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# Significance of Ethnicity in the Risk of Acute Graft-versus-Host Disease and Leukemia Relapse after Unrelated Donor Hematopoietic Stem Cell Transplantation

Yasuo Morishima [1,2,*], Takakazu Kawase [1,2], Mari Malkki [3], Satoko Morishima [1,4], Stephen Spellman [5], Koichi Kashiwase [1,6], Shunichi Kato [1,7], Anne Cesbron [8], Jean-Marie Tiercy [9], David Senitzer [10], Andrea Velardi [11], Effie W. Petersdorf [3], on behalf of the International Histocompatibility Working Group in Hematopoietic Cell Transplantation

[1] *Japan Marrow Donor Program, Tokyo, Japan*
[2] *Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan*
[3] *Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, Washington*
[4] *Department of Hematology, Fujita Health University School of Medicine, Toyoake, Japan*
[5] *Center for International Blood and Marrow Transplant Research, Minneapolis, Minnesota*
[6] *Japanese Red Cross Kanto-Koshinetsu Block Blood Center, Tokyo, Japan*
[7] *Department of Cell Transplantation & Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan*
[8] *Laboratoire d'Histocompatibilité, EFS Pays de la Loire, Nantes, France*
[9] *LNRH/Transplantation Immunology Unit, University Hospital, Geneva, Switzerland*
[10] *City of Hope National Cancer Center, Duarte, California*
[11] *European Group for Blood and Marrow Transplantation, Perugia, Italy*

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A B S T R A C T

The significance of patient and donor ethnicity on risk of acute graft-versus-host disease (GVHD) and disease relapse after unrelated donor hematopoietic cell transplantation (HCT) is not known. A total of 4335 patient–donor pairs from the International Histocompatibility Working Group in HCT met the following 3 criteria: (1) HLA-A, -B, -C, -DRB1, and -DQB1 allele matched donor, (2) diagnosis of leukemia, and (3) non–T cell depleted GVHD prophylaxis. Posttransplantation risks of acute GVHD and leukemia relapse were defined in Asian/Pacific Islander, white, African American, Hispanic, and Native American patients that underwent transplantation from donors with the same self-described background. Asian patients had a significantly lower incidence of acute GVHD (Japanese patients: 40.0% grades II to IV and 15.3% grades III to IV; non-Japanese Asian patients: 42.1% grades II to IV and 15.7% grades III to IV) compared with white patients (56.5% grades II to IV and 22.6% grades III to IV) ($P < .001$). The hazard ratio of acute GVHD for white patients was significantly higher than for Japanese patients. Unexpectedly, the hazard ratio of leukemia relapse in white patients with early disease status was also significantly higher than that in Japanese patients. These results provide a platform for future investigation into the genetic factors for unrelated donor HCT and clinical implications of diverse ethnic background.



## INTRODUCTION

Patients who lack a matched sibling to serve as the transplantation donor benefit from hematopoietic cell transplantation (HCT) from an HLA matched unrelated donor [1-6]. In both settings, donor recognition of recipient polymorphisms may lead to acute graft-versus-host disease (GVHD), a potentially life-threatening complication that necessitates long-term immunosuppressive therapy. HLA identical siblings are identical by descent; hence, acute GVHD arises from donor recognition of non-HLA polymorphisms located outside the HLA region [7]. Criteria for unrelated donor selection is based on compatibility for alleles of HLA-A, -C, -B, -DRB1, and -DQB1 genes because matching is associated with lower risks of acute GVHD than HLA mismatching. Acute GVHD after HLA matched unrelated donor HCT may result from donor recognition of genome-wide polymorphisms, including undetected variation within the HLA region [8,9].

Clinical outcome after HCT depends on many factors contributed by the patient, the donor, and the specific transplantation procedures used. In HLA matched sibling transplantation, patient ethnicity has been reported to influence the incidence of GVHD where white Americans, African Americans, and Irish cohorts were at significantly higher risk for acute GVHD than were Japanese or Scandinavian cohorts [10]. The genetic basis that might explain these different outcomes has not been defined. The distribution of HLA alleles is reflected in the ethnicity of the population [11], and the probability of finding an HLA matched unrelated donor is highest when the donor and patient share the same ethnicity. The results of transplantation from selected populations have been extensively reported. In the US population of white patients receiving a transplantation from a white unrelated donor [4], the incidence of grades III to IV acute GVHD is 28% compared with 11.8% observed in the Japanese experience [5]. These data individually suggest that the risk of acute GVHD after unrelated donor HCT depends on the patient's and donor's ethnic backgrounds; however, a formal comparison of outcomes among patients with different ethnic backgrounds has never been undertaken. If the complications after

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* Correspondence and reprint requests: Yasuo Morishima, MD, Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan.
*E-mail address:* ymorisim@aichi-cc.jp (Y. Morishima).

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**Table 1**
Characteristics of Hematopoietic Cell Transplantation from Unrelated Donors by Ethnicity

| | Total Number of Pairs | Ethnicity of Patient–Donor Pair | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Japanese* | Asian Excluding Japanese | White | African American | Hispanic | Native American | Mismatched Ethnicity | Unknown Ethnicity |
| Number of pairs | 4335 | 1734 | 19 | 1794 | 34 | 27 | 1 | 171 | 555 |
| HLA-DPB1 matching status (GVH direction) | | | | | | | | | |
| Match | 1053 | 622 | 8 | 320 | 2 | 9 | 0 | 27 | 65 |
| One allele mismatch | 1530 | 798 | 3 | 553 | 12 | 3 | 0 | 45 | 116 |
| Two allele mismatch | 818 | 307 | 1 | 383 | 3 | 5 | 1 | 38 | 80 |
| Unknown | 934 | 7 | 7 | 538 | 17 | 10 | 0 | 61 | 294 |
| Patient age, mean | 33.8 | 30.4 | 33.9 | 36.5 | 34.1 | 30.4 | — | 35.3 | 35.4 |
| Donor age, mean | 34.7 | 34.3 | 35.4 | 35.1 | 38.9 | 34.0 | — | 35.1 | 34.6 |
| Disease | | | | | | | | | |
| ALL | 1232 | 620 | 11 | 424 | 8 | 9 | 0 | 41 | 119 |
| AML | 1758 | 709 | 4 | 703 | 11 | 8 | 0 | 83 | 240 |
| CML | 1345 | 405 | 4 | 667 | 15 | 10 | 1 | 47 | 196 |
| Disease risk† | | | | | | | | | |
| Low | 938 | 273 | 3 | 480 | 5 | 7 | 1 | 29 | 140 |
| Intermediate | 2441 | 1093 | 10 | 910 | 19 | 16 | 0 | 94 | 299 |
| High | 931 | 352 | 6 | 399 | 10 | 4 | 0 | 46 | 114 |
| Unknown | 25 | 16 | 0 | 5 | 0 | 0 | 0 | 2 | 2 |
| Patient–donor sex | | | | | | | | | |
| Female–male | 756 | 302 | 3 | 308 | 9 | 10 | 1 | 33 | 90 |
| Male–female | 944 | 341 | 6 | 435 | 5 | 4 | 0 | 45 | 108 |
| Female–female | 811 | 335 | 3 | 324 | 9 | 3 | 0 | 39 | 98 |
| Male–male | 1789 | 752 | 7 | 725 | 11 | 10 | 0 | 54 | 230 |
| Unknown | 35 | 4 | 0 | 2 | 0 | 0 | 0 | 0 | 29 |
| GVHD prophylaxis | | | | | | | | | |
| Cyclosporine based | 2593 | 964 | 9 | 1167 | 19 | 12 | 1 | 97 | 324 |
| Tacrolimus based | 1536 | 757 | 10 | 592 | 15 | 14 | 0 | 70 | 78 |
| Other | 78 | 8 | 0 | 23 | 0 | 1 | 0 | 4 | 42 |
| Unknown | 128 | 5 | 0 | 12 | 0 | 0 | 0 | 0 | 111 |
| Conditioning regimen | | | | | | | | | |
| Myeloablative | 3687 | 1631 | 18 | 1545 | 27 | 25 | 1 | 149 | 291 |
| Nonmyeloablative/ reduced intensity | 381 | 103 | 1 | 219 | 7 | 2 | 0 | 21 | 28 |
| Unknown | 267 | 0 | 0 | 30 | 0 | 0 | 0 | 1 | 236 |
| Total body irradiation | | | | | | | | | |
| No | 949 | 315 | 1 | 423 | 10 | 5 | 0 | 44 | 151 |
| Yes | 3371 | 1419 | 18 | 1360 | 23 | 22 | 1 | 125 | 403 |
| Unknown | 15 | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 1 |
| Stem cell source | | | | | | | | | |
| Bone marrow | 3481 | 1734 | 9 | 1272 | 24 | 19 | 1 | 105 | 317 |
| Peripheral blood stem cells | 854 | 0 | 10 | 522 | 10 | 8 | 0 | 66 | 238 |
| Transplanted yr (median) | — | 1993-2005 (2000) | 1991-2005 (2000) | 1984-2007 (1999) | 1991-2005 (2000) | 1996-2006 (2001) | — | — | — |

* Japanese include only individuals from the Japan Marrow Donor Program.
† Disease status before transplantation is categorized as low (CP of CML); intermediate (the first or second CR of ALL, AML, or the second CP or accelerated phase of CML); high risk (more advanced stage than intermediate risk).

transplantation depend on donor–recipient ethnicity, then this information will facilitate future mapping of polymorphisms responsible for complications such as acute GVHD and provide insight into the genetic basis of acute GVHD. Furthermore, the information may have practical value in the search for suitable unrelated donors.

We undertook a large-scale international study to define risks after HLA matched unrelated donor HCT performed for patients with different ethnic backgrounds within the International Histocompatibility Working Group (IHWG) in HCT [12]. These data provide a unique opportunity to elucidate the clinical effects of ethnicity on risk of acute GVHD and leukemia relapse in HLA matched unrelated HCT.

## METHODS

### *Study Population*

A total of 4335 patients from the IHWG database met the following criteria and were included in the current analysis: (1) transplantation from an HLA-A, -B, -C, -DRB1, and -DQB1 allele compatible unrelated donor; (2) patient diagnosis of acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), or chronic myeloid leukemia (CML); and (3) non–T cell depleted stem cell source without the use of antithymocyte globulin for GVHD prophylaxis.

Patient characteristics by ethnicity are described in Table 1. Among these subjects, 1232 patients carried the diagnosis of ALL, 1758 AML, and 1345 CML. Low-risk disease was defined as a CML chronic phase (CP) at the time of transplantation. The definition of disease risk was comparable among clinical centers within IHWG. Intermediate risk was defined as transplantation in the first or second complete remission (CR) of ALL, AML, or the second CP or accelerated phase of CML. High risk was defined as transplantation in a more advanced stage than intermediate risk. Early status of disease included patients in first and second CR of ALL or AML at transplantation or first CP of CML at transplantation.

For GVHD prophylaxis, a tacrolimus-based regimen was used in 1536 patients, a cyclosporine-based regimen in 2593, and other regimens in 78. Patients were conditioned for transplantation using either a myeloablative (n = 3687) or a nonmyeloablative/reduced-intensity regimen (n = 381). A total of 3481 patients was transplanted with bone marrow and 854 with peripheral blood stem cells.

Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki in each registry or institution, and consent

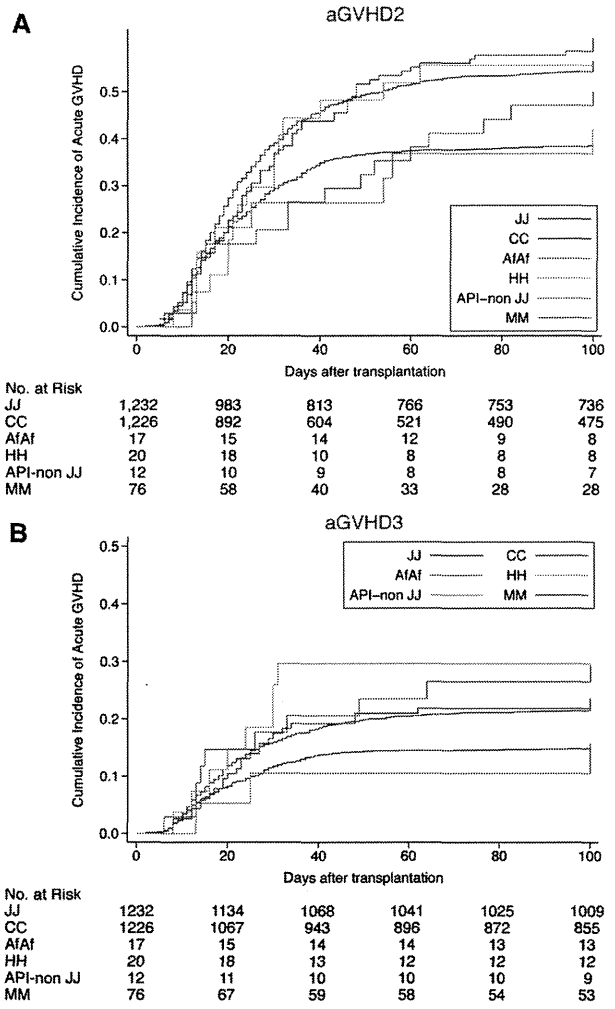


**Figure 1.** Cumulative incidence of acute GVHD by ethnicity. (A) Grades II to IV acute GVHD; (B) grades III to IV acute GVHD. JJ, Japanese donor and patient pair; CC, white donor and patient pair; AfAf, African American donor and patient pair; HH, Hispanic donor and patient pair; API non-JJ, Asian/Pacific Islander donor and patients excluding Japanese pair; MM, mismatch race/ethnicity pair between donor and patients. Incidence of acute GVHD is shown in Supplemental Table S1.

of participation with the IHWG was obtained from each participating registry or institution before registration of the data or materials. Participating registries and institutions are listed in the Supplemental Table 6.

#### *Donor–Recipient Ethnicity*

Patients were grouped as having the same, different, or unknown ethnicity from their HLA matched unrelated donor according to self-designated descriptions as defined elsewhere [13]. A total of 3609 patients had the same ethnicity as their donors ("matched ethnicity"); of these pairs, 1753 were of Asian/Pacific Islander background (of which 1734 were transplantations for Japanese pairs performed by the Japan Marrow Donor Program in Japan), 1794 white, 34 African American, 27 Hispanic, and 1 Native American (Table 1). A total of 171 patients had a different background from their unrelated donor ("mismatched ethnicity"). Most pairs with mismatched ethnicity (162 of 171) were of non-Asian backgrounds. Ethnicity information of either patient or donor was not available for 555 pairs ("unknown ethnicity").

#### *HLA Typing and HLA Matching*

High-resolution HLA typing of patient and donor pairs was performed in HLA typing laboratories or registries participating within the IHWG or in the Division of Clinical Research of Fred Hutchinson Cancer Research Center as described elsewhere [6,12]. HLA-DPB1 allele mismatching among donor–recipient pairs was scored when the recipients' alleles were not shared by the donor in GVH direction for all analyses.

#### *Biostatistical Methods*

Cumulative incidences of acute GVHD and relapse were assessed by a method described elsewhere to eliminate the effect of competing risk [14]. Overall survival was calculated using the Kaplan-Meier method. A competing event regarding acute GVHD was defined as death without acute GVHD, and a competing event regarding relapse was defined as death without relapse. A log-rank test was applied to assess the impact by the factor of interest. Multivariable Cox regression analyses were conducted to evaluate the impact of acute GVHD, leukemia relapse, and mortality after transplantation. Confounders considered were combinations of ethnicity between patient and donor, sex (donor–recipient pair), patient age (linear), donor age (linear), risk of leukemia relapse (low, intermediate, and high), GVHD prophylaxis (cyclosporine-based regimen, tacrolimus-based regimen, and the other regimen without cyclosporine and tacrolimus), source of stem cell, and preconditioning (myeloablative and nonmyeloablative/reduced intensity). Missing events were treated as an unknown group.

## RESULTS

### *Risk of Acute GVHD among Ethnicity Matched Donor–Recipient Pairs*

The cumulative incidence of acute GVHD in HLA-A, -B, -C, -DRB1, and -DQB1 matched pairs was lower in patients of Asian/Pacific Islander background (Japanese pairs: 40.0% grades II to IV and 15.3% grades III to IV; non-Japanese pairs: 42.1% grades II to IV and 15.7% grades III to IV) compared with white background (56.5% grades II to IV and 22.6% grades III to IV). The incidence of grades II to IV and III to IV acute GVHD was 50.0% and 29.6% in African American pairs, 55.5% and 29.6% in Hispanic pairs, and 60.0% and 23.5% in patients of mismatched ethnicity, respectively (Figure 1, Supplemental Table S1).

Of the 4335 pairs, 1053 were matched for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles (12/12 match). Japanese pairs (n = 622) showed a significantly lower incidence of acute GVHD than white pairs (n = 320): 30.7% and 57.0% of grades II to IV ($P < .001$) and 12.1% and 19.4% grades III to IV ($P = .001$), respectively.

Multivariate models adjusted for HLA-DPB1 match status and clinical factors (see Methods) revealed that the hazard ratio (HR) of acute GVHD in white pairs was significantly higher compared with Japanese pairs (reference group): HR 1.59 for grades II to IV ($P < .001$) and HR 1.48 for grades III to IV ($P < .001$). The HR for acute GVHD in non-Japanese Asian pairs was not significantly higher compared with Japanese pairs (reference group): HR 1.07 for grades II to IV ($P = .84$) and HR 1.03 for grades III to IV ($P = .96$) (Table 2).

### *Risk of Leukemia Relapse among Ethnicity Matched Donor–Recipient Pairs*

Patients who experience acute GVHD may have a lower risk of disease recurrence compared with patients without GVHD, a phenomenon termed the graft-versus-leukemia (GVL) effect.[15] To better define GVHD-associated risks after transplantation, we investigated the probability of disease recurrence among the ethnicity-matched group. Using the Japanese pairs as the reference, we unexpectedly found that the HR for relapse in white patients was significantly higher compared with Japanese patients (HR 1.63, $P < .001$) (Table 3). The rates of acute GVHD (grades II to IV and grades III to IV) were not associated with differences in relapse rates either in Japanese pairs or in white pairs (Supplemental Table S2).

The cumulative incidence of relapse at 5 years after transplantation was assessed according to myeloid or lymphoid lineage of the leukemia and disease risk at the time

**Table 2**
HR of Acute GVHD, Leukemia Relapse, and Mortality by Ethnicity

| | N | Grades II-IV Acute GVHD HR (95% CI) | P | Grades III-IV Acute GVHD HR (95% CI) | P | Relapse HR (95% CI) | P | Mortality HR (95% CI) | P |
|---|---|---|---|---|---|---|---|---|---|
| Ethnicity | | | | | | | | | |
| Japanese pair | 1734 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| Asian pair excluding Japanese | 19 | 1.07 (.55-2.17) | .84 | 1.03 (.32-3.23) | .96 | 2.92 (1.59-5.36) | .001 | 1.25 (.68-2.28) | .46 |
| White pair | 1794 | 1.59 (1.42-1.77) | <.001 | 1.48 (1.25-1.76) | <.001 | 1.63 (1.40-1.90) | <.001 | 1.46 (1.32-1.62) | <.001 |
| African pair | 34 | 1.21 (.74-1.98) | .43 | 1.81 (.94-3.45) | .07 | 2.35 (1.24-4.47) | .04 | 2.51 (1.67-3.76) | <.001 |
| Hispanic pair | 27 | 1.61 (.96-2.64) | .07 | 2.23 (1.09-4.53) | .03 | 3.77 (2.00-7.13) | <.001 | 2.87 (1.85-4.45) | <.001 |
| Mismatched race/ethnicity | 171 | 1.71 (1.38-2.11) | <.001 | 1.57 (1.12-2.22) | .009 | 1.80 (1.34-2.42) | <.001 | 1.62 (1.32-1.98) | <.001 |
| Unknown ethnicity | 555 | 2.38 (2.01-2.83) | <.001 | 1.48 (1.10-1.98) | .008 | 1.56 (1.21-2.00) | .005 | 1.33 (1.11-1.58) | .001 |
| HLA-DPB1 matching (GVH direction) | | | | | | | | | |
| Match | 1053 | 1.00 | | 1.00 | | 1.00 | | 1.00 | |
| One-allele mismatch | 1530 | 1.34 (1.19-1.51) | <.001 | 1.40 (1.15-1.69) | .001 | .66 (.57-.77) | <.001 | 1.02 (.91-1.13) | .69 |
| Two-allele mismatch | 818 | 1.48 (1.29-1.69) | <.001 | 1.45 (1.16-1.80) | <.001 | .54 (.44-.66) | <.001 | 1.01 (.89-1.15) | .78 |
| Unknown | 934 | 1.20 (1.03-1.40) | .01 | 1.12 (.87-1.43) | .30 | .62 (.51-.75) | <.001 | .74 (.64-.85) | <.001 |

Multivariate analysis adjusted for clinical factors listed in Table 1. Data from 1 North American pair is not shown in this table.

of transplantation (Table 3). In patients that underwent transplantation in early status of their disease (AML and ALL in the first CR or second CR, CML in the first CP), white patients had a significantly higher relapse rate than Japanese patients. In patients that underwent transplantation for more advanced status of disease, relapse rates for white patients and Japanese patients were not significantly different.

### *Risk of Survival among Ethnicity Matched Donor–Recipient Pairs*

Compared with Japanese patients, patients of white, African American, or Hispanic backgrounds and patients who received a transplantation from donors with a different background had higher mortality, with the exception of non-Japanese Asian patients (Table 2, Figure 2).

### *Factors Other than Ethnicity among Ethnicity Matched Donor–Recipient Pairs*

The increased HRs of acute GVHD and the decreased HRs of relapse in 1 or 2 HLA-DPB1 mismatched transplantations did not translate to any difference in survival by ethnicity (Table 2). The HRs of the other factors for acute GVHD, leukemia relapse, and mortality by multivariate analysis are shown in Supplemental Table S3.

Because all Japanese patients received bone marrow for the grafting source, the association of ethnicity with the HRs of acute GVHD, leukemia relapse, and mortality for patients receiving bone marrow transplantation are shown in Supplemental Tables S4 and S5. The HRs of acute GVHD, leukemia relapse, mortality, and relapse at 5 years after transplantation between Japanese pairs and white pairs

**Table 3**
Leukemia Relapse Rate by Ethnicity, Leukemia Cell Type, and Status at Transplantation

| | N | ALL | N | AML | N | CML |
|---|---|---|---|---|---|---|
| Early status* | | | | | | |
| Japanese pair | 469 | 23.9† | 465 | 20.9 | 269 | 7.3 |
| | | $P = .004$ | | $P < .001$ | | $P = .02$ |
| White pair | 298 | 30.5 | 436 | 30.7 | 478 | 10.7 |
| Advanced status‡ | | | | | | |
| Japanese pair | 124 | 54.5 | 205 | 43.7 | 123 | 24.3 |
| | | $P = .54$ | | $P = .12$ | | $P = .30$ |
| White pair | 118 | 51.0 | 249 | 48.4 | 187 | 25.8 |

* First and second CR of ALL or AML at transplantation, first CP of CML at transplantation.

† Cumulative incidence (%) of leukemia relapse at 5 yrs after transplantation.

‡ More advanced status than early status.

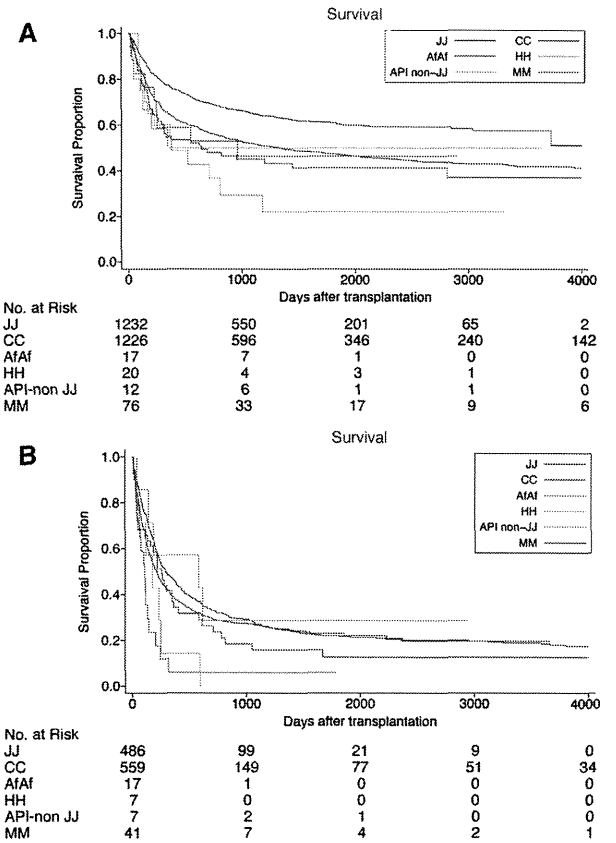


**Figure 2.** Survival after transplantation by ethnicity. (A) Patients with early status of leukemia at transplantation; (B) patients with advanced status of leukemia at transplantation. Early status: first and second CR of ALL or AML at transplantation, first CP of CML. Advanced status: more advanced status than early status. JJ, Japanese donor and patient pair; CC, white donor and patient pair; AfAf, African American donor and patient pair; HH, Hispanic donor and patient pair; API non-JJ, Asian/Pacific Islander donor and patients excluding Japanese pair; MM, mismatch ethnicity pair between donor and patients. Five-year survival rate after transplantation by ethnicity is provided in Supplemental Table S1.

were similar for recipients of bone marrow and peripheral blood stem cell transplantation.

## DISCUSSION

Donor HLA matching lowers risks of graft failure, acute GVHD, morbidity, and mortality [2-6] and remains the standard for the selection of unrelated donors for HCT. Clinical experience demonstrates that the incidence of GVHD after HLA matched unrelated HCT is not the same across different populations [4,5]. The frequency of HLA phenotypes and haplotypes differs worldwide [11,12], and information regarding the content of HLA region variation is available for selected populations [15,16]. We hypothesized that the risks of GVHD depend on population-specific genetic variation. We undertook the current analysis to compare the risks of GVHD among patients of different ethnic backgrounds. This information provides the necessary basis for future investigation of the impact of specific genetic variation on clinical outcome.

HLA antigens and haplotypes are population specific. The IHWG database is a unique resource for the comparative analysis of transplantation outcomes and HLA genetics. To analyze acute GVHD and GVL effects clinically, it is essential that nongenetic factors that also influence these transplantation-related immunological events are taken into consideration. Because HLA compatibility between the patient and donor is a well-recognized risk factor for acute GVHD and leukemia relapse in unrelated donor HCT [1-6,17,18], we restricted the study population to HLA-A, -B, -C, -DRB1, and -DQB1 allele matched pairs. GVHD prophylaxis is also another important factor for acute GVHD. Therefore, only leukemia patients that underwent transplantation from non–T cell depleted stem cell sources without antithymocyte globulin were included in the analysis. White and Asian patients whose donors were also of the same background were particularly well represented, which permitted us to assess risks between these 2 populations. A larger clinical experience will be needed to fully evaluate clinical outcome in patients and donors of African American, Hispanic, and Native American heritage.

This study shows that the incidence of acute GVHD in Japanese patients is lower than that in white patients. Although HLA-DPB1 mismatching is associated with risk of acute GVHD [2,19], ethnicity was an independent risk factor in HLA-A, -B, -C, -DRB1, and -DQB1 matched transplantations; furthermore, among pairs matched at all 6 classical loci, including HLA-DPB1, we observed a significantly higher incidence of acute GVHD in white compared with Japanese patients. Multi-single nucleotide polymorphism (SNP) analysis of the HLA region has been performed in Japanese [9,16] and white [8] populations. We surmise that the lowered risk of acute GVHD observed in the Japanese patients might be explained in part by the high degree of conservation of the HLA region for common HLA haplotypes in the Japanese population, and/or the absence of GVHD risk determinants on haplotypes in the Japanese population, and/or presence of GVHD risk determinants on haplotypes in white populations. Although our study population was matched for HLA, unrelated donors are only matched for HLA alleles; hence, HLA matched pairs may have the same or different HLA haplotypes, which could be a source of disparity for undetected haplotype-linked variation [17].

In contrast to the homogeneity of the Japanese transplantation population in which 3 extended HLA haplotypes were observed at very high frequency [16], only 37 white donor–recipient pairs in the current study were homozygous for the 4 most commonly observed white haplotypes. Formal analysis of HLA haplotype-associated variation has uncovered novel untyped variation responsible for GVHD in white patients [18,19], and comparable analyses are underway for Japanese pairs. Examination of MHC resident variation should shed light on haplotype-linked polymorphisms unique to each ethnic group that could be investigated in the future.

In the HLA region, several candidate genes exist in which SNPs may be related to immune responses. Genetic variants of TNF-$\alpha$ gene located in the HLA region might influence the risk of developing GVHD [20-22]. In addition, TAP1/TAP2 and LMP2/LMP7 genes encode subunit components of the proteasome implicated in the processing of class I HLA-bound peptides, and polymorphisms of these genes may affect antigen presentation on recipient tissues, thus leading to different susceptibility to GVHD.

In HCT from HLA-identical siblings, white Americans, African Americans, and Irish cohorts were reported to be at significantly higher risk for acute GVHD than Japanese or Scandinavian cohorts [10]. In unrelated donor HCT, there are polymorphisms other than HLA. These data suggest that other genetic differences might exist outside the HLA region. Microbe-associated molecules [23], innate immune receptors associated with GVHD, IL-10 [24], and heparanase [25] are all candidates. The HLA alleles of each haplotype might present different immunodominant peptides to T cells and evoke different alloreactivity in HLA-matched unrelated donor HCT [9]. The critical, but as yet unidentified, minor histocompatibility antigens restricted by major histocompatibility antigens such as common gene deletion polymorphisms and the differences in ethnically diverse populations should shed light [26]. Other intriguing candidate factors for acute GVHD might include environmental factors, foods, and drug sensitivity of chemotherapeutic regimens used specifically in transplantation and of other pharmacological agents for health maintenance [27]. These factors could be explored in unrelated as well as HCT from HLA identical related donors.

In the current study, significantly lower incidence of relapse in Japanese pairs than that in white pairs was observed in patients with early disease status. Japanese patients had a lower relapse rate than white pairs regardless of leukemia type and occurrence of acute GVHD. HLA-DPB1 mismatching was also an independent hazard risk for leukemia relapse and lowered leukemia relapse in both Japanese and white populations [28,29]. Although these results might be induced in part by GVL effects, GVL effects cannot entirely explain the difference of the risk of leukemia relapse between the 2 ethnic populations. Although information on pretransplantation induction/consolidation regimens was not available, disease risk stage at the time of transplantation is a surrogate for the risk of relapse. That Japanese patients had both lower relapse and lower GVHD risk compared with white patients suggests more complex factors beyond the classic GVL effect, including GVHD-dependent and GVHD-independent pathways. Yang et al. [30] reported that leukemia relapse risk after chemotherapy differed by ethnicity in children with ALL in a North American population, showing that Native Americans and Hispanic ethnicity had a higher incidence of relapse rate. The polymorphisms of chemotherapy resistance for relapse specific to ethnicity might exist among ethnic groups. Identification of non-HLA genes and alleles within the MHC and

polymorphisms outside of the HLA region responsible for leukemia relapse remains an important research goal.

Genome-wide analysis of world populations shows intriguing genetic differences that are population-specific [31]; however, genetics may be only 1 of several factors that lead to differences in GVHD and mortality between Japanese and white patients. The overwhelming majority of Japanese patients included in this analysis were treated in Japan and were therefore exposed to a relatively homogeneous "environment." The impact of sociocultural and/or dietary factors that could also influence posttransplantation risks merits evaluation in future studies of ethnically diverse populations, particularly transplantation outcomes for patients of Japanese heritage who reside outside Japan.

Our study highlights the need for comprehensive analysis of HLA region genetics as a starting point for identification of GVHD-associated polymorphisms. We used broad terms to classify white, Hispanic, and African Americans; however, each of these populations may include individuals of very different ethnic backgrounds and is 1 limitation of our study at present. Critical to achieving this goal is the need to define the genetic diversity of all human populations given the highly polymorphic nature of the HLA system. In the United States, a larger clinical experience will aid our understanding of the immunogenicity of specific HLA phenotypes representing African Americans, Hispanics, and Native Americans [13,32]. Because most Asian/Pacific Islander pairs in our current study (1734 of 1753) were Japanese pairs registered from the Japan Marrow Donor Program, the data are representative of the Japanese population rather than Asian/Pacific ethnicities as a whole.

Contribution of data from other populations in the future will aid in the effort to mapping transplantation determinants, information that will benefit all patients. The results of our analysis provide a platform for future international analyses of unrelated HCT outcomes and for international exchange of unrelated donors.

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## SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2013.05.020.

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# 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

Makoto Murata [1,2,*], Hideki Nakasone [2,3], Junya Kanda [2,3], Takahiko Nakane [3,4], Tatsuo Furukawa [2,5], Takahiro Fukuda [6], Takehiko Mori [7], Shuichi Taniguchi [8], Tetsuya Eto [9], Kazuteru Ohashi [10], Masayuki Hino [4], Masami Inoue [11], Hiroyasu Ogawa [12], Yoshiko Atsuta [13], Tokiko Nagamura-Inoue [14], Hiromasa Yabe [15], Yasuo Morishima [16], Hisashi Sakamaki [10], Ritsuro Suzuki [13]

[1] *Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[2] *GVHD Working Group of the Japan Society for Hematopoietic Cell Transplantation*
[3] *Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan*
[4] *Department of Hematology, Graduate School of Medicine, Osaka City University, Osaka, Japan*
[5] *Division of Bone Marrow Transplantation, Niigata University Medical and Dental Hospital, Niigata, Japan*
[6] *Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan*
[7] *Division of Hematology, Keio University School of Medicine, Tokyo, Japan*
[8] *Department of Hematology, Toranomon Hospital, Tokyo, Japan*
[9] *Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan*
[10] *Hematology Division, Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital, Tokyo, Japan*
[11] *Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan*
[12] *Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan*
[13] *Department of HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[14] *Department of Cell Processing and Transfusion, Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[15] *Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan*
[16] *Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan*

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A B S T R A C T

Systemic corticosteroid therapy is recommended as a first-line treatment for acute graft-versus-host disease (GVHD). We performed a retrospective study to identify the factors affecting the response of grade II to IV acute GVHD to systemic corticosteroid therapy using the Japanese national registry data for patients who received first allogeneic hematopoietic cell transplantation with bone marrow (BM) (n = 1955), peripheral blood stem cells (PBSCs) (n = 642), or umbilical cord blood (UCB) (n = 839). Of 3436 patients, 2190 (63.7%) showed improvement of acute GVHD to first-line therapy with corticosteroids. Various factors were identified to predict corticosteroid response. Interestingly, UCB (versus HLA-matched related BM) transplantation was significantly associated with a higher probability of improvement, whereas HLA-matched unrelated BM and HLA-mismatched stem cell sources other than UCB were significantly associated with a lower probability of improvement. HLA-matched related PBSC transplantation was not significantly different from HLA-matched related BM transplantation. Patients without improvement from corticosteroid therapy had a 2.5-times higher nonrelapse mortality and a .6-times lower overall survival rate. The present study demonstrated, for the first time, a higher probability of improvement in grade II to IV acute GVHD with systemic corticosteroid therapy in patients after UCB transplantation than in those after BM and PBSC transplantation. A prospective study is warranted.



## INTRODUCTION

Despite prophylactic treatment with immunosuppressive agents, acute graft-versus-host disease (GVHD) remains a major problem after allogeneic hematopoietic cell transplantation (HCT). Several studies have evaluated a variety of agents added to prednisone [1-7], but the use of prednisone or methylprednisolone alone is recommended as a standard first-line treatment for acute GVHD [8]. The response rate is approximately 40% to 60%, and patients unresponsive or resistant to corticosteroid therapy have an increased risk of mortality related to uncontrolled GVHD [2,9-16]. Some clinical factors are reported to be statistically predictive of a response to systemic corticosteroid therapy: HLA-mismatched donor transplantation, unrelated donor transplantation, combination of male recipient and female donor, early onset of GVHD, higher grade of GVHD, and liver or gut involvement of GVHD have lower response rates [2,9,10,14].

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* Correspondence and reprint requests: Makoto Murata, MD, PhD, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa, Nagoya, Aichi 466-8550, Japan.
*E-mail address:* mmurata@med.nagoya-u.ac.jp (M. Murata).

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These significant factors were identified in retrospective studies in which most or all patients underwent bone marrow (BM) transplantation. However, stem cell sources for allogeneic HCT have changed dramatically with the frequent use of peripheral blood stem cells (PBSCs) and umbilical cord blood (UCB), and no study has compared the response rates of corticosteroid therapy among stem cell sources.

To identify the factors affecting the response to systemic corticosteroid therapy as a first-line treatment for patients with grade II to IV acute GVHD, a retrospective study was conducted using the national registry data on 3436 patients who received first allogeneic HCT in Japan with BM (n = 1955), PBSCs (n = 642), or UCB (n = 839).

## PATIENTS AND METHODS

### *Patients*

Clinical data for patients who received the first allogeneic HCT in Japan, achieved neutrophil engraftment (>.5 × $10^9$/L), developed grade II to IV acute GVHD, and received systemic corticosteroid therapy as a first-line treatment for acute GVHD were extracted from the Transplant Registry Unified Management Program system, which is a registry of the outcomes of Japanese transplantation patients [17]. Patients who relapsed before GVHD development were excluded, as were patients who received other agents as initial therapy in addition to systemic corticosteroid therapy. This study was approved by the Data Management Committee of the Japan Society for Hematopoietic Cell Transplantation and by the ethical committee of the Nagoya University School of Medicine.

### *Definitions*

Acute GVHD was diagnosed and graded according to established criteria [18]. Persistent nausea with histologic evidence of GVHD but no diarrhea was included as stage 1 gut GVHD. Responses of acute GVHD to corticosteroid therapy were defined as *improved* if the grade was improved without additional systemic treatment. Responses were evaluated without time limitation, and therefore were considered improved even if the GVHD was improved later than day 28 of corticosteroid therapy, although response by day 28 is proposed as the best endpoint to define need for second-line treatment [16]. Responses were also considered improved even if acute GVHD was improved and then a new immunosuppressant was added to treat chronic GVHD. Responses were defined as *stable or progressive* if the grade was unchanged or worsened after first-line corticosteroid therapy or if second-line systemic treatment for acute GVHD was added regardless of responsiveness to first-line corticosteroid therapy. Thus, all patients who received second-line treatment for acute GVHD were considered stable or progressive even if the GVHD was improved temporarily after corticosteroid therapy.

Acute myeloid leukemia in the first or second remission, acute lymphoblastic leukemia in the first remission, chronic myelogenous leukemia in the first chronic phase, and myelodysplastic syndromes with refractory anemia or refractory anemia with ringed sideroblasts were defined as *standard-risk malignancies*, and other malignant diseases were defined as *high-risk malignancies*.

BM transplantation from serological HLA-A, B, and DR 6/6 matched related donors was defined as *MRD-BM*, and BM transplantation from serological HLA-A, B, and DR at least 3/6 matched, but not 6/6 matched related donors, was defined as *MMRD-BM*. PBSC transplantation from serological HLA-A, B, and DR 6/6 matched related donors was defined as *MRD-PB*, and PBSC transplantation from serological HLA-A, B, and DR at least 3/6 matched, but not 6/6 matched related donors, was defined as *MMRD-PB*. For unrelated BM transplantation, all patient–donor pairs were HLA-typed to allele level for at least 3 loci (HLA-A, B, and DRB1) during the coordination process. BM transplantation from HLA-A, B, and DRB1 alleles 6/6 matched unrelated donors was defined as *MUD-BM*, and BM transplantation from HLA-A, B, and DRB1 alleles 5/6 or 4/6 matched unrelated donors was defined as *MMUD-BM*. UCB transplantation from serological HLA-A, B, and DR at least 4/6 matched donors was defined as *UCB*.

Based on the report by the Center for International Blood and Marrow Transplant Research [19], the conditioning regimens were classified as *myeloablative* if total body irradiation >8 Gy, oral busulfan ≥9 mg/kg, intravenous busulfan ≥7.2 mg/kg, or melphalan >140 mg/m$^2$ was included in the conditioning regimen, whereas other conditioning regimens were classified as *nonmyeloablative*.

Onset of acute GVHD was classified into 3 groups: day ≤28, day ≥29, and unknown; however, acute GVHD that occurred earlier than day 4, which might be an error at the time of registration, was classified into unknown.

### *Endpoints*

The primary endpoint of this study was to identify the factors affecting the response to systemic corticosteroid therapy as a first-line treatment for grade II to IV acute GVHD. The secondary endpoints were to identify factors associated with nonrelapse mortality (NRM) after corticosteroid therapy and to evaluate the impact of response to corticosteroid therapy on the overall survival (OS) rate after corticosteroid therapy.

### *Statistical Analysis*

Univariate and multivariate logistic regression analyses were used to identify factors associated with the response to corticosteroid therapy. The probability of NRM after systemic corticosteroid therapy stratified by response to corticosteroid therapy was estimated on the basis of cumulative incidence curves in which relapse was treated as a competing event [20]. The probability of OS after systemic corticosteroid therapy stratified by response to corticosteroid therapy was estimated according to the Kaplan-Meier method [21]. The groups were compared using the log-rank test. Competing risk regression analysis was used to identify factors associated with NRM after corticosteroid therapy. The adjusted probability of OS after corticosteroid therapy was estimated using the Cox proportional hazards model, with consideration of other significant clinical variables in the final multivariate models [22]. *P* values were 2 sided, and $P < .05$ was considered significant. The following covariates were considered for the multivariate models: patient age, patient sex, sex mismatch between patient and donor, disease, stem cell source, cytomegalovirus serostatus, preconditioning, GVHD prophylaxis, in vivo T cell depletion, year of transplantation, onset of acute GVHD, grade of acute GVHD, organ involvement of acute GVHD, and response to systemic corticosteroid therapy (improved or stable/progressive). The data were analyzed by STATA version 12 statistical software (StataCorp, TX).

## RESULTS

### *Patient, Transplantation, and GVHD Characteristics*

A total of 3436 patients met the inclusion criteria. Patient and transplantation characteristics are shown in Table 1. Patient age at transplantation ranged from 0 to 82 years (median, 40 years); the number of patients age <18, 18 to 49, and ≥50 years was 672, 1626, and 1138, respectively. Stem cell sources were BM (n = 1955), PBSC (n = 642), and UCB (n = 839). All UCB transplantation was performed with a single unit. In vivo T cell depletion was performed in 168 (5%) patients by either antithymocyte globulin or antilymphocyte globulin. No other drugs, such as alemtuzumab, were used for in vivo T cell depletion, nor was ex vivo T cell depletion used in any patients. The year of transplantation ranged from 1984 to 2009; the majority of cases (94%) were performed in 2000 or later.

Characteristics of acute GVHD cases are shown in Table 2. The numbers of patients who developed acute GVHD at day ≤28 and day ≥29 were 2344 and 994, respectively. Of 3436 patients who received systemic corticosteroid therapy as the first-line treatment for grade II to IV acute GVHD, 2190 (63.7%) showed improvement of acute GVHD.

### *Factors Associated with Improvement of GVHD by Corticosteroid Therapy*

MUD-BM, HLA-mismatched stem cell source other than UCB (MMRD-BM, MMRD-PB, and MMUD-BM), more severe acute GVHD, and multiple organ involvement of acute GVHD, including gut, were significantly associated with a lower probability of improvement by corticosteroid therapy (Table 3). On the other hand, adult patient (ages 18 to 49 years) and UCB were significantly associated with a higher probability of improvement by corticosteroid therapy (Table 3). Although some factors, such as disease, cytomegalovirus serostatus, and preconditioning, were significant for corticosteroid response in univariate analysis, they were not significant in multivariate analysis. Additional analysis in which onset of acute GVHD was modeled as a continuous variable could not detect a significant association between

**Table 1**
Patient and Transplantation Characteristics (N = 3436)

| Characteristic | Total (N = 3246) | MRD-BM/PB (n = 926) | MUD-BM + mm* (n = 1671) | UCB (n = 839) |
|---|---|---|---|---|
| Patient age at transplantation | | | | |
| <18 yr | 672 (20) | 99 (11) | 310 (19) | 263 (31) |
| 18 to 49 yr | 1626 (47) | 520 (56) | 836 (50) | 270 (32) |
| ≥50 yr | 1138 (33) | 307 (33) | 525 (31) | 306 (37) |
| Patient sex | | | | |
| Female | 1393 (41) | 380 (41) | 668 (40) | 345 (41) |
| Male | 2043 (59) | 546 (59) | 1003 (60) | 494 (59) |
| Sex mismatch between patient and donor | | | | |
| Female donor to male patient | 815 (24) | 251 (27) | 348 (21) | 216 (26) |
| Other combinations | 2525 (73) | 662 (72) | 1321 (79) | 542 (64) |
| Unknown | 96 (3) | 13 (1) | 2 (0) | 81 (10) |
| Disease | | | | |
| Standard-risk malignancies | 1320 (38) | 372 (40) | 686 (41) | 262 (31) |
| High-risk malignancies | 1926 (57) | 509 (55) | 900 (54) | 517 (62) |
| Nonmalignancies | 154 (4) | 40 (4) | 80 (5) | 34 (4) |
| Unknown | 36 (1) | 5 (1) | 5 (0) | 26 (3) |
| Stem cell source | | | | |
| MRD-BM | 445 (13) | 445 (48) | 0 (0) | 0 (0) |
| MRD-PB | 481 (14) | 481 (52) | 0 (0) | 0 (0) |
| MUD-BM | 783 (23) | 0 (0) | 783 (47) | 0 (0) |
| UCB | 839 (24) | 0 (0) | 0 (0) | 839 (100) |
| MMRD-BM | 155 (4) | 0 (0) | 155 (9) | 0 (0) |
| MMRD-PB | 161 (5) | 0 (0) | 161 (10) | 0 (0) |
| MMUD-BM | 572 (17) | 0 (0) | 572 (34) | 0 (0) |
| Cytomegalovirus serostatus | | | | |
| Negative donor to negative patient | 322 (9) | 53 (6) | 112 (7) | 159 (19) |
| Positive donor to negative patient | 215 (6) | 64 (7) | 149 (9) | 0 (0) |
| Negative donor to positive patient | 899 (26) | 107 (12) | 290 (17) | 509 (61) |
| Positive donor to positive patient | 1541 (46) | 574 (61) | 960 (57) | 0 (0) |
| Unknown | 459 (13) | 128 (14) | 160 (10) | 171 (20) |
| Preconditioning | | | | |
| Myeloablative | 2094 (61) | 578 (62) | 1030 (62) | 486 (58) |
| Nonmyeloablative | 1307 (38) | 323 (35) | 636 (38) | 348 (41) |
| Unknown | 35 (1) | 25 (3) | 5 (0) | 5 (1) |
| GVHD prophylaxis | | | | |
| Cyclosporine A–based | 1676 (49) | 800 (87) | 417 (25) | 459 (55) |
| Tacrolimus-based | 1691 (49) | 103 (11) | 1227 (73) | 361 (43) |
| Others | 56 (2) | 20 (2) | 26 (2) | 10 (1) |
| Unknown | 13 (0) | 3 (0) | 1 (0) | 9 (1) |
| In vivo T cell depletion | | | | |
| No | 3251 (95) | 876 (94) | 1556 (93) | 819 (98) |
| Yes | 168 (5) | 34 (4) | 115 (7) | 19 (2) |
| Unknown | 17 (0) | 16 (2) | 0 (0) | 1 (0) |
| Year of transplantation | | | | |
| 1984 to 1999 | 200 (6) | 103 (11) | 63 (4) | 34 (4) |
| 2000 to 2004 | 721 (21) | 182 (20) | 221 (13) | 318 (38) |
| 2005 to 2009 | 2515 (73) | 641 (69) | 1387 (83) | 487 (58) |

MRD-BM indicates HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow; GVHD, graft-versus-host disease.
Data presented are n (%).
* mm indicates MMRD-BM, MMRD-PB, and MMUD-BM.

onset of acute GVHD and response to corticosteroid therapy. Response rates to corticosteroid therapy in each stem cell source are summarized in Table 4.

### *Impact of the Response to Corticosteroid Therapy on NRM*

The cumulative incidence rates of NRM after systemic corticosteroid therapy for grade II to IV acute GVHD are shown in Figure 1. Patients who did not achieve improvement of acute GVHD by corticosteroid therapy had a significantly higher NRM compared with those who achieved improvement ($P < .0001$).

To identify factors associated with NRM after corticosteroid therapy for grade II to IV acute GVHD, competing risk regression analysis was performed. The patients with a stable or progressive response to corticosteroid therapy were approximately 2.5 times more likely to have NRM than patients with an improved response to corticosteroid therapy (Table 5). Other factors associated with significantly worse NRM included older patient age (18 to 49 years and ≥50 years), higher grades of acute GVHD (grades III and IV), and liver or multiple organ involvement including liver of acute GVHD (Table 5). Although some factors such as patient sex, disease, and preconditioning were significant for NRM in univariate analysis, they were not significant in multivariate analysis. Additional analysis in which onset of acute GVHD was modeled as a continuous variable could not detect a significant association between onset of acute GVHD and NRM.

### *Impact of the Response to Corticosteroid Therapy on the OS Rate*

The Kaplan-Meier estimates of OS rates after systemic corticosteroid therapy for grade II to IV acute GVHD are shown in Figure 2. Patients who did not achieve improvement of acute GVHD by corticosteroid therapy had

**Table 2**
Acute GVHD Characteristics

| Characteristic | Total (N = 3436) | MRD-BM/PB (n = 926) | MUD-BM + mm* (n = 1671) | UCB (n = 839) |
|---|---|---|---|---|
| Onset of acute GVHD | | | | |
| Day ≤28 | 2344 (68) | 560 (60) | 1221 (73) | 563 (67) |
| Day ≥29 | 994 (29) | 351 (38) | 434 (26) | 209 (25) |
| Unknown | 98 (3) | 15 (2) | 16 (1) | 67 (8) |
| Grade of acute GVHD | | | | |
| II | 2049 (59) | 584 (63) | 973 (58) | 492 (58) |
| III | 1015 (30) | 259 (28) | 482 (29) | 274 (33) |
| IV | 372 (11) | 83 (9) | 216 (13) | 73 (9) |
| Organ involvement | | | | |
| Skin only | 1110 (32) | 288 (31) | 579 (34) | 243 (29) |
| Gut only | 310 (9) | 125 (13) | 129 (8) | 55 (7) |
| Liver only | 35 (1) | 8 (1) | 16 (1) | 11 (1) |
| Skin and gut, no liver | 1178 (34) | 316 (34) | 576 (34) | 286 (34) |
| Skin and liver, no gut | 177 (5) | 56 (6) | 72 (4) | 49 (6) |
| Gut and liver, no skin | 87 (3) | 26 (3) | 42 (3) | 19 (2) |
| Skin, gut, and liver | 487 (14) | 107 (12) | 256 (16) | 124 (15) |
| Unknown | 52 (2) | 0 (0) | 1 (0) | 51 (6) |

GVHD indicates graft-versus-host disease; MRD-BM/PB, HLA-matched related donor bone marrow and HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood.
Data are presented as n (%).
* mm indicates HLA-mismatched related donor bone marrow, HLA-mismatched related donor peripheral blood stem cells and HLA-mismatched unrelated donor bone marrow.

a significantly lower OS rate than those who achieved improvement ($P < .0001$).

To evaluate the impact of the response to corticosteroid therapy on the OS rate, the Cox proportional hazards model was used with all of the clinical features listed in Tables 1 and 2. On univariate analysis, the OS rate was significantly lower in patients with a stable or progressive response to corticosteroid therapy than in patients with an improved response (hazard ratio, 2.18; 95% confidence interval, 1.97 to 2.40). After adjustment by patient age, disease, preconditioning, grade of acute GVHD, and organ involvement of acute GVHD, which were significant on univariate analysis, the OS rate

**Table 3**
Factors Associated with Improvement of GVHD by Corticosteroid Therapy

| Factor (n) | Univariate Analysis Relative Risk* (95% CI) | *P* Value | Multivariate Analysis Relative Risk* (95% CI) | *P* Value |
|---|---|---|---|---|
| Patient age | | | | |
| <18 yr (672) | 1 | | 1 | |
| 18 to 49 yr (1626) | 1.33 (1.10 to 1.60) | .003 | 1.48 (1.18 to 1.85) | <.002 |
| ≥50 yr (1138) | 1.06 (.88 to 1.30) | .509 | 1.11 (.88 to 1.40) | .385 |
| Stem cell source | | | | |
| MRD-BM (445) | 1 | | 1 | |
| MRD-PB (481) | .66 (.50 to .87) | .004 | .81 (.59 to 1.12) | .201 |
| MUD-BM (783) | .53 (.41 to .68) | <.001 | .57 (.43 to .76) | <.001 |
| UCB (839) | .97 (.75 to 1.26) | .839 | 1.36 (1.01 to 1.83) | .042 |
| MMRD-BM (155) | .26 (.18 to .39) | <.001 | .37 (.24 to .57) | <.001 |
| MMRD-PB (161) | .34 (.23 to .49) | <.001 | .41 (.27 to .63) | <.001 |
| MMUD-BM (572) | .47 (.36 to .61) | <.001 | .57 (.42 to .77) | <.001 |
| GVHD prophylaxis | | | | |
| Cyclosporine A-based (1676) | 1 | | 1 | |
| Tacrolimus-based (1691) | .80 (.69 to .92) | .002 | 1.02 (.82 to 1.26) | .851 |
| Other (56) | .38 (.22 to .64) | <.001 | .61 (.31 to 1.22) | .164 |
| In vivo T cell depletion | | | | |
| No (3251) | 1 | | 1 | |
| Yes (168) | 1.47 (1.08 to 2.01) | .015 | 1.06 (.68 to 1.65) | .787 |
| Onset of acute GVHD | | | | |
| Day ≤28 (2344) | 1 | | 1 | |
| Day ≥29 (994) | 1.20 (1.03 to 1.40) | .023 | 1.10 (.91 to 1.34) | .336 |
| Grade of acute GVHD | | | | |
| II (2049) | 1 | | 1 | |
| III (1015) | .34 (.29 to .39) | <.001 | .45 (.37 to .55) | <.001 |
| IV (372) | .04 (.03 to .06) | <.001 | .07 (.05 to .10) | <.001 |
| Organ involvement | | | | |
| Skin only (1110) | 1 | | 1 | |
| Gut only (310) | .69 (.52 to .92) | .011 | .91 (.66 to 1.24) | .541 |
| Liver only (35) | .22 (.11 to .43) | <.001 | .56 (.25 to 1.25) | .157 |
| Skin and gut, no liver (1178) | .55 (.45 to .66) | <.001 | .77 (.62 to .96) | .021 |
| Skin and liver, no gut (177) | .39 (.28 to .54) | <.001 | .78 (.53 to 1.15) | .214 |
| Gut and liver, no skin (87) | .17 (.11 to .26) | <.001 | .36 (.21 to .59) | <.001 |
| Skin, gut, and liver (487) | .13 (.10 to .17) | <.001 | .38 (.28 to .51) | <.001 |

GVHD indicates graft-versus-host disease; MRD-BM, HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow; CI, confidence interval.
* Values >1.0 indicate higher probability of improvement; values <1.0 indicate lower probability.

**Table 4**
Response to Corticosteroid Therapy in Each Stem Cell Source

| Stem Cell Source | No. of Cases | Patients with Improved Response, n (%) |
|---|---|---|
| MRD-BM | 445 | 328 (73.7) |
| MRD-PB | 481 | 312 (64.9) |
| MUD-BM | 783 | 468 (59.8) |
| UCB | 839 | 614 (73.2) |
| MMRD-BM | 155 | 66 (42.9) |
| MMRD-PB | 161 | 78 (48.4) |
| MMUD-BM | 572 | 324 (56.6) |
| Total | 3436 | 2190 (63.7) |

MRD-BM indicates HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow.

was still significantly lower in patients with a stable or progressive response to corticosteroid therapy than in patients with an improved response (hazard ratio, 1.66; 95% confidence interval, 1.49 to 1.85).

## DISCUSSION

The present nationwide study revealed that the response rate of grade II to IV acute GVHD to systemic corticosteroid therapy in Japanese patients was approximately 64%, which is comparable to that in Caucasian patients. In a retrospective analysis of 456 patients who were treated with methylprednisolone 2 mg/kg/day for grade II to IV acute GVHD after allogeneic BM transplantation at the Fred Hutchinson Cancer Research Center, 59% of the patients experienced a complete, partial, or mixed response [10]. In another retrospective analysis of 864 patients who were treated with prednisone 60 mg/m$^2$/day for grade II to IV acute GVHD after BM, PBSC, or UCB transplantation at the University of Minnesota, 65% of the patients experienced a complete, very good partial, or partial response [16].

The factors associated with poor response to corticosteroid therapy were MUD-BM, HLA-mismatched stem cell sources other than UCB (MMRD-BM, MMRD-PB, and MMUD-BM), more severe acute GVHD, and multiple organ involvement including gut of acute GVHD (Table 3). The previous studies also found these features as risk factors for an increased treatment failure rate [9,10], suggesting that these subgroups may be targets for alternate first-line immunosuppressive therapies.

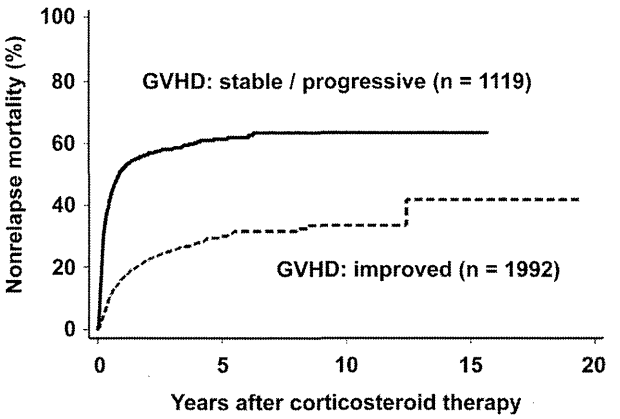


**Figure 1.** Nonrelapse mortality (NRM) after systemic corticosteroid therapy for patients with grade II to IV acute GVHD. Cumulative incidence rates of NRM after systemic corticosteroid therapy in patients (n = 1992) with an improved response to corticosteroid therapy (dashed line, 22.2% [95% confidence interval, 20.1% to 24.4%] at 2 years, 30.1% [27.1% to 33.0%] at 5 years, 33.5% [29.4% to 37.6%] at 10 years, and 41.8% [26.2% to 56.7%] at 15 years) and patients (n = 1119) with a stable or progressive response to corticosteroid therapy (solid line, 56.3% [53.1% to 59.5%] at 2 years, 61.4% [57.7% to 64.9%] at 5 years, 63.4% [59.2% to 67.3%] at 10 years, and 63.4% [59.2% to 67.3%] at 15 years) are shown ($P < .0001$).

On the other hand, UCB was identified as a factor associated with a higher response to first-line corticosteroid therapy in the present study (Table 3). Although several studies have demonstrated a significantly lower incidence of acute GVHD in UCB transplantation than in unrelated BM transplantation [23-29], no study has compared the response to treatment of acute GVHD between them. The present study demonstrated, for the first time, a higher response of grade II to IV acute GVHD to systemic corticosteroid therapy in patients after UCB transplantation than in those after BM or PBSC transplantation.

Nevertheless, UCB transplantation had no impact on NRM after corticosteroid therapy in the multivariate analysis and, in fact, had higher NRM than MRD-BM transplantation in the univariate analysis (Table 5). Thus, even though there was a higher response of acute GVHD to systemic corticosteroid therapy in patients after UCB transplantation, careful management is required for patients who suffer from grade II to IV acute GVHD after UCB transplantation, as well as those after transplantation with other stem cell sources.

Unexpectedly, adult patient (ages 18 to 49 years) was predictive of a good response to systemic corticosteroid therapy compared with child patient (age <18 years). Additional analysis was performed, and it was found that patients with grade II acute GVHD accounted for 61.4% of adult patient group, whereas 56.1% of child patient group (Fisher exact test, $P = .019$). This difference might affect the above result because severity of acute GVHD was the most significant factor associated with response to corticosteroid therapy (Table 3). Nonetheless, adult patients were likely to have higher NRM than child patients (Table 5). Our data indicate that although adult patients may be more responsive to corticosteroid therapy for acute GVHD, they have a higher risk of transplant-related toxicity than children with acute GVHD.

Despite the fact that multivariate analysis showed a significantly higher response rate to corticosteroid therapy in UCB transplantation than MRD-BM transplantation, the actual percentage was similar between UCB (73.2%) and MRD-BM (73.7%) transplantations (Table 4). Additional analysis found that patients in the age group 18 to 49 years (predictive factor of good response) accounted for only 32.2% of UCB transplantation, but constituted 58.4% of the MRD-BM population (Fisher exact test, $P < .001$) and that patients with grade II acute GVHD (predictive factor of good response) accounted for only 58.6% of UCB transplantation, but constituted 70.1% of the MRD-BM population (Fisher exact test, $P < .001$). These data suggested that the UCB population included fewer patients having predictive factors of good response to corticosteroid therapy compared with the MRD-BM population. This could explain why the actual percentage of patients with an improved response in UCB transplantation was almost the same as the percentage of patients with an improved response in MRD-BM transplantation.

Interestingly, multiorgan involvement that includes the gut was less likely to respond to first-line therapy with corticosteroids (Table 3); however, patients with liver involvement are more likely to have higher NRM (Table 5). Further study is required to elucidate the mechanisms of the difference in the effect of gut and liver GVHD on

**Table 5**
Factors Associated with Nonrelapse Mortality after Corticosteroid Therapy

| Factor (n) | Univariate Analysis Hazard Ratio* (95% CI) | *P* Value | Multivariate Analysis Hazard Ratio* (95% CI) | *P* Value |
|---|---|---|---|---|
| Patient age | | | | |
| <18 yr (554) | 1 | | 1 | |
| 18 to 49 yr (1503) | 1.50 (1.21 to 1.85) | <.001 | 1.72 (1.38 to 2.14) | <.001 |
| ≥50 yr (1054) | 2.74 (2.22 to 3.38) | <.001 | 3.34 (2.67 to 4.17) | <.001 |
| Stem cell source | | | | |
| MRD-BM (402) | 1 | | 1 | |
| MRD-PB (447) | 1.43 (1.11 to 1.83) | .005 | .88 (.68 to 1.15) | .344 |
| MUD-BM (726) | 1.40 (1.11 to 1.77) | .004 | 1.02 (.80 to 1.30) | .866 |
| UCB (720) | 1.35 (1.06 to 1.71) | .014 | 1.15 (.90 to 1.48) | .265 |
| MMRD-BM (141) | 1.63 (1.16 to 2.28) | .005 | 1.15 (.82 to 1.62) | .415 |
| MMRD-PB (153) | 1.74 (1.26 to 2.39) | .001 | .97 (.69 to 1.37) | .882 |
| MMUD-BM (522) | 1.79 (1.41 to 2.27) | <.001 | 1.25 (.97 to 1.60) | .082 |
| GVHD prophylaxis | | | | |
| Cyclosporine A-based (1528) | 1 | | | |
| Tacrolimus-based (1520) | 1.06 (.94 to 1.21) | .332 | | |
| Other (50) | 1.28 (.81 to 2.04) | .296 | | |
| In vivo T cell depletion | | | | |
| No (3004) | 1 | | | |
| Yes (91) | .98 (.66 to 1.44) | .919 | | |
| Onset of acute GVHD | | | | |
| Day ≤28 (2212) | 1 | | | |
| Day ≥29 (899) | 1.05 (.92 to 1.20) | .476 | | |
| Grade of acute GVHD | | | | |
| II (1864) | 1 | | 1 | |
| III (917) | 2.21 (1.92 to 2.56) | <.001 | 1.56 (1.31 to 1.86) | <.001 |
| IV (330) | 7.93 (6.67 to 9.43) | <.001 | 3.53 (2.84 to 4.38) | <.001 |
| Organ involvement | | | | |
| Skin only (1010) | 1 | | 1 | |
| Gut only (266) | 1.11 (.84 to 1.47) | .448 | .80 (.59 to 1.08) | .139 |
| Liver only (28) | 4.11 (2.20 to 7.69) | <.001 | 2.22 (1.19 to 4.16) | .013 |
| Skin and gut, no liver (1083) | 1.27 (1.06 to 1.51) | .008 | .97 (.79 to 1.18) | .753 |
| Skin and liver, no gut (160) | 2.42 (1.83 to 3.21) | <.001 | 1.54 (1.13 to 2.08) | .006 |
| Gut and liver, no skin (75) | 3.64 (2.57 to 5.16) | <.001 | 1.88 (1.29 to 2.73) | .001 |
| Skin, gut, and liver (448) | 4.82 (4.03 to 5.77) | <.001 | 2.07 (1.64 to 2.62) | <.001 |
| Response to systemic corticosteroid therapy | | | | |
| Improved (1992) | 1 | | 1 | |
| Stable/progressive (1119) | 3.63 (3.20 to 4.12) | <.001 | 2.45 (2.14 to 2.82) | <.001 |

MRD-BM indicates HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow; GVHD, graft-versus-host disease; CI, confidence interval.
* Values >1.0 indicate higher probability of non relapse mortality; values <1.0 indicate lower probability.

transplantation outcome. Nevertheless, lack of response to initial therapy is an important risk factor in predicting high NRM in patients with grade II to IV acute GVHD (Table 5).

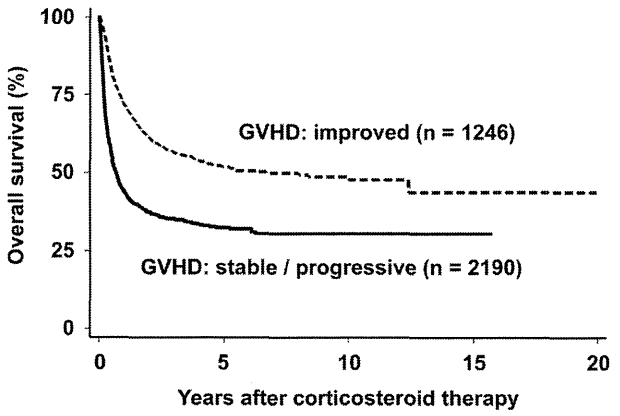


**Figure 2.** Overall survival (OS) for patients with grade II to IV acute GVHD. OS for patients (n = 2190) with an improved response (dashed line; 61.3% [95% confidence interval, 59.0% to 63.5%] at 2 years, 51.9% [49.2% to 54.5%] at 5 years, 47.8% [44.0% to 51.5%] at 10 years, and 43.8% [35.5% to 51.8%] at 15 years) and OS for patients (n = 1246) with a stable or progressive response (solid line; 37.4% [34.6% to 40.3%] at 2 years, 32.5% [29.5% to 35.6%] at 5 years, 30.6% [27.3% to 34.1%] at 10 years, and 30.6% [27.3% to 34.1%] at 15 years) are shown ($P < .0001$).

The patients who did not achieve improvement of acute GVHD by corticosteroid therapy had approximately 2.5-times higher NRM and approximately .6-times lower OS rates. It is well known that the incidence of acute GVHD in Japanese patients is lower than that in Caucasian patients [30,31]. However, the present data clearly demonstrate that, if the systemic corticosteroid therapy is ineffective, even Japanese patients cannot achieve a satisfactory survival rate. Another important message of this study is that the establishment of second-line treatment for corticosteroid-refractory acute GVHD is required for not only Caucasian, but also for Japanese patients.

This study had several limitations. First, the sort and dose of corticosteroids are not collected in the Japan Society for Hematopoietic Cell Transplantation database. In patients with grade II to IV acute GVHD, initial treatment with prednisone-equivalent steroid doses higher than 2.5 mg/kg has not been shown to provide better outcomes [32], although in patients with grade II acute GVHD, lower-dose initial treatment at 1.0 mg/kg has not been shown to provide worse outcomes [33]. The intensity of corticosteroid therapy may differ by each transplantation team or each patient, as shown by a survey in Europe [34], and this information may give us additional findings. Second, criteria for improvement, or for stable or progressive acute GVHD, had been previously defined in the

database, which did not allow for analysis by outcomes such as complete, partial, or mixed response, as has been performed in previous studies [10,16]. Third, the time of the evaluation of GVHD is not defined in the database. Thus, the response was evaluated using a nonfixed time point, although GVHD sometimes shows a waxing and waning course. This also prevented us from analyzing the speed of the response to therapy. A recent study has reported that the day-28 response to corticosteroid therapy can predict the outcomes for patients with acute GVHD [16]. Fourth, this study was a retrospective analysis, which is challenging given the heterogeneous background. Multivariate analysis was used to attempt to reduce statistical bias, but a prospective study is required to validate the present findings.

The results of this large retrospective study showed a higher response of acute GVHD to systemic corticosteroid therapy in patients after UCB transplantation than for patients after BM and PBSC transplantation, and confirmed the factors previously reported. These results should be considered in the design of future clinical trials of acute GVHD treatment.

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*Conflict of interest statement:* The authors declare no competing financial interests.

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# Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia

Yoshiko Atsuta,[1] Junya Kanda,[2] Minoko Takanashi,[3] Yasuo Morishima,[4] Shuichi Taniguchi,[5] Satoshi Takahashi,[6] Hiroyasu Ogawa,[7] Kazuteru Ohashi,[8] Yuju Ohno,[9] Yasushi Onishi,[10] Nobuyuki Aotsuka,[11] Tokiko Nagamura-Inoue,[12] Koji Kato,[13] and Yoshinobu Kanda,[2] on behalf of the HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation

[1]Department of Hematopoietic Stem Cell Transplantation Data Management / Biostatistics, Nagoya University Graduate School of Medicine, Nagoya; [2]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama; [3]The Japanese Red Cross Tokyo Blood Center, Tokyo; [4]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya; [5]Department of Hematology, Toranomon Hospital, Tokyo; [6]Department of Molecular Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo; [7]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo; [8]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo; [9]Department of Internal Medicine, Kitakyushu Municipal Medical Center, Kitakyushu; [10]Department of Hematology and Rheumatology, Tohoku University Hospital, Sendai; [11]Department of Hematology and Oncology, Japanese Red Cross Narita Hospital, Narita; [12]Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, and Tokyo Cord Blood Bank, Tokyo; and [13]Department of Pediatrics, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

## ABSTRACT

Recent advances in unrelated cord blood transplantation have increased chances and options available in allogeneic stem cell transplantation. The effect of HLA disparity on outcomes after cord blood transplantation was studied recently in mainly pediatric populations. Results showed that HLA matching in combination with total nucleated cell dose positively affects survival. The effect of HLA disparity after single-unit cord blood transplantation may be different in adults because their total nucleated cell dose is much lower compared to pediatric patients. We investigated the effect of HLA disparity on the outcome of single-unit unrelated cord blood transplantation separately in 498 children aged 15 years or under (HLA-A, HLA-B low-resolution, and HLA-DRB1 high-resolution matched [6/6], n=82, and one locus- [5/6], n=222, two loci- [4/6], n=158, three loci- [3/6] mismatched, n=36) and 1,880 adults (6/6, n=71; 5/6, n=309; 4/6, n=1,025; 3/6, n=475) with leukemia. With adjusted analyses, in children, 4/6 showed significantly increased risks of overall mortality (relative risk [RR]=1.61, $P$=0.042) and transplant-related mortality (RR=3.55, $P$=0.005) compared to 6/6. The risk of grade 2 to 4 acute GVHD was increased in 5/6 (RR=2.13, $P$=0.004) and 4/6 (RR=2.65, $P$<0.001). In adults, the risk of mortality did not increase with the number of mismatched loci (RR=0.99, $P$=0.944 for 5/6; RR=0.88, $P$=0.436 for 4/6). The risk of relapse was significantly decreased in 4/6 (RR=0.67, $P$=0.034). The risk of transplant-related mortality (TRM) or acute GVHD was not increased in 5/6 or 4/6. The effect of HLA disparity on transplant outcome differed between children and adults. In children, an increased number of mismatched HLA loci correlated with an increased risk of mortality. In adults, there was no increase in mortality with an increase in the number of mismatched HLA loci.

## Introduction

Recent advances in unrelated cord blood transplantation (UCBT) have provided increased opportunities for patients with hematologic malignancies to receive hematopoietic stem cell transplantation (HSCT). This has led to an increased number of UCBT procedures over the past decade.[1,2] Clinical comparison studies of cord blood and bone marrow from unrelated donors have shown comparable results, which indicates that cord blood is a reasonable alternative donor / stem cell source.[3-12] These studies support the use of HLA-A, HLA-B, low-resolution and HLA-DRB1 zero- to two-loci-mismatched UCB for patients with leukemia in the absence of an HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched unrelated adult donor, and the use of UCB as a first-line option when a transplant is urgently required.

The effect of HLA mismatches after bone marrow transplantation from unrelated donors (UBMT) has been well studied, and HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched bone marrow is currently the first alternative for HLA-identical sibling donors.[13-16] An increase in the number of HLA mismatches, antigen-level, or high-resolution, at HLA-A, HLA-B, HLA-C, or HLA-DRB1 loci from 8/8 to 7/8, or 7/8 to 6/8 was associated with higher mortality with an approximately 10% reduction in survival in UBM recipients.[12,13,15] Since HLA mismatches are better tolerated after UCB with a lower incidence of severe graft-*versus*-host disease (GVHD), up to two HLA antigen mismatches of HLA-A, HLA-B, low resolution and HLA-DRB1 high resolution are considered in the current CB selection algorithm. Several reports have recently described the effect of HLA disparity on the transplant outcomes after UCBT.[9,17,18] Eapen *et al.* reported the pos-

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Correspondence: y-atsuta@med.nagoya-u.ac.jp

sibility of a better outcome in HLA 6/6 matched UCB in 35 recipients, and Barker *et al.* confirmed these results with a larger number of UCB recipients.[9,18] However, these studies, which assessed the effect of HLA disparity on the outcome of single-unit CBT, were mainly conducted in pediatric populations in which the infused cell dose is much greater than that in adult recipients.

The aim of this study was to assess the effect of HLA disparity on the transplant outcomes after single-unit UCBT in pediatric and adult recipients. The accumulation of single-unit CBT in adult recipients has enabled us to assess separately the effect of HLA disparity on CBT outcomes in children and adults.

## Design and Methods

### Study design and data source

For this retrospective observational study, recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN). All 11 cord blood banks in Japan are affiliated with the JCBBN. JCBBN collected the recipients' clinical information at 100 days post-transplant through the Transplant Registry Unified Management Program (TRUMP) of the Japan Society of Hematopoietic Cell Transplantation (JSHCT).[19] Information on survival, disease status, and long-term complications including chronic graft-*versus*-host disease and second malignancies is renewed annually. Patient consent is not required for TRUMP registration of the JSHCT for the registry data consists of anonymized clinical information. This study was approved by the data management committees of the JSHCT and the JCBBN, and by the institutional review boards of Saitama Medical Center, Jichi Medical University and Nagoya University Graduate School of Medicine, Japan.

### Patients

The subjects were patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), or myelodysplastic syndrome (MDS), who were recipients of their first UCBT between January 2000 and December 2009. Among 2,461 recipients of single-unit UCB with complete HLA-A, HLA-B, low-resolution and HLA-DRB1 high-resolution data, 51 recipients with 4 HLA mismatches were excluded. Thirty recipients who did not receive GVHD prophylaxis and 2 recipients for whom information regarding the conditioning regimen was missing were excluded. A total of 2378 single-unit UCB recipients (498 children aged 15 years or under at transplant, and 1880 adults aged 16 years or over at transplant) were subjects for analysis.

### HLA typing

Histocompatibility data for low-resolution typing for the HLA-A, HLA-B, and HLA-DR loci and high-resolution typing for HLA-DRB1 were obtained from the TRUMP database which includes HLA information provided by cord blood banks or transplant centers. The level of HLA typing in the present study was HLA-A, HLA-B, low-resolution, and HLA-DRB1 high-resolution, as in other studies in Europe and North America. However, according to current practice in Japan, mismatches in HLA-DR loci were counted at the low-resolution level at UCB unit selection. Therefore, results regarding the effect of HLA mismatches in HLA-A, HLA-B, and HLA-DR low-resolution are also provided (*Online Supplementary Table S1*). Analyses from the Japan Marrow Donor Program (JMDP) showed better survival in HLA class II mismatched recipients compared to HLA class I mismatched recipients. Thus, in Japan, a single-DRB1-mismatched UBM donor is preferred over a single-A-mismatched UBM or single-B-mismatched UBM donor.[15,20] This background affected HLA typing strategy of HLA-DR low-resolution typing instead of high-resolution typing for selection of cord blood units in Japan. This observation may explain the fact that the frequency of 4/6 grafts is higher in this cohort than in cohorts in Europe and the USA.

### Definitions

The primary outcome of the analyses was overall survival, defined as time from transplant to death from any cause. Several secondary end points were also analyzed. Neutrophil recovery was defined as an absolute neutrophil count of at least $0.5x10^9$/L cells per cubic millimeter for three consecutive points; platelet recovery was defined as a count of at least $50x10^9$ platelets per cubic millimeter without transfusion support. The recipients of reduced-intensity conditioning were also defined with the criteria above, according to the previous report that confirmed complete donor chimeras of all engrafted patients after CBT with reduced-intensity conditioning.[21] Diagnosis and clinical grading of acute GVHD were performed according to the established criteria.[22,23] Relapse was defined as the recurrence of underlying hematologic malignant diseases. Transplant-related death was defined as death during a continuous remission.

### Statistical analysis

Descriptive statistical analysis was performed to assess patient baseline characteristics, diagnosis, disease status at conditioning, donor-patient ABO mismatches, preparative regimen, and GVHD prophylaxis. Medians and ranges are provided for continuous variables and percentages are shown for categorical variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of acute and chronic GVHD, relapse and transplant-related mortality (TRM).[24] Gray's test was used for group comparisons of cumulative incidences.[25] An adjusted comparison of the groups with regard to overall survival (OS) was performed with the use of the Cox's proportional-hazards regression model.[26] For other outcomes with competing risks, Fine and Gray's proportional-hazards model for the subdistribution of a competing risk was used.[27] For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event. For GVHD, death without GVHD and relapse were competing events. For relapse, death without relapse was the competing event, and for transplant-related mortality (TRM), relapse was the competing event.[28] For acute GVHD, subjects were limited to those who engrafted, and for chronic GVHD, subjects were limited to those who engrafted and survived at least 100 days after transplantation.

The variables considered were the patient's age at transplant (5 years or over *vs.* under 5 years for pediatric recipients, and 50 years or over *vs.* under 50 years for adult recipients; cut-off points were around the median in each group), patient's sex, donor-patient sex mismatch (matched *vs.* male to female *vs.* female to male), donor-patient ABO mismatch (major mismatch *vs.* matched or minor mismatch), diagnosis (AML, ALL, CML or MDS), disease status at conditioning (first or second complete remission (CR) of AML, 1CR of ALL, first chronic phase of CML, and refractory anemia or refractory anemia with ringed sideroblasts as standard-risk diseases *vs.* advanced for all others), the conditioning regimen (reduced-intensity conditioning *vs.* myeloablative conditioning), and the type of prophylaxis against GVHD (tacrolimus-based *vs.* cyclosporine-based). Conditioning regimens were classified as myeloablative if total-body irradiation >8 Gy, oral busulfan ≥9 mg/kg, intravenous busulfan ≥7.2 mg/kg, or melphalan >140 $mg/m^2$ was used based on the report from the Center for International Blood and Marrow Transplant Research.[29,30] We cat-

egorized patients for whom there was insufficient information regarding the doses of agents or radiation used for the conditioning regimen according to information on the conditioning intensity (i.e. whether or not the conditioning regimen was intended to be myeloablative) as reported by the treating clinicians. The cryopreserved total nucleated cell dose was categorized as >10.0x10$^7$/kg, 5.0-9.9 x 10$^7$/kg, 2.5-4.9x10$^7$/kg, or <2.5 x 10$^7$/kg for children, and >3.0x10$^7$/kg, 2.5-2.9x10$^7$/kg, 2.0-2.4x10$^7$/kg, or <2.0x10$^7$/kg for adults. HLA disparity and nucleated cell dose were maintained in the model. Since patient age was highly correlated with the total nucleated cell dose in children, age was excluded from multivariate analyses for pediatric recipients. Other variables were selected in a backward stepwise manner with a variable retention criterion of $P$<0.05. Interaction between HLA disparity and adult (patient age at transplant 16 years or over) or child (patient age at transplant 15 years or under) was tested for overall survival by using a Cox's proportional-hazards regression model adjusted by other significant covariates in the final model for adult and pediatric recipients except for patient age. All $P$ values were two-sided.

## Results

### Patients' characteristics

Table 1 shows patients' characteristics, their disease, and transplant regimens. Median age at transplant was five years (range 0-15) in 498 pediatric and 49 years (range 16-82) in 1880 adult recipients of single-unit CBT. The proportion of females was 45% in both children and adults. Among children, the proportion of patients with ALL was greatest (58%) followed by that of patients with AML (34%). Among adults, the most frequent disease was AML (59%), followed by ALL (22%) and MDS (13%). The median number of cryopreserved total nucleated cells received in children was 5.30 x 10$^7$/kg, which was significantly greater (approximately double) than the number of nucleated cells received in adult patients (2.52 x 10$^7$/kg). In adults, only 33 patients (2%) received CB with a total nucleated cell dose greater than or equal to 5.0 x 10$^7$/kg. In children, 82 patients (16%) received HLA-matched (6/6) UCB, 222 (45%) received one-locus-mismatched (5/6), 158 (32%) received two-loci-mismatched (4/6), and 36 (7%) received three-loci-mismatched (3/6) UCB. For adults, the numbers and proportions of recipients were 71 (4%) for 6/6, 309 (16%) for 5/6, 1025 (55%) for 4/6, and 475 (25%) for 3/6. Among those who received 3/6 UCB, only 2 pediatric and 11 adult patients received three HLA-A, HLA-B, HLA-DR low-resolution mismatched UCB. Eighty-eight percent (TBI regimen 62%, non-TBI regimen 26%) and 62% (TBI regimen 56%, non-TBI regimen 6%) of children and adults, respectively, received myeloablative conditioning. Fludarabine-based reduced-intensity conditioning was given to 34% of adult recipients. T-cell depletion *in vivo* with antithymocyte globulin or antilymphocyte globulin was performed in only 6 (2%) child recipients and 26 (1%) adult recipients. The median follow-up period for survivors was 2.4 years (range 0.1-9.5) for pediatric recipients and 2.1 (range 0.1-9.0) years for adult recipients.

### Outcome

Overall survival, relapse, and transplant-related mortality: among children, overall mortality in 4/6 UCB recipients was significantly higher than that in 6/6 UCB recipients (RR=1.61, 95% confidence interval [CI], 1.02-2.56, $P$=0.042) (Table 2). Overall mortality increased with the number of mismatched loci in children ($P$ for trend 0.043). The increased mortality in 4/6 UCB recipients was mainly affected by increased transplant-related mortality (TRM) (RR=3.55, 95%CI: 1.47-8.58, $P$=0.005) ($P$ for trend 0.002) but not by the risk of relapse (RR=0.77, 95%CI: 0.48-1.24, $P$=0.392) in children. Among children, there were no differences in the risks of mortality and relapse between 5/6 UCB recipients (RR=1.07, $P$=0.765 for overall mortality; RR=1.06, $P$=0.794 for relapse; and RR=1.29, $P$=0.58 for TRM) and 6/6 UCB recipients (Table 2).

In adults, the number of HLA mismatches was not significantly associated with increased mortality (for overall mortality: RR=0.99, $P$=0.944 for 5/6; RR=0.88, $P$=0.436 for 4/6; RR=0.95, $P$=0.751 for 3/6; for TRM, RR=1.41, $P$=0.205 for 5/6; RR=1.24, $P$=0.408 for 4/6; RR=1.29, $P$=0.339 for 3/6). A two-loci mismatch was associated with a decreased risk of relapse in adult recipients (RR=0.70, $P$=0.075 for 5/6; RR=0.67, $P$=0.034 for 4/6; RR=0.70, $P$=0.07 for 3/6) (Table 2). The risks of mortality were similar when subjects were limited to those with standard risk disease status or to those with advanced risk disease status at transplant, to those who received myeloablative conditioning or to those who received reduced-intensity conditioning (*Online Supplementary Table S2*). A decreased risk of relapse was more prominent in patients with acute myeloid leukemia, and those who received reduced-intensity conditioning (*Online Supplementary Table S2*).)

Figure 1 shows unadjusted overall survival curves in children and adults. In children, the unadjusted probabilities of survival at three years post-transplant were 66% for 6/6, 62% for 5/6, 45% for 4/6, and 62% for 3/6 ($P$=0.032) (Figure 1A). In adults, the survival probabilities in all of the HLA disparity groups were similar (38% for 6/6, 37% for 5/6, 39% for 4/6, and 40% for 3/6 at three years post-transplant, $P$=0.567) (Figure 1B). A similar trend was seen when subjects were limited to standard-risk disease status at transplant (81% for 6/6, 76% for 5/6, 57% for 4/6, and 81% for 3/6 at three years post-transplant, $P$=0.035, for children; 51% for 6/6, 57% for 5/6, 58% for 4/6, and 55% for 3/6 at three years post-transplant, $P$=0.375, for adults) (*Online Supplementary Figure S1*).

A test of the interaction between HLA disparity and age (adult *vs.* child) revealed that the effect of HLA disparity on overall survival differed significantly between the pediatric and adult patient groups ($P$=0.009 for HLA disparity of 0-1 mismatches *vs.* 2-3 mismatches).

### Hematologic recovery

The cryopreserved total nucleated cell dose significantly affected neutrophil and platelet recovery in children and neutrophil recovery in adults (Table 3). HLA disparity did not significantly affect neutrophil or platelet recovery in adults or children for neutrophil recovery: RR=1.03, $P$=0.823 for 5/6; RR=0.96, $P$=0.799 for 4/6; RR=0.67, $P$=0.068 for 3/6 in children; RR=0.89, $P$=0.436 for 5/6; RR=0.92, $P$=0.576 for 4/6; RR=0.84, $P$=0.243 for 3/6 in adults; for platelet recovery: RR=0.89, $P$=0.438 for 5/6; RR=0.75, $P$=0.09 for 4/6; RR=0.71, $P$=0.164 for 3/6 in children; RR=1.05, $P$=0.775 for 5/6; RR=1.05, $P$=0.791 for 4/6; RR=0.99, $P$=0.951 in 3/6 in adults (Table 3).

Table 1. **Patients', disease, and transplant characteristics of pediatric and adult recipients of single-unit cord blood.**

| Characteristics | Children (age<16) N. | (%) | Adult (age>16) N. | (%) |
|---|---|---|---|---|
| N. of transplants | 498 | | 1880 | |
| Patient age at transplant | | | | |
| Median (range) | 5 (0-15) | | 49 (16-82) | |
| 0-9 years | 378 | (76) | | |
| 10-19 years | 120 | (24) | 88 | (5) |
| 20-29 years | | | 236 | (13) |
| 30-39 years | | | 317 | (17) |
| 40-49 years | | | 351 | (19) |
| 50-59 years | | | 492 | (26) |
| ≥60 years or older | | | 396 | (21) |
| Patient sex | | | | |
| Male | 275 | (55) | 1039 | (55) |
| Female | 223 | (45) | 841 | (45) |
| Sex matching | | | | |
| Matched | 207 | (42) | 696 | (37) |
| Male to female | 114 | (23) | 391 | (21) |
| Female to male | 125 | (25) | 485 | (26) |
| Unknown | 52 | (10) | 308 | (16) |
| Diagnosis | | | | |
| AML | 170 | (34) | 1115 | (59) |
| ALL | 290 | (58) | 418 | (22) |
| CML | 7 | (1) | 106 | (6) |
| MDS | 31 | (6) | 241 | (13) |
| Disease status | | | | |
| Standard | 247 | (50) | 673 | (36) |
| Advanced | 236 | (47) | 1127 | (60) |
| Unknown | 15 | (3) | 80 | (4) |
| ABO matching | | | | |
| Matched | 182 | (37) | 602 | (32) |
| Minor mismatch | 127 | (26) | 522 | (28) |
| Major mismatch | 113 | (23) | 451 | (24) |
| Bidirectional | 75 | (15) | 301 | (16) |
| Unknown | 1 | (<1) | 4 | (<1) |
| HLA mismatched number | | | | |
| Matched (6/6) | 82 | (16) | 71 | (4) |
| One locus mismatched (5/6) | 222 | (45) | 309 | (16) |
| Two loci mismatched (4/6) | 158 | (32) | 1025 | (55) |
| Three loci mismatched (3/6) | 36 | (7) | 475 | (25) |
| N. of cryopreserved nucleated cells (x10$^7$/kg) | | | | |
| Median | 5.30 | | 2.52 | |
| Range | 0.81-38.7 | | 0.71-9.98 | |
| N. of cryopreserved CD34-positive cells (x10$^5$/kg) | | | | |
| Median | 1.68 | | 0.83 | |
| Range | 0.072-65.66 | | 0.07-14.02 | |
| Preparative regimen* | | | | |
| MAST | | | | |
| CY+TBI | 216 | (43) | 891 | (47) |
| Other TBI regimen | 93 | (19) | 162 | (9) |
| BU+CY | 86 | (17) | 65 | (3) |
| Other non-TBI regimen | 41 | (8) | 47 | (3) |
| RIST | | | | |
| FL+BU+other | 6 | (1) | 172 | (9) |
| FL+CY+other | 12 | (2) | 119 | (6) |
| FL+Mel+other | 21 | (4) | 357 | (19) |
| Other RIST | 23 | (5) | 67 | (4) |
| T-cell depletion *in vivo*** | | | | |
| ATG or ALG use | 9 | (2) | 26 | (1) |
| GVHD prophylaxis*** | | | | |
| Cyclosporine A + sMTX | 157 | (32) | 748 | (40) |
| Cyclosporine A + MMF/steroid | 37 | (7) | 99 | (5) |
| Cyclosporine A alone | 31 | (6) | 142 | (8) |
| Tacrolimus + sMTX | 216 | (43) | 434 | (23) |
| Tacrolimus + MMF/steroid | 24 | (5) | 132 | (7) |
| Tacrolimus alone | 20 | (4) | 304 | (16) |
| Others | 13 | (3) | 21 | (1) |

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**CY: cyclophosphamide; CA: citarabine; BU: busulfan; TBI: total body irradiation; FL: fludarabine; Mel: melphalan, **ATG: antithymocyte globulin; ALG: antilymphocyte globulin; ***sMTX: short-term methotrexate; MMF: mycophenolate mofetil.*

### *Acute and chronic graft-versus-host disease*

The risk of grade 2 to 4 acute GVHD was significantly higher in HLA-mismatched UCB pediatric recipients (RR=2.13, *P*=0.004 for 5/6; RR=2.65, *P*<0.001 for 4/6; RR=2.39, *P*=0.0015 for 3/6; P for trend 0.001) (Table 4). The risk of chronic GVHD and extensive-type chronic GVHD was also significantly higher in 4/6 UCB recipients (RR=2.99, *P*=0.005 for chronic GVHD, and RR=7.62, *P*=0.047 for extensive-type chronic GVHD), and the risks increased according to the number of mismatches (P for trend, 0.002 for chronic GVHD, 0.005 for extensive-type chronic GVHD). In adults, in contrast to the results in children, there were no differences in the risks of grade 2 to 4 acute GVHD in 5/6 and 4/6 UCB recipients (for grade 2 to 4 acute GVHD, RR=1.03, *P*=0.916 for 5/6, RR=1.27, *P*=0.276 for 4/6). The risk of grade 2 to 4 acute GVHD was higher for 3/6 (RR=1.72, *P*=0.017). In adult recipients, the risk of chronic GVHD was increased in recipients of 4/6 UCB (RR=1.90, *P*=0.04), however, there were no differences in the risk of extensive-type chronic GVHD (RR=1.15, *P*=0.758 for 5/6; RR=1.62, *P*=0.253 for 4/6; RR=1.28, *P*=0.574 for 3/6) (Table 4).

### *Effect of total nucleated cell dose on outcome*

An increase in the cryopreserved total nucleated cell dose increased the incidence of neutrophil recovery in both children and adults, as well as the incidence of platelet recovery in children (Table 3). The cumulative incidences of neutrophil recovery were 94% for >10 x $10^7$/kg, 88% for 5.0-9.9 x $10^7$/kg, 82% for 2.5-4.9 x $10^7$/kg, and 86% for <2.5 x $10^7$/kg in children (*P*<0.001) (Figure 2A). The cell dose was significantly correlated with the recipient's age at transplant in children (the median ages were one year for >10 x $10^7$/kg, 3 years for 5.0-9.9 x $10^7$/kg, 8 years for 2.5-4.9 x $10^7$/kg, and 12 years for <2.5 x $10^7$/kg). The cumulative incidences of neutrophil recovery were 76% for >2.5 x $10^7$/kg and 74% for <2.5 x $10^7$/kg in adults (*P*=0.007) (Figure 2B). The cumulative incidences of TRM at three years post-transplant were 13% for >10 x $10^7$/kg, 14% for 5.0-9.9 x $10^7$/kg, 14% for 2.5-4.9 x $10^7$/kg, and 14% for <2.5x$10^7$/kg in children (*P*=0.98) and 29% for >2.5x$10^7$/kg and 28% for <2.5 x $10^7$/kg in adults (*P*=0.77) (*Online Supplementary Figure S2*). The probabilities of overall survival at three years post-transplant were 68% for >10x$10^7$/kg, 53% for 5.0-9.9 x $10^7$/kg, 57% for 2.5-4.9 x $10^7$/kg, and 55% for <2.5x$10^7$/kg in children (*P*=0.30) and 36% for >2.5 x 107/kg and 41% for <2.5x$10^7$/kg in adults (*P*=0.13). A lower total nucleated cell dose was neither associated with increased mortality in children or adults in multivariate analyses (Table 2). Thus, there was no combined effect of HLA disparity and total nucleated cell dose on mortality neither in children nor in adults (cumulative

incidence of TRM at three years post-transplant, 8% for 6/6, 11% for 5/6 and >5 x $10^7$/kg, 11% for 5/6 and 2.5-4.9 x $10^7$/kg, 0% for 5/6 and <2.5 x $10^7$/kg, 23% for 4/6 and >5 x $10^7$/kg, 24% for 4/6 and 2.5-4.9 x $10^7$/kg, 25% for 4/6 and <2.5 x $10^7$/kg in children, and 23% for 6/6, 29% for 5/6 and >2.5 x 107/kg, 30% for 5/6 and <2.5 x $10^7$/kg, 27% for 4/6 and >2.5 x $10^7$/kg, 27% for 4/6 and <2.5 x $10^7$/kg in adults (*Online Supplementary Figure S3*).

### Association of outcomes with the type of HLA mismatches for 4/6 adult recipients

The large number of adult recipients of 4/6 CB enabled us to analyze association of outcomes with the type of HLA mismatches in this population. The number of recipients were 7 for HLA-A double mismatch, 170 for HLA-A and HLA-B mismatch, 190 for HLA-A and HLA-DRB1 mismatch, 36 for HLA-B double mismatch, 581 for HLA-B and HLA-DRB1 mismatch, and 41 for HLA-DRB1 double mismatch. With adjusted analyses, adjusted with same variables in the final model of all adult recipients, there was no significant effect of HLA mismatch types on overall mortality with HLA-A and HLA-B mismatch as the reference (*Online Supplementary Table S3*). The risk of relapse was significantly decreased in HLA-A and HLA-DRB1

**Table 2. Multivariate analyses of overall survival, relapse, and transplant-related mortality.**

| Outcome | N. | Overall mortality RR | 95%CI | P | Relapse RR | 95%CI | P | Transplant-related mortality RR | 95%CI | P |
|---|---|---|---|---|---|---|---|---|---|---|
| Children 15 years or younger | | | | | | | | | | |
| HLA disparity | | | | | | | | | | |
| Matched (6/6) | 82 | 1.00 | | | 1.00 | | | 1.00 | | |
| 5/6 | 222 | 1.07 | (0.68-1.69) | 0.765 | 1.06 | (0.68-1.65) | 0.794 | 1.29 | (0.52-3.23) | 0.58 |
| 4/6 | 158 | 1.61 | (1.02-2.56) | 0.042 | 0.77 | (0.48-1.24) | 0.282 | 3.55 | (1.47-8.58) | 0.005 |
| 3/6 | 36 | 1.25 | (0.65-2.42) | 0.498 | 0.91 | (0.45-1.86) | 0.802 | 1.56 | (0.43-5.63) | 0.497 |
| Total nucleated cell dose | | | | | | | | | | |
| ≥10.0x$10^7$/kg | 85 | 1.00 | | | 1.00 | | | 1.00 | | |
| 5.0-9.9x$10^7$/kg | 169 | 1.14 | (0.72-1.79) | 0.579 | 1.10 | (0.69-1.75) | 0.684 | 0.82 | (0.40-1.68) | 0.592 |
| 2.5-4.9x$10^7$/kg | 190 | 0.92 | (0.58-1.45) | 0.707 | 0.90 | (0.56-1.44) | 0.651 | 0.90 | (0.45-1.80) | 0.77 |
| <2.5x$10^7$/kg | 43 | 0.88 | (0.47-1.67) | 0.701 | 0.98 | (0.53-1.83) | 0.961 | 0.67 | (0.24-1.88) | 0.443 |
| Adults 16 years or older | | | | | | | | | | |
| HLA disparity | | | | | | | | | | |
| Matched (6/6) | 71 | 1.00 | | | 1.00 | | | 1.00 | | |
| 5/6 | 309 | 0.99 | (0.71-1.38) | 0.944 | 0.70 | (0.47-1.04) | 0.075 | 1.41 | (0.83-2.41) | 0.205 |
| 4/6 | 1025 | 0.88 | (0.65-1.21) | 0.436 | 0.67 | (0.47-0.97) | 0.034 | 1.24 | (0.75-2.04) | 0.408 |
| 3/6 | 475 | 0.95 | (0.69-1.31) | 0.751 | 0.70 | (0.48-1.03) | 0.07 | 1.29 | (0.77-2.16) | 0.339 |
| Total nucleated cell dose | | | | | | | | | | |
| ≥3.0x$10^7$/kg | 439 | 1.00 | | | 1.00 | | | 1.00 | | |
| 2.5-2.9x$10^7$/kg | 492 | 0.99 | (0.83-1.17) | 0.876 | 0.86 | (0.70-1.06) | 0.167 | 1.10 | (0.86-1.42) | 0.445 |
| 2.0-2.4x$10^7$/kg | 705 | 0.86 | (0.72-1.01) | 0.06 | 0.79 | (0.65-0.97) | 0.021 | 1.05 | (0.83-1.33) | 0.694 |
| <2.0x$10^7$/kg | 183 | 0.93 | (0.73-1.18) | 0.562 | 0.79 | (0.59-1.07) | 0.126 | 1.00 | (0.70-1.45) | 0.983 |

*For overall mortality, other predictive variables were advanced disease status at transplant in children, and age at transplant over 50 years, male sex, advanced disease status at transplant, chronic myeloid leukemia (associated with a lower risk of mortality), and reduced-intensity conditioning in adults. For relapse, other predictive variables were advanced disease status at transplant, and acute lymphoblastic leukemia or myelodysplastic syndrome (associated with a lower risk of relapse) in children, and advanced disease status at transplant and myelodysplastic syndrome (associated with a lower risk of relapse) in adults. For transplant-related mortality, there was no other predictive variable in children. Other predictive variables for adults were age at transplant over 50 years and female to male donor-recipient sex mismatch.*

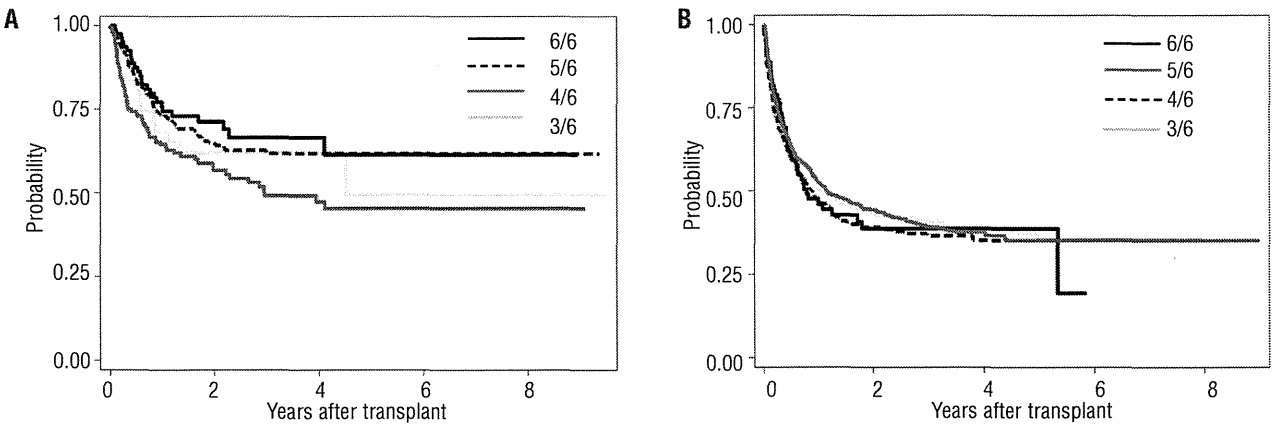


**Figure 1. Unadjusted probabilities of overall survival in HLA disparity groups for pediatric (A) and adult (B) recipients with leukemia. (A) In children, the unadjusted probabilities of survival at three years post-transplant were 66% for recipients of HLA matched (6/6), 62% for one-locus-mismatched (5/6), 45% for two-loci-mismatched (4/6), and 62% for three-loci-mismatched (3/6) single-unit unrelated cord blood (*P*=0.032). (B) In adults, these probabilities were 38% 37%, 39%, and 40% respectively (*P*=0.567) (B).**

mismatch, HLA-B and HLA-DRB1 mismatch, and HLA-DRB1 double mismatch recipients (RR=0.70, *P*=0.045; RR=0.76, *P*=0.047; and RR=0.46, *P*=0.03, respectively). The risk of transplant-related mortality was significantly increased in HLA-DRB1 double mismatch recipients (RR=2.06, *P*=0.025). There was no significant effect of HLA mismatch types for risks of grade 2 to 4 and grade 3 to 4 acute GVHD (*Online Supplementary Table S3*).

## Discussion

Our main objective was to assess the effect of HLA disparity on survival after single-unit UCBT in children and adults, and to obtain data that could be useful for the selection of an appropriate cord blood unit for patients with leukemia. Our study is the first to assess the effect of UCB HLA-matching on the transplant outcome in a large

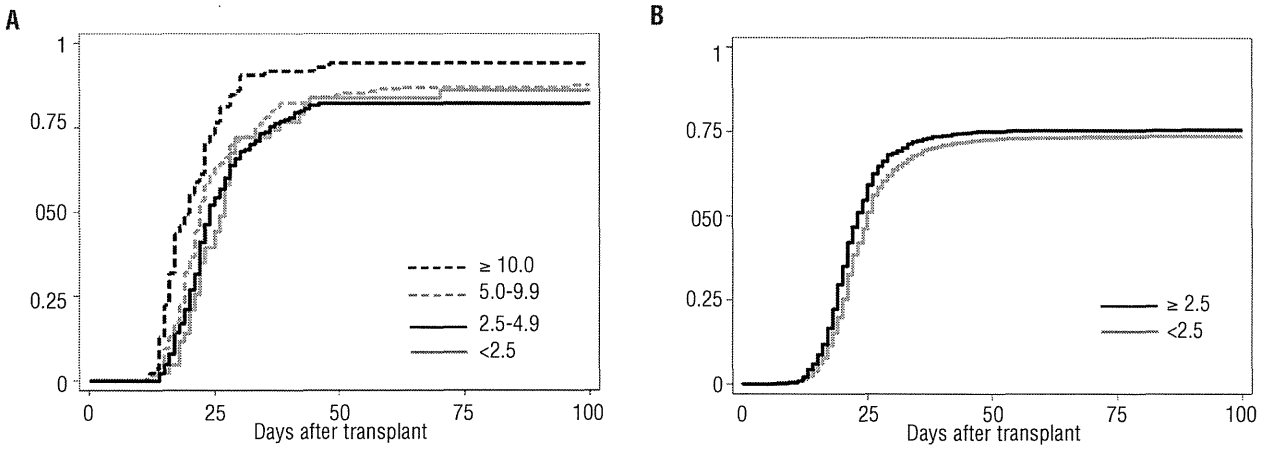


**Figure 2. Unadjusted cumulative incidences of neutrophil recovery in total nucleated cell dose groups for pediatric (A) and adult (B) recipients with leukemia.** (A) In children, the unadjusted cumulative incidences of neutrophil recovery were 94% for >10x10$^7$/kg, 88% for 5.0-9.9 x10$^7$/kg, 82% for 2.5-4.9x10$^7$/kg, and 86% for <2.5x10$^7$/kg (*P*<0.001). (B) In adults, these incidences were 76% for >2.5x10$^7$/kg and 74% for <2.5 x10$^7$/kg (*P*=0.007).

**Table 3. Multivariate analyses of neutrophil and platelet recovery.**

| Outcome | Children ≤15 years or younger N | RR | 95%CI | *P* value | | Adults ≥16 years or older N | RR | 95%CI | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Neutrophil recovery | | | | | | | | | |
| HLA disparity | | | | | | | | | |
| Matched (6/6) | 82 | 1.00 | | | | 71 | 1.00 | | |
| 5/6 | 222 | 1.03 | (0.77-1.39) | 0.823 | | 309 | 0.89 | (0.66-1.19) | 0.436 |
| 4/6 | 158 | 0.96 | (0.71-1.30) | 0.799 | | 1025 | 0.92 | (0.70-1.22) | 0.576 |
| 3/6 | 36 | 0.67 | (0.44-1.03) | 0.068 | | 475 | 0.84 | (0.64-1.12) | 0.243 |
| Total nucleated cell dose | | | | | | | | | |
| ≥>10.0x10$^7$/kg | 85 | 1.00 | | | ≥3.0x10$^7$/kg | 439 | 1.00 | | |
| 5.0-9.9x10$^7$/kg | 169 | 0.66 | (0.49-0.89) | 0.007 | 2.5-2.9x10$^7$/kg | 492 | 0.84 | (0.72-0.97) | 0.021 |
| 2.5-4.9x10$^7$/kg | 190 | 0.50 | (0.37-0.67) | <0.001 | 2.0-2.4x10$^7$/kg | 705 | 0.79 | (0.68-0.90) | 0.001 |
| <2.5x10$^7$/kg | 43 | 0.54 | (0.38-0.77) | 0.001 | <2.0x10$^7$/kg | 183 | 0.78 | (0.64-0.94) | 0.009 |
| Platelet recovery | | | | | | | | | |
| HLA disparity | | | | | | | | | |
| Matched (6/6) | 82 | 1.00 | | | | 71 | 1.00 | | |
| 5/6 | 222 | 0.89 | (0.66-1.20) | 0.438 | | 309 | 1.05 | (0.73-1.52) | 0.775 |
| 4/6 | 158 | 0.75 | (0.54-1.05) | 0.09 | | 1025 | 1.05 | (0.74-1.48) | 0.791 |
| 3/6 | 36 | 0.71 | (0.44-1.15) | 0.164 | | 475 | 0.99 | (0.69-1.41) | 0.951 |
| Total nucleated cell dose | | | | | | | | | |
| ≥10.0x10$^7$/kg | 85 | 1.00 | | | ≥3.0x10$^7$/kg | 439 | 1.00 | | |
| 5.0-9.9x10$^7$/kg | 169 | 0.93 | (0.68-1.29) | 0.681 | 2.5-2.9x10$^7$/kg | 492 | 0.84 | (0.70-1.01) | 0.058 |
| 2.5-4.9x10$^7$/kg | 190 | 0.70 | (0.51-0.97) | 0.03 | 2.0-2.4x10$^7$/kg | 705 | 0.86 | (0.73-1.02) | 0.078 |
| <2.5x10$^7$/kg | 43 | 0.70 | (0.45-1.07) | 0.101 | <2.0x10$^7$/kg | 183 | 0.72 | (0.57-0.91) | 0.007 |

*For neutrophil recovery, other predictive variables were acute lymphoblastic leukemia in children (with a higher neutrophil recovery), and advanced disease status at transplant in adults. For platelet recovery, other predictive variables were advanced disease status at transplant in children, and age at transplant over 50 years, male sex, and advanced disease status at transplant in adults.*