Graft-versus-host disease prophylaxis after single-unit reduced intensity conditioning cord blood transplantation in adults with acute leukemia

Seitaro Terakura[1], Yachiyo Kuwatsuka[2], Satoshi Yamasaki[3], Atsushi Wake[4,5], Junya Kanda[6], Yoshihiro Inamoto[7], Shuichi Mizuta[8], Takuhiro Yamaguchi[9], Naoyuki Uchida[5,10], Yasuji Kouzai[11], Nobuyuki Aotsuka[12], Hiroyasu Ogawa[13], Heiwa Kanamori[14], Kaichi Nishiwaki[15], Shigesaburo Miyakoshi[16], Makoto Onizuka[17], Itsuto Amano[18], Takahiro Fukuda[7], Tatsuo Ichinohe[19], Yoshiko Atsuta[20,21], Makoto Murata[1], and Takanori Teshima[22] for The Japan Society for Hematopoietic Cell Transplantation GVHD working group

[1] Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan

[2] Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan

[3] Department of Hematology and Clinical Research Institute, National Hospital Organization Kyushu Medical Center, Fukuoka, Japan

[4] Department of Hematology, Toranomon Hospital Kajigaya, Kawasaki, Japan

[5] Okinaka Memorial Institute of Medical Research, Tokyo, Japan

[6] Department of Hematology and Oncology, Kyoto University Graduate School of Medicine, Kyoto, Japan

[7] Division of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan

[8] Department of Internal Medicine, National Hospital Organization Toyohashi Medical Center, Toyohashi, Japan

[9] Division of Biostatistics, Tohoku University Graduate School of Medicine, Sendai, Japan

[10] Department of Hematology, Toranomon Hospital, Tokyo, Japan

[11] Department of Transfusion Medicine, Tokyo Metropolitan Tama Medical Center, Fuchu, Japan

[12] Division of Hematology-Oncology, Japanese Red Cross Narita Hospital, Narita, Japan

[13] Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan

[14] Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan

[15] Division of Oncology and Hematology, Department of Internal Medicine, Jikei University School of Medicine, Kashiwa Hospital, Kashiwa, Japan

[16] Department of Hematology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan

[17] Department of Hematology and Oncology, Tokai University School of Medicine, Isehara, Japan

[18] The Second Department of Internal Medicine, Nara Medical University Hospital, Kashihara, Japan

[19] Department of Hematology and Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

[20] Japanese Data Center for Hematopoietic Cell Transplantation, Nagoya, Japan

[21] Department of Healthcare Administration, Nagoya University Graduate School of Medicine, Nagoya, Japan

[22] Department of Hematology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Correspondence: Seitaro Terakura, MD, PhD,

Department of Hematology and Oncology, Nagoya University Graduate School of Medicine,

65 Tsurumai, Showa-ku, Nagoya, Aichi 466-8560, Japan

Tel.: +81-52-744-2145

Fax: +81-52-744-2161

E-mail: tseit@med.nagoya-u.ac.jp

Running head: GVHD prophylaxis in reduced intensity UCBT

Word counts: 2940 words for the manuscript, 198 words for the abstract

Figures: 3; Tables: 4; References: 47

Supplemental Figure: 1; Supplemental Tables: 2

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

Key words

Graft-versus-host disease, GVHD prophylaxis, immunosuppressant selection, reduced intensity conditioning, single-unit umbilical cord blood transplantation

Abstract

To investigate better GVHD prophylaxis in reduced intensity conditioning umbilical cord blood transplantation (RIC-UCBT), we compared transplant outcomes after UCBT among GVHD prophylaxes using registry data. We selected patients transplanted for AML or ALL with a calcineurin inhibitor and methotrexate (MTX)/mycophenolate mofetil (MMF) combination. A total of 748 first RIC-UCBT between 2000 and 2012 (MTX+ group, 446, MMF+ group, 302) were included. The cumulative incidence of neutrophil and platelet counts higher than 50000/μl was significantly better in the MMF+ group (Relative risk [RR], 1.55; $P<0.001$: RR, 1.34; $P=0.003$, respectively). In multivariate analyses, the risk of grade II-IV and III-IV acute GVHD was significantly higher in the MMF+ group than in the MTX+ group (RR, 1.75; $P<0.001$: RR, 1.97; $P=0.004$, respectively). In disease-specific analyses of AML, the risk of relapse of high-risk disease was significantly lower in the MMF+ group (RR, 0.69; $P=0.009$), whereas no significant difference was observed in the risk for relapse-free and overall survival in high-risk disease. In patients with standard-risk disease, no significant differences were noted in the risk of relapse or survival between the MTX+ and MMF+ groups. Collectively, these results suggest that MMF-containing prophylaxis may be preferable in RIC-UCBT, particularly for high-risk disease.

Introduction

Umbilical cord blood transplantation (UCBT) has been established as an alternative donor source for hematopoietic stem cell transplantation.[1-6] Since a new combination for a reduced intensity conditioning (RIC) regimen and UCBT was introduced, transplant outcomes with RIC-UCBT have improved in recent years, and RIC-UCBT has been recognized as a reasonable alternative donor source to unrelated bone marrow (BM).[7, 8] As a part of conditioning and GVHD prophylaxis, anti-thymocyte globulin (ATG) has been used in Europe and US; however, the use of ATG in UCBT is reportedly associated with detrimental immunological recovery and worse survival outcomes.[9-14] Since the incidence of severe acute GVHD after UCBT is similar to that of matched unrelated donor transplantation,[15-17] the choice of GVHD prophylaxis is important for optimal transplant outcomes. In historical data on RIC-UCBT, a high incidence of pre-engraftment immunological reactions and subsequent GVHD was observed with single-agent GVHD prophylaxis using only a calcineurin inhibitor (CNI).[18, 19] The combination of methotrexate (MTX) or mycophenolate mofetil (MMF) with CNI has since been attempted. Comparative studies on CNI alone and CNI plus MTX or MMF demonstrated that both combinations of prophylaxis resulted in significant improvements in survival after UCBT.[20, 21] The standard GVHD prophylaxis in UCBT is a combination of CNI and MTX or MMF in Japan.[22, 23] However, a detailed comparison has not yet been conducted between MTX and MMF in RIC-UCBT; therefore, we performed the present study in order to compare the CNI plus MTX regimen with the CNI plus MMF regimen.

Subjects and Methods

Data collection and source

All transplantation data for the present study were obtained from the Transplant Registry Unified Management Program database.[24-26] Inclusion criteria for the present study were: 1) patients aged 16 years or older with AML or ALL, 2) first allogeneic transplant between 2000 and 2012, 3) RIC regimen, 4) UCB as the donor source, and 5) data for GVHD prophylaxis were available and GVHD prophylaxis consisted of CNI (tacrolimus [Tac] or cyclosporine A [CyA]) with either MTX or MMF. Exclusion criteria were patients with: 1) double-unit UCBT, 2) *in vivo* T-cell depletion with ATG, and 3) data missing regarding the survival status or the date of last contact. Conditioning intensities were classified as reported previously.[27] The retrospective study protocol was approved by the Institutional Review Board of Nagoya University Graduate School of Medicine, and written informed consent was obtained from each patient in accordance with the Declaration of Helsinki.

CB selection strategy

A common UCB unit selection strategy is to choose a UCB unit with total nucleated cell count > 2.0×10^7/kg recipient weight within a 2-loci mismatch among HLA-A, -B, and -DR loci at the antigen level. Among them, the UCB unit with the higher CD34 cell dose is typically selected.[28]

Definitions

Neutrophil recovery was defined as an absolute neutrophil count of at least 500/μl for three

consecutive time points. Platelet recovery was defined as a count of 50,000/μl without transfusion support. The diagnosis and clinical grading of acute and chronic GVHD were performed according to established criteria.[29, 30] Relapse was defined as the recurrence of the underlying hematological disease. Non-relapse mortality (NRM) was defined as death during continuous remission. Relapse-free survival (RFS) was defined as survival in a state of continuous remission.

Statistical analysis

All categorical variables such as patients, diseases, and transplantation characteristics were compared using χ^2 statistics, and all quantitative variables such as ages, weights, and cell doses were compared using Mann-Whitney U test. The probabilities of OS and RFS were calculated using the Kaplan-Meier survival estimate.[31] The probabilities of neutrophil and platelet recovery, acute and chronic GVHD, NRM, and relapse were calculated using the cumulative incidence estimate to consider competing risks.[32] Relapse was the competing risk for NRM, while that for relapse was NRM. Death without an event was the competing risk for hematopoietic recovery and acute and chronic GVHD. In the analysis of OS, death from any cause was considered an event. In the analysis of RFS, relapse or death from any cause was considered an event. The Log-rank test was used for group comparisons.

Cox's proportional hazards univariate and multivariate regression models were applied to identify significant risk factors for RFS and OS.[33] Competing risk regression models using Fine-Gay method were applied for NRM, relapse, and acute and chronic GVHD.[34] By utilizing risk factors, a final multivariate regression model was constructed to assess differences in the GVHD prophylaxis

methods at each endpoint. Multivariate models were built using a backward stepwise selection method with a threshold *P*-value less than 0.1. Results are expressed as relative risk (RR) with the 95% confidence interval (95%CI). The proportional hazards assumption was tested for all variables considered in the multivariate analysis, and no violations occurred. Regardless of the level of significance, the main variable of interest, GVHD prophylaxis (MTX-containing vs. MMF-containing), was considered in all steps of model construction. Other variables tested were patient age (continuous variable), patient sex (male vs. female), donor sex (male vs. female), the use of TBI (TBI-regimen vs. non-TBI regimen), conditioning regimen (Flu + Bu ± regimen vs. Flu + CY ± regimen vs. Flu + Mel ± regimen vs. others), HLA disparity in -A/B/DR loci (HLA serological mismatch equal to or more than 2 antigens vs. less than 2 antigens), disease status at transplantation (standard risk vs. high risk), and transplant period (before 2009 vs. 2010–2012). The standard risk for AML was defined as first and second CR, with all others being considered high risk. The standard risk for ALL included CR1 alone, with CR2, further CR, and non-remission being defined as high risk. Differences were considered significant when $P<0.05$. All statistical analyses were performed with Stata software version 12 (College Station, TX, USA) and EZR statistical software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[35]

Results

Patient and UCB graft characteristics

A total of 748 patients were included after the application of inclusion and exclusion criteria (Table 1). The median patient age was 60 years (range, 17-82 years); patient age was significantly older in the MMF+ group (median age: MTX+ group, 59; MMF+ group, 61; p<0.001, Mann-Whitney U test). The median patient weight was 55 kg (range, 34-88 kg). Transplantation year was not significantly different between MTX+ group and MMF+ group (p=0.71). Conditioning regimens mostly consisted of fludarabine and another cytotoxic agent with low-dose TBI. The median TBI dose was 2-4 Gy in each group. Approximately 60% of UCBT were 2/6 antigen mismatches among HLA-A/B/DR antigens. The median follow-up period for surviving patients was 26.1 months (range, 2.7-143.8 months).

Hematopoietic recovery

Neutrophil recovery was significantly better in the MMF+ groups (median days for recovery [cumulative incidence]: MTX+ group, 25 days [66.8%; 95%CI, 62.3-71.0]; MMF+ group, 21.5 days [81.7%; 95%CI, 76.8-85.6]; *P*<0.0001, Fine-Gray) (Figure 1a). In a multivariate competing risk regression analysis, the likelihood of recovery was significantly greater in the MMF+ group than in the MTX+ group (Table 2). Furthermore, platelet recovery of greater than 50,000/μl was significantly better in the MMF+ groups (median days for recovery [cumulative incidence]: MTX+ group, 45.5 days [51.4%; 95%CI, 46.7-56.0]; MMF+ group, 45.5 days [60.3%; 95%CI, 54.4-65.6]) (Figure 1b; *P*=0.001, Fine-Gray). The likelihood of recovery was significantly greater in the MMF+ group than in

the MTX+ group (Table 2). Conditioning regimen was not significant factor in the final model of multivariate competing risk regression analysis.

Acute and chronic GVHD

In multivariate analyses, the risk of grade II-IV acute GVHD was significantly higher in the MMF+ group than in the MTX+ group (Table 2). The cumulative incidence curve of grade II-IV acute GVHD was depicted for a dichotomous comparison between the MTX+ and MMF+ groups (Figure 2a).

In multivariate analyses, the risk of grade III-IV acute GVHD was higher in the MMF+ group than in the MTX+ group (Table 2). The cumulative incidence curve of grade III-IV acute GVHD was depicted for a dichotomous comparison between the MTX+ and MMF+ groups (Figure 2b).

The risk of developing extensive chronic GVHD was not significantly affected by the GVHD prophylaxis method in multivariate analyses using the competing risk regression model (Table 2). A similar RR was observed for the MTX+ and MMF+ groups (RR, 1.21; 95% CI [0.70-2.08]; *P*=0.50, the MTX+ group as a reference).

NRM

In multivariate analyses using the competing risk regression model, the risk of NRM in the MMF+ group was slightly higher compared with the MTX+ group in AML (RR; MMF+ group; 1.33, 95% CI [1.00-1.78], *P*=0.054; MTX+ group as a reference), whereas there was no significant difference between the MTX+ group and MMF+ group in ALL (RR; MMF+ group; 0.82, 95% CI [0.41-1.64], *P*=0.57; MTX+ group as a reference) (supplemental Table S1).

Relapse

In terms of relapse and survival, AML and ALL were analyzed separately in the multivariate competing risk regression model, because the disease was significant factor in the multivariate analyses when the disease factor (AML vs. ALL) was involved as a covariate. Disease status at transplantation (standard-risk vs. high-risk) had a significant impact as a primarily important factor for the risk of relapse (RR, 4.04, 95% CI [3.04-5.38], $P<0.0001$ for the MTX+ vs. MMF+ comparison). Therefore, we adopted risk-stratified analyses for the relapse and survival of AML and ALL (Table 3 and supplemental Table S2). In standard-risk AML, no significant differences were observed in the MTX+ vs. MMF+ comparison (Table 3). In high-risk AML, the risk of relapse was significantly lower in the MMF+ group than in the MTX+ group (Table 3). The cumulative incidence curve of relapse was shown for each GVHD prophylaxis group for standard-risk disease (MTX+, 19.0% [95% CI, 13.5-25.3%]; MMF+, 18.3% [95% CI, 11.2-26.8%]; $P=0.65$, Fine-Gray) (Figure 3a) and high-risk disease (MTX+, 56.8% [95% CI, 49.7-63.3%]; MMF+, 44.9% [95% CI, 36.9-52.5%]; $P=0.013$, Fine-Gray) (Figure 3b).

In multivariate analyses of ALL, no significant differences were observed in the risk of relapse among each categorized group or in the MTX+ vs. MMF+ group comparison (supplemental Table S2).

CNS complications and causes of death

In our previous study on GVHD prophylaxis in myeloablative UCBT, we observed a significant difference in the incidence of central nervous system (CNS) complications between GVHD prophylaxes, which was significantly higher after Tac plus MMF prophylaxis. In the present study, the incidences of CNS complications after RIC-UCBT were 31 out of 446 (7.0%) for the MTX group, 27

out of 302 (8.9%) for the MMF group (χ^2-test, P=0.32). No significant differences were noted in the incidence of CNS complications between the MTX+ and MMF+ groups. Although details on CNS complications were not obtained due to the lack of data, most of CNS complications would consist of HHV6 encephalitis syndrome, which reportedly associated with the incidence of acute GVHD[36]. Relapse was the leading cause of death after UCBT in the MTX and MMF groups, whereas no rejection/graft failure was observed in the MMF group (Table 4).

Survival

AML

In multivariate analyses using Cox's proportional hazard model, RFS was similar in the MTX+ vs. MMF+ group comparison for standard-risk disease (Table 3). In the high-risk disease, the risk of RFS was slightly lower in the MMF+ group than in the MTX+ group (Table 3). RFS was depicted by Kaplan-Meier estimates for standard-risk and high-risk disease. There was no significant difference between MTX+ and MMF+ group in standard-risk disease, whereas there was a tendency of superior RFS in the MMF+ group in the high-risk disease (Figure 3c, d).

In multivariate analyses, OS was similar in the MTX+ vs. MMF+ group comparison for standard-risk and high-risk disease (Table 3). OS was depicted by Kaplan-Meier estimates for standard-risk and high-risk disease, however there was no significant difference between MTX+ and MMF+ group (supplemental Figure S1).

ALL

In multivariate analyses of ALL, no significant difference was observed in the risk of RFS in the MTX+ vs. MMF+ group comparison for standard-risk disease (supplemental Table S2). Regarding high-risk disease, the MMF+ group was associated with a marginally lower risk of RFS than the MTX+ group (RR, 0.51; 95% CI [0.26-1.01]; *P*=0.053) (supplemental Table S2).

In multivariate analyses, OS was similar in the MTX+ vs. MMF+ group comparison for standard-risk disease (supplemental Table S2). In high-risk disease, the MMF+ group was associated with a significantly lower risk of OS (RR, 0.50; 95% CI [0.25-0.99]; *P*=0.048) (supplemental Table S2).

Discussion

In the present study, we investigated better GVHD prophylaxis methods after adult single-unit RIC-UCBT. As we already demonstrated in a previous study on myeloablative UCBT, CNI plus MTX prophylaxis showed a significantly lower incidence of severe GVHD.[37] However, in terms of relapse, CNI plus MMF prophylaxis was better, particularly for high-risk AML.[37] In contrast to the previous findings, the engraftment of neutrophils and platelets was significantly better in the MMF+ group than in the MTX+ group in the present study. Although MMF+ group was associated with the significantly lower relapse of high-risk disease, we did not observe significant differences for RFS and OS. We speculate that the reason for this was that the potential benefit of lower relapse may have been cancelled out by the marginally higher risk of NRM.

The cumulative incidence of neutrophil and platelet engraftment was significantly higher in the MMF+ group than in the MTX+ group. In a previous study on myeloablative UCBT, we did not observe significant differences in the cumulative incidence of engraftment between the MTX+ and MMF+ groups.[37] The reason why we observed this dissociation between myeloablative UCBT and RIC-UCBT may be as follows. Recipient lymphocytes are supposed to be more profoundly depleted by myeloablative conditioning than by the RIC setting, and recipient lymphocytes may survive RIC. These recipient lymphocytes may then initiate immunological rejection or inhibit engraftment, and potentially increase the incidence of graft failure.[38] Given the effects of MTX against proliferating lymphocytes, MTX+ prophylaxis may excessively suppress donor-derived lymphocytes, which are expected to facilitate engraftment.[39-41] The effects of facilitating cells may be important in the RIC

setting because the depletion of host-derived lymphocytes in the RIC setting may be incomplete and, thus, immunological competition between host- and donor-derived cells may also occur more frequently. On the other hand, MMF is given continuously preventing proliferation by inhibiting enzymes utilized by proliferating T- and B-lymphocytes.[42] Therefore, MMF does not kill facilitating cells, and it merely prevents proliferation. These differences in the mode of action may translate into the outcomes observed in the present study. Also the canine experiments of Seattle group demonstrated similar observation, which was that the 2 Gy TBI conditioning led sustained engraftment only together with MMF but not with MTX due to an increased rejection after MTX.[43] Therefore, preventing the activation and proliferation of donor-derived immune cells without killing lymphocytes may be a future direction for GVHD prophylaxis, in which successful engraftment is assured and severe GVHD is simultaneously controlled.[44]

The use of MMF correlated with a higher incidence of grade II-IV and III-IV acute GVHD. Nevertheless, the increase observed in the incidence of severe GVHD did not dramatically enhance NRM or lead to inferior survival. A recent study reported that the incidence of grade I-II acute GVHD was associated not only with a low risk of relapse, but also with a low risk of NRM, and provides a survival benefit in UCBT.[45] Although we observed a possible association between an increase in the incidence of severe GVHD and reductions in relapse after the use of MMF in high-risk AML recipient, we only noted a marginal increase in NRM in AML. These results indicate that severe GVHD after UCBT may be more manageable than that after other stem cell sources.[46, 47]

In terms of RFS and OS, we did not observe significant differences between the MTX+ group and MMF+ group in a multivariate analysis on AML patients. Nevertheless, in the risk-stratified analysis, the MTX+ and MMF+ groups demonstrated similar risks for survival in standard-risk disease, whereas the MMF+ group had a slightly lower risk of RFS in high-risk disease. In a previous study on myeloablative UCBT, we found a similar pattern of outcomes for OS; however, the MTX+ group was associated with a significantly lower incidence of CNS complications than the MMF+ group in despite the similar survival outcome between the MTX+ group and the MMF+ group. Thus, we concluded that MTX is preferable for standard-risk disease, whereas MMF is better for high-risk disease in myeloablative UCBT.[37] Herein we did not observe a clear difference in the incidence of CNS complications between the MTX+ and MMF+ groups in the current study. Since we demonstrated superior engraftment and similar survival in the MMF+ group, we concluded that MMF+ prophylaxis might be preferable, particularly for high-risk disease, in RIC-UCBT. Several dosing protocols have been described for short-term MTX, and the total dose of MTX markedly varies among different dose schedules. Therefore, careful comparisons of MTX+ prophylaxis groups including doses and schedules are needed in order to draw any solid conclusions. We are currently planning another study to investigate the possible effects of the doses of MTX and MMF for GVHD prophylaxis after UCBT.

There were some limitations to the present study. Since this is a retrospective study, the results obtained must be carefully interpreted. We observed significant differences in the patient age and conditioning regimen between the MTX+ and MMF+ groups. Although we conducted multivariate analyses to consider background differences, we still have to be aware of unrecognized bias because

of the retrospective nature of this study. Furthermore, there were several missing data points due to the lack of data.

In summary, our results suggest that MMF+ prophylaxis may be preferable for single-unit RIC-UCBT, because engraftment was significantly better after MMF+ prophylaxis. On the other hand, due to the increase in the incidence of severe GVHD in the MMF+ prophylaxis, the use of MTX might be occasionally suitable choice for standard-risk disease.

Acknowledgments:

The authors would like to thank all the physicians and data managers at the institutes that contributed valuable data on transplantation to the JSHCT and all the members of the data management committees of the JSHCT. This study was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI 15K09497 to ST) from the Japan Society for the Promotion of Science (JSPS).

Authorship contributions:

ST designed the research, analyzed data, and wrote the manuscript. Y Kuwatsuka, SY, AW, JK, YI, and TY analyzed data and helped write the manuscript. S Mizuta, NU, Y Kozai, NA, HO, HK, YN, S Miyakoshi, MO, and IA collected patient data. TF, TI, and YA supervised data management. MM and TT designed and supervised the research. All authors reviewed and approved the final version of the manuscript.

Disclosure of conflicts of interest: The authors declare no competing financial interests.

References

1. Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A *et al.* Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 2004; **351**(22): 2276-85.

2. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE *et al.* Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 2004; **351**(22): 2265-75.

3. Ooi J. Cord blood transplantation in adults. *Bone Marrow Transplant* 2009; **44**(10): 661-6.

4. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood* 2013; **122**(4): 491-8.

5. Takahashi S, Iseki T, Ooi J, Tomonari A, Takasugi K, Shimohakamada Y *et al.* Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood* 2004; **104**(12): 3813-20.

6. Eapen M, Rocha V, Sanz G, Scaradavou A, Zhang MJ, Arcese W *et al.* Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a

retrospective analysis. *Lancet Oncol* 2010; **11**(7): 653-60.

7. Tanaka M, Miyamura K, Terakura S, Imai K, Uchida N, Ago H *et al.* Comparison of cord blood transplantation with unrelated bone marrow transplantation in patients older than fifty years. *Biol Blood Marrow Transplant* 2015; **21**(3): 517-25.

8. Majhail NS, Brunstein CG, Shanley R, Sandhu K, McClune B, Oran B *et al.* Reduced-intensity hematopoietic cell transplantation in older patients with AML/MDS: umbilical cord blood is a feasible option for patients without HLA-matched sibling donors. *Bone Marrow Transplant* 2012; **47**(4): 494-8.

9. Pascal L, Tucunduva L, Ruggeri A, Blaise D, Ceballos P, Chevallier P *et al.* Impact of ATG-containing reduced-intensity conditioning after single- or double-unit allogeneic cord blood transplantation. *Blood* 2015; **126**(8): 1027-32.

10. Mohty M, Gaugler B. Advances in umbilical cord transplantation: the role of thymoglobulin/ATG in cord blood transplantation. *Best practice & research. Clinical haematology* 2010; **23**(2): 275-82.

11. Pascal L, Mohty M, Ruggeri A, Tucunduva L, Milpied N, Chevallier P *et al.* Impact of rabbit ATG-containing myeloablative conditioning regimens on the outcome of patients undergoing unrelated

single-unit cord blood transplantation for hematological malignancies. *Bone Marrow Transplant* 2015; **50**(1): 45-50.

12. Lindemans CA, Chiesa R, Amrolia PJ, Rao K, Nikolajeva O, de Wildt A *et al.* Impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune reconstitution and clinical outcome. *Blood* 2014; **123**(1): 126-32.

13. Admiraal R, van Kesteren C, Jol-van der Zijde CM, Lankester AC, Bierings MB, Egberts TC *et al.* Association between anti-thymocyte globulin exposure and CD4+ immune reconstitution in paediatric haemopoietic cell transplantation: a multicentre, retrospective pharmacodynamic cohort analysis. *The Lancet. Haematology* 2015; **2**(5): e194-203.

14. Admiraal R, Lindemans CA, van Kesteren C, Bierings MB, Versluys AB, Nierkens S *et al.* Excellent T-cell reconstitution and survival provided ATG exposure is low or absent after pediatric cord blood transplantation. *Blood* 2016.

15. Sakai R, Taguri M, Oshima K, Mori T, Ago H, Adachi S *et al.* A comparison of tacrolimus and cyclosporine combined with methotrexate for graft-versus-host disease prophylaxis, stratified by stem cell source: a retrospective nationwide survey. *Int J Hematol* 2016; **103**(3): 322-33.

16. Terakura S, Atsuta Y, Tsukada N, Kobayashi T, Tanaka M, Kanda J *et al.* Comparison of Outcomes of 8/8 and 7/8 Allele-Matched Unrelated Bone Marrow Transplantation and Single-Unit Cord Blood Transplantation in Adults with Acute Leukemia. *Biol Blood Marrow Transplant* 2016; **22**(2): 330-8.

17. Barker JN, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. *Blood* 2001; **97**(10): 2957-61.

18. Miyakoshi S, Yuji K, Kami M, Kusumi E, Kishi Y, Kobayashi K *et al.* Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. *Clin Cancer Res* 2004; **10**(11): 3586-92.

19. Kishi Y, Kami M, Miyakoshi S, Kanda Y, Murashige N, Teshima T *et al.* Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. *Transplantation* 2005; **80**(1): 34-40.

20. Uchida N, Wake A, Nakano N, Ishiwata K, Takagi S, Tsuji M *et al.* Mycophenolate and tacrolimus for graft-versus-host disease prophylaxis for elderly after cord blood transplantation: a matched

pair comparison with tacrolimus alone. *Transplantation* 2011; **92**(3): 366-71.

21. Narimatsu H, Terakura S, Matsuo K, Oba T, Uchida T, Iida H *et al.* Short-term methotrexate could reduce early immune reactions and improve outcomes in umbilical cord blood transplantation for adults. *Bone Marrow Transplant* 2007; **39**(1): 31-9.

22. Murata M. Prophylactic and therapeutic treatment of graft-versus-host disease in Japan. *Int J Hematol* 2015; **101**(5): 467-86.

23. Kanda J, Atsuta Y, Wake A, Ichinohe T, Takanashi M, Morishima Y *et al.* Impact of the direction of HLA mismatch on transplantation outcomes in single unrelated cord blood transplantation. *Biol Blood Marrow Transplant* 2013; **19**(2): 247-54.

24. Atsuta Y. Introduction of Transplant Registry Unified Management Program 2 (TRUMP2): scripts for TRUMP data analyses, part I (variables other than HLA-related data). *Int J Hematol* 2016; **103**(1): 3-10.

25. Atsuta Y, Suzuki R, Yoshimi A, Gondo H, Tanaka J, Hiraoka A *et al.* Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol* 2007; **86**(3): 269-74.

26. Kanda J. Scripts for TRUMP data analyses. Part II (HLA-related data): statistical analyses specific for hematopoietic stem cell transplantation. *Int J Hematol* 2016; **103**(1): 11-9.

27. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V *et al.* Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant* 2009; **15**(12): 1628-33.

28. Schoemans H, Theunissen K, Maertens J, Boogaerts M, Verfaillie C, Wagner J. Adult umbilical cord blood transplantation: a comprehensive review. *Bone Marrow Transplant* 2006; **38**(2): 83-93.

29. Przepiorka D, Weisdorf D, Martin P, Klingemann H, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**(6): 825-8.

30. Lee SJ, Vogelsang G, Flowers MED. Chronic graft-versus-host disease. *Biology of Blood and Marrow Transplantation* 2003; **9**(4): 215-233.

31. Kaplan E, Meier. P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958; **53:** 457-481.

32. 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**(6): 695-706.

33. Cox DR. Regression Models and Life-Tables. *Journal of the Royal Statistical Society. Series B (Methodological)* 1972; **34**(2): 187-220.

34. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. *Journal of the American Statistical Association* 1999; **94**(446): 496-509.

35. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**(3): 452-8.

36. Ogata M, Fukuda T, Teshima T. Human herpesvirus-6 encephalitis after allogeneic hematopoietic cell transplantation: what we do and do not know. *Bone Marrow Transplant* 2015; **50**(8): 1030-6.

37. Terakura S, Wake A, Inamoto Y, Murata M, Sakai R, Yamaguchi T *et al.* Exploratory research for optimal GvHD prophylaxis after single unit CBT in adults: short-term methotrexate reduced the incidence of severe GvHD more than mycophenolate mofetil. *Bone Marrow Transplant* 2016.

38. Koyama M, Hashimoto D, Nagafuji K, Eto T, Ohno Y, Aoyama K *et al.* Expansion of donor-reactive host T cells in primary graft failure after allogeneic hematopoietic SCT following reduced-intensity

conditioning. *Bone Marrow Transplant* 2014; **49**(1): 110-5.

39. Martin P. Donor CD8 cells prevent allogeneic marrow graft rejection in mice: potential implications for marrow transplantation in humans. *J Exp Med* 1993; **178**(2): 703-12.

40. Moscardo F, Sanz J, Carbonell F, Sanz MA, Larrea L, Montesinos P *et al.* Effect of CD8 Cell Content on Umbilical Cord Blood Transplantation in Adults with Hematological Malignancies. *Biol Blood Marrow Transplant* 2014.

41. Terakura S, Azuma E, Murata M, Kumamoto T, Hirayama M, Atsuta Y *et al.* Hematopoietic engraftment in recipients of unrelated donor umbilical cord blood is affected by the CD34+ and CD8+ cell doses. *Biol Blood Marrow Transplant* 2007; **13**(7): 822-30.

42. 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**(12): 1255-62.

43. Storb R, Yu C, Wagner JL, Deeg HJ, Nash RA, Kiem H-P *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**(8): 3048-3054.

44. Teshima T, Reddy P, Zeiser R. Acute Graft-versus-Host Disease: Novel Biological Insights. *Biol Blood Marrow Transplant* 2016; **22**(1): 11-6.

45. Kanda J, Morishima Y, Terakura S, Wake A, Uchida N, Takahashi S *et al.* Impact of graft-versus-host disease on outcomes after unrelated cord blood transplantation. *Leukemia* 2016.

46. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; **75**(3): 555-62.

47. Storb R, Gyurkocza B, Storer BE, Sorror ML, Blume K, Niederwieser D *et al.* Graft-versus-host disease and graft-versus-tumor effects after allogeneic hematopoietic cell transplantation. *J Clin Oncol* 2013; **31**(12): 1530-8.

Figure Legends

Figure 1. Cumulative incidence of neutrophil and platelet engraftment for MTX+ and MMF+ group. Cumulative incidence curves of neutrophil and platelet counts greater than 50,000/μl are shown for the MTX+ and MMF+ groups (a, b). The cumulative incidences of neutrophil engraftment at day 60 were 66.8% and 81.7% for the MTX+ and MMF+ group, respectively, in (a). The cumulative incidences of platelet engraftment at day 150 were 51.4% and 60.3% for the MTX+ and MMF+ group, respectively, in (b).

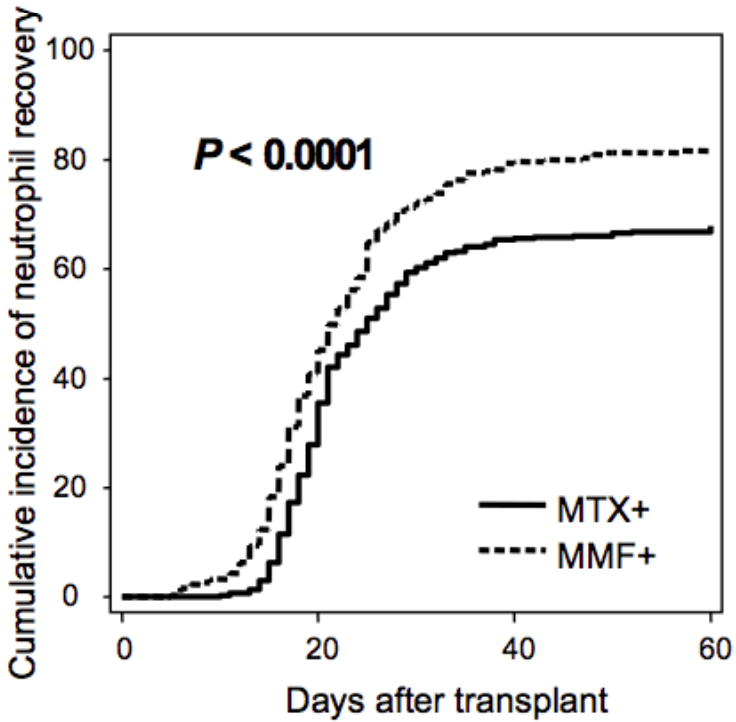
Figure 2. Cumulative incidence of severe acute GVHD for MTX+ and MMF+ group. (a) Grade II-IV acute GVHD and (b) grade III-IV acute GVHD. The cumulative incidences of acute GVHD at day 100 for the MTX+ group and MMF+ group were 24.9% [95% CI, 20.9-29.0] and 36.3% [95% CI, 30.8-41.8] (P<0.001, Fine-Gray), respectively, in (a), and 7.3% [95% CI, 5.1-10.0] and 13.4% [95% CI, 9.8-17.6] (P=0.003, Fine-Gray), respectively, in (b).

Figure 3. Cumulative incidences of relapse and probabilities of RFS in AML according to the GVHD prophylaxis group. Cumulative incidence curves of relapse (a, b) and probabilities of RFS (c, d) in AML are shown for each GVHD prophylaxis group. (a, c) Standard-risk disease and (b, d) high-risk disease. The 2-year cumulative incidences of relapse for the MTX+ group and MMF+ group were

18.7% and 16.9%, respectively, in (a), and 55.1% and 44.9%, respectively, in (b). The 4-year probabilities of RFS for the MTX+ group and MMF+ group were 49.4% and 42.7%, respectively, in (c), and 13.1% and 17.3%, respectively, in (D).

Figure 1

a


Cumulative incidence of neutrophil recovery
100
80
60
40
20
0
P < 0.0001
MTX+
MMF+
0
20
40
60
Days after transplant

b

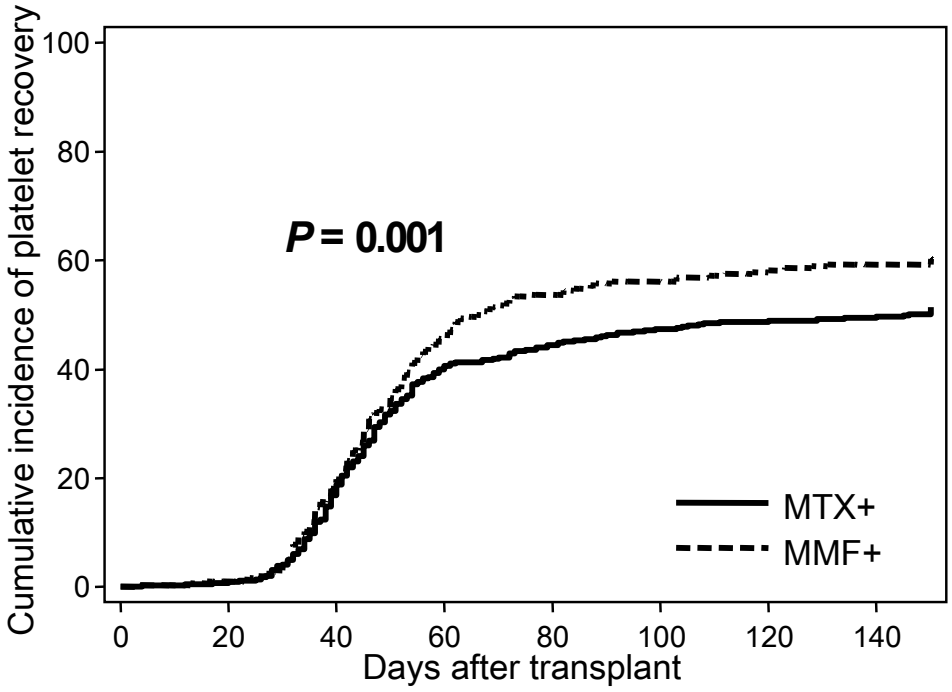

Cumulative incidence of platelet recovery
100
80
60
40
20
0
P = 0.001
MTX+
MMF+
0
20
40
60
80
100
120
140
Days after transplant

Figure 2

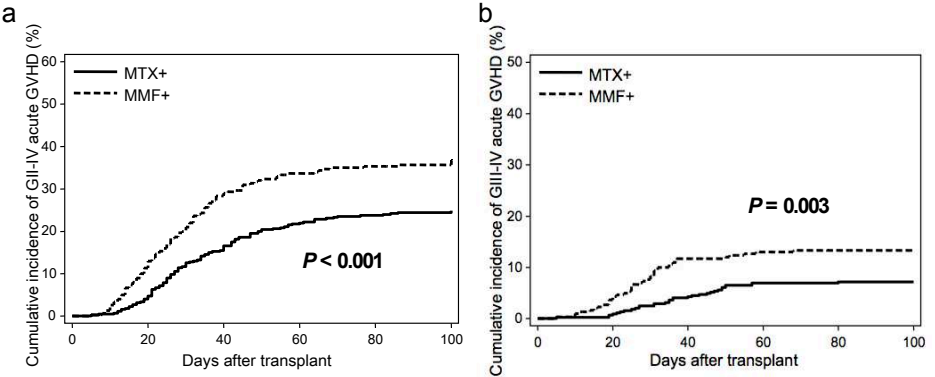

a
Cumulative incidence of GII-IV acute GVHD (%)
MTX+
MMF+
P < 0.001
Days after transplant
b
Cumulative incidence of GIII-IV acute GVHD (%)
MTX+
MMF+
P = 0.003
Days after transplant

Figure 3

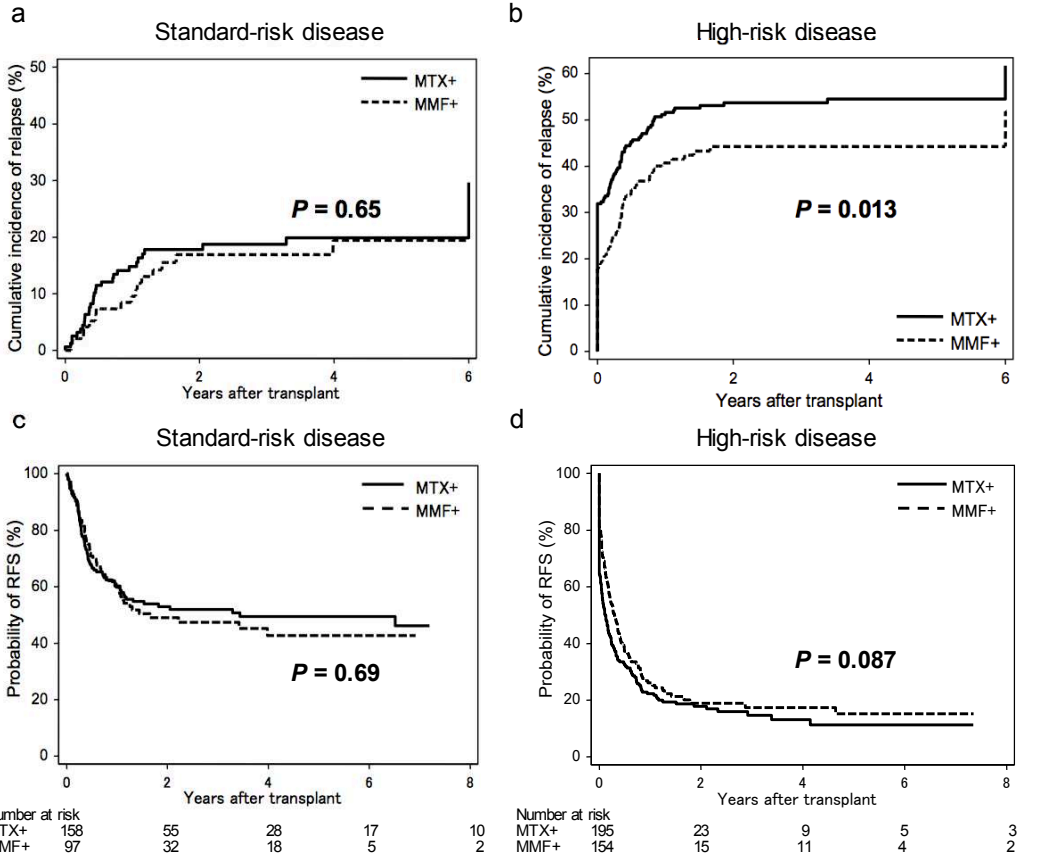

a
Standard-risk disease
Cumulative incidence of relapse (%)
MTX+
MMF+
P = 0.65
Years after transplant
b
High-risk disease
Cumulative incidence of relapse (%)
P = 0.013
MTX+
MMF+
Years after transplant
c
Standard-risk disease
Probability of RFS (%)
MTX+
MMF+
P = 0.69
Years after transplant
Number at risk
MTX+ 158 55 28 17 10
MMF+ 97 32 18 5 2
d
High-risk disease
Probability of RFS (%)
MTX+
MMF+
P = 0.087
Years after transplant
Number at risk
MTX+ 195 23 9 5 3
MMF+ 154 15 11 4 2

Table 1. Patient and transplant characteristics.

	MTX-containing	MMF-containing	P-value
Number	446	302	
Patient age, years (median, range)	59 (17-73)	61 (20-82)	<0.001
Body weight, kg (median, range)	55 (34-88)	55 (35-86)	0.35
Patient sex (Male: Female)	246:200	191:111	0.028
Donor sex (Male: Female: missing)	147:162:137	107:130:58	0.57
Transplantation year			
2000-2009	236	164	
2010-2012	210	138	0.71
Diagnosis			
AML	355	255	
ALL	91	47	0.094
AML			
Disease status at transplant			
Standard risk	158	97	
High risk	195	154	
Data missing	2	4	0.13
ALL			
Disease status at transplant			
Standard risk	53	26	
High risk	37	20	
Data missing	1	1	0.79
Conditioning			
Flu+Bu+- regimen	114	55	
Flu+CY+- regimen	77	82	
Flu+Mel+- regimen	205	147	
Others	50	18	<0.0001
TBI (+/-)	372/74	279/23	<0.0001
TBI dose, Gy (median, range)	4 (2-8)	4 (2-8)	0.78
GVHD prophylaxis			
CyA plus MTX	164	0	
Tac plus MTX	282	0	
CyA plus MMF	0	134	
Tac plus MMF	0	168	
Number of antigen-level mismatches			
0	57	25	
1	122	96	
2	263	177	
3	3	0	
Data missing	1	4	0.087
TNCC ($\times 10^7$/kg), median (range)	2.63 (1.68-5.62)	2.56 (1.55-9.98)	0.42
Median follow-up, months (range)	28.0 (3.2-143.8)	24.6 (2.7-115.8)	0.10

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; Flu, fludarabine; Bu, busulfan; CY, cyclophosphamide; Mel, melphalan; TBI, total body irradiation; CyA, cyclosporine A; MTX, methotrexate; Tac, tacrolimus; MMF, mycophenolate mofetil; TNCC, total nucleated cell count;

Table 2. Adjusted comparison of hematopoietic recovery and acute and chronic GVHD.

		Neutrophil recovery			Platelet recovery			Grade II–IV acute GvHD			Grade III–IV acute GvHD			extensive chronic GvHD		
	n	RR	95%CI	*P*	RR	95%CI	*P*	RR	95%CI	p-value	RR	95%CI	p-value	RR	95%CI	p-value
MTX-containing	446	1.00			1.00			1.00			1.00			1.00		
MMF-containing	302	1.55	1.31–1.83	<0.001	1.34	1.10–1.63	0.003	1.75	1.33–2.31	<0.001	1.97	1.24–3.13	0.004	1.21	0.70–2.08	0.50

Analyses of neutrophil recovery were adjusted for patient age, TNCC, and disease status at transplantation.

Analyses of platelet recovery were adjusted for disease status at transplantation.

Platelet recovery was defined by a platelet count higher than 50,000/μl.

Analyses of each GVHD outcome were adjusted with the following values: Grade II–IV GvHD, HLA disparity, and disease status at transplantation; Grade III–IV GvHD, no variable; extensive chronic GvHD, no variable.

Table 3. Adjusted comparison of relapse and survival for AML according to disease status at transplantation.

		Relapse			RFS			OS		
	n	RR	95%CI	*P*	RR	95%CI	*P*	RR	95%CI	*P*
Standard risk										
MTX-containing	158	1.00			1.00			1.00		
MMF-containing	97	1.03	0.72-1.48	0.56	1.03	0.72-1.48	0.88	0.99	0.69-1.42	0.95
High risk										
MTX-containing	195	1.00			1.00			1.00		
MMF-containing	154	0.69	0.52-0.91	0.009	0.81	0.64-1.03	0.091	0.87	0.68-1.10	0.25

In analyses of relapse and survival, disease status at transplantation was a significant variable in multivariate analyses. There was no other significant variable for relapse, whereas being female significantly correlated with better outcomes in RFS and OS.

Table 4. Causes of death according to GVHD prophylaxis.

		MTX-containing		MMF-containing	
Total	no. (%)	274		187	
	Bleeding	16	(5.8)	13	(7.0)
	Rejection/Graft failure	19	(6.9)	0	(0.0)
	Relapse	99	(36.1)	69	(36.9)
	Acute GVHD with or without infection	4	(1.5)	9	(4.8)
	Chronic GVHD	2	(0.7)	4	(2.1)
	Infection	61	(22.3)	45	(24.1)
	Pulmonary complication	17	(6.2)	10	(5.3)
	Other organ failure	26	(9.5)	17	(9.1)
	Others	30	(11.0)	20	(10.7)

Others include acute respiratory distress syndrome, thrombotic microangiopathy, accidents, and secondary malignancy.