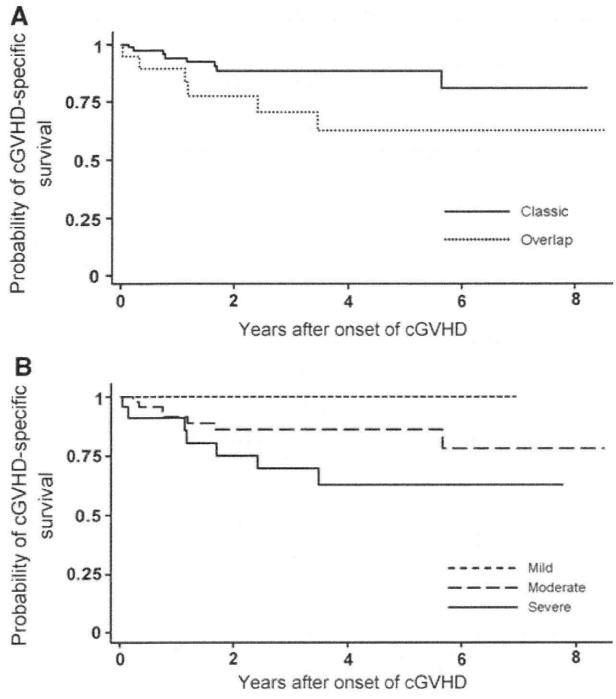

Fig. 1 Chronic GVHD-specific survival in patients with chronic GVHD diagnosed by the NIH consensus criteria. **a** Probability of chronic GVHD-specific survival (cGSS) among patients who developed classic chronic GVHD (*solid line*) and overlap syndrome (*dotted line*). **b** Probability of cGSS among patients who developed mild (*short dashed line*), moderate (*long dashed line*), and severe chronic GVHD (*solid line*)

improved grading scales for established cGVHD. A retrospective analysis of data on HLA-identical sibling transplantation reported to the International Bone Marrow Transplant Registry identified five variables independently associated with worse survival of those who developed historic cGVHD: low Karnofsky performance status at cGVHD diagnosis (<80), chronic diarrhea, weight loss, presence of cutaneous manifestation, and lack of oral involvement [15]. The Seattle group also proposed a revised classification for distinguishing limited and extensive cGVHD by the use of 16 clinical criteria [16]. Although these new classifications do not clearly discriminate between cGVHD and delayed onset GVHD with features resembling aGVHD, they have been shown to be at least useful for identifying patients at higher risk of NRM. Future studies are strongly warranted to compare the prognostic values of NIH cGVHD subcategories with those determined by other cGVHD grading system [21].

So far, several groups have reported the prognostic relevance of cGVHD severity graded by the NIH criteria and consistently found the inferior survival of patients with severe cGVHD [20–23], although such association was not observed in one earlier study [19]. While only a few of these studies focused on the significance of distinction between "overlap syndrome" and "classic cGVHD", our study revealed a trend toward worse survival in patients with overlap syndrome compared to those with classic GVHD, as was recently reported by Kim et al. [23]. In the present study, patients with overlap syndrome had a significantly shorter median time to the development of cGVHD than patients with classic cGVHD and were more likely to receive corticosteroid treatment for prior aGVHD at the onset of cGVHD. Intriguingly, these observations were very similar to the findings by Arora et al. [22], who reported that most of patients with overlap syndrome had a history of prior aGVHD and a progressive cGVHD onset, although they did not observe worse survival of this subgroup of patients compared to those with classic cGVHD. Given that nearly all patients who developed overlap syndrome had a prior history of aGVHD in our study cohort, NIH overlap syndrome in most instances could be considered as a flare of preexisting aGVHD, concomitant with development of classic cGVHD. In this context, it is important to note that early flare of cGVHD or early treatment change for exacerbation of cGVHD has been reported to be associated with increased NRM and inferior cGSS [34, 35]. It is also of note that a significantly higher proportion of patients with overlap syndrome had thrombocytopenia less than $100 \times 10^3/\mu L$ at cGVHD onset in our study. Since the progressive cGVHD onset and the presence of thrombocytopenia were consistently associated with an increased NRM across various studies [16, 36], more effective management of patients with overlap syndrome and thrombocytopenia might be needed.

Duration of systemic immunosuppressive therapy is suggested to be a useful surrogate endpoint to evaluate the response to specific treatment for cGVHD [26]. Although we could not find significant association of NIH cGVHD subtypes with duration of systemic IST, patients who had been given ongoing systemic corticosteroids at the onset of cGVHD were found to receive significantly prolonged systemic IST in multivariable analysis, consistent with the findings of Vigorito et al. [37]. In our study, the duration of systemic IST was also prolonged in patients who had high-risk underlying disease compared with those who had standard-risk disease. If the activity of cGVHD were likely to worsen in the high-risk subgroup of patients, one possible explanation might be the preference of physicians to taper systemic IST faster for patients at higher risk of relapse.

The present study, however, has several limitations; the retrospective study design, small cohort size, recording bias, and heterogeneity of underlying diseases and transplantation procedures might substantially influence the results. In addition, diagnostic cGVHD manifestations of affected organs or sites might have originated from other causes, including drug reactions, infection, and

Table 3 Univariable and multivariable analysis of factors potentially associated with chronic GVHD-specific survival among patients who developed chronic GVHD defined by the National Institutes of Health criteria

Variable	*n* (%)	Univariable analysis		Multivariable analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Patient age					
Less than 50 years	51 (53)	1.00		–	
50 years or more	45 (47)	1.40 (0.49–4.05)	0.53	–	
Donor/recipient sex combination					
Other than female/male	69 (72)	1.00		–	
Female/male	27 (28)	1.03 (0.32–3.28)	0.97	–	
Disease status at transplant					
Standard risk	51 (53)	1.00		1.00	
High risk	45 (47)	3.03 (0.95–9.68)	0.061	2.75 (0.86–8.80)	0.088
Donor/recipient HLA compatibility					
Matched	80 (83)	1.00		–	
Mismatched	16 (17)	0.33 (0.04–2.53)	0.29	–	
Conditioning regimen					
Myeloablative intensity	54 (56)	1.00		–	
Reduced intensity	42 (44)	1.04 (0.36–3.00)	0.95	–	
Stem cell source					
Bone marrow	67 (70)	1.00		–	
Peripheral blood	24 (25)	2.07 (0.69–6.19)	0.19	–	
Cord blood	5 (5)	1.63 (0.57–4.68)	0.37	–	
Prior aGVHD					
Grade 0–1	47 (49)	1.00		–	
Grade 2–4	49 (51)	1.16 (0.40–3.37)	0.78	–	
Subcategory of cGVHD					
Classic cGVHD	77 (80)	1.00		–	
Overlap syndrome	19 (20)	2.76 (0.96–7.97)	0.060	–	
Severity of cGVHD at onset					
Mild to moderate	73 (76)	1.00		1.00	
Severe	23 (24)	3.10 (1.09–8.86)	0.034	2.58 (0.90–7.39)	0.077
Platelet count at cGVHD onset					
$100 \times 10^3/\mu L$ or more	65 (68)	1.00		1.00	
Less than $100 \times 10^3/\mu L$	31 (32)	4.19 (1.40–12.5)	0.010	4.05 (1.35–12.1)	0.013
Eosinophil count at cGVHD onset					
Less than 500/μL	68 (71)	1.00		–	
500/μL or more	28 (29)	0.90 (0.28–2.88)	0.86	–	
Systemic corticosteroids at cGVHD onset					
Not received	63 (66)	1.00		–	
Received	33 (34)	1.74 (0.61–4.97)	0.30	–	

CI confidence interval, *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease

comorbidity before transplantation. Furthermore, genital tract involvement might be underestimated because female patients do not always report about their genital symptoms to physicians.

In conclusion, our present study suggests that both the subcategory and global severity of cGVHD proposed by NIH consensus criteria have effects on cGSS and the risk of NRM among patients who develop NIH cGVHD. Future prospective studies are warranted to more precisely characterize the clinical significance of the subcategory and severity of cGVHD evaluated by the NIH consensus criteria.

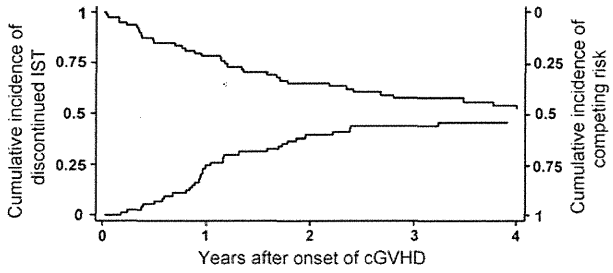

Fig. 2 Cumulative incidence of discontinued systemic immunosuppressive treatment. The *lower curve* shows the cumulative incidence of discontinued systemic immunosuppressive treatment (IST) in the absence of death, recurrent primary disease, or secondary malignancy among 81 patients who developed NIH cGVHD and received systemic IST (*left-hand scale*). The *upper curve* shows the competing risks of death or recurrent/secondary malignancy during systemic IST (*right-hand scale*). At the onset of cGVHD, 69 patients had been already given ongoing systemic IST consisting of calcineurin inhibitors alone ($n = 36$), calcineurin inhibitors plus corticosteroids ($n = 27$), corticosteroids alone ($n = 4$), or corticosteroids plus mycophenolate mofetil ($n = 2$)

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Conflict of interest The authors have no conflict of interest to declare.

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ORIGINAL ARTICLE

Unrelated cord blood transplantation vs related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction

J Kanda[1], T Ichinohe[2], S Kato[3], N Uchida[4], S Terakura[5], T Fukuda[6], M Hidaka[7], Y Ueda[8], T Kondo[9], S Taniguchi[4], S Takahashi[10], T Nagamura-Inoue[11], J Tanaka[12], Y Atsuta[13], K Miyamura[14] and Y Kanda[1] on behalf of the Donor/Source Working Group and HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation

Little information is available regarding whether an unrelated cord blood (UCB) unit or a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the graft-versus-host direction (RD/1AG-MM-GVH) should be selected as an alternative donor for patients without an HLA-matched related/unrelated donor. Therefore, we conducted a retrospective study using national registry data on patients with leukemia or myelodysplastic syndrome who received transplantation using a single UCB ($n = 2288$) unit or an RD/1AG-MM-GVH ($n = 525$). We found that the survival rate in the UCB group was comparable to that in the RD/1AG-MM-GVH group, although the RD/1AG-MM-GVH group with an HLA-B mismatch showed significantly higher overall and non-relapse mortality. Neutrophil and platelet engraftment were significantly faster, whereas the incidence of acute or chronic graft-versus-host disease (GVHD) was significantly higher in the RD/1AG-MM-GVH group. The incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group with *in vivo* T-cell depletion was comparable to that in the UCB group, which translated into a trend toward better overall survival, regardless of the presence of an HLA-B mismatch. In conclusion, UCB and RD/1AG-MM-GVH are comparable for use as an alternative donor, except for RD/1AG-MM-GVH involving an HLA-B mismatch.

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Keywords: cord blood transplantation; related transplantation; HLA mismatch; alternative donor

INTRODUCTION

For patients who lack an HLA-identical sibling, an HLA-matched unrelated donor (MUD) is considered to be the preferred alternative donor in allogeneic hematopoietic cell transplantation (HCT).[1–5] However, it is difficult to find an MUD for patients with rare HLA haplotypes. Furthermore, it takes at least a few months from the start of an unrelated donor search to actually receive a graft. Therefore, there is a large demand for an alternative source to an HLA-identical sibling or MUD, particularly for patients who have a rare haplotype or who need immediate transplantation.

Unrelated cord blood (UCB) has emerged as a promising alternative source for pediatric and adult patients.[6–17] In UCB transplantation, up to two antigen/allele mismatches between a recipient and cord blood unit are acceptable without an increased risk of acute graft-versus-host disease (GVHD). The clinical outcome in UCB transplantation is improving, and is almost comparable to that in HLA 8/8 allele MUD transplantation, although a high risk of graft failure and early treatment-related complications are still major issues.[15–17]

Another alternative source is an HLA-mismatched related donor, particularly when a related donor with a 1-antigen mismatch at the HLA-A, HLA-B, or HLA-DR locus in the graft-versus-host (GVH) direction (RD/1AG-MM-GVH) is available. HCT from an RD/1AG-MM-GVH results in a higher but acceptable incidence of acute GVHD.[18–20] In previous studies, HLA mismatches in the host-versus-graft (HVG) direction were associated with a higher incidence of graft failure and lower overall survival (OS).[18,19,21] However, the risk of graft failure might have been improved by the use of conditioning regimens that strongly suppress the recipient's immune system.[22] Therefore, in current clinical practice in Japan, stem cell transplantation from an RD/1AG-MM-GVH is being performed while accepting multiple antigen mismatches in the HVG direction without specific *ex vivo* stem cell manipulation.[18,19,23] We have recently reported that OS in transplantation from an RD/1AG-MM-GVH involving an HLA-B antigen mismatch was inferior, whereas that from an RD/1AG-MM-GVH involving an HLA-A or -DR antigen mismatch was comparable to that from an 8/8-MUD in standard-risk diseases.[23]

Unlike transplantation from an MUD, transplantation using a UCB unit or an RD/1AG-MM-GVH can be performed immediately when necessary. However, little information is available regarding the priority in selecting these alternative donors. Therefore, we conducted a retrospective study using national registry data on 2813 patients with leukemia or myelodysplastic syndrome (MDS)

[1]Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan; [2]Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga University, Saga, Japan; [3]Department of Cell Transplantation & Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan; [4]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [5]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [6]Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan; [7]Department of Internal Medicine, National Hospital Organization, Kumamoto Medical Center, Kumamoto, Japan; [8]Department of Haematology/Oncology, Kurashiki Central Hospital, Kurashiki, Japan; [9]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [10]Department of Molecular Therapy, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [11]Department of Cell Processing & Transfusion, Research Hospital, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [12]Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [13]Department of Hematopoietic Stem Cell Transplantation Data Management/Biostatistics, Nagoya University School of Medicine, Nagoya, Japan and [14]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan. Correspondence: Dr J Kanda, Division of Hematology, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma-cho, Omiya-ku, Saitama city, Saitama, Japan.
E-mail: jkandajp@gmail.com
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who received transplantation using a single UCB or an RD/1AG-MM-GVH.

MATERIALS AND METHODS

Data collection

Data for patients (age: ⩾16 years) with acute myeloid leukemia, acute lymphoblastic leukemia, MDS and chronic myelogenous leukemia who received a first HCT using a single HLA 0–2 antigen-mismatched UCB unit or an RD/1AG-MM-GVH between 1 January 1998 and 31 December 2009 were obtained from the Transplant Registry Unified Management Program (TRUMP),[24] which includes data from the Japan Cord Blood Bank Network (JCBBN) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT). Our analysis included 2306 patients who received a single UCB graft (UCB group) and 541 patients who received a graft from an RD/1AG-MM-GVH (RD/1AG-MM-GVH group). As of January 2012, double UCB grafts for HCT are not available in Japan. The following patients were excluded: 26 patients who lacked data on survival status, survival date, sex of recipient, or GVHD prophylaxis and 8 patients who received stem cells that had been manipulated by *ex vivo* T-cell depletion or CD34 selection. Overall, 2288 patients who received a UCB unit and 525 who received a graft from an RD/1AG-MM-GVH fulfilled the criteria. The study was approved by the data management committees of TRUMP and by the institutional review boards of Japanese Red Cross Nagoya First Hospital and Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for the HLA-A, HLA-B and HLA-DR loci were obtained from reports from the institution where the transplantation was performed or from cord blood banks. To reflect current practice in Japan, HLA matching in UCB or RD/1AG-MM-GVH transplantation was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci. An HLA mismatch in the GVH direction was defined as when the recipient's antigens or alleles were not shared by the donor, whereas a mismatch in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient.

End points

The primary end point of the study was to compare OS rates between the UCB and RD/1AG-MM-GVH groups. Other end points were the cumulative incidences of neutrophil and platelet engraftment, acute and chronic GVHD, relapse, and non-relapse mortality (NRM). Neutrophil recovery was considered to have occurred when the absolute neutrophil count exceeded 0.5×10^9/l for 3 consecutive days following transplantation. Platelet recovery was considered to have occurred when the absolute platelet count exceeded 50×10^9/l without platelet transfusion. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to the traditional criteria.[25,26] The incidence of chronic GVHD was evaluated in patients who survived for at least 100 days.

Statistical analysis

Descriptive statistics were used to summarize variables related to the patient characteristics. Comparisons between groups were performed with the χ^2-test or extended Fisher's exact test as appropriate for categorical variables and the Mann–Whitney *U*-test for continuous variables. The probability of OS was estimated according to the Kaplan–Meier method, and the groups were compared with the log-rank test. The adjusted probability of OS was estimated according to the Cox proportional-hazards model, with other significant variables considered in the final multivariate model. The probabilities of neutrophil and platelet engraftment, acute and chronic GVHD, NRM, and relapse were estimated on the basis of cumulative incidence methods, and the groups were compared with the Gray test;[27,28] competing events were death without engraftment for neutrophil and platelet engraftment, death or relapse without GVHD for acute and chronic GVHD, death without relapse for relapse, and relapse for NRM. The Cox proportional-hazards model was used to evaluate variables that may affect OS, whereas the Fine and Gray proportional-hazards model was used to evaluate variables that may affect engraftment, GVHD, NRM and relapse.[29] We classified the conditioning regimen as myeloablative if either total body irradiation >8 Gy, oral busulfan ⩾9 mg/kg, intravenous busulfan ⩾7.2 mg/kg, or melphalan >140 mg/m^2 was used in the conditioning regimen, and otherwise classified it as reduced intensity, based on the report by the Center for International Blood and Marrow Transplant Research.[30] For patients for whom the doses of agents used in the conditioning regimen were not available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by the treating clinicians. Acute leukemia in the first or second remission, chronic myelogenous leukemia in the first or second chronic phase or accelerated phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts were defined as standard-risk diseases, and other conditions were defined as high-risk diseases. The following variables were considered when comparing the UCB and RD/1AG-MM-GVH groups: the recipient's age group (⩽50 years or >50 years at transplantation), sex of recipient, disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia or MDS), disease status before transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced intensity), type of GVHD prophylaxis (calcineurin inhibitor and methotrexate, calcineurin inhibitor only, or other), year of transplantation (1998–2004, 2005–2009), and the time from diagnosis to transplantation (<6 months or ⩾6 months). In the analysis within the RD/1AG-MM-GVH group, the use of *in vivo* T cell depletion (no vs yes), stem cell source (peripheral blood (PB) stem cells vs bone marrow (BM)), and the number of HLA mismatches in the HVG direction (0–1 vs 2–3) were also considered. Factors without a variable of main interest were selected in a stepwise manner from the model with a variable retention criterion of $P<0.05$. We then added a variable of main interest to the final model. All tests were two-sided, and $P<0.05$ was considered to indicate statistical significance. All statistical analyses were performed with Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[31] EZR is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria). More precisely, it is a modified version of R commander (version 1.6–3) that was designed to add statistical functions that are frequently used in biostatistics.

RESULTS

Characteristics of patients and transplants

Table 1 shows the patient and transplant characteristics. Recipients of an RD/1AG-MM-GVH were younger than recipients of a UCB unit. Approximately half of the recipients in the RD/1AG-MM-GVH group received PB. The number of HLA mismatches in the GVH direction between a UCB unit and recipient was 0 in 10%, 1 in 33% and 2 in 57%. In the RD/1AG-MM-GVH group, the number of antigen mismatches in the HVG direction was 0 in 12%, 1 in 68%, 2 in 18% and 3 in 3%. Most of the recipients of an RD/1AG-MM-GVH received a calcineurin inhibitor with methotrexate for GVHD prophylaxis, whereas 25% of UCB recipients received only calcineurin inhibitor. *In vivo* T-cell depletion including antithymocyte globulin (ATG) or alemtuzumab was used in 10% of the RD/1AG-MM-GVH group, but in only 1% of the UCB group. Alemtuzumab was used in only one patient, who received transplantation from an RD/1AG-MM-GVH. Information regarding the dose and type of ATG was missing in two-third of the patients who received ATG. Available data showed that the median dose of thymoglobulin was 2.5 (range 2.5–9.0, $n=9$) and 2.5 (range 1.25–5.0, $n=10$) mg/kg and the median dose of ATG-Fresenius was 8.0 (range 5.0–10.0, $n=3$) and 8.0 (range 5.0–10.0, $n=7$) mg/kg, in the UCB and RD/1AG-MM-GVH groups, respectively. Two-third of UCB transplantations were performed between 2005 and 2009. The median duration of follow-up for survivors was 2 and 4 years in the UCB and RD/1AG-MM-GVH groups, respectively.

Neutrophil and platelet engraftment

The incidence of neutrophil engraftment at day 50 in the RD/1AG-MM-GVH group was higher than that in the UCB group (UCB group, 73%, 95% confidence interval (CI), 71–75%; RD/1AG-MM-GVH group, 93%, 95% CI, 91–95%; Gray test, $P<0.001$; Figure 1a). The incidence of platelet engraftment at day 150 in the

Table 1. Patient characteristics

Variable	*UCB (n = 2288)*	*RD/1AG-MM-GVH (n = 525)*	P
Age at transplant, median (range)	49 (16–82)	43 (16–74)	<0.001
Recipient sex			
Female	1004 (44%)	239 (46%)	0.494
Male	1284 (56%)	286 (54%)	
Disease			
Acute myelogenous leukemia	1365 (60%)	269 (51%)	0.003
Acute lymphoblastic leukemia	498 (22%)	137 (26%)	
Chronic myelogenous leukemia	124 (5%)	42 (8%)	
Myelodysplastic syndrome	301 (13%)	77 (15%)	
Duration from diagnosis to transplant			
Median time (range), months	7.9 (0.2–768.5)	7.6 (0–251.7)	0.233
Disease risk			
Standard	959 (42%)	249 (47%)	0.050
High	1217 (53%)	257 (49%)	
Unknown	112 (5%)	19 (4%)	
Source of stem cells			
Bone marrow	—	251 (48%)	—
Peripheral blood	—	274 (52%)	
Cord blood	2288 (100%)	—	
HLA compatibility in the graft-versus-host direction			
Matched	225 (10%)	—	<0.001
One-antigen mismatch	753 (33%)	525 (100%)	
Two-antigen mismatch	1310 (57%)	—	
HLA compatibility in the host-versus-graft direction			
Matched	233 (10%)	62 (12%)	<0.001
One-antigen mismatch	716 (31%)	355 (68%)	
Two-antigen mismatch	1339 (59%)	94 (18%)	
Three-antigen mismatch	—	14 (3%)	
Conditioning regimen			
Myeloablative	1390 (61%)	253 (48%)	<0.001
CY + TBI ±	1062	164	
Other TBI regimen	130	20	
BU + CY ±	88	45	
Other non-TBI regimen	110	24	
Reduced intensity	894 (39%)	162 (31%)	
FLU ± TBI ±	840	138	
Other regimen	54	24	
Unclassifiable	4 (0.2%)	110 (21%)	
GVHD prophylaxis			
CSA/TAC + MTX	1410 (62%)	448 (85%)	<0.001
CSA/TAC + MMF	246 (11%)	12 (2%)	
CSA/TAC + Steroid	28 (1%)	13 (2%)	
CSA/TAC only	571 (25%)	45 (9%)	
Unknown	33 (1%)	7 (1%)	
Use of in vivo *T-cell depletion*			
No	2258 (99%)	472 (90%)	<0.001
Yes	30 (1%)	53 (10%)	
Year at transplant			
1998–2004	760 (33%)	260 (50%)	<0.001
2005–2009	1528 (67%)	265 (50%)	
Follow-up of survivors			
Median time (range), years	2.1 (0.0–10.0)	4.0 (0.1–12.2)	<0.001

Abbreviations: BU, busulfan; CSA, cyclosporine; CY, cyclophosphamide; FLU, fludarabine; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus; TBI, total body irradiation; UCB, unrelated cord blood.

RD/1AG-MM-GVH group was also higher than that in the UCB group (UCB group, 53%, 95% CI, 51–55%; RD/1AG-MM-GVH group, 70%, 95% CI, 66–74%; Gray test, $P<0.001$; Figure 1b). The use of RD/1AG-MM-GVH was significantly associated with a higher incidence of neutrophil and platelet engraftment in the multivariate analysis (neutrophil engraftment, hazard ratio (HR), 3.46,

95% CI, 3.00–3.98, $P<0.001$; platelet engraftment, HR 2.20, 95% CI, 1.89–2.57, $P<0.001$; Supplementary Table 1). As our previous study revealed that an HLA-B mismatch had an adverse effect on OS in transplantation from an RD/1AG-MM-GVH, patients in the RD/1AG-MM-GVH group with an HLA-A, -B, or -DR mismatch were separately compared with the UCB group. We consistently observed superior neutrophil and platelet engraftment in each RD/1AG-MM-GVH group as compared with the UCB group (Supplementary Table 1).

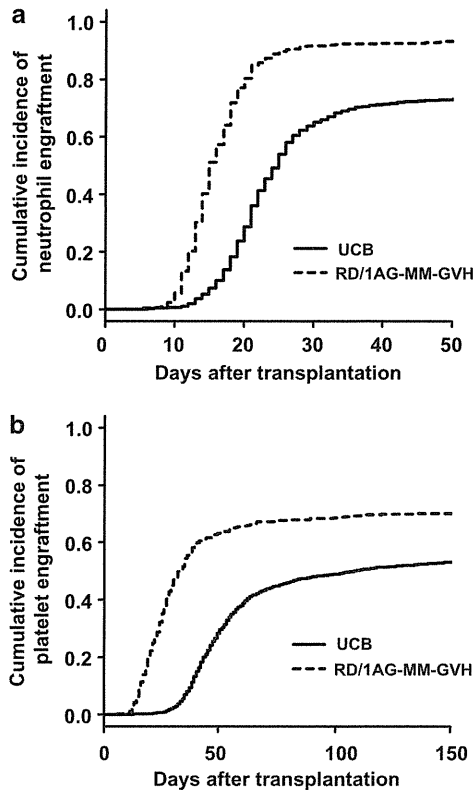

Figure 1. Neutrophil (**a**) and platelet engraftment (**b**).

Acute and chronic GVHD

The incidence of grade II–IV or grade III–IV acute GVHD in the RD/1AG-MM-GVH group was significantly higher than that in the UCB group (grade II–IV acute GVHD at day 100: UCB group, 34%, 95% CI, 32–36%; RD/1AG-MM-GVH group, 50%, 95% CI, 45–54%; Gray test, $P<0.001$; grade III–IV acute GVHD at day 100: UCB group, 11%, 95% CI, 10–13%; RD/1AG-MM-GVH group, 21%, 95% CI, 17–24%; Gray test, $P<0.001$; Figures 2a and b). The incidence of chronic GVHD or extensive type of chronic GVHD in the RD/1AG-MM-GVH group was also significantly higher than that in the UCB group (chronic GVHD at 3 years: UCB group, 25%, 95% CI, 23–27%; RD/1AG-MM-GVH group, 42%, 95% CI, 38–47%; Gray test, $P<0.001$; extensive chronic GVHD at 3 years: UCB group, 11%, 95% CI, 10–13%; RD/1AG-MM-GVH group, 29%, 95% CI, 25–34%; Gray test, $P<0.001$; Figures 2c and d). A multivariate analysis confirmed a higher risk of grade II–IV or grade III–IV acute GHVD, chronic or extensive chronic GVHD in the RD/1AG-MM-GVH group than in the UCB group (grade II–IV acute GVHD; HR 1.64, 95% CI, 1.43–1.90, grade III–IV acute GVHD; HR 2.28, 95% CI, 1.80–2.88, chronic GVHD; HR 1.47, 95% CI, 1.24–1.73, extensive chronic GVHD; HR 2.35, 95% CI, 1.90–2.91, Supplementary Table 2).

OS

The 3-year unadjusted OS rates in the UCB and RD/1AG-MM-GVH groups were 38% (36–41%) and 39% (34–43%), respectively ($P=0.115$). The use of either UCB or RD/1AG-MM-GVH was not associated with OS rates in the multivariate analysis (UCB vs RD/1AG-MM-GVH, HR, 0.99, 95% CI, 0.87–1.12, $P=0.833$) in all-risk patients, or either standard-risk ($P=0.588$) or high-risk patients ($P=0.639$; Table 2), after adjusting for the following significant risk factors: age >50 years, male recipient, acute myeloid leukemia vs MDS, high-risk disease, GVHD prophylaxis using only calcineurin inhibitor vs calcineurin inhibitor + methotrexate, and earlier year

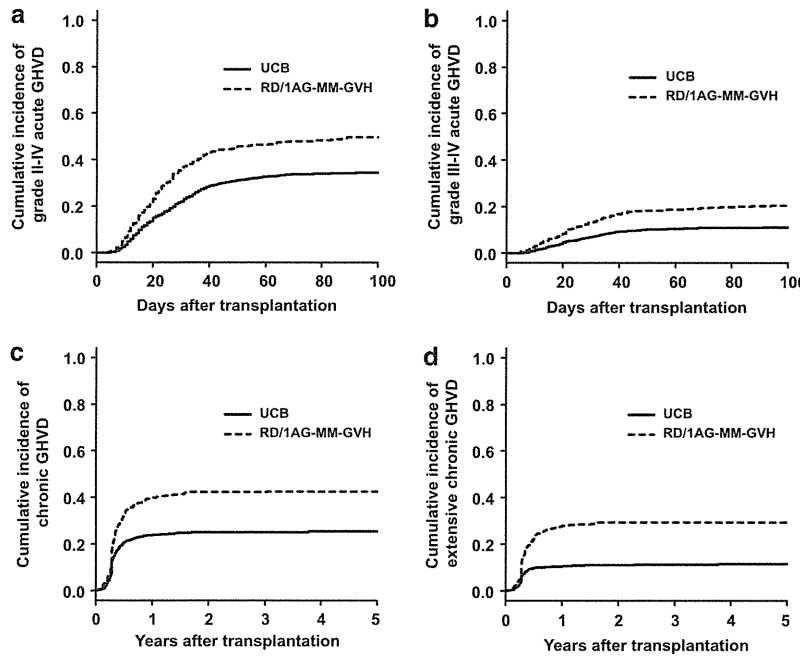

Figure 2. Acute and chronic GVHD. Cumulative incidences of grade II–IV (**a**) and grade III–IV acute GVHD (**b**) and chronic (**c**) and extensive chronic GVHD (**d**) are shown.

Table 2. Multivariate analysis of overall mortality

Variable	*Total*[a]		*Standard risk*[b]		*High risk*[c]	
	HR (95% CI)	P *value*	*HR (95% CI)*	P *value*	*HR (95% CI)*	P *value*
(A)						
UCB	1.00	reference	1.00	reference	1.00	reference
RD/1AG-MM-GVH	0.99 (0.87–1.12)	0.833	1.06 (0.86–1.31)	0.588	0.96 (0.81–1.13)	0.639
(B)						
UCB	1.00	reference	1.00	reference	1.00	reference
RD/HLA-A-MM-GVH	0.92 (0.72–1.18)	0.519	0.99 (0.66–1.48)	0.959	0.90 (0.64–1.26)	0.551
RD/HLA-B-MM-GVH	1.20 (1.01–1.44)	0.043	1.44 (1.05–1.96)	0.023	1.12 (0.89–1.41)	0.326
RD/HLA-DR-MM-GVH	0.85 (0.70–1.02)	0.084	0.88 (0.66–1.19)	0.411	0.84 (0.65–1.08)	0.170

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CI, confidence interval; CML, chronic myelogenous leukemia; CSA, cyclosporine; HR, hazard ratio; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus. [a]Other significant variables in model A were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.50, 95% CI, 1.35–1.66, $P<0.001$); sex of recipient, female (reference, 1.00), male (HR, 1.12; 95% CI, 1.02–1.24; $P=0.023$); diagnosis, AML (reference, 1.00),ALL (HR, 1.11, 95% CI, 0.98–1.26, $P=0.112$), CML (HR, 0.90, 95% CI, 0.72–1.13, $P=0.374$), MDS (HR, 0.81, 95% CI, 0.68–0.95, $P=0.001$); disease risk, standard risk (reference, 1.00), high risk (HR, 2.24; 95% CI, 2.00–2.50; $P<0.001$), status not known, (HR, 1.59; 95% CI, 1.21–2.09; $P=0.001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00),CSA/TAC only (HR, 1.23; 95% CI, 1.09–1.39; $P=0.001$), CSA/TAC + steroid/MMF (HR, 1.02; 95% CI, 0.86–1.21; $P=0.820$), other/missing (HR, 1.21; 95% CI, 0.82–1.78; $P=0.342$); year of transplantation, 1998–2004 (reference, 1.00), 2005–2009 (HR, 0.89; 95% CI, 0.80–0.99; $P=0.038$). [b]Other significant variables in model A were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.72, 95% CI, 1.42–2.07, $P<0.001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 1.43; 95% CI, 1.14–1.78; $P=0.002$), CSA/TAC + steroid/MMF (HR, 1.00; 95% CI, 0.73–1.37; $P=0.995$), other/missing (HR, 1.51; 95% CI, 0.67–3.39; $P=0.319$). [c]Other significant variables were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.41, 95% CI, 1.23–1.61, $P<0.001$); diagnosis, AML (reference, 1.00), ALL (HR, 1.13, 95% CI, 0.95–1.34, $P=0.183$), CML (HR, 0.94, 95% CI, 0.70–1.27, $P=0.704$), MDS (HR, 0.73, 95% CI, 0.60–0.89, $P=0.002$).

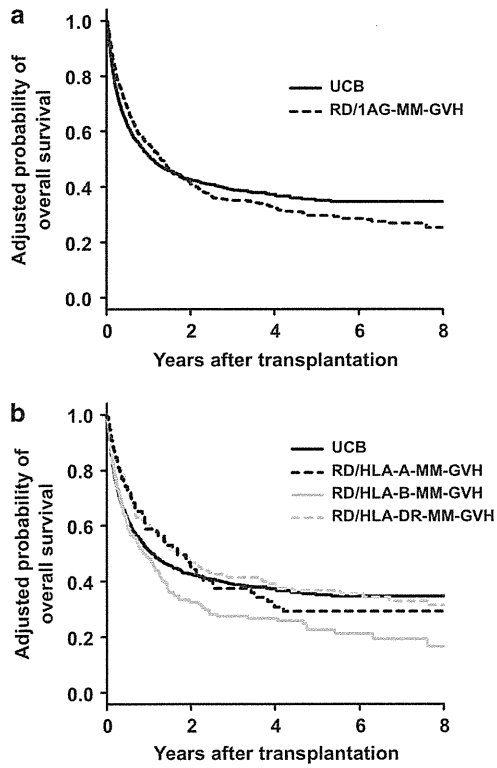

Figure 3. Overall survival. Overall survival rates in the transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**) or with an HLA-A, -B, or -DR antigen mismatch in the GVH direction (**b**) are shown.

of transplantation (1998–2004). Figure 3a shows the adjusted survival curves of the two groups. Next, the HLA-A, HLA-B and HLA-DR mismatched groups in transplantation from an RD/1AG-MM-GVH were compared with the UCB group. The OS rate of patients who received transplantation from an RD/1AG-MM-GVH involving an HLA-B mismatch was significantly lower than that in the UCB group ($P=0.043$; Figure 3b and Table 2), and a subgroup analysis revealed that the adverse effect of an HLA-B mismatch was significant only in standard-risk patients (standard-risk, $P=0.023$; high-risk, $P=0.326$; Table 2).

Relapse and NRM

The 3-year relapse rates in the UCB and RD/1AG-MM-GVH groups were 35% (95%CI, 33–37%) and 32% (95% CI, 28–36%), respectively (Gray test; $P=0.041$; Figure 4a), and a significant decrease in the incidence of relapse was found in the RD/1AG-MM-GVH group in the multivariate analysis (RD/1AG-MM-GVH vs UCB, HR, 0.78, 95%CI, 0.64–0.95, $P=0.012$; Table 3). The impact of reducing the incidence of relapse did not differ according to the HLA mismatch antigen in the RD/1AG-MM-GVH group (Table 3 and Figure 4b). The 3-year NRM rates in the UCB and RD/1AG-MM-GVH groups were 30% (95% CI, 28–32%) and 32% (95% CI, 28–36%), respectively (Gray test; $P=0.474$; Figure 4c), and a significant increase in the NRM rate was observed in the RD/1AG-MM-GVH group in the multivariate analysis (RD/1AG-MM-GVH vs UCB, HR, 1.24, 95% CI, 1.04–1.47, $P=0.016$; Table 3). In particular, the NRM rate of patients who received transplantation from an RD/1AG-MM-GVH with an HLA-B mismatch was significantly higher than that in the UCB group (RD/1AG-MM-GVH vs UCB, HR, 1.50, 95% CI, 1.17–1.92, $P=0.001$; Figure 4d and Table 3).

The causes of death in patients who died without relapse are shown in Supplementary Table 3. The rates of GVHD and organ failure in the RD/1AG-MM-GVH group were higher than those in the UCB group (GVHD, 18 vs 10%, organ failure, 28 vs 19%), whereas the rates of graft failure and infection were lower in the RD/1AG-MM-GVH group (graft failure, 1 vs 5%; infection, 26 vs 38%).

The impact of the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group

Based on the fact that the leading causes of death in the RD/1AG-MM-GVH group were GVHD and organ failure, we analyzed the risk factors for the development of acute GVHD in this group.

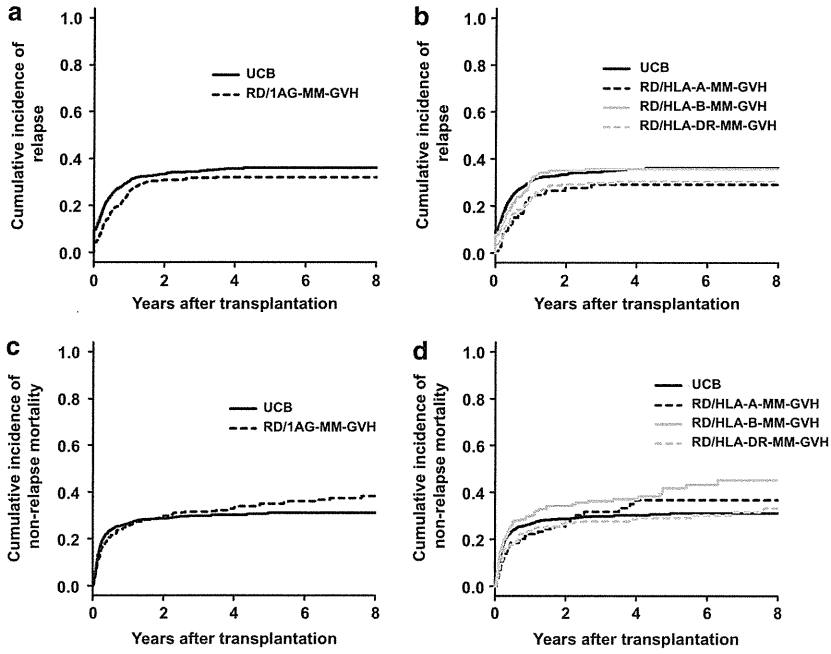

Figure 4. Relapse and non-relapse mortality. Cumulative incidence of relapse and non-relapse mortality after transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**, **c**) or with an HLA-A, -B, or -DR antigen mismatch in the GVH direction (**b**, **d**) are shown.

Table 3. Multivariate analysis of relapse and non-relapse mortality

Variable	*Relapse*[a]		*Non-relapse mortality*[b]	
	HR (95% CI)	P *value*	*HR (95% CI)*	P *value*
(A)				
UCB	1.00	reference	1.00	reference
RD/1AG-MM-GVH	0.78 (0.64–0.95)	0.012	1.24 (1.04–1.47)	0.016
(B)				
UCB	1.00	reference	1.00	reference
RD/HLA-A-MM-GVH	0.70 (0.49–1.00)	0.050	1.28 (0.93–1.76)	0.130
RD/HLA-B-MM-GVH	0.81 (0.62–1.07)	0.134	1.50 (1.17–1.92)	0.001
RD/HLA-DR-MM-GVH	0.80 (0.61–1.04)	0.096	1.02 (0.78–1.32)	0.901

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CI, confidence interval; CML, chronic myelogenous leukemia; CSA, cyclosporine; HR, hazard ratio; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus. [a]Other significant variables in model A were; diagnosis, AML (reference, 1.00), ALL (HR, 1.09, 95% CI, 0.92–1.29, $P=0.336$), CML (HR, 1.39, 95% CI, 1.05–1.82, $P=0.019$), MDS (HR, 0.59, 95% CI, 0.46–0.76, $P<0.001$); time from diagnosis to transplantation, <6 months (reference, 1.00), ⩾6 months (HR, 0.80; 95% CI, 0.70–0.92; $P=0.002$); disease risk, standard risk (reference, 1.00), high risk (HR, 2.81; 95% CI, 2.41–3.27; $P<0.001$), status not known, (HR, 2.17; 95% CI, 1.45–3.23; $P<0.001$); conditioning intensity, myeloablative (reference, 1.00), reduced intensity (HR, 1.22; 95% CI, 1.04–1.44; $P=0.014$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00), CSA/TAC only (HR, 0.65; 95% CI, 0.53–0.78; $P<0.001$), CSA/TAC + steroid/MMF (HR, 0.75; 95% CI, 0.59–0.96; $P=0.024$), other/missing (HR, 0.94; 95% CI, 0.55–1.61; $P=0.825$). [b]Other significant variables in model A were; patient age, 16–49 (reference, 1.00), 50–(HR, 1.70, 95% CI, 1.47–1.98, $P<0.001$); GVHD prophylaxis, CSA/TAC + MTX (reference, 1.00),CSA/TAC only (HR, 1.70; 95% CI, 1.44–2.01; $P<0.001$), CSA/TAC + steroid/MMF (HR, 1.18; 95% CI, 0.94–1.49; $P=0.158$), other/missing (HR, 1.47; 95% CI, 0.86–2.51; $P=0.154$); year of transplantation, 1998–2004 (reference, 1.00), 2005–2009 (HR, 0.76; 95% CI, 0.66–0.88; $P<0.001$).

In multivariate analysis, two factors were found to be significantly associated with the risk of developing grade II–IV acute GVHD in the RD/1AG-MM-GVH group: the use of *in vivo* T-cell depletion and source of stem cells (use of *in vivo* T-cell depletion, yes vs no, HR 0.40, $P=0.002$, PB vs BM, HR 1.61, $P<0.001$).

Because the use of *in vivo* T-cell depletion significantly lowered the risk of acute GVHD, we re-compared the RD/1AG-MM-GVH group and the UCB group while focusing on the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group. The incidence of grade II–IV or grade III–IV acute GVHD or chronic or extensive chronic GVHD in the RD/1AG-MM-GVH group using *in vivo* T-cell depletion was comparable to that in the UCB group (Supplementary Figure 1 and Supplementary Table 4), whereas the incidences of neutrophil and platelet engraftment were significantly higher in the RD/1AG-MM-GVH group using *in vivo* T-cell depletion than in the UCB group (neutrophil engraftment, HR, 5.52, 95% CI, 3.36–9.05, $P<0.001$; platelet engraftment, HR 2.01, 95% CI, 1.26–3.21, $P<0.001$). Compared to the UCB group, the RD/1AG-MM-GVH group with T-cell depletion showed lower overall and NRM, albeit these differences were not significant, which suggests that the use of *in vivo* T-cell depletion may improve the outcome of transplantation from an RD/1AG-MM-GVH (Figure 5, Supplementary Table 5). It is interesting to note that the adverse impact of an HLA-B mismatch vs HLA-A or -DR

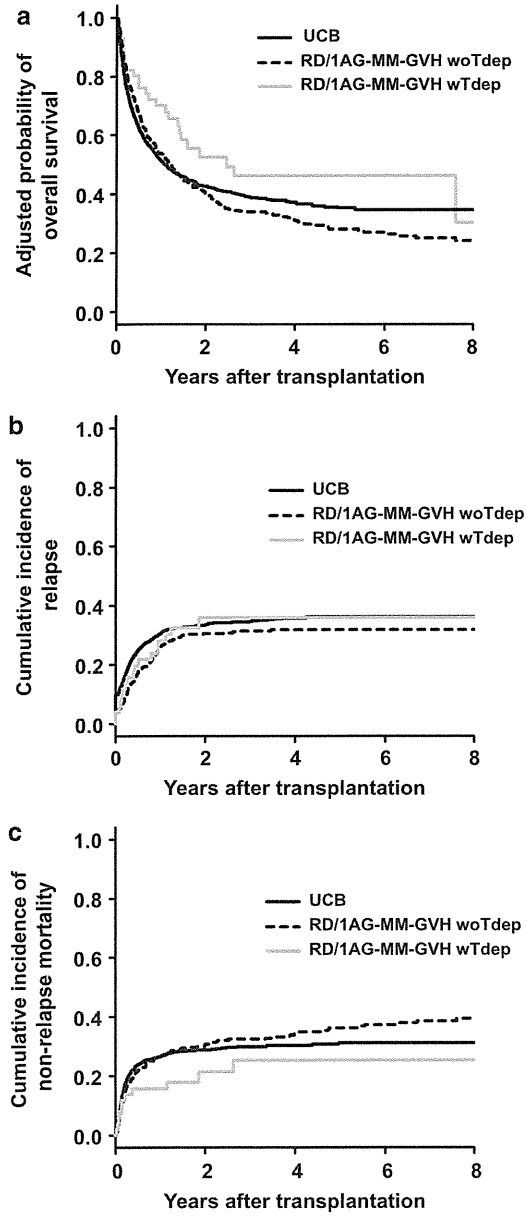

Figure 5. OS (**a**), relapse (**b**) and NRM (**c**) according to the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group.

mismatch in the RD/1AG-MM-GVH group disappeared with the use of *in vivo* T-cell depletion (with *in vivo* T-cell depletion; HLA-B vs HLA-A/DR mismatch; HR 1.08, 95% CI, 0.45–2.62, $P = 0.864$, without *in vivo* T-cell depletion; HLA-B vs HLA-A/DR mismatch; HR 1.59, 95% CI, 1.25–2.01, $P < 0.001$).

With regard to the effect of stem cell source, the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group using BM was lower than that with PB but higher than that with UCB (Supplementary Figure 2). The use of PB or BM did not affect OS, relapse, or NRM (Supplementary Table 5).

DISCUSSION

In this nationwide retrospective study, we found that the survival rate in the UCB group was comparable to that in the RD/1AG-MM-GVH group regardless of the disease risk. The RD/1AG-MM-GVH group with an HLA-B mismatch showed significantly higher overall and NRM, whereas the RD/1AG-MM-GVH group with an HLA-A or HLA-DR mismatch showed an OS comparable to that in the UCB group. Neutrophil and platelet engraftment in the RD/1AG-MM-GVH group were significantly faster than those in the UCB group, whereas the incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group was significantly higher. However, the incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group with *in vivo* T-cell depletion was comparable to that in the UCB group, which translated into a better, but not significantly better, OS than that in the UCB group.

In Japan, unrelated BM donor coordination (from donor search to transplantation) takes a median of 4 months, whereas much less time is required for UCB or RD/1AG-MM-GVH transplantation if there is a candidate. This was reflected in the longer duration from diagnosis to transplantation in unrelated BM transplantation.[32] In contrast, UCB and RD/1AG-MM-GVH transplantation show a similar and shorter duration (Table 1 ; 7.9 months vs 7.6 months). Therefore, in cases where both UCB and RD/1AG-MM-GVH are available, donors should be chosen based on their advantages and disadvantages. Compared with UCB, the use of RD/1AG-MM-GVH has a great advantage in neutrophil and platelet engraftment, which is not inconsistent with a previous finding that engraftment in the UCB group was significantly delayed comparing with that in MUD.[33] This translated into a lower rate of death from graft failure or infection in the RD/1AG-MM-GVH group. However, these advantages were offset by a substantial increase in the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group. The risk of grade III–IV acute GVHD and extensive chronic GVHD in the RD/1AG-MM-GVH group was twice that in the UCB group. If UCB units containing adequate total nucleated cell doses (ex. $> 2.5 \times 10^7$/kg) are available,[34] the selection of UCB would be appropriate to avoid the risk of chronic GVHD. In contrast, RD/1AG-MM-GVH would be more appropriate when early neutrophil engraftment should be prioritized, such as for a patient with an active infectious disease at transplantation.

The high incidences of GVHD and GVHD-related death in the RD/1AG-MM-GVH group indicate the need for stronger immunosuppression to improve the clinical outcome. The use of T-cell depletion, mostly by ATG, was significantly associated with a lower incidence of grade III–IV acute GVHD and extensive chronic GVHD in the RD/1AG-MM-GVH group. Although this effect was not statistically significant, the RD/1AG-MM-GVH group with *in vivo* T-cell depletion showed lower overall and treatment-related mortality, which would outweigh a possible increased risk of relapse. These findings in our cohort suggest that ATG may be effective, and the addition of ATG in the RD/1AG-MM-GVH group should be assessed in a prospective study.

As shown in our previous study,[23] overall mortality in the RD/1AG-MM-GVH group involving an HLA-B mismatch was significantly higher than that in the RD/1AG-MM-GVH group with an HLA-A or -DR mismatch, probably because of an additional HLA-C antigen mismatch as expected from linkage disequilibrium between HLA-B and HLA-C and available data on HLA-C antigen.[23,35] The incidence of grade III–IV acute GVHD in the HLA-B mismatch group was higher than that in the HLA-DR mismatch group, but was comparable to that in the HLA-A mismatch group. In addition, the incidence of death from GVHD was similar in the HLA-B and HLA-A/DR mismatch groups (data not shown). Therefore, the reason for the lower overall morality in the RD/1AG-MM-GVH group with an HLA-B mismatch remains unclear. However, the adverse effect of an HLA-B mismatch disappeared when *in vivo* T-cell depletion was used, which suggests that an immunological effect is involved in this mechanism.

This study has several limitations. First, in clinical practice in Japan, matching of HLA-DR is counted at a low resolution, as with HLA-A and HLA-B, whereas it is counted at a high resolution in the

United States and Europe. To evaluate the impact of this difference, we divided patients in the UCB group with two antigen mismatches into two groups by using available HLA-DRB1 allele information: a group with two antigen mismatches with one additional HLA-DRB1 allele mismatch ($n = 609$) and another group with two antigen mismatches without an additional HLA-DRB1 mismatch ($n = 295$). We did not find a significant difference in OS between these two groups ($P = 0.758$), which suggests that HLA-matching using HLA-DR antigen or allele information will not affect OS in the present study. Second, the findings in the present study are based on Asian cohort who received a 'single' UCB or RD/1AG-MM-GVH transplantation. Lighter body weight in Asian population than Caucasian population may make it easy to find a suitable single UCB unit that contains adequate total nucleated cell doses. In addition, as suggested by Oh *et al.*,[36] limited heterogeneity of Japanese population may affect the outcomes of transplantation. Therefore, the findings should be externally validated in the non-Asian cohort or transplantation using double UCB units. Third, information on the dose and type of ATG was missing in two-third of the patients who received ATG. However, the available data showed that the median dose of thymoglobulin (2.5 mg/kg) or ATG-F (8 mg/kg) was equivalent to the dose that is widely used in our daily practice. Lastly, heterogeneous backgrounds may have resulted in a bias, although we tried to adjust for possible confounders by multivariate analyses. Lastly, the effect of multiple testing should be taken into account for the interpretation of secondary end points.

In conclusion, our findings suggest that both UCB and RD/1AG-MM-GVH are suitable as alternative donors for patients without an HLA-matched sibling or unrelated donor. However, the presence of an HLA-B-antigen mismatch in the GVH direction has an adverse effect on OS because of treatment-related complications. Neutrophil and platelet engraftment in the RD/1AG-MM-GVH group were significantly faster than those in the UCB group, whereas the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group was significantly higher, which translated into a high incidence of death from GVHD. Donor selection between UCB and RD/1AG-MM-GVH should be determined based on the presence of an HLA-B mismatch in RD/1AG-MM-GVH and from the risks and benefits derived from the risk of graft failure and infection in the UCB group and acute or chronic GVHD in the RD/1AG-MM-GVH group. Additional immune suppression using *in vivo* T-cell depletion may improve the clinical outcome in the RD/1AG-MM-GVH group by decreasing the incidences of GVHD and NRM and may also overcome the adverse effect of an HLA-B mismatch. This approach should be assessed in a prospective study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

JK and YK designed the research, organized the project and wrote the manuscript; JK, YA, and YK performed the statistical analysis and analyzed the data; KK and TN-I collected data from JCBBN; and all of the authors interpreted the data and reviewed and approved the final manuscript.

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ORIGINAL ARTICLE

Feasibility of unmanipulated haploidentical stem cell transplantation using standard GVHD prophylaxis for HLA-homozygous patients

Kazuhiro Ikegame · Katsuji Kaida · Satoshi Yoshihara · Masayuki Fujiwara · Kyoko Taniguchi · Ruri Kato · Takayuki Inoue · Tatsuya Fujioka · Hiroya Tamaki · Masaya Okada · Toshihiro Soma · Norihiko Kamikonya · Hiroh Saji · Shozo Hirota · Hiroyasu Ogawa

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Abstract HLA-haploidentical hematopoietic stem cell transplantation (haplo-SCT) in HLA-homozygous patients is accompanied by HLA mismatches only in the host-versus-graft vector, and thus theoretically could be performed with standard graft-versus-host disease (GVHD) prophylaxis. However, the risk of GVHD remains uncertain, and graft failure could be a problem. In this study, we assessed nine HLA-homozygous patients who underwent haplo-SCT. Preparative treatment was cyclophosphamide/total body irradiation-based regimen in five patients, fludarabine/busulfan-based regimen in two, and other regimens in two. GVHD prophylaxis consisted of cyclosporine and methotrexate in seven patients, cyclosporine and mycophenolate mofetil in one, and cyclosporine alone in one. Seven patients achieved neutrophil engraftment and platelet recovery. The median times to neutrophil engraftment and platelet recovery were 15 and 44 days, respectively. Two patients developed graft failure, including one who achieved engraftment with a second SCT from the same donor. Grade II GVHD was observed in half of the evaluable patients; grades III and IV were not observed. Two patients died from treatment-related causes. Five patients were alive after a median follow-up period of 563 days. The probability of overall survival at 5 years was 65 %. These findings may serve as a rationale for considering haplo-SCT as a treatment option for HLA-homozygous patients.

Keywords Haploidentical stem cell transplantation · HLA-homozygous patients · Hetero-to-homo transplantation · GVHD · Graft failure

K. Ikegame · K. Kaida · S. Yoshihara (✉) · K. Taniguchi · R. Kato · T. Inoue · T. Fujioka · H. Tamaki · M. Okada · T. Soma · H. Ogawa
Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan
e-mail: yoshihar@hyo-med.ac.jp

M. Fujiwara · N. Kamikonya · S. Hirota
Department of Radiology, Hyogo College of Medicine, Hyogo, Japan

H. Tamaki · H. Ogawa
Laboratory of Cell Transplantation, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Hyogo, Japan

H. Saji
HLA Laboratory, Kyoto, Japan

H. Ogawa
Department of Molecular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan

Introduction

The role of alternative stem cell sources in allogeneic hematopoietic stem cell transplantation (SCT) is currently expanding because of the reduced chance of finding a matched sibling donor, due to the elevation of the age limit for SCT and the low birth rates, particularly in Japan. HLA-haploidentical SCT (haplo-SCT) has substantial advantages, including the immediate availability of donors—which enables urgent SCT where necessary—and the availability of donor lymphocyte infusions after SCT [1–3]. However, earlier studies of haplo-SCT with a standard preparative regimen and graft-versus-host disease (GVHD) prophylaxis have shown high risks of graft failure and GVHD [4, 5]. Notably, Anasetti et al. demonstrated that HLA disparities in the host-versus-graft (HVG) vector and graft-versus-host (GVH) vector are correlated with the

risks of graft failure and GVHD, respectively. HLA homozygous patients inherently have no HLA mismatches in the GVH vector, whereas they usually have mismatches in the HVG vector. In fact, HLA homozygous patients who underwent 1 locus-mismatched haplo-SCT in the HVG vector had an incidence of GVHD similar to that of patients who underwent HLA-matched SCT [5]. Meanwhile, homozygous patients are predisposed to have natural killer (NK) cell alloreactivity in the GVH vector based on the killer cell immunoglobulin-like receptors (KIR) ligand incompatibility model. KIR ligand incompatibility in the GVH vector has been shown to be associated with a reduction of graft failure, GVHD, and relapse in several previous studies [6, 7].

Although these previous findings have indicated the feasibility of haplo-SCT for HLA-homozygous patients with standard GVHD prophylaxis, there have been scarce reports focusing on this treatment option. Therefore, the place of haplo-SCT in an algorithm of donor selection in homozygous patients remains unclear. One of the major reasons for the lack of the reports is probably the small number of HLA-homozygous patients. In the Japanese population, however, several haplotypes are quite common and well conserved [8–10]. Consequently, the number of homozygous patients with those common haplotypes is not negligible in Japan. Here, we describe the outcomes of 9 HLA-homozygous patients who underwent haplo-SCT from HLA-heterozygous donors ("hetero-to-homo SCT").

Subjects and methods

Patients

This study is a retrospective analysis of 9 consecutive HLA-homozygous patients who underwent haplo-SCT between May 1998 and September 2010 with a single transplant team at Osaka University Hospital (May 1998–March 2006) or Hyogo College of Medicine Hospital (January 2006–September 2010). Selection of donor source was based on its availability, disease status, and patient's request. While HLA-allele matched unrelated donors were given precedence for patients who remain in complete remission, haploidentical-related donors were given precedence for patients with active disease or those with impending relapse, which was suggested by minimal residual disease monitoring. Informed consent was obtained from all the patients, and they were treated according to our institutionally approved protocols.

The median age of the patients was 43 years (range 29–58 years) at the time of SCT (Table 1). Of 9 patients, 5 patients had acute myeloid leukemia (AML) or refractory lymphoma in no remission, including 1 who had a relapse after HLA-matched unrelated bone marrow transplantation (BMT), 3 who had AML in CR (2 had minimal residual disease), and 1 who had transfusion-dependent severe aplastic anemia.

HLA study and assessment of KIR ligand incompatibility

Generally, the patients and donors were tested for the allele type of HLA-A, B, C, and DRB1 loci. However, several patients who underwent SCT in earlier part of the study period were tested only for the serotype of HLA-A, B, and DR loci. KIR2DL ligand incompatibility in the GVH vector was scored when the KIR2DL epitope of HLA-C was present in donors and absent in recipients (that is, when recipients had Cw3 and donors had Cw3/Cw4 or Cw4/Cw4 or when recipients had Cw4 and donors had Cw3/Cw3 or Cw3/Cw4). KIR3DL ligand incompatibility in the GVH vector was scored when the HLA-Bw4 epitope including A24 was present in donors and absent in recipients. For the 5 donors or recipients who were typed only for HLA-A, B, and DR loci, those with A24-B52-DR15 were presumed to have Cw12, and those with A24-B7-DR1 were presumed to have Cw7, because Cw locus can be predicted with more than 99 % accuracy for these haplotypes in the Japanese population according to our database, which covers more than 4700 families in Japan. In patients who underwent SCT after January 2008 (no. 6–9), HLA antibodies were examined as part of the pretransplant work-up. The methodology used for the measurement of HLA antibodies was previously described [11].

Preparative regimen and stem cell sources

The preparative treatment consisted of cyclophosphamide/total body irradiation (CY/TBI, CY 60 mg/kg for 2 days and TBI 12 Gy divided in 4 fractions)-based myeloablative regimen in 5 patients, fludarabine/busulfan (Flu/BU, Flu 30 mg/m^2 for 6 days and BU 3.2 mg/kg for 4 days)-based myeloablative regimen in 2 patients, and other regimens in 2 patients (Table 2). Overall, in an attempt to overcome HLA disparity in the HVG vector, Flu was used in all 6 patients who underwent SCT after the approval of Flu in Japan. High-dose cytarabine (Ara-C, 2 g/m^2 for 4 days) was added to the CY/TBI-based regimen or to the Flu/BU-based regimen in 4 patients, mainly in an attempt to reduce tumor burden at the time of SCT. Bone marrow was used as a stem cell source in 6 patients, including all 5 who received the CY/TBI-based regimen. Peripheral blood stem cell (PBSC) were used for 3 patients, including the 2 patients who received Flu/BU-based regimen and the other patient with severe aplastic anemia, who received a reduced-intensity conditioning regimen consisting of Flu

Table 1 Patients characteristics

Patient no.	UPN	Age (years)/ sex	Diagnosis	Disease stage	Donor	HLA typing		No. of HLA mismatch[a]		KIR ligand mismatch		
						Recipient HLA	Donor HLA	GVH vector	HVG vector	GVH vector		HVG vector
										KIR2DL ligand	KIR3DL ligand	
1	174	43/F	AML	CR1	Daughter	A24-B52-(Cw12)-DR15	A24-B52-(Cw12)-DR15 A24-B7-(Cw7)-DR1	0	2	No	No	No
2	209	51/F	DLBCL	Relapse after auto-SCT	Daughter	A24-B7-Cw7-DR1	A24-B7-Cw7-DR1 A2-B13-Cw10-DR12	0	3	No	No	No
3	312	36/F	AML	CR1 (MRD positive)	Sibling	A*02:06-B*40:02-DRB1*14:05 A*02:01-B*40:01-DRB1*14:05	A*02:06-B*40:02-DRB1*14:05 A*31:01-B*40:01-DRB1*04:03	0	2	Not evaluable	No	No
4	444	44/M	FL (grade 3)	Refractory	Sibling	A24-B52-(Cw12)-DR15	A*24:02-B*52:01-Cw*12:02-DRB1*15:02 A*26:01-B*56:03-Cw*01:02-DRB1*12:01	0	3	No	No	No
5	490	50/M	DLBCL	Relapse after auto-SCT	Sibling	A*31:01-B*15:07-Cw*03:03-DRB1*04:05 A*31:01-B*15:07-Cw*03:04-DRB1*04:05	A*31:01-B*15:07-Cw*03:03-DRB1*04:05 A*24:02-B*55:02-Cw*01:02-DRB1*09:01	0	3	No	Yes (A24 = Bw4)	No
6	536	35/F	AML	Relapse after uBMT	Sibling	A24-B52-(Cw12)-DR15	A*24:02-B*52:01-Cw*12:02-DRB1*15:02 A*26:02-B*15:01-Cw*03:03-DRB1*14:06	0	3	No	No	No
7	617	58/M	MDS-AML	No treatment	Daughter	A*02:01-B*54:01-Cw*01:02-DRB1*04:05	A*02:01-B*54:01-Cw*01:02-DRB1*04:05 A*24:02-B*07:02-Cw*07:02-DRB1*01:01	0	3	No	Yes (A24 = Bw4)	No
8	654	36/M	AML	CR2 (MRD positive)	Sibling	A*24:02-B*07:02-Cw*07:02-DRB1*01:01	A*24:02-B*07:02-Cw*07:02-DRB1*01:01 A*02:06-B*54:01-Cw*08:03-DRB1*04:05	0	3	No	No	No
9	681	29/M	AA	Severe	Sibling	A*24:02-B*52:01-Cw*12:02-DRB1*15:02	A*24:02-B*52:01-Cw*12:02-DRB1*15:02 A*02:06-B*35:01-Cw*03:03-DRB1*15:01	0	2	No	No	No

UPN unique patient number, *GVH* graft-versus-host, *HVG* host-versus-graft, *AML* acute myeloid leukemia, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *MDS-AML* AML evolving from myelodysplastic syndrome, *AA* aplastic anemia, *auto-SCT* autologous stem cell transplantation, *MRD* minimal residual disease

[a] Number of serological mismatches in A, B, or DR loci

Table 2 Transplantation protocols and grafts

Patient no.	Transplant no.	Preparative regimen	Stem cell source	Infused cell dose		GVHD prophylaxis
				NCC ($\times 10^8$/kg)	$CD34^+$ ($\times 10^6$/kg)	
1	1	CY/TT	BM	3.5	–	CsA/MTX
2	2	CY/TBI (12)	BM	2.2	–	CsA/MTX
3	3	Flu/CY/TBI (12)	BM	4.8	–	CsA/MTX
4	4	Flu/CA/CY/TBI (12)	BM	2.2	–	CsA
5	5	Flu/CY/TBI (12)	BM	2.4	–	CsA/MTX
6	6-1	Flu/CA/BU4	PBSC	–	8.9	CsA/MTX
	6-2	TBI (2)	BM	1.2		CsA
7	7	Flu/CA/BU4/TBI (4)	PBSC	–	3.3	CsA/MTX
8	8-1	Flu/CA/CY/TBI (12)	BM	2.2	–	CsA/MTX
	8-2	Flu/CY/ATG	PBSC	–	7.0	CsA
9	9	Flu/CY/TBI (3)/ATG	PBSC	–	3.1	CsA/MMF

CY cyclophosphamide, *TT* thiotepa, *TBI (12)* total body irradiation 12 Gy, *Flu* fludarabine, *BU* busulfan, *CA* cytosine arabinoside, *BU4* once-daily BU for 4 days, *ATG* antithymocyte globulin, *MMF* mycophenolate mofetil

(30 mg/m^2 for 6 days), CY (50 mg/kg for 2 days), TBI (3 Gy), and antithymocyte globulin (thymoglobulin, 1 mg/kg for 4 days). Granulocyte-colony stimulating factor-mobilized PBSC were collected from the donor for 3 days, on days 0–2 when possible, to obtain as many stem cells as possible. The median number of infused nuclear cells in BMT was 2.3 $\times$ 10^8/kg (range 2.2–4.8 $\times$ 10^8/kg), and the median number of infused $CD34^+$ cells in peripheral blood stem cell transplantation (PBSCT) was 5.2 $\times$ 10^6/kg (range 3.1–8.9 $\times$ 10^6/kg).

GVHD prophylaxis and treatment

GVHD prophylaxis consisted of cyclosporine and short-term methotrexate on days 1, 4, and 8 in 7 patients; cyclosporine and mycophenolate mofetil (15 mg/kg/day) in 1 patient; and cyclosporine alone in 1 patient who had a bulky lymphoma at the time of SCT (Table 2). In the second transplantation following primary graft failure in 1 patient (no. 8), cyclosporine alone was used as GVHD prophylaxis.

Supportive care

Patients were hospitalized in single rooms ventilated with high-efficiency particulate air filtration systems. All patients received broad-spectrum antibiotics and either amphotericin B or azoles (itraconazole or voriconazole) during the neutropenic period before and after SCT. Following neutrophil engraftment, patients received trimethoprim–sulfamethoxazole or aerosolized pentamidine for prophylaxis against pneumocystis pneumonia for at least 12 months post-transplantation. Acyclovir (200 mg/day) was continued until the discontinuation of immunosuppressant. Patients received intravenous immunoglobulin (100 mg/kg) weekly for 2 months after transplantation. Cytomegalovirus was monitored weekly by a pp65 antigenemia test. Documented cytomegalovirus reactivation was treated with either ganciclovir or foscarnet. Granulocyte-colony stimulating factor (300 μg/m^2) was administered from days 1 or 5 until the neutrophil count was greater than 2500/μL for 2 consecutive tests.

Chimerism analysis

In patients who underwent SCT after April 2005 (no. 4–9), donor chimerism was examined serially in the T-cell- or neutrophil-enriched cell fractions of peripheral blood and bone marrow. The methodology used for cell separation and chimerism analysis has been detailed elsewhere [12, 13]. Briefly, T cells were enriched by a negative selection system (RosetteSep; StemCell, Vancouver, Canada) to a purity of >95 %, and granulocytes were recovered from the Ficoll-red blood cell interface with a purity of >99 %. Chimerism analysis involved quantitative polymerase chain reaction (PCR) of informative short tandem repeats in the recipient and donor. DNA was amplified with fluorescent PCR primers for markers that would distinguish the donor and recipient alleles. Fluorescent PCR products were separated with an Applied Biosystems 310 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA), and GeneScan software (Applied Biosystems) was used to correlate allele peak areas with the percentage of donor or recipient DNA.

Definitions and statistical analysis

Donor-specific HLA antibodies were defined as HLA antibodies that correspond to the mismatched donor HLA antigen with median fluorescence intensity >5000 in the LABScreen Single Antigen analysis (One Lambda, Canoga Park, CA, USA). Neutrophil engraftment was defined by an absolute neutrophil count of at least 500/μL for 3 consecutive tests,

whereas platelet recovery was defined by a platelet count of at least 20,000/μL without transfusion support. Primary graft failure was defined by an absence of neutrophil recovery associated with no appearance or a decrease of donor cells in chimerism analysis by day 18 or an absence of neutrophil recovery by day 60. Diagnosis of acute and chronic GVHD was based on the standard clinical criteria [14], with histopathologic confirmation where possible. Overall survival was calculated using the Kaplan–Meier method.

Results

HLA and KIR ligand incompatibility

Four patients (no. 1, 4, 6, 9) were homozygous for the most common haplotype in Japan (HLA A*24:02-B*52:01-Cw*12:02-DRB1*15:02), which is possessed by approximately 8.4 % of the Japanese population (Table 1). Two patients (no. 2, 8) were homozygous for the third most common haplotype in Japan (A*24:02-B*07:02-Cw*07:02-DRB1*01:01), which is possessed by approximately 3.5 % of the Japanese population. In 2 patients (no. 3, 5), the haplotypes were serologically identical, but genotypically different. Thus, these patients were not homozygous, as stringently defined, but were included in this analysis because they also had no serological mismatches in the GVH vector. The other patient (no. 7) was homozygous for a less-frequent haplotype, which is possessed by approximately 0.42 % of the Japanese population. In 6 patients, the donors were siblings, and in 3 patients, the donors were daughters. The number of HLA mismatches in the HVG vector in A, B, and DR loci was 2 in 3 patients and 3 in the remaining 6 patients. KIR ligand incompatibility in the GVH vector was found in only 2 (no. 5, 7) of 8 evaluable patients. Both were KIR3DL-ligand incompatible, with A24 present in the donors and absent in the recipients. None of the 4 evaluated patients (no. 6–9) had donor-specific HLA antibodies.

Engraftment

Of 9 patients, 7 achieved neutrophil engraftment and platelet recovery. The median times to neutrophil engraftment and platelet recovery were 15 days (range 11–30 days) and 44 days (range 15–189 days), respectively. Two patients (no. 6, 8) developed primary graft failure.

One patient (no. 6), who underwent haplo-SCT as second SCT, showed no signs of neutrophil recovery, and donor chimerism in the T cell fraction started to decline by day 17. Salvage SCT (BMT) from the same donor following low dose TBI (2 Gy) was performed 21 days after haplo-SCT, followed by donor lymphocyte infusion, including 1.8×10^7 $CD3^+$ cells/kg. However, donor chimerism in the T cell fraction continued to decline and completely disappeared 31 days after the first haplo-SCT. The patient died with bacterial pneumonia 36 days after first haplo-SCT, as a consequence of prolonged neutropenia.

Another patient (no. 8) also showed a gradual increase of donor chimerism in the T cell fraction, up to 88.3 % on day 13. However, following a high fever beginning on day 11 and a 10-fold elevation of serum soluble interleukin 2 receptor levels from the baseline (from 548 U/ml on day 2 to 5163 U/ml on day 14), donor chimerism in the T cell fraction was suddenly completely lost on day 17. Consequently, the lymphocyte count rapidly increased from 10 cells/μl on day 16 to 440 cells/μl on day 20. Based on the diagnosis of graft failure with the mechanism of immune rejection, a second SCT (PBSCT) from the same donor with a highly immunosuppressive nonmyeloablative conditioning regimen (Flu 30 mg/m^2 for 4 days, CY 50 mg/kg for 1 day, Thymoglobulin 2 mg/kg for 3 days) was performed 26 days after the first SCT and achieved donor engraftment on day 12 after the second SCT. Chimerism analysis on day 12 showed complete donor chimerism in both the T cell and myeloid fractions. Chimerism analysis in all 4 patients who were evaluated for chimerism serially and achieved engraftment showed complete donor chimerism in both the T cell and myeloid lineages by 4 weeks after SCT.

GVHD

Of 8 evaluable patients, including 1 who achieved engraftment after a second SCT, 4 patients (50 %) developed grade II acute GVHD. One patient developed grade I GVHD, and the remaining 4 patients had no clinical GVHD. None of the evaluable patients died from acute GVHD-related complications. Chronic GVHD was observed in 4 patients (extensive type in 3 and limited type in 1 patient). Of the 2 patients with KIR ligand incompatibility in the GVH vector, 1 patient developed grade I acute GVHD and extensive chronic GVHD, and the other patient developed grade II acute GVHD but had no signs of chronic GVHD.

Outcomes

The outcomes of the patients are shown in Table 3. In all, 2 of the 9 patients died from treatment-related causes: 1 from *Pneumocystis jirovecii* pneumonia and 1, who had primary graft failure, from bacterial pneumonia. One patient died more than 9 years after SCT from repeated pancreatitis and encephalopathy of unknown etiology. One patient had a relapse of lymphoma 77 days after SCT and died with disease progression. Five patients were alive at a median

Table 3 The outcomes of haplo-SCT in HLA-homozygous patients

Patient No.	Transplant No.	Donor engraftment	Time to engraftment (days)		GVHD		Relapse	Current status	Cause of death
			Neutrophil	Platelet	Acute	Chronic			
1	1	Yes	23	139	0	Extensive	No	Dead, day 286	*Pneumocystis jirovecii* pneumonia
2	2	Yes	19	189	II	Extensive	No	Dead, day 3532	Pancreatitis, encephalopathy
3	3	Yes	15	15	0	No	No	Alive, day 2822	
4	4	Yes	30	60	II	No	Yes (day 77)	Dead, day 172	Relapse
5	5	Yes	15	28	I	Extensive	No	Alive, day 1331	
6	6-1	No	NA	NA	NE	NE	NE		
	6-2	NE	NA	NA	NE	NE	NE	Dead, day 36[a]	Bacterial pneumonia
7	7	Yes	13	141	II	No	No	Alive, day 563	
8	8-1	No	NA	NA	NE	NE	NE		
	8-2	Yes	12	23	II	Limited	No	Alive, day 365[a]	
9	9	Yes	11	19	0	No	No	Alive, day 221	

NE not evaluable, *NA* not achieved

[a] Counted from the date of first SCT

follow-up of 563 days (range 221–2822 days). The probability of overall survival at 5 years was 65 %.

Discussion

The present study had several significant findings regarding the feasibility of unmanipulated haplo-SCT for HLA-homozygous patients. First, we found that primary graft failure remains a major obstacle for those patients; 2 of 9 patients developed primary graft failure. Two major mechanisms are thought to be involved in primary graft failure after HLA-mismatched SCT: T cell-mediated cellular immune rejection [15, 16] and HLA antibody-mediated humoral immune rejection [11, 17–19]. Because donor-specific HLA antibodies were absent, the latter mechanism was unlikely to be involved in the 2 patients who had graft failure in the present study. The former mechanism occurs as a result of the balance between residual host T cells and donor-derived T cells. Several previous studies have supported this mechanism by demonstrating that host T cells that recognize donor HLA antigens emerge at the time of graft failure [20, 21]. In this respect, haplo-SCT in homozygous patients from heterozygous donors is inherently predisposed to cellular immune rejection, because T cell-derived alloreactivity occurs only in the HVG direction. In fact, the clinical course of patient no. 8—who developed a high fever simultaneous with the rapid decline of donor chimerism in the T cell fraction, followed by an increase of lymphocytes—suggested the emergence of host-derived alloreactive T cells during the process of immure rejection.

Because the preparative regimen affects only the residual host immunity (with the exception of antithymocyte globulin or alemtuzumab), it promotes engraftment by changing the balance between host residual T cells and donor-derived T cells. One of the 2 patients who failed to achieve engraftment underwent haplo-SCT (PBSCT) as a second SCT for relapse after unrelated BMT. Because the patient had received the conventional dose of TBI (12 Gy) at the time of unrelated BMT, haplo-SCT was performed with a non-TBI regimen consisting of Flu, BU (4 days), and Ara-C. One of the previous studies in the settings of cord blood transplantation has shown that Flu/BU regimen provided donor-derived neutrophil engraftment in only 2 of 10 patients [22]. This suggests that Flu/Bu regimen is less immunosuppressive than regimens containing CY or TBI and has less potential to facilitate engraftment. The other patient, who developed primary graft failure despite a highly myeloablative and lymphoablative conditioning regimen with Flu, CY, TBI, and Ara-C, was used BM as a stem cell source. Collectively, considering the substantial

risk of graft failure, a combination of a highly lymphoablative regimen (such as Flu/CY with low or conventional dose of TBI) and PBSCT should be used for future studies.

Our second major finding was that the incidence of GVHD with haplo-SCT in homozygous patients using standard GVHD prophylaxis was comparable to that with HLA-matched SCT. Although grade II GVHD was observed in half of the evaluable patients, none had grade III or IV GVHD, and there were no GVHD-related mortalities. These findings support the hypothesis that haplo-SCT in HLA-homozygous patients generates a GVH response comparable to that from HLA-matched SCT.

Our third finding was that the incidence of KIR ligand incompatibility in the GVH vector was low in the Japanese population, even in the combination of HLA-heterozygous donors and HLA-homozygous patients. In fact, only 2 of 8 evaluable patients had incompatibility in the present study. This is probably attributable to the remarkably biased frequency of the HLA-Cw groups in Japanese population (92.4 % of the population has the Cw3 group and 7.6 % has the Cw4 group) [23]. KIR ligand incompatibility in the GVH vector has been shown to be associated with a reduction of graft failure, GVHD, and relapse in patients who underwent T-cell-depleted haplo-SCT with CD34 positive cell selection [6, 7]. These favorable effects are delivered by alloreactive NK cells that are differentiated from the engrafted stem cells [24]. However, several studies in the settings of on unmanipulated unrelated BMT have shown KIR ligand incompatibility in the GVH vector to be associated with a high incidence of GVHD and poor overall survival [23, 25]. The use of antithymocyte globulin and/or the T-cell depletion was suggested to be a major reason for the discrepancy [19]. In this respect, KIR ligand incompatibility in the GVH vector could negatively affect outcomes in our transplant settings, although this was not evaluable due to the small number of patients with this incompatibility.

The present study had several inherent limitations. First, as a retrospective review, our case series was subject to a possible selection bias. Second, the number of patients was small, and the duration of follow-up was short in some patients. Nevertheless, our case series suggests the usefulness of this approach, which warrants further clinical study.

In conclusion, we showed the feasibility of unmanipulated haploidentical transplantation for HLA-homozygous patients using standard GVHD prophylaxis. While HLA-allele matched unrelated donors can be found in the majority of the HLA-homozygous patients, the major drawback associated with unrelated transplantation is a delay in provision of unrelated donor [26]. Previous studies have indeed shown that significant proportion of the patients became medically unfit while waiting for an unrelated transplantation [27]. Taken together with our findings, haploidentical transplantation can be considered to be a viable treatment option particularly for patients in need of an urgent transplant.

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Conflict of Interest The authors declare no conflict of interest.

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