feasible and is performed in routine clinical practice. However, several complications have been reported in ABO-mismatched SCT. Major mismatched transplantation, characterized by the presence of preformed anti-donor hemagglutinin, is sometimes complicated by delayed red blood cell (RBC) engraftment and pure red cell aplasia[2-7] and by hemolytic anemia.[8,9] In minor mismatched transplantation, characterized by the ability of donor B lymphocytes to produce anti-recipient hemagglutinin, acute hemolytic anemia, known as passenger lymphocyte syndrome, can occur shortly after SCT.[9-12] In bidirectional mismatched transplantation, characterized by the combination of major and minor characteristics, both sets of complications can occur. Owing to these reasons, clinicians are very interested in determining whether ABO mismatching affects the final outcome of SCT, especially when several donor candidates with various ABO-matching pairs are available. To resolve these issues, the impact of ABO mismatching on overall survival (OS) in SCT settings has been evaluated in many studies; however, all these studies obtained conflicting results. Some studies reported the association of poorer OS,[13-16] increased nonrelapse mortality,[17] or increased incidence of acute graft-versus-host disease (GVHD) with a single or any type of ABO mismatch compared with ABO-matched SCT.[16,18] In contrast, one report indicated better OS and decreased relapse rate in ABO-mismatched transplantation.[19] In addition to these contradictory reports, many studies reported that ABO mismatching had no impact on OS, incidence of acute GVHD, or relapse rate in SCT.[2,20-26] These contradictory results could have originated due to the following reasons: 1) in many studies, each ABO-mismatched pair is not analyzed independently; 2) the number of bidirectional mismatched transplants is often small; 3) transplant centers may employ differing treatment and supportive care regimes; and 4) the background of the studied populations is heterogeneous. To obtain more robust results, a few large retrospective studies analyzing more than 1000 patients have recently been performed. Seebach and coworkers[18] showed no impact of ABO mismatching on OS in an analysis of 3103 patients who had received bone marrow transplantation from a human leukocyte antigen (HLA)-identical sibling for early-stage acute leukemia and chronic myelogenous leukemia (CML). On the other hand, Michallet and colleagues[27] demonstrated an adverse impact of a minor mismatch on OS by analyzing 1108 patients who received SCT with a reduced-intensity conditioning regimen. Therefore, these results need further evaluation with other methods or populations. To reevaluate and summarize conflicting results from previously published studies and to provide better evidence, we designed a meta-analysis based on individual patient data (IPD) with a pooled data set. IPD-based meta-analysis is a relatively new approach to systemic reviews, aimed to reduce the bias in systemic reviews compared to meta-analysis based on abstracted data without IPD retrieval during central collection and reanalysis of IPD from each study.[28,29] We conducted the IPD-based meta-analysis using data sets, including those obtained from six previously published articles as well as an unpublished data set from one center that did not participate in previous studies.

MATERIALS AND METHODS

Study design

An IPD-based meta-analysis was designed to evaluate the impact of donor-recipient ABO matching on clinical outcomes after peripheral blood and marrow SCT for hematologic malignancies. The primary endpoint was OS, which was compared among patients receiving an ABO-matched graft and those receiving a major, minor, or bidirectional ABO-mismatched graft. The other endpoints analyzed were treatment-related mortality (TRM); GVHD-related mortality; and engraftment of reticulocytes, neutrophils, and platelets (PLTs).

Selection of studies for meta-analysis

Inclusion criteria for the selection of studies were as follows: 1) the studies were original articles published in English after 1995 and 2) the endpoints considered by the studies included the comparison of OS between ABO-matched and any mismatched SCTs. Exclusion criteria were as follows: 1) the studies included 80 or fewer SCT subjects and 2) the median follow-up period of the studies was less than 6 months. An initial literature search of the PubMed database was conducted using the following free-text terms: ABO blood-group system* and ("blood grouping and crossmatching"[Mesh] or blood group incompatibility*[Mesh]) and (bone marrow transplantation*[Mesh] or hematopoietic stem cell transplantation*[Mesh] or peripheral blood stem cell transplantation*[Mesh]). The date of the last search was June 30, 2007. The initial PubMed literature search identified 194 articles published between 1970 and 2007; 11 articles were found to be eligible for the analysis (Fig. 1).[13-16,18-24] Letters were sent to the corresponding authors of these 11 articles asking them to join the IPD-based meta-analysis and 6 of the corresponding authors agreed to participate. The 6 participating studies included 2 multicenter studies,[13,14,20,22-24] and the other 5 nonparticipating studies included 3 multicenter retrospective studies.[15,16,18,19,21] Patients receiving SCT from unrelated donors were present in 4 of the 6 participating studies and in 4 of the 5 nonparticipating studies. Two of the nonparticipating studies were relatively large, analyzing data of more than 1000 patients. In addition, Kyoto University, where this study was designed, participated in the study,

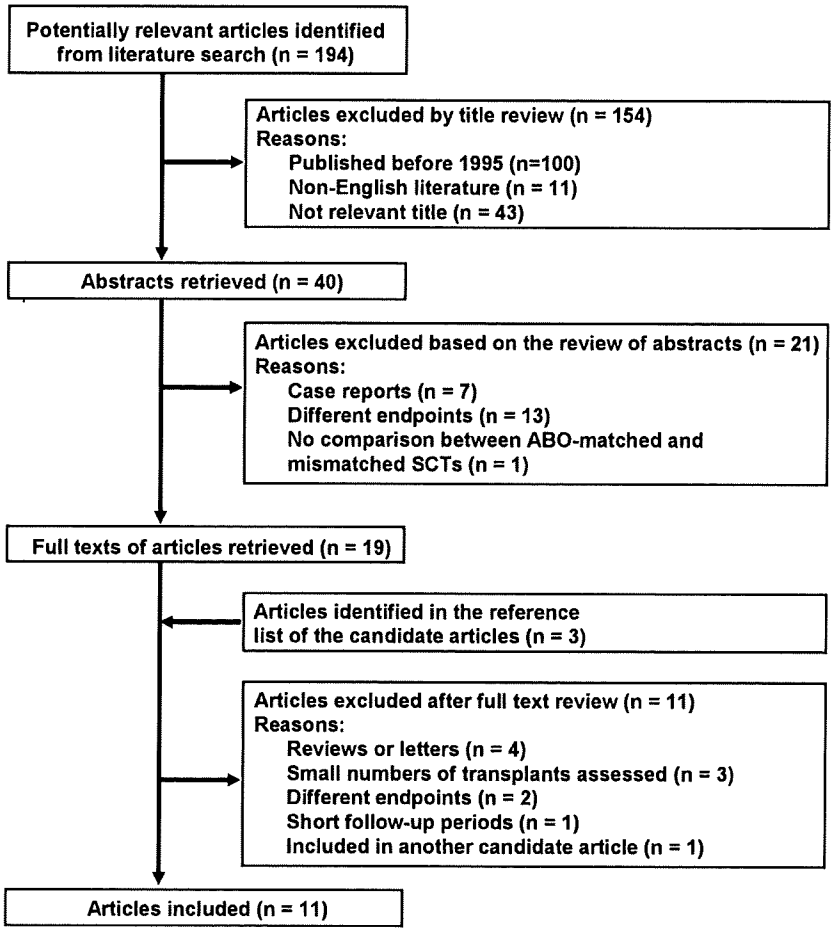

Fig. 1. A flow chart illustrating the process of article selection.

providing its data set on SCT that had not been subjected to survival analysis with reference to ABO matching.

Data collection

We first established the following exclusion criteria for IPD collection: 1) patients who did not meet the minimum data requirements in the following criteria, 2) patients who received SCT for diseases other than hematologic malignancies, 3) patients who received cord blood graft or both peripheral blood and marrow graft, and 4) patients who had experienced prior SCT or had no information regarding their SCT history. Further, we also excluded patients enrolled in the other pooled cohort studies so that the results of our study can be interpreted independently. Second, we defined all the variables required in the present study and made a report form for this data. We then asked the corresponding authors of the participating studies to fill the forms with data. Some authors sent all the raw data sets, which were converted to the report format of our study at the center. Ambiguous definitions were discussed and resolved with the principal investigators, corresponding authors, or data managers. Data from each study were verified against the reported results in some centers, and queries were resolved with the principal investigator, corresponding authors, data managers, or statisticians. The minimum data requirements for participation in this study were data on age and sex of recipients, diagnosis (acute myelogenous leukemia [AML], acute lymphoblastic leukemia [ALL], acute biphenotypic or unclassifiable leukemia [AL], CML, chronic lymphocytic leukemia [CLL], myelodysplastic syndrome [MDS], malignant lymphoma [ML], or multiple myeloma [MM]), type of stem cell source (marrow or peripheral blood stem cell), type of donor (related or unrelated), status of survival (alive, dead, or censored), days of survival after transplantation at the latest follow-up period, and donor-recipient ABO matching (matched or major, minor, or bidirectional mismatched pairs). Additional information requested included donor-recipient compatibility of HLA-A, HLA-B, and HLA-DR antigens by low-resolution typing (matched or mismatched); intensity of conditioning regimen (reduced intensity or myeloablative intensity); GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other prophylaxes); primary cause of death (disease progression or treatment-related death or detailed information regarding primary cause of death); disease status at SCT; and days to reticulocyte, neutrophil, and PLT engraftment. Data were excluded for patients who met any of the following criteria: patients undergoing SCT for other than hematologic malignancies, those receiving cord blood transplant, those with a history of prior SCT, or those included in a previous large multicenter study published before June 30, 2007. This study was approved by the institutional review board of Kyoto University and other institutions.

Definition of disease risks, engraftment, and primary cause of death

Complete remission in AML, ALL, AL, CLL, ML, and MM; chronic phase in CML; and untreated or complete remission in MDS were considered indicative of standard-risk diseases. Statuses other than complete remission in AML, ALL, AL, CLL, ML, and MM; accelerated phase and blastic crisis in CML; and statuses other than complete remission

in MDS after treatment were considered indicative of high-risk diseases. As described in previous studies,[2,5] the day of reticulocyte engraftment was defined as the first day when the percentage of reticulocytes in peripheral blood exceeded 1 percent. The day of neutrophil engraftment was defined as the first day of 3 consecutive days when the absolute neutrophil count exceeded 0.5×10^9 per L and that of PLT engraftment, the first day of 3 consecutive days when the PLT count exceeded 20×10^9 per L without PLT transfusions. The primary cause of death was classified into two categories: disease-associated death or treatment-related death. Among patients who experienced treatment-related death, GVHD-related death was defined as death primarily associated with acute or chronic GVHD.

Statistical analysis

Patient and transplant characteristics among ABO matching groups were compared by using Kruskal-Wallis test or chi-square analysis, as appropriate. Survival was estimated according to Kaplan-Meier product limit methods. Cumulative incidences of TRM, GVHD-related mortality, and engraftment were assessed using methods described elsewhere to eliminate the effect of competing risk.[30] The competing event in cumulative incidence analyses was defined as death without an event of interest. Disease-associated death was considered a competing risk in the analysis of cumulative incidence of TRM. Death other than GVHD-related death was considered a competing risk in the analysis of cumulative incidence of GVHD-related death. When appropriate, Gray's test was applied to assess the impact of the factor of interest. Multivariate proportional hazard modeling of subdistribution functions in competing risks was applied to assess the impact of potential prognostic factors.[31] Cox regression analysis was used to determine the impact of ABO matching on the primary endpoint with adjustment for age (continuous), sex (male or female), and center effects in the seven data sets. When appropriate, the following items were added as confounders in addition to age, sex, and center effects: diagnosis (acute leukemia or others), risk (standard-risk, high-risk, or unknown), donor (related or unrelated), stem cell source (bone marrow or peripheral blood), conditioning regimen (reduced intensity, myeloablative intensity, or unknown), GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or unknown), transplant year (1990-1997, 1998-2007, or unknown), and transplant centers (Asian or non-Asian centers). All of the confounders were also considered in the multivariate analysis of TRM, GVHD-related mortality, and engraftment. p Values of less than 0.05 were considered significant for the comparison of baseline characteristics and the primary endpoint. With regard to secondary endpoints, p values of less than 0.001 were considered significant to eliminate false-positive associations possibly induced by multiple testing, and p values of less than 0.05 and equal to 0.001 or more were defined as marginally significant. All analyses were conducted using computer software (STATA, Version 10, STATA Corp., College Station, TX; R, Version 2.6.3, The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Collection of data

Seven data sets containing data on a total of 1424 SCT patients were collected from six published data sets and one unpublished data set from one center. A total of 133 patients not meeting the minimum data requirements or those who received SCT for diseases other than hematologic malignancies were excluded. Twenty-eight patients who received cord blood graft or both peripheral blood and marrow graft were also excluded. In addition, 6 patients enrolled in the other pooled studies were excluded. Forty-nine patients who had experienced prior SCT or had no information regarding their SCT history were also excluded. In the end, 1208 transplants, including 697 ABO-matched cases and 202 major, 228 minor, and 81 bidirectional mismatched cases, were included in the study. With regard to the additional data requests, data on disease status at transplant were obtained for five data sets; type of conditioning regimen, GVHD prophylaxis, and transplant year for six data sets; reticulocyte engraftment for two data sets; neutrophil and PLT engraftment for five data sets; and binary data on either disease-associated death or treatment-related death for one data set and for five data sets with detailed information on the primary cause of death.

Characteristics of patients and transplants

Table 1 shows the patient characteristics. The cases included 709 related SCTs and 184 unrelated SCTs from Western centers as well as 214 related SCTs and 101 unrelated SCTs from Asian centers. The median age of the recipients was 39 years (range, 1-69 years). Marrow and peripheral blood stem cell was used for 915 and 293 cases, respectively. There were no significant differences among ABO-matched and mismatched groups for any category except for the type of donors and centers of transplantation. With regard to donor type, bidirectional ABO-mismatched grafts were more frequently used among unrelated SCTs when compared to the ABO-matched group. With regard to transplant centers, SCTs from bidirectional mismatched donors were more frequently performed in Asian centers.

OS

The median follow-up period of survivors was 37 months (range, 3-268 months). The unadjusted probabilities of OS

TABLE 1. Characteristics of patients and transplants

Characteristic	Match (%) (n = 697)	Major mismatch (%) (n = 202)	Minor mismatch (%) (n = 228)	Bidirectional mismatch (%) (n = 81)	p Value
Age					
Median (range)	39 (1-67)	39 (1-66)	39 (2-69)	43 (4-62)	0.074
Sex					
Male	393 (56.4)	129 (63.9)	118 (51.8)	45 (55.6)	0.087
Female	304 (43.6)	73 (36.1)	110 (48.3)	36 (44.4)	
Diagnosis					
AML/MDS	323 (46.3)	70 (34.7)	102 (44.7)	37 (45.7)	0.115
ALL	102 (14.6)	36 (17.8)	45 (19.7)	14 (17.3)	
AL	6 (0.9)	1 (0.5)	0 (0.0)	0 (0.0)	
CML	168 (24.1)	58 (28.4)	50 (21.4)	17 (21.0)	
CLL	5 (0.7)	6 (3.0)	4 (1.8)	0 (0.0)	
ML	67 (9.6)	26 (12.9)	18 (7.9)	10 (12.4)	
MM	26 (3.7)	5 (2.5)	9 (4.0)	3 (3.7)	
Risk					
Standard	341 (48.9)	75 (37.1)	91 (39.9)	39 (48.2)	0.597
High	112 (16.1)	31 (15.4)	50 (21.9)	17 (21.0)	
Unknown	244 (35.0)	96 (47.5)	87 (38.2)	25 (30.9)	
Type of donors					
Related					<0.001
HLA-matched	374 (53.7)	83 (41.1)	103 (45.2)	31 (38.3)	
HLA-mismatched	31 (4.5)	8 (4.0)	9 (4.0)	5 (3.7)	
HLA matching unknown	168 (24.1)	49 (24.3)	51 (22.4)	11 (13.6)	
Unrelated					
HLA-matched	121 (17.4)	62 (30.7)	63 (27.6)	31 (38.3)	
HLA-mismatched	3 (0.4)	0 (0.0)	2 (0.9)	3 (3.7)	
Stem cell source					
BM	519 (74.5)	155 (76.7)	177 (77.6)	64 (79.0)	0.649
PB	178 (25.5)	47 (23.3)	51 (22.4)	17 (21.0)	
Conditioning regimens					
Reduced intensity	101 (14.5)	27 (13.4)	41 (18.0)	8 (9.9)	0.209
Myeloablative intensity	515 (73.9)	144 (71.3)	158 (69.3)	69 (85.2)	
Unknown	81 (11.6)	31 (15.4)	29 (12.7)	4 (4.9)	
GVHD prophylaxis regimen					
CyA based	413 (59.3)	120 (59.4)	122 (53.6)	44 (56.8)	0.052
FK based	153 (22.0)	44 (21.8)	69 (30.3)	29 (35.8)	
Others	3 (0.4)	0 (0.0)	0 (0.0)	1 (1.2)	
Unknown	128 (18.4)	38 (18.9)	37 (16.2)	5 (6.2)	
Transplant year					
1990-1994	123 (17.7)	32 (15.8)	30 (13.2)	8 (9.9)	0.065
1995-1997	189 (27.1)	74 (36.6)	74 (32.5)	25 (30.9)	
1998-2000	147 (21.1)	40 (19.8)	36 (15.8)	18 (22.2)	
2001-2003	102 (14.6)	30 (14.9)	36 (15.8)	15 (18.5)	
2004-2007	58 (8.3)	15 (7.4)	31 (13.6)	12 (14.8)	
Unknown	78 (11.2)	11 (5.5)	21 (9.2)	3 (3.7)	
Transplant centers					
Asian centers	169 (24.3)	46 (22.8)	67 (29.4)	33 (40.7)	0.007
Non-Asian centers	528 (75.8)	156 (77.2)	161 (70.6)	48 (59.3)	

BM = bone marrow; CyA = cyclosporine; FK = tacrolimus; PB = peripheral blood.

(95% confidence interval [CI]) at 5 years among patients receiving ABO-matched grafts and major, minor, and bidirectional mismatched grafts were 48% (44%-52%), 48% (40%-56%), 45% (38%-51%), and 37% (26%-49%), respectively (Fig. 2A). Because different backgrounds and heterogeneity of results in stem cell sources were found, the impact of ABO matching among recipients of either related or unrelated SCT in each stratified category was assessed (Figs. 2B and 2C and 3A and 3B).

Among recipients of related SCT, no significant difference in OS was observed between the ABO-matched group and any other mismatched group. These results were consistent across each stratified group. In contrast, minor and bidirectional mismatched groups among unrelated SCT recipients tended to be associated with poorer OS when adjusted for age and sex (adjusted hazard ratio [HR]: minor, 1.71 [95% CI, 1.15-2.53], p = 0.008; bidirectional, 1.73 [95% CI, 1.05-2.86], p = 0.031). The adverse impact of minor and bidirectional mismatched grafts on OS in unrelated SCT was strongly observed in the following stratified categories: patients with acute leukemia, patients who received SCT after 1998, and patients who underwent transplants at Asian centers.

In multivariate regression analysis of OS adjusted for potential confounders listed in Table 2, no adverse impact of ABO matching on OS was observed among all or the

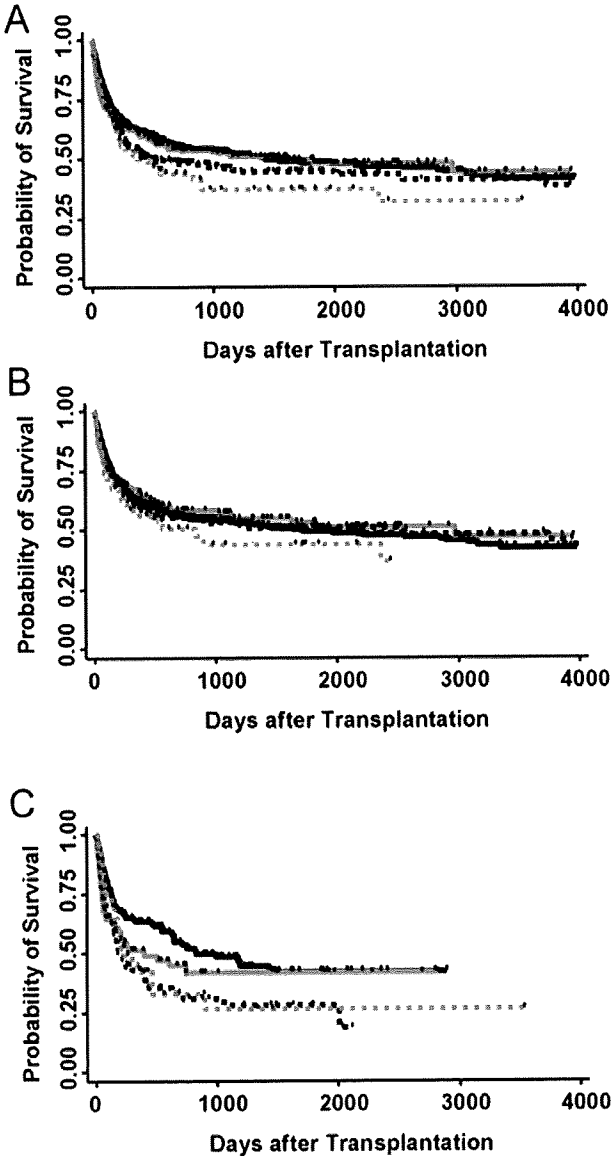

Fig. 2. Kaplan-Meier survival estimates of OS in all patients (A), those who received a related graft (B), and those who received an unrelated graft (C). (—) ABO-matched transplantation; (−) major mismatched; (· · ·) minor mismatched; (···) bidirectional mismatched.

subset of related SCTs, while minor and bidirectional mismatched groups showed tendency of poorer OS among the subset of unrelated SCT (adjusted HR: major, 1.38 [95% CI, 0.87-2.17], p = 0.17, minor, 1.68 [95% CI, 1.12-2.51], p = 0.012; bidirectional, 1.81 [95% CI, 1.08-3.00], p = 0.023) (Table 2).

TABLE 2. Impact of ABO mismatching on OS

Category	OS (n = 1208) HRs (95% CI)*	p Value
Overall		
Match	1.00	
Major	1.03 (0.82-1.30)	0.81
Minor	1.19 (0.97-1.47)	0.10
Bidirectional	1.25 (0.91-1.72)	0.17
Related SCT		
Match	1.00	
Major	0.93 (0.70-1.23)	0.62
Minor	1.02 (0.79-1.32)	0.88
Bidirectional	1.09 (0.71-1.68)	0.70
Unrelated SCT		
Match	1.00	
Major	1.38 (0.87-2.17)	0.17
Minor	1.68 (1.12-2.51)	0.012
Bidirectional	1.81 (1.08-3.00)	0.023

* HRs were adjusted for age, sex, diagnosis, risk, stem cell source, conditioning regimen, GVHD prophylaxis, transplant year, transplant centers, and donor, if appropriate.

TRM

Data on the primary cause of death were available for 1026 patients (85%). To evaluate the effect of ABO mismatch on treatment-related complications, we analyzed overall TRM at 5 years and early TRM within 100 days of transplantation. Although the cumulative incidences of overall TRM among the ABO-matched group and any mismatched groups did not show any significant difference in multivariate regression analysis, an increased risk of early TRM was observed among the bidirectional mismatched group (adjusted HR: 2.08 [95% CI, 1.14-3.79], p = 0.017; Table 3). This impact remained marginally significant among recipients of related SCTs (adjusted HR: 2.08 [95% CI, 1.04-4.15], p = 0.038). To evaluate whether early TRM was associated with acute GVHD, GVHD-related mortality within 100 days was analyzed using the available data sets (964 patients, 80%). Based on multivariate regression analysis adjusted for the confounding factors, the risk of acute GVHD-related mortality was significantly higher for the bidirectional mismatched group (adjusted HR, 9.35 [95% CI, 3.24-26.93], p < 0.001); however, further stratification by donor type could not be performed due to insufficient number of the data sets.

Engraftment

The data on days to reticulocyte, neutrophil, and PLT engraftment were available for 269 (24%), 667 (55%), and 662 (55%) patients, respectively. As shown in Table 4, multivariate regression analysis adjusted for confounders revealed no impact of ABO mismatching on reticulocyte, neutrophil, or PLT engraftment among patients who received related SCTs. In contrast, there was a marginally significant impact of ABO matching among recipients of unrelated SCTs. This analysis demonstrated a marginally significant impact of minor and bidirectional mismatched grafts on delay in reticulocyte engraftment compared to matched grafts among unrelated SCT recipients (major,

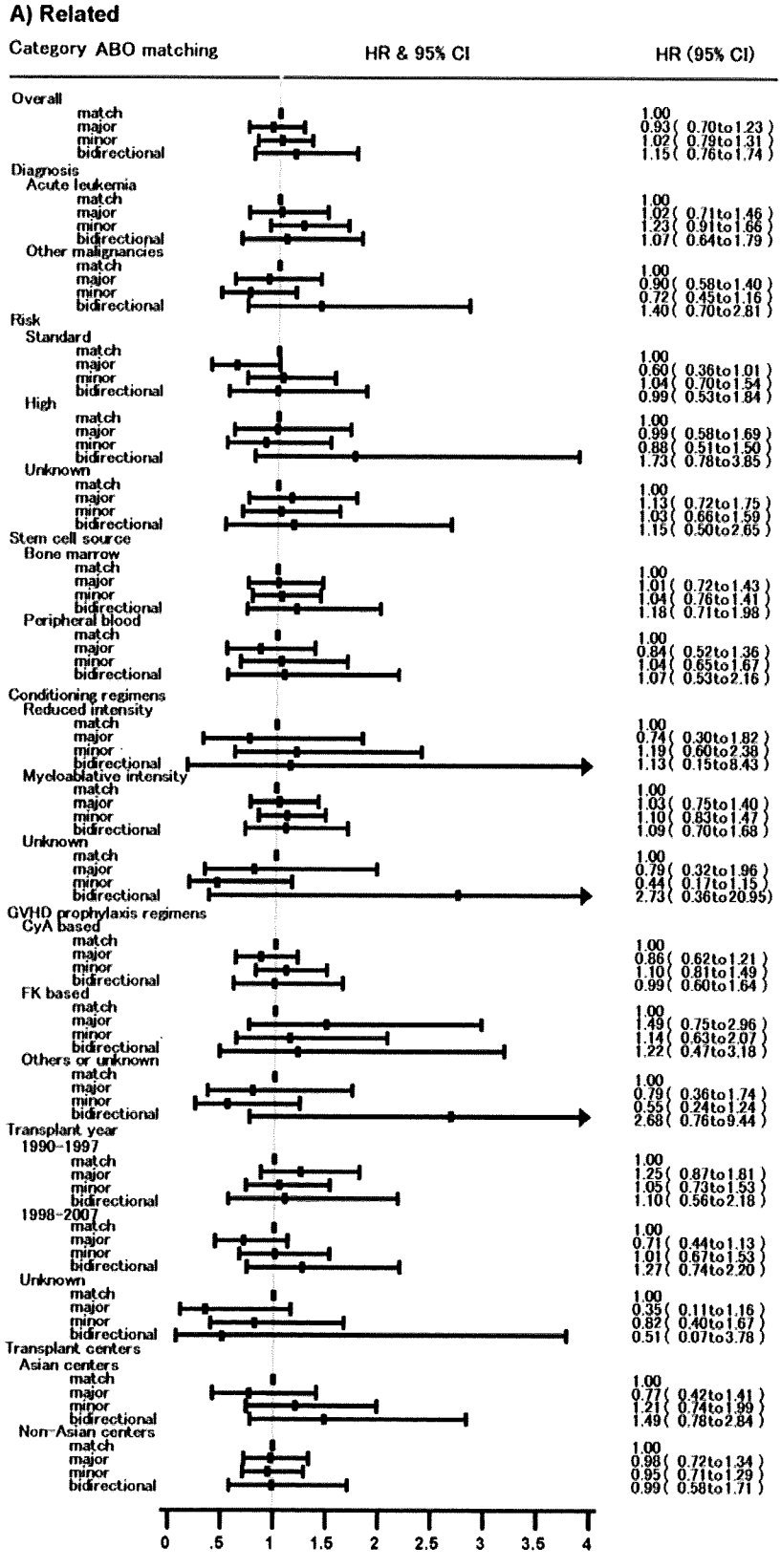

Fig. 3. Impact of ABO mismatching on OS in each stratified category among related (n = 923) (A) and unrelated stem cell transplantation (n = 285) (B). HRs were adjusted for age and sex. Square boxes on lines indicate HRs, and horizontal lines represent 95% CI.

p = 0.010; bidirectional, p = 0.012). Among recipients of unrelated SCTs, neutrophil engraftment tended to be delayed in the bidirectional mismatched group compared to the matched group (p = 0.019), and PLT engraftment tended to be delayed in the minor and bidirectional mismatched groups when compared to the matched group (minor mismatch, p = 0.023; bidirectional, p = 0.024).

DISCUSSION

To integrate the previous contradictory results, and to provide new data regarding the impact of ABO matching on survival after allogeneic blood and marrow SCTs, we performed an IPD-based meta-analysis using seven independent data sets including more than 1200 ABO-matched and mismatched transplants. Consistent with the results of the previous large retrospective analyses, our study confirmed and externally validated a lack of association between the use of ABO-mismatched grafts and OS among patients who underwent related SCTs. In contrast, we found marginally significant impact of minor and bidirectional mismatch among those who received unrelated SCTs. This observation suggested the need for larger studies focusing on unrelated SCTs that include various ethnic backgrounds as the next step in assessing the clinical significance of ABO mismatching in SCTs.

In this study, the adverse impact of minor and bidirectional mismatch on OS after unrelated SCTs was observed in the following stratified categories: patients with acute leukemia, patients who received SCT after 1998, and patients who underwent transplants at Asian centers. These associations might

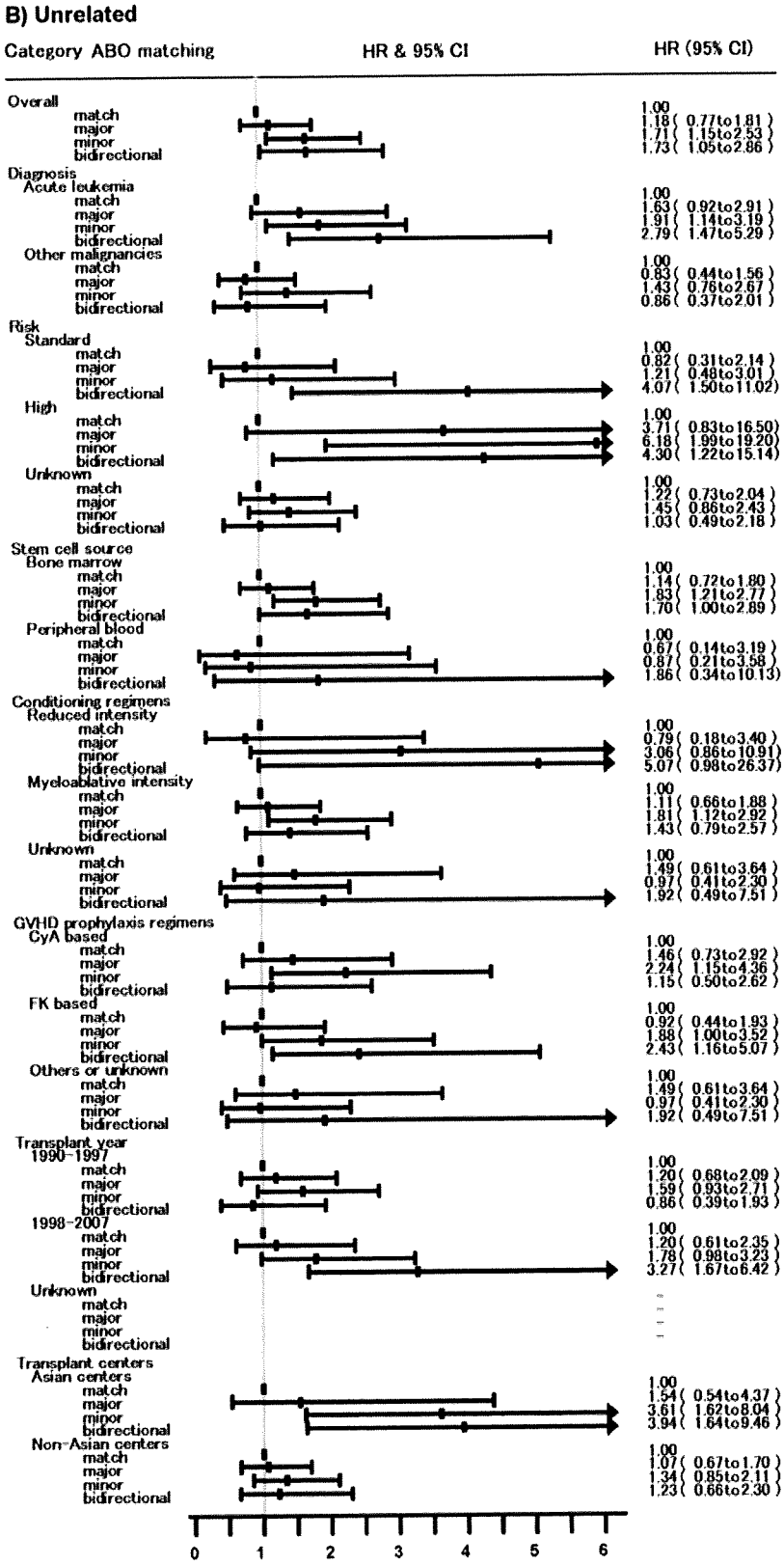

Fig. 3. Continued.

be biased by the relatively small size of unrelated transplant recipients in our analysis, because the previous study on the effect of ABO compatibility in unrelated SCTs among non-Asian populations reported that OS was not influenced by ABO mismatching.[21] However, more recently, a retrospective analysis of more than 5000 HLA-matched or mismatched unrelated SCTs facilitated by the Japan Marrow Donor Program revealed that the major ABO-mismatched group as well as minor mismatched group had inferior OS when compared to the ABO-matched group.[32] These varying results may partly be attributable to differences in the genetic backgrounds between Asian and non-Asian populations, such as cytokine gene polymorphisms and minor histocompatibility antigens:[33] it might be possible that the impact of minor and bidirectional mismatch is amplified by the increased immune dysregulation more likely to be seen in unrelated transplants compared with related transplants. Otherwise, ABO mismatching may exacerbate any underlying tendency toward complications seen in allogeneic transplantation, and these effects might be more prominent in unrelated SCTs. Recently, Michallet and coworkers[27] reported the results of a large retrospective study using the transplant data registered at the Société Française de Greffe de Moëlle et de Thérapie Cellulaire registry. The study analyzed 1108 patients who received related or unrelated SCTs after reduced-intensity conditioning for hematologic malignancies and it showed that minor ABO-mismatched grafts were associated with poorer OS. Although the background of patient characteristics in their study was different from that in this study, these results partly support our observation that minor and bidirectional mismatched grafts could have an adverse impact on OS.

However, the mechanism that underlies inferior survival after minor and bidirectional mismatched SCTs is presently unknown. In minor or bidirectional mismatched SCTs with marrow or peripheral blood grafts, passenger

TABLE 3. Impact of ABO mismatching on early TRM within 100 days and overall TRM

	Treatment-related death within 100 days (n = 1026)		Treatment-related death (n = 1026)	
Category	HRs (95% CI)*	p Value	HRs (95% CI)*	p Value
Overall				
Match	1.00		1.00	
Major	1.40 (0.84-2.32)	0.19	0.85 (0.57-1.28)	0.45
Minor	0.91 (0.52-1.59)	0.71	0.94 (0.65-1.34)	0.73
Bidirectional	2.08 (1.14-3.79)	0.017	1.45 (0.91-2.29)	0.11
Related SCT				
Match	1.00		1.00	
Major	1.10 (0.59-2.06)	0.75	0.81 (0.51-1.27)	0.36
Minor	0.81 (0.41-1.62)	0.56	0.85 (0.54-1.31)	0.45
Bidirectional	2.08 (1.04-4.15)	0.038	1.58 (0.95-2.64)	0.08
Unrelated SCT				
Match	1.00		1.00	
Major	2.10 (0.70-6.29)	0.19	0.84 (0.33-2.18)	0.72
Minor	1.17 (0.36-3.84)	0.79	1.15 (0.53-2.50)	0.72
Bidirectional	3.35 (0.95-11.80)	0.059	1.57 (0.63-3.92)	0.33

* HRs were adjusted for age, sex, diagnosis, risk, stem cell source, conditioning regimen, GVHD prophylaxis, transplant year, transplant centers, and donor, if appropriate.

donor B lymphocytes are known to often produce anti-recipient hemagglutinin 1 or 2 weeks after SCT.[10-12,34] For certain periods of time, such hemagglutinin could be continuously absorbed on widely expressed A/B antigens in tissues and residual RBCs of the recipient. Therefore, in addition to complication of delayed hemolysis, production of immune complexes on the surfaces of recipient tissues shortly after SCT could be a target for alloreaction or could dysregulate immunity. In addition, different transfusion policies may affect survival in minor and bidirectional mismatched transplants, because Benjamin and Antin[35] suggested that the transfusion of plasma containing anti-A,B antibodies in group O PLTs and RBC may exacerbate the cytokine storm that follows allogeneic transplant. Assessing the number of components transfused and the presence and/or development of anti-A/B antibodies would be a worthwhile consideration in future studies.

Subgroup analyses regarding TRM and engraftment were performed with available data sets to evaluate other effects of ABO mismatching. Those analyses showed that the use of bidirectional mismatched grafts was associated with an increased risk of early TRM when compared with matched grafts ($p = 0.017$), while the overall TRM was similar. The higher TRM observed in the early period after bidirectional ABO-mismatched SCTs may be due to the combination of major and minor ABO mismatching with additive or synergistic enhancement of single adverse effects. Theoretically, major ABO mismatching leads to antidonor cell damage and release of cytokines soon after transplantation. That may enhance the subsequent activation of antihost donor-derived lymphocytes in the minor mismatch direction. Therefore, fatal transplant complications such as severe acute GVHD may occur more often among the bidirectional mismatched group.[18] This hypothesis was supported by our observation that the incidence of GVHD-related death within 100 days was significantly higher among recipients of bidirectional mismatched SCTs ($p < 0.001$). Furthermore, delayed engraftment of neutrophils and PLTs could potentially affect early transplant complications, such as infection and bleeding, although we could not clearly identify an increased risk of such complications among a subgroup of patients who received bidirectional mismatched grafts from an unrelated donor. To assess the effect of immunologic reactions between ABO-mismatched pairs, the genotype of genes regulating the secretor status of ABO substances and glycosyltransferases are worth exploring in future studies. First, it is well known that only "secretors," that is, individuals who possess the appropriate secretor genotype, can secrete the soluble H and ABO substances into the body fluids and plasma. In secretor patients, hemagglutinin may form immune complexes with secreted ABO substances in circulation. In contrast, in nonsecretor patients, it may react with the endothelial compartment as well as blood cells. These different immune reactions can modify treatment-related complications. Second, Eiz-Vesper and coworkers[36] have recently demonstrated that a genotype mismatch with regard to glycosyltransferases among phenotypically ABO-matched donor-recipient pairs can induce an alloreaction in vitro. Therefore, the genotypic difference may be a source of minor histocompatibility antigens and affect the risk of GVHD in addition to ABO mismatching.

Reticulocyte engraftment tended to be delayed for the major and bidirectional mismatched groups among recipients of unrelated SCTs ($p = 0.010$ and 0.012, respectively), consistent with previous reports.[2-6] The delay in reticulocyte engraftment may become more evident through the enhanced host-versus-graft reactions in some unrelated SCTs than in related SCTs. In addition, neutrophil and PLT recovery tended to be delayed among patients receiving bidirectional mismatched unrelated grafts ($p = 0.019$). Late recovery of neutrophils after ABO-mismatched transplantation was also observed in the major mismatched group of both related and unrelated SCTs,[18,24,37] although these findings were not confirmed in the present study. Rozman and colleagues[24] hypothesized that immune complexes formed after ABO-mismatched transplantation can cause a pseudo-delay in neutrophil engraftment because immune complexes can be constantly recognized by the Fc receptors on immune cells,

TABLE 4. Impact of ABO mismatching on reticulocyte, neutrophil, and PLT engraftment

	Reticulocytes (>1%) (n = 269)			Neutrophils (0.5×10^9/L) (n = 667)			PLTs ($>20 \times 10^9$/L) (n = 662)		
	Median (day)	HRs (95% CI)*	p Value	Median (day)	HRs (95% CI)*	p Value	Median (day)	HRs (95% CI)*	p Value
Overall	10	1.00		16	1.00		18	1.00	
Match	26	0.67 (0.47-0.96)	0.029	16	1.01 (0.83-1.23)	0.92	21	0.91 (0.75-1.11)	0.37
Major	20	0.91 (0.64-1.30)	0.61	16	0.93 (0.73-1.17)	0.51	19	0.85 (0.69-1.06)	0.15
Minor	21	0.84 (0.58-1.21)	0.35	17	0.76 (0.56-1.03)	0.079	20	0.66 (0.45-0.96)	0.031
Bidirectional									
Related SCT	18	1.00		16	1.00		17	1.00	
Match	23	0.89 (0.57-1.39)	0.61	17	1.05 (0.83-1.31)	0.70	21	0.92 (0.74-1.14)	0.44
Major	19	0.81 (0.51-1.29)	0.37	16	0.90 (0.68-1.19)	0.47	19	0.90 (0.70-1.16)	0.43
Minor	18	1.17 (0.75-1.84)	0.49	16.5	1.02 (0.70-1.47)	0.93	17.5	0.78 (0.48-1.29)	0.34
Bidirectional									
Unrelated SCT	22	1.00		16	1.00		21.5	1.00	
Match	30	0.42 (0.21-0.81)	0.010	16	0.85 (0.54-1.33)	0.47	22	0.98 (0.65-1.48)	0.92
Major	20	0.85 (0.47-1.53)	0.58	15.5	0.93 (0.62-1.40)	0.74	24.5	0.61 (0.40-0.93)	0.023
Minor	26	0.43 (0.22-0.83)	0.012	18	0.52 (0.30-0.90)	0.019	25	0.47 (0.24-0.91)	0.024
Bidirectional									

* HRs were adjusted for age, sex, diagnosis, risk, stem cell source, conditioning regimen, GVHD prophylaxis, transplant year, transplant centers, and donor, if appropriate.

including neutrophils, which are subsequently removed from circulation. Finally, it should be mentioned that the presence of HLA antibodies, HLA allelic mismatching, or infused stem cell doses in unrelated donor SCTs could affect engraftment. It is desirable to include these factors in future studies of unrelated SCTs.

Limitations of this study should be noted. First, our data sets included heterogeneous diseases and various transplant methods, which made it difficult to elucidate the factors potentially associated with OS among minor and bidirectional mismatched transplantations. Second, the existence of missing data may have biased the results. In addition, data regarding the secondary endpoints were not available in some data sets. Therefore, these endpoints should be cautiously interpreted. Third, since we collected IPD from 6 of 11 candidate studies, there might be a potential selection bias. The findings of the meta-analysis should be interpreted in reference to the other large studies. Fourth, we performed the meta-analysis of non-randomized cohort studies, which might limit our interpretation due to the potential selection bias. However, truly randomized control trials for SCT have rarely been conducted. Fifth, generally speaking, the effect of multiple testing should be taken into account when we interpret secondary endpoints. Finally, missing data on HLA matching between related donors and recipients might reduce the statistical power in the analysis of related SCTs. However, with regard to unrelated SCTs (n = 285), exclusion of patients receiving SCT from HLA-mismatched unrelated donors (n = 8) did not alter the main result (data not shown).

In conclusion, our IPD-based meta-analysis demonstrates no adverse association between any type of ABO mismatching and survival in allogeneic SCTs for hematologic malignancies, although the possible association of minor or bidirectional ABO mismatching with lower OS was observed among recipients of unrelated SCTs. Larger studies focusing on the effects of ABO matching in unrelated SCTs from various ethnic backgrounds with complete HLA allele information are warranted.

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REFERENCES

1. Rydberg L. ABO-incompatibility in solid organ transplantation. Transfus Med 2001;11:325-42.
2. Canals C, Muniz-Diaz E, Martinez C, Martino R, Moreno I, Ramos A, Arilla M, Boto N, Pastoret C, Remacha A, Sierra J, Madoz P. Impact of ABO incompatibility on allogeneic peripheral blood progenitor cell transplantation after reduced intensity conditioning. Transfusion 2004;44:1603-11.
3. Helbig G, Stella-Holowiecka B, Wojnar J, Krawczyk M, Krzemien S, Wojciechowska-Sadus M, Markiewicz M, Wylezol I, Kopera M, Holowiecki J. Pure red-cell aplasia following major and bi-directional ABO-incompatible allogeneic stem-cell transplantation: recovery of donor-derived erythropoiesis after long-term treatment using different therapeutic strategies. Ann Hematol 2007;86:677-83.
4. Malfuson JV, Amor RB, Bonin P, Rodet M, Boccaccio C, Pautas C, Kuentz M, Cordonnier C, Noizat-Pirenne F, Maury S. Impact of nonmyeloablative conditioning regimens on the occurrence of pure red cell aplasia after ABO-incompatible allogeneic haematopoietic stem cell transplantation. Vox Sang 2007;92:85-9.
5. Mueller RJ, Stussi G, Odermatt B, Halter J, Schanz U, Seebach JD. Major ABO-incompatible hematopoietic stem cell transplantation: study of post-transplant pure red cell aplasia and endothelial cell chimerism. Xenotransplantation 2006;13:126-32.
6. Zhu KE, Li JP, Zhang T, Zhong J, Chen J. Clinical features and risk factors of pure red cell aplasia following major ABO-incompatible allogeneic hematopoietic stem cell transplantation. Hematology 2007;12:117-21.
7. Benjamin RJ, Connors JM, McGurk S, Churchill WH, Antin JH. Prolonged erythroid aplasia after major ABO-mismatched transplantation for chronic myelogenous leukemia. Biol Blood Marrow Transplant 1998;4:151-6.
8. Sniecinski IJ, Oien L, Petz LD, Blume KG. Immunohematologic consequences of major ABO-mismatched bone marrow transplantation. Transplantation 1988;45:530-4.
9. Yazer MH, Triulzi DJ. Immune hemolysis following ABO-mismatched stem cell or solid organ transplantation. Curr Opin Hematol 2007;14:664-70.
10. Worel N, Greinix HT, Keil F, Mitterbauer M, Lechner K, Fischer G, Mayr W, Hocker P, Kalhs P. Severe immune hemolysis after minor ABO-mismatched allogeneic peripheral blood progenitor cell transplantation occurs more frequently after nonmyeloablative than myeloablative conditioning. Transfusion 2002;42:1293-301.
11. Salmon JP, Michaux S, Hermanne JP, Baudoux E, Gerard C, Sontag-Thull D, Fillet G, Beguin Y. Delayed massive immune hemolysis mediated by minor ABO incompatibility after allogeneic peripheral blood progenitor cell transplantation. Transfusion 1999;39:824-7.
12. Laurencet FM, Samii K, Bressoud A, Tajeddin M, Easton J, Stelling MJ, Chapuis B. Massive delayed hemolysis following peripheral blood stem cell transplantation with minor ABO incompatibility. Hematol Cell Ther 1997;39:159-62.
13. Benjamin RJ, McGurk S, Ralston MS, Churchill WH, Antin JH. ABO incompatibility as an adverse risk factor for survival after allogeneic bone marrow transplantation. Transfusion 1999;39:179-87.
14. Mehta J, Powles R, Sirohi B, Treleaven J, Kulkarni S, Saso R, Tait D, Singhal S. Does donor-recipient ABO incompatibility protect against relapse after allogeneic bone marrow transplantation in first remission acute myeloid leukemia? Bone Marrow Transplant 2002;29:853-9.
15. Erker CG, Steins MB, Fischer RJ, Kienast J, Berdel WE, Sibrowski W, Cassens U. The influence of blood group differences in allogeneic hematopoietic peripheral blood progenitor cell transplantation. Transfusion 2005;45:1382-90.
16. Stussi G, Muntwyler J, Passweg JR, Seebach L, Schanz U, Gmur J, Gratwohl A, Seebach JD. Consequences of ABO incompatibility in allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2002;30:87-93.
17. Resnick IB, Tsirigotis PD, Shapira MY, Aker M, Bitan M, Samuel S, Abdul-Hai A, Ackerstein A, Or R, Slavin S. ABO incompatibility is associated with increased non-relapse and GVHD related mortality in patients with malignancies treated with a reduced intensity regimen: a single center experience of 221 patients. Biol Blood Marrow Transplant 2008;14:409-17.
18. Seebach JD, Stussi G, Passweg JR, Loberiza FR Jr, Gajewski JL, Keating A, Goerner M, Rowlings PA, Tiberghien P, Elfenbein GJ, Gale RP, van Rood JJ, Reddy V, Gluckman E, Bolwell BJ, Klumpp TR, Horowitz MM, Ringden O, Barrett AJ. ABO blood group barrier in allogeneic bone marrow transplantation revisited. Biol Blood Marrow Transplant 2005;11:1006-13.
19. Kalaycioglu M, Copelan E, Avalos B, Klein J, Goormastic M, Bolwell B. Survival after ABO-incompatible allogeneic bone marrow transplant after a preparative regimen of busulfan and cyclophosphamide. Bone Marrow Transplant 1995;15:105-10.
20. Klumpp TR, Herman JH, Ulicny J, Emmons RV, Martin ME, Mangan KF. Lack of effect of donor-recipient ABO mismatching on outcome following allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2006; 38:615-20.
21. Mielcarek M, Leisenring W, Torok-Storb B, Storb R. Graft-versus-host disease and donor-directed hemagglutinin titers after ABO-mismatched related and unrelated marrow allografts: evidence for a graft-versus-plasma cell effect. Blood 2000;96:1150-6.
22. Kim JG, Sohn SK, Kim DH, Baek JH, Lee KB, Min WS, Kim CC, Lee MH, Lee JJ, Chung IJ, Kim HJ, Lee JW. Impact of

ABO incompatibility on outcome after allogeneic peripheral blood stem cell transplantation. Bone Marrow Transplant 2005;35:489-95.
23. Goldman J, Liesveld J, Nichols D, Heal J, Blumberg N. ABO incompatibility between donor and recipient and clinical outcomes in allogeneic stem cell transplantation. Leuk Res 2003;27:489-91.
24. Rozman P, Kosir A, Bohinjec M. Is the ABO incompatibility a risk factor in bone marrow transplantation? Transpl Immunol 2005;14:159-69.
25. Worel N, Kalhs P, Keil F, Prinz E, Moser K, Schulenburg A, Mitterbauer M, Mannhalter C, Mayr WR, Schwarzinger I, Hocker P, Lechner K, Greinix HT. ABO mismatch increases transplant-related morbidity and mortality in patients given nonmyeloablative allogeneic HPC transplantation. Transfusion 2003;43:1153-61.
26. Helming AM, Brand A, Wolterbeek R, van Tol MJ, Egeler RM, Ball LM. ABO incompatible stem cell transplantation in children does not influence outcome. Pediatr Blood Cancer 2007;49:313-7.
27. Michallet M, Le QH, Mohty M, Prebet T, Nicolini F, Boiron JM, Esperou H, Attal M, Milpied N, Lioure B, Bordigoni P, Yakoub-Agha I, Bourhis JH, Rio B, Deconinck E, Renaud M, Chir Z, Blaise D. Predictive factors for outcomes after reduced intensity conditioning hematopoietic stem cell transplantation for hematological malignancies: a 10-year retrospective analysis from the Société Française de Greffe de Moëlle et de Thérapie Cellulaire. Exp Hematol 2008;36:535-44.
28. Stewart LA, Parmar MK. Meta-analysis of the literature or of IPD: is there a difference? Lancet 1993;341:418-22.
29. Piedbois P, Buyse M. Meta-analyses based on abstracted data: a step in the right direction, but only a first step. J Clin Oncol 2004;22:3839-41.
30. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med 1999;18:695-706.
31. Fine J, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496-7.
32. Kimura F, Sato K, Kobayashi S, Ikeda T, Sao H, Okamoto S, Miyamura K, Mori S, Akiyama H, Hirokawa M, Ohto H, Ashida H, Motoyoshi K; Japan Marrow Donor Program. Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan marrow Donor Program. Haematologia 2008;93:1686-93.
33. Mullally A, Ritz J. Beyond HLA: the significance of genomic variation for allogeneic hematopoietic stem cell transplantation. Blood 2007;109:1355-62.
34. Snell M, Chau C, Hendrix D, Fox R, Downes KA, Creger R, Meyerson H, Telen MJ, Laughlin MJ, Lazarus HM, Yomtovian R. Lack of isohemagglutinin production following minor ABO incompatible unrelated HLA mismatched umbilical cord blood transplantation. Bone Marrow Transplant 2006;38:135-40.
35. Benjamin RJ, Antin JH. ABO-incompatible bone marrow transplantation: the transfusion of incompatible plasma may exacerbate regimen-related toxicity. Transfusion 1999;39:1273-4.
36. Eiz-Vesper B, Seltsam A, Blasczyk R. ABO glycosyltransferases as potential source of minor histocompatibility antigens in allogeneic peripheral blood progenitor cell transplantation. Transfusion 2005;45:960-8.
37. Remberger M, Watz E, Ringden O, Mattsson J, Shanwell A, Wikman A. Major ABO blood group mismatch increases the risk for graft failure after unrelated donor hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2007;13:675-82.

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ORIGINAL ARTICLE

Mycophenolate mofetil combined with tacrolimus and minidose methotrexate after unrelated donor bone marrow transplantation with reduced-intensity conditioning

Chisaki Mizumoto · Junya Kanda · Tatsuo Ichinohe · Takayuki Ishikawa · Masashi Matsui · Norimitsu Kadowaki · Tadakazu Kondo · Kazunori Imada · Masakatsu Hishizawa · Hiroshi Kawabata · Momoko Nishikori · Kouhei Yamashita · Akifumi Takaori-Kondo · Toshiyuki Hori · Takashi Uchiyama

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Abstract We evaluated the efficacy of a post-grafting immunosuppressive regimen consisting of tacrolimus, methotrexate, and mycophenolate mofetil (MMF) in 21 adults (median age, 55 years) with poor-risk hematologic malignancy who underwent unrelated bone marrow transplantation after fludarabine-based reduced-intensity conditioning (RIC). In combination with intravenous tacrolimus and minidose methotrexate (5 mg/m^2 on days 1, 3, and 6), MMF was orally administered at 30 mg/kg daily in three divided doses between days 7 and 27. All patients achieved neutrophil recovery with donor-type chimerism at a median of 19 days (range, 13–35). Cumulative incidences of grades II–IV and III–IV acute graft-versus-host disease (GVHD) were 33% (95% CI, 15–53%) and 5% (95% CI, 0.3–20%), respectively. Five of 20 evaluable patients developed extensive chronic GVHD. Toxicities associated with the use of MMF were acceptable, although one patient experienced intractable GVHD immediately after the cessation of MMF. With a median follow-up of 24 months, overall survival at 3 years was 38% (95% CI, 14–63%). No late graft failure was observed. In conclusion, post-transplant MMF combined with tacrolimus and methotrexate was well tolerated and conferred stable donor cell engraftment, low risk of severe acute GVHD, and encouraging overall survival in unrelated donor marrow transplantation after RIC regimens.

Keywords Mycophenolate mofetil · Reduced-intensity conditioning · Unrelated donor · Bone marrow transplantation · Graft-versus-host disease

C. Mizumoto · J. Kanda · T. Ichinohe (✉) · T. Ishikawa · M. Matsui · N. Kadowaki · T. Kondo · M. Hishizawa · H. Kawabata · M. Nishikori · K. Yamashita · A. Takaori-Kondo · T. Hori · T. Uchiyama
Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan
e-mail: nohe@kuhp.kyoto-u.ac.jp

K. Imada
Department of Hematology, Kokura Memorial Hospital, Kita-kyushu, Japan

1 Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) with reduced-intensity conditioning (RIC) regimens is increasingly employed as a treatment option for various hematologic disorders. RIC transplantations using cytokine-mobilized peripheral blood stem cells (PBSC) have been reported to yield comparable outcomes with conventional myeloablative HSCT at least in selected patients [1–4]. However, previous reports have consistently shown that RIC transplantations using bone marrow (BM) graft, especially from an unrelated donor, are associated with an increased risk of graft failure and treatment-related toxicity as compared with those using PBSC, although confirmatory data from randomized-controlled trials are currently unavailable [5–8]. To improve outcomes after unrelated BM transplantation conditioned with non-myeloablative or reduced-intensity regimens, it would be beneficial to introduce a newer post-transplant immunosuppressive protocol which can effectively prevent both graft rejection and severe graft-versus-host disease (GVHD).

Mycophenolate mofetil (MMF) is an esterified prodrug of mycophenolic acid (MPA), which has pleiotropic immunosuppressive actions [9, 10]. MPA preferentially inhibits de novo purine nucleotide synthesis in T-cells and B-cells via inhibition of inosine-5′-monophosphate

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dehydrogenase, interfering with their proliferation. MPA also suppresses dendritic cell maturation and can induce T-cell apoptosis. The use of MMF in combination with cyclosporine or tacrolimus was proven to be active in promoting hematopoietic stem cell engraftment after non-myeloablative HSCT using fludarabine and low-dose total-body irradiation (TBI) conditioning [11, 12], and was also shown to be as effective as the standard post-grafting immunosuppression with cyclosporine and methotrexate (MTX) in preventing severe acute GVHD after myeloablative HSCT from an HLA-matched related donor [13, 14].

A combination of tacrolimus and minidose MTX has been widely used as GVHD prophylaxis in RIC transplantation as well as in conventional HSCT from adult unrelated donors [15, 16]. Because MMF and tacrolimus are shown to have synergistic immunosuppressive actions in experimental and clinical organ transplantations [17, 18], we hypothesized that MMF in conjunction with tacrolimus and minidose MTX would be more efficacious than a combination of tacrolimus/MTX alone. In this single-center study, we retrospectively evaluated the efficacy of such triple combination as an alternative peritransplant immunosuppressive protocol in unrelated donor RIC transplantation using exclusively BM as a stem cell source.

2 Patients and methods

2.1 Patients

Among 61 consecutive adult patients who received BM transplantation from an unrelated donor between 2003 and 2006 at Kyoto University Hospital, those who fulfilled the following criteria were selected for the study: having a hematologic malignant disease; having an unrelated donor who was serologically matched at HLA-A, -B, and -DR antigens, allowing a single-allele mismatch identified by high-resolution DNA typing; receiving fludarabine-based RIC because of having a history of chemoradiotherapy precluding the use of myeloablative conditioning or having 55 through 69 years of age; receiving GVHD prophylaxis consisted of intravenous tacrolimus, minidose MTX and oral MMF. All patients had an adequate cardiac, pulmonary, hepatic, and renal function at the time of transplantation and did not have therapy-resistant central nervous system involvement or active infectious disease. A total of 21 patients fulfilled these criteria and considered evaluable for the study. With respect to disease status at transplant, patients who received transplant without prior cytotoxic chemotherapy or in first complete remission were considered to have an early disease, while those who underwent transplantation in all the other conditions were considered to have an advanced disease. All the patients with early disease were considered to have resistance to conventional chemotherapy or to have a high risk of relapse: those included two cases with untreated high-risk myelodysplastic syndrome, one with chronic active Epstein-Barr virus infection and one with adult T-cell leukemia/lymphoma in first remission. This study was approved by the Institutional Review Board and Ethic Committee of Kyoto University; written informed consent for transplantation was obtained from all participating patients.

2.2 Study end points

The primary end points of the study were donor cell engraftment and the occurrence of grade II–IV acute GVHD. Secondary end points included the neutrophil and platelet recovery, the occurrence of extensive chronic GVHD, progression or relapse of primary disease, and death from any cause.

Donor cell engraftment was defined as the detection of donor-type chimerism among unfractionated BM-nucleated cells with concomitant neutrophil recovery. Date of neutrophil recovery was defined as the first 3 consecutive days with the absolute neutrophil count (ANC) higher than 0.5×10^9/L. Date of platelet recovery was defined as the first 7 consecutive days with platelet count exceeding 20×10^9/L without transfusion. Acute GVHD was diagnosed and graded according to the conventional criteria [19]. Chronic GVHD was diagnosed and staged as limited or extensive on the basis of traditional criteria among patients who survived more than 90 days after transplantation [20]. Disease response and progression were defined by the standard criteria [21–25]. Toxicity observed between days 0 and 100 after transplantation was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events ver 3.0. Non-infectious pulmonary complications were diagnosed on the basis of clinical manifestations, radiologic findings, and the results of pulmonary function tests if available [26].

2.3 HLA typing and chimerism analysis

Compatibility at *HLA-A*, *-B*, and *-DRB1* loci between patients and donors was determined by standard serologic technique and high-resolution DNA typing as described elsewhere [27]. *HLA-C* compatibility was not included as a criterion for donor selection because routine *HLA-C* allele typing for the screening of unrelated donors was not available before April 2004. Donor cell chimerism levels among unfractionated BM-nucleated cells were evaluated on day 28 and thereafter at the appropriate time point by polymerase chain reaction-based analysis of polymorphic microsatellite regions for recipients of sex-matched graft or fluorescent in situ hybridization analysis of sex chromosomes for sex-mismatched pairs as described previously [28].

2.4 Transplantation procedure

Preparative regimens were assigned according to diagnosis and disease status at transplantation. Fourteen patients received fludarabine 25 mg/m^2/day for 5 consecutive days (days −6 to −2) in combination with oral busulfan 1 mg/kg every 6 h for 2 days (days −3 and −2) followed by 400 cGy of TBI in 2 fractions (on day −1 and/or day 0). One patient who had a history of TBI-based myeloablative allogeneic transplantation received the same dose schedule of fludarabine plus busulfan regimen without 400 cGy TBI. Four patients received fludarabine at the same daily dose from days −8 through −4 combined with melphalan 70 mg/m^2/day on days −3 and −2. The remaining two patients without a history of cytotoxic chemotherapy received 200 cGy TBI in a single fraction in addition to the fludarabine plus melphalan regimen.

All BM collections from unrelated donors were facilitated through the Japan Marrow Donor Program [27]. On day 0, BM graft was infused without T-cell depletion; ABO major-mismatched or bidirectionally mismatched graft was processed to isolate mononuclear cell suspension using COBE Spectra (Gambro BCT, Lakewood, CO, USA) or CS-3000 Plus (Baxter Corp., Deerfield, IL, USA) according to the manufacture's instruction, while ABO minor-mismatched graft was plasma depleted before infusion. Eleven patients who were suspected to develop bacterial infection during the first week after transplantation or who were considered to be at high risk for infectious complications because of prior history of allogeneic transplantation received infusional or subcutaneous granulocyte colony-stimulating factor 5 μg/kg/day from day 7 until ANC exceeded 0.5 × 10^9/L.

Continuous intravenous administration of tacrolimus in a dose of 0.02 mg/kg/day was started on day −3 in patients receiving busulfan-based conditioning or on day −1 in patients receiving melphalan-based conditioning with therapeutic monitoring which targeted blood levels of 10–15 ng/ml at least until day 28 after transplantation, converted to twice-daily oral administration at an appropriate time to maintain trough levels between 5 and 10 ng/ml until day 100, followed by stepwise tapering over 3–6 months if active GVHD was absent. MTX at a dose of 5 mg/m^2 was intravenously injected on days 1, 3, and 6; MMF 30 mg/kg/day was orally administered in three divided doses from days 7 to 27. After day 28, MMF was discontinued without tapering if acute GVHD was absent or gradually tapered if ongoing acute GVHD was present. Patients who developed grade II–IV acute GVHD were initially treated with methylprednisolone or prednisolone at a dose of 1–2 mg/kg/day. All patients received supportive care including blood product transfusion and prophylaxis against opportunistic infections according to our institutional protocols [29].

2.5 Statistical analysis

Probabilities of neutrophil recovery, platelet recovery, and grade II–IV or grade III–IV acute GVHD were calculated by cumulative incidence estimates, treating death without the respective event as a competing risk [30]. Overall survival from the date of transplantation until the date of death from any cause was estimated by the Kaplan–Meier method; progression-free survival was estimated from the date of transplantation until the date of disease progression, relapse, or death from any cause. Data on patients who were alive at the time of last follow-up were censored. All statistical analyses were performed using STATA version 10 software (Stata Corp., College Station, TX, USA) based on dataset available on 10 January 2008.

3 Results

3.1 Patient and transplant characteristics

Table 1 shows the characteristics of the patients and transplantation procedures. A total of 17 patients (86%) had an advanced disease at transplantation, while the remaining 4 patients had an early disease. With respect to the compatibility at *HLA-A*, *-B*, and *-DRB1*, five patients (24%) received a single-allele mismatched graft, three had a mismatch at *HLA-A* and two at *HLA-DRB1*. Two of these five patients were found to have an additional allele mismatch at *HLA-C*. The median total number of nucleated cells included in the collected BM graft was 3.0 (range, 1.2–4.0) × 10^8 per kg of the recipient's body weight.

3.2 Engraftment

All patients achieved successful donor cell engraftment. The cumulative incidence of neutrophil recovery >0.5 × 10^9/L by day 35 was 100%, with a median time of 19 days (range, 13–35 days) (Fig. 1a). The cumulative probability of platelet recovery >20 × 10^9/L by day 42 was 81%, with a median time of 26 days (range, 13–91 days) (Fig. 1b). Two patients had experienced relapse on days 39 and 67 after transplantation without platelet recovery. No secondary graft failure was observed.

3.3 Acute and chronic GVHD

Acute GVHD was evaluable in all the patients. A total of seven patients developed grade II–IV acute GVHD: grade II in 6 and grade IV in 1. Cumulative incidence of developing grade II–IV acute GVHD at day 100 after transplantation was 33% (95% CI, 15–53%), and that of grade III–IV acute GVHD was 5% (95% CI, 0.3–20%)

Table 1 Patient and transplant characteristics

	$n = 21$
Median recipient age (range) (years)	52 (24–66)
Recipient sex, *n*	
Female/male	12/9
Diagnosis, *n*	
Acute myeloid leukemia	5
Myelodysplastic syndrome	4
Adult T-cell leukemia/lymphoma	4
Follicular lymphoma	3
Hodgkin lymphoma	1
Plasma cell myeloma	2
Chronic active EBV infection	1
Extranodal NK/T-cell lymphoma	1
Disease status at transplantation, *n*	
Early disease	
CR1	1
Untreated	3
Advanced disease	
CR > 1	4
PR	8
Progressive disease	5
Median donor age (range) (years)	34 (20–48)
HLA matching (at HLA-A, -B, -DRB1), *n*	
Match	16
Single-allele mismatch	5
ABO incompatibility, *n*	
Match	9
Minor	3
Major	5
Bidirectional	4
Conditioning, *n*	
Fludarabine + busulfan + 4 Gy TBI	14
Fludarabine + busulfan	1
Fludarabine + melphalan + 2 Gy TBI	2
Fludarabine + melphalan	4

EBV Epstein-Barr virus, *CR* complete remission, *PR* partial remission, *TBI* total-body irradiation

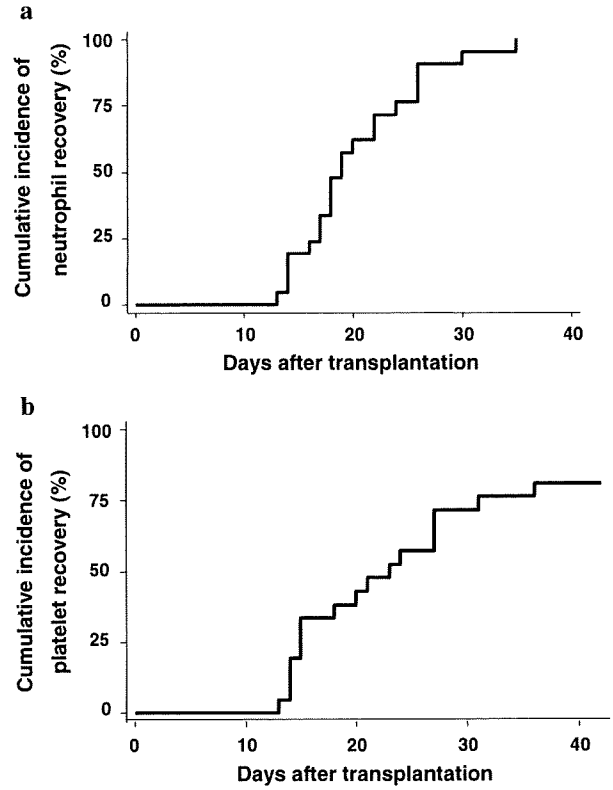

Fig. 1 Cumulative incidence of neutrophil recovery (**a**) and platelet recovery (**b**)

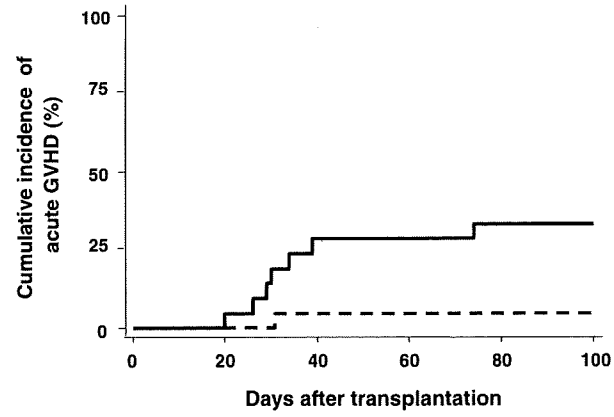

Fig. 2 Cumulative incidences of grade II–IV (*solid line*) and grade III–IV (*dashed line*) acute GVHD

(Figs. 2, 3). Chronic GVHD was observed in 11 of 20 (55%) evaluable patients who survived 100 days after transplantation: limited type in 6 and extensive type in 5.

3.4 Transplant-related toxicities and infectious complications

Transplant-related organ toxicities during the first 100 days after transplantation are shown in Table 2. Mild to moderate gastrointestinal symptoms considered to be associated with preparative regimens were frequently observed, although other adverse events were mostly moderate. One patient was required to discontinue MMF on day 9 because of grade III diarrhea.

Eighteen patients (86%) experienced 38 episodes of documented or suspected infectious complications (Table 3). Fourteen episodes of culture-negative neutropenic fever were reported. Five episodes of microbiologically documented bacterial infection were observed in four patients:

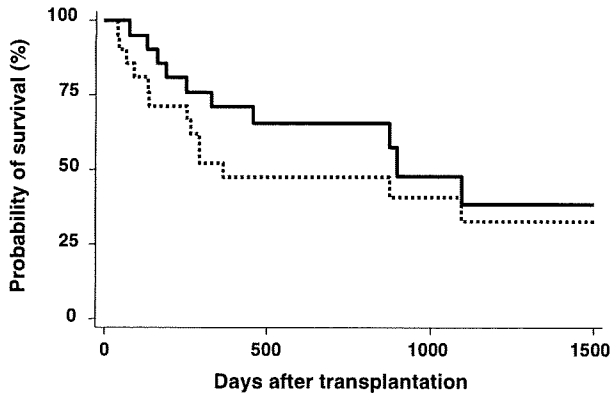

Fig. 3 Probabilities of overall survival (*solid line*) and progression-free survival (*dotted line*) after transplantation

Table 2 Transplant-related toxicities

	CTCAE grade I–II	CTCAE grade III
Vomiting	3 (14%)	6 (28%)
Stomatitis	5 (24%)	6 (28%)
Diarrhea	9 (43%)	3 (14%)
Liver dysfunction	5 (24%)	3 (14%)
Renal dysfunction	3 (14%)	0 (0%)
Headache	0 (0%)	2 (10%)
Pleural effusion	2 (10%)	0 (0%)
Myalgia	2 (10%)	0 (0%)

CTCAE common terminology criteria for adverse events

Table 3 Infectious complications

	No. of episodes
Culture-negative febrile neutropenia	14
Bacteremia	4
Clostridium difficile-associated diarrhea	1
Phlegmone	1
Neutropenic enterocolitis	1
CMV antigenemia	11
Cystitis	5
Aseptic meningitis	1
Total	38

CMV cytomegalovirus

bloodstream infection ($n = 4$) and *Clostridium difficile*-associated enteritis ($n = 1$). Two episodes of suspected bacterial infections were reported: phlegmone ($n = 1$) and neutropenic enterocolitis ($n = 1$). Eleven patients became positive for cytomegalovirus (CMV) antigenemia; one of them developed CMV-associated hepatitis and enteritis. Adenovirus was detected from the urine from one of five patients who developed cystitis. One patient experienced aseptic meningitis. No varicella-zoster virus infection was observed. There was no death directly attributable to infectious events until day 100.

Six patients developed non-infectious pulmonary complications between 3 and 14 months after transplantation: bronchiolitis obliterans ($n = 2$), bronchiolitis obliterans-organizing pneumonia ($n = 1$), diffuse alveolar hemorrhage ($n = 1$), and idiopathic interstitial pneumonia ($n = 2$). One patient developed secondary gastric cancer at 34 months after transplantation.

3.5 Survival and treatment-related mortality

Eleven patients were alive and 10 of them were disease-free at a median follow-up of 24 months (range, 3–31 months). Among four patients who had an early disease at transplant, one experienced relapse on day 292. Fifteen of 17 patients who had an advanced disease maintained or attained remission after transplantation, but seven of them eventually relapsed between days 67 and 363. Two of the relapsed patients received donor lymphocyte infusion after chemotherapy, and durable remission lasting more than 16 months was observed in one patient.

Ten patients were deceased between 76 and 1093 days after transplantation. Six patients succumbed to disease progression and four patients died of treatment-related complications including interstitial pneumonia ($n = 1$), bronchiolitis obliterans followed by diffuse alveolar damage ($n = 1$), intracranial hemorrhage during exacerbation of bronchiolitis obliterans ($n = 1$), and secondary gastric cancer ($n = 1$). The probabilities of overall survival and progression-free survival at 3 years after transplantation were 38% (95% CI, 14–63%) and 33% (95% CI, 12–55%), respectively.

4 Discussion

In this study, we evaluated the efficacy of a combination of tacrolimus, minidose MTX, and MMF as post-transplant immunosuppression in RIC transplantations using BM grafts from an HLA-A, -B, -DR antigen compatible unrelated donor. This triple regimen conferred stable donor cell engraftment, low risk of severe acute GVHD, and encouraging overall survival with acceptable toxicity profiles.

Recent introduction of RIC has provided the opportunity to enjoy long-term disease-free survival in patients with hematologic malignancies who were previously ineligible for allogeneic HSCT because of elder age or pre-existing comorbidity. It has been shown that RIC HSCT using alternative stem cell source is a feasible treatment option when an HLA-matched related donor is not available, albeit at the expense of substantial risk of more serious

transplant-related complications. Among the first 285 patients who underwent unrelated RIC HSCT through the National Marrow Donor Program, the respective incidence rates of primary graft failure, grade III–IV acute GVHD, and treatment-related mortality at 3 months after transplantation were 11, 22, and 19%, respectively [7].

It should also be noticed that RIC transplantations using BM as a stem cell source have been reported to be associated with a higher risk of graft failure when compared with those using cytokine-mobilized PBSC, especially in the unrelated donor setting [6, 7]. As compared with BM, PBSC grafts usually contain more than ten times higher number of T-cells and 2–4 times greater number of $CD34^+$ cells, which would have a beneficial impact on successful engraftment after RIC [31]. In a study which compared the engraftment kinetics after transplantation of PBSC and BM with an identical non-myeloablative conditioning, the number of patients who achieved full donor chimerism was significantly lower in the BM group [32]. These observations suggested that, to improve the outcomes after RIC transplantation using BM grafts from unrelated donors, it is important to develop more optimal post-transplant immunosuppressive protocol which can effectively prevent graft rejection as well as severe GVHD.

In preclinical canine models and clinical experiences of HSCT after truly non-myeloablative regimen using low-dose TBI with or without fludarabine as pre-transplant conditioning, post-transplant administration of MMF was shown to improve the rate of successful donor cell engraftment [11, 33]. Therefore, we hypothesized that the addition of MMF to the standard immunosuppression with tacrolimus plus minidose MTX might facilitate engraftment after unrelated BM allografting with RIC. In support of this hypothesis, all the patients in this study achieved durable donor cell engraftment without experiencing serious morbidity associated with delayed hematopoietic recovery or late graft failure. However, this promising result awaits further validation because the probability of engraftment can also be influenced by the type and intensity of RIC regimens or by the use of pre-transplant anti-thymocyte globulins or T-cell-depleting monoclonal antibodies. Onishi et al. reported the outcomes of unrelated BM transplantation after RIC with fludarabine, busulfan, and 4 Gy TBI among a cohort of 17 patients with various hematologic malignant diseases. Although all the patients in their report initially achieved successful engraftment with the use of conventional post-transplant immunosuppression composed of cyclosporine and MTX, 2 of them subsequently developed secondary graft failure [8]. This observation suggests that the intensification of conditioning with 4 Gy TBI does not always confer sustained engraftment, at least in the setting of unrelated marrow transplantation.

In contrast, the role of MMF in ameliorating acute GVHD has been controversial at least when administered solely with calcineurin inhibitors. Recently, Koh et al. [34] reported that the post-grafting MTX combined with cyclosporine and MMF significantly reduced the risk of grade III–IV acute GVHD as compared with a combination of cyclosporine and MMF. Consistent with their experience, the cumulative incidence of severe acute GVHD after our triple combination was 5%, encouragingly lower than those previously reported in the analysis of unrelated BMT through the Japan Marrow Donor Program, while the incidence of extensive chronic GVHD was apparently similar [27, 35]. However, an important concern regarding the intensification of post-transplant immunosuppressive regimen is an increased risk of infection or relapse. In this study, a substantial proportion of patients developed manageable infections and experienced disease progression within 1 year after transplantation. Although the incidence rates of these events might be adversely affected by the high proportion of patients who had an advanced disease at transplantation, further studies are needed to elucidate whether our triple immunosuppressive regimen may increase the risk of infectious complications or may compromise the graft-versus-tumor effect after RIC HSCT [36, 37].

An unresolved issue in the present study is a pharmacokinetic/pharmacodynamic profile of MMF when combined with tacrolimus and MTX. The increased mean total plasma MPA concentrations at steady state were reported to be associated with higher donor cell chimerism after unrelated non-myeloablative transplantation, while the lower MPA levels were shown to be a predictor of graft rejection [38]. We administered MMF in three divided doses rather than in twice-daily doses because the former is more likely to confer higher mean total MPA concentrations [38, 39]. Because it is speculated that the bioavailability of oral MMF is highly variable depending on the degree of gastrointestinal mucosal damage and donor-recipient pharmacogenomic backgrounds [40], it is important in the future studies to evaluate the association of MPA pharmacodynamics with the risk of post-transplant immunologic complications such as graft rejection, acute GVHD, and infections. Furthermore, appropriate dosing of MMF would be affected by the type of combined calcineurin inhibitor: cyclosporine is reported to decrease MPA exposure due to delay of the excretion of the MPA metabolites, while tacrolimus is less likely to cause drug interaction with MPA [41, 42].

In conclusion, our study demonstrated the feasibility and efficacy of using a triple combination of tacrolimus, minidose MTX and MMF as post-grafting immunosuppression after RIC BM transplantation from unrelated donors. Because this triple regimen conferred high

probability of sustained donor engraftment with an acceptable risk of transplant-related complications, further studies are warranted to confirm its efficacy in a larger population including patients who receive HLA-mismatched family donor grafts or unrelated cord blood units with dose-reduced conditioning.

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References

1. Aoudjhane M, Labopin M, Gorin NC, Shimoni A, Ruutu T, Kolb HJ, et al. Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT). Leukemia. 2005;19:2304–12. doi:10.1038/sj.leu.2403967.
2. Alyea EP, Kim HT, Ho V, Cutler C, Gribben J, DeAngelo DJ, et al. Comparative outcome of nonmyeloablative and myeloablative allogeneic hematopoietic cell transplantation for patients older than 50 years of age. Blood. 2005;105:1810–4. doi:10.1182/blood-2004-05-1947.
3. Martino R, Iacobelli S, Brand R, Jansen T, van Biezen A, Finke J, et al. Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. Blood. 2006;108:836–46. doi:10.1182/blood-2005-11-4503.
4. Rodriguez R, Nademanee A, Ruel N, Smith E, Krishnan A, Popplewell L, et al. Comparison of reduced-intensity and conventional myeloablative regimens for allogeneic transplantation in non-Hodgkin's lymphoma. Biol Blood Marrow Transplant. 2006;12:1326–34. doi:10.1016/j.bbmt.2006.08.035.
5. Bornhäuser M, Thiede C, Platzbecker U, Jenke A, Helwig A, Plettig R, et al. Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. Clin Cancer Res. 2001;7:2254–62.
6. Maris MB, Niederwieser D, Sandmeier BM, Storer B, Stuart M, Maloney D, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood. 2003;102:2021–30. doi:10.1182/blood-2003-02-0482.
7. Giralt S, Logan B, Rizzo D, Zhang MJ, Ballen K, Emmanoulides C, et al. Reduced-intensity conditioning for unrelated donor progenitor cell transplantation: long-term follow-up of the first 285 reported to the national marrow donor program. Biol Blood Marrow Transplant. 2007;13:844–52. doi:10.1016/j.bbmt.2007.03.011.
8. Onishi Y, Mori S, Kusumoto S, Sugimoto K, Akahane D, Morita-Hoshi Y, et al. Unrelated-donor bone marrow transplantation with a conditioning regimen including fludarabine, busulfan, and 4 Gy total body irradiation. Int J Hematol. 2007;85:256–63. doi:10.1532/IJH97.06199.
9. Vogelsang GB, Arai S. Mycophenolate mofetil for the prevention and treatment of graft-versus-host disease following stem cell transplantation: preliminary findings. Bone Marrow Transplant. 2001;27:1255–62. doi:10.1038/sj.bmt.1703076.
10. Allison AC, Eugui EM. Mechanism of action of mycophenolate mofetil in preventing acute and chronic allograft rejection. Transplantation. 2005;80(Suppl 2):S181–90. doi:10.1097/01.tp.0000186390.10150.66.
11. McSweeney PA, Niederwieser D, Shizuru JA, Sandmeier BM, Molina AJ, Maloney DG, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001;97:3390–400. doi:10.1182/blood.V97.11.3390.
12. Nieto Y, Patton N, Hawkins T, Spearing R, Bearman SI, Jones RB, et al. Tacrolimus and mycophenolate mofetil after nonmyeloablative matched-sibling donor allogeneic stem-cell transplantations conditioned with fludarabine and low-dose total body irradiation. Biol Blood Marrow Transplant. 2006;12:217–25. doi:10.1016/j.bbmt.2005.10.012.
13. Bolwell B, Sobecks R, Pohlman B, Andresen S, Rybicki L, Kuczkowsky E, et al. A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. Bone Marrow Transplant. 2004;34:621–5. doi:10.1038/sj.bmt.1704647.
14. Nash RA, Johnston L, Parker P, McCune JS, Storer B, Slattery JT, et al. A phase I/II study of mycophenolate mofetil in combination with cyclosporine for prophylaxis of acute graft-versus-host disease after myeloablative conditioning and allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2005;11:495–505. doi:10.1016/j.bbmt.2005.03.006.
15. Przepiorka D, Ippoliti C, Khouri I, Woo M, Mehra R, Le Bherz D, et al. Tacrolimus and minidose methotrexate for prevention of acute graft-versus-host disease after matched unrelated donor marrow transplantation. Blood. 1996;88:4383–9.
16. Wong R, Giralt SA, Martin T, Couriel DR, Anagnostopoulos A, Hosing C, et al. Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years. Blood. 2003;102:3052–9. doi:10.1182/blood-2003-03-0855.
17. Tanabe M, Todo S, Murase N, Irish W, Miyazawa H, Fujisaki S, et al. Therapeutic synergism between low-dose FK506 and antimetabolites in rat allogeneic heart transplantation. Transplant Proc. 1995;27:364–5.
18. Eckhoff DE, McGuire BM, Frenette LR, Contreras JL, Hudson SL, Bynon JS. Tacrolimus (FK506) and mycophenolate mofetil combination therapy versus tacrolimus in adult liver transplantation. Transplantation. 1998;65:180–7. doi:10.1097/00007890-199801270-00006.
19. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15:825–8.
20. Sullivan KM, Agura E, Anasetti C, Appelbaum FR, Badger C, Bearman S, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. Semin Hematol. 1991;28:250–9.
21. Cheson BD, Cassileth PA, Head DR, Schiffer CA, Bennett JM, Bloomfield CD, et al. Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. J Clin Oncol. 1990;8:813–9.
22. Cheson BD, Bennett JM, Kantarjian H, Pinto A, Schiffer CA, Nimer SD, et al. Report of an international working group to standardize response criteria for myelodysplastic syndrome. Blood. 2000;96:3671–4.
23. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, et al. Report of an International workshop to standardize response criteria for non-Hodgkin's lymphomas NCI sponsored International Working Group. J Clin Oncol. 1999;17:1244–53.

24. Bladé J, Samson D, Reece D, Apperley J, Björkstrand B, Gahrton G, et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and hematopoietic stem cell transplantation. Br J Haematol. 1998;102:1115–23. doi:10.1046/j.1365-2141.1998.00930.x.
25. Yamada Y, Tomonaga M, Fukuda H, Hanada S, Utsunomiya A, Tara M, et al. A new G-CSF-supported combination chemotherapy, LSG15, for adult T-cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. Br J Haematol. 2001;113:375–82. doi:10.1046/j.1365-2141.2001.02737.x.
26. Sakaida E, Nakaseko C, Harima A, Yokota A, Cho R, Saito Y, et al. Late-onset noninfectious pulmonary complications after allogeneic stem cell transplantation are significantly associated with chronic graft-versus-host disease and with the graft-versus-leukemia effect. Blood. 2003;102:4236–42. doi:10.1182/blood-2002-10-3289.
27. Morishima Y, Sasazuki K, Inoko H, Juji T, Akaza T, Yamamoto K, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. Blood. 2002;99:4200–6. doi:10.1182/blood.V99.11.4200.
28. Kitawaki T, Kadowaki N, Ishikawa T, Ichinohe T, Uchiyama T. Compromised recovery of natural interferon-α/β-producing cells after allogeneic hematopoietic stem cell transplantation complicated by acute graft-versus-host disease and glucocorticoid administration. Bone Marrow Transplant. 2003;32:187–94. doi:10.1038/sj.bmt.1704093.
29. Saito T, Yoshioka S, Iinuma Y, Takakura S, Fujihara N, Ichinohe T, et al. Effects on spectrum and susceptibility patterns of isolates causing bloodstream infection by restriction of fluoroquinolone prophylaxis in a hematology–oncology unit. Eur J Clin Microbiol Infect Dis. 2008;27:209–16. doi:10.1007/s10096-007-0428-8.
30. Gooley TA, Leisenring W, Crowley JA, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representation of old estimators. Stat Med. 1999;18:665–706. doi:10.1002/(SICI)1097-0258(19990330)18:6<695::AID-SIM60>3.0.CO;2-O.
31. Körbling M, Anderlini P. Peripheral blood stem cell versus bone marrow allotransplantation: does the source of hematopoietic stem cells matter? Blood. 2001;98:2900–8. doi:10.1182/blood.V98.10.2900.
32. Dey BR, Shaffer J, Yee AJ, McAfee S, Caron M, Power K, et al. Comparison of outcomes after transplantation of peripheral blood stem cells versus bone marrow following an identical nonmyeloablative conditioning regimen. Bone Marrow Transplant. 2007;40:19–27. doi:10.1038/sj.bmt.1705688.
33. Storb R, Yu C, Wagner JL, Deeg HJ, Nash RA, Kiem HP, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. Blood. 1997;89:3048–54.
34. Koh LP, Chen CS, Tai BC, Hwang WY, Tan LK, Ng HY, et al. Impact of postgrafting immunosuppressive regimens on nonrelapse mortality and survival after nonmyeloablative allogeneic hematopoietic stem cell transplant using the fludarabine and low-dose total-body irradiation 200-cGy. Biol Blood Marrow Transplant. 2007;13:790–805. doi:10.1016/j.bbmt.2007.03.002.
35. Ozawa S, Nakaseko C, Nishimura M, Maruta A, Cho R, Ohwada C, et al. Chronic graft-versus-host-disease after allogeneic bone marrow transplantation from an unrelated donor: incidence, risk factors and association with relapse. A report from the Japan Marrow Donor Program. Br J Haematol. 2007;137:142–51. doi:10.1111/j.1365-2141.2007.06543.x.
36. Hambach L, Stadler M, Dammann E, Ganser A, Hertenstein B. Increased risk of complicated CMV infection with the use of mycophenolate mofetil in allogeneic stem cell transplantation. Bone Marrow Transplant. 2002;29:903–6. doi:10.1038/sj.bmt.1703583.
37. Baudard M, Vincent A, Moreau P, Kergueris MF, Harousseau JL, Milpied N, et al. Mycophenolate mofetil for the treatment of acute and chronic GVHD is effective and well tolerated but induces a high risk of infectious complications: a series of 21 BM or PBSC transplant patients. Bone Marrow Transplant. 2002;30:287–95. doi:10.1038/sj.bmt.1703633.
38. Giaccone L, McCune JS, Maris MB, Gooley TA, Sandmeier BM, Slattery JT, et al. Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. Blood. 2005;106:4381–8. doi:10.1182/blood-2005-06-2217.
39. Lange S, Mueller SC, Altmann S, Dahlhaus M, Drewelow B, Freund M, et al. Pharmacokinetics of oral mycopheolate mofetil in combination with CsA in dogs after nonmyeloablative allogeneic stem cell transplantation. Bone Marrow Transplant. 2008;41:667–74. doi:10.1038/sj.bmt.1705958.
40. Jacobson P, Green K, Rogosheske J, Brunstein C, Ebeling B, DeFor T, et al. Highly variable mycophenolate mofetil bioavailability following nonmyeloablative hematopoietic cell transplantation. J Clin Pharmacol. 2007;47:6–12. doi:10.1177/0091270006295064.
41. van Gelder T, Klupp J, Barten MJ, Christians U, Morris RE. Comparison of the effects of tacrolimus and cyclosporine on the pharmacokinetics of mycophenolic acid. Ther Drug Monit. 2001;23:119–28. doi:10.1097/00007691-200104000-00005.
42. Kagaya H, Miura M, Satoh S, Inoue K, Saito M, Inoue T, et al. No pharmacokinetic interactions between mycophenolic acid and tacrolimus in renal transplant recipients. J Clin Pharm Ther. 2008;33:193–201. doi:10.1111/j.1365-2710.2008.00906.x.

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LETTER TO THE EDITOR

Long-term survival after HLA-haploidentical SCT from noninherited maternal antigen-mismatched family donors: impact of chronic GVHD

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Allo-SCT from an HLA-haploidentical family donor has been the treatment of choice in patients with high-risk hematologic malignancies, who are expected to have a better prognosis with SCT but lack immediate access to a conventional stem cell source.[1,2] With intent to minimize the risk of severe GVHD, most haploidentical SCT protocols employ *ex vivo* or *in vivo* T-cell depletion, albeit at the expense of an increased risk of infection or relapse as a result of poor post-transplant immune reconstitution. To develop an alternative strategy to perform haploidentical SCT that confers improved immune recovery and acceptable risk of GVHD, we have explored the feasibility of T-cell-replete SCT from family donors mismatched for noninherited maternal HLA antigens (NIMA); NIMA-mismatched donor selection is based on the hypothesis that the detection of long-term maternal or fetal microchimerism in the donor's peripheral circulation is associated with immunological hyporesponsiveness against NIMA (in the case of NIMA mismatch in the graft-vs-host direction) or against inherited paternal HLA antigens (IPA) (in the case of NIMA mismatch in the host-vs-graft direction).[3] According to this scenario, we and other groups showed that T-cell-replete HLA-haploidentical SCT from a NIMA-mismatched family donor is feasible in selected patients with poor-risk hematologic malignancies.[3,4] However, late complications and long-term outcomes in patients undergoing such transplantations have been so far largely unknown. Therefore, we retrospectively studied the severity of chronic GVHD, requirement for immunosupressive treatment, and status of primary disease in long-term survivors who received T-cell-replete NIMA-mismatched haploidentical SCT.

We collected data on 16 consecutive patients who had survived more than 3 years after NIMA-mismatched SCT performed between January 2001 and July 2004 at 11 institutions that participated in our previous nationwide study (Table 1).[3] At the time of SCT, they had a median age of 19 years (range, 2–56) and received BM ($n=5$) or G-CSF-mobilized peripheral blood ($n=11$) as treatment for acute myeloid leukemia ($n=6$), acute lymphoblastic leukemia ($n=3$), chronic myeloid leukemia ($n=4$) and other B-cell neoplasms ($n=3$); 6 patients had a chemosensitive disease and 10 had a refractory disease. Early outcomes of 14 of these transplantations have been described elsewhere.[3–5] All patients received tacrolimus-based GVHD prophylaxis after myeloablative ($n=10$) or reduced-intensity conditioning ($n=6$). The type of donor was NIMA-mismatched sibling in 9 cases, mother in 6 and daughter in 1; all patient-donor pairs had two or three serologic mismatches at HLA-A, HLA-B and HLA-DR antigens in the graft-versus-host direction. The presence of long-term maternal or fetal microchimerism was detected in all donors through NIMA- or IPA-specific nested PCR as described earlier.[3] Karnofsky score was employed to record the performance status for patients who were 16 years or

Table 1 Characteristics of long-term survivors after NIMA-mismatched haploidentical SCT

Median age at transplant, years (range)	19 (2–56)
Sex	
Male	10
Female	6
Diagnosis	
Acute leukemia[a]	9
Chronic myeloid leukemia	4
Diffuse large B-cell lymphoma	1
Plasma cell myeloma	2
Disease status	
CR or chronic phase	6
Chemorefractory or blastic phase	10
Donor type	
NIMA-mismatched sibling	9
Mother	6
Daughter	1
No. of mismatched HLA antigens[b]	
Two antigens	10
Three antigens	6
Stem cell source	
BM	5
Peripheral blood	11
Conditioning	
Myeloablative	10
Reduced-intensity	6
GVHD prophylaxis	
Tacrolimus alone	1
Tacrolimus + MTX	13
Tacrolimus + MTX + corticosteroids	2
Acute GVHD	
None or grade 1	9
Grade 2	6
Grade 3	1

[a]One patient had secondary acute myeloid leukemia developed after treatment for acute lymphoblastic leukemia.
[b]The number of serologic mismatch at HLA-A, HLA-B and HLA-DR antigens in the graft-versus-host vector was shown.