Cord Blood

Effects of Haplotype Matching on Outcomes after Adult Single-Cord Blood Transplantation

Junya Kanda1,*, Takakazu Kawase2, Hidenori Tanaka3, Hiroto Kojima3, Yasuo Morishima4,5,6, Naoyuki Uchida7, Koji Nagafuji8, Yoshiko Matsushita9, Takanori Ohta10, Makoto Onizuka11, Toru Sakura12, Satoshi Takahashi13, Shigesaburo Miyakoshi14, Hikaru Kobayashi15, Tetsuya Eto16, Junji Tanaka17, Tatsuochinoh’e, Yoshiko Atsuta18,19, Satoko Morishima20, on behalf of the HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation

1 Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan
2 Department of Hematology and Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan
3 HLA Foundation Laboratory, Kyoto, Japan
4 Central Japan Cord Blood Bank, Seto, Japan
5 Department of Hematology and Oncology, Nagakoami Hospital, Okinawa, Japan
6 Department of Promotion for Blood and Marrow Transplantation, Aichi Medical University School of Medicine, Nagakute, Japan
7 Department of Hematology, Federation of National Public Service Personnel Mutual Aid Associations Toranomon Hospital, Tokyo, Japan
8 Division of Hematology and Oncology, Department of Medicine, Kurume University Hospital, Kurume, Japan
9 Department of Hematology, Kawasaki Medical School Hospital, Kurashiki, Japan
10 Department of Internal Medicine, Kitakyushu Municipal Medical Center, Kitakyushu, Japan
11 Department of Hematology/Oncology, Tokai University School of Medicine, Isehara, Japan
12 Leukemia Research Center, Saiseikai Maebashi Hospital, Maebashi, Japan
13 Department of Molecular Therapy, Advanced Clinical, Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan
14 Department of Hematology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan
15 Department of Hematology, Nagano Red Cross Hospital, Nagano, Japan
16 Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan
17 Department of Hematology, Tokyo Women’s Medical University, Tokyo, Japan
18 Japanese Data Center for Hematopoietic Cell Transplantation, Nagoya, Japan
19 Department of Healthcare Administration, Nagoya University Graduate School of Medicine, Nagoya, Japan
20 Department of Endocrinology, Diabetes and Metabolism, Hematology, Rheumatology (Second Department of Internal Medicine), Graduate School of Medicine, University of the Ryukyus, Nishihara, Japan

Article history:
Received 9 July 2019
Accepted 30 September 2019

ABSTRACT
It remains unclear whether the HLA haplotype of unrelated cord blood (UCB) should be matched to that of the patient in single UCB transplantation. Thus, using data from a Japanese registry, we analyzed the effect of haplotype matching on outcomes. Patients with hematologic diseases aged 16 years or older who had undergone their first transplant were included (N = 1347). The effects of haplotype matching and high-frequency HLA haplotype on outcomes were analyzed. Median patient age was 55 years. The cumulative incidences of neutrophil engraftment among groups with 0, 1, and 2 HLA haplotype matches were 79%, 82%, and 88%, respectively (P = .008). In a multivariate analysis, the group with 0 haplotype matches was marginally associated with worse neutrophil engraftment (P = .087) and significantly associated with platelet engraftment (P = .044) compared with the group with 1 haplotype match. Two-haplotype matches were associated with a higher risk of relapse. In the group with 1 haplotype match, the top 3 shared haplotypes were “A*24:02-B*52:01-C*12:02-DRB1*15:02” (HP-P1), “A*33:03-B*44:03-C*14:03-DRB1*13:02” (HP-P2), and “A*24:02-B*07:02-C*07:02-DRB1*01:01” (HP-P3). The presence of HP-P2 but not HP-P1 or HP-P3 was associated with a decreased risk of grades II to IV acute graft-versus-host disease (hazard ratio, 0.56; P = .001) but an increased risk of relapse (hazard ratio, 1.35; P = .045). HLA haplotype matching might be considered to improve engraftment. Two-haplotype matches should be avoided if the relapse risk is high. The haplotype itself may have an effect on the risk of acute graft-versus-host disease and relapse.

© 2019 American Society for Transplantation and Cellular Therapy. Published by Elsevier Inc.

Keywords:
Cord blood transplantation
HLA haplotype
Neutrophil engraftment
Graft-versus-host disease

Financial disclosure: See Acknowledgments on page 517.
*Correspondence and reprint requests: Junya Kanda, MD, Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, Japan 606-8507.
E-mail address: jkanda16@kuhp.kyoto-u.ac.jp (J. Kanda).

https://doi.org/10.1016/j.bbmt.2019.09.035
1083-8791/© 2019 American Society for Transplantation and Cellular Therapy. Published by Elsevier Inc.
INTRODUCTION

Unrelated cord blood (UCB) has been established as an alternative source of hematopoietic stem cells for adult and pediatric allogeneic hematopoietic cell transplant [1-15]. One of the advantages of UCB transplantation (UCBT) is the less stringent requirement for HLA matching compared with that in bone marrow or peripheral blood stem cell transplant under standard graft-versus-host disease (GVHD) prophylaxis, which makes it easier to find candidate UCB units. Another advantage of UCBT is lower incidences of acute and chronic GVHD despite multiple HLA mismatches and better responses to corticosteroid treatment for acute GVHD compared with peripheral blood stem cell transplant. This compensates for the risk of early transplant mortality and provides a favorable quality of life as well as long-term overall survival after UCBT [16-18].

On the other hand, the disadvantages of UCBT include risks of graft failure and infectious complications associated with delayed neutrophil engraftment in the early period after UCBT. The median time for neutrophil engraftment was reportedly 21 days and the engraftment rate 80% to 90%, which are much slower and lower, respectively, than those in peripheral blood stem cell transplant. This is why physicians sometimes hesitate to choose UCB units as stem cell sources. To increase the probability of engraftment, UCB units with higher total nucleated cell (TNC) count and CD34 cell count and fewer HLA mismatches between the UCB units and patients are usually selected [10-13,19-21]. Improved conditioning regimens and GVHD prophylaxis and the use of double UCB units in patients for whom it is difficult to find a single UCB unit that contains sufficient TNCs have significantly decreased early transplant mortality [5,6,22]. The avoidance of UCB units against which the recipient has antidonor HLA antibodies is also crucial to reduce the risk of graft failure [23,24].

Although these changes have improved the incidence of engraftment after UCBT over the years, the results are still not satisfactory. To achieve better engraftment, a question is whether the HLA haplotypes of UCB units and patients should be matched, which has not yet been examined. Using data from a Japanese registry, we analyzed the effects of haplotype matching and the haplotype itself on outcomes after single UCBT.

METHODS

Data Collection

Transplant data were obtained from the Transplant Registry Unified Management Program [25-27]. We included 3659 patients aged 16 years or older with hematologic diseases who received a first allogeneic stem cell transplant using a single UCB unit between 2004 and 2015 and for whom recipient and donor HLA-A, -B, -C, and -DRB1 allelic information was available. We excluded patients who lacked data on survival status (n = 3).

The study was approved by the data management committees of Transplant Registry Unified Management Program and by the institutional review board of Kyoto University, where this study was organized. The study was conducted in accordance with the Declaration of Helsinki.

Haplotypes Estimation and Categorization

Haplotypes of HLA-A, -B, -C, and -DRB1 loci were estimated using a maximum probability algorithm (Supplementary Figure 1). Eight possible haplotype combinations were determined based on the results of HLA-A, -B, -C, and -DRB1 genotyping in each patient. The probabilities of the 8 haplotype combinations were calculated using haplotype frequency data from a family study in a Japanese population [28]. The haplotype combination with the highest probability among the 8 combinations was used as the predicted haplotype of the patient. Only haplotypes that were determined to have a likelihood ratio of 80% for both donors and recipients were included. This estimation was validated in actual donor and recipient pairs whose haplotypes were predetermined based on family HLA, and 95% of donor and recipient pairs were correctly identified by our haplotype estimation. Haplotypes of 1443 donors and recipient pairs were determined.

Because more than 4 allele mismatches were matched, and the number of HLA mismatches was associated with grades III to IV acute GVHD, higher nonrelapse mortality, and lower overall survival and, most importantly, by the presence of more than 4 allele mismatches meant that no haplotype matched in these groups, we excluded 96 patients with more than 4 allele mismatches from the analysis. Finally, 1347 patients were included in the analysis.

We divided these patients into 3 groups according to the number of matching haplotypes. In the 2-haplotype match group (n = 82), both haplotypes of 4 allele donors and recipients were matched. In the 1-haplotype match group (n = 985), 1 haplotype was shared. The 0-haplotype match group did not share any haplotype (n = 280). The 0-haplotype match group was further divided into 2 groups with and without double mismatches at any locus of HLA-A, -B, -C, and -DRB1 (0-haplotype match with double mismatch versus 0-haplotype match without double mismatch).

Endpoints and Definitions

The primary endpoint of the study was the impact of haplotype matching on neutrophil and platelet engraftment. Other assessed endpoints included the impact on overall survival, relapse, nonrelapse mortality, and acute and chronic GVHD. Neutrophil recovery was defined as an absolute neutrophil count exceeding 500/µl for 3 consecutive days after UCBT. Platelet recovery was defined as an absolute platelet count exceeding 20,000/µl without a platelet transfusion. Physicians who performed the transplants at each center diagnosed and graded acute and chronic GVHD based on traditional criteria [29,30]. The intensity of the conditioning regimen was classified as myeloablative or reduced intensity based on criteria outlined by the Center for International Blood and Marrow Transplant Research and information from a questionnaire, as previously described [31-33]. We defined acute myeloid leukemia and acute lymphocytic leukemia in complete remission, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts, chronic myelogenous leukemia in the chronic and accelerated phase, adult T cell leukemia in complete remission, other leukemia in complete remission, lymphoma in complete remission/partial remission, and nonmalignant disease as standard-risk diseases and other conditions as high-risk diseases.

Statistical Analysis

The probabilities of overall and disease-free survival were estimated according to the Kaplan-Meier method, and groups were compared using the log-rank test. The probabilities of neutrophil and platelet engraftment, acute and chronic GVHD, relapse, and nonrelapse mortality were estimated on the basis of cumulative incidence curves [34]. Competing events were deaths without events. The groups were compared using Gray’s test [35]. The Cox proportional hazards model was used to evaluate the effect of haplotype matching on overall survival and disease-free survival, whereas the competing regression model was used to evaluate the effects on other endpoints [36].

The main effect assessed was the haplotype matching or the haplotype itself and the number of allele mismatches at HLA-A, -B, -C, and -DRB1 loci. The following possible confounding variables were included: donor and recipient sex, recipient age at transplant, year of transplant (2005 to 2009 or 2010 to 2014), performance status (0 to 1 versus 2 to 4), body type matching between the recipient and cord blood unit (match versus mismatch), type of conditioning regimen (myeloablative or reduced intensity), TNC dose category (≥2.00 to 2.49, ≥2.50 to 2.99, ≥3.00 to 3.99, ≥4.00 to 4.99, ≥5.00 to 9.99, ≥10.00 to 19.99, ≥20.00 to 100.00, ≥100.00 kg), TNC dose category (≥1.00 to 2.49, ≥2.50 to 4.99, ≥5.00 to 9.99, ≥10.00 to 19.99, ≥20.00 to 29.99, ≥30.00 to 99.99, ≥100.00 kg), disease status before transplant (standard or high risk), GVHD prophylaxis (calcineurin inhibitor only, calcineurin inhibitor plus mycophenolate mofetil, or calcineurin inhibitor plus methotrexate), and diagnosis (myeloid disease, lymphoid disease, or nonmalignant disease). Factors other than haplotype matching and the number of HLA mismatches were selected in a stepwise manner from the model with a variable retention criterion of P < 0.05. We then added the main variables to the final model. All tests were 2-sided, and P < 0.05 was considered to be statistically significant. All statistical analyses were performed with Stata version 13 software (StataCorp, College Station, TX) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [37].

RESULTS

Table 1 shows patient and transplant characteristics. Median patient age was 55 years (range, 16 to 79). Median TNC and CD34 doses were 2.7 × 10^6/kg (range, 1.4 to 8.1) and 8 × 10^6/kg (range, 1 to 5.2), respectively. The number of allele mismatches was 0 in 82 patients, 1 in 154, 2 in 252, 3 in 565, and 4 in 294. GVHD prophylaxis consisted of calcineurin inhibitor in combination with methotrexate in 707 patients, calcineurin inhibitor in combination with mycophenolate mofetil in 430, and other prophylaxes in 208. Number of transplant centers was 176. Eleven cord blood banks provided cord blood units.

The median age of the recipients at transplant was significantly older in the 2-haplotype match group. TNCs and CD34
cells were highest among the 1-haplotype match group, although the differences were small. The number of allele mismatches for the 1-haplotype match group was 1 (n = 148), 2 (n = 201), 3 (n = 471), and 4 (n = 165), whereas the number of allele mismatches for the 0-haplotype match group was 1 (n = 6), 2 (n = 51), 3 (n = 94), and 4 (n = 129). The median follow-up period of survivors was 2.0 years (range, .1 to 10.2).

Neutrophil and Platelet Engraftment

The cumulative incidence of neutrophil engraftment at day 42 among groups with 2, 1, and 0 HLA haplotype matches was 88% (95% confidence interval [CI], 78% to 93%), 82% (95% CI, 79% to 84%), and 79% (95% CI, 73% to 83%), respectively (Gray’s test, P = .008) (Figure 1). In the multivariate analysis the group with 0-haplotype matches was marginally associated with worse neutrophil engraftment compared with the group with 1-haplotype match (0 matches versus 1 match: hazard ratio [HR], .88; P = .087), whereas the group with 2-haplotype matches was significantly associated with better neutrophil engraftment (2 matches versus 1 match: HR, 1.39; P = .005).

Other significant variables were recipient sex, CD34 cell dose, transplant year, performance status, ABO matching, GVHD prophylaxis, and disease status (Table 2). The tendency for worse neutrophil engraftment in the group with 0-haplotype matches was more apparent in patients with double mismatches at the same locus (0 matches / no double mismatch versus 1 match: HR, .93 [P = .429]; 0 matches + double mismatch versus 1 match: HR, .80 [P = .050]; trend P = .046 for these 3 groups) (Supplementary Table 1).

The cumulative incidence of platelet engraftment at day 100 among groups with 2, 1, and 0 HLA haplotype matches was 72% (95% CI, 61% to 81%), 66% (95% CI, 63% to 69%), and

| Table 1  | Patient and Transplant Characteristics |
|----------|----------------------------------------|
| Characteristics | Subclass | Total (N = 1347) | 2-Haplotype Matches (n = 82) | 1-Haplotype Match (n = 985) | 0-Haplotype Matches (n = 280) | P |
| Patient sex | Female | 572 | 45 | 412 | 115 | .062 |
| | Male | 775 | 37 | 573 | 165 | .236 |
| Patient age | Median (range) | 55 (16-79) | 59.5 (16-75) | 55 (16-79) | 54 (16-75) | .310 |
| Diagnosis | Myeloid disease | 1132 | 67 | 831 | 234 | .236 |
| | Lymphoid disease | 179 | 10 | 134 | 35 | .377 |
| | Nonmalignant disease | 36 | 5 | 20 | 11 | .101 |
| Disease risk | Standard risk | 593 | 36 | 418 | 139 | .518 |
| | High risk | 751 | 46 | 564 | 141 | .236 |
| | Missing | 3 | 0 | 3 | 0 | .236 |
| Performance status | 0-1 | 1109 | 65 | 816 | 228 | .518 |
| | 2-4 | 223 | 17 | 159 | 47 | .518 |
| | Missing | 15 | 0 | 10 | 5 | .518 |
| TNC count x 10^7/kg | Median (range) | 2.7 (1.4-8.1) | 2.5 (1.8-4.3) | 2.7 (1.4-6.3) | 2.6 (1.4-8.1) | .051 |
| | <2 | 78 | 5 | 53 | 20 | .164 |
| | ≥2, <2.5 | 417 | 36 | 294 | 87 | .164 |
| | ≥2.5 | 815 | 39 | 612 | 164 | .164 |
| | Missing | 37 | 2 | 26 | 9 | .164 |
| CD34 cells (x10^5/kg) | Median (range) | .8 (1.5-2.5) | .7 (1.2-2.5) | .9 (1.5-2.5) | .8 (1.4-2.5) | .001 |
| | <.5 | 162 | 12 | 112 | 38 | .001 |
| | ≥.5, <1.0 | 679 | 53 | 476 | 150 | .001 |
| | ≥1.0 | 482 | 15 | 381 | 86 | .001 |
| | Missing | 24 | 2 | 16 | 6 | .001 |
| ABO matching | Match | 492 | 32 | 361 | 99 | .413 |
| | Minor mismatch | 331 | 21 | 243 | 67 | .413 |
| | Major mismatch | 348 | 25 | 251 | 72 | .413 |
| | Bidirectional mismatch | 176 | 4 | 130 | 42 | .413 |
| Number of allele mismatches at HLA-A, -B, -C, -DRB1 | 0 | 82 | 82 | 0 | 0 | .518 |
| | 1 | 154 | 0 | 148 | 6 | .518 |
| | 2 | 252 | 0 | 201 | 51 | .518 |
| | 3 | 565 | 0 | 471 | 94 | .518 |
| | 4 | 294 | 0 | 165 | 129 | .518 |
| Conditioning regimen | Myeloablative | 776 | 33 | 574 | 169 | .565 |
| | Reduced intensity | 570 | 49 | 410 | 111 | .565 |
| | Missing | 1 | 0 | 1 | 0 | .565 |
| GVHD prophylaxis | Calcineurin inhibitor + MTX | 707 | 42 | 513 | 152 | .565 |
| | Calcineurin inhibitor + MMF | 430 | 23 | 314 | 93 | .565 |
| | Others | 208 | 17 | 157 | 34 | .565 |
| | Missing | 1 | 0 | 1 | 0 | .565 |
| Year of transplant | 2005-2009 | 278 | 18 | 197 | 63 | .630 |
| | 2010-2014 | 1069 | 64 | 788 | 217 | .630 |

MTX indicates methotrexate; MMF, mycophenolate mofetil.
62% (95% CI, 56% to 67%), respectively (Gray’s test, *P* = .016) (Figure 1). In the multivariate analysis, the group with 0-haplotype matches was associated with worse platelet engraftment compared with the group with 1-haplotype match (0 matches versus 1 match: HR, .84; *P* = .044), whereas the group with 2-haplotype matches was significantly associated with better platelet engraftment (2 matches versus 1 match: HR, 1.49; *P* = .007) (Table 3, Supplementary Table 2). Worse platelet engraftment in the group with 0-haplotype matches was more apparent in patients with double mismatches at the same locus (0 matches + no double mismatch versus 1 match: HR, .89 [95% CI, 0.76 to 1.02] *P* = .222; 0 matches + double mismatch versus 1 match: HR, .76 [95% CI, 0.67 to 0.88] *P* = .016; trend *P* = .033 for these 3 groups) (Supplementary Table 1).

**Table 2**
Effect of Haplotype Matching on Neutrophil Engraftment

| Variable            | Subclass                          | HR (95% CI)       | *P*  |
|---------------------|-----------------------------------|-------------------|------|
| Haplotype           | 0 matches (vs. 1 match)           | .88 (.76-.102)    | .087 |
|                     | 2 matches (vs. 1 match)           | 1.29 (1.10-1.52)  | <.001|
| HLA mismatch        | 0-1 mismatches (vs. 2 mismatches) | 1.12 (.98-1.27)   | .086 |
| Patient sex         | Male (vs. female)                 | .82 (.73-.93)     | .01  |
| CD34 cells, \(x10^5/\)kg | \(\geq 5\) (vs. \(< 5\)) | 1.29 (1.10-1.52)  | .002 |
| Year of transplant  | 2010-2014 (vs. 2005-2009)         | 1.20 (1.03-1.40)  | .018 |
| Performance status  | 2-4 (vs. 0-1)                     | .65 (.55-.78)     | <.001|
| ABO matching        | Mismatch (vs. match)              | 1.14 (1.01-1.29)  | .034 |
| GVHD prophylaxis    | Calcineurin inhibitor + MMF       | 1.24 (1.09-1.42)  | .001 |
|                     | (vs. calcineurin inhibitor + MTX) |                   |      |
|                     | Others (vs. calcineurin inhibitor + MTX) | 1.14 (.95-1.38) | .156 |
| Disease risk        | High risk (vs. standard risk)     | .83 (.73-.93)     | .002 |

**Overall and Disease-Free Survival**

Overall survival at 2 years was 34% (95% CI, 22% to 45%), 45% (95% CI, 42% to 49%), and 41% (95% CI, 35% to 47%) for the haplotype 2, 1, and 0 match groups, respectively (log-rank test, *P* = .214) (Figure 2). Haplotype matching did not have any effect on overall survival in the multivariate analysis (Table 3, Supplementary Table 2). Likewise, disease-free survival at 2 years was 27% (95% CI, 17% to 38%), 38% (95% CI, 35% to 41%), and 38% (95% CI, 32% to 44%) for the haplotype 2, 1, and 0 match groups, respectively (log-rank test, *P* = .106) (Figure 2). Complete haplotype matching was associated with worse disease-free survival in the multivariate analysis (Table 3, Supplementary Table 2).

**Relapse and Nonrelapse Mortality**

Relapse incidence at 2 years was 54% (95% CI, 42% to 64%), 37% (95% CI, 33% to 40%), and 32% (95% CI, 27% to 38%) for the
haplotype 2, 1, and 0 match groups, respectively (Gray’s test, $P = .001$) (Figure 3). In the multivariate analysis, the group with 2-haplotype matches was significantly associated with a higher risk of relapse than the group with 1-haplotype match (HR, 1.69; $P = .001$), whereas there was no difference in the risk of relapse between the groups with 0 matches and 1 match (Table 3, Supplementary Table 2).

Nonrelapse mortality incidence at 2 years was 19% (95% CI, 11% to 29%), 25% (95% CI, 23% to 28%), and 30% (95% CI, 24% to 36%) for the haplotype 2, 1, and 0 match groups, respectively (Gray’s test, $P = .071$) (Figure 3). In the multivariate analysis, the group with 2-haplotype matches was significantly associated with a lower risk of nonrelapse mortality than the group with 1-haplotype match, whereas there was no difference in the risk of nonrelapse mortality between the groups with no match and 1 match (Table 3, Supplementary Table 2).

TABLE 3
Effect of Haplotype Matching on Transplant Outcomes

| Outcome                  | Haplotype Matching | HR (95% CI)          | $P$  |
|--------------------------|--------------------|----------------------|------|
| Neutrophil engraftment   |                    |                      |      |
| 0 matches (vs. 1 match)  | .88 (.76-1.02)     | .067                 |      |
| 2 matches (vs. 1 match)  | 1.39 (1.10-1.76)   | .005                 |      |
| Overall mortality        |                    |                      |      |
| 0 matches (vs. 1 match)  | 1.15 (.96-1.38)    | .128                 |      |
| 2 matches (vs. 1 match)  | 1.22 (.90-1.63)    | .195                 |      |
| Relapse                  |                    |                      |      |
| 0 matches (vs. 1 match)  | .99 (.79-1.25)     | .950                 |      |
| 2 matches (vs. 1 match)  | 1.69 (1.25-2.30)   | .001                 |      |
| Nonrelapse mortality     |                    |                      |      |
| 0 matches (vs. 1 match)  | 1.21 (.94-1.56)    | .146                 |      |
| 2 matches (vs. 1 match)  | .66 (.38-1.17)     | .157                 |      |
| Grades II-IV acute GVHD  |                    |                      |      |
| 0 matches (vs. 1 match)  | 1.12 (0.89-1.40)   | .344                 |      |
| 2 matches (vs. 1 match)  | .63 (0.40-0.99)    | .043                 |      |
| Grades III-IV acute GVHD |                    |                      |      |
| 0 matches (vs. 1 match)  | .88 (0.58-1.34)    | .560                 |      |
| 2 matches (vs. 1 match)  | .36 (0.13-0.97)    | .043                 |      |
| Chronic GVHD             |                    |                      |      |
| 0 matches (vs. 1 match)  | 1.30 (0.98-1.73)   | .067                 |      |
| 2 matches (vs. 1 match)  | .60 (0.32-1.11)    | .104                 |      |

**Acute and Chronic GVHD**

The cumulative incidence of grades II to IV and III to IV acute GVHD at day 100 was 23% (95% CI, 15% to 33%) and 5% (95% CI, 2% to 11%) for the 2-haplotype match, 35% (95% CI, 32% to 38%) and 11% (95% CI, 10% to 14%) for the 1-haplotype match, and 38% (95% CI, 32% to 44%) and 10% (95% CI, 7% to 14%) for the 0-haplotype match groups, respectively (Gray’s test, grades II to IV acute GVHD, $P = .033$; grades III to IV acute GVHD, $P = .176$) (Figure 4). In the multivariate analysis, the group with 2-haplotype matches was significantly associated with a lower risk of grades II to IV acute GVHD than the group with 1-haplotype match, whereas there was no difference in the risk of acute GVHD between the groups with 0 matches and 1 match (Table 3, Supplementary Table 2).
The cumulative incidence of chronic GVHD at 2 years was 17% (95% CI, 9% to 28%) for 2-haplotype matches, 27% (95% CI, 24% to 31%) for 1-haplotype match, and 33% (95% CI, 27% to 40%) for 0-haplotype matches, respectively (chronic GVHD, P = .035) (Figure 4). In the multivariate analysis, the group with 0-haplotype matches was marginally associated with a higher risk of chronic GVHD than the group with 1-haplotype match, whereas there was no difference in the risk of chronic GVHD between the groups with 2 matches and 1 match (Table 3, Supplementary Table 2).

Impact of Haplotype on Transplant Outcomes

We further investigated the impact of a high-frequency haplotype shared between donors and recipients on transplant outcomes in the 1-haplotype match group. In the group with 1-haplotype match (n = 985), the top 3 shared haplotypes were “A*24:02-B*52:01-C*12:02-DRB1*15:02” (HP-P1, n = 289), “A*33:03-B*44:03-C*14:03-DRB1*13:02” (HP-P2, n = 167), and “A*24:02-B*07:02-C*07:02-DRB1*01:01” (HP-P3, n = 144). The impacts of the HP-P1, HP-P2, and HP-P3 groups on transplant outcomes were compared with non-HP P1 to P3 groups. Multivariate analysis showed that the risk of grades II to IV acute GVHD was significantly lower in patients having HP-P2. The risk of relapse was significantly higher in those having HP-P1 and HP-P3 (Figure 5, Table 4).

DISCUSSION

In the present study, haplotype matching between donors and recipients was marginally associated with better neutrophil engraftment and significantly associated with better platelet engraftment. Particularly, 0-haplotype matches with double mismatches at any locus showed the worst neutrophil engraftment. In addition to HLA allele matching and CD34 cell counts, haplotype matching and presence of double mismatches should be considered in UCB selection to achieve better engraftment. To our knowledge, this is the first study to evaluate the impact of haplotype matching in single UCBT.

HLA allele matching was shown to affect overall mortality and neutrophil engraftment in both the present and previous studies [11,12]. Because more than 4 allele mismatches at HLA-A, -B, -C, and -DRB1 loci preclude the possibility of haplotype sharing, these cases were excluded from the analysis. Even among groups with 1 to 4 allele mismatches, haplotype matching showed better neutrophil and platelet engraftment. There are 2 possible underlying mechanisms for better engraftment with shared haplotypes: avoiding double mismatches at the same locus by matching 1 haplotype and/or the effect of common haplotypes that encode genes/single-nucleotide polymorphisms that reduce the risk of graft failure. First, to evaluate the impact of double mismatches at the same locus on outcomes, we divided the group with 0-haplotype matches into 2 groups according to the presence of double mismatches.
This classification revealed that among the 0-haplotype match group, the presence of a double mismatch at any locus may have had a mild impact on neutrophil and platelet engraftment.

We further evaluated the impact of a common haplotype itself on transplant outcomes. In the 1-haplotype match group, 3 common haplotypes were found in 61% of patients. Interestingly, the second most frequent haplotype (HP-P2) in Japan was associated with a lower risk of acute GVHD compared with less common haplotypes. Further, the relapse risk was high in patients with HP-P1, HP-P2, or HP-P3 (HP-P1-3) compared with less common haplotypes. Morishima et al. [38] showed that common Japanese HLA haplotypes were extraordinarily conserved from HLA-A to -DPB1, and HP-P2 was significantly associated with a lower risk of grades II to IV acute GVHD and marginally associated with a higher risk of relapse in unrelated bone marrow transplant. A potential explanation for the common findings regarding the effect of haplotype in UCBT and unrelated bone marrow transplant may be that there are important genes in addition to HLA or single-nucleotide polymorphisms in the haplotype related to the immune response, although no genes or single-nucleotide polymorphisms have

---

**Figure 4.** Cumulative incidences of grades II to IV (A and B) and III to IV acute GVHD (C and D) and chronic GVHD (E and F) according to haplotype matching.
been reported to regulate immune responses related to high-frequency haplotypes. Combinations of alleles of the HLA haplotype may also be related to GVHD. Specific alleles are shown to be associated with GVHD and mortality in Japanese populations [39], although the HLA alleles in HP-P2 have not been reported. The higher relapse risk for HP-P1 to P3 than for less common haplotypes might be explained by HLA-DPB1 matching. A previous study showed that an HLA-DPB1 mismatch was associated with a low risk of relapse in single UCBT [40]. Because common Japanese HLA haplotypes were conserved from HLA-A to -DPB1 [38], HLA-DPB1 matching might be more frequently observed in patients with HP-P1 to P3 than in those with less common haplotypes among the 1-haplotype match group.

In this study, the group with 2-haplotype matches showed the highest rates of neutrophil and platelet engraftment and the lowest rate of acute GVHD. However, surprisingly, this group showed the highest risk of relapse and a tendency of low overall mortality. Eight of 8 matching is considered to be most important because it increases the probability of neutrophil engraftment and decreases the risk of acute GVHD [11-13]. HLA matching showed the lowest overall mortality among previous studies in Western countries. Previous studies

### Figure 5

Cumulative incidences of neutrophil (A) and platelet engraftment (B) and grades II to IV acute GVHD (C), probability of overall survival (D), and cumulative incidences of relapse (E) and nonrelapse mortality (F) according to high-frequency haplotype.
Table 4
Effect of High-Frequency Haplotypes on Outcomes

| Outcome                  | Haplotype Matching | HR (95% CI) | P     |
|--------------------------|--------------------|-------------|-------|
| Neutrophil engraftment   |                    |             |       |
| HP-P1 (vs. non-HP-P1-P3) | .85 (.72-1.01)     | .062        |
| HP-P2 (vs. non-HP-P1-P3) | .91 (.76-1.09)     | .332        |
| HP-P3 (vs. non-HP-P1-P3) | .84 (.67-1.05)     | .117        |
| Platelet engraftment     |                    |             |       |
| HP-P1 (vs. non-HP-P1-P3) | .85 (.70-1.03)     | .105        |
| HP-P2 (vs. non-HP-P1-P3) | .86 (.70-1.07)     | .173        |
| HP-P3 (vs. non-HP-P1-P3) | .81 (.64-1.03)     | .090        |
| Grades II-IV acute GVHD  |                    |             |       |
| HP-P1 (vs. non-HP-P1-P3) | .88 (.69-1.13)     | .311        |
| HP-P2 (vs. non-HP-P1-P3) | .56 (.40-0.79)     | .001        |
| HP-P3 (vs. non-HP-P1-P3) | .49 (.34-1.22)     | .457        |
| Overall mortality        |                    |             |       |
| HP-P1 (vs. non-HP-P1-P3) | 1.19 (.97-1.47)    | .099        |
| HP-P2 (vs. non-HP-P1-P3) | 1.05 (.82-1.35)    | .696        |
| HP-P3 (vs. non-HP-P1-P3) | 1.09 (.84-1.41)    | .504        |
| Relapse                  |                    |             |       |
| HP-P1 (vs. non-HP-P1-P3) | 1.26 (.97-1.64)    | .078        |
| HP-P2 (vs. non-HP-P1-P3) | 1.36 (1.01-1.83)   | .045        |
| HP-P3 (vs. non-HP-P1-P3) | 1.30 (.95-1.77)    | .105        |
| Nonrelapse mortality     |                    |             |       |
| HP-P1 (vs. non-HP-P1-P3) | .91 (.67-1.24)     | .553        |
| HP-P2 (vs. non-HP-P1-P3) | .83 (.57-1.21)     | .334        |
| HP-P3 (vs. non-HP-P1-P3) | .92 (.63-1.36)     | .682        |

HP-P1, most frequent haplotype of “A*24:02-B*52:01-C*07:02-DRB1*15:02”; HP-P2, the second most frequent haplotype of “A*33:03-B*44:03-C*14:03-DRB1*13:02”; HP-P3, the third most frequent haplotype of “A*24:02-B*07:02-C*07:02-DRB1*01:01”.  

showed that the risk of GVHD in Japan is lower than those in the white population or other countries after HLA-matched sibling or unrelated transplants [41-43]. Less immunologic reaction in HLA-matched UCBT could affect the risk of relapse in Japanese populations, and complete haplotype matching should be avoided, particularly for patients with hematologic malignancies with a high risk of relapse.

This study has several limitations that are inherent to a retrospective analysis. First, haplotype was estimated based on the haplotype frequency in Japan [28]. Although the actual accuracy was 95%, there will still be misclassifications, and this would reduce the power to detect true differences between the groups. Although haplotypes were estimated with high probability for 46% of the total cohorts, this could limit the external validation for cases in which the donor or recipient HLA haplotype cannot be determined. Therefore, we evaluated neutrophil engraftment and survival between cases where haplotypes for both the recipient and donor were determined (n = 1347) and those where haplotypes for either the recipient or donor were not determined (n = 1611). We did not find a significant difference between the 2 groups (data not shown), which suggested that the bias arising from haplotype estimation could be minimal. In future studies, haplotypes should be determined using next-generation sequencing. Second, the heterogeneous backgrounds of patients may have resulted in a statistical bias, although we tried to reduce this bias by adjusting the impact in multivariate analyses. Third, the information on the presence of HLA antibody was available in only 18% of the cohort. Therefore, we could not assess the impact of donor-specific HLA antibody. Fourth, common haplotypes and haplotype frequencies differ according to region and race. It may be important to assess and compare the impact of haplotype matching on outcomes among various regions and races. Because haplotypes are more diverse in Western countries, this observation will probably have a little practical application for the criteria of cord blood donor selection in other populations than Japanese. Fifth, the number of allele mismatches or CD34 cell doses could influence the effect of haplotype matching, but a stratified analysis and its interpretation could be limited because of the smaller sample size in each category. However, in a stratified analysis the effect of haplotype matching was evident in the 3 or 4 allele mismatched groups (data not shown), which might indicate the importance of haplotype matching, particularly in multiple allele mismatched UCBT. Further, the effect of haplotype matching was not observed in patients in the highest quartiles of CD34 cell dose (data not shown). This suggested that the adverse effect of haplotype mismatching could be overcome by increasing the dose of CD34 cells. Finally, we excluded patients with more than 4 allele mismatches and those whose haplotype was not determined, which could reduce the power to evaluate the impact of a double mismatch or combinations on outcomes. This should be evaluated in a future study.

In conclusion, the present study revealed that in addition to HLA allele matching and CD34 cell counts, HLA haplotype matching may influence engraftment. Double mismatches at some loci might also impact outcomes. Further, haplotypes themselves may be associated with better transplant outcomes. These points should be considered when selecting appropriate UCB units.

ACKNOWLEDGMENTS
The authors are indebted to all physicians and data managers who contributed valuable transplant data to the Japan Society for Hematopoietic Cell Transplantation (JSHCT). The authors also thank the members of the data management committee of the JSHCT for their assistance.

Financial disclosure: This work was supported in part by the Takeda Science Foundation (to J.K.) and the Practical Research Project for Allergic Diseases and Immunology (Research Technology of Medical Transplantation) from the Japan Agency for Medical Research and Development, AMED (to Y.A. and J.K.).

Conflict of interest statement: J.T.: Research funding, Bristol-Myers Squibb; honoraria, Novartis Pharma, Bristol-Myers Squibb, Otsuka, Pfizer. T.I.: Research funding, Astellas Pharma, Chugai Pharmaceutical Co., CSL Behring, Eisai Co., Kyowa Hakko Kirin Co., Ono Pharmaceutical Co., Pfizer, Nippon Shiroyaku Co., MSD, Otsuka Pharmaceutical Co., Repertoire Genesis Inc., Sumitomo Dainippon Pharma Co., Taiho Pharmaceutical Co., Takeda Pharmaceutical Co., Zenyaku Kogyo Co.; honoraria, Alexion Pharmaceuticals, Bristol-Myers Squibb, Celgene, JCR Pharmaceuticals, Janssen Pharmaceutical K.K., Mundipharma, Novartis.

Authorship statement: J.K. designed the research, organized the project, performed the statistical analysis, and analyzed the data. T.K., H.T., H.K,Y.M., and S.M. analyzed and interpreted the data. N.U., K.N., Y.M., T.O., M.O., T.K., S.T., S.M., H.K., T.E., J.T., T.I., and Y.A. gathered and organized the data. J.K. wrote the first draft, and all other authors contributed to the final version.

SUPPLEMENTARY MATERIALS
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.09.035.
REFERENCES

1. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med*. 2004;351:2276–2285.
2. Laughlin MJ, Eapen M,Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med*. 2004;351:2265–2275.
3. Atsuta Y, Suzuki K, Nagamura-Inoue T, et al. Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. *Blood*. 2009;113:1631–1638.
4. Cohen YC, Scaradavou A, Stevens CE, et al. Factors affecting mortality following myeloablative cord blood transplantation in adults: a pooled analysis of three international registries. *Bone Marrow Transplant*. 2011;46:70–76.
5. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Biol Blood Marrow Transplant*. 2013;19:491–498.
6. Terakura S, Atsuta Y, Tsukada N, et al. Comparison of outcomes of 8/8 and 7/8 allele-matched unrelated bone marrow transplantation and single-unit cord blood transplantation in adults with acute leukemia. *Biol Blood Marrow Transplant*. 2016;22:330–338.
7. Gratwohl A, Pasquini MC, Aljurf M, et al. One million haemopoietic stem-cell transplants: a retrospective observational study. *Lancet Haematol*. 2015;2:e91–e100.
8. Atsuta Y, Kanda J, Takanashi M, et al. Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia. *Haematologica*. 2013;98:814–822.
9. Rocha V, Gluckman E. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. *Br J Haematol*. 2009;147:262–274.
10. Barker JN, Kurtzberg J, Ballen K, et al. Double unrelated reduced-intensity donor hematopoietic stem cell transplantation in ethnic populations. *Blood*. 2010;115:1685–1694.
11. Eapen M, Klein JP, Sanz G, et al. Effect of donor-recipient HLA matching at HLA A, B, C, and DRB1 on outcomes after umbilical-cord blood transplantation for leukemia and myelodysplastic syndrome: a retrospective analysis. *Lancet Oncol*. 2011;12:1214–1221.
12. Eapen M, Klein JP, Ruggeri A, et al. Impact of allele-level HLA matching on outcomes after myeloablative single unit umbilical cord blood transplantation for hematologic malignancy. *Blood*. 2014;123:133–140.
13. Barker JN, Scaradavou A, Stevens CE. Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1081 cord blood recipients with hematologic malignancies. *Blood*. 2010;115:1843–1849.
14. Wagner Jr, JE, Eapen M, Carter S, et al. One-unit versus two-unit cord-blood transplantation for hematologic cancers. *N Engl J Med*. 2014;371:1685–1694.
15. Sanz J, Wagner JE, Sanz MA, et al. Myeloablative cord blood transplantation in adults with acute leukemia: comparison of two different transplant platforms. *Biol Blood Marrow Transplant*. 2013;19:1725–1730.
16. Eapen M, Rocha V, Sanz G, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. *Lancet Oncol*. 2010;11:653–660.
17. Kanda J, Nakasone H, Atsuta Y, et al. Risk factors and organ involvement of chronic GVHD in Japan. *Bone Marrow Transplant*. 2014;49:228–235.
18. Murata M, Nakasone H, Kanda J, et al. Clinical factors predicting the response of acute graft-versus-host disease to corticosteroid therapy: an analysis from the GVHD Working Group of the Japan Society for Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant*. 2013;19:1183–1189.
19. Konuma T, Kato S, Ohwa-Monma M, et al. Cryopreserved CD34+ cell dose, but not total nucleated cell dose, influences hematopoietic recovery and extensive chronic graft-versus-host disease after single-unit cord blood transplantation in adult patients. *Biol Blood Marrow Transplant*. 2017;23:1142–1150.
20. Nakasone H, Tabuchi K, Uchida N, et al. Which is more important for the selection of cord blood units for hematopoietic stem cell transplantation: the number of CD34-positive cells or total nucleated cells? *Br J Haematol*. 2019;185:166–169.
21. Hough R, Daniil R, Russell N, et al. Recommendations for a standard UK approach to incorporating umbilical cord blood into clinical transplantation practice: an update on cord blood unit selection, donor selection algorithms and conditioning protocols. *Br J Haematol*. 2016;172:360–370.
22. Ballen KK, Spitzer TR, Yeap BY, et al. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. *Biol Blood Marrow Transplant*. 2007;13:82–89.
23. Takashita M, Atsuta Y, Fujikawa K, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. *Blood*. 2010;116:2839–2846.
24. Yamamoto H, Uchida N, Matsuho N, et al. 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. *Biol Blood Marrow Transplant*. 2014;20:1634–1640.
25. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol*. 2007;86:269–274.
26. Atsuta Y. Introduction of Transplant Registry Unified Management Program 2 (TRUMP2): scripts for TRUMP data analyses, part I (variables other than HLA-related data). *Int J Hematol*. 2016;103:3–10.
27. Kanda J. Scripts for TRUMP data analyses. Part II (HLA-related data): statistical analyses specific for hematopoietic stem cell transplantation. *Int J Hematol*. 2016;103:11–19.
28. Ikeda N, Kojima H, Nishikawa M, et al. Determination of HLA-A, -C, -DRB1 allele and haplotype frequency in Japanese population based on family study. *Tissue Antigen*. 2015;85:252–259.
29. Przepiórka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading, *Bone Marrow Transplant*. 1995;15:825–828.
30. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol*. 1991;28:250–259.
31. Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimens for chronic GVHD: wide dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Blood Marrow Transplant*. 2009;15:367–369.
32. Kanda J, Ichinose T, Fuji S, et al. Impact of HLA mismatch direction on the outcome of unrelated bone marrow transplantation: a retrospective analysis from the Japan Society for Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant*. 2015;21:305–311.
33. Kanda J, Atsuta Y, Wake A, et al. Impact of the direction of HLA mismatch on transplantation outcomes in single unrelated cord blood transplantation. *Biol Blood Marrow Transplant*. 2013;19:247–254.
34. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695–708.
35. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141–1154.
36. Fine JP, Gray RJ. A proportional hazards model for subdividing of a competing risk. *J Am Stat Assoc*. 1999;94:566–590.
37. Kanda Y. Investigation of the freely available easy-to-use software “EZR” for medical statistics. *Bone Marrow Transplant*. 2013;48:452–458.
38. Morishima S, Ogawa S, Matsubara A, et al. Impact of highly conserved HLA haplotype on acute graft-versus-host disease. *Blood*. 2010;115:4644–4670.
39. Morishima S, Kashikawa K, Matsuo K, et al. High-risk HLA alleles for severe acute graft-versus-host disease and mortality in unrelated donor bone marrow transplantation. *Haematologica*. 2016;101:491–498.
40. Yabe T, Azuma F, Kashikawa K, et al. HLA-DPB1 mismatch induces a graft-versus-leukemia effect without severe acute GVHD after single-unit umbilical cord blood transplantation. *Leukemia*. 2018;32:168–175.
41. Kanda J, Brazauskas R, Hu ZH, et al. Graft-versus-host disease after HLA-mismatch sibling bone marrow or peripheral blood stem cell transplantation: comparison of Northern American Caucasian and Japanese populations. *Biol Blood Marrow Transplant*. 2016;22:744–751.
42. Morishima Y, Kawase T, Malik K, et al. Significance of ethnicity in the risk of acute graft-versus-host disease and leukemia relapse after unrelated donor hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2013;19:1197–1203.
43. Oh H, Iberzina Jr, FR, Zhang MJ, et al. Comparison of graft-versus-host-disease and survival after HLA-identical sibling bone marrow transplantation in ethnic populations. *Blood*. 2005;105:1408–1416.