

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 **Title**

2 Favorable outcomes after single cord blood transplantation for patients with high-risk  
3 hematological diseases; a single institute retrospective analysis

4

5 Fumiya Wada<sup>1</sup>, Junya Kanda<sup>1</sup> †, Mizuki Watanabe<sup>1</sup>, Yasuyuki Arai<sup>1</sup>, Masakatsu  
6 Hishizawa<sup>1</sup>, Tadakazu Kondo<sup>1</sup>, Kouhei Yamashita<sup>1</sup>, and Akifumi Takaori-Kondo<sup>1</sup>

7

8 <sup>1</sup>Department of Hematology and Oncology, Graduate School of Medicine, Kyoto  
9 University, Kyoto, Japan

10

11 †Correspondence: Junya Kanda, M.D.

12 Department of Hematology and Oncology, Graduate School of Medicine, Kyoto  
13 University, 54 Kawaharacho, Shogoin, Sakyo-ku, Kyoto, Japan, 606-8507.

14 Tel: +81-75-751-3152; Fax: +81-75-751-3153; e-mail: jkanda16@kuhp.kyoto-u.ac.jp

15

16 **Short title**

17 Favorable outcomes of cord blood transplantation

18

19 **Conflict of Interest Disclosure**

20 The authors declare no competing financial interests.

21

22

23

24

## 1    **Structured Abstract**

2    **Background:** The donor selection algorithm for cord blood (CB) with regards to  
3    matched related and unrelated donors has not been fully investigated.

4    **Objective:** To assess the potential of CB transplantation (CBT) in patients with  
5    hematological malignancies, especially for high-risk patients, we performed a single-  
6    institute retrospective analysis and compared the clinical outcomes of CBT with those  
7    of HLA-matched sibling and unrelated donor transplantation.

8    **Study Design:** We included 394 patients aged 16 years and older with hematological  
9    diseases who received their first allogeneic hematopoietic cell transplantation between  
10    1990 and 2018 at Kyoto University Hospital. These included 394 recipients of single  
11    unrelated cord blood units (UCB, n=108), HLA-matched sibling donors (MSDs,  
12    n=143), or HLA-matched unrelated donors (MUDs, n=143).

13    **Results:** There was no significant difference in relapse-free survival (RFS) between  
14    UCB, MSD, and MUD recipients ( $P=0.975$ ). However, we found a significant  
15    interaction between transplant year and CBT outcomes ( $P=0.010$ ), with significantly  
16    better outcomes observed in the more recent years. Furthermore, we found that CBT  
17    showed better RFS than matched donor transplantation (hazard ratio [HR], 0.50; 95%  
18    confidence interval [CI], 0.30–0.84). This impact was more prominent in high-risk  
19    patients (HR, 0.35; 95% CI, 0.16–0.77), with lower relapse rates (HR, 0.25; 95% CI,  
20    0.11–0.54) and comparable non-relapse mortality (NRM) compared to matched donor  
21    transplantation. Extensive chronic GVHD was less frequently observed in CBT (HR,  
22    0.58; 95% CI, 0.26–1.28).

23    **Conclusions:** CBT associated with favorable outcomes, particularly in high-risk  
24    patients, with good RFS and low relapse rates without an increase in NRM in the single  
25    institute study. Although the findings should be externally validated, CBT might serve  
26    as a reasonable donor choice, particularly in high-risk patients.

1     **Introduction**

2     Hematopoietic stem cell transplantation (HSCT) is a potentially curative strategy for a  
3     wide variety of hematological diseases. With the expansion of donor sources, unrelated  
4     cord blood (UCB) and haploidentical transplantations, especially those with post-  
5     transplant cyclophosphamide (Haplo-PTCY), have been used as alternatives when  
6     matched sibling donors (MSDs) and matched unrelated donors (MUDs) are  
7     unavailable<sup>1, 2</sup>. In particular, UCB has become an established source for HSCT because  
8     of its widespread availability, rapid accessibility, increased tolerance to human  
9     leukocyte antigen (HLA) mismatches, and decreased incidence of chronic graft-versus-  
10    host disease (GVHD)<sup>3</sup>. Serious concerns after cord blood transplantation (CBT) include  
11    graft failure and early transplant mortality. However, significant progress has been  
12    made in the implementation of CBT, particularly regarding the appropriate infused  
13    dosages of CD34+ cells and the degree of HLA disparity, the avoidance of donor-  
14    specific anti-HLA antibody (DSA) development in recipients, advances in conditioning  
15    regimens and GVHD prophylaxis, the management of severe pre-engraftment immune  
16    reactions (PIR), and center experience<sup>4-6</sup>. Such improvements over the past decade have  
17    decreased the risk of early mortality after CBT, which has led to an improvement in  
18    long-term overall survival (OS)<sup>7, 8</sup>. Several studies have demonstrated that CBT offers  
19    outcomes comparable to transplantation of other stem cell sources<sup>9-14</sup>. However, the rate  
20    of CBT has recently decreased in both Europe and the USA<sup>15</sup>. This is apparently not  
21    due to comparability between CBT and transplantation of other stem cell sources, but  
22    rather due to the increase in medical expenses associated with CBT, and the increasing  
23    use of Haplo-PTCY. CBT has shown better outcomes in patients with minimal residual  
24    diseases, potentially reflecting the potent graft-versus-leukemia (GVL) effects of this

1 procedure<sup>16</sup>. In the future, it might be important to select for donor sources according to  
2 the relapse risk of a patient. With the aim to assess the potential of CBT in patients with  
3 hematological malignancies, especially for high-risk patients, we performed a single-  
4 institute retrospective analysis and compared the clinical outcomes of CBT with those  
5 of HLA-matched sibling and unrelated donor transplantation.

## 1 **Methods**

### 2 **Data source**

3 A total of 571 consecutive patients aged 16–73 years who received their first  
4 allogeneic HSCT for hematological diseases at Kyoto University Hospital between  
5 1990 and 2018 were reviewed. These included 394 recipients of single unrelated cord  
6 blood units (UCB, n=108), HLA-matched sibling donors (MSDs, n=143), or HLA-  
7 matched unrelated donors (MUDs, n=143). MSDs have remained our first choice of  
8 donor source if available, followed by MUDs. In the absence of these donor sources,  
9 HLA-1 allele mismatched unrelated donors would be considered. In the case of  
10 emergencies, UCB, followed by HLA-haploidentical donors, would be opted for instead  
11 of unrelated donors. For UCB donors, we prioritize units with total nucleated cell doses  
12  $>2.0 \times 10^7/\text{kg}$ , and select those with the maximum available number of CD34 positive  
13 cells. HLA typing with 4/6 matches (HLA-A, HLA-B, and HLA-DRB1) was considered  
14 acceptable in our institute. We have consistently utilized such practices over the years.  
15 Double-unit UCBTs (dUCBTs), were not included in the present study, as they are  
16 currently only under clinical trial in Japan. This study was approved by the institutional  
17 review board of Kyoto University, where this study was organized. This study was  
18 conducted in accordance with the Declaration of Helsinki.

19

### 20 **Definitions**

21 Relapse-free survival (RFS) was defined as the time from transplant to relapse, death, or  
22 the last date of follow-up. OS was defined as the time from transplant to the last date of  
23 follow-up or death. GVHD-free and relapse-free survival (GRFS) was defined as the  
24 time from transplant to grade III–IV acute GVHD, extensive chronic GVHD, relapse,

1 death, or the last date of follow-up. Relapse was defined based on morphological and  
2 clinical evidence of disease activity, and non-relapse mortality (NRM) was defined as  
3 the time to death without relapse. Neutrophil engraftment was defined as the first of 3  
4 consecutive days with a neutrophil count of 500/ $\mu$ l, without evidence of autologous  
5 reconstitution or graft rejection within the first 100 days. Acute and chronic GVHD  
6 were diagnosed and graded using standard criteria<sup>17, 18</sup>. The intensity of conditioning  
7 regimens was classified as myeloablative if either total body irradiation >8 Gy, oral  
8 busulfan  $\geq$ 9 mg/kg, intravenous busulfan  $\geq$ 7.2 mg/kg, melphalan >140 mg/m<sup>2</sup>, or  
9 thiotepa  $\geq$ 10 mg/kg was used, and otherwise as reduced intensity<sup>19</sup>. We defined HLA  
10 matching based on HLA-A, HLA-B, and HLA-DR antigen levels in UCB and sibling  
11 donors, and based on HLA-A, HLA-B, and HLA-DRB1 allele levels in unrelated  
12 donors. Standard-risk diseases were defined as acute myeloid leukemia (AML) and  
13 acute lymphoid leukemia (ALL) in complete remission, myelodysplastic syndrome  
14 (MDS) with refractory anemia or refractory anemia with ringed sideroblasts, chronic  
15 myelogenous leukemia (CML) in the chronic and accelerated phases, adult T-cell  
16 leukemia (ATL) in complete remission, lymphoma in complete or partial remission,  
17 multiple myeloma in complete remission, and non-malignant hematological diseases; all  
18 other conditions were considered high-risk diseases. In the subgroup analysis, AML,  
19 ALL, MDS, CML, myeloproliferative neoplasm, and ATL were classified as very-high-  
20 risk in the presence of  $\geq$ 20% blasts in the bone marrow or  $\geq$ 10% blasts in the peripheral  
21 blood, and lymphoma size of  $\geq$ 5 cm, spread of over 30 regions, or infiltration of the  
22 bone marrow and spleen.

23

## 24 **Endpoints**

1 The primary endpoint of the study was the impact of donor source on RFS. Secondary  
2 endpoints were OS, GRFS, relapse, NRM, neutrophil engraftment, grade III–IV acute  
3 GVHD, and extensive chronic GVHD.

#### 4 5 **Statistical analysis**

6 The probabilities of RFS, OS, and GRFS were estimated using the Kaplan-Meier  
7 method, and groups were compared using the log-rank test. The incidence of relapse,  
8 NRM, neutrophil engraftment, and acute and chronic GVHD were estimated using the  
9 cumulative incidence curve<sup>20</sup>. Competing events included death without relapse for  
10 relapse, relapse for NRM, and death for both neutrophil engraftment and acute and  
11 chronic GVHD. The groups were compared using the Gray's test<sup>21</sup>. Cox proportional  
12 hazards models were used to evaluate the effects of donor source and other variables on  
13 RFS, OS, and GRFS, while Fine and Gray's proportional hazards models were used for  
14 all other endpoints<sup>22</sup>. Chronic GVHD was assessed in patients who survived at least 100  
15 days. The following covariates were considered: patient sex, age (<50 or ≥50 years  
16 old), disease diagnosis (myeloid malignancies, lymphoid malignancies, or non-  
17 malignant disease), performance status (0–1 or >1), disease status (standard risk or high  
18 risk), conditioning regimen intensity (reduced intensity or myeloablative), and year of  
19 transplantation (1990–2010 or 2011–2018). GVHD prophylaxis was not considered,  
20 given that it is donor source-dependent. The effects of ATG were also not considered  
21 because it was only used in 2% of the patients in the cohort. If a variable had >5%  
22 missing values, missing data were included in the analysis as a separate category.  
23 Confounding variables were selected with a variable retention criterion of  $P < 0.10$  in the  
24 univariate analysis of the total cohort. Significant variables, in addition to donor source,

1 were subsequently included in the multivariate analysis of both the total cohort and the  
2 subgroup cohort. All statistical analyses were performed using EZR (Saitama Medical  
3 Center, Jichi Medical University, Saitama, Japan)<sup>23</sup>.

4

5

## 1 **Results**

### 2 **Patient characteristics for the total cohort**

3 **Table 1** summarizes the patient and transplant characteristics of the total cohort.

4 Among the 394 patients, 108 received single UCB units, 143 received MSD  
5 transplantation (89 bone marrow grafts and 54 peripheral-blood stem cell grafts), and  
6 143 received MUD transplantation (bone marrow grafts in all). Median age of the UCB,  
7 MSD, and MUD groups were 49, 46, and 48 years, respectively (P=0.097). Similar rates  
8 of high-risk disease were observed in each group (UCB, 36.1%; MSD, 32.9%; and  
9 MUD, 29.4%; P=0.26). The median follow-up of survivors was 3.9, 7.6, and 6.4 years,  
10 respectively. In terms of UCB unit characteristics, the median total nucleated cells  
11 (TNCs) cryopreserved and infused ( $\times 10^7/\text{kg}$ ), and CD34-positive cells infused ( $\times$   
12  $10^5/\text{kg}$ ) were 2.63 (1.51–6.32) and 0.67 (0.18–1.91), respectively. Although 16  
13 recipients (11 of UCB, 1 of MSD, and 4 of MUD) had HLA antibodies, none of them  
14 had donor-specific HLA antibodies.

15

### 16 **RFS, OS, and GRFS of the total cohort**

17 The 3-year RFS rates in the UCB, MSD, and MUD groups were 53.1% (95% CI,  
18 42.8–62.3%), 50.9% (95% CI, 42.3–58.9%), and 47.9% (95% CI, 39.3– 56.1%),  
19 respectively (P=0.975, **Figure 1a**). The 3-year OS rates were 60.8% (95% CI, 50.5 –  
20 69.6%), 59.4% (95% CI, 50.6–67.2%), and 56.6% (95% CI, 47.7–64.5%), respectively  
21 (P=0.924, **Figure 1b**). The 3-year GRFS rates were 43.0% (95% CI, 33.1–52.4%),  
22 38.2% (95% CI, 29.7–46.6%), and 37.4% (95% CI, 29.1–45.6%), respectively  
23 (P=0.934, **Figure 1c**). Since the MSD and MUD groups showed comparable RFS, OS,  
24 and GRFS in the multivariate analysis (**Table 2**), they were treated as a single group

1 (matched donor transplantation, MDT) in later analyses. Furthermore, a significant  
2 interaction between the transplant period and stem cell source was demonstrated (RFS,  
3 P=0.010; OS, P=0.159; GRFS, P<0.001), indicating that RFS and GRFS of CBT  
4 significantly improved in recent years. Therefore, we decided to perform a separate  
5 analysis for each period (1990–2010 and 2011–2018), and focused on the more recent  
6 period to reflect current practices.

7

### 8 **RFS in the old period**

9 Patient and transplant characteristics according to donor source in the older period are  
10 summarized in **Supplementary Table 1**. The 3-year RFS rate was 37.9% (95% CI,  
11 21.6–54.2%) after CBT and 51.4% (95% CI, 44.3–48.1%) after MDT (P=0.0942,  
12 **Figure 2a**).

13

### 14 **RFS in the recent period**

15 Patient and transplant characteristics according to donor source in the recent period are  
16 summarized in **Supplementary Table 2**. Among them, 75 received CBT and 81  
17 received MDT (60 bone marrow transplants and 21 peripheral blood transplants). The  
18 performance status in the CBT cohort was higher than that in the MDT cohort  
19 (P=0.056). In CBT, the fludarabine and melphalan (Flu/Mel) regimen was most  
20 frequently used in reduced intensity conditioning (RIC), while the combination of  
21 busulfan and cyclophosphamide (BU/CY) regimen was less frequently used in  
22 myeloablative conditioning (MAC). The median follow-up periods of survivors were  
23 3.3 and 3.5 years, respectively.

1 The 3-year RFS rate was 60.4% (95% CI, 47.9–70.9%) after CBT and 43.3% (95% CI,  
2 31.4–54.6%) after MDT (P=0.11, **Figure 2b**). Multivariate analysis showed that CBT  
3 associated with significantly better outcomes than MDT in the recent period. Since  
4 disease risk was considered the strongest prognostic factor for transplant outcomes and  
5 of clinical importance, outcomes of the standard- and high-risk groups were analyzed.  
6 The 3-year RFS rates in standard-risk patients were 65.6% (95% CI, 49.0–77.9%) after  
7 CBT and 55.7% (95% CI, 40.8–68.2%) after MDT (P=0.354, **Figure 2c**). In high-risk  
8 patients, the 3-year RFS rates were 49.7% (95% CI, 29.5–67.0%) and 8.2% (95% CI,  
9 0.7–28.3%), respectively (P=0.0256, **Figure 2d**). Multivariate analysis showed that  
10 CBT was significantly associated with better RFS in high-risk patients (**Table 3**),  
11 although no significant interaction was observed, probably due to insufficient power  
12 (P=0.278). Even after exclusion of HLA-C allele mismatched transplantations from the  
13 available data, CBT remained significantly associated with better RFS (data not shown).  
14 In the subgroup analysis, we further categorized the high-risk patients into high and  
15 very high-risk groups. A total of 26 patients after CBT and 21 patients after MDT were  
16 evaluated. The 2-year RFS rates in very high-risk patients were 45.5% (95% CI, 16.7–  
17 70.7%) after CBT and 15.0% (95% CI, 1.0 – 45.7%) after MDT (P=0.106), while those  
18 in high-risk patients were 53.3% (95% CI, 26.3–74.4%) and 18.2% (95% CI, 2.9 –  
19 44.2%), respectively (P=0.155). In the multivariate analysis, the HRs of CBT were  
20 consistently low in both the very high- and high-risk groups (HR 0.38, P=0.09; and HR  
21 0.48, P=0.19, respectively).

22

23 **OS and GRFS in the recent period**

1 According to donor source, the 3-year OS rates in standard-risk patients were 80.8%  
2 (95% CI, 66.2–89.5%) after CBT and 65.5% (95% CI, 50.1–77.1%) after MDT  
3 (P=0.601, **Figure 3a**), while those in high-risk patients were 49.7% (95% CI, 29.5–  
4 67.0%) and 28.0% (95% CI, 9.7–49.9%), respectively (P=0.45, **Figure 3b**). In the  
5 multivariate analysis, OS after CBT trended better than that after MDT in high-risk  
6 patients (HR 0.52, P=0.13), while OS after CBT was comparable to that after MDT in  
7 standard-risk patients (HR 0.78, P=0.54) (**Table 3**). The 3-year GRFS rates in standard-  
8 risk patients were 57.4% (95% CI, 41.2–70.7%) after CBT and 34.2% (95% CI, 21.0–  
9 47.8%) after MDT (P=0.0827, **Figure 3c**), while those in high-risk patients were 42.3%  
10 (95% CI, 23.5–60.0%) and 9.5% (95% CI, 1.6–26.1%), respectively (P=0.043, **Figure**  
11 **3d**). In the multivariate analysis, GRFS after CBT was significantly better than that after  
12 MDT in high-risk patients (HR, 0.39, P=0.013), and was also better in standard-risk  
13 patients (HR, 0.59, P=0.078), although significance level of 0.05 was not reached  
14 (**Table 3**).

15

### 16 **Relapse and NRM in the recent period**

17 According to the donor source, the 3-year relapse rates in standard-risk patients were  
18 19.8% (95% CI, 9.0–33.6%) after CBT and 26.0% (95% CI, 15.0–38.5%) after MDT  
19 (P=0.251, **Figure 4a**), while those in high-risk patients were 23.1% (95% CI, 9.1–  
20 40.7%) and 53.9% (95% CI, 26.3–75.1%), respectively (P=0.0145, **Figure 4b**). In the  
21 multivariate analysis, relapse risk after CBT was significantly lower than that after  
22 MDT in high-risk patients (HR, 0.25; P<0.001), while the relapse risks after CBT and  
23 MDT were comparable in standard-risk patients (HR, 0.62; P=0.29) (**Table 4**).

1 The 3-year NRM rates in standard-risk patients were 14.7% (95% CI, 6.4–26.3%) after  
2 CBT and 18.3% (95% CI, 8.8–30.4%) after MDT (P=0.99, **Figure 4c**), while those in  
3 high-risk patients were 27.2% (95% CI, 11.7–45.5%) and 25.9% (95% CI, 8.5–47.6%),  
4 respectively (P=0.737, **Figure 4d**). In the multivariate analysis, the risks of NRM after  
5 CBT and MDT were comparable in both high-risk (HR, 1.53; P=0.55) and standard-risk  
6 groups (HR, 1.03; P=0.95) (**Table 4**).

7

### 8 **Neutrophil engraftment, aGVHD, and cGVHD in the more recent period**

9 Neutrophil engraftment, grade III–IV acute GVHD, and extensive chronic GVHD were  
10 examined for all patients during the recent period. The rates of neutrophil engraftment  
11 at 56 days after CBT and MSD transplantation were 93.3% and 96.3%, respectively  
12 (P=0.0065, **Figure 5a**). The rates of Grade III–IV acute GVHD at 100 days after CBT  
13 and MSD were 12.0% and 6.3%, respectively (P=0.644, **Figure 5b**). The rate of  
14 extensive chronic GVHD at 2 years after CBT was lower (15.5%) than that after MSD  
15 transplantation (26.6%), although significance was not reached (P=0.131, **Figure 5c**).  
16 Neutrophil engraftment was significantly delayed after CBT as compared to MDT in the  
17 multivariate analysis (**Supplemental Table 3**). No significant difference in acute or  
18 chronic GVHD was observed, although the risk of chronic GVHD seemed to be lower  
19 in the CBT group (**Supplemental Table 3**).

20

## 1 **Discussion**

2 As in previous reports, RFS of CBT was comparable to that of transplantation with  
3 other donor sources for the total cohort<sup>9-14</sup>. In the old period, RFS after CBT and MDT  
4 were comparable, whereas in the recent period, RFS after CBT was significantly better.  
5 This impact was more prominent in patients with high-risk diseases. Furthermore, the  
6 superiority of CBT persisted regardless of the degree of disease risk in high-risk  
7 patients. To further explore this finding, we evaluated the impact of donor source on  
8 OS, relapse, and NRM, and found that relapse risk was significantly lower in the CBT  
9 group, particularly in patients with high-risk disease. These results were consistent with  
10 those of previous studies. Milano et al. found that the risk of relapse was significantly  
11 lower after CBT than after unrelated donor transplantation among patients with minimal  
12 residual disease<sup>16</sup>. A potential graft-versus-leukemia (GVL) effect after CBT is highly  
13 anticipated. In dUCBT, CD4+ T-cell-mediated graft-vs-graft (GVG) alloreactivity may  
14 occur and enhance the GVL effect, thereby providing an explanation for the relatively  
15 low relapse rates associated with dUCBT<sup>24</sup>. However, only single-unit UCBTs were  
16 included in our study, as dUCBTs are currently only under clinical trial in Japan. Our  
17 results support the notion that CBT can provide a GVL effect, although the  
18 immunological potential of CBT has not been elucidated. Kanda et al. demonstrated that  
19 mild acute and chronic GVHD associated with not only a low risk of relapse, but also a  
20 low risk of NRM following CBT<sup>25</sup>. Moreover, robust CD4+ T-cells and T-cell function  
21 after CBT associated with improved survival despite the high rates of acute GVHD<sup>26</sup>.  
22 These results may provide a better understanding of why UCB offers better outcomes in  
23 high-risk patients than other sources.

24

1 High rates of engraftment failure and NRM after CBT have been a serious concern.  
2 The higher NRM observed following CBT may be due to delayed neutrophil recovery  
3 or increased mortality from infections. Because anti-HLA antibodies were not  
4 necessarily checked in the earlier period, the presence of donor-specific HLA antibody,  
5 which can affect delayed neutrophil engraftment, could not be determined. However,  
6 significant progress has been made, and our results showed acceptable neutrophil  
7 engraftment rates (93.3% in the recent period, and 81.8% in the old period) and  
8 comparable NRM compared to transplantation with other stem cell sources. Infections  
9 during prolonged neutropenia are the main cause of NRM. In our study, mortality from  
10 infections after CBT decreased from 15.1% (5/33) in the old period, to 5.3% (4/75) in  
11 the recent period. No cell content disparities were observed between each period, with  
12 units in the old period consisting of  $0.72 \times 10^7/\text{kg}$  TNCs and  $2.58 \times 10^5/\text{kg}$  CD34-  
13 positive cells, while those in the recent period containing  $0.67 \times 10^7/\text{kg}$  TNCs and  $2.72$   
14  $\times 10^5/\text{kg}$  CD34-positive cells (**Supplemental Figure 1**). Better conditioning regimens  
15 and supportive care during prolonged neutropenic periods have helped decrease NRM  
16 following CBT. Notably, Uchida et al. demonstrated the effectiveness of combined  
17 mycophenolate mofetil (MMF) and tacrolimus as GVHD prophylaxis in elderly  
18 recipients after CBT, which showed superior engraftment rates and a decrease in early  
19 NRM through better control of pre-engraftment immune reactions<sup>27, 28</sup>. In addition,  
20 several studies have demonstrated the importance of MMF monitoring for effective  
21 prophylaxis of acute GVHD after CBT. Since 2011, we have introduced MMF as an  
22 adjunct to tacrolimus for GVHD prophylaxis in CBT<sup>29, 30</sup>, and have regularly monitored  
23 the plasma levels of MMF and modified its dosages throughout the clinical course after  
24 CBT<sup>31</sup>. In fact, 85% of our cases have received dose-monitoring of MMF as GVHD

1 prophylaxis since 2011. This may be one of the reasons for the better CBT outcomes  
2 observed in the more recent period.

3

4 Chronic GVHD is a serious complication that affects the OS and quality of life of  
5 long-term survivors after HSCT. Extensive chronic GVHD was observed less frequently  
6 after CBT than after MSD transplantation in this study. This advantage of CBT has  
7 been discussed in previous studies, and has been found to associate with frequent organ  
8 involvement such as the oral cavity, eye, liver, lung, and joints<sup>32, 33</sup>. A low incidence of  
9 chronic GVHD allows for easier discontinuation of immunosuppressive agents, and  
10 consequently earlier immune reconstitution. This may be one of the reasons for the  
11 better long-term outcomes observed after CBT. GRFS after CBT was significantly  
12 better than that after MDT. The low incidence of chronic GVHD and relapse, and the  
13 comparable NRM and grade III–IV acute GVHD rates, may reflect the better GRFS  
14 associated with CBT.

15

16 This study has several limitations. First, this is a retrospective study involving a small  
17 population with heterogeneous backgrounds from a single transplant center. The  
18 heterogeneous background of patients may have resulted in a statistical bias, although  
19 attempts were made to reduce this bias by adjusting the impact in multivariate analyses.  
20 Further, the findings should be externally validated. Second, we utilized the original  
21 disease risk definition to classify our heterogenous populations comprehensively. The  
22 refined disease risk index (rDRI) has often applied to risk stratification analyses<sup>34</sup>.  
23 However, it was difficult to divide our cohort using the rDRI because of the  
24 heterogeneity of the population, which included both ATL and non-malignant

1 hematological diseases. RFS was found to be consistent regardless of whether the rDRI  
2 was used, and significant correlation ( $P < 0.010$ ) between our staging approach and the  
3 rDRI was observed (data not shown). We therefore chose to retain our original disease  
4 risk classification system for this study. Third, we only analyzed HLA-A, HLA-B, and  
5 HLA-DRB1 alleles because of the incomplete data on HLA-C, particularly in the old  
6 period. However, our results were almost the same even after the exclusion of HLA-C  
7 mismatched cases and cases without HLA-C locus information in the recent cohort.  
8 Finally, we did not consider the effects of donor allocation time. Earlier search for  
9 unrelated donors and earlier transplantation could have helped improve the outcomes of  
10 matched unrelated transplantation.

11

12 In conclusion, our study demonstrated that CBT associated with better RFS than MDT  
13 in recent years. Notably, patients with high-risk disease benefitted more from CBT, as  
14 reflected by the significantly better RFS, and the low relapse rates without an increase  
15 in NRM observed, suggesting the safety and high potential of a GVL effect after single-  
16 unit CBT. CBT might hence serve as a reasonable donor choice, particularly for high-  
17 risk patients.

18

19

## 20 **Authors' Contribution**

21 FW and JK designed the research; JK organized the project; FW and JK performed the  
22 statistical analysis; MH, TK, KY and AT-K interpreted the data; FW wrote the first  
23 draft; and all other authors critically reviewed the draft and approved the final version  
24 for publication.

1

## 2 **Acknowledgments**

3 The authors thank Emi Furusaka, Tomoko Okuda, Miki Shirasu, and Megumi Oka for  
4 their expert data management and secretarial assistance, and the transplantation team  
5 members at Kyoto University Hospital for their dedicated care of the patients and  
6 donors.

7

## 8 **Funding**

9 This work was supported in part by the Takeda Science Foundation (JK) and JSPS  
10 KAKENHI Grant Number 18K08325 (JK).

11

## 12 **References**

- 13 1. Dehn J, Spellman S, Hurley CK, et al. Selection of unrelated donors and cord  
14 blood units for hematopoietic cell transplantation: guidelines from the  
15 NMDP/CIBMTR. *Blood*. 2019;134:924–934.
- 16 2. Fuchs EJ, O'Donnell PV, Eapen M, et al. Double unrelated umbilical cord  
17 blood versus HLA-haploidentical bone marrow transplantation (BMT CTN  
18 1101). *Blood*. 2020.
- 19 3. Barker JN, Kurtzberg J, Ballen K, et al. Optimal Practices in Unrelated Donor  
20 Cord Blood Transplantation for Hematologic Malignancies. *Biology of Blood  
21 and Marrow Transplantation*. 2017;23:882–896.
- 22 4. Gluckman E. Role of HLA Matching in Single Umbilical Cord Blood  
23 Transplantation Outcomes. *Biology of Blood and Marrow Transplantation*.  
24 2020;26:e53–e54.
- 25 5. Barker JN, Mazis CM, Devlin SM, et al. Evaluation of Cord Blood Total  
26 Nucleated and CD34(+) Cell Content, Cell Dose, and 8-Allele HLA Match by  
27 Patient Ancestry. *Biol Blood Marrow Transplant*. 2020;26:734–744.
- 28 6. Kanda J, Hayashi H, Ruggeri A, et al. Prognostic factors for adult single cord  
29 blood transplantation among European and Japanese populations: the

- 1 Eurocord/ALWP-EBMT and JSHCT/JDCHCT collaborative study. *Leukemia*.  
2 2020;34:128-137.
- 3 7. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation:  
4 the first 25 years and beyond. *Blood*. 2013;122:491-498.
- 5 8. Konuma T, Kanda J, Inamoto Y, et al. Improvement of early mortality in  
6 single-unit cord blood transplantation for Japanese adults from 1998 to 2017.  
7 *Am J Hematol*. 2019.
- 8 9. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of  
9 cord blood or bone marrow from unrelated donors in adults with leukemia. *N*  
10 *Engl J Med*. 2004;351:2265-2275.
- 11 10. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or  
12 bone marrow from unrelated donors in adults with acute leukemia. *N Engl J*  
13 *Med*. 2004;351:2276-2285.
- 14 11. Eapen M, Rocha V, Sanz G, et al. Effect of graft source on unrelated donor  
15 haemopoietic stem-cell transplantation in adults with acute leukaemia: a  
16 retrospective analysis. *Lancet Oncol*. 2010;11:653-660.
- 17 12. Atsuta Y, Suzuki R, Nagamura-Inoue T, et al. Disease-specific analyses of  
18 unrelated cord blood transplantation compared with unrelated bone marrow  
19 transplantation in adult patients with acute leukemia. *Blood*. 2009;113:1631-  
20 1638.
- 21 13. Terakura S, Atsuta Y, Tsukada N, et al. Comparison of Outcomes of 8/8 and  
22 7/8 Allele-Matched Unrelated Bone Marrow Transplantation and Single-Unit  
23 Cord Blood Transplantation in Adults with Acute Leukemia. *Biol Blood*  
24 *Marrow Transplant*. 2016;22:330-338.
- 25 14. Sharma P, Purev E, Haverkos B, et al. Adult cord blood transplant results in  
26 comparable overall survival and improved GRFS vs matched related  
27 transplant. *Blood Adv*. 2020;4:2227-2235.
- 28 15. Niederwieser D, Baldomero H, Szer J, et al. Hematopoietic stem cell  
29 transplantation activity worldwide in 2012 and a SWOT analysis of the  
30 Worldwide Network for Blood and Marrow Transplantation Group including the  
31 global survey. *Bone Marrow Transplant*. 2016;51:778-785.
- 32 16. Milano F, Gooley T, Wood B, et al. Cord-Blood Transplantation in Patients  
33 with Minimal Residual Disease. *N Engl J Med*. 2016;375:944-953.
- 34 17. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on  
35 Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.

- 1 18. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease  
2 and other late complications of bone marrow transplantation. *Semin Hematol.*  
3 1991;28:250–259.
- 4 19. Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimen  
5 workshop: defining the dose spectrum. Report of a workshop convened by  
6 the center for international blood and marrow transplant research. *Biol Blood*  
7 *Marrow Transplant.* 2009;15:367–369.
- 8 20. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure  
9 probabilities in the presence of competing risks: new representations of old  
10 estimators. *Stat Med.* 1999;18:695–706.
- 11 21. Gray RJ. A Class of  $K-S$ -Sample Tests for Comparing the Cumulative  
12 Incidence of a Competing Risk. *The Annals of Statistics.* 1988;16:1141–1154.
- 13 22. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a  
14 Competing Risk. *Journal of the American Statistical Association.*  
15 1999;94:496–509.
- 16 23. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for  
17 medical statistics. *Bone Marrow Transplant.* 2013;48:452–458.
- 18 24. Verneris MR, Brunstein CG, Barker J, et al. Relapse risk after umbilical cord  
19 blood transplantation: enhanced graft-versus-leukemia effect in recipients of  
20 2 units. *Blood.* 2009;114:4293–4299.
- 21 25. Kanda J, Morishima Y, Terakura S, et al. Impact of graft-versus-host disease  
22 on outcomes after unrelated cord blood transplantation. *Leukemia.*  
23 2017;31:663–668.
- 24 26. Politikos I, Lavery JA, Hilden P, et al. Robust CD4+ T-cell recovery in adults  
25 transplanted with cord blood and no antithymocyte globulin. *Blood Adv.*  
26 2020;4:191–202.
- 27 27. Uchida N, Wake A, Nakano N, et al. Mycophenolate and tacrolimus for graft-  
28 versus-host disease prophylaxis for elderly after cord blood transplantation:  
29 a matched pair comparison with tacrolimus alone. *Transplantation.*  
30 2011;92:366–371.
- 31 28. Minagawa K, Yamamori M, Katayama Y, Matsui T. Mycophenolate mofetil: fully  
32 utilizing its benefits for GvHD prophylaxis. *Int J Hematol.* 2012;96:10–25.
- 33 29. Arai Y, Kondo T, Kitano T, et al. Monitoring mycophenolate mofetil is  
34 necessary for the effective prophylaxis of acute GVHD after cord blood  
35 transplantation. *Bone Marrow Transplant.* 2015;50:312–314.

- 1   **30.**   Wakahashi K, Yamamori M, Minagawa K, et al. Pharmacokinetics-based  
2           optimal dose prediction of donor source-dependent response to  
3           mycophenolate mofetil in unrelated hematopoietic cell transplantation. *Int J*  
4           *Hematol.* 2011;94:193–202.
- 5   **31.**   Muranushi H, Kanda J, Arai Y, et al. Drug monitoring for mycophenolic acid in  
6           graft-versus-host disease prophylaxis in cord blood transplantation. *Br J Clin*  
7           *Pharmacol.* 2020.
- 8   **32.**   Kanda J, Nakasone H, Atsuta Y, et al. Risk factors and organ involvement of  
9           chronic GVHD in Japan. *Bone Marrow Transplantation.* 2014;49:228–235.
- 10   **33.**   Kanda J, Ichinohe T, Kato S, et al. Unrelated cord blood transplantation vs  
11           related transplantation with HLA 1-antigen mismatch in the graft-versus-  
12           host direction. *Leukemia.* 2013;27:286–294.
- 13   **34.**   Armand P, Kim HT, Logan BR, et al. Validation and refinement of the Disease  
14           Risk Index for allogeneic stem cell transplantation. *Blood.* 2014;123:3664–  
15           3671.
- 16

1 **Figure legends**

2 **Figure 1 Kaplan-Meier estimates of RFS (a), OS (b), and GRFS (c) of the entire**  
3 **cohort according to donor source.**

4

5 **Figure 2 Kaplan-Meier estimates of RFS according to each donor source,**  
6 **transplant period and the disease risk in the recent period.**

7 (a) RFS in the old period, (b) RFS in the recent period, (c) RFS for standard-risk  
8 patients in the recent period, and (d) RFS for high-risk patients in the recent period.

9

10 **Figure 3 Kaplan-Meier estimates of OS and GRFS according to disease risk in the**  
11 **recent period.**

12 (a) OS of standard-risk patients, (b) OS of high-risk patients, (c) GRFS of standard-risk  
13 patients, and (d) GRFS of high-risk patients.

14

15 **Figure 4 Cumulative incidence of relapse and NRM according to disease risk in the**  
16 **recent period.**

17 (a) Relapse in standard-risk patients, (b) relapse in high-risk patients, (c) NRM in  
18 standard-risk patients, and (d) NRM in high-risk patients.

19

20 **Figure 5 Cumulative incidence of neutrophil engraftment (a), grade III–IV acute**  
21 **GVHD (b), and extensive chronic GVHD (c) in the recent period.**

22

23

24

1 Table 1 Patient characteristics of the total cohort

2

	Donor source (n=394)			
Characteristics	UCB (n=108)	MSD (n=143)	MUD (n=143)	P-value
Sex-no. (%)				0.071
Female	42(38.9)	53(37.1)	71(49.7)	
Male	66(61.1)	90(62.9)	72(50.3)	
Age-yr				0.097
Median	49	46	48	
Range	20-68	16-73	18-67	
Diagnosis-no. (%)				0.955
Leukemia	81(75.0)	104(72.7)	106(74.1)	
Lymphoma	22(20.4)	27(18.9)	29(20.3)	
Myeloma	2(1.9)	6(4.2)	3(2.1)	
AA/PRCA/PNH	3(2.8)	5(3.5)	4(2.8)	
other	0(0.0)	1(0.7)	1(0.7)	
Stem Cell Source-no. (%)				<0.001
Peripheral Blood	0(0.0)	54(37.8)	0(0.0)	
Bone Marrow	0(0.0)	89(62.2)	143(100.0)	
Cord Blood	108(100.0)	0(0.0)	0(0.0)	
ECOG PS-no. (%)				0.001
0-1	91(84.3)	108(75.5)	111(77.6)	
>1	14(13.0)	7(4.9)	13(9.1)	
missing	3(2.8)	28(19.6)	19(13.3)	
Stage-no. (%)				0.26
standard	69(63.9)	92(64.3)	95(66.4)	
high	39(36.1)	47(32.9)	42(29.4)	
missing	0(0.0)	4(2.8)	6(4.2)	
Conditioning Regimen-no. (%)				<0.001
RIC	52(48.1)	39(27.3)	60(42.0)	

MAC	56(51.9)	46(54.1)	80(55.9)	
missing	0(0.0)	58(40.6)	3(2.1)	
GVHD Prophylaxis-no. (%)				<0.001
CI+MTX	14(13.0)	130(90.9)	128(89.5)	
CI+MMF (+MTX/MMF)	66(61.0)	0(0.0)	12(8.4)	
CI only	28(25.9)	13(9.1)	3(2.1)	
ATG				0.199
contained	0(0.0)	2(1.4)	4(2.8)	
not contained	108(100.0)	141(98.6)	139(97.2)	
HLA-mismatch				<0.001
0	6(5.6)	143(0)	143(100)	
1	30(27.8)	0(0.0)	0(0.0)	
2	56(51.9)	0(0.0)	0(0.0)	
3	1(0.9)	0(0.0)	0(0.0)	
missing	15(13.9)	0(0.0)	0(0.0)	
Transplant Period-no. (%)				<0.001
1990-2003	2(1.9)	70(49.0)	35(24.5)	
2004-2010	31(28.7)	38(26.6)	62(43.4)	
2011-2018	75(69.4)	35(24.5)	46(32.2)	
Median Follow-up of Survivors (range)	3.9 years (0.2-13.4)	7.6 years (0.3-27.1)	6.4 years (0.1-19.0)	<0.001

- 1 Abbreviations: AA, aplastic anemia; PRCA, pure red cell aplasia; PNH, paroximal
- 2 nocturnal hemoglobinuria; RIC, reduced-intensity conditioning; MAC, myeloablative
- 3 conditioning; GVHD, graft-versus host disease; CI, calcineurin inhibitor; MTX,
- 4 methotrexate; MMF, mycophenolate mofetil; UCB, unrelated cord blood; MSD,
- 5 matched sibling donor; MUD, matched unrelated donor.

1 Table 2 Adjusted comparison of transplant outcomes with UCB, MSD, and MUD

Outcome	Donor source	HR (95% CI)	P-value
<b>RFS</b>			
	UCB (n=108)	1.00	Reference
	MSD (n=143)	1.24(0.85-1.81)	0.27
	MUD (n=143)	1.22(0.84-1.77)	0.29
<b>OS</b>			
	UCB (n=108)	1.00	Reference
	MSD (n=143)	1.10(0.72-1.66)	0.67
	MUD (n=143)	1.14(0.77-1.70)	0.52
<b>GRFS</b>			
	UCB (n=108)	1.00	Reference
	MSD (n=143)	1.07(0.75-1.52)	0.7
	MSD (n=143)	1.16(0.82-1.62)	0.4

2

3 Other significant variables included for adjustment in RFS, OS and GRFS were patient  
 4 age, disease diagnosis, performance status, and disease status.

5 Abbreviations: HR, hazard ratio; RFS, relapse-free survival; OS, overall survival;  
 6 GRFS, GVHD-free and relapse-free survival; MSD, matched sibling donor; MUD,  
 7 matched unrelated donor; UCB, unrelated cord blood.

1 Table 3

2 Adjusted comparison of outcomes following CBT and MDT in the recent period

Outcome	HR (95% CI)	P-value
<b>RFS</b>		
All risk		
MDT (n=81)	1.00	Reference
CBT (n=75)	0.50(0.30-0.84)	0.008
High risk		
MDT (n=21)	1.00	Reference
CBT (n=26)	0.35(0.16-0.77)	0.009
Standard risk		
MDT (n=60)	1.00	Reference
CBT (n=49)	0.68(0.34-1.33)	0.25
<b>OS</b>		
All risk		
MDT (n=81)	1.00	Reference
CBT (n=75)	0.65(0.37-1.20)	0.14
High risk		
MDT (n=21)	1.00	Reference
CBT (n=26)	0.52(0.22-1.22)	0.13
Standard risk		
MDT (n=60)	1.00	Reference
CBT (n=49)	0.78(0.35-1.72)	0.54
<b>GRFS</b>		
All		
MDT (n=81)	1.00	Reference
CBT (n=75)	0.50(0.32-0.79)	0.003
High risk		
MDT (n=21)	1.00	Reference
CBT (n=26)	0.39(0.18-0.82)	0.013
Standard risk		
MDT (n=60)	1.00	Reference

CBT (n=49)	0.59(0.33-1.06)	0.078
------------	-----------------	-------

1

2 Other variables included for adjustment in RFS, OS and GRFS were patient age,

3 disease diagnosis, performance status, and disease status.

1 Table 4 Adjusted comparison of relapse and NRM following CBT and MDT in the  
 2 recent period

Outcome	HR (95% CI)	P-value
<b>Relapse*</b>		
All risk		
MDT (n=81)	1.00	Reference
CBT (n=75)	0.37(0.19-0.72)	0.004
High risk		
MDT (n=21)	1.00	Reference
CBT (n=26)	0.25(0.11-0.54)	<0.001
Standard risk		
MDT (n=60)	1.00	Reference
CBT (n=49)	0.62(0.25-1.50)	0.29
<b>NRM**</b>		
All risk		
MDT (n=81)	1.00	Reference
CBT (n=75)	1.08(0.51-2.29)	0.83
High risk		
MDT (n=21)	1.00	Reference
CBT (n=26)	1.53(0.38-6.06)	0.55
Standard risk		
MDT (n=60)	1.00	Reference
CBT (n=49)	1.03(0.39-2.80)	0.95

3 \* Other variables included for adjustment were disease diagnosis, performance status,  
 4 and disease status.

5 \*\* Other variables included for adjustment were patient sex, patient age, performance  
 6 status, and disease status

7









