Table 2. Multivariate analysis of overall mortality

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

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

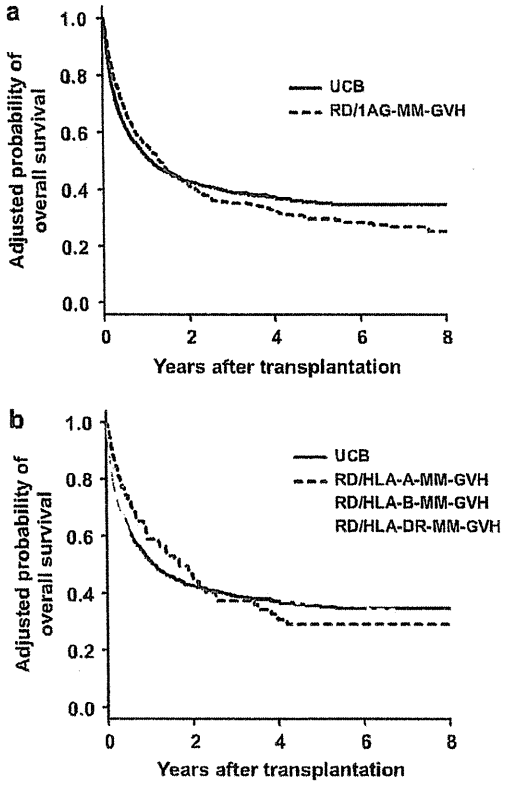

Figure 3. Overall survival. Overall survival rates in the transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**) or with an HLA-A, -B, or -DR antigen mismatch in the GVH direction (**b**) are shown.

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

Relapse and NRM

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

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

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

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

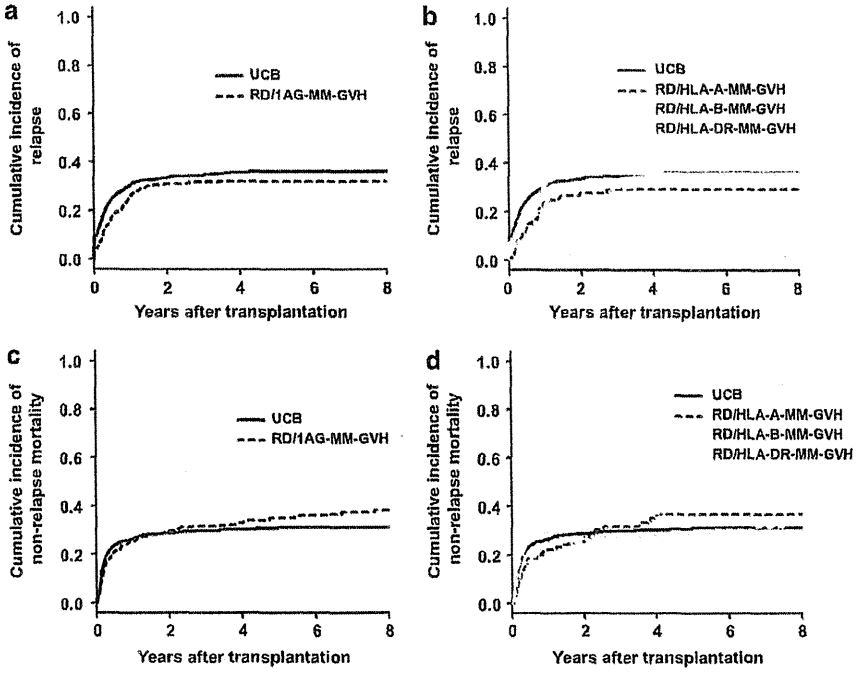

Figure 4. Relapse and non-relapse mortality. Cumulative incidence of relapse and non-relapse mortality after transplantation using an unrelated cord blood vs a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the GVH direction (**a**, **c**) or with an HLA-A, -B, or -DR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

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

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

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

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

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

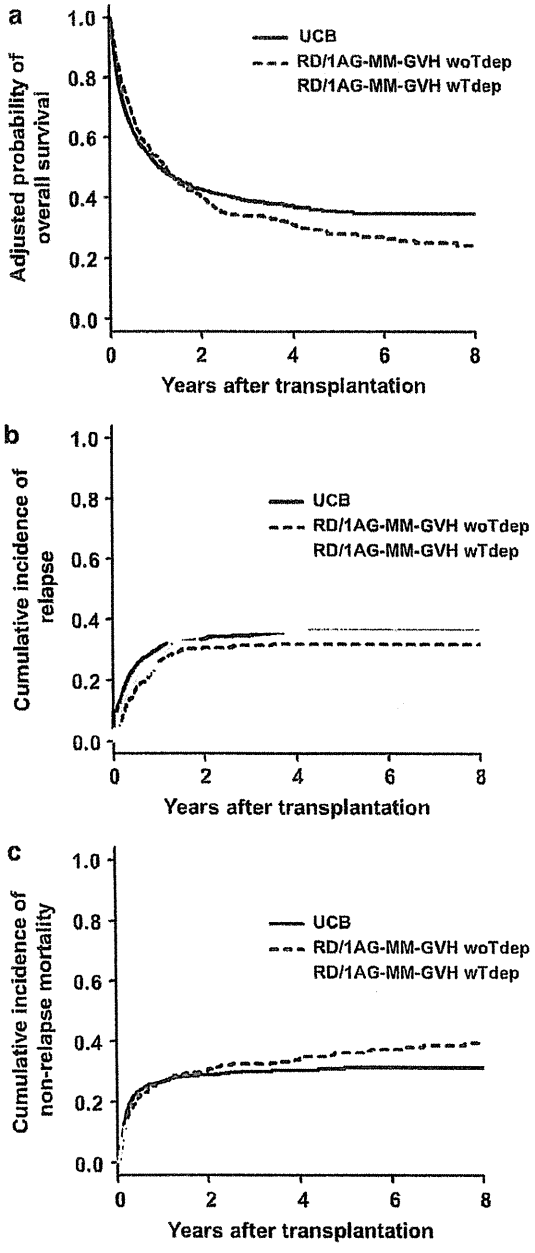

Figure 5. OS (**a**), relapse (**b**) and NRM (**c**) according to the use of *in vivo* T-cell depletion in the RD/1AG-MM-GVH group.

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

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

DISCUSSION

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

In Japan, unrelated BM donor coordination (from donor search to transplantation) takes a median of 4 months, whereas much less time is required for UCB or RD/1AG-MM-GVH transplantation if there is a candidate. This was reflected in the longer duration from diagnosis to transplantation in unrelated BM transplantation.[32] In contrast, UCB and RD/1AG-MM-GVH transplantation show a similar and shorter duration (Table 1 ; 7.9 months vs 7.6 months). Therefore, in cases where both UCB and RD/1AG-MM-GVH are available, donors should be chosen based on their advantages and disadvantages. Compared with UCB, the use of RD/1AG-MM-GVH has a great advantage in neutrophil and platelet engraftment, which is not inconsistent with a previous finding that engraftment in the UCB group was significantly delayed comparing with that in MUD.[33] This translated into a lower rate of death from graft failure or infection in the RD/1AG-MM-GVH group. However, these advantages were offset by a substantial increase in the incidence of acute and chronic GVHD in the RD/1AG-MM-GVH group. The risk of grade III–IV acute GVHD and extensive chronic GVHD in the RD/1AG-MM-GVH group was twice that in the UCB group. If UCB units containing adequate total nucleated cell doses (ex. $>2.5\times10^7$/kg) are available,[34] the selection of UCB would be appropriate to avoid the risk of chronic GVHD. In contrast, RD/1AG-MM-GVH would be more appropriate when early neutrophil engraftment should be prioritized, such as for a patient with an active infectious disease at transplantation.

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

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

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

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

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

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

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

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ORIGINAL ARTICLE

Allogeneic hematopoietic stem cell transplantation for intermediate cytogenetic risk AML in first CR

N Imahashi[1], R Suzuki[2], T Fukuda[3], K Kakihana[4], H Kanamori[5], T Eto[6], T Mori[7], N Kobayashi[8], K Iwato[9], T Sakura[10], K Ikegame[11], M Kurokawa[12], T Kondo[13], H Iida[14], H Sakamaki[4], J Tanaka[15], K Kawa[16], Y Morishima[17], Y Atsuta[2] and K Miyamura[1]

Allogeneic hematopoietic SCT (allo-HCT) from matched sibling donor (MSD) is recommended for younger patients with intermediate cytogenetic risk AML in first CR (CR1), whereas the role of alternative donor transplants in these patients is unknown. We retrospectively analyzed 605 patients with intermediate-risk AML, who received myeloablative allo-HCT in CR1. The 4-year OS for MSD ($n=290$) and matched unrelated donor (MUD; $n=141$) was 65% and 68% ($P=0.50$), respectively. In multivariate analysis, MUD had a similar risk of overall mortality as MSD (hazard ratio = 0.90; 95% confidence interval, 0.62–1.30; $P=0.58$), whereas older age, female donor/male recipient (FDMR) combination, and requiring more than one course of induction chemotherapy to achieve CR1 were poor prognostic factors for OS. Thus, OS after MUD HCT with sex combinations other than FDMR was significantly higher than that after MSD HCT from female donors to male recipients (4-year OS 72% versus 55%, $P=0.04$). These results suggest that HCT, not only from MSD, but also from MUD, should be considered in younger patients with intermediate-risk AML in CR1, and that the donor–recipient sex combination is more important than the donor type in donor selection.

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INTRODUCTION

The current standard treatment strategy for young patients with AML consists of induction chemotherapy and subsequent post-remission therapy. The post-remission therapy includes intensive consolidation chemotherapy and allogeneic hematopoietic SCT (allo-HCT). Although the toxicity of consolidation chemotherapy is relatively low, a substantial proportion of patients relapse, and the risk of relapse depends on cytogenetic risk.[1,2] On the other hand, allo-HCT as a post-remission therapy is associated with the lowest relapse rates. However, this benefit is limited by the high nonrelapse mortality (NRM) and the donor type has a significant impact on NRM.[3] The risk of NRM associated with allo-HCT needs to be balanced with the risk of relapse, and hence, the indication for allo-HCT among patients with AML in the first CR (CR1) depends on the cytogenetic risk and available donor type.[4]

Regarding those patients with favorable cytogenetic risk AML, who achieved CR1, the long-term disease-free survival after intensive consolidation chemotherapy of approximately 60% is reported, and they did not benefit from allo-HCT in CR1.[5–7] Thus, these patients are not considered candidates for allo-HCT in CR1.[8]

As for patients with unfavorable cytogenetic risk AML in CR1, previous prospective studies that assigned allo-HCT versus alternative post-remission therapies, on an intent-to-treat donor versus no-donor basis showed significant disease-free survival and OS benefit with allo-HCT, not only from a matched sibling donor (MSD), but also from a matched unrelated donor (MUD).[5–7,9] Accordingly, allo-HCT in CR1 from MSD or MUD is recommended for unfavorable risk AML.[8]

The indication for allo-HCT in CR1 depends on the available donor type in patients with intermediate cytogenetic risk AML. As meta-analyses of prospective studies showed that allo-HCT in CR1 from MSD offered significant disease-free survival and OS benefit,[5,6] allo-HCT in CR1 from MSD is recommended. In contrast, the indication for allo-HCT from alternative donors among these patients is unknown, because higher NRM may offset therapeutic benefits.[3] Although several studies reported comparable outcome after MUD or MSD transplantation,[10–13] these studies included only a small number of patients with intermediate-risk AML in CR1, and information regarding the outcome of allo-HCT from alternative donors in this group of patients is limited. Collectively, further investigation of the outcome of allo-HCT from alternative donors in patients with intermediate-risk AML in CR1 is warranted. In the present study, we retrospectively analyzed the impact of donor type on

[1]Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [2]Department of Hematopoietic Stem Cell Transplantation Data Management/Biostatistics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3]Department of Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan; [4]Department of Hematology, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan; [5]Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan; [6]Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; [7]Department of Medicine, Division of Hematology, Keio University School of Medicine, Tokyo, Japan; [8]Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan; [9]Department of Hematology, Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital, Hiroshima, Japan; [10]Department of Hematology, Saiseikai Maebashi Hospital, Gunma, Japan; [11]Department of Internal Medicine, Divison of Hematology, Hyogo College of Medicine, Nishinomiya, Japan; [12]Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [13]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [14]Department of Hematology, Meitetsu Hospital, Nagoya, Japan; [15]Department of Hematology, Hokkaido University Hospital, Sapporo, Japan; [16]Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan and [17]Department of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan. Correspondence: Dr N Imahashi, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan.
E-mail: nimahashi@med.nagoya-u.ac.jp
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transplant outcomes among patients with intermediate-risk AML in CR1.

MATERIALS AND METHODS

Collection of data and data source

The recipients' clinical data were provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP). The registry data is managed using the 'Transplant Registry Unified Management Program' system.[14] Both JSHCT and JMDP collect recipients' clinical data at 100 days after allo-HCT. The patient's data on survival, disease status and long-term complications, including chronic GVHD and second malignancies, are renewed annually by follow-up forms. This study was approved by the data management committees of JSHCT. Informed consent was provided according to the Declaration of Helsinki.

Patients

Between January 1996 and December 2008, a total of 682 adult patients aged 16 to 70 years, with intermediate cytogenetic risk AML in CR1, received first BM or PBSC transplantation with myeloablative conditioning regimens. Excluding 66 patients without complete HLA data and 11 patients whose follow-up data were not available, we analyzed 605 patients. Only BM grafts were used in unrelated HCT, because the PBSC donation from unrelated donors was not permitted in Japan. HLA compatibility was determined by serological typing for HLA-A, -B and -DR in related donor (RD) HCT, and by high-resolution typing for HLA-A, -B, -C and -DRB1 in unrelated donor HCT. A MSD was defined as a serologically MSD, whereas other RDs were defined as RDs other than MSD. A MUD was defined as an eight/eight identical unrelated donor, whereas a mismatched unrelated donor (MMUD) was defined as an unrelated donor who had at least one locus mismatch.

Definitions

Neutrophil recovery was defined by an ANC of at least 500 cells per mm^3 for three consecutive points. Acute and chronic GVHD were diagnosed and graded according to defined criteria.[15,16] Relapse was defined as a recurrence of underlying hematological malignant diseases. NRM was defined as death during continuous remission. For OS, failure was death due to any cause, and surviving patients were censored at the last follow-up. The date of transplantation was the starting time point for calculating all outcomes. Cytogenetic risk-group assignment was done according to the Southwest Oncology Group/Eastern Cooperative Oncology Group classification.[2]

Statistical analysis

The two-sided χ^2-test was used for categorical variables, and the two-sided Wilcoxon rank sum test was used for continuous variables. OS was calculated using the Kaplan–Meier method. The log-rank test was used for group comparisons. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of acute and chronic GVHD, relapse and NRM.[17] For GVHD, death without GVHD and relapse were the competing events; for relapse, death without relapse was the competing event; and for NRM, relapse was the competing event. Gray's test was used for group comparison of cumulative incidence.[18] The Cox proportional hazards regression model was used to test the statistical significance of several potential prognostic factors for relapse, NRM and OS. Variables with a significance level less than 0.1 in univariate analysis were entered into multivariable models and sequentially eliminated in a stepwise backward fashion. Each step of model building contained the main effect of donor type. Factors with a significance level less than 0.05 were kept in the final model. The median value was used as a cut-off point for year of transplant. For WBC counts at diagnosis, 50×10^9/L was used as a cut-off point according to the previous report.[10] All *P*-values were two-sided, and *P*-values of less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

Characteristics of the patients are summarized in Table 1. Among the 605 patients analyzed, 290 had MSD HCT, 53 had other RD HCT, 141 had MUD HCT and 121 had MMUD HCT. Of 53 patients with other RD, HLA was matched in 14 and mismatched in 39 patients. Of 121 patients with MMUD, 69 were one locus mismatched and 52 were two or more loci mismatched. The median age of patients was 37 (range, 16–59) years, and median time from diagnosis to HCT was 7.43 (range, 0.43–54.3) months. The median follow-up period of survivors was 4.2 (range, 0.1–13) years. The proportions of male patients, normal karyotype, conditioning regimens, including TBI, and BMT were significantly higher, whereas those of M1/M2/M3/M4/M5 FAB classification and CYA-based GVHD prophylaxis were significantly lower in the unrelated HCT than in the related HCT. The time from diagnosis to HCT was longer in the unrelated HCT compared with related HCT. Other characteristics were not significantly different between related and unrelated HCT.

Acute and chronic GVHD

The unadjusted cumulative incidences of grade II–IV acute GVHD for the MSD and MUD HCT were 26% and 25% at 100 days ($P=0.89$), respectively, and those of grade III–IV acute GVHD were 10% and 7% at 100 days ($P=0.46$), respectively (Table 2). The unadjusted cumulative incidences of chronic GVHD for the MSD and MUD HCT were 45% and 44% at 2 years ($P=0.98$), respectively, and those of extensive chronic GVHD were 28% and 23% at 2 years ($P=0.37$), respectively (Table 2).

Survival

OS rates for the MSD and MUD HCT were 65% and 68% at 4 years, respectively ($P=0.50$; Table 2, Figure 1a). Univariate analysis of risk factors for overall mortality showed that the following factors were significant at the 0.1 level: patient age $\geqslant$40 years, female donor/male recipient (FDMR) combination, and requiring more than one course of induction chemotherapy to achieve CR1 (Table 3). In multivariate analysis, MUD was not a significant factor for overall mortality (hazard ratio (HR) = 0.90; 95% confidence interval (CI), 0.62–1.30; $P=0.58$). Significant factors for overall mortality were patient age $\geqslant$40 years (HR = 1.55; 95% CI, 1.17–2.06; $P<0.01$), FDMR combination (HR = 1.42; 95% CI, 1.03–1.95; $P=0.03$) and requiring more than one course of induction chemotherapy to achieve CR1 (HR = 1.81; 95% CI, 1.36–2.41; $P<0.01$) (Table 4). As the donor–recipient sex combination, but not donor type, was a significant factor for overall mortality, OS after MUD HCT with sex combinations other than FDMR was significantly higher than that after MSD HCT from female donors to male recipients (4-year OS 72% versus 55%, $P=0.04$) (Figure 1b).

Nonrelapse mortality

The cumulative incidences of NRM for the MSD and MUD HCT were 17% and 19% at 4 years, respectively ($P=0.52$) (Table 2, Figure 2a). Univariate analysis of risk factors for NRM showed that the following factors were significant at the 0.1 level: patient age $\geqslant$40 years, FDMR combination and MMUD (Table 3). In multivariate analysis, MUD HCT was not a significant factor for NRM compared with MSD HCT (HR = 1.26; 95% CI, 0.77–2.06; $P=0.35$; Table 4). Significant factors for higher NRM were patient age $\geqslant$40 years (HR = 1.71; 95% CI, 1.17–2.50; $P<0.01$), FDMR combination (HR = 1.68; 95% CI, 1.12–2.52; $P=0.01$) and MMUD (HR = 1.83; 95% CI, 1.16–2.86; $P<0.01$).

Relapse

The cumulative incidences of relapse for the MSD and MUD HCT were 24% and 19% at 4 years, respectively ($P=0.25$; Table 2, Figure 2b). Univariate analysis of risk factors for relapse showed that the following factors were significant at the 0.1 level: longer interval between diagnosis and transplantation, peripheral blood

Table 1. Patient characteristics

Characteristics	*MSD*	*Other RD*	*MUD*	*MMUD*	*P-values*[a]
No. of patients	290	53	141	121	
Median patient age at HCT, years	39	36	35	37	0.09
Range	16–58	17–58	16–59	16–59	
Patient sex, n (%)					0.02
Male	155 (53)	24 (45)	86 (61)	75 (62)	
Female	135 (47)	29 (55)	55 (39)	46 (38)	
Sex matching, n (%)					0.61
Others	202 (77)	45 (87)	112 (79)	98 (81)	
Female to male	61 (23)	7 (13)	29 (21)	23 (19)	
Not available	27	1	0	0	
FAB classification, n (%)					<0.01
M1–M5	227 (82)	39 (80)	90 (70)	83 (74)	
M0, M6, M7	51 (18)	10 (20)	39 (30)	29 (26)	
Others, not available	12	4	12	9	
Prior myelodysplastic syndrome, n (%)					0.52
No	279 (97)	49 (92)	134 (98)	116 (96)	
Yes	10 (3)	4 (8)	3 (2)	5 (4)	
Not available	1	0	4	0	
Cytogenetics, n (%)					0.03
Normal	272 (94)	49 (92)	138 (98)	117 (97)	
+8, +6, -Y, del(12p)	18 (6)	4 (8)	3 (2)	4 (3)	
Conditioning regimen					<0.01[b]
CY + TBI	94 (32)	25 (47)	65 (46)	64 (53)	
CY + CA + TBI	40 (14)	3 (6)	18 (13)	10 (8)	
CY + BU + TBI	12 (4)	1 (2)	13 (9)	5 (4)	
Other TBI regimen	36 (12)	8 (15)	12 (9)	16 (13)	
BU + CY	102 (35)	12 (23)	31 (22)	17 (14)	
Other non-TBI regimen	6 (2)	4 (8)	2 (1)	9(7)	
GVHD prophylaxis, n (%)					<0.01[c]
CsA-based	268 (94)	29 (55)	55 (39)	40 (34)	
FK-based	9 (3)	21 (40)	79 (56)	69 (59)	
Others[d]	9 (3)	3 (6)	7 (5)	8 (9)	
Not available	4	0	0	4	
Time from diagnosis to HCT[e]					
Median	5.79	7.60	8.62	10.2	<0.01
Range	0.43–47.6	2.83–27.6	2.50–54.3	3.49–27.7	
<6 months	153 (54)	17 (33)	20 (14)	10 (8)	<0.01
6 to < 9 months	97 (34)	21 (41)	53 (38)	35 (29)	
9 months or longer	34 (12)	13 (25)	68 (48)	75 (63)	
Not available	6	2	0	1	
Year of transplant, n (%)					0.76
1996–2003	156 (54)	23 (43)	74 (52)	66 (55)	
2004–2008	134 (46)	30 (57)	67 (48)	55 (45)	
Stem cell source, n (%)					<0.01
BM	175 (60)	33 (62)	141 (100)	121 (100)	
Peripheral blood	115 (40)	20 (38)	0 (0)	0 (0)	
WBC counts at diagnosis, $\times 10^9$/L					
<50	196 (71)	36 (75)	108 (79)	82 (75)	0.14
⩾50	79 (29)	12 (25)	29 (21)	27 (25)	
Not available	15	5	4	12	
No. of induction courses to achieve CR, n (%)					0.43
1	187 (68)	31 (62)	88 (67)	68 (60)	
⩾2	88 (32)	19 (38)	43 (33)	45 (40)	
Not available	15	3	10	8	

Abbreviations: CA = cytarabine; FK = tacrolimus; HCT = hematopoietic SCT; MMUD = mismatched unrelated donor; MSD = matched sibling donor; MUD = matched unrelated donor; RD = related donor. [a]*P*-value between related and unrelated donors. [b]*P*-value between TBI regimen and non-TBI regimen. [c]*P*-value between CsA-based prophylaxis and FK-based prophylaxis. [d]Others include T-cell depletion. [e]The median time from diagnosis to transplant was 7.43 months for the whole group.

Table 2. Clinical outcomes

	MSD	*Other RD*		*MUD*		*MMUD*	
	% (95% CI)	*% (95% CI)*	*P-values*[a]	*% (95% CI)*	*P-values*[a]	*% (95% CI)*	*P-values*[a]
Acute GVHD, grades II–IV at 100 days	26 (21–31)	38 (25–51)	0.04	25 (18–32)	0.89	51 (42–59)	<0.01
Acute GVHD, grades III–IV at 100 days	10 (6–13)	15 (7–26)	0.19	7 (4–12)	0.46	14 (9–21)	0.16
Chronic GVHD at 2 years	45 (39–51)	48 (33–62)	0.75	44 (35–53)	0.98	41 (32–51)	0.55
Extensive chronic GVHD at 2 years	28 (23–34)	31 (18–44)	0.73	23 (16–31)	0.37	23 (15–31)	0.25
OS at 4 years	65 (59–71)	53 (37–68)	0.26	68 (59–76)	0.50	61 (51–70)	0.25
Nonrelapse mortality at 4 years	17 (12–22)	18 (9–30)	0.73	19 (13–27)	0.52	25 (18–34)	<0.01
Relapse at 4 years	24 (19–29)	29 (17–42)	0.45	19 (13–27)	0.25	12 (7–19)	0.02

Abbreviations: CI = confidence interval; MSD = matched sibling donor; RD = related donor; MUD = matched unrelated donor; MMUD = mismatched unrelated donor. [a]*P*-values for comparison with MSD.

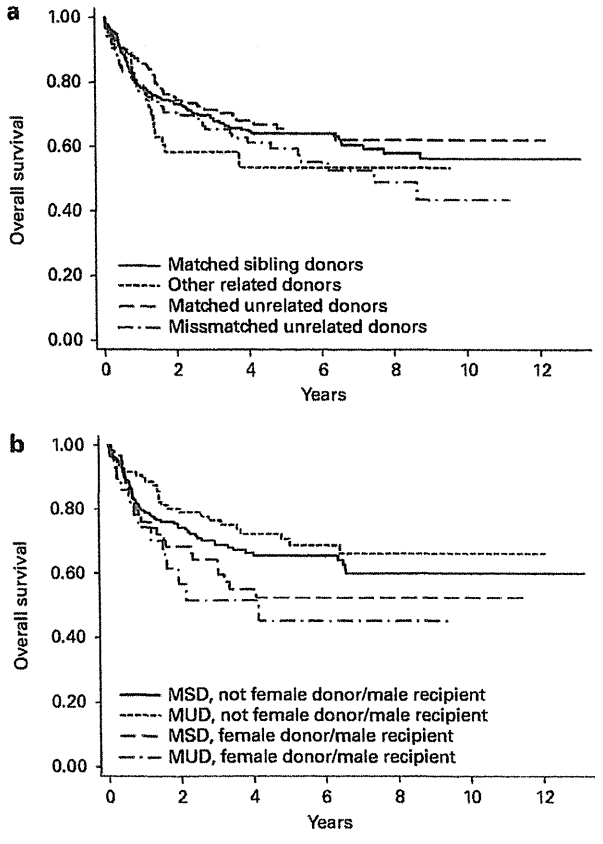

Figure 1. OS. (**a**) Comparison of MSD, other RD, MUD and MMUD transplantation. (**b**) Comparison according to the donor–recipient sex combination and donor type among patients with MSD and MUD.

as stem cell source, WBC counts at diagnosis $\geqslant 50 \times 10^9$/L, requiring more than one course of induction chemotherapy to achieve CR1, and MMUD (Table 3). In multivariate analysis, MUD HCT was not a significant factor for relapse compared with MSD HCT (HR = 0.98; 95% CI, 0.58–1.64; $P = 0.93$; Table 4). Significant factors for relapse were WBC counts at diagnosis $\geqslant 50 \times 10^9$/L (HR = 1.77; 95% CI, 1.20–2.63; $P < 0.01$) and requiring more than one course of induction chemotherapy to achieve CR1 (HR = 2.24; 95% CI, 1.54–3.27; $P < 0.01$), and 9 months or longer interval between diagnosis and transplantation (HR = 0.56; 95% CI, 0.32–0.98; $P = 0.04$).

DISCUSSION

We retrospectively analyzed the impact of donor type on transplant outcomes among patients with intermediate-risk AML in CR1. We observed comparable survival after MSD or MUD HCT, but the donor–recipient sex combination had a significant impact on transplant outcomes. The prognosis of older patients was poorer than that of younger patients because of higher NRM. These findings have important implications for the treatment of intermediate-risk AML in CR1.

The prognosis of younger patients with intermediate-risk AML could be improved by performing allo-HCT in CR1 when MSD is available.[5,6] On the other hand, it is unknown whether these patients without MSD may benefit from alternative donor transplantation, because higher NRM associated with alternative donor transplantation may offset therapeutic benefits.[3] In our study, NRM for a MUD HCT was 19% at 4 years, which was similar to that for a MSD HCT and appeared acceptable. The comparable outcomes after a MSD or a MUD HCT observed in our study suggest that HCT, not only from MSD, but also from MUD, should be considered in younger patients with intermediate-risk AML in CR1.

The FDMR combination had a crucial negative impact on transplant outcome in the present study, whereas it had no or a modest effect on transplant outcome in other studies.[19–21] We suggest two possible explanations for this discrepancy. First, it has been reported that the negative effect of the FDMR combination on survival was more pronounced in the standard-risk disease group than in the high-risk disease group, because the negative impact of the FDMR combination on NRM was stronger in the former than in the latter group, whereas the GVL effect associated with the FDMR combination becomes less important in the standard-risk disease group.[21,22] In the current study, subjects were restricted to patients with intermediate-risk AML in CR1. This may have resulted in a pronounced impact of the FDMR combination on transplant outcome in the current study. Second, as the impact of the FDMR combination on NRM is reported to be at least partially independent from that of GVHD on NRM,[21] and Japanese patients have lower incidence of GVHD,[23] the impact of sex combination on transplant outcome may be more evident in the Japanese than in the western populations.[22] The results of the present study suggest that the donor–recipient sex combination is a more important factor than the donor type in donor selection, in a certain subgroup of patients. As this may alter the current strategies in donor selection, verification in future studies is warranted.

Regarding older patients with intermediate-risk AML, a recent retrospective study showed that patients who underwent allo-HCT in CR1 had better survival than those who were treated with conventional chemotherapy alone, because the latter patients were associated with high relapse rates.[24] On the other hand, previous prospective studies, including patients with AML of all

Table 3. Univariate analysis of OS, nonrelapse mortality and relapse

Variables	N	*OS*		*NRM*		*Relapse*	
		HR (95% CI)	*P-values*	*HR (95% CI)*	*P-values*	*HR (95% CI)*	*P-values*
Patient age							
20–39	290	1.00		1.00		1.00	
<20	45	0.83 (0.47–1.46)	0.52	0.67 (0.29–1.57)	0.36	1.05 (0.53–2.06)	0.89
⩾40	270	1.47 (1.11–1.95)	<0.01	1.65 (1.14–2.41)	<0.01	1.13 (0.78–1.65)	0.52
Sex matching							
Others	457	1.00		1.00		1.00	
Female to male	120	1.39 (1.01–1.91)	0.04	1.68 (1.12–2.53)	0.01	0.80 (0.49–1.31)	0.38
FAB classification							
M1–M5	439	1.00		1.00		1.00	
M0, M6, M7	129	0.89 (0.63–1.25)	0.51	1.01 (0.65–1.56)	0.97	0.87 (0.56–1.37)	0.55
Prior MDS							
No	578	1.00		1.00		1.00	
Yes	22	0.67 (0.28–1.64)	0.39	0.46 (0.11–1.86)	0.28	0.70 (0.22–2.19)	0.54
Cytogenetics							
Normal	576	1.00		1.00		1.00	
+8, +6, -Y, del(12p)	29	0.72 (0.35–1.46)	0.36	1.11 (0.52–2.38)	0.80	0.31 (0.08–1.25)	0.10
TBI							
Yes	422	1.00		1.00		1.00	
No	183	1.06 (0.80–1.42)	0.68	1.01 (0.69–1.50)	0.94	1.01 (0.68–1.49)	0.97
GVHD prophylaxis							
CsA-based	392	1.00		1.00		1.00	
FK-based	178	1.13 (0.84–1.53)	0.42	1.14 (0.77–1.71)	0.51	1.10 (0.73–1.64)	0.65
Others	27	1.19 (0.63–2.27)	0.59	1.06 (0.43–2.63)	0.89	1.48 (0.68–3.20)	0.32
Time from diagnosis to HCT							
< 6 months	200	1.00		1.00		1.00	
6 to <9 months	206	0.86 (0.62–1.20)	0.37	0.92 (0.58–1.48)	0.74	0.77 (0.51–1.17)	0.23
9 months or longer	190	0.88 (0.63–1.22)	0.45	1.26 (0.81–1.96)	0.31	0.48 (0.29–0.77)	<0.01
Year of transplant							
2004–2008	286	1.00		1.00		1.00	
1996–2003	319	0.91 (0.69–1.21)	0.53	1.08 (0.73–1.59)	0.69	0.83 (0.57–1.19)	0.31
Stem cell source							
BM	470	1.00		1.00		1.00	
Peripheral blood	135	1.08 (0.78–1.49)	0.64	0.76 (0.47–1.23)	0.27	1.64 (1.11–2.42)	0.01
WBC counts at diagnosis							
$<50 \times 10^9$/L	422	1.00		1.00		1.00	
$\geqslant 50 \times 10^9$/L	147	1.15 (0.84–1.57)	0.38	0.77 (0.49–1.24)	0.28	1.86 (1.27–2.74)	<0.01
No. of induction courses							
1	374	1.00		1.00		1.00	
⩾2	195	1.76 (1.32–2.33)	<0.01	1.36 (0.92–2.01)	0.12	2.25 (1.55–3.26)	<0.01
Donor							
MSD	290	1.00		1.00		1.00	
Other RD	53	1.34 (0.84–2.15)	0.23	1.17 (0.58–2.39)	0.66	1.31 (0.73–2.33)	0.36
MUD	141	0.88 (0.61–1.26)	0.49	1.12 (0.69–1.79)	0.65	0.77 (0.48–1.23)	0.28
MMUD	121	1.21 (0.86–1.71)	0.27	1.73 (1.11–2.67)	0.02	0.56 (0.32–0.99)	0.046

Abbreviations: CI = confidence interval; FK = tacrolimus; HCT = hematopoietic SCT; HR = hazard ratio; MDS = myelodysplastic syndrome; MSD = matched sibling donor; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; NRM = nonrelapse mortality; RD = related donor.

cytogenetic risk groups, showed that the beneficial effect of allo-HCT in CR1 on OS was absent in patients older than 35–40 years, because the benefits of the reduced relapse rate were offset by a higher NRM.[6,25] In accordance with these prospective studies, older patients had higher NRM and overall mortality than younger patients in the current study. Our study revealed that a substantial number of older patients received allo-HCT in CR1, but the results of our study and others indicate that prospective studies to evaluate the efficacy of allo-HCT in CR1 for older patients with intermediate-risk AML are necessary before it becomes a general practice.

The proportion of patients who received TBI regimens tended to be lower in the older patients than in the younger patients in the current study (data not shown), perhaps in an attempt to

Table 4. Significant factors in multivariate analysis for OS, nonrelapse mortality and relapse

Variables	N	*OS*		*NRM*		*Relapse*	
		HR (95% CI)	*P-values*	*HR (95% CI)*	*P-values*	*HR (95% CI)*	*P-values*
Patient age							
20–39	290	1.00		1.00		—	—
<40	45	0.85 (0.48–1.50)	0.58	0.67 (0.28–1.57)	0.35	—	—
⩾40	270	1.55 (1.17–2.06)	<0.01	1.71 (1.17–2.50)	<0.01	—	—
Sex matching							
Others	457	1.00		1.00		—	—
Female to male	120	1.42 (1.03–1.95)	0.03	1.68 (1.12–2.52)	0.01	—	—
WBC counts at diagnosis							
$<50 \times 10^9$/L	422	—	—	—	—	1.00	
$\geqslant 50 \times 10^9$/L	147	—	—	—	—	1.77 (1.20–2.63)	<0.01
No. of induction courses							
1	374	1.00	—	—	—	1.00	
⩾2	195	1.81 (1.36–2.41)	<0.01	—	—	2.24 (1.54–3.27)	<0.01
Time from diagnosis to HCT							
<6 months	200	—	—	—	—	1.00	
6 to <9 months	206	—	—	—	—	0.85 (0.55–1.31)	0.45
9 months or longer	190	—	—	—	—	0.56 (0.32–0.98)	0.04
Donor							
MSD	290	1.00		1.00		1.00	
Other RD	53	1.35 (0.84–2.18)	0.21	1.31 (0.64–2.68)	0.47	1.44 (0.80–2.61)	0.22
MUD	141	0.90 (0.62–1.30)	0.58	1.26 (0.77–2.06)	0.35	0.98 (0.58–1.64)	0.93
MMUD	121	1.17 (0.83–1.67)	0.37	1.83 (1.16–2.86)	<0.01	0.71 (0.38–1.32)	0.28

Abbreviations: CI = confidence interval; HCT = hematopoietic SCT; HR = hazard ratio; MMUD = mismatched unrelated donor; MSD = matched sibling donor; MUD = matched unrelated donor; NRM = nonrelapse mortality; RD = related donor.

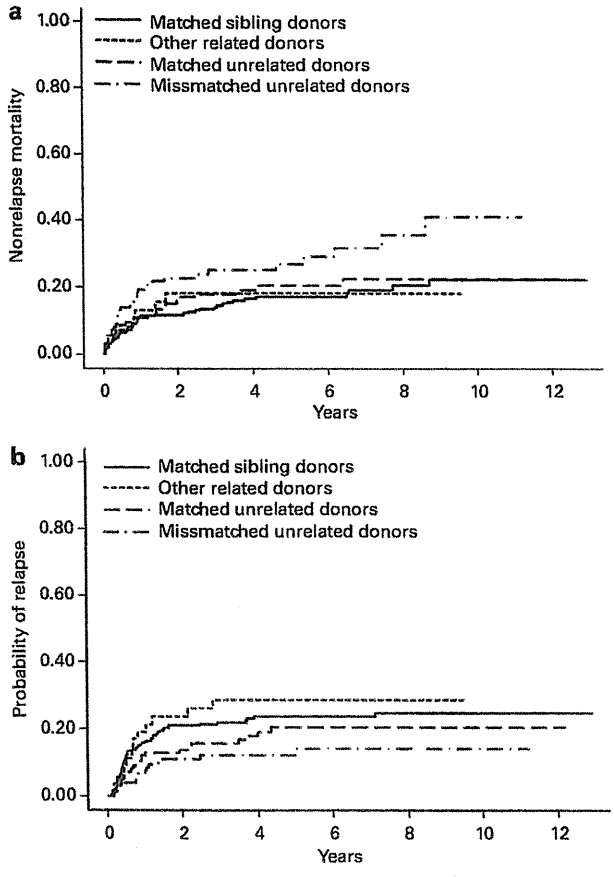

Figure 2. Comparison of MSD, other RD, MUD, and MMUD transplantation. (**a**) Cumulative incidence of NRM. (**b**) Cumulative incidence of relapse.

reduce toxicity. However, there was no significant difference in NRM between TBI and non-TBI regimens among older patients (data not shown). Recently, reduced toxicity myeloablative regimens, such as the combination of fludarabine with myeloablative doses of BU, were developed with an aim to decrease toxicity without compromising antileukemic effects.[26] These regimens might be beneficial for older patients, especially for those with standard-risk disease.[27] The optimal conditioning regimens for older patients need to be determined in the future studies.

OS after other RD and MMUD HCT did not differ significantly from that after MSD HCT in the current study, but these results need to be interpreted with caution. First, the small number of patients with other RD limited the power to detect significant differences in survival between MSD and other RD HCT. Second, other RD and MMUD included donors with various degrees of HLA incompatibilities. Thus, it is difficult to draw firm conclusions regarding the role of other RD and MMUD HCT from this study. Nonetheless, considering that other RD and MMUD HCT yielded a 4-year OS of 53% and 61%, respectively, allo-HCT from these donors might be an option for patients with unfavorable features. For example, as patients who required more than one course of induction therapy to achieve CR1 have poor outcomes with conventional chemotherapy,[8] they might benefit from allo-HCT from other RD or MMUD, when MSD and MUD are not available.

Our study has several limitations. First, this is a non-randomized, retrospective observational study using registry data, which would allow for the introduction of bias. To minimize bias, we conducted multivariate analyses to adjust for baseline differences. However, some factors which might have influenced transplant outcomes (such as performance score and extramedullary disease) could not be included in the Cox proportional hazards regression model due to a high frequency of missing values. Second, a time-censoring effect might have influenced the results.[28] Patients who undergo transplantation late after achievement of CR may be at a lower risk of relapse, by virtue of having remained in remission a time long enough for a transplantation to be performed.[28] This effect might have favorably affected the outcome of unrelated donor HCT. However, there was no significant difference in OS between MSD

and MUD HCT, even when the time from diagnosis to transplantation was included in the final model of multivariate analyses (data not shown). Third, although the role of allo-HCT according to genetic mutations, such as *FLT3-ITD*, *NPM1* and *CEBPA*, is now being explored,[29] the information about these mutations was not available and this was beyond the scope of the present study. However, the results of our study do support the inclusion of not only MSD HCT, but also MUD HCT, in the prospective studies, which evaluate the role of allo-HCT according to these genetic mutations.

In conclusion, the results of our study suggest that HCT, not only from MSD, but also from MUD ,should be considered in younger patients with intermediate-risk AML in CR1, and that the donor–recipient sex combination is more important than the donor type in donor selection. Prospective studies to evaluate the role of allo-HCT in CR1 for older patients are warranted.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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The recipient CXCL10 +1642C>G variation predicts survival outcomes after HLA fully matched unrelated bone marrow transplantation☆,☆☆

Katsuya Nakata [a], Akiyoshi Takami [a,*], J. Luis Espinoza [a], Keitaro Matsuo [b], Yasuo Morishima [b], Makoto Onizuka [c], Takahiro Fukuda [d], Yoshihisa Kodera [e], Hideki Akiyama [f], Koichi Miyamura [g], Takehiko Mori [h], Shinji Nakao [a] for the Japan Marrow Donor Program

[a] *Department of Hematology and Oncology, Kanazawa University Hospital, Kanazawa, Japan*
[b] *Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan*
[c] *Department of Hematology and Oncology, Tokai University School of Medicine, Isehara, Japan*
[d] *Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo, Japan*
[e] *Department of Promotion for Blood and Marrow Transplantation, Aichi Medical University, Nagoya, Japan*
[f] *Department of Internal Medicine, Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan*
[g] *Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan*
[h] *Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan*

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KEYWORDS
CXCL10;
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Bone marrow

Abstract CXCL10 is a chemoattractant for immune cells that is involved in several immune-inflammatory disorders. This study retrospectively examined the impact of a single nucleotide variation (rs3921, +1642C>G) in the CXCL10 gene on transplant outcomes in a cohort of 652 patients who underwent unrelated HLA-matched bone marrow transplantation (BMT) for hematologic malignancies. The recipient C/G or G/G genotype was found to be associated with a

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* Corresponding author at: Department of Hematology and Oncology, Kanazawa University Hospital, 13–1 Takaramachi, Kanazawa 920–8641, Japan. Fax: 81 76 234 4277.
E-mail addresses: bn6a-tkm@asahi-net.or.jp (K. Nakata), takami@staff.kanazawa-u.ac.jp (A. Takami), luis@staff.kanazawa-u.ac.jp (J.L. Espinoza), kmatsuo@aichi-cc.jp (K. Matsuo), ymorisim@aichi-cc.jp (Y. Morishima), moni5@mac.com (M. Onizuka), tafukuda@ncc.go.jp (T. Fukuda), ykodera@river.ocn.ne.jp (Y. Kodera), hakiyama-e@ebara-hp.ota.tokyo.jp (H. Akiyama), miyamu@nagoya-1st.jrc.or.jp (K. Miyamura), tmori@sc.itc.keio.ac.jp (T. Mori), snakao8205@staff.m.kanazawa-u.ac.jp (S. Nakao).

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transplantation;
Single nucleotide variation;
Organ failure

significantly better 5-year overall survival (OS) rate and a lower transplant-related mortality (TRM) rate than the recipient C/C genotype. The recipient C/G or G/G genotype also predicted a reduced incidence of death due to organ failure. The multivariate analysis showed the recipient C/G or G/G genotype to exhibit statistical trends toward beneficial effects on OS but not on TRM. CXCL10 genotyping could therefore be useful in predicting prognoses and creating therapeutic strategies for improving the final outcomes of patients who undergo allogeneic BMT.

1. Introduction

C-X-C motif chemokine 10 (CXCL10), also known as interferon-γ-inducible protein 10, is a chemoattractant for various immune cells such as activated type 1 T helper (Th1) cells, natural killer (NK) cells, dendritic cells (DCs), γδ T cells and macrophages and critically regulates immune-inflammatory responses against microbes and cancer [1,2]. CXCL10 also plays pivotal roles in the initiation and progression of chronic inflammation, autoimmune diseases and allograft rejection [2–8]. A single nucleotide variation in the 3′-untranslated region (3′UTR) of the CXCL10 gene, rs3921 (+1642C>G), is associated with the development of invasive aspergillosis after allogeneic hematopoietic stem-cell transplantation (HSCT) [9] and the disease progression of multiple sclerosis [10] in European populations. The +11 101C>T (rs1554013) and +908A>G (rs4859588) variations in the CXCL10 gene, which also has been reported to correlate with invasive aspergillosis after HSCT, are at a near-perfect disequilibrium with the +1642C>G variation [9]. The role of CXCL10 in anti-infection and anti-tumor immunity and the association between the CXCL10 variant and autoimmunity prompted us to investigate the impact of donor and recipient +1642C>G variation in the CXCL10 gene on the clinical outcomes of patients who undergo allogeneic bone marrow transplantation (BMT) using HLA allele-matched unrelated donors through the Japan Marrow Donor Program (JMDP). The data showed that the recipient C/G or G/G genotype is associated with a significantly better survival rate in patients with hematologic malignancies.

Table 1 Donor and recipient characteristics (first table).

Variable	No.	Ratio
No. of cases	652	
Recipient age, years		
Median	35	
Range	1–67	
Donor age, years		
Median	34	
Range	20–57	
Year of transplant		
Median	2001	
Range	1993–2007	
Recipient CXCL10 genotype		
C/C	562	86%
C/G	85	13%
G/G	5	1%
Donor CXCL10 genotype		
C/C	569	87%
C/G	81	12%
G/G	2	<1%
Recipient sex		
Male	390	60%
Female	262	40%
Donor sex		
Male	410	63%
Female	241	37%
Donor/recipient sex		
Sex matched	419	64%
Female/male	106	16%
Male/female	126	19%
Missing	1	0%

Table 2 Donor and recipient characteristics (second table)
Abbreviation: TNC: total nucleated cell count harvested.

Variable	No.	Ratio
Disease		
Acute myeloid leukemia	208	32%
Acute lymphoblastic leukemia	161	25%
Myelodysplastic syndrome	87	13%
Malignant lymphoma	74	11%
Chronic myeloid leukemia	118	18%
Multiple myeloma	4	1%
Disease stage		
Standard-risk	397	61%
High-risk	255	39%
ABO matching		
Major or/and minor mismatch	252	39%
Major mismatch	143	22%
Minor mismatch	127	19%
Bidirectional	18	3%
Missing	9	1%
Conditioning regimen		
Myeloablative	572	88%
Reduced intensity	80	12%
With total body irradiation	515	79%
Pretransplant CMV serostatus		
CMV positive recipient	444	68%
Missing	70	12%
GVHD prophylaxis		
With cyclosporine	373	57%
With tacrolimus	277	42%
Missing	2	0%
TNC, $\times 10^8$ per kg		
Median	5.1	
Range	0.1–87.0	

Table 3 The results of the univariate analysis regarding the association between CXCL10 variations and the clinical outcomes after transplantation.

Variable	No.	5-year OS	P	5-year TRM	P	5-year relapse/ progression	P	II-IV acute GVHD	P	III-IV acute GVHD	P	Chronic GVHD	P
Recipient CXCL10 genotype													
C/C	562	47%		28%		29%		32%		13%		44%	
C/G or G/G	90	59%	0.02	17%	0.03	29%	0.78	31%	0.29	11%	0.69	51%	0.31
Donor CXCL10 genotype													
C/C	569	48%		26%		30%		32%		12%		44%	
C/C or G/G	83	53%	0.86	27%	0.85	27%	0.97	29%	0.64	14%	0.72	50%	0.32

2. Materials and methods

2.1. Patients

CXCL10 genotyping was performed on 652 transplantation recipients with hematological malignancies and their unrelated donors who underwent BMT through the JMDP with T-cell-replete marrow from HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 allele-matched donors between January 1993 and December 2007. None of the present patients had a history of any prior transplantation. The final clinical survey of these patients was completed by November 1, 2008. The diagnoses included acute myeloid leukemia (AML) in 208 patients (32%), acute lymphoblastic leukemia (ALL) in 161 patients (25%), chronic myeloid leukemia (CML) in 118 patients (18%), myelodysplastic syndrome (MDS) in 87 patients (13%), malignant lymphoma (ML) in 74 patients (11%) and multiple myeloma (MM) in four patients (1%) (Tables 1 and 2). Data on the classification of MDS as well as the prognostic scoring system were not available in the present cohort. Of the 74 patients with ML, 68 had non-Hodgkin lymphoma and six had Hodgkin lymphoma; however, the data on a refined lymphoma classification were not available. The disease response was evaluated according to the previously reported criteria [11–15]. The median follow-up duration in the cohort was 2,085 days in the survivors (range: 124–5,136 days), and 176 recipients (27%) relapsed or progressed and 316 (48%) died. Sixteen patients (2%) died before undergoing engraftment. The recipients were defined as having standard-risk disease if they had AML or ALL in a first complete remission, CML in chronic phase, ML in any complete remission or MDS [16]. All others were designated as having high-risk disease. The observed myeloid malignancies included AML, CML and MDS and the observed lymphoid malignancies included ALL, ML and MM. Cyclosporine- or tacrolimus-based regimens were used in all patients for GVHD prophylaxis, while anti-T cell therapies such as anti-thymocyte globulin or ex vivo T cell depletion were not used in any of the patients. All patients and donors provided their written informed consent to participate in molecular studies of this nature at the time of transplantation according to the declaration of Helsinki. This project was approved by the Institutional Review Board of Kanazawa University Graduate School of Medicine and the JMDP.

2.2. CXCL10 genotyping

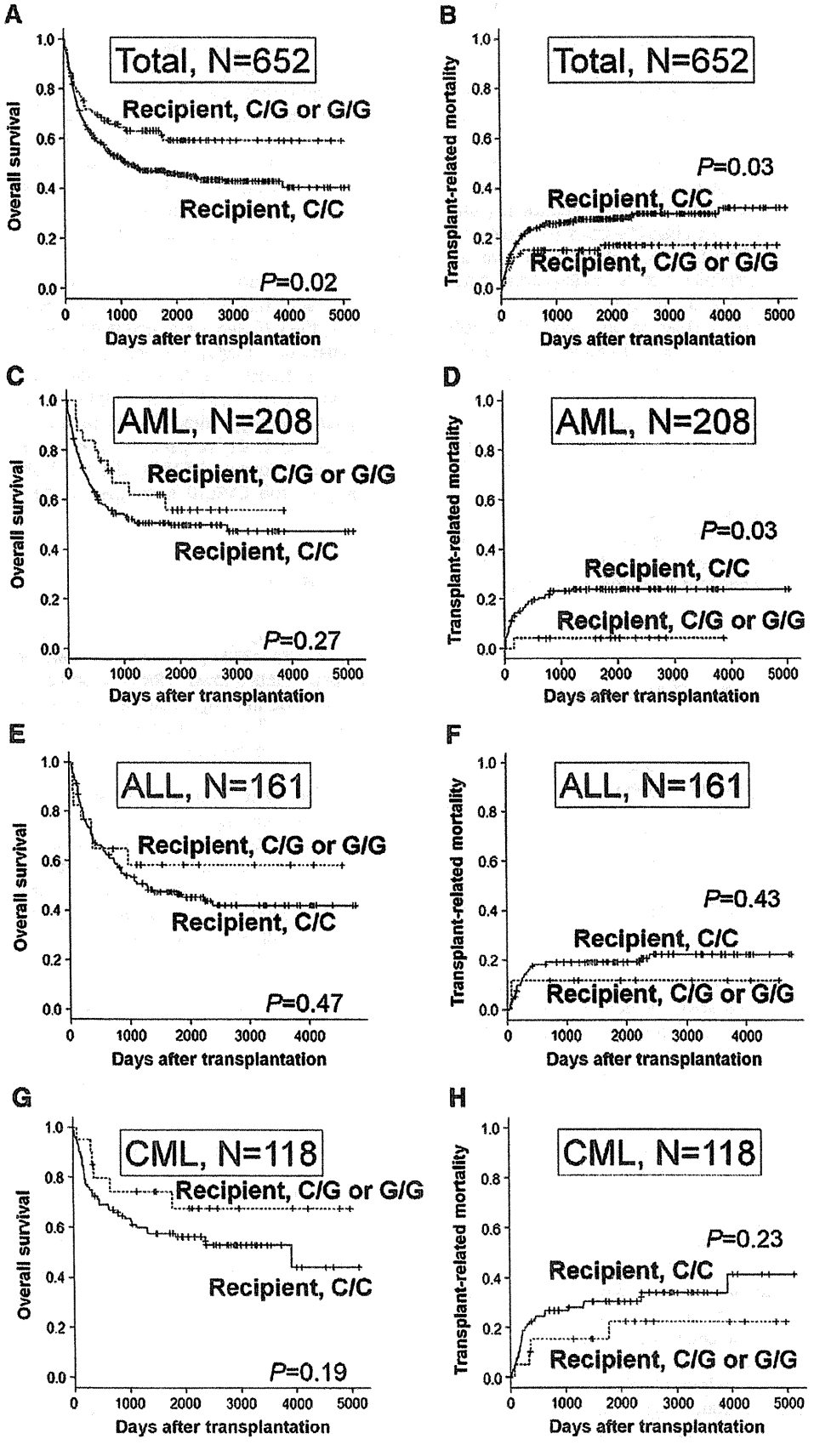
The genotyping of CXCL10 was performed using the TaqMan-Allelic discrimination method, as previously described [17]. The genotyping assay was conducted in 96-well PCR plates using specific TaqMan probes for the CXCL10 gene SNPs rs3921 (catalog C_497062_1) in a StepOne Plus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA).

2.3. Data management and statistical analysis

The data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year and annually after transplantation. Pre-transplant cytomegalovirus (CMV) serostatus was routinely tested in patients only and not in donors. Engraftment was confirmed with absolute neutrophil counts of more than 0.5×10^9/L for at least three consecutive days. After collecting the data, acute and chronic cases of GVHD were diagnosed and graded based on classically defined criteria [18,19], namely, acute GVHD was defined as GVHD that developed within the first 100 days post-transplant, while the manifestations of GVHD occurring after day 100 were classified as chronic GVHD. Data using the updated criteria for assessment of GVHD [20,21] were not available in our cohort. The overall survival (OS) rate was defined as the number of days from transplantation to death from any cause. Disease relapse or progression was defined as the number of days from transplantation to disease relapse or progression. Transplant-related mortality (TRM) was defined as death without disease relapse or progression. Data regarding causative microbes of infections, postmortem changes in causes of death and supportive care, including prophylaxis for infections and therapy for GVHD given on an institutional basis, were not available for this cohort.

All statistical analyses were performed with the EZR software package (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) [22], as described in a previous report [23]. The probability of OS was calculated using the Kaplan–Meier method and compared using the log-rank test. Any patients who were alive on the last follow-up date were censored. The probabilities of TRM,

Figure 1 The Kaplan–Meier analysis of the overall survival rates (A, C, E, G) and the estimated cumulative incidence curves of transplant-related mortality (B, D, F, H) after transplantation according to the recipient CXCL10 genotype in all patients (A, B) or patients with AML (C, D) or ALL (E, F) or CML (G, H). The solid lines represent the recipient C/C genotype and the dashed lines represent the recipient G/C or G/G genotype.


A
Total, N=652
Recipient, C/G or G/G
Recipient, C/C
P=0.02
Overall survival
Days after transplantation
B
Total, N=652
P=0.03
Recipient, C/C
Recipient, C/G or G/G
Transplant-related mortality
Days after transplantation
C
AML, N=208
Recipient, C/G or G/G
Recipient, C/C
P=0.27
Overall survival
Days after transplantation
D
AML, N=208
P=0.03
Recipient, C/C
Recipient, C/G or G/G
Transplant-related mortality
Days after transplantation
E
ALL, N=161
Recipient, C/G or G/G
Recipient, C/C
P=0.47
Overall survival
Days after transplantation
F
ALL, N=161
P=0.43
Recipient, C/C
Recipient, C/G or G/G
Transplant-related mortality
Days after transplantation
G
CML, N=118
Recipient, C/G or G/G
Recipient, C/C
P=0.19
Overall survival
Days after transplantation
H
CML, N=118
P=0.23
Recipient, C/C
Recipient, C/G or G/G
Transplant-related mortality
Days after transplantation

disease relapse or progression, acute GVHD, chronic GVHD and engraftment were compared using the Gray test [24] and analyzed using a cumulative incidence analysis while considering disease relapse or progression, death without disease relapse or progression, death without acute GVHD, death without chronic GVHD and death without engraftment as respective competing risks. The variables were the recipient's age at the time of transplantation, sex, disease characteristics (disease type, disease lineage and disease risk at transplantation), donor characteristics (age, sex, sex compatibility and ABO compatibility), transplant characteristics (conventional or reduced-intensity conditioning [25], total body irradiation-containing regimens, tacrolimus versus cyclosporine, total nucleated cell counts harvested per recipient weight [TNC]) and year of transplantation. The median was used as the cutoff point for continuous variables. The chi-square test and the Mann–Whitney *U* test were used to compare data between two groups.

Multivariate analyses were performed using Cox proportional hazards regression models or competing risk regression models [26] as appropriate to evaluate the relative risk associated with the CXCL10 variation. Covariates found to be significant in the univariate analyses ($P \leq 0.10$) were used to adjust the relative risk. For both the univariate and multivariate analyses, the *P* values were two-sided and the outcomes were considered to be significant for values of $P \leq 0.05$.

3. Results

3.1. Frequencies of the CXCL10 genotypes

The rs3921 SNP in the CXCL10 gene was genotyped in 652 unrelated bone marrow donor-transplant recipient pairs (Tables 1 and 2). The genotype frequencies of C/C, C/G and G/G were 86%, 13% and 1% in the recipients and 87%, 12% and <1% in the donors, respectively. These results are similar to HapMap data in the Japanese population [27].

3.2. Effects of the CXCL10 genotype on transplant outcomes

The donor and recipient CXCL10 genotype did not significantly influence the cumulative incidence of engraftment (data not shown). Considering the low frequency of the CXCL10 G/G genotype in the Japanese population, differences in effects between the CXCL10 C/G or G/G genotype and the CXCL10 C/C genotype were analyzed. The transplant outcomes according to the CXCL10 genotype are summarized in Table 3. The recipient C/G or G/G genotype was associated with a significantly better 5-year OS rate (59% vs. 47%, $P=0.02$; Fig. 1A) and a lower TRM rate (17% vs. 28%, $P=0.03$; Fig. 1B) than the recipient C/C genotype. Separate analyses for patients with AML, ALL and CML also showed trends in the recipient C/G or G/G genotype toward better OS and TRM, although most of the differences were not significant (Fig. 1C–H). When patients who survived 6 months or more were analyzed to reduce the effects of the conditioning regimen and early inevitable toxicities, the association between the recipient C/G or G/G genotype and superior OS and TRM remained significant (Supplementary Fig. 1A and B). This was also true when the study population was made more homogenous by excluding four patients with MM (Supplementary Fig. 1C and D). No difference was noted in disease relapse/progression or GVHD in relation to the recipients' genotype (Table 3).

After adjusting for clinical factors in the multivariate model, statistical trends toward beneficial effects of the recipient C/G or G/G genotype were observed on OS (relative risk [RR]: 0.70; 95% confidence interval [CI]: 0.48–1.02; $P=0.06$) but not on TRM (Table 4). The donor CXCL10 genotype did not significantly influence the transplant outcomes.

When the main causes of death were analyzed according to the CXCL10 genotype, the recipient C/G or G/G genotype resulted in a statistically reduced incidence of death attributed to organ failure (Fig. 2). Forty-seven (9%) of the patients possessing the C/C genotype died due to organ failure of the liver (14 patients), lungs (11 patients), kidneys (nine patients), heart (five patients), gut (two patients) and multiple organs (six patients), while two patients (2%; $P=0.048$) possessing the C/G or G/G genotype died due to failure of the liver and gastrointestinal tract, respectively. Other causes of death, including infections and GVHD, did not significantly differ according to the CXCL10 genotype of the recipients and donors.

4. Discussion

The present study showed that the recipient C/G or G/G genotype at the rs3921 (+1642C>G) variant of the CXCL10 3′ UTR gene predicts a lower TRM rate and a higher OS rate in patients with hematologic malignancies receiving unrelated HLA-matched BMT. The recipient C/G or G/G genotype also contributes to decreased rates of death due to organ failure.

The mechanisms through which the recipient +1642G allele of the CXCL10 gene improves TRM and OS remain unclear. The CXCL10 +1642C>G variation is located in 3′UTR, at which genetic alterations potentially influence the protein levels by affecting the stability and location of mRNA [28,29], thus leading to a hypothesis that this variation may correlate with the CXCL10 expression. This hypothesis is supported by a previous report [9] that showed that immature DCs carrying the +1642G allele produce CXCL10 less efficiently than those carrying the +1642C allele. In addition to monocytes, neutrophils and DCs, CXCL10 is produced in response to interferon-γ by various non-hematopoietic cells, including endothelial cells, vascular pericytes, keratinocytes, mesangial cells, fibroblasts and astrocytes. CXCL10 is a critical mediator contributing to the injury of transplanted grafts, including the liver, lungs, kidneys, heart and gut. Additionally, an association between the intragraft CXCL10 expression and acute rejection and the effects of CXCL10 monoclonal antibodies in the treatment of acute rejection have been demonstrated [2,4–7]. The level of CXCL10 is also increased in the serum of patients receiving HSCT, indicating a potential role of CXCL10 in organ injury and inflammation [9,30]. Based on these findings, one may hypothesize that higher CXCL10 secretion by intraorgan non-hematopoietic cells of +1642C/C HSCT recipients possibly accounts for the increased risk for organ failure after transplantation observed in these patients. This hypothesis may be supported by the findings of an earlier study [31] using a solid organ rejection model, which showed

Table 4 The results of the multivariate analysis of the association between CXCL10 variations and the clinical outcomes after transplantation.

Variable	OS			TRM			Relapse			II–IV acute GVHD			III–IV acute GVHD			Chronic GVHD		
	Adjusted RR	95% CI	P	Adjusted RR	95% CI	P	Adjusted RR	95% CI	P	Adjusted RR	95% CI	P	Adjusted RR	95% CI	P	Adjusted RR	95% CI	P
Recipient CXCL10 genotype, C/G or G/G vs. C/C	0.70	0.48–1.02	0.06	0.85	0.48–1.53	0.59	0.93	0.55–1.55	0.77	0.98	0.65–1.47	0.91	0.85	0.43–1.69	0.64	1.11	0.80–1.55	0.53
Donor CXCL10 genotype, C/G or G/G vs. C/C	0.98	0.69–1.39	0.91	0.81	0.45–1.45	0.48	0.96	0.57–1.63	0.89	0.90	0.58–1.40	0.64	1.10	0.56–2.14	0.79	1.13	0.79–1.63	0.49

the intragraft endothelial CXCL10 expression to play a pivotal role in initiating multiple effector pathways and inducing graft injury.

Since immunosuppressive agents can alter the serum levels of Th1-type chemokines, including CXCL10 [32], immunosuppressive therapy against GVHD might have exerted some impact on the findings of this study. Data on GVHD therapy were not available in the present cohort. However, in the patients who developed grades II to IV acute GVHD, which is usually an indication for treatment with systemic steroids, the superior 5-year OS rate associated with the recipient C/G or G/G genotype remained significant (68% vs. 39%, $P = 0.02$, data not shown), thus implying that the influence of immunosuppressive therapy on the effects of the CXCL10 genotype was minimal.

The current data are not consistent with those of a previous European study [9] that found an association between the recipient CXCL10 +1642G allele and the development of invasive aspergillosis. In the present study, the death rate due to fungal infection associated with the recipient +1642C/G or G/G genotype was comparable to that associated with the recipient +1642C/C genotype (2% vs. 2%, $P = 0.72$), although no data on the incidence of fungal infections or the types of fungi were available. Prospective studies should be conducted to investigate whether the CXCL10 +1642C>G variation correlates with the development of invasive aspergillosis after HSCT.

In conclusion, the present data suggest that the recipient CXCL10 +1642C>G variation affects prognoses after BMT from unrelated donors. Therefore, CXCL10 genotyping in transplant recipients may be a potentially useful tool in the development of models that predict pretransplantation risks for matched unrelated allotransplantion in well-defined patient populations with hematological malignancies. However, care should be taken in drawing conclusions because experimental evidence is required to substantiate the effects of CXCL10 variations. Second, transplant outcomes are multifactorial, and single nucleotide variations in one chemokine gene are unlikely to determine the majority of transplant outcomes. Finally, the lack of significant effects in relation to the donor CXCL10 genotype may suggest that the beneficial effects of CXCL10 genotyping are limited. Further studies are warranted to ascertain whether the findings of this study can be extended to other stem cell sources or to HLA-mismatched transplantation and to also validate the present data in other ethnic groups.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clim.2012.11.009.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Authorship

Contributions: K.N., J.L.E. and A.T. equally contributed to the study. J.L.E., K.N. and A.T. designed the study. J.L.E.

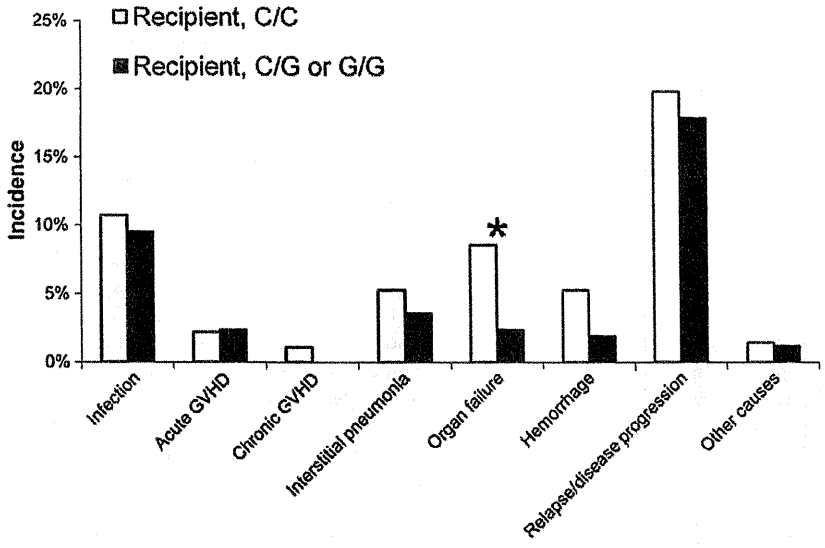

Figure 2 The main causes of death after transplantation according to the recipient CXCL10 genotype. The asterisk denotes $P < 0.05$.

and K.N. performed the experimental analyses. A.T. and K.M. performed the statistical analysis. A.T. and J.L.E. wrote the paper. The remaining authors contributed to data collection and sample management.

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