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ORIGINAL ARTICLE

Impact of the presence of HLA 1-locus mismatch and the use of low-dose antithymocyte globulin in unrelated bone marrow transplantation

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HLA 1-locus-mismatched unrelated donors (1MMUD) have been used in allogeneic hematopoietic stem cell transplantation (allo-HCT) for patients who lack an HLA-matched donor. We retrospectively analyzed 3313 patients with acute leukemia or myelodysplastic syndrome who underwent bone marrow transplantation from an HLA allele-matched unrelated donor (MUD) or 1MMUD between 2009 and 2014. We compared the outcomes of MUD ($n=2089$) and 1MMUD with antithymocyte globulin (ATG) (1MM-ATG(+); $n=109$) with those of 1MMUD without ATG (1MM-ATG(−); $n=1115$). The median total dose of ATG (thymoglobulin) was 2.5 mg/kg (range 1.0–11.0 mg/kg) in the 1MM-ATG(+) group. The rates of grade III–IV acute GvHD, non-relapse mortality (NRM) and overall mortality were significantly lower in the MUD group than in the 1MM-ATG(−) group (hazard ratio (HR) 0.77, $P=0.016$; HR 0.74; $P<0.001$; and HR 0.87, $P=0.020$, respectively). Likewise, the rates of grade III–IV acute GVHD, NRM and overall mortality were significantly lower in the 1MM-ATG(+) group than in the 1MM-ATG(−) group (HR 0.42, $P=0.035$; HR 0.35, $P<0.001$; and HR 0.71, $P=0.042$, respectively). The outcome of allo-HCT from 1MM-ATG(−) was inferior to that of allo-HCT from MUD even in the recent cohort. However, the negative impact of 1MMUD disappeared with the use of low-dose ATG without increasing the risk of relapse.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HCT) from unrelated donors has been established as a curative therapy for various malignant and nonmalignant hematological disorders in the absence of an HLA-identical sibling. The development of high-resolution HLA typing and GvHD prophylaxis has greatly contributed to this success. The transplant outcomes from an HLA-A, -B, -C and -DRB1 allele-matched unrelated donor (MUD) are now comparable to those from an HLA-matched related donor.[1–3] However, it is difficult to find MUD for patients who have rare HLA haplotypes. Therefore, an HLA 1-locus-mismatched unrelated donor (1MMUD) has been considered as an alternative donor in allo-HCT when an HLA-matched related or unrelated donor is unavailable. However, the outcome has been shown to be inferior to that of allo-HCT from MUD mainly because of higher rates of GvHD and graft failure.[4–7] Therefore, an effective intervention is necessary to overcome this drawback.

Antithymocyte globulin (ATG) has been used as part of conditioning in allo-HCT to decrease the incidences of acute and chronic GvHD. Some previous studies reported that the use of ATG significantly reduced the incidence of GvHD,[8–14] but a high dose of ATG increased the risks of relapse and infections.[9,15–17] Unfortunately, the optimal dose of ATG remains unclear. The optimal ATG dose should be determined based on a fine balance between the reduction of GvHD and the increase in relapse and infections. In addition, the optimal ATG dose may differ according to the number of HLA mismatches, donor source, conditioning regimen, disease type, disease stage and race. Therefore, the aims of the current study were to compare the transplant outcomes among 1MMUD without ATG, 1MMUD with ATG and MUD in allogeneic bone marrow transplantation (BMT) and to evaluate the effectiveness of ATG in BMT from 1MMUD using a recent cohort. Finally, we investigated the optimal dose of ATG for Japanese patients with acute leukemia or myelodysplastic syndrome who received BMT from 1MMUD.

MATERIALS AND METHODS

Patients

Patients aged at least 16 years with AML, ALL or myelodysplastic syndrome who underwent a first BMT from MUD or 1MMUD (one allele or Ag mismatch in the graft-versus-host direction) through the Japan Marrow

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Donor Program between 2009 and 2014 were included in this study. Clinical data for these patients were obtained from the Transplant Registry Unified Management Program (TRUMP),[18,19] which includes clinical data of HCT performed in Japan. Of the 3404 patients who fulfilled these selection criteria, the following patients were excluded: 56 patients who received ATG in BMT from MUD; 16 patients who received stem cells manipulated by *ex vivo* T-cell depletion or CD34 selection; 1 patient who lacked data on survival status; and 18 patients who lacked data on the brand or dose of ATG, or who received any brand of ATG other than thymoglobulin (Sanofi, Paris, France), which is the only brand of ATG approved for GvHD prophylaxis under the Japanese National Health Insurance system. Finally, 1115 patients who underwent BMT from 1MMUD without ATG (1MM-ATG (−)), 109 patients who did so from 1MMUD with ATG (1MM-ATG(+)) and 2089 patients who did so from MUD were included in the study. This study was planned by the HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation and approved by the data management committees of TRUMP and the Institutional Review Board of Saitama Medical Center, Jichi Medical University.

Histocompatibility

Histocompatibility data for serological and genomic typing for the HLA-A, HLA-B, HLA-C and HLA-DR loci were obtained from the TRUMP database, which includes HLA allele data that were determined retrospectively by Japan Marrow Donor Program using frozen samples.[20,21] An HLA mismatch in the graft-versus-host direction was defined as when the recipient's Ags or alleles were not shared by the donor, and an HLA mismatch in the host-versus-graft direction was defined as when the donor's Ags or alleles were not shared by the recipient. In this study, patients who received BMT from an HLA 1MMUD in only the host-versus-graft direction were excluded.

End points and definitions

The primary end point was overall survival (OS) after BMT. Secondary end points were disease-free survival (DFS), GvHD-free, relapse-free survival (GRFS), and the cumulative incidences of neutrophil and platelet engraftment, acute and chronic GvHD, relapse and non-relapse mortality (NRM). Neutrophil recovery was defined as an absolute neutrophil count of at least 500 cells/mm^3 for 3 consecutive days after transplantation. Platelet recovery was defined as an absolute platelet count of at least 5×10^4 cells/mm^3 without platelet transfusion. Acute and chronic GvHD were graded according to previously published criteria.[22,23] The incidence of chronic GvHD was evaluated in patients who survived for at least 100 days. NRM was defined as death without relapse, DFS was defined as survival without disease progression or relapse, and GRFS was defined as survival without grade III–IV acute GvHD, chronic GvHD requiring systemic treatment, relapse or death from any cause.[24] We classified the conditioning regimen as either myeloablative or reduced intensity according to the operational definitions of the National Marrow Donor Program/Center for International Blood and Marrow Transplant Research.[25] Acute leukemia in first or second remission, and myelodysplastic syndrome excluding refractory anemia with excess blasts or leukemic transformation were defined as standard-risk diseases, and others were defined as high-risk diseases.

Statistical analysis

Categorical variables were compared between groups with the χ^2-test or Fisher's exact test, and continuous variables were compared with the Kruskal–Wallis test. The probabilities of DFS, GRFS and OS were estimated according to the Kaplan–Meier method, and compared among groups with the log-rank test. The probabilities of neutrophil and platelet engraftment, acute and chronic GvHD, relapse and NRM were estimated on the basis of cumulative incidence methods, and compared among groups with the Gray test, considering death without engraftment as a competing event for neutrophil and platelet engraftment, death or relapse without GvHD as a competing event for acute and chronic GvHD, death without relapse as a competing event for relapse, and relapse as a competing event for NRM.[26,27] Multivariate analyses for DFS, GRFS and OS were performed using the Cox proportional hazards model, whereas multivariate analyses for acute and chronic GvHD, relapse and NRM were performed using the Fine and Gray regression model.[28] The following variables were considered; the patient's age at transplantation ($\leqslant$50 years or $>$50 years), patient sex, disease type (AML, ALL or myelodysplastic syndrome), disease risk (standard risk or high risk), Eastern Cooperative Oncology Group Performance Status (0–1 or 2–4), HCT-Specific Comorbidity Index (0, 1–2 or $\geqslant$3), intensity of the conditioning regimen (myeloablative or reduced intensity), GvHD prophylaxis (cyclosporine-based or tacrolimus-based), year of transplantation (2009–2011 or 2012–2014) and donor type (MUD, 1MM-ATG(−) or 1MM-ATG(+)). Factors other than donor type were deleted from the model in a stepwise manner to exclude factors with a *P*-value of 0.05 or higher. All *P*-values were two-sided and *P*-values of 0.05 or less were considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University),[29,30] which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.0.2, Vienna, Austria). More precisely, it is a modified version of R commander (version 2.0-3) that was designed to add statistical functions that are frequently used in biostatistics.

RESULTS

Patient characteristics

The characteristics of the patients in each group are shown in Table 1. The median ages of the recipients at transplantation were 49 (16–74), 53 (16–71) and 50 (16–77) years in the 1MM-ATG(−), 1MM-ATG(+) and MUD groups, respectively. The proportion of patients with performance status 2–4 in the 1MM-ATG(+) group (16.5%) was significantly higher than those in the 1MM-ATG(−) (7.5%) and MUD groups (6.5%). On the other hand, there were no significant differences among the three groups with respect to recipient sex, disease, disease risk, HCT-Specific Comorbidity Index or conditioning regimen. In the 1MM-ATG(+) group, 64 (5.7%), 12 (1.1%), 328 (29.4%) and 711 (63.8%) patients underwent single HLA-A, HLA-B, HLA-C and HLA-DR Ag-/allele-mismatched BMT, respectively, whereas 8 (7.3%), 3 (2.8%), 17 (15.6%) and 81(74.3%) patients in the 1MM-ATG(−) group, respectively (Supplementary Table 1). The median total dose of ATG (thymoglobulin) in the 1MM-ATG(+) group was 2.5 mg/kg (range 1.0–11.0 mg/kg) and about 96% of patients received ATG at a total dose of 5.0 mg/kg or lower.

OS, DFS and GRFS

The 3-year unadjusted OS, DFS and GRFS rates were 52.4% (95% CI, 49.0–55.6%), 47.3% (95% CI, 44.0–50.6%) and 28.2% (95% CI, 25.2–31.2%) in the 1MM-ATG(−) group; 56.3% (95% CI, 44.2–66.8%), 60.2% (95% CI, 49.1–69.6%) and 38.9% (95% CI, 28.3–49.4%) in the 1MM-ATG(+) group; and 55.7% (95% CI, 53.2–58.0%), 51.6% (95% CI, 49.2–54.0%) and 33.0% (95% CI, 30.8–35.2%) in the MUD group ($P=0.020$, Figure 1a; $P=0.0092$, Figure 1b; and $P=0.0019$, Figure 1c). In the multivariate analysis, OS, DFS and GRFS in the MUD group were significantly superior to those in the 1MM-ATG(−) group (HR 0.87, 95% CI, 0.78–0.98, $P=0.020$; HR 0.88, 95% CI, 0.79–0.98, $P=0.021$; and HR 0.89, 95% CI, 0.81–0.97, $P=0.0088$, respectively; Table 2). Likewise, OS, DFS and GRFS in the 1MM-ATG(+) group were also significantly superior to those in the 1MM-ATG(−) group (HR 0.71, 95% CI, 0.50–0.99, $P=0.042$; HR 0.67, 95% CI, 0.48–0.94, $P=0.021$; and HR 0.73, 95% CI, 0.56–0.96, $P=0.023$, respectively; Table 2).

NRM and relapse

The cumulative incidence of NRM at 3 years in the 1MM-ATG(−) group (26.6%; 95% confidence interval (CI), 23.7–29.5) was significantly higher than those in the 1MM-ATG(+) (11.9%; 95% CI, 5.9–20.2) and MUD groups (21.4%; 95% CI, 19.5–23.4; $P<0.001$; Figure 1d). A multivariate analysis confirmed that the risks of NRM in the 1MM-ATG(+) (HR, 0.35; 95% CI, 0.19–0.65; $P<0.001$) and MUD groups (HR, 0.74; 95% CI, 0.63–0.87; $P<0.001$) were lower than that in the 1MM-ATG(−) group (Table 2). On the other hand, there were no significant differences in relapse rate (Figure 1e), and the relapse risk was comparable among the three groups (Table 2). The relapse rate tended to be higher in the 1MM-ATG(+) group among standard-risk patients, but did not significantly differ among the three groups among high-risk patients (Supplementary Figure 1). The causes of death are summarized

Table 1. Patient characteristics

Variable	*1MM-ATG(-)* [a] (n = *1115)*	*1MM-ATG(+)* [a] (n = *109)*	*MUD* [a] (n = *2089)*	P *value*
Age, median (range)	49 (16-74)	53 (16-71)	50 (16-77)	0.080
Recipient sex (%)				
Female	461 (41.3)	45 (41.3)	848 (40.6)	0.92
Male	654 (58.7)	64 (58.7)	1241 (59.4)	
Disease (%)				
AML	618 (55.4)	57 (52.3)	1160 (55.5)	0.54
ALL	290 (26.0)	25 (22.9)	515 (24.7)	
MDS	207 (18.6)	27 (24.8)	414 (19.8)	
Disease risk (%)				
Standard risk	684 (61.3)	73 (67.0)	1347 (64.5)	0.14
High risk	429 (38.5)	35 (32.1)	737 (35.3)	
Unknown	2 (0.2)	1 (0.9)	5 (0.2)	
ECOG PS (%)				
0-1	1029 (92.3)	91 (83.5)	1956 (93.6)	< 0.001
2-4	84 (7.5)	18 (16.5)	130 (6.2)	
Unknown	2 (0.2)	0 (0.0)	3 (0.1)	
HCT-CI (%)				
0	679 (60.9)	60 (55.0)	1268 (60.7)	0.72
1-2	277 (24.8)	29 (26.6)	510 (24.4)	
$\geqslant 3$	157 (14.1)	20 (18.3)	308 (14.7)	
Unknown	2 (0.2)	0 (0.0)	3 (0.1)	
Conditioning regimen (%)				
Myeloablative	817 (73.3)	73 (67.0)	1540 (73.7)	0.29
BU+CY ±	144	7	242	
CY+TBI ±	449	29	794	
Other TBI regimens	35	2	68	
Other non-TBI regimens	189	35	436	
Reduced-intensity	296 (26.5)	36 (33.0)	546 (26.1)	
FLU+BU ±	138	17	238	
FLU+MEL ±	133	15	270	
FLU+CY ±	4	3	10	
Other RIC regimens	21	1	28	
Unknown	2 (0.2)	0 (0.0)	3 (0.1)	
GVHD prophylaxis (%)				
CSA with MTX	119 (10.7)	12 (11.0)	310 (14.8)	0.0011
CSA without MTX	3 (0.3)	2 (1.8)	8 (0.4)	
TAC with MTX	926 (83.0)	89 (81.7)	1610 (77.1)	
TAC without MTX	41 (3.7)	3 (2.8)	122 (5.8)	
Others/Unknown	26 (2.3)	3 (2.8)	39 (1.9)	
Year of transplantation (%)				
2009-2011	499 (44.8)	33 (30.3)	957 (45.8)	0.0056
2012-2014	616 (55.2)	76 (69.7)	1132 (54.2)	

Abbreviations: ATG = antithymocyte globulin; BU = busulfan; CSA = cyclosporine; CY = cyclophosphamide; ECOG PS = Eastern Cooperative Oncology Group Performance Status; FLU = fludarabine; HCT-CI = Hematopoietic Cell Transplantation-Specific Comorbidity Index; MDS = myelodysplastic syndrome; MEL = melphalan; RIC = reduced-intensity conditioning; TAC = tacrolimus. [a]HLA compatibility was defined according to HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci.

in Supplementary Table 2. Disease relapse was the most common cause of death, and accounted for 31%, 44% and 40% of all deaths in the 1MM-ATG(−), 1MM-ATG(+) and MUD groups, respectively. GvHD and infections accounted for 10% and 22%, 3% and 15%, and 8% and 18% of all deaths in the 1MM-ATG(−), 1MM-ATG(+) and MUD groups, respectively.

Engraftment and GvHD

The cumulative incidences of neutrophil engraftment at day 50 and platelet engraftment at day 150 in the MUD group (96.7%, 95% CI, 95.8–97.4%; and 80.4%, 95% CI, 78.6–82.0%) were both higher than those in the 1MM-ATG(−) group (95.0%, 95% CI, 93.5–96.1%; and 70.9%, 95% CI, 68.1–73.5%) or the 1MM-ATG(+) group (93.6%, 95% CI, 86.6–97.0%; and 75.3%, 95% CI, 65.9–82.4%; Figures 2a and b), although there was no statistically significant difference in the cumulative incidence of neutrophil engraftment between the 1MM-ATG(+) and MUD groups. There were no significant differences in chimerism after allo-HCT between the 1MM-ATG(−) and 1MM-ATG(+) groups (702 and 83 in the complete donor chimerism, 115 and 8 in dominant donor chimerism ($\geqslant$80%), 70 and 9 in mixed chimerism, 5 and 1 in the autologous recovery, and 223 and 8 in others/unknown, respectively, $P = 0.40$).

The cumulative incidences of grade II–IV and III–IV acute GvHD at 100 days were 44.5% (95% CI, 41.5– 47.4%) and 13.1% (95% CI,

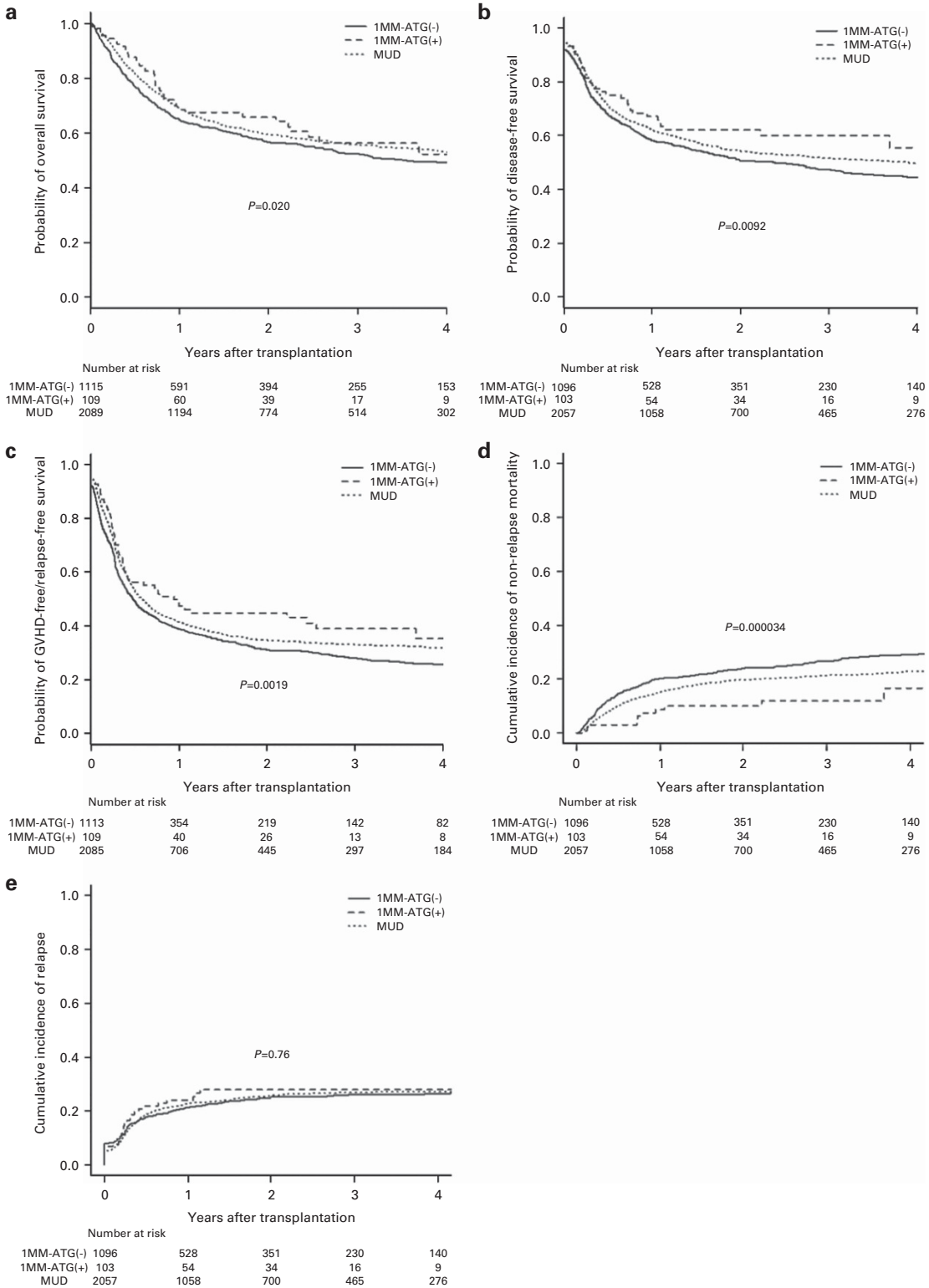

Figure 1. Probability of OS (**a**), DFS (**b**) GRFS (**c**) and cumulative incidences of non-relapse mortality (**d**) and relapse (**e**) in the 1MM-ATG(−), 1MM-ATG(+) and MUD groups.

11.2–15.2%) in the 1MM-ATG(−) group, 33.4% (95% CI, 24.5–42.5%) and 5.8% (95% CI, 2.4–11.6%) in the 1MM-ATG(+) group, and 36.1% (95% CI, 34.1–38.2%) and 10.5% (95% CI, 9.2–11.9%) in the MUD group ($P < 0.001$ and $P = 0.0085$; Figures 3a and b). The risks of grade II–IV and III–IV acute GvHD in the 1MM-ATG(+) (hazard ratio (HR), 0.69; 95% CI, 0.49–0.98; $P = 0.040$; and HR, 0.42; 95% CI, 0.19–0.94; $P = 0.035$) and MUD groups (HR, 0.75; 95% CI, 0.67–0.84; $P < 0.001$; and HR, 0.77; 95% CI, 0.63–0.95; $P = 0.016$) were significantly lower than those in the 1MM-ATG(−) group, after adjusting for other significant factors in multivariate analyses (Table 2). The cumulative incidences of chronic and extensive chronic GvHD at 3 years were 38.1% (95% CI, 34.8–

Table 2. Results of a multivariate analysis of outcomes

	HR (95% CI)	P-*value*
Overall survival[a]		
1MM-ATG(−)	1	Reference
1MM-ATG(+)	0.71 (0.50–0.99)	0.042
MUD	0.87 (0.78–0.98)	0.020
Disease-free survival[b]		
1MM-ATG(−)	1	Reference
1MM-ATG(+)	0.67 (0.48–0.94)	0.021
MUD	0.88 (0.79–0.98)	0.021
GvHD-free/relapse-free survival[c]		
1MM-ATG(−)	1	Reference
1MM-ATG(+)	0.73 (0.56–0.96)	0.023
MUD	0.89 (0.81–0.97)	0.0088
Non-relapse mortality[d]		
1MM-ATG(−)	1	Reference
1MM-ATG(+)	0.35 (0.19–0.65)	0.00081
MUD	0.74 (0.63–0.87)	0.00021
Relapse[e]		
1MM-ATG(−)	1	Reference
1MM-ATG(+)	1.22 (0.80–1.88)	0.35
MUD	1.09 (0.94–1.27)	0.25
II–IV acute GvHD[f]		
1MM-ATG(−)	1	Reference
1MM-ATG(+)	0.69 (0.49–0.98)	0.040
MUD	0.75 (0.67–0.84)	< 0.0001
III–IV acute GvHD[g]		
1MM-ATG(−)	1	Reference
1MM-ATG(+)	0.42 (0.19–0.94)	0.035
MUD	0.77 (0.63–0.95)	0.016
Chronic GvHD[h]		
1MM-ATG(−)	1	Reference
1MM-ATG(+)	0.87 (0.59–1.29)	0.49
MUD	0.98 (0.86–1.13)	0.82
Extensive chronic GvHD[i]		
1MM-ATG(−)	1	Reference
1MM-ATG(+)	0.75 (0.43–1.31)	0.31
MUD	1.02 (0.85–1.22)	0.83

Abbreviations: ATG = antithymocyte globulin; CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group Performance Status; HR = hazard ratio; HCT-CI = Hematopoietic Cell Transplantation-Specific Comorbidity Index; MUD = matched unrelated donor. [a]Other significant variables were recipient age more than 50 years, recipient sex, disease, disease risk, ECOG PS, HCT-CI and GvHD prophylaxis. [b]Other significant variables were recipient age more than 50 years, disease, disease risk, ECOG PS, HCT-CI and GvHD prophylaxis. [c]Other significant variables were recipient age more than 50 years, recipient sex, disease, disease risk, ECOG PS, HCT-CI and GvHD prophylaxis. [d]Other significant variables were recipient age more than 50 years, recipient sex, disease, ECOG PS, HCT-CI and GvHD prophylaxis. [e]Other significant variables were disease, disease risk, ECOG PS and HCT-CI. [f]Other significant variables were disease and ECOG PS. [g]Other significant variables were disease and disease risk. [h]Another significant variable was disease risk. [i]No other significant variables were identified.

41.4%) and 21.5% (95% CI, 18.8–24.4%) in the 1MM-ATG(−) group; 34.0% (95% CI, 24.1–44.0%) and 16.3% (95% CI, 9.3–25.0%) in the 1MM-ATG(+) group; and 38.4% (95% CI, 36.1–40.8%) and 22.2% (95% CI, 20.2–24.2%) in the MUD group ($P = 0.85$ and 0.44; Figures 3 and d). In a multivariate analysis, no significant difference was found among the three groups (Table 2).

Impact of ATG dose in the 1MMUD-ATG(+) group

Next, we divided patients in the 1MM-ATG(+) group into three groups according to the total dose of ATG (b: < 2 mg/kg; c: 2–4 mg/kg; and d: > 4 mg/kg), and compared the outcomes in the three groups with those in the 1MM-ATG(−) group (a: 0 mg/kg). The patient characteristics of these groups are shown in Supplementary Table 3. The median total dose of ATG was 1.0 mg/kg (range 1.0–1.5 mg/kg), 2.5 mg/kg (range 2.0–3.75mg/kg) and 5.0 mg/kg (range 5.0–11.0 mg/kg) in the b: < 2mg/kg, c: 2–4 mg/kg and d: > 4 mg/kg groups, respectively. The cumulative incidences of grade III–IV acute GvHD at 100 days and extensive chronic GvHD at 2 years were 13.1% (95% CI, 11.2–15.2%) and 21.1% (95% CI, 18.5–23.9%) in the a: 0 mg/kg group; 5.3% (95% CI, 0.3–22.0%) and 52.1% (95% CI, 23.4–74.6%) in the b: < 2 mg/kg group; 3.8% (95% CI, 0.7–11.6%) and 6.8% (95% CI, 1.7–16.8%) in the c: 2–4 mg/kg group; and 9.7% (95% CI, 2.4–23.2%) and 11.8% (95% CI, 2.8–28.0%) in the d: > 4 mg/kg group (Figures 4a and b). The incidence of NRM at 2 years and the rate of 1-year GRFS were 24.1% (95% CI, 21.5–26.8%) and 38.8% (95% CI, 35.9–41.8%) in the a: 0 mg/kg group; 16.1% (95% CI, 3.7–36.3%) and 28.3% (95% CI, 10.4–49.6%) in the b: < 2 mg/kg group; 6.4% (95% CI, 1.6–16.0%) and 52.6% (95% CI, 38.4–64.9%) in the c: 2–4 mg/kg group; and 12.7% (95% CI, 3.0–29.6%) and 49.4% (95% CI, 30.2–66.0%) in the d: > 4 mg/kg group ($P = 0.014$ and 0.055; Figures 4d and e).

DISCUSSION

This study showed that OS in the 1MM-ATG(−) group was inferior to that in the MUD group even in a recent cohort (2009–2014). The difference in OS between the two groups was mainly due to the higher NRM in the 1MM-ATG(−) group, which was most likely associated with a higher incidence of acute GvHD. On the other hand, the risks of acute GvHD and NRM in the 1MM-ATG(+) group were significantly lower than those in the 1MM-ATG(−) group without an increase in relapse, although there was no statistically significant difference in chronic GvHD between the two groups. Accordingly, OS in the 1MM-ATG(+) group was significantly superior to that in the 1MM-ATG(−) group. In addition, most transplant outcomes in the 1MM-ATG(+) group were comparable to those in the MUD group. In fact, in a multivariate analysis using the MUD group as a reference, no significant differences in OS, DFS, GRFS or relapse rate were found between the two groups, and NRM in the 1MM-ATG(+) group was superior to that in the MUD group (data not shown). Therefore, the appropriate use of ATG as part of conditioning for GvHD prophylaxis should be considered in BMT from 1MMUD.

Most previous studies have reported that the use of ATG reduced the incidence of acute GvHD and/or chronic GvHD.[8–14] On the other hand, several studies have noted the drawbacks of high doses of ATG, such as increased rates of relapse or infection.[9,15–17] In this study, however, the cumulative incidence of relapse and the proportion of infection-related mortality did not significantly differ between the 1MM-ATG(−) group and the 1MM-ATG(+) group. This difference might result from differences in the dose of ATG or differences in risk factors for GvHD and relapse, that is, the number of HLA mismatches, donor source, disease stage and so on. Finke *et al.*[10] reported that the addition of ATG-Fresenius (total dose 60 mg/kg) to standard GvHD prophylaxis with cyclosporin and methotrexate reduced the incidences of acute and chronic GvHD without increasing the risk of relapse in patients who underwent allo-HCT from MUD. Likewise, Walker *et al.*[31] showed that the use of thymoglobulin (total dose of 4.5 mg/kg) in allo-HCT from an MUD or 1MMUD markedly decreased both the need for immunosuppressive treatment and the symptoms of chronic GvHD in comparison with the no-ATG group. In these two large prospective, randomized trials, however,

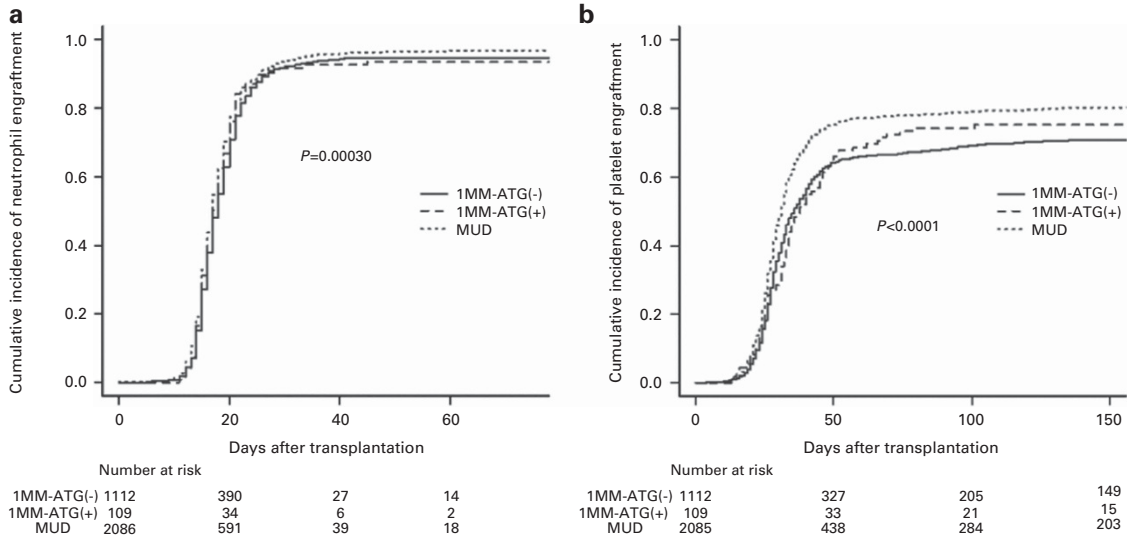

Figure 2. Cumulative incidences of neutrophil (**a**) and platelet engraftment (**b**) in the 1MM-ATG(−), 1MM-ATG(+) and MUD groups.

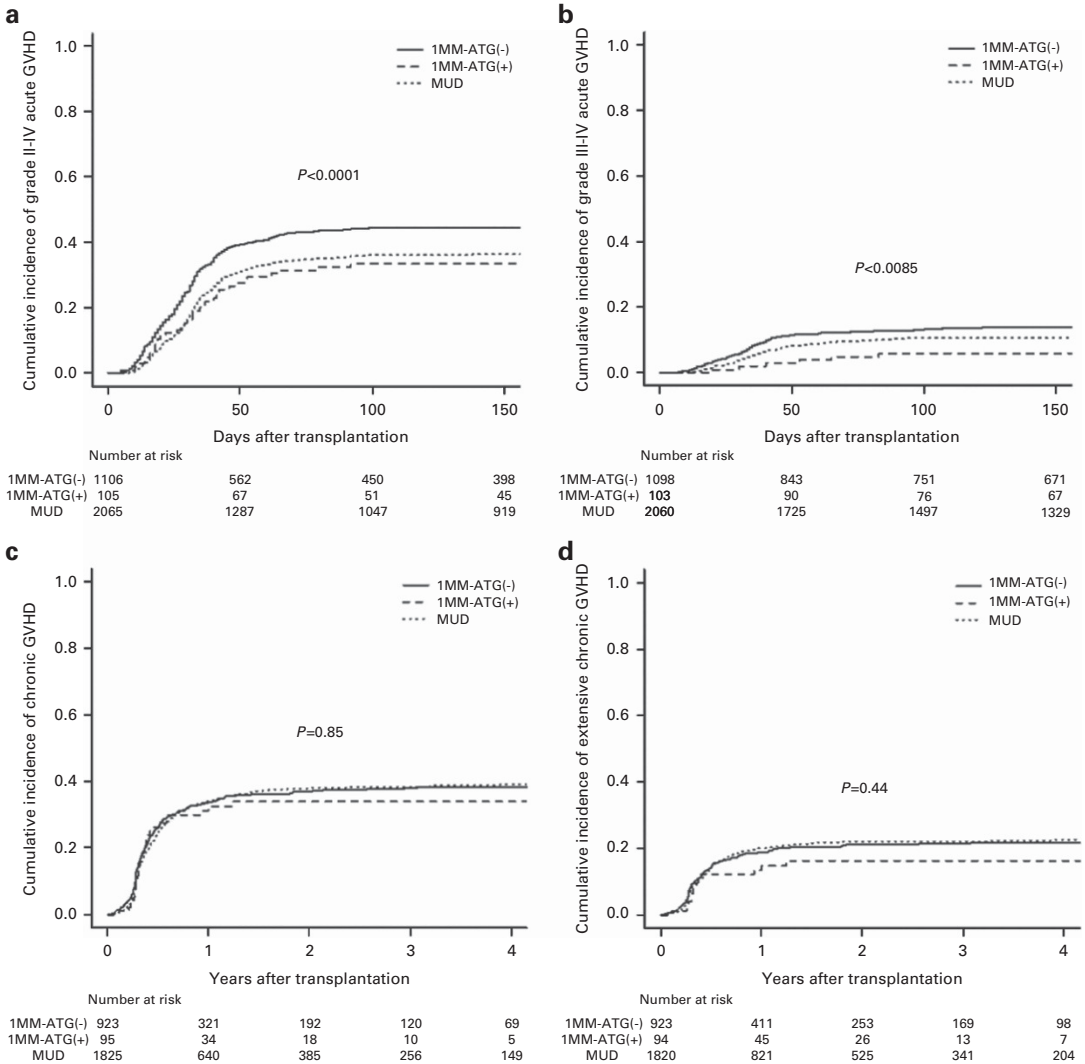

Figure 3. Cumulative incidences of grade II–IV acute GvHD (**a**), III–IV acute GvHD (**b**), chronic GvHD (**c**) and extensive chronic GvHD (**d**) in the 1MM-ATG(−), 1MM-ATG(+) and MUD groups.

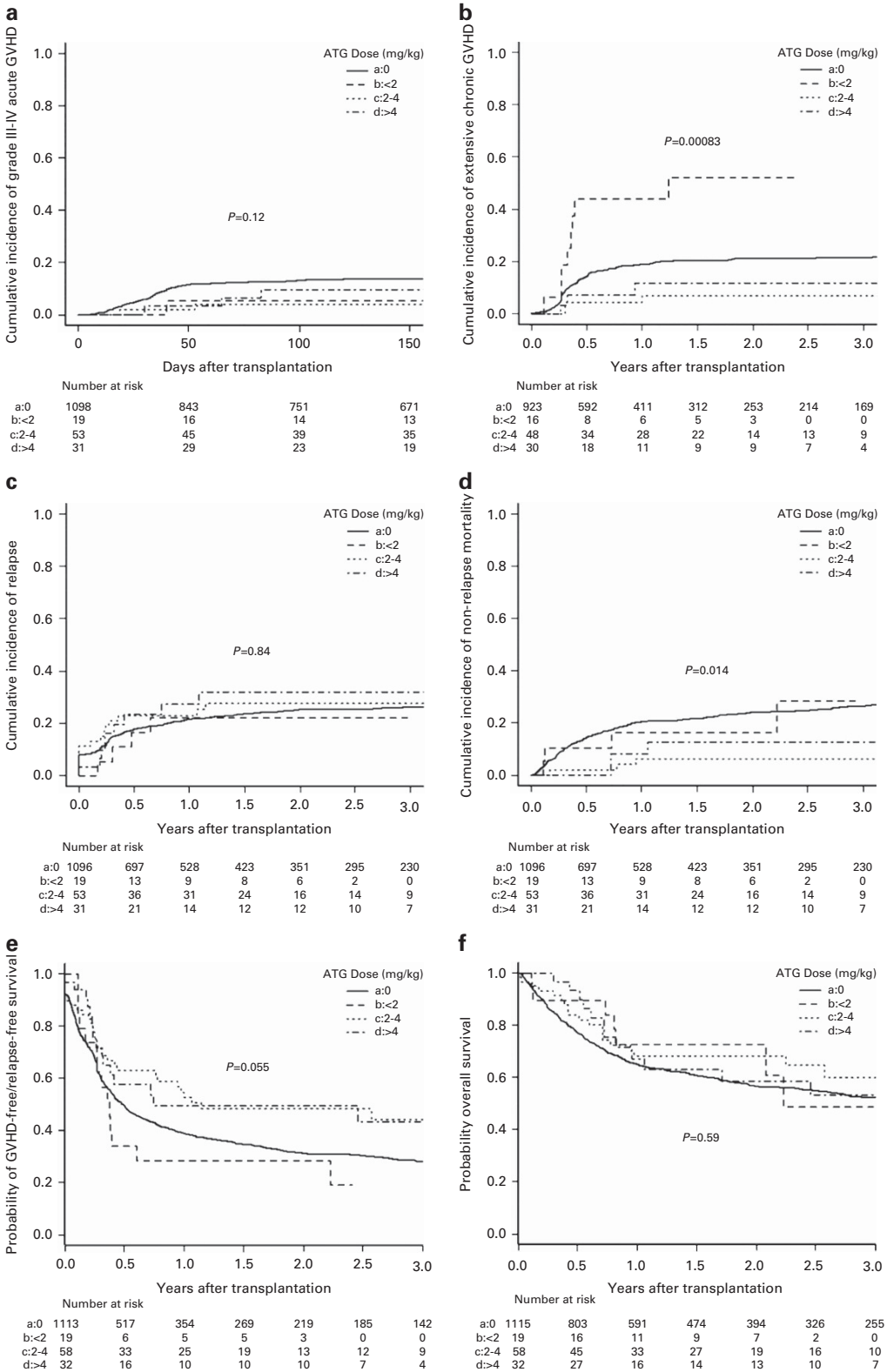

Figure 4. Comparison of ATG (thymoglobulin) dose in recipients of 1MMUD-GVH (a: ATG 0 mg/kg; b: ATG < 2 mg/kg; c: ATG 2–4 mg/kg; and d: ATG > 4 mg/kg); III–IV acute GvHD (**a**), extensive chronic GvHD (**b**), relapse (**c**), non-relapse mortality (**d**), GRFS (**e**) and OS (**f**).

there were no significant differences in OS between the ATG group and the no-ATG group, although the follow-up duration in the latter trial might have been insufficient for the evaluation of OS.

The current study suggested that the use of ATG might improve OS in patients who received BMT from 1MMUD. The major difference between this study and previous studies, other than the presence or absence of HLA mismatch, was the dose of ATG. In the current study, most patients received a relatively lower dose of ATG (median 2.5 mg/kg). As shown by the comparison of ATG doses (Figure 4), a total dose of 2–4 mg/kg might be sufficient to

prevent severe acute GvHD in BMT from 1MMUD, although it might be insufficient to decrease the incidence of chronic GvHD. Kim *et al.*[12] also reported that a low dose of thymoglobulin (2.5 mg/kg) significantly decreased acute GvHD and NRM in allo-HCT from HLA-mismatched unrelated donors in Korea. Their results were quite similar to our findings. In contrast, in a randomized trial from an Italian group Gruppo Italiano Trapianti di Midollo Osseo (GITMO), thymoglobulin at 7.5 mg/kg did not significantly reduce acute GvHD compared with standard GvHD prophylaxis in allo-HCT from an unrelated donor, whereas a higher dose (15 mg/kg) reduced severe acute GvHD but increased the risk of infection, although this study was performed before the establishment of current criteria of HLA matching.[9] Therefore, the suitable dose of ATG may be different between Asian and Caucasian patients. Several studies have shown that the incidence of GvHD in Asian patients is lower than that in Caucasian patients.[32–34] Thus, race should also be considered when we examine the optimal dose of ATG.

The reduction of chronic GvHD may improve the quality of life (QOL) of the patients, as severe chronic GvHD is the leading cause of late morbidity and mortality in long-term survivors. However, there was no statistically significant difference in the incidence of chronic GvHD between the 1MM-ATG(−) and 1MM-ATG(+) groups in this study. This might be because of the lower dose of ATG or because there was insufficient statistical power to detect a difference. A lower dose of ATG might be able to significantly inhibit the proliferation of T cells, but not B cells. In addition, several studies have reported that another possible benefit of ATG is the early discontinuation of immunosuppressive drugs,[14,31,35] which would also lead to improved QOL. A lower dose of ATG might allow patients to discontinue immunosuppressive drugs early even if there is no difference in the incidence of chronic GvHD, although we could not assess the duration of immunosuppressive drug use due to the lack of data. GRFS, which is considered to reflect QOL early after allo-HCT, in the 1MM-ATG(+) group was superior to that in the 1MM-ATG(−) group. Unfortunately, however, we could not precisely evaluate QOL because relevant data were not included in the database.

This study has several limitations. First, data on the timing of ATG infusion were unavailable. The duration between ATG infusion and allo-HCT may affect the degree of donor T-cell depletion and the incidence of GvHD. Second, data on cytogenetic or genetic abnormalities in hematological malignancies were insufficient, and therefore, it was impossible to accurately assess the impact of the use of ATG on relapse. Finally, there were fewer patients in the 1MM-ATG(+) group than in the other two groups, and therefore, the statistical power of the analysis of ATG dose was limited. In addition, according to the policy of the TRUMP database, we could not obtain information about the transplant center for each patient, and therefore, we could not directly assess a center effect. With regard to the factors that may be affected by the centers' policy, there were no significant differences in the use of G-CSF in AML patients or the brand of calcineurin inhibitors among the groups, and, therefore, we suppose that there was not a strong center effect. However, it was impossible to completely deny the presence of a center effect.

In conclusion, the outcome of allo-HCT from 1MM-ATG(−) was inferior to that of allo-HCT from MUD even in a recent cohort. However, the negative impact of 1MMUD disappeared with the use of low-dose ATG without increasing the risk of relapse. A large prospective study is warranted to confirm the role of low-dose ATG in allo-HCT from 1MMUD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

KK, JK and YK designed the study and performed the statistical analysis; KK, JK, SF, MM, K Ikegame and YK interpreted the data and wrote the manuscript; KY, TF, TH, YO, NU, YK, K Iwato, TS, MH and HH provided the patient data; TF, TI and YA collected the patient data; and all of the authors interpreted the data, and reviewed and approved the final manuscript.

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