 HLA–matched UBMT recipients (HR, .98 [95% CI, .77 to 1.25]; $P = .88$) (Table 2). According to primary disease, NRM by 2 years after the 4/6 to 6/6 HLA–matched UCBT was likely higher than that after the 8/8 HLA–matched UBMT only among patients with MDS; however, the difference was not significant regardless of primary diseases (Supplemental Table 4). On multivariate analysis of subgroup analysis according to conditioning regimen, NRM after the 8/8 HLA–matched UBMT was significantly lower than that after the 7/8 HLA–matched UBMT and 4/6 to 6/6 HLA–matched UCBT only among recipients of RIC (Supplemental Table 5).
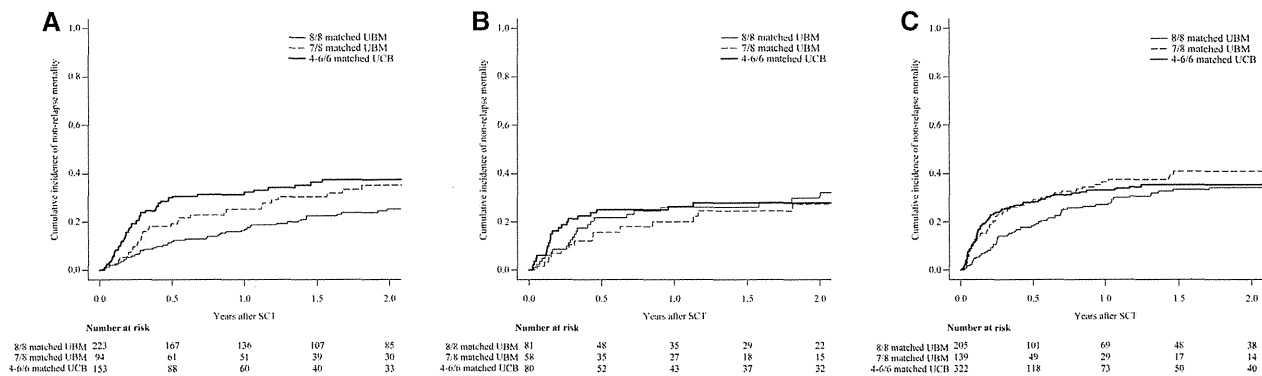

Figure 2. Cumulative incidence of NRM in patients with early phase disease, in those with intermediate phase disease, and in those with advanced phase disease according to hematopoietic stem cell source and donor-recipient HLA match. (A) The cumulative incidences of NRM in patients with early phase disease by 2 years after an 8/8 HLA–matched unrelated bone marrow transplantation (UBMT), a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched umbilical cord blood transplantation (UCBT) were 25% (95% CI, 95% CI, 19% to 32%), 35% (95% CI, 95% CI, 25% to 45%), and 37% (95% CI, 29% to 46%), respectively. (B) The cumulative incidences of NRM in patients with intermediate phase disease by 2 years after an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 32% (95% CI, 31% to 43%), 27% (95% CI, 16% to 40%), and 28% (95% CI, 18% to 38%), respectively. (C) The cumulative incidences of NRM in patients with advanced phase disease by 2 years after an 8/8 HLA–matched UBMT, a 7/8 HLA–matched UBMT, and a 4/6 to 6/6 HLA–matched UCBT were 34% (95% CI, 27% to 41%), 41% (95% CI, 32% to 50%), and 36% (95% CI, 30% to 41%), respectively.

Survival

The 2-year unadjusted probabilities of OS after the 8/8 HLA—matched UBMT (51% [95% CI, 46% to 56%]) were significantly higher than those of the 7/8 HLA—matched UBMT (39% [95% CI, 32% to 45%]) and 4/6 to 6/6 HLA—matched UCBT (35% [95% CI, 31% to 39%]) recipients, respectively. The adjusted probabilities of OS at 2 years were also significantly better in recipients of the 8/8 HLA—matched UBMT than in the recipients of the 7/8 HLA—matched UBMT or 4/6 to 6/6 HLA—matched UCBT (49% [95% CI, 44% to 54%], 38% [95% CI, 32% to 45%], 39% [95% CI, 35% to 44%], respectively). This finding was also observed in the subgroup analysis for disease status (at early phase: the adjusted probabilities of OS at 2 years after the 8/8 HLA—matched UBMT, 7/8 HLA—matched UBMT, and 4/6 to 6/6 HLA—matched UCBT were 69% [95% CI, 62% to 76%], 54% [95% CI, 44% to 66%], and 46% [95% CI, 38% to 56%]; at intermediate phase: 53% [95% CI, 42% to 67%], 55% [95% CI, 42% to 72%], and 62% [95% CI, 52% to 74%], respectively; at advanced phase: 31% [95% CI, 24% to 39%], 24% [95% CI, 17% to 33%], and 25% [95% CI, 21% to 31%], respectively) (Figure 3).

According to the multivariate analysis, the 4/6 to 6/6 HLA—matched UCBT recipients had a significantly higher risk of overall mortality than did the 8/8 HLA—matched UBMT recipients (HR, 1.47 [95% CI, 1.24 to 1.74]; $P < .001$) (Table 2). However, the 4/6 to 6/6 HLA—matched UCBT recipients had a similar risk of overall mortality when compared with the 7/8 HLA—matched UBMT recipients (HR, 1.03 [95% CI, .86 to 1.24]; $P = .75$) (Table 2). The adjusted probabilities of OS at 2 years after 8/8 HLA—matched UBMT were superior to those after 4/6 to 6/6 HLA—matched UCBT, regardless of primary disease and conditioning regimen, especially in the patients with MDS (Supplemental Figure 2, Supplemental Tables 4 and 5).

To identify the population of UCBT recipients who had a similar OS to those of 8/8 HLA—matched UBMT, we evaluated the impact of cell dose, HLA matching, and GVHD prophylaxis on the OS of UCBT recipients. The 2-year unadjusted OS of UCBT recipients who received $\geq$.84 × 10^5 CD34$^+$ cells/kg, which was median cell dose, was significantly higher than those who received <.84 × 10^5 CD34$^+$ cells/kg (Supplemental Figure 3A). HLA matching did not have an effect on OS (Supplemental Figure 3B). GVHD prophylaxis

with calcineurin inhibitor (CNI) and other agents improved OS compared with that with CNI alone (Supplemental Figure 3C). Therefore, we compared the OS of 4/6 to 6/6 HLA—matched UCBT recipients who received umbilical cord blood units containing $\geq$.84 × 10^5 CD34$^+$ cells/kg with 8/8 HLA—matched UBMT recipients, among those with AML and those with ALL who received GVHD prophylaxis with CNI and other agent. The unadjusted 2-year OS after 8/8 HLA—matched UBMT was higher than 4/6 to 6/6 HLA—matched UCBT in patients with early phase disease. Among those with intermediate phase disease, the unadjusted 2-year OS after 4/6 to 6/6 HLA—matched UCBT was likely higher than 8/8 HLA—matched UBMT. Among those with advanced phase disease, the 2-year OS were similar between 2 groups (8/8 HLA—matched UBMT versus 4/6 to 6/6 HLA—matched UCBT; the unadjusted OS of early phase disease, 67% [95% CI, 59% to 74%] versus 55% [95% CI, 40% to 67%], $P = .044$; the unadjusted OS of intermediate disease, 52% [95% CI, 39% to 64%] versus 77% [95% CI, 56% to 89%], $P = .08$; the unadjusted OS of advanced phase disease, 25% [95% CI, 17% to 33%] versus 26% [95% CI, 16% to 36%], $P = .82$) (Figure 4A,C). The adjusted probability of OS were similar between 2 groups (8/8 HLA—matched UBMT versus 4/6 to 6/6 HLA—matched UCBT; the adjusted OS, 49% [95% CI, 43% to 55%] versus 49% [95% CI, 41% to 58%], $P = .74$, respectively) (Figure 4D).

DISCUSSION

The primary objectives of this study were to compare OS after 4/6 to 6/6 HLA—matched UCBT with those after 8/8 and 7/8 HLA—matched UBMT in patients with hematologic malignancies ages 50 years or older and to provide useful data for the selection of an appropriate unrelated stem cell source for those patients who do not have an available HLA-identical sibling. Our findings suggested that an 8/8 HLA allele—matched unrelated donor is the best alternative to a HLA-identical sibling donor. Four of 6 to 6/6 HLA—matched UCBT had a similar OS to 8/8 HLA—matched UBMT for patients with AML and for those with ALL when the umbilical cord blood unit containing $\geq$.84 × 10^5 CD34$^+$ cells/kg is available.

Neutrophil and platelet recovery were significantly slower after the 4/6 to 6/6 HLA—matched UCBT than after the
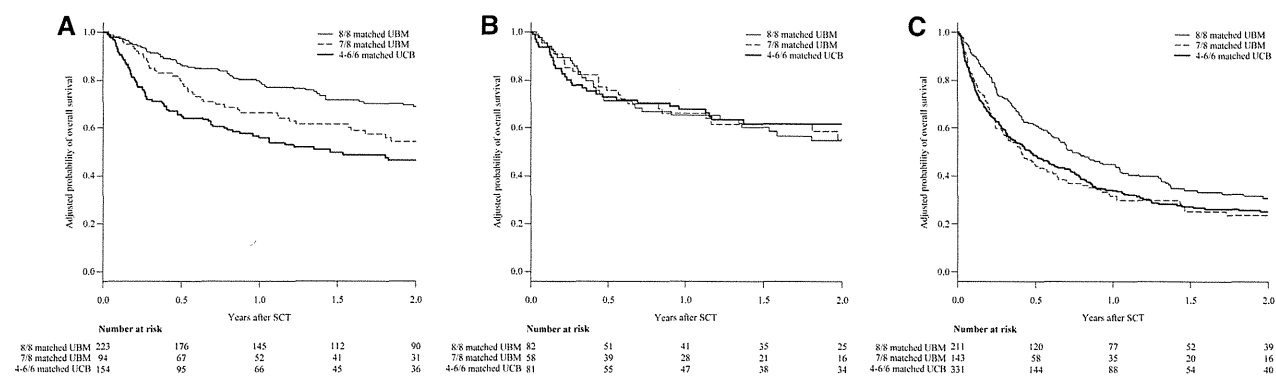

Figure 3. Adjusted probabilities of OS in patients with early phase disease, in those with intermediate phase disease, and in those with advanced phase disease according to hematopoietic stem cell source and donor-recipient HLA match. (A) The adjusted probabilities of the 2-year OS after transplantation in patients with early phase disease who received an 8/8 HLA—matched unrelated bone marrow transplantation (UBMT), a 7/8 HLA—matched UBMT, and a 4/6 to 6/6 HLA—matched umbilical cord blood transplantation (UCBT) were 69% (95% CI, 62% to 76%), 54% (95% CI, 44% to 66%), and 46% (95% CI, 38% to 56%), respectively. (B) The adjusted probabilities of the 2-year OS after transplantation in patients with intermediate phase disease who received an 8/8 HLA—matched UBMT, a 7/8 HLA—matched UBMT, and a 4/6 to 6/6 HLA—matched UCBT were 53% (95% CI, 42% to 67%), 55% (95% CI, 42% to 72%), and 62% (95% CI, 52% to 74%), respectively. (C) The adjusted probabilities of the 2-year OS after transplantation in patients with intermediate phase disease who received an 8/8 HLA—matched UBMT, a 7/8 HLA—matched UBMT, and a 4/6 to 6/6 HLA—matched UCBT were 31% (95% CI, 24% to 39%), 24% (95% CI, 17% to 33%), and 25% (95% CI, 21% to 31%), respectively.
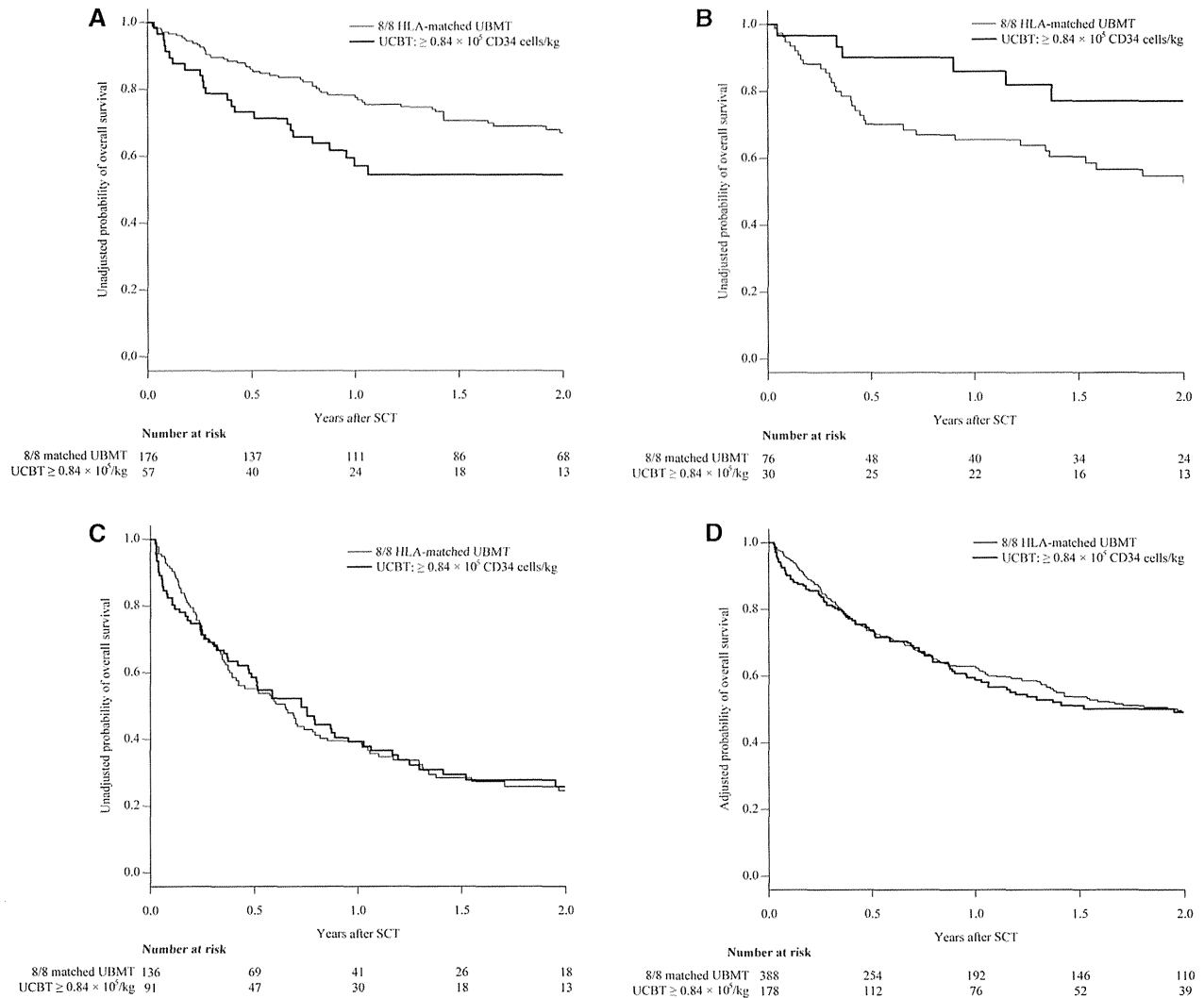
Figure 4. OS in UCBT recipient who received $\geq$ .84 × 10^5/kg CD34 cells compared with 8/8 HLA–matched UBMT recipients, among those with AML and ALL who prevented graft-versus-host disease with CNI and other agents. (A) The unadjusted probabilities of the 2-year OS after transplantation in patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) at early phase disease and prevented GVHD with CNI and other agent who received an 8/8 HLA–matched unrelated bone marrow transplantation (UBMT) and a 4/6 to 6/6 HLA–matched umbilical cord blood transplantation (UCBT) receiving $\geq$.84 × 10^5/kg CD34 cells were 67% (95% CI, 59% to 74%) and 55% (95% CI, 40% to 67%), respectively, $P = .044$. (B) The unadjusted probabilities of the 2-year OS after transplantation in patients with AML and ALL at intermediate phase disease and prevented GVHD with CNI and other agent who received an 8/8 HLA–matched UBMT and a 4/6 to 6/6 HLA–matched UCBT receiving $\geq$.84 × 10^5/kg CD34 cells were 52% (95% CI, 39% to 64%) and 77% (95% CI, 56% to 89%), respectively, $P = .08$. (C) The unadjusted probabilities of the 2-year OS after transplantation in patients with AML and ALL at advanced phase disease and prevented GVHD with CNI and other agent who received an 8/8 HLA–matched UBMT and a 4/6 to 6/6 HLA–matched UCBT receiving $\geq$.84 × 105/kg CD34 cells were 25% (95% CI, 17% to 33%) and 26% (95% CI, 16% to 36%), respectively, $P = .82$. (D) The adjusted probabilities of the 2-year OS after transplantation in patients with AML and ALL prevented GVHD with CNI and other agent who received an 8/8 HLA–matched UBMT and a 4/6 to 6/6 HLA–matched UCBT receiving $\geq$.84 × 10^5/kg CD34 cells were 49% (95% CI, 43% to 55%) and 49% (95% CI, 41% to 58%), respectively, $P = .74$.

8/8 and 7/8 HLA–matched UBMT, which was consistent with findings from previous studies [6-9,11,15]. Neutrophil recovery in patients with early phase disease and intermediate phase disease at transplantation was significantly faster than in those with advanced phase disease, which was consistent with the findings in allogeneic peripheral blood stem cell transplantation that had been previously reported [26]. This may be associated with the fact that patients with advanced phase disease were likely pretreated more heavily than those with early phase disease and intermediate phase disease and that they had damage in the microenvironment of the bone marrow.

UCBT recipients had a lower risk of extensive chronic GVHD and a higher risk of relapse compared with 8/8 HLA–matched UBMT recipients. These findings suggested that the graft-versus-leukemia effect in the UCBT recipients was lower than that in the recipients of 8/8 HLA–matched UBMT.

Several studies comparing transplantation outcomes after UBMT versus after UCBT have been reported [6-9]. In some studies, serological HLA class I typing was used for UBMT [6-8]. In another study, UCBT recipients were significantly younger than UBMT recipients, and all patients received a MAC regimen. As a result, only a small number of patients aged 50 years or older were included [9], so direct comparisons of our findings with previous studies are difficult. We had previously demonstrated that HR of overall mortality after a 4/6 to 6/6 HLA–matched UCBT was significantly

higher than that after an 8/8 HLA–matched UBMT among AML patients but not among ALL patients [15]. By contrast, this study showed that the overall survival after an 8/8 HLA–matched UBMT was superior to that after a 4/6 to 6/6 HLA–matched UCBT for patients with AML and for patients with ALL. The present study included patients 50 years or older who received HSCT between 2000 and 2009 regardless of intensity of the conditioning regimen, whereas our previous study had included the recipients of MAC between 2000 and 2005 ages 16 years or older. Therefore, 20% of the 8/8 HLA–matched UBMT recipients and 10% of the 4/6 to 6/6 HLA–matched UCBT recipients in the present study were also included in our previous study. The discrepancy of the results for ALL may be partly due to differences in conditioning regimens (only recipients of MAC regimens were described in our previous report, whereas more than one half of the patients in this study received RIC regimen). Older patients with ALL had a higher risk of relapse and tended to receive RIC when compared with younger patients [27]; therefore, these patients would need a strong graft-versus-leukemia effect. In addition, short-term methotrexate improved OS in the UCBT recipients [28]. In our cohort, approximately 30% of UCBT recipients received GVHD prophylaxis with cyclosporine or tacrolimus alone, and this reduced OS in UCBT recipients. As previously described [29], UCBT recipients receiving higher CD34$^+$ cells had a higher OS than those receiving lower CD34$^+$ cells. For patients with AML and for patients with ALL, UCBT recipients receiving $\geq$.84 $\times$ 10^5 CD34$^+$ cells/kg had a similar adjusted and unadjusted OS to 8/8 HLA–matched UBMT recipients. These findings suggest that the outcomes of UCBT may improve with graft selection based on CD34$^+$ cell dose. The HR of overall mortality after a 4/6 to 6/6 HLA–matched UCBT was similar to that after a 7/8 HLA–matched UBMT, regardless of disease status at transplantation. To the best of our knowledge, this is the first report to compare transplantation outcomes in patients 50 years or older who received a 4/6 to 6/6 HLA–matched UCBT with those who received a 7-8/8 HLA–matched UBMT in a large cohort.

This study had several limitations. Although we adjusted for known risk factors using multivariate analysis, we could not exclude selection bias because this was a retrospective study based on registry data. Further, donor selection was influenced by several factors that were not statistically adjustable. Some patients with urgent disease who could not wait for the preparation of UBMT received UCBT; in other cases, a suitable UCB unit with enough cell doses was not available, and these patients therefore received UBMT. Patients who planned to receive UBMT and could not receive transplantation because of disease progression during the donor coordination were not included in this analysis. In addition, only 5% of recipients of UBMT received GVHD prophylaxis using only a CNI; on the other hand, approximately 30% of UCBT recipients employed the same protocol, which may have influenced the occurrence of GVHD and overall survival. A randomized controlled trial comparing UCBT with UBMT is needed to validate the findings from the present study; however, a study of that design is very difficult to conduct. Clinical decision analysis may help to address any selection bias caused by the donor search process. From 2000 onwards, UPBSCT was more common than UBMT [5]; however, we could not compare the transplantation outcomes of the 4/6 to 6/6 HLA–matched UCBT with the UPBSCT because more than 99% of the unrelated donors from Japan Marrow Donor Program were harvested bone marrow. A

randomized controlled trial comparing UPBSCT with UBMT had shown similar outcomes for OS, NRM, and relapse rate [30]. Taken together, UCBT may also be an alternative stem cell source when a HLA-matched peripheral blood stem cell donor is not available.

In conclusion, UCB is a reasonable alternative donor/stem cell source for elderly patients with AML and for those with ALL with similar outcomes compared with UBM from a 8/8 HLA–matched unrelated donor when UCB unit containing $\geq$.84 $\times$ 10^5 CD34$^+$ cells/kg is available. If urgently needed or if there is no 8/8 HLA–matched unrelated donor, a 4/6 to 6/6 HLA–matched UCBT is an acceptable treatment.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbmt.2014.11.685

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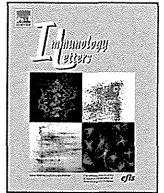
Letter to the Editor

Successful unrelated cord blood transplantation for adult acquired aplastic anemia using reduced intensity conditioning without ATG

Acquired aplastic anemia (aAA) patients who are transfusion-dependent and who have failed or relapsed after immunosuppressive therapy need further treatment. In cases in which a human leukocyte antigen (HLA)-identical sibling donor is not available, the use of alternative donor including HLA-matched unrelated donor and unrelated cord blood (CB) are commonly considered, but this strategy is associated with worse outcomes [1]. Because of the abundant availability of acceptable CB units, the use of cord blood transplantation (CBT) has been increasing. Although the use of CBT in patients with aAA has recently been evaluated [2], relatively little information is available on how to achieve proper engraftment with a reduced intensity conditioning (RIC) regimen in aAA patients undergoing CBT. We describe here three adult patients with aAA who underwent transplantation with unrelated CB after a RIC regimen without ATG. The patient characteristics are shown in Table 1. All three patients received single-unit CB containing more than 2.2×10^7/kg of total nucleated cell (TNC) with no more than two of six HLA-mismatches. The conditioning regimen consisted of six doses of fludarabine (Flu) 30 mg/m^2, two doses of cyclophosphamide (CY) 60 mg/kg and total body irradiation (TBI) $2 \text{ Gy} \times 2$ with no use of anti-thymocyte globulin (ATG). The graft-versus-host disease (GVHD) prophylaxis regimen was a combination of short-term methotrexate (15, 10, and 10 mg/m^2 on days 1, 3, 6, respectively) and tacrolimus. Because patient #3 had a high titer of anti-HLA antibody, a CB unit that was not cross-reactive with this antibody was chosen.

All three patients rapidly exhibited sustained CB engraftment (Table 2). Chimerism analyses of the CD3$^+$ fraction using various numbers of tandem repeats showed initial full-donor conversion from the first point of analysis in all patients. No secondary graft-failure was observed. Acute GVHD was observed in patient #2 (skin only stage 2, Grade I) and resolved spontaneously. Chronic GVHD was observed in patient #3 (skin, oral involvement) and symptoms resolved quickly after the administration of 0.5 mg/kg oral prednisolone. The regimen was generally well tolerated, and no significant organ damage or severe toxicity occurred. The patients remain alive without transfusion dependence at 68, 44 and 9 months, with Karnofsky scores of 70% (due to postherpetic neuralgia), 100% and 100%, respectively.

Here we report three CBT recipients who received successful single-unit CBT after a RIC regimen. All three patients exhibited sustained full donor-type hematopoiesis without further intervention to increase donor-type chimerism. The conditioning regimen included 180 mg/m^2 Flu and 120 mg/kg CY with $2 \text{ Gy} \times 2$ TBI, which may be regarded as a relatively strong regimen in terms of immunosuppressive and cytotoxic ability. Thus, one might think this regimen too potent for the induction of sustained engraftment of CB. However, Liu et al. reported that RIC regimen, consisting of Flu 120 mg/m^2, CY 1200 mg/m^2 (equivalent to 40 mg/kg if the patient's body weight was 50 kg) and rabbit ATG 30 mg/kg, was not sufficiently potent enough to induce engraftment after CBT in patients with aAA. They reported two early deaths and 16 graft-failures among the 18 CBT recipients conditioned with the above regimen [3]. Thus, it is reasonable to use a CY dose $>40 \text{ mg/kg}$, and further study to determine the optimal CY dose between 40 and 120 mg/kg is warranted.

To ensure rapid and proper CB engraftment, graft cell contents, such as TNC, CD34$^+$ cell count and CD8$^+$ cell count, are important factors [4]. In Western countries, ATG is commonly used as the conditioning regimen for CBT. Nevertheless, the use of ATG will decrease lymphocytes, including graft-facilitating CD8$^+$ lymphocytes, which may lead to attenuation of total potency for the facilitation of engraftment in exchange for the beneficial effect of reducing the incidence of severe acute GVHD. Indeed, only one of seven CB recipients for aAA who received ATG-containing regimen achieved engraftment in a previous retrospective study in Japan [5]. Thus, we replaced ATG with 4 Gy TBI in our regimen, which may be another reason for successful engraftment.

One of the biggest differences in CBT between Western countries and Japan may be the attitude toward the use of ATG. In the recent protocol of European group, two doses of ATG 2.5 mg/kg and a single agent GVHD prophylaxis are recommended [6]. To reduce the incidence of severe acute GVHD, physicians in Europe and US would be likely to use ATG more frequently, which might result in failure to observe better engraftment. In fact, it is reported that a conditioning regimen without ATG provided a low incidence of graft-failure [7]. Taken together, we believe that ATG should not be included in the conditioning regimen for CBT, not only for a single-unit CBT but also for a double-unit CBT. We also have shown the superiority of two-drug GVHD prophylaxis (including methotrexate) over single-drug prophylaxis in CBT [8]. To compensate prophylactic effect of ATG to control severe GVHD, it would be preferable to develop the GVHD prophylaxis after transplantation without ATG. Further study to determine whether or not ATG should be used in order to achieve prompt engraftment and subsequent higher quality of life and survival after RIC-CBT is warranted.

Table 1
Patient demographics and CB unit characteristics.

Pt no.	Age/sex	BW (kg)	Disease status at transplant	Interval from diagnosis to CBT (year)	Transfusion dependency	ABO mismatch	HLA serological mismatch	HLA allele mismatch	HLA-antibody	Donor-specific antibody	TNCC (10^7/kg)	CD34$^+$ (10^5/kg)
1	48/M	51	Severe	1.2	RBC	Match	2/6	3/8	–	–	3.67	0.50
2	53/M	65	Severe	22.1	RBC/PC	Major/minor	1/6	4/8	–	–	2.79	0.44
3	37/F	51	Non-severe	26.9	RBC/PC	Major/minor	2/6	3/8	+	–	2.24	0.55

Pt, patient; M, male; F, female; BW, body weight; RBC, red blood cell concentration; PC, platelet concentration; HLA, human leukocyte antigen; TNCC, total nucleated cell count.

Table 2
Engraftment, chimerism and other outcomes.

Pt no.	Days to ANC >500/µl	Days to reticulocyte >1%	Days to plt > 20,000/µl	Days to plt > 50,000/µl	Chimerism after CBT	Acute GVHD	Chronic GVHD	Other complications	Survival, mo	KS (%)
1	19	30	25	191	Day 20, 95% donor	No	No	Postherpetic neuralgia	Alive, 68	70
2	21	28	37	44	Day 19, 100% donor	Grade I (skin 2)	No	Polymyalgia rheumatica	Alive, 44	100
3	22	37	32	43	Day 25, 100% donor	No	Yes (skin, oral)	No	Alive, 9	100

Pt, patient; ANC, absolute neutrophil count; plt, platelet; KS, Karnofsky score.

Conflict of interest

All authors declare that there are no competing financial interests.

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Anti-HLA Antibodies Other than Against HLA-A, -B, -DRB1 Adversely Affect Engraftment and Nonrelapse Mortality in HLA-Mismatched Single Cord Blood Transplantation: Possible Implications of Unrecognized Donor-specific Antibodies

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A B S T R A C T

The impact of anti-HLA antibodies, except for donor-specific anti-HLA-A, -B, -DRB1 antibodies, on engraftment was retrospectively evaluated in 175 single cord blood transplantations (CBT). Patients and donors had been typed at HLA-A, -B, and -DRB1 antigens, and anti-HLA antibodies had been screened before transplantation to avoid the use of cord blood (CB) units with corresponding antigens. The median age was 59 (range, 17 to 74) years. Overall, 61% were male, 89% had high-risk disease status, 77% received myeloablative conditioning regimens, and over 80% were heavily transfused patients. Sixty-nine of the 175 (39.4%) were positive for anti-HLA antibodies. Thirty-nine patients had antibodies only against HLA-A, -B, or -DRB1, 13 had antibodies only against HLA-C, -DP, -DQ, or -DRB3/4/5, and 17 had antibodies both against HLA-C, -DP, -DQ, or -DRB3/4/5 and against HLA-A, -B, or -DRB1. Because CB units had not been typed at HLA-C, -DP, -DQ, or -DRB3/4/5, it was possible that antibodies against them were unrecognized donor-specific antibodies. Patients with antibodies only against HLA-A, -B, or -DRB1 showed comparable neutrophil engraftment rates to those without antibodies (89.7% versus 83%, $P = .65$), whereas patients having antibodies against C, DP, DQ, or -DRB3/4/5 showed lower engraftment rate (66.7%, $P = .12$), which became statistically significant in a subgroup of HLA-mismatched donor-recipient pairs (50%, $P = .01$). Our results demonstrated that the presence of donor nonspecific anti-HLA-A, -B, -DRB1 antibodies had no significant influence on engraftment, whereas anti-HLA-C, -DP, -DQ, or -DRB3/4/5 antibodies adversely affect engraftment, possibly because of unrecognized donor-specific anti-HLA antibodies against them, especially in HLA-mismatched CBT.

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INTRODUCTION

Cord blood transplantation (CBT) has become a valuable alternative for patients who require allogeneic stem cell transplantation (Allo-SCT) but who lack HLA-identical sibling or a matched unrelated donor [1]. Although the outcomes of CBT are almost comparable to those of Allo-SCT using unrelated donor, graft failure (GF), or engraftment delay, is one of the major concerns after CBT, leading to

increased early nonrelapse mortality (NRM) [2-6]. The pathogenesis of GF is likely multifactorial, and to date, several factors, including cell dose infused, HLA disparities, the type of conditioning regimens, and chemo-naïve status of recipient, have been identified as risk factors associated with GF after CBT [1,7-10].

Recently, the impact of anti-HLA antibodies on engraftment in Allo-SCT has drawn increasing attention because of an increasing number of patients who undergo Allo-SCT using HLA-mismatched donors [11]. Recent clinical data demonstrated that the presence of donor-specific anti-HLA antibodies (DSA) in the recipient is significantly associated with GF in unrelated Allo-SCT and related haploidentical stem cell transplantation (Haplo-SCT) [11], as well as in CBT. In the setting of

CBT, the impact of DSA on engraftment appears to be strong because the majority of the patients receive HLA-mismatched units with a relatively lower cell dose compared with that of unrelated allo-SCT or haploidentical-SCT recipients. Takanashi et al. clearly demonstrated that only 32% of patients with DSA achieve engraftment, compared with 83% of patients without DSA, in their retrospective analysis of 386 patients who underwent myeloablative single-unit CBT [12]. The negative impact of DSA on engraftment in CBT was also confirmed in the setting of reduced-intensity conditioning (RIC) and/or double CBT [13,14], although another study showed no significant effect of DSA on engraftment, probably because of the different thresholds for the definition of DSA positivity [15]. Based on the growing body of evidence, there has been a consensus that we should avoid selecting cord blood (CB) units when the anti-HLA antibodies are directed against the mismatched HLA of the CB unit. However, all studies performed in the CBT field have specifically focused on anti-HLA-A, -B, and -DRB1 antibodies in the recipient. So far, the impact of anti-HLA antibodies against HLA-C, -DP -DQ, or DRB3/4/5 has not entirely been evaluated. Further, clinical significance of anti-HLA antibodies not corresponding to HLA antigens expressed on CB remains to be determined. In this study, we retrospectively evaluated the impact of a presence of these anti-HLA antibodies on outcomes in CBT, with a special focus on its association with engraftment.

PATIENTS AND METHODS

Patients and Cord Blood Transplantation

This study included 175 consecutive adult patients who underwent CBT as their first Allo-SCT at Toranomon Hospital from March 2008 through July 2011. All patients received a single CB unit after either myeloablative or RIC regimens. Conditioning regimens were classified based on the report by the Center for International Blood and Marrow Transplant Research [16]. Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus (TAC) plus mycophenolate mofetil (MMF) or TAC alone. Granulocyte colony-stimulating factor was administered intravenously from around day 3 until neutrophil recovery. For disease status, those with hematologic malignancies in first or second complete remission at the time of transplantation, those in the chronic phase or accelerated phase of chronic myeloid leukemia, and those with refractory anemia of myelodysplastic syndrome were defined as being at standard risk, whereas those in other situations were defined as being at high risk. Details of supportive care during transplantation was performed as previously reported [17,18]. All patients gave written informed consent, and this study was approved by the institutional review board.

Cord Blood Units and Anti-HLA Antibodies

CB units were obtained from the Japanese Cord Blood Bank Network. In the Japanese Cord Blood Bank Network, CB units were serologically typed only at HLA-A, -B, and -DRB1 locus before selection. The CB unit was selected from those with no more than 2 antigen-mismatches to recipients and principally contained 2 × 10^7 and more total nucleated cells (TNC) counts per kilogram of recipient body weight. Since March 2008, we prospectively screened anti-HLA antibodies in recipients before CBT to select proper units of CB without corresponding HLA-A, -B, and -DRB1 antigens to DSA. In this study, CB unit with DSAs against HLA-A, -B or -DRB1 were not selected for transplantation. Anti-HLA antibodies were tested using LAB Screen PRA and Single Antigen (One Lambda, Canoga Park, CA) for class I (HLA-A/-B/-C) and class II (HLA-DR/-DP/-DQ) anti-HLA antibodies [19,20]. Median fluorescence intensity (MFI) of ≥ 1000 was defined to be positive. In this analysis, patients positive for anti-HLA antibodies were divided into 2 groups: group A included patients with antibodies only against HLA-A, -B, or -DRB1 without those against HLA-C, -DP, -DQ, or -DRB3/4/5, whereas group B included patients having antibodies against HLA-C, -DP, -DQ, or DRB3/4/5 with or without those against HLA-A, -B, or -DRB1. Because CB units had not been typed at HLA-C, -DP, -DQ, or -DRB3/4/5, it was possible that patients in group B harbored DSA against them.

Definitions and Statistical Analysis

Neutrophil engraftment was defined as the first of 3 consecutive days with absolute neutrophil count of at least 500 cells/mm³ by 60 days after transplantation. Platelet engraftment was defined as the first day of a platelet count of 20,000/mm³ without transfusion support by 100 days after transplantation. Chimerism was assessed using fluorescence in situ hybridization in sex-mismatched donor-recipient pairs, and in sex-matched pairs, PCR for

a variable number of tandem repeats was used with donor cells detected at a sensitivity of 10%. Whole blood, CD3⁺ cells, or bone marrow cells were assessed at the time of neutrophil engraftment and repeated as indicated, according to the patients' condition. Acute and chronic GVHD were diagnosed and graded according to the standard criteria [21,22]. A pretransplantation hematopoietic cell transplantation-specific comorbidity index score was calculated retrospectively for each patient using previously reported scoring system [23]. For statistical analysis, categorical variables were compared by the chi-square test or Fisher exact test, whereas continuous variables were compared by Wilcoxon rank-sum test. The probability of overall survival (OS) were estimated using the Kaplan-Meier method and the groups were compared using the log-rank test. The probabilities of neutrophil and platelet engraftment, relapse, and NRM were estimated based on cumulative incidence curves [24]. Competing events were death or relapse without engraftment for neutrophil and platelet engraftment, death without relapse for relapse, and relapse for NRM. The groups were compared using Gray's test. The Cox proportional hazard model and the Fine-Gray proportional hazards model were used to determine the significance of multiple variables in determining these outcomes.

RESULTS

Patient Characteristics

The patients' characteristics are summarized in Table 1. Their median age was 59 (range, 17 to 74). One hundred and seven (61.1%) were male and 156 (89.1%) had high-risk disease status. The majority of the patients had extensive prior history of transfusion, both in red blood cell and platelet concentrate. One hundred thirty-five patients (77.1%) were conditioned with myeloablative regimens, whereas 40 patients received RIC regimens. One hundred forty-four (82.2%) received TAC plus MMF for GVHD prophylaxis. The median TNC and CD34⁺ cells infused were 2.55 (range, 1.67 to 5.65) × 10⁷/kg and .91 (.27 to 2.97) × 10⁵/kg, respectively. HLA mismatch at HLA-A, -B, and -DRB1 in host-versus-graft (HVG) direction was 0/6 (n = 4), 1/6 (n = 48), and 2/6 (n = 123).

Anti-HLA Antibodies

Sixty-nine of the 175 (39.4%) patients were positive for anti-HLA antibodies. The median number of anti-HLA specificities was 2 (range, 1 to 73), and the median value of maximum MFI was 2150 (range, 1004 to 16,402). Among the antibody-positive group, 39 patients had antibodies only against HLA-A or -B, or -DRB1 (categorized as group A), 13 had antibodies only against HLA-C or -DP, or -DQ, or -DRB3/4/5, and 17 had antibodies both against HLA-C, -DP, -DQ, or -DRB3/4/5 and against HLA-A, -B, or -DRB1 (the latter 2 were categorized as group B). Among the 30 patients who were categorized as group B, 17 had antibodies against HLA-C, 5 against HLA-DP, 12 against HLA-DQ, and 6 against HLA-DRB3/4/5, including overlapping cases. Characteristics of patients with or without anti-HLA antibodies are summarized in Table 1. The antibody-positive group included older patients than the negative group (P = .01). Patients in the positive group received a higher TNC dose than the antibody-negative group (P = .04). The degree of HLA-mismatch in the HVG direction in the antibody-positive group was suggestively lower than those in antibody-negative group, although it was not statistically significant (P = .06). More intensive GVHD prophylaxis using TAC plus MMF and RIC regimes were used in the antibody-positive group, although it was not statistically significant (P = .06 in both).

Effect of Anti-HLA Antibodies on Hematopoietic Recovery

Among the 175 patients, 143 achieved neutrophil engraftment. In the 32 who did not achieve engraftment, 8 had graft failure and proceeded to second transplantation, 5 had early disease progression, and 19 had NRM. The cumulative incidences of neutrophil and platelet engraftment

Table 1

Characteristics of All Patients and Those with or without Anti-HLA Antibodies

Characteristic	All	Ab Positive	Ab Negative	P Value
No. of patients	n = 175	n = 69	n = 106	
Age, median (range), yr	59 (17-74)	60 (21-73)	57.5 (17-74)	.01
Gender				
Male	107 (61.1%)	39 (56.5%)	68 (64.1%)	.34
Female	68 (38.8%)	30 (43.4%)	38 (35.8%)	
Diagnosis				
AML	51 (29.1%)	18 (26%)	33 (31.1%)	.19
MDS/MPN overt AML	62 (35.4%)	32 (46.3%)	30 (28.3%)	
MDS	10 (5.7%)	3 (4.3%)	7 (6.6%)	
CML	7 (4%)	1 (1.4%)	6 (5.6%)	
ALL	11 (6.2%)	5 (7.2%)	6 (5.6%)	
ML	20 (11.4%)	4 (5.7%)	16 (15%)	
ATLL	8 (4.5%)	4 (5.7%)	4 (3.7%)	
SAA	5 (2.8%)	2 (2.8%)	3 (2.8%)	
MM	1 (.5%)		1 (.9%)	-
Disease status*				
Standard risk	19 (10.8%)	8 (11.5%)	11 (10.3%)	.80
High risk	156 (89.1%)	61 (88.4%)	95 (89.6%)	
Prior history of RBC transfusion				
≥20 times	149 (85.1%)	55 (79.7%)	94 (88.6%)	.11
< 20 times	16 (9.1%)	7 (10.1%)	9 (8.4%)	
Unknown	10 (5.7%)	7 (10.1%)	3 (2.8%)	
Prior history of PC transfusion				
≥ 20 times	155 (88.5%)	58 (84%)	97 (91.5%)	.23
< 20 times	14 (8%)	7 (10.1%)	7 (6.6%)	
Unknown	6 (3.4%)	4 (5.7%)	2 (1.8%)	
HCT-CI score				
0	33 (18.8%)	11 (15.9%)	22 (20.7%)	.41
1	25 (14.2%)	10 (14.1%)	15 (14.1%)	
2	38 (21.7%)	20 (28.9%)	18 (16.9%)	
3	37 (21.1%)	12 (17.3%)	25 (23.5%)	
≥ 4	42 (24%)	16 (23.1%)	26 (24.5%)	
Conditioning regimen				
RIC	40 (22.8%)	21 (30.4%)	19 (17.9%)	.06
Myeloablative	135 (77.1%)	48 (69.5%)	87 (82.0%)	
GVHD prophylaxis				
TAC	28 (16%)	6 (8.6%)	22 (20.7%)	.06
TAC + MMF	144 (82.2%)	62 (89.8%)	82 (77.3%)	
TAC + sMTX	3 (1.7%)	1 (1.4%)	2 (1.8%)	
Number of TNC infused				
Median (range), × 10^7/kg	2.55 (1.67-5.65)	2.70 (1.83-5.09)	2.49 (1.67-5.65)	.04
Number of CD34$^+$ cells infused				
Median (range), × 10^5/kg	.91 (.27-2.97)	.91 (.32-2.97)	.91 (.27-2.35)	.41
HLA antigen mismatch				
HVG direction				
0	4 (2.2%)	2 (2.8%)	2 (1.8%)	.06
1	48 (27.4%)	25 (36.2%)	23 (21.6%)	
2	123 (70.2%)	42 (60.8%)	81 (76.4%)	

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; ATLL, adult T cell leukemia/lymphoma; SAA, severe aplastic anemia; MM, multiple myeloma; PC, platelet concentrate; HCT-CI, Hematopoietic Cell Transplantation–Specific Comorbidity Index; RIC, reduced-intensity conditioning; TAC, tacrolimus; MMF, mycophenolate mofetil; sMTX, short-term methotrexate; TNC, total nucleated cells; HVG, host-versus-graft.

Data presented are n (%), unless otherwise indicated.

* Acute leukemia in first or second complete remission, CML in chronic phase, MDS in refractory anemia, and severe aplastic anemia were defined as standard risk. All the others were considered high risk.

were 81.7% (95% confidence interval [CI], 75.0% to 86.8%) and 53.5% (95% CI, 45.7% to 60.6%), respectively. The median time to neutrophil and platelet engraftment were 19 days (range, 11 to 60) and 43 days (range, 24 to 94) after transplantation, respectively. The presence of anti-HLA antibodies itself had no significant effect on neutrophil and platelet engraftment (positive versus negative: 79.7% versus 83.0%. P = .44, in neutrophil [Figure 1A]; 50.7% versus 55.2%, P = .53, in platelet). The number (≥5 versus < 5) and intensity (MFI ≥ 2000 versus < 2000) of anti-HLA antibodies also had no significant effect on engraftment. Patients with a higher degree of HLA-antigen mismatch in the HVG direction (2 versus 0 to 1 antigen mismatch) showed an inferior neutrophil engraftment rate (79.7% versus 86.5%, P = .02),

whereas TNC (≥2.5 × 10^7/kg versus < 2.5 × 10^7/kg) did not affect engraftment in our CBT setting (78.3% versus 85.5%, P = .62). In multivariate analysis, a higher degree of HLA mismatch in the HVG direction was the only negative factor for neutrophil engraftment (hazard ratio [HR], .82; 95% CI, .68 to .9; P = .03), and presence of anti-HLA antibodies did not show a statistical significance. Regarding the impact of anti-HLA antibodies other than against HLA-A, -B, -DRB1 on neutrophil engraftment, group B tended to show lower engraftment rates (66.7%, n = 30) compared with group A (89.7%, n = 39) or the negative group (83%, n = 106), although the difference did not reach statistical significance (Figure 1B) (P = .12). In fact, among the 30 patients who were categorized as group B, only 20 patients (66%) achieved
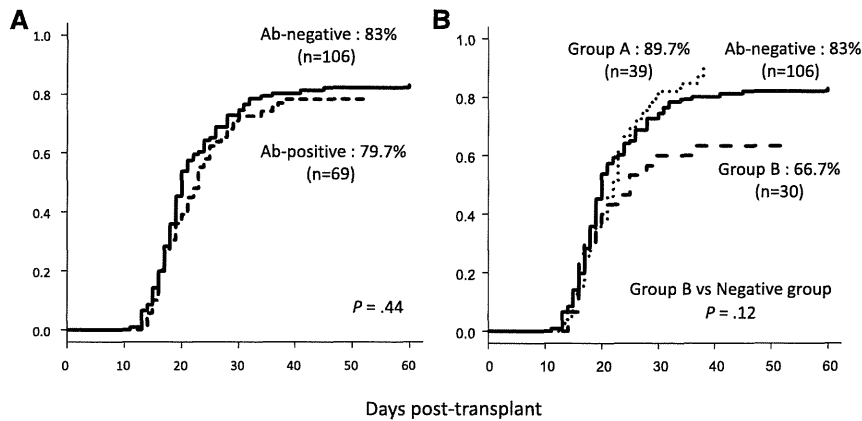
Figure 1. Cumulative incidence of neutrophil engraftment in all the studied patients (n = 175). (A) The incidence was 79.7% (95% confidence interval [CI], 67.2% to 87.9%) for the antibodies-positive group (n = 69) and 83.0% (95% CI, 73.8% to 89.2%) for the antibodies-negative group (n = 106), respectively (P = .44). Positive group represented by dashed line and the negative group represented by solid line. (B) The incidence according to the type of anti-HLA antibodies. Group B tended to show lower engraftment rates (66.7% [95% CI, 43.8% to 81.9%], n = 30) compared with group A (89.7% [95% CI, 70.6% to 96.7%], n = 39) or the antibodies-negative group (83.0% [95% CI, 73.8% to 89.2%], n = 106). (P = .12; group B versus negative group). Group A is represented by dotted line, group B by dashed line, and negative group by solid line.

engraftment. Among 13 patients who had antibodies only against HLA-C, -DP, -DQ, or DRB3/4/5, only 8 (61.5%) patients achieved engraftment.

Among 8 patients who had engraftment failure, 5 had anti-HLA antibodies (4 in group B and 1 in group A). In the chimerism analysis, 2 of the 3 patients without antibodies showed transient donor-dominant chimerism, whereas recipient cell dominance was observed consistently in all 5 patients with anti-HLA antibodies.

In the subgroup analysis in patients who received 2 antigen-mismatched CB in the HVG direction (n = 123), overall, the HLA-antibodies—positive group tended to show lower engraftment rates compared with the negative group (Figure 2A) (positive group [n = 81]: 71.4%, negative group [n = 42]: 84%, P = .07). In this analysis, group A showed comparable engraftment rates to the negative group, whereas group B showed extremely low engraftment rates with statistical significance (Figure 2B) (group A [n = 24]: 87.5%, negative group [n = 81]: 84%, group B [n = 18]: 50%. Group B versus negative group, P = .01). Multivariate analysis in this subgroup showed that group B and patients older than

55 years were identified as the negative factor for neutrophil engraftment (HR, 2.79; 95% CI, 1.39 to 5.62; P ≤ .01 in group B; HR, .66; 95% CI, .44 to .97; P = .03 in elderly patients).

Effect of Anti-HLA Antibodies on Survival

At a median follow up of 18 (range, 5.1 to 46) months, the cumulative incidence of NRM at 2 years was 34.0% (95% CI, 26.8% to 41.2%). The cumulative incidence of NRM at 2 years was almost comparable between the anti-HLA antibody-positive and -negative groups (Figure 3A) (positive group [n = 69]: 38.3%, negative group [n = 106]: 31.2%; P = .28), whereas group B tended to show a higher incidence of NRM compared with the negative group (Figure 3B) (group B [n = 30]: 47.9%; P = .07). NRM in group A was almost identical to that in the antibodies-negative group (Figure 3B) (group A [n = 39]: 31.5%). Early NRM within 28 days after transplantation was significantly higher in group B compared with other groups (20% in group B, 7.7% in group A, 6.6% in negative group; group B versus negative, P = .03). Two-year OS for the entire study population was 45.1% (95% CI, 37.1% to 52.7%). The OS at 2 years was almost comparable between
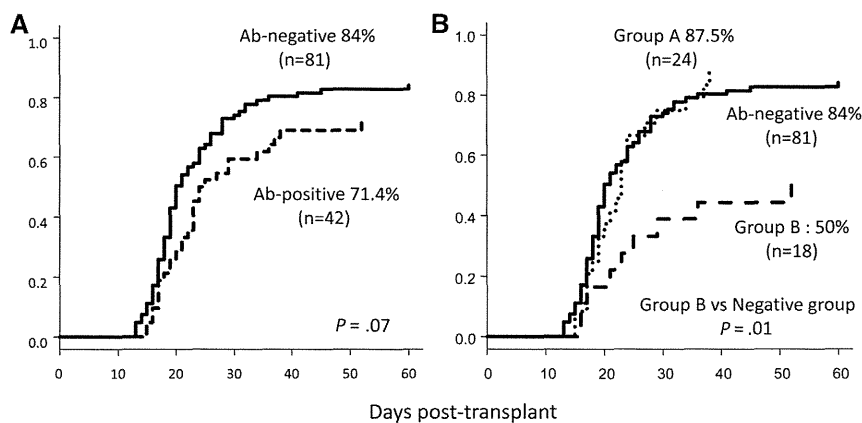

Figure 2. Cumulative incidence of neutrophil engraftment in patients who received 2 antigen-mismatched CB (n = 123). (A) The incidence was 71.4% (95% CI, 53.5% to 83.5%) for the antibodies-positive group (n = 42) and 84.0% (95% CI, 73.1% to 90.7%) for the antibodies-negative group (n = 81), respectively (P = .07). Positive group represented by dashed line and the negative group represented by solid line. (B) The incidence according to the type of anti-HLA antibodies. Group B showed lower engraftment rates (50% [95% CI, 21.6% to 73.1%], n = 18) compared with group A (87.5% [95% CI, 57.3% to 96.9%], n = 24) or the antibodies-negative group (84.0% [95% CI, 73.1% to 90.7%], n = 81) with statistical significance (P = .01; group B versus negative group). Group A is represented by dotted line, group B by dashed line, and negative group by solid line.
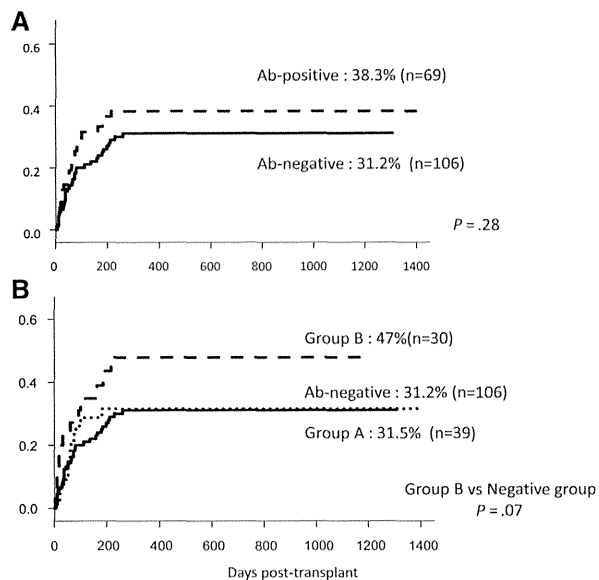
Figure 3. Cumulative incidence of nonrelapse mortality (NRM) (n = 175). (A) NRM at 2 years was 38.3% (95% CI, 26.4% to 50.0%) for the antibodies-positive group (n = 69) and 31.2% (95% CI, 22.4% to 40.3%) for the antibodies-negative group (n = 106), respectively (P = .28). Positive group represented by dashed line and the negative group represented by solid line. (B) NRM according to the type of anti-HLA antibodies. Group B showed a significantly higher incidence of NRM (47.9% [95% CI, 27.4% to 65.9%], n = 30) compared with group A (31.5% [95% CI, 17.5% to 46.5%], n = 39) or the antibodies-negative group (31.2% [95% CI, 22.4% to 40.3%], n = 106). (P = .07; group B versus negative group). Group A is represented by dotted line, group B by dashed line, and negative group by solid line.

the antibodies-positive and -negative groups (Figure 4A) (positive group [n = 69]: 42.9%, negative group [n = 106]: 46.2%; P = .47), although group B had a tendency of decreased OS rate compared with the negative group (Figure 4B) (group B [n = 30]: 35.1%; P = .26).

DISCUSSION

The aim of this study was to evaluate the effect of anti-HLA antibodies, except for donor-specific anti-HLA-A, -B, -DRB1 antibodies, on engraftment after single CBT. In this study, we demonstrated that the presence of non-DSA had no significant influence on engraftment, NRM, and OS after

CBT. In particular, among patients with antibodies only against HLA-A, -B, or -DRB1 (group A), the results were comparable with those without antibodies. These observations strongly support that necessity to screen for DSA before the selection of CB units, and indicated that CBT is an available option.

On the other hand, patients with antibodies including HLA-C, -DP, -DQ, or -DRB3/4/5 (group B) showed a lower incidence of engraftment compared with those only having donor nonspecific anti-HLA-A, -B, -DRB1 antibodies, "true non-DSA," or those without antibodies, especially in HLA-mismatched CBT. The findings suggest that unrecognized DSA against for HLA-C, -DP, -DQ, or -DRB3/4/5 might adversely affect engraftment after CBT. All previous studies of anti-HLA antibodies performed in the CBT field have specifically looked at anti-HLA-A, -B, and -DRB1 antibodies in the recipient [12-15], and the clinical significance of anti-HLA antibodies against HLA-C, -DP, -DQ, or -DRB3/4/5 has not been investigated so far. In the largest study of anti-HLA antibodies after CBT reported by Takanashi et al., patients who had anti-HLA antibodies, except for donor-specific anti-HLA-A, -B, -DRB1 antibodies, showed significantly lower neutrophil engraftment compared with those without anti-HLA antibodies (73% versus 83%) [12]. One possible reason for the lower engraftment rate in that study might be the existence of unrecognized DSA against HLA-C, -DP, -DQ, or -DRB3/4/5, as suggested in the present study. In unrelated Allo-SCT, anti-DPB1 DSA has been recognized to be associated with increased risk for engraftment failure [25,26]. In the setting of HLA-mismatched CBT, additional mismatch in the HLA-C, -DP, -DQ, or -DRB3/4/5 antigens could likely be present because of linkage disequilibrium, especially between the HLA-B and -C locus, or the -DRB1 and -DQ locus, respectively [27,28]. In this study, there were 12 patients who had antibodies against HLA-DQ, and 7 were matched and 5 were mismatched for DRB1. Neutrophil recovery was observed in all 7 HLA-DRB1—matched and 3 of 5 in the mismatched group. The results further strengthen the association of DSA against HLA-DQ with engraftment failure. Moreover, HLA-DP and -DQ antigens are also known to be expressed on hematopoietic precursor cells [26,29-31]. Our results, combined with previous findings, strongly suggest the possibility of graft rejection associated with unrecognized DSA against HLA-C, -DP, -DQ, -DRB3/4/5 antigens of an
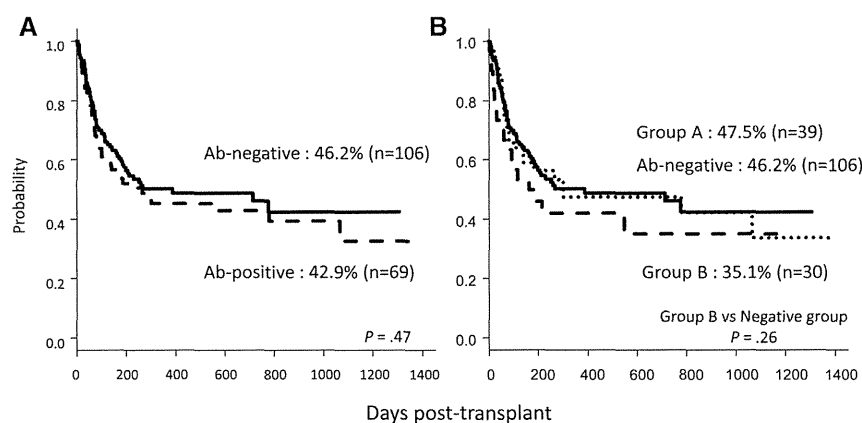

Figure 4. Probability of overall survival (OS) (n = 175). (A) OS at 2 years was 42.9% (95% CI, 30.5% to 54.7%) for the antibodies-positive group (n = 69) and 46.2% (95% CI, 35.5% to 56.3%) for the antibodies-negative group (n = 106), respectively (P = .47). Positive group represented by dashed line and the negative group represented by solid line. (B) OS according to the type of anti-HLA antibodies. Group B had tendency to decreased OS rate (35.1% [95% CI, 16.8% to 54.1%], n = 30) compared with group A (47.5% [95% CI, 31.0% to 62.3%], n = 39) or the antibodies-negative group (46.2% [95% CI, 35.5% to 56.3%], n = 106). (P = .26; group B versus negative group). Group A is represented by dotted line, group B by dashed line, and negative group by solid line.

infused CB unit. Multivariate analysis showed that a higher degree of HLA-mismatch in HVG direction had a significantly negative effect on engraftment. This result suggests that the HLA-specific cellular immunity plays a critical role in engraftment process, as previously reported [32,33]. However, in multivariate analysis for patients who received 2 antigen-mismatched CB, the presence of anti-HLA-C, -DP, -DQ, -DRB3/4/5 antibodies was the negative factor for neutrophil engraftment. Thus, in the setting of transplantation using HLA-mismatched grafts, humoral immunity could adversely affect engraftment.

Because this is a retrospective analysis, there is no information available on HLA-DP, -DQ, or -DRB3/4/5 antigens of CB units, negating direct assessment whether these antigens were DSAs. We have done HLA-C typing retrospectively in 16 patients who had anti–HLA-C antibodies. There were 2 patients who had DSA against HLA-C; 1 engrafted and the other died early before engraftment. Among the remaining 14 who did not have DSA against HLA-C, 2 developed engraftment failure, 3 died early before engraftment, and 9 engrafted, and with such a small sample size, we were not able to assess the impact of DSA against HLA-C on engraftment. It remains to be determined whether more mismatches in HLA-C, -DP, -DQ, or -DRB3/4/5 antigens, or the combined effect of mismatches and DSA, have a negative effect on engraftment. Furthermore, patients in group B showed a higher incidence of NRM before engraftment, which could be associated with the low engraftment rate. The high rate of early NRM observed in group B became clear in the subgroup analysis for patients who received 2 antigen-mismatched CB (Supplementary Table S1). In the subgroup analysis for patients who survived for 28 days or longer after transplantation and who received 2 antigen-mismatched CB (n = 110), group B (n = 12) still tended to show a lower engraftment rate compared with other groups (75% in group B, 95.5% in group A, and 89.5% in negative group; group B versus negative; P = .30). Lower engraftment or delayed neutrophil recovery observed in group B could have affected this higher NRM. The presence of other factors that have an impact on engraftment or early morality in group B cannot be excluded, although background characteristics among group B were almost comparable to the others (Supplementary Table S1). In this study, the frequency of patients with anti-HLA antibodies is higher compared with other previous series [11,12]. Our patient characteristics, including elderly or heavily transfused patients, might have possibly affected the result. The existence of a "natural antibody" is also one of the factors to be considered [34,35]. The recent use of more sensitive methods has resulted in the detection of low-level antibodies not contributable to allogeneic antigens exposure. When we re-evaluated our results based on cross-reactive group [36,37], some antibodies seem to be classified as natural antibodies; however, we could not show their relevance to the clinical outcomes.

In conclusion, our results demonstrate that the presence of donor nonspecific anti-HLA-A, -B, -DRB1 antibodies had no significant influence on engraftment, NRM, and OS in HLA-mismatched CBT. On the other hand, a high rate of engraftment failure was seen in the presence of anti-HLA-C, -DP, -DQ, or -DRB3/4/5 antibodies, suggesting unrecognized DSA against HLA-C, -DP, -DQ, or -DRB3/4/5 antigens adversely affect engraftment. Because this is a retrospective study including a small and heterogeneous group of patients with various characteristics, the results should be confirmed by larger scale studies involving information on HLA-C, -DP, -DQ, or -DRB3/4/5, which will establish the optimal strategies for selecting CB units.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.06.024.

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LETTER TO THE EDITOR

I.v. BU/fludarabine plus melphalan or TBI in unrelated cord blood transplantation for high-risk hematological diseases

Although a myeloablative dose of i.v. BU/fludarabine (i.v. BU/Flu) has been widely accepted as a reduced toxicity myeloablative regimen for Allo-SCT from a related or unrelated donor,[1] little information is available on the feasibility of the i.v. BU/Flu regimen in cord blood transplantation (CBT). Studies from Duke University, in which i.v. BU/Flu was used as the conditioning regimen followed by double CBT, demonstrated that only 2 of 10 patients engrafted with donor hematopoietic cells.[2] To evaluate the safety and efficacy of the i.v. BU/Flu-conditioning regimen in CBT, we retrospectively reviewed 62 consecutive adult CBT recipients with i.v. BU/Flu-based conditioning regimens at our institute from February 2007 to March 2010.

The patients' characteristics are summarized in Table 1. Their median age was 59 years (range, 21–72), with a median hematopoietic cell transplant-specific comorbidity index score of 2 (0–5). All were considered not to be proper candidates for conventional myeloablative therapy. Fifty-seven (92%) patients were not in remission and 16 (36%) had a prior history of Allo-SCT. All were transplanted with a single cord blood unit, and all received fludarabine (Flu; 25–30 mg/m^2 once daily for 5–6 days, 125–180 mg/ m^2 per total) and i.v. BU (0.8 mg/kg four times a day for 2–4 days, 6.4–12.8 mg/kg per total). Melphalan (Mel; 40–70 mg/m^2 once daily for 2 days, 80–140 mg/m^2 per total) or TBI (2–8 Gy per total) were used in combination with i.v. BU/Flu regimens in all the patients. Pretransplant conditioning was determined by each attending physician according to the patient's condition, disease status and history of prior therapy. Engraftment, regimen-related toxicities (RRT), chimerism, GVHD, nonrelapse mortality (NRM), OS and disease-free survival (DFS) were assessed as previously reported.[3,4] All patients gave written informed consent for transplant, and this study was approved by the institutional review board.

Forty-eight patients achieved neutrophil recovery on a median of day 21 (range, 12–52) post transplant. Among 14 who failed to achieve neutrophil recovery, 5 experienced early disease progression, 6 showed graft rejection, and 3 died before engraftment. The cumulative incidences of neutrophil and platelet recoveries were 77.4% and 56.5%, respectively. All patients who achieved engraftment showed complete donor chimerism within 28 days post transplant. In multivariate analysis, more mismatches in HLA (2/6 vs 1/6) was the only significant factor negatively affecting neutrophil engraftment rate (Hazard ratio (HR) = 0.65, 95% confidence interval (CI), 0.44–0.96, P = 0.03). RRT and GVHD were summarized in Table 1. At the time of analysis, 27 of 62 patients survived for a median of 33.5 (range, 21–55) months after transplant. Seventeen patients died of nonrelapse causes. Six of them were from infections, four from GVHD, two from engraftment failure and five from idiopathic pneumonia syndrome. The cumulative incidences of NRM at day 100 and 2 years were 23.4% (13.5–34.8%) and 28.6% (17.6–40.4%), respectively. Twenty-five patients relapsed at a median of 7.5 months (range; 1–21) after transplant. The cumulative incidences of relapse at day 100 and 2 years were 15.1% (7.4–25.4%) and 40.9% (28.1–53.3%), respectively. Two-year actual OS and DFS were 46.8% (34.0–58.5%) and 32.1% (20.9–43.8%), respectively.

Patients were divided into two groups: those received a myeloablative doses of i.v. BU (12.8 mg/kg, FB 4 group), and those received lower doses of i.v. BU (6.4–9.6 mg/kg, FB 2–3 group). No statistically significant differences were observed in patients' characteristics between the FB 4 and FB 2–3 groups (Table 1). The incidences of neutrophil engraftment, RRT and GVHD did not differ between the FB 4 and FB 2–3 groups, as summarized in Table 1. Remarkably, the incidences of NRM did not differ between the two groups (Figure 1a). In contrast, relapse rate at 2 years for the patients in the FB 4 group was 31.0% (15.9–47.5%), whereas 53.8% (32.3–71.3%) in the FB 2–3 group (P = 0.03, Figure 1b). Interestingly, patients who received 8 Gy of TBI (n = 8) showed a significantly higher incidence of relapse compared with those who had 0–4Gy of TBI (75 vs 35.5% at 2 years, P = < 0.01), which could be a reflection of the fact that the TBI 8 Gy group included patients who received a lower dose of i.v. BU. Other pretransplant factors including age (⩾55 vs < 55 years), disease status (high vs standard), HCT–CI (⩾2 vs < 2 score), HLA disparities (2/6 vs 1/6), number of TNC infused (⩾2.5x10*7/kg vs < 2.5x10*7/ kg), GVHD prophylaxis (TAC alone vs TAC+MMF) and dose of Mel (80 vs 120–140 mg/m^2) did not correlate with incidence of NRM and relapse (data not shown). Prior Allo-SCT was the only significant factor associated with a higher NRM in univariate analysis (P < 0.01) and in multivariate analysis (HR = 6.66, 95% CI 2.29–19.3, P < 0.01). FB 4 group was the only significant factor associated with a lower relapse rate even in multivariate analysis (HR = 0.88, 95% CI 0.80–0.97, P = 0.01). Two-year OS of FB 4 and FB 2–3 were 58.3% (40.7–72.4%) and 30.8% (14.6–48.5%), respectively, and 2-year DFS of FB 4 and FB 2–3 were 41.3% (25.2–56.7%) and 19.2% (7.0–36.0%), respectively (Figure 1c and d). The differences reached statistical significance for both OS (P = 0.04) and DFS (P = 0.04) in univariate analysis, but not in multivariate analysis. Prior Allo-SCT was the only significant factor for poor OS rate in multivariate analysis (HR = 2.72, 95% CI 1.09–6.79, P = 0.03).

This study clearly demonstrated feasibility of i.v. BU/Flu+Mel or TBI regimen in CBT. In the setting of CBT, a lower engraftment rate using BU/Flu-based regimen was reported,[2,5,6] likely owing to insufficient immunoablation associated with BU.[7] As cord blood contains functionally immature lymphocytes and the total number of T cells are small,[8] more intensive immune suppression before transplant would be crucial to ensure engraftment compared with BM or PB SCT.[9] Our data here demonstrated that addition of Mel or TBI to BU/Flu regimen, expected to increase lymphoablative effect, is sufficient for engraftment following CBT. Secondly, our data confirmed that no additional toxicities were observed by increasing dose of i.v. BU from 6.4–9.6 to 12.8 mg/kg, even for patients having high-risk disease with old age and/or comorbidities. Some investigators confirmed its safety even in patients with higher age (up to 70 years old) and/or comorbidities.[10–12] Finally, FB 4 regimens could also have an impact on reducing relapse rate. Despite the fact that higher doses of TBI or Mel were added to patients in the FB 2–3 group than to those in the FB 4 group, the relapse rate was lower in the FB 4 group, which could possibly indicate that a myeloablative dose of i.v. BU is more potent in disease control. Previous attempts to decrease the intensity of conditioning regimens have not resulted in improved survival,

because any reduction in NRM was offset by higher relapse.[13] Myeloablative doses of i.v. BU could be an ideal in this regard.

In conclusion, our results clearly demonstrated that an i.v. BU/Flu +Mel or TBI regimen is feasible in CBT. Myeloablative dose of i.v. BU does not increase NRM even in patients having high-risk disease with old age and/or comorbidities, and could possibly be

associated with decrease of relapse. As this is a retrospective study including small and heterogeneous groups of patients with various characteristics, the results should be re-confirmed by larger-scale prospective studies, which are now going on throughout Japan (Japan Study Group for Cell Therapy and Transplantation; FB09/10 studies, UMIN000002426, UMIN000004211/000004213)).

Table 1. Patients' characteristics, regimen-related toxicity and GVHD

	All (n = 62)	FB 4 (n = 36)	FB 2-3 (n = 26)	P-value
Age (years)				
Median(range)	59 (21-72)	59 (21-72)	62 (21-71)	0.52
Gender				
Male/female	39/23	20/16	19/7	0.19
Diagnosis				
AML/MDS	52 (84%)	32 (89%)	20 (80%)	0.35
CML	3 (5%)	2 (6%)	1 (4%)	
ALL	6 (10%)	2 (6%)	4 (15%)	
ATLL	1 (2%)	–	1 (4%)	
Disease status[a]				
Standard risk	5 (8%)	1 (3%)	4 (15%)	0.15
High risk	57 (92%)	35 (97%)	22 (85%)	
Prior Allo-SCT				
Yes	16 (26%)	8 (22%)	8 (31%)	0.55
ECOG PS				
0	4 (6%)	3 (8%)	1 (4%)	0.61
1	39 (63%)	21 (58%)	18 (69%)	
2	19 (31%)	12 (33%)	7 (27%)	
HCT-CI score				
0	15 (24%)	8 (22%)	7 (27%)	0.90
1	10 (16%)	5 (14%)	5 (19%)	
2	19 (31%)	11 (31%)	8 (31%)	
⩾3	18 (29%)	12 (33%)	6 (23%)	
Additional agents to i.v. BU/Flu				
TBI (2-4 Gy)	30 (48%)	25 (69%)	5 (19%)	< 0.001
TBI (8 Gy)	8 (13%)	1 (3%)	7 (27%)	
Mel 80 mg/m^2	20 (32%)	10 (28%)	10 (38%)	
Mel 120-140 mg/m^2	4 (6%)	–	4 (15%)	
GVHD prophylaxis				
TAC	13 (21%)	7 (19%)	6 (23%)	0.76
TAC+MMF	49 (79%)	29 (81%)	20 (77%)	
Number of TNC infused				
Median × 10^7/kg (range)	2.57 (1.58-4.31)	2.57 (1.58-4.21)	2.60 (1.81-4.31)	0.98
Number of CD34$^+$ cells infused				
Median ×10^5/kg (range)	0.98 (0.45-2.25)	0.99 (0.45-2.25)	0.99 (0.47-1.98)	0.98
HLA-antigen mismatched				
1/6	9 (15%)	5 (14%)	4 (15%)	0.33
2/6	53 (84%)	31 (86%)	22 (85%)	
Regimen-related toxicity (grade 3 or greater)				
Total	57 (92%)	33 (92%)	24 (92%)	1
Infections	57 (92%)	33 (92%)	24 (92%)	1
Stomatitis	24 (39%)	16 (44%)	8 (31%)	0.30
Diarrhea	18 (29%)	11 (31%)	7 (27%)	0.76
Liver	2 (3%)	1 (3%)	1 (4%)	1
Renal/genitourinary	2 (3%)	2 (6%)	–	0.50
Cardiac arrhythmia	1 (2%)	1 (3%)	–	1
Pulmonary	9 (16%)	4 (11%)	5 (19%)	0.47
Neurological	3 (5%)	2 (6%)	1 (4%)	1
Cumulative incidence of acute GVHD (n = 45)				
Grade II-IV (95% CI)	50% (36.9-61.8)	52.8% (35.0-67.8)	46.2% (26.1-64.1)	0.46
Grade III-IV (95% CI)	16.1% (8.2-26.4)	16.7% (6.6-30.6)	15.4% (4.7-31.8)	0.88
Cumulative incidence of chronic GVHD at 2 years (n = 36)				
(95% CI)	29.2% (17.4-42.1)	32.0% (15.6-49.8)	26.1% (10.2-45.3)	0.71

Abbreviations: ATLL = adult T-cell leukemia/lymphoma; ECOG PS = Eastern Cooperative Oncology Group performance status; Flu = fludarabine; HCT-CI = Hematopoietic Cell Transplantation-Specific Comorbidity Index; MDS = myelodysplastic syndrome; Mel = melphalan; MMF = mycophenolate mofetil; Tac = tacrolimus; TNC = total nucleated cells. [a]Acute leukemia in first or second CR, CML in chronic phase, MDS in refractory anemia and ATLL in CR were defined as standard risk. All the others were considered as high-risk.
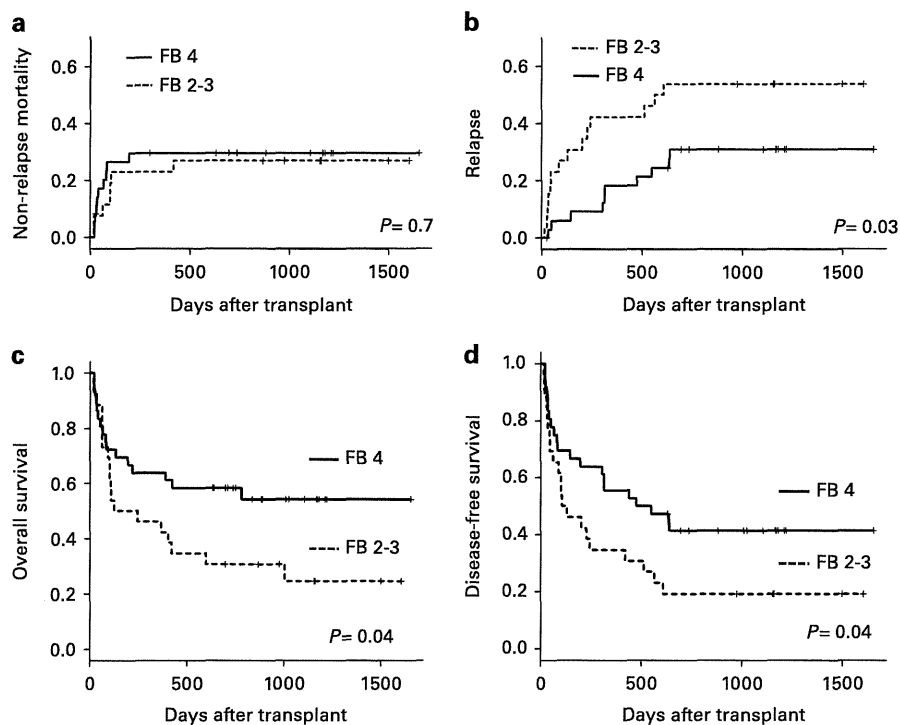
Figure 1. (a and b) Cumulative incidence of nonrelapse mortality (NRM) and relapse. (a) NRM according to the dose of i.v. BU (FB 4 ($n = 36$) vs FB 2-3 ($n = 26$)). (b) Relapse according to the dose of i.v. BU (FB 4 ($n = 36$) vs FB 2-3 ($n = 26$)). Solid curves represent patients in the FB 4 group, whereas the dashed curves represent patients in the FB 2-3 group. (c and d) Probabilities of OS and disease-free survival (DFS). (c) OS according to the dose of i.v. BU (FB 4 ($n = 36$) vs FB 2-3 ($n = 26$)). (d) DFS according to the dose of i.v. BU (FB 4 ($n = 36$) vs FB 2-3 ($n = 26$)). Solid curves represent patients in the FB 4 group, whereas the dashed curves represent patients in the FB 2-3 group.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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