

Figure 4. The cumulative incidence of grade III to IV acute GVHD (A) and adjusted overall survival (B) grouped according to the HLA mismatch loci between the donor and recipient in the mid or late time period. AB-HR MM, high-risk mismatch at the HLA-A or -B locus; C-HR MM, high-risk mismatch at the HLA-C locus; DR-HR MM, high-risk mismatch at the DRB1 locus; LR-MM, low-risk mismatch; MUD, matched unrelated donor.

the 3 time periods but statistically significant only in the mid period (HR, .83; 95% CI, .69 to .98; $P = .032$). Figure 2 shows the overall survival curves grouped according to the HLA-mismatch groups in each time period, adjusted for other significant factors by the mean of covariates method.

Disease-specific Effects of HR-MM in the Early Period

The number of patients with CML was significantly higher in the early period than in the mid and late periods. Therefore, we evaluated the disease-specific impact of HR-MM in the early period. As shown in Figures 3A and B, the presence

of HR-MM had an adverse impact on overall survival only in patients with CML, although HR-MM showed a similar adverse impact on the incidence of grade III to IV acute GVHD regardless of the underlying disease (Figure 3C, D). Of the 24 CML patients who died after HSCT with HR-MM, 23 died without relapse of CML, and 10 of these patients died without grade III to IV acute GVHD.

Impact of HR-MM at Each Locus

To evaluate the impact of HR-MM at each locus in the mid and early periods, we combined the 2 periods together to

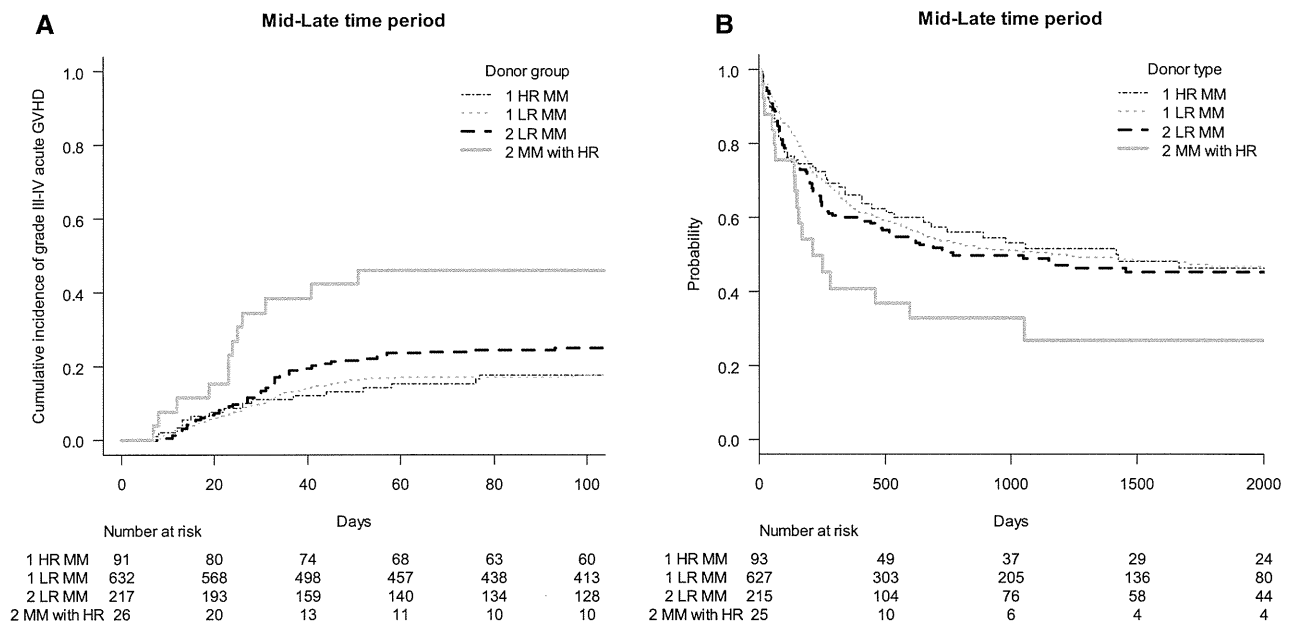


Figure 5. The cumulative incidence of grade III to IV acute GVHD (A) and adjusted overall survival (B) grouped according to the HLA mismatch between the donor and recipient in the mid or late time period. 1HR-MM, 1 high-risk mismatch; 1LR-MM, 1 low-risk mismatch; 2LR-MM, 2 low-risk mismatches; 2MM with HR, 2 allele mismatches including at least 1 HR-MM.

increase statistical power because the impact of HR-MM on acute GVHD and survival tended to be similar in these 2 time periods. The presence of HR-MMs at the HLA-A/B (HLA-A or -B), HLA-C, and HLA-DRB1 loci was not associated with significantly different survival compared with the LR-MM group (HR, 1.23; 95% CI, .76 to 1.98; $P = .41$; HR, .96; 95% CI, .65 to 1.44; $P = .86$; and HR, .95; 95% CI, .45 to 2.02; $P = .89$, respectively. Figure 4A). However, the incidence of grade III to IV acute GVHD was higher in patients who had HR-MM at the HLA-A/B locus than in those with LR-MM, although this difference was not statistically significant (HR, 1.78; 95% CI, .86 to 3.66; $P = .12$; HR, .63; 95% CI, .28 to 1.41; $P = .26$; and HR, .69; 95% CI, .15 to 3.12; $P = .63$ for HLA-A/B, HLA-C, and HLA-DRB1, respectively.) (Figure 4B).

Comparison of One HR-MM and Two LR-MMs

To evaluate whether a donor with 1 HR-MM or a donor with 2 LR-MMs should be preferred, we added patients with 2 LR-MMs and those with 2 allele mismatches including at least 1 HR-MM to the dataset, and we compared the outcome of HSCT from these donors with that of HSCT from a donor with 1 LR-MM as a reference in the combined mid and late periods.

The presence of 2 LR-MMs was associated with a significantly higher incidence of grade III to IV acute GVHD (HR, 1.44; 95% CI, 1.04 to 2.00; $P = .030$), but the impact of 1 HR-MM was not statistically significant (HR, .94; 95% CI, .56 to 1.59; $P = .83$) (Figure 5A). However, the impact of 2 LR-MMs was not associated with inferior survival. The HR for survival of 1 HR-MM and 2 LR-MMs were 1.05 (95% CI, .78 to 1.42; $P = .75$) and 1.12 (95% CI, .90 to 1.39; $P = .33$), respectively (Figure 5B).

On the other hand, the presence of 2 allele mismatches including at least 1 HR-MM was associated with an extremely poor outcome; HR, 3.61 (95% CI, 1.96 to 6.66; $P < .001$) for grade III to IV acute GVHD and HR, 2.02 (95% CI, 1.25 to 3.26; $P = .0040$) for overall survival. These results suggested that the impact of HR-MM may change according to the presence or absence of an additional allele mismatch. In fact, there was a statistically significant interaction between the presence of HR-MM and the presence of an additional allele mismatch ($P = .020$). The likelihood ratio test revealed that the prognostic value of Fine and Gray's proportional hazards model for acute GVHD was significantly improved by adding the interaction term to the model ($P = .024$).

DISCUSSION

In this study, we reevaluated the clinical impact of HR-MMs in unrelated HSCT. We confirmed that the presence of HR-MMs was associated with a significantly higher incidence of grade III to IV acute GVHD and significantly inferior survival in the early transplantation time period. However, in the mid and late periods, ie, after 2002, there was no statistically significant difference in overall survival or the incidence of grade III to IV acute GVHD between patients with HR-MMs and those with LR-MMs. The methods used for the statistical analyses were somewhat different than those in a previous study, but this is not the major reason for the different results, as the significant impact of HR-MMs on survival and acute GVHD was reproduced in the early time period. Another possible explanation is a bias caused by the availability of information about HR-MMs. After the publication of a paper that reported the importance of HR-MM, physicians may have tended to intensify prophylaxis against GVHD in unrelated HSCT with HR-MMs, and, thereby, the impact of HR-MMs might have become less significant. However, this is not the case because the impact of HR-MMs

was already not apparent in the mid time period, before the paper was published. We also considered that the difference in the underlying disease might have influenced the effect of HR-MMs. The proportion of patients with CML decreased from 30.7% in the early period to 10.4% and 3.6% in the mid and late periods, respectively. Therefore, we analyzed the impact of HR-MMs grouped according to the underlying disease in the early period. The effect of HR-MMs on survival was observed only in patients with CML (Figure 3A,B). However, HR-MMs had an adverse effect on the incidence of grade III to IV acute GVHD regardless of the underlying disease (Figure 3C,D). Therefore, the different effects of HR-MMs on the incidence of grade III to IV acute GVHD among the time periods could not be explained solely by the underlying diseases. We could not clarify the reason for this different effect, but the changes in the transplantation procedure, including prophylaxis against GVHD, might have reduced the clinical impact of HR-MM. In fact, the incidence of grade III to IV acute GVHD decreased from 42.6%, 16.8%, and 14.5% in the HR-MM, LR-MM, and MUD groups, respectively, in the early time period to 17.6%, 17.7%, and 10.6% in the mid or late period. Improved survival in patients who developed severe acute GVHD might also reduce the effect of HR-MMs on survival. The 1-year survival in patients who developed grade III to IV acute GVHD improved from 32.1% in the early period to 44.4% in the mid and late time periods. This change may have resulted from the progress in supportive care, including strategies against fungal or viral infections.

Another important finding is that the impact of HR-MM was significantly enhanced by the presence of an additional allele mismatch in the mid and late time periods. This fact may be explained by a hypothesis that the HR-MM biologically increases the graft-versus-host (GVH) reaction, but the recent improvement in GVHD prophylaxis has masked its effect, if HR-MM exists as a single allele mismatch, whereas the adverse impact of HR-MM is not suppressed even by recent methods of GVHD prophylaxis when an additional allele mismatch is present. Based on these findings, interaction terms should be incorporated into the statistical model when the impact of HR-MMs is analyzed in datasets that include HSCT with multiple allele mismatches.

A major limitation of this study is the small number of patients with HR-MMs, especially in the late time period. We cannot deny the possibility that an important effect of HR-MMs might be overlooked because of the poor statistical power. The lack of a significant difference in the incidence of grade III to IV acute GVHD between unrelated HSCT with HR-MMs at the HLA-A/B locus and HSCT with LR-MM should be interpreted with caution, because of the small number of patients. Furthermore, it was impossible to evaluate the effect of each mismatch combination, as the number of patients with each mismatch combination was most often fewer than 10. HR-MMs associated with at least a 20% incidence of grade III to IV acute GVHD in the mid and late periods included A*0206-A*0201 (4 of 14), A*0206-A*0207 (3 of 4), B*1501-B*1507 (1 of 1), C*0801-C*0303 (4 of 15), and C*1402-C*0304 (1 of 5), but the number of patients in each pair was too small to draw any definitive conclusions.

When we consider the impact of HR-MMs, especially at the HLA-C locus, we should also consider the effect of a killer immunoglobulin-like receptor ligand (KIR) mismatch [13,14]. Among the 50 patients with HR-MMs at the HLA-C locus in the mid and late periods, 20 had a KIR mismatch in the GVH direction, whereas 30 did not. The incidence of grade III to IV acute GVHD was 5% and 16.7%, respectively, but this

difference was not statistically significant ($P = .24$). The incidence of grade III to IV acute GVHD in the 21 patients who had LR-MMs and a KIR mismatch in the GVH direction was 15.0%. We could not conclude that a KIR mismatch had an impact in this study because of the small number of patients with a KIR mismatch in the GVH direction.

We should note that the results of the current study are applicable to patients who receive bone marrow graft after a myeloablative conditioning regimen. The impact of HR-MMs may change according to the stem cell source or the conditioning regimen. Therefore, further analyses are required to evaluate the impact of HR-MMs in peripheral blood stem cell transplantation and reduced-intensity conditioning transplantation.

In conclusion, this retrospective study revealed that the clinical impact of HR-MMs became less significant after 2002. Although HR-MMs may have a biological impact, their effect may be controlled by recent methods for GVHD prophylaxis when they exist as a single allele mismatch. It may still be prudent to avoid a donor with HR-MMs, especially at the HLA-A or -B locus, if a donor with the other mismatch combination is available. However, in the absence of MUD or an unrelated donor with a LR-MM, a donor with a single HR-MM could be a viable option for unrelated HSCT, and it is preferred over a donor with 2 LR-MMs. In addition, we should be aware that the clinical impact of risk factors may change over time periods, and therefore, we should repeatedly confirm the validity of risk factors.

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