



Full Length Article

Histocompatibility

Epitope Mismatch at HLA-DRB1 Associates with Reduced Relapse Risk in Cord Blood Transplantation for Standard-Risk Hematologic Malignancy



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Cord blood transplantation (CBT) is an attractive therapeutic option for patients with hematologic malignancies. CBT tolerates HLA mismatches between donors and recipients, but the HLA mismatches that generate graft-versus-tumor (GVT) effects remain unknown. Given that HLA molecules contain epitopes comprising polymorphic amino acids that determine their immunogenicity, we investigated associations between epitope-level HLA mismatches and relapse following single-unit CBT. A total of 492 patients with hematologic malignancies who underwent single-unit, T cell-replete CBT were included in this multicenter retrospective study. HLA epitope mismatches (EMs) were quantified using HLA matchmaker software from donor and recipient HLA-A, -B, -C, and -DRB1 allele data. Patients were dichotomized by median EM value and divided into 2 groups: patients who underwent transplantation in complete/partial remission (standard stage: 62.4%) and others (advanced stage: 37.6%). The median number of EMs in the graft-versus-host direction (GVH-EM) was 3 (range, 0 to 16) at HLA class

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I and 1 (range, 0 to 7) at HLA-DRB1. Higher HLA class I GVH-EM was associated with increased nonrelapse mortality (NRM) in the advanced stage group (adjusted hazard ratio [HR], 2.12; $P = .021$), with no significant advantage for relapse in either stage. In contrast, higher HLA-DRB1 GVH-EM was associated with better disease-free survival in the standard stage group (adjusted HR, .63; $P = .020$), which was attributed to lower relapse risk (adjusted HR, .46; $P = .014$). These associations also were observed even within HLA-DRB1 allele-mismatched transplantations in the standard stage group, indicating that EM might have an impact on relapse risk independent of allele mismatch. High HLA-DRB1 GVH-EM did not increase NRM in either stage. High HLA-DRB1 GVH-EM may lead to potent GVT effects and a favorable prognosis following CBT, especially in patients who underwent transplantation at the standard stage. This approach may facilitate appropriate unit selection and improve the overall prognosis of patients with hematologic malignancies who undergo CBT.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) can deliver long-term survival for patients with high-risk hematologic malignancies through graft-versus-tumor (GVT) effects; however, fatal immunopathology, such as graft-versus-host disease (GVHD) and opportunistic infections, has limited the indications for and development of allo-HSCT in this context [1,2]. To achieve rapid immunologic reconstitution and avoid nonrelapse mortality (NRM), HLA-matched donors are desirable [3]. Nevertheless, some patients lack suitable donors and cord blood transplantation (CBT) is an alternative option, given that mismatches at up to 2 loci within HLA-A, -B, and -DRB1 can be tolerated in achieving engraftment [4]. Although most patients have a range of available cord blood donors, the optimal donor HLA characteristics remain elusive.

Because post-transplantation relapse of underlying malignancy is suppressed in recipients of HLA-mismatched CBT [5–7], HLA mismatch likely contributes to the GVT effects in this context; however, to date, which HLA mismatches lead to GVT effects and improve survival are unclear. Higher numbers of allele mismatches (AMs) are associated with reduced relapse [6] but also carry a risk of NRM [8,9], which offsets the advantage. Regarding locus mismatch, retrospective studies from Japan showed that the number of AMs at HLA-DRB1 is associated with a lower relapse rate but also with an elevated risk of NRM [10,11]. In addition, no association between locus mismatch and relapse rate was detected in Caucasians [5]. Therefore, deeper investigation of whether HLA mismatches can more precisely predict GVT effects and identify patients who can benefit from substantial GVT effects via HLA mismatch are warranted.

HLA epitopes are defined as small clusters of polymorphic amino acids that are assumed to have immunogenic potentials [12]. HLA epitopes are determined *in silico* by structure prediction from amino acid sequences and functionally categorized into antibody-verified (AbV) and other epitopes, depending on their antibody reactivity *in vitro* [13,14]. HLA epitopes can provide clues to the immunogenicity of polymorphic HLA molecules. HLA matchmaker is an algorithm for determining the number of EMs between donors and recipients, depending on their HLA alleles [15]. The number of EMs is an established indicator of donor-specific antibody development in solid organ transplantation [16,17]. In allo-HSCT, a high number of EMs at HLA class II is associated with lower relapse rate following HLA-haploidentical HSCT with post-transplantation cyclophosphamide (haplo-PTCy) [18], suggesting that EM may also be a benchmark for selecting optimal HLA mismatch in allo-HSCT; however, this hypothesis has not been investigated in CBT recipients.

Here we report a multicenter retrospective study of patients with hematologic malignancies who underwent

single unit T cell-replete CBT to investigate whether the number of EMs is associated with post-transplantation relapse of the underlying malignancy, focusing on experimentally proven immunogenic epitopes (ie, AbV epitopes). As previous reports of GVT effects of CBT have mainly focused on patients who underwent transplantation while in remission [19–22], we hypothesized that such patients may be more susceptible to GVT effects through HLA-EM. Therefore, we also performed subgroup analysis in patients who underwent transplantation in complete remission (CR) or partial remission (PR) and others, to define which patients can benefit from HLA-EM in CBT.

METHODS

Inclusion Criteria

Data on adult patients age ≥ 16 years who underwent their first HSCT between January 2000 and December 2019 at hospitals belonging to the Kyoto Stem Cell Transplantation Group (KSCTG) were obtained through the Japanese Transplant Registry Unified Management Program, established by the Japanese Society for Transfusion Medicine and Cell Therapy and the Japanese Data Center for Hematopoietic Cell Transplantation [23]. Data from recipients of unrelated CBT were screened, and those who met the following criteria were excluded: (1) non-malignant underlying disease, (2) primary graft failure, (3) early relapse before engraftment, or (4) incomplete typing data for HLA-A, -B, -C, and -DRB1 loci. The study protocol complied with the standards outlined in the Declaration of Helsinki and was approved by the Ethical Committee of Kyoto University. Written informed consent was obtained from each patient or from a parent and/or legal guardian for patients age < 18 years.

HLA Genotyping and Determination of EM Number

Allelic genotyping of the HLA-A, -B, -C, and -DRB1 loci was performed as described previously [24]. A standard method using PCR-sequence specific oligonucleotide probes, which can detect HLA alleles with a frequency $> .1\%$ in the Japanese population, was used [25]. The number of HLA class I EMs was determined from donor and patient HLA allele data using HLA Matchmaker (version 4.0 for HLA class I eplet). Mismatches in both graft-versus-host (GVH) and host-versus-graft (HVG) directions were evaluated. The number of EMs specific to HLA-A, -B, and -C was determined as well. HLA class I epitopes shared among the HLA-A, B, and C molecules (interlocus epitopes) were not included. EMs in HLA-DRB1 were determined from HLA-DRB1 allele data using the HLA Matchmaker for HLA class II eplet (version 3.1). All EM calculations included only AbV epitopes. Patients were categorized into 2 groups—EM high and EM low—for analysis, using cutoff values of the median EM for the whole cohort ($\leq$ median and $>$ median).

Definitions of Covariates

Data on basic pretransplantation characteristics and post-transplantation clinical course were extracted from the KSCITG database. Disease stage at the time of HSCT was determined based on the Disease Risk Index with minor modifications [26]. Standard disease was defined as acute myelogenous leukemia/acute lymphoblastic leukemia in CR, myelodysplastic syndrome/myeloproliferative neoplasia in CR/PR/treatment naïve, lymphoma in CR/PR, myeloma in CR/PR, or chronic myelogenous leukemia in chronic phase; others were classified as advanced disease. Myeloablative conditioning and reduced-intensity conditioning were defined based on consensus criteria [27]. Infections were diagnosed by attending physicians, based on clinical symptoms and laboratory tests (generally, culture test for bacterial and fungal infections and seropositivity or PCR analysis for viral infections). Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and graded using consensus criteria [28,29].

Statistical Analyses

Patient characteristics were compared between groups using the chi-square test for categorical variables and the Mann-Whitney *U* test for continuous variables. Clinical endpoints included overall survival (OS), disease-free survival (DFS), relapse of underlying malignancy, NRM, aGVHD, cGVHD, and infections. DFS included relapse or NRM. Bacterial, fungal, and viral infections were assessed separately. All endpoints were measured from the date of HSCT; patients without events were censored at the last follow-up visit. For OS and DFS, survival curves were generated using the Kaplan-Meier method, and groups were compared using the log-rank test. For other endpoints, cumulative incidence curves were compared using the Gray test. Relapse and NRM were treated as reciprocal competing risks, and death without an event was treated as a competing risk for aGVHD, cGVHD, and infections.

Statistical analysis of prognostic factors was performed using a Cox proportional hazards regression model and the Fine-Gray proportional hazards models. Multivariate analyses included significant variables from univariate analyses and clinically relevant variables: age, patient sex, Hematopoietic Cell Transplantation Comorbidity Index, total nucleated cell count, ABO mismatch, GVHD prophylaxis, conditioning intensity, and year of HSCT, as well as disease stage for analysis of whole cohort. Allele match/mismatch was not included in covariates for adjustment because it was correlated with low/high EM status.

All statistical analyses were performed using the R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). All *P* values are 2-sided, and *P* < .05 was considered to indicate statistical significance.

RESULTS

Patient Characteristics and Determination of EM

A total of 492 patients were enrolled in this study. Patient characteristics are shown in Table 1. The median age at the time of CBT was 55 years (range, 16 to 77 years). Underlying diseases included acute myelogenous leukemia/myelodysplastic syndrome (*n* = 316; 64.2%), acute lymphoblastic leukemia (*n* = 76; 15.4%), lymphoma (*n* = 84; 17.1%), and chronic myelogenous leukemia/myeloproliferative neoplasia (*n* = 16; 3.3%). The majority of patient (62.4%; *n* = 307) had standard stage disease, and the other 37.6% (*n* = 185) had advanced stage disease. For the whole cohort, OS and DFS at 5 years were 52% (95% confidence interval [CI], 47% to 57%) and 48% (95% CI, 43%

Table 1

Patient Characteristics (N = 492)

Variable	Value	
Patient age, yr, median (range)	55 (16–77)	
Patient age, yr, n (%)		
16–55 yr	253	(51.4)
≥56 yr	239	(48.6)
Patient sex, n (%)		
Female	194	(39.4)
Male	298	(60.6)
HCT-CI, n (%)		
0	304	(61.8)
≥1	171	(34.8)
Diagnosis, n (%)		
AML/MDS	316	(64.2)
Others (ALL, ML, CML/MPN)	176	(35.8)
Disease stage, n (%)		
Standard	307	(62.4)
Advanced	185	(37.6)
TNCs, × 10 ⁷ /kg, n (%)		
<2.5	155	(31.5)
≥2.5	301	(61.2)
Sex mismatch, n (%)		
Matched	213	(43.3)
Male to female	92	(18.7)
Female to male	133	(27.0)
ABO mismatch, n (%)		
Matched	161	(32.7)
Minor mismatch	133	(27.0)
Major mismatch	122	(24.8)
Both	74	(15.0)
GVHD prophylaxis, n (%)		
CNI + MMF	291	(59.1)
CNI + MTX	154	(31.3)
Conditioning regimen, n (%)		
MAC	300	(61.0)
RIC	192	(39.0)
Year of HSCT, n (%)		
2000–2014	247	(50.2)
2015–	245	(49.8)
Follow-up, mo, median (range)	33 (2–164)	

HCT-CI indicates Hematopoietic Cell Transplantation Comorbidity Index; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; CML, chronic myelogenous leukemia; MPN, myeloproliferative neoplasia; TNC, total nucleated cells; CNI, calcineurin inhibitor; MTX, methotrexate; MMF, mycophenolate mofetil; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

to 53%), respectively. The cumulative incidence of relapse and NRM at 5 years were 26% (95% CI, 22% to 30%) and 27% (95% CI, 22% to 31%), respectively.

Among the whole cohort, the median number of EMs in the GVH direction (GVH-EM) was 3 (range, 0 to 16) at HLA class I and 1 (range, 0 to 7) at HLA-DRB1 (Figure 1A,B). The median EM in the HVG direction (HVG-EM) was 3 (range, 0 to 13) at HLA class I and 1 (range, 0 to 7) at HLA-DRB1 (Figure 1C,D). GVH-EM was weakly correlated with HVG-EM (HLA class I EM, *r* = .27; HLA-DRB1 EM, *r* = .28). HLA class I EM did not correlate with HLA-DRB1 EM (GVH-EM, *r* = −.2; HVG-EM, *r* = −.06). EM showed a wide range of distribution, even in patients with the same number of AMs (Figure 1A to D). Among transplant

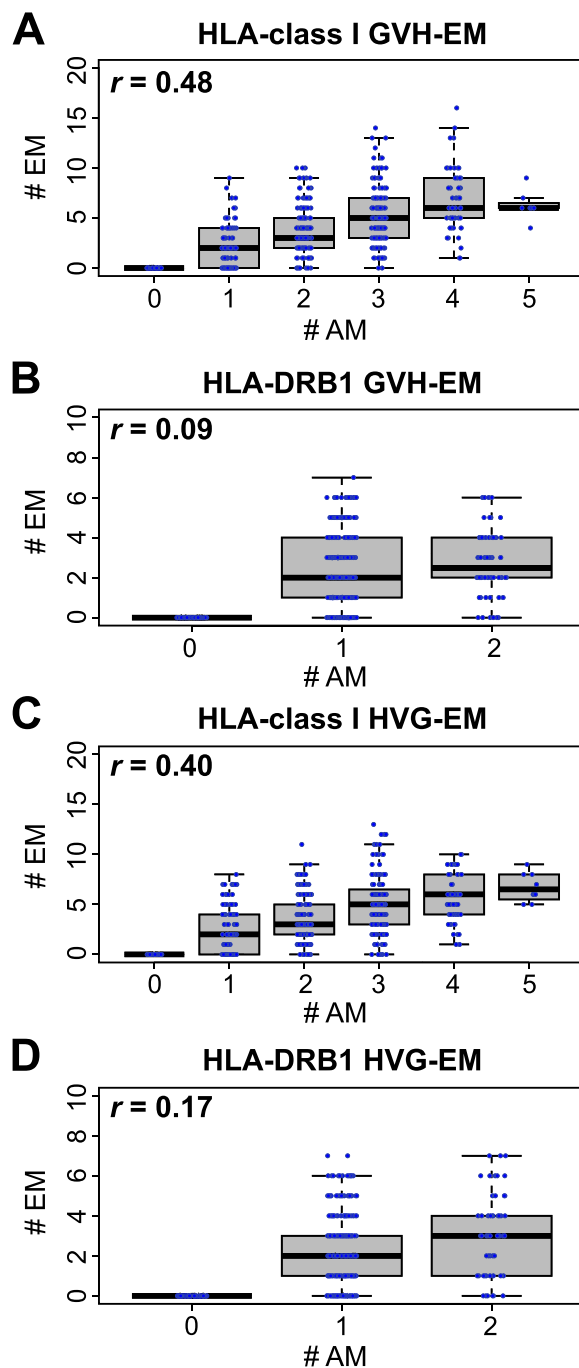


Figure 1. The relationships between EMs and AMs. Boxplots of numbers of EMs against different AMs for HLA class I GVH-EM (A), HLA-DRB1 GVH-EM (B), HLA class I HVG-EM (C), and HLA-DRB1 HVG-EM (D). Dots denote EM values. Within each box, horizontal black lines represent median values, and boxes extend from the 25th to the 75th percentile of each group's distribution of values. Pearson's correlation coefficient values (r) are shown.

recipients with at least 1 AM, EMs correlated with AMs in HLA class I (GVH-EM, $r = .48$; HVG-EM, $r = .40$) (Figure 1A,C), whereas little correlation was observed in HLA-DRB1 (GVH-EM, $r = .09$; HVG-EM, $r = .17$) (Figure 1B,D). Distribution of EM did not differ according to disease stage (Supplementary Figure S1). These results provide a rationale for investigating the clinical significance of EMs compared with AMs in CBT.

HLA-Class I GVH-EM is not Associated with Prognostic Advantages

First, we investigated whether HLA class I GVH-EM is associated with patient outcomes (Figure 2, Tables 2 and 3, Supplementary Table S1). DFS was similar between the high and low HLA class I GVH-EM groups in the whole cohort (hazard ratio [HR], 1.04; 95% CI, .80 to 1.35; $P = .77$) (Figure 2A). This was consistent for both standard stage and advanced stage patients (Figure 2B,C).

We next compared the incidence of relapse and NRM by disease stage (Figure 2D to G). In standard stage patients, relapse and NRM rate were similar in those with high HLA class I GVH-EM and those with low HLA class I GVH-EM (Figure 2D,E). Interestingly, in patients in the advanced stage group, high HLA class I GVH-EM was associated with lower relapse rate, although the difference did not reach statistical significance (HR, .59; 95% CI, .35 to 1.00; $P = .051$) (Figure 2F). High HLA class I GVH-EM also was associated with higher NRM (HR, 2.29; 95% CI, 1.32 to 4.00; $P < .01$) (Figure 2G), which was confirmed in univariate and multivariate analyses (adjusted HR, 2.12; 95% CI, 1.12 to 4.02; $P = .021$) (Tables 2 and 3).

We also performed univariate analysis for GVH-EM at HLA-A, -B, and -C separately (Supplementary Table S2). HLA-B GVH-EM was significantly associated with higher NRM rate in the whole cohort (HR, 1.54; 95% CI, 1.07 to 2.22; $P = .019$) and in patients in the advanced stage group (HR, 1.94; 95% CI, 1.16–3.24; $P = .012$). No individual HLA class I loci were correlated with relapse rate.

These results imply that HLA class I GVH-EM may be associated with GVT effects but also has negative impacts on NRM, resulting in no overall prognostic advantage.

HLA-DRB1 GVH-EM is Associated with Lower Relapse Rate in Standard Stage Patients

We then investigated the impacts of GVH-EM at HLA-DRB1 (Figure 3, Tables 2 and 3, Supplementary Table S1). There was no skewed distribution of patient characteristics between the lower and higher HLA-DRB1 GVH-EM groups (Supplementary Table S3). Slightly higher DFS was observed in the high HLA-DRB1 GVH-EM group in the whole cohort (HR, .80; 95% CI, .62 to 1.05; $P = .11$) (Figure 3A). Notably, the difference was significant only in standard stage patients (HR, .69; 95% CI, .47 to 1.00; $P = .049$) (Figure 3B,C), where high HLA-DRB1 GVH-EM was significantly associated with lower relapse rate (HR, .49; 95% CI, .28 to .87; $P = .015$), but patients with high and low HLA-DRB1 GVH-EM had similar NRM rates (Figure 3D,E, Table 2). Furthermore, multivariate analysis identified high HLA-DRB1 EM as an independent favorable factor for DFS (adjusted HR, .63; 95% CI, .42 to .93; $P = .020$) and relapse (adjusted HR, .46; 95% CI, .25 to .86; $P = .014$) in standard stage patients, although the impact on OS was not statistically significant (adjusted HR, .69; 95% CI, .46 to 1.04; $P = .078$) (Table 3). Even when the number of HLA-DRB1 AMs (0 or 1 versus 2) was included in multivariate analysis, high HLA-DRB1 GVH-EM remained an independent prognostic factor for DFS (adjusted HR, .67; 95% CI, .45 to .99; $P = .046$) and relapse (adjusted HR, .50; 95% CI, .27 to .93; $P = .028$) in standard stage patients.

We also performed a multivariate analysis using EM as a continuous variable (Supplementary Table S4). The mismatch-dependent association between HLA-DRB1 GVH-EM and relapse was of borderline significance (adjusted HR, .85; 95% CI, .72 to 1.01; $P = .073$). Moreover, the association between high HLA-DRB1 GVH-EM and lower relapse rate was not

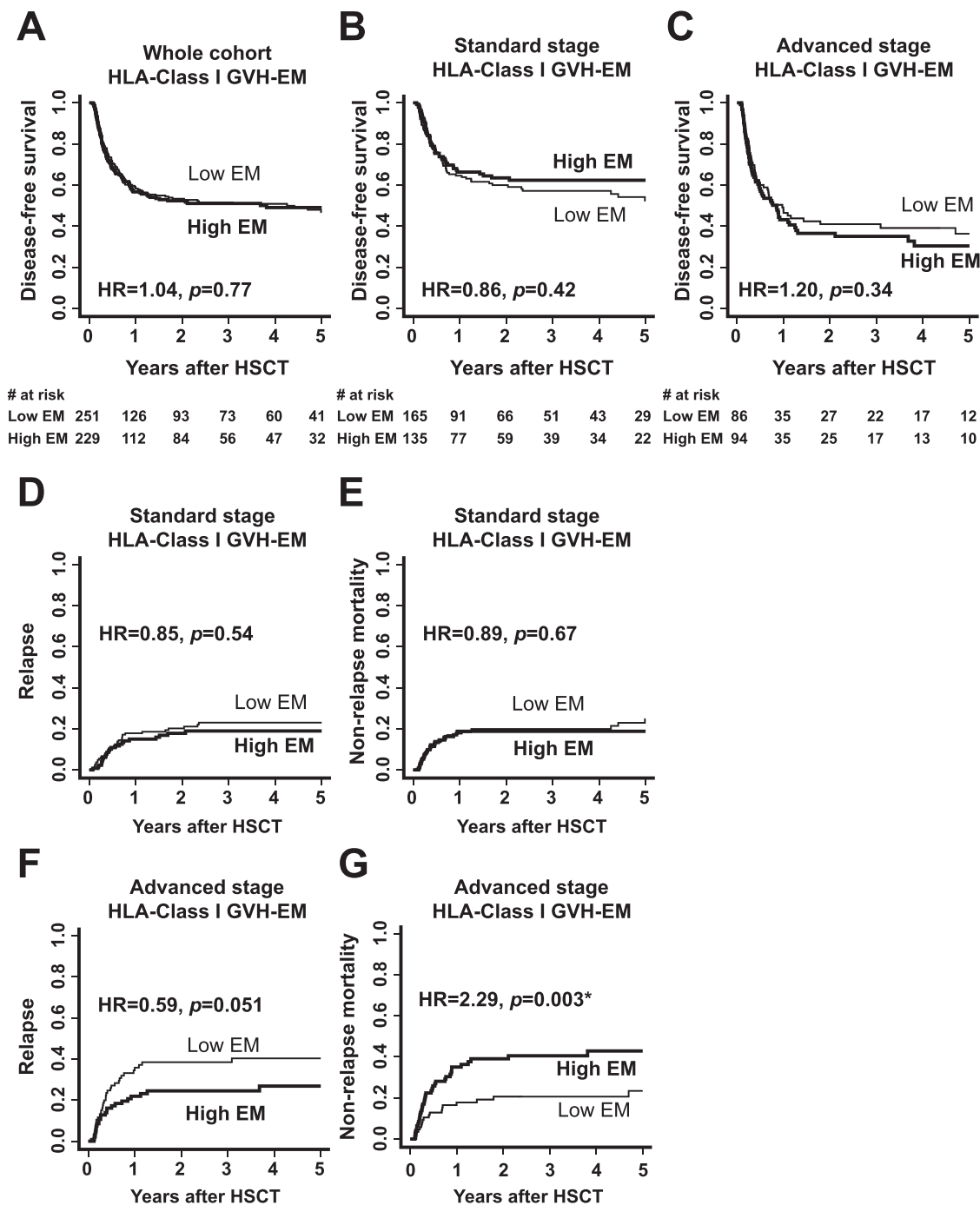


Figure 2. Outcomes following CBT according to HLA class I GVH-EM. DFS in low and high HLA class I GVH-EM groups for the whole cohort ($n = 492$) (A), patients who underwent transplantation in standard stage ($n = 307$) (B), and patients who underwent transplantation in advanced stage ($n = 185$) (C). (D-G) Relapse and NRM according to HLA class I GVH-EM by disease stage: standard stage (D,E), advanced stage (F,G).

evident in advanced stage patients (Figure 3F). We performed subgroup analysis stratified by disease stage and underlying disease (Supplementary Table S5) and found a significant association between high HLA-DRB1 GVH-EM and decreased relapse in the patients with acute myelogenous leukemia/myelodysplastic syndrome who underwent transplantation in the standard stage (adjusted HR, .22; 95% CI, .08 to .63; $P = .004$) (Supplementary Table S5, Supplementary Figure S2C). This subgroup also had better DFS (adjusted HR, .42; 95%

CI, .24 to .73; $P = .002$) and OS (adjusted HR, .47; 95% CI, .26 to .83; $P = .010$) with a similar rate of NRM (Supplementary Figure S2A,B,D). High HLA-DRB1 GVH-EM was not associated with decreased relapse in the patients with other diseases who underwent transplantation in the standard stage and patients who did so in the advanced stage, regardless of the underlying disease. In contrast to HLA class I GVH-EM, high HLA-DRB1 EM was not significantly associated with increased NRM in advanced stage patients (Figure 3G). Thus, high HLA-

Table 2
Univariate Analysis of EM in the GVH Direction

Variable	All Patients (N = 492)		Standard Stage (N = 307)		Advanced Stage (N = 185)	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
HLA class I GVH-EM						
OS	1.24 (.94-1.62)	.12	.98 (.67-1.44)	.93	1.53 (1.04-2.26)	.032*
DFS	1.04 (.80-1.35)	.77	.86 (.60-1.24)	.42	1.20 (.83-1.73)	.34
Relapse	.74 (.51-1.08)	.12	.85 (.50-1.43)	.54	.59 (.35-1.00)	.051
NRM	1.41 (.98-2.02)	.06	.89 (.54-1.49)	.67	2.29 (1.32-4.00)	.003*
aGVHD grade II-IV	1.20 (.91-1.58)	.19	1.40 (.99-1.98)	.059	.93 (.60-1.44)	.73
aGVHD grade III-IV	1.51 (.92-2.48)	.10	1.58 (.82-3.02)	.17	1.37 (.64-2.93)	.41
cGVHD	1.16 (.82-1.64)	.41	1.36 (.88-2.11)	.17	.89 (.50-1.58)	.69
HLA-DRB1 GVH-EM						
OS	.83 (.63-1.09)	.18	.72 (.48-1.07)	.11	.94 (.64-1.38)	.75
DFS	.80 (.62-1.05)	.11	.69 (.47-1.00)	.049*	.96 (.66-1.39)	.81
Relapse	.79 (.54-1.15)	.21	.49 (.28-.87)	.015*	1.24 (.74-2.09)	.41
NRM	.87 (.60-1.26)	.46	.98 (.59-1.63)	.94	.76 (.45-1.28)	.30
aGVHD grade II-IV	1.40 (1.07-1.84)	.015*	1.30 (.92-1.85)	.13	1.57 (1.01-2.44)	.043*
aGVHD grade III-IV	1.44 (.89-2.34)	.14	1.11 (.58-2.11)	.76	2.06 (.97-4.37)	.060
cGVHD	1.24 (.88-1.76)	.22	1.30 (.84-2.01)	.25	1.15 (.65-2.04)	.64

* Significant P value.

Table 3
Multivariate Analysis of EM in the GVH Direction

Variable	All Patients (N = 492)		Standard Stage (N = 307)		Advanced Stage (N = 185)	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
HLA class I GVH-EM						
OS	1.16 (.88-1.54)	.30	.89 (.59-1.34)	.58	1.52 (1.00-2.31)	.049*
DFS	.97 (.74-1.27)	.81	.81 (.54-1.20)	.29	1.19 (.8-1.77)	.40
Relapse	.70 (.47-1.05)	.088	.79 (.42-1.48)	.46	.62 (.35-1.12)	.12
NRM	1.39 (.95-2.02)	.087	.94 (.56-1.57)	.80	2.12 (1.12-4.02)	.021*
HLA-DRB1 GVH-EM						
OS	.87 (.65-1.15)	.34	.69 (.46-1.04)	.078	1.09 (.73-1.63)	.68
DFS	.82 (.62-1.07)	.14	.63 (.42-.93)	.020*	1.03 (.7-1.52)	.88
Relapse	.79 (.53-1.17)	.23	.46 (.25-.86)	.014*	1.20 (.68-2.13)	.53
NRM	.92 (.63-1.34)	.67	.96 (.58-1.59)	.87	.83 (.48-1.44)	.52

Univariate analysis of other covariates is presented in [Supplementary Table S1](#).

* Significant P value.

DRB1 GVH-EM may be associated with potent GVT effects, particularly in standard stage patients, while not leading to increased NRM.

We next investigated whether specific HLA-DRB1 EMs were associated with the outcomes among standard stage patients. The univariate analysis was performed for 16 HLA-DRB1 EMs observed at a frequency >5% ([Supplementary Table S6](#)). In the epitope notation system, a number indicates the sequence position of the polymorphic amino acid residues, and associated residues are listed with a standard single-letter code [30]. With P values adjusted for multiple testing using the Benjamini-Hochberg false discovery rate control at .05 [31], we found no significant association. However, notably, mismatch at 47F epitope showed a trend toward better DFS (HR, .46; 95% CI, .22 to .99; $P = .048$) and lower relapse rate (HR, .27; 95% CI, .06 to 1.09; $P = .066$) ([Supplementary Figure S3](#)).

HLA-DRB1 GVH-EM Is Independent of AM for Stratification of Relapse Risk

Whereas HLA class I GVH-EM number increased moderately in proportion to the number of AMs, HLA-DRB1 GVH-EM

numbers were highly variable in both 1- and 2-allele-mismatched transplants ([Figure 1A,B](#)). Therefore, we investigated whether HLA-DRB1 GVH-EM had a superior prognostic impact compared with HLA-DRB1 GVH-AM. Patients with HLA-DRB1 GVH-AM were divided into 2 groups by the EM cutoff value as in the above analysis (≤ 1 and > 1). Notably, even in the patients with HLA-DRB1 GVH-AM, the high HLA-DRB1 GVH-EM group showed better DFS (HR, .62; 95% CI, .40 to .95; $P = .027$) and a lower relapse rate (HR, .42; 95% CI, .23 to .79, $P = .007$) compared with the low HLA-DRB1 GVH-EM group ([Figure 4A,B](#)). Furthermore, higher HLA-DRB1 GVH-EM did not influence the NRM rate (HR, .96; 95% CI, .52 to 1.77; $P = .91$) ([Figure 4C](#)). Whereas the relapse rate was higher in AM(+)/EM(-) group compared with the AM(-)/EM(-) group ([Figure 4](#)), the difference was not statistically significant ($P = .27$, Gray's test). Thus, the impact of HLA-DRB1 GVH-EM on the GVT effect may be independent of the effect of AM.

Impacts of GVH-EM on Immunologic Complications

To elucidate the differential impacts of HLA class I and HLA-DRB1 GVH-EM on NRM, we compared the incidence rates of

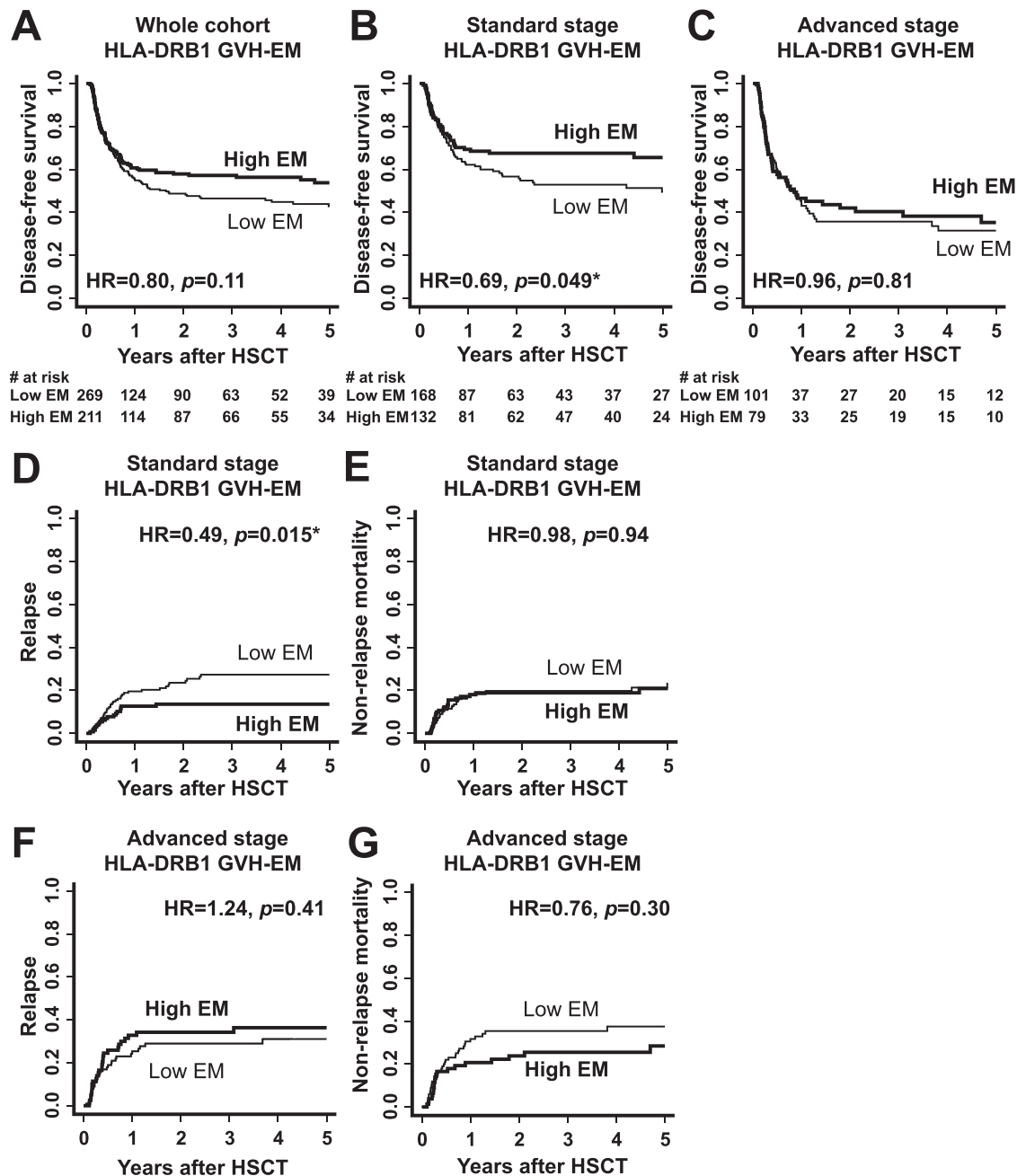


Figure 3. Outcomes following CBT depending on HLA-DRB1 GVH-EM. DFS in low and high HLA-DRB1 GVH-EM groups for the whole cohort (A), patients who underwent transplantation in standard stage (B), and patients who underwent transplantation in advanced stage (C). (D-G) Relapse and NRM according to HLA-DRB1 GVH-EM by disease stage: standard stage (D,E); advanced stage (F,G).

GVHD and infectious complications between patients receiving high GVH-EM transplants and those receiving low GVH-EM transplants (Figures 5 and 6). High HLA class I GVH-EM was associated with a slightly higher incidence of grade II-IV and grade III-IV aGVHD ($P = .19$ and $.10$, respectively) (Figure 5A,B). Accordingly, GVHD-related mortality was greater in patients with high HLA class I GVH-EM ($P = .019$), although the numbers included in this analysis were limited (Supplementary Table S7). High HLA-DRB1 GVH-EM was associated with a significantly higher incidence of grade II-IV aGVHD ($P = .015$), whereas the incidence of grade III-IV aGVHD was similar in patients with high HLA-DRB1 GVH-EM and patients with low HLA-DRB1 GVH-EM (Figure 5C,D). These

results indicate that high HLA-DRB1 GVH-EM is correlated only with moderate aGVHD. Neither HLA class I nor HLA-DRB1 GVH-EM was correlated with the incidence of cGVHD (Figure 5E,F).

Regarding infectious complications, high HLA class I GVH-EM was significantly associated with a higher incidence of bacterial infections ($P = .006$), whereas high HLA-DRB1 GVH-EM was not (Figure 6A,B). The incidence of fungal infections did not differ according to either HLA class I or HLA-DRB1 GVH-EM (Figure 6C,D). The incidence of viral infections was slightly higher in both HLA class I and HLA-DRB1 GVH-EM patients, although the difference was not significant (Figure 6E,F).

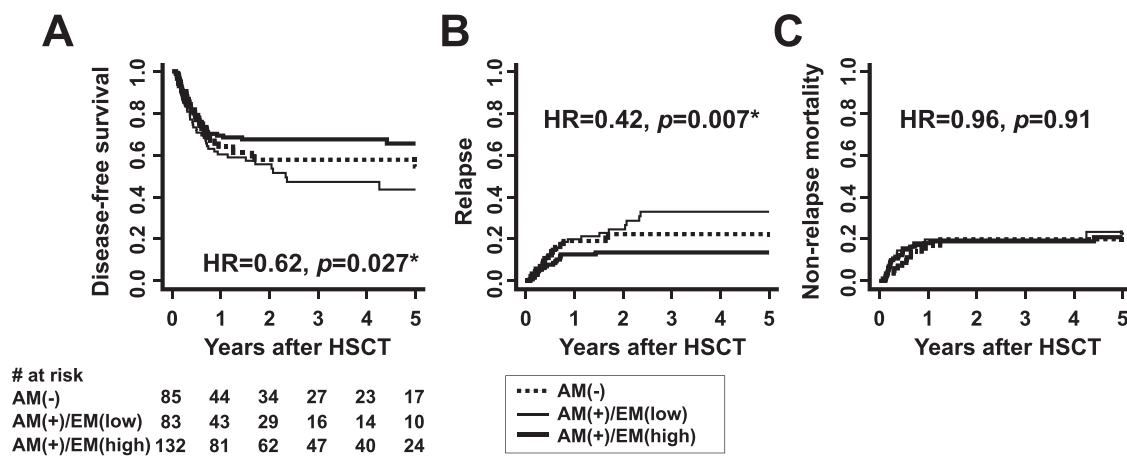


Figure 4. Outcomes depending on HLA-DRB1 GVH-EM in HLA-DRB1 allele-mismatched CBT. Comparisons of DFS (A), relapse (B), and NRM (C) in 3 groups of standard stage patients: those who received allele-mismatched and low HLA-DRB1 GVH-EM CBT [AM(+)/EM(low)], allele-mismatched and high HLA-DRB1 GVH-EM CBT [AM(+)/EM(high)], and allele-matched CBT [AM(-)/EM(low)]. HRs and *P* values of the comparison between the AM(+)/EM(low) and AM(+)/EM(high) groups are shown.

Although higher GVH-EM may interfere with immune reconstitution following transplantation, the negative impacts of HLA-DRB1 GVH-EM were not significant.

HVG-EM and Major Outcomes

Given the association of HVG-EM with relapse risk and survival in haplo-PTCy [32], we evaluated the impacts of HVG-EM on major patient outcomes (Supplementary Table S8). High HLA-B HVG-EM was associated with lower OS (HR, 1.56; 95% CI, 1.06 to 2.29; *P* = .023) and DFS (HR, 1.45; 95% CI, 1.01 to 2.10; *P* = .046) in standard stage patients; however, no other significant associations specific for relapse or NRM were observed.

DISCUSSION

This multicenter retrospective cohort study revealed 2 major findings. First, high HLA-DRB1 GVH-EM was associated with a lower relapse rate without increased severe immunopathology, resulting in improved DFS, particularly in standard stage patients. Second, high HLA class I GVH-EM was associated with higher risk of NRM, particularly in advanced stage patients, and showed no survival advantage. Our study is the first to highlight the clinical relevance of HLA-EM in CBT.

A positive association between high HLA class II GVH-EM and lower relapse rate was previously reported in haplo-PTCy [18]. Another study showed a trend toward relapse protection with HLA-DRB1 GVH-EM (HR, .78; 95% CI, .63 to .96; *P* = .019), although the authors did not conclude a significant association because the significance level was set as .01 [33]. Based on these observations, we speculate that high HLA class II GVH-EM can exert potent GVT effects in any type of allo-HSCT. CD4⁺ T cells may contribute to GVT effects by HLA class II EM, given that donor-derived CD4⁺ T cells can show cross-restriction to mismatched HLA class II in the early phase after allo-HSCT [34,35]. Further, although HLA epitopes were discovered primarily as B cell epitopes, they also can activate T cells through T cell receptor-HLA interaction [36,37]. It is assumed that the immunogenicity of HLA epitopes is nonuniform [38]. Our results support this paradigm in that GVT effects by HLA EM might not increase in proportion to the number of EMs (Supplementary Table S4). Therefore, identification of highly immunogenic epitopes that can specifically lead to potent T cell alloreactivity might improve prediction sensitivity and

selection algorithms for cord blood, depending on HLA-EM. Our results suggest that 47F epitope on tumor cells might favor donor-derived T cell reactivation, as 47F epitope is located in the pocket 7 of peptide-binding groove, and amino acid polymorphism in this position affects T-cell recognition and is known to be associated with susceptibility to autoimmune diseases [39–41]. However, as the study size was limited to sort out the degree of contribution of each epitopes taking linkage disequilibrium between epitopes into account, further investigation with future studies is needed. In this study, a clear association between high HLA-DRB1 GVH-EM and reduced relapse was detected only in patients who underwent transplantation in standard stage and not in those who did so in advanced stage. These results suggest that GVT effects through HLA EM can suppress minor populations of tumor cells, whereas substantial residual disease at transplant cannot be extinguished. This hypothesis warrants further exploration in a larger study, which would provide precise evaluation on the strength of GVT effects through HLA EM.

There is a correlation between HLA-EM and HLA-AM, and our findings have similarities with those of several previous Japanese studies based on HLA-AM. Atsuta et al. [10] and Kanda et al. [11] reported that double HLA-DRB1 mismatch was associated with both a lower relapse rate and a higher risk of NRM. Konuma et al. [42] reported that single or double HLA-DRB1-mismatched recipients showed decreased relapse with a similar NRM rate, although their sample size was small (*n* = 107). However, our study further demonstrates that HLA-DRB1 GVH-EM can be used to stratify the relapse risk of HLA-DRB1-mismatched recipients, and that high HLA-DRB1 GVH-EM was permissive in terms of NRM. Compared with HLA-DRB1 AM, the association of HLA-DRB1 GVH-EM with relapse in CBT may be more sensitive.

Our results indicate that high HLA class I GVH-EM may have negative effects on patient prognosis after CBT. A higher incidence of bacterial and viral infections indicates that high HLA class I GVH-EM may be associated with delayed immune reconstitution [43]. In addition, the associations between high HLA class I EM and severe aGVHD may contribute to increased NRM. No relationship between cGVHD and EM was detected in our cohort, possibly attributable to the low risk of cGVHD in patients receiving CBT [44]. Further studies with larger numbers of events are needed to verify these associations.

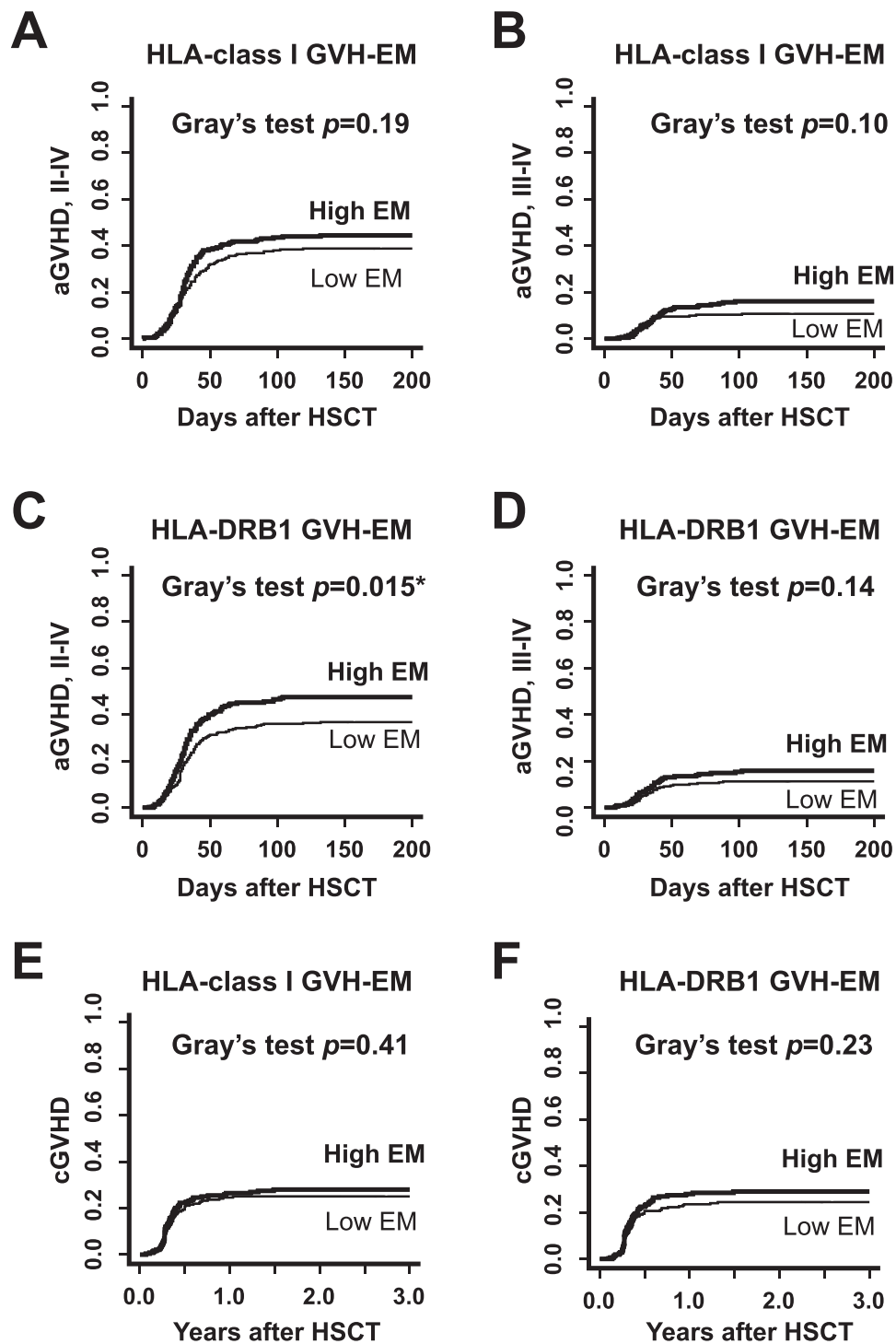


Figure 5. Incidence rates of GVHD depending on GVH-EM. Comparisons of the incidence rates of acute GVHD in the whole cohort, according to HLA ;class I and HLA-DRB1 GVH-EM, for all grades (II-IV) (A,C) and severe (grade III-IV) (B,D). (E,F) Comparisons of incidence rates of all severity of chronic GVHD according to HLA class I GVH-EM (E) and HLA-DRB1 GVH-EM (F).

A major limitation of our study is that we could not evaluate HLA-DQB1 or -DPB1 owing to a lack of typing data. Because the matching probability for HLA-DRB1 and -DQB1 is up to 89% in unrelated bone marrow transplantation in Japan [45], HLA-DQB1 EM is an important confounding factor that may underscore the effect of HLA-DRB1 EM. In addition, HLA-DPB1 mismatch is independently associated with reduced relapse in

CBT [46]. Further investigations including HLA-DQB1 and DPB1 are needed. Second, given the substantial diversity in HLA alleles and haplotypes among populations, whether our results are applicable in different populations requires verification. A third limitation is resolution of HLA typing. The method used in this study is considered low-to-intermediate resolution typing. Although optimized for alleles with frequencies of

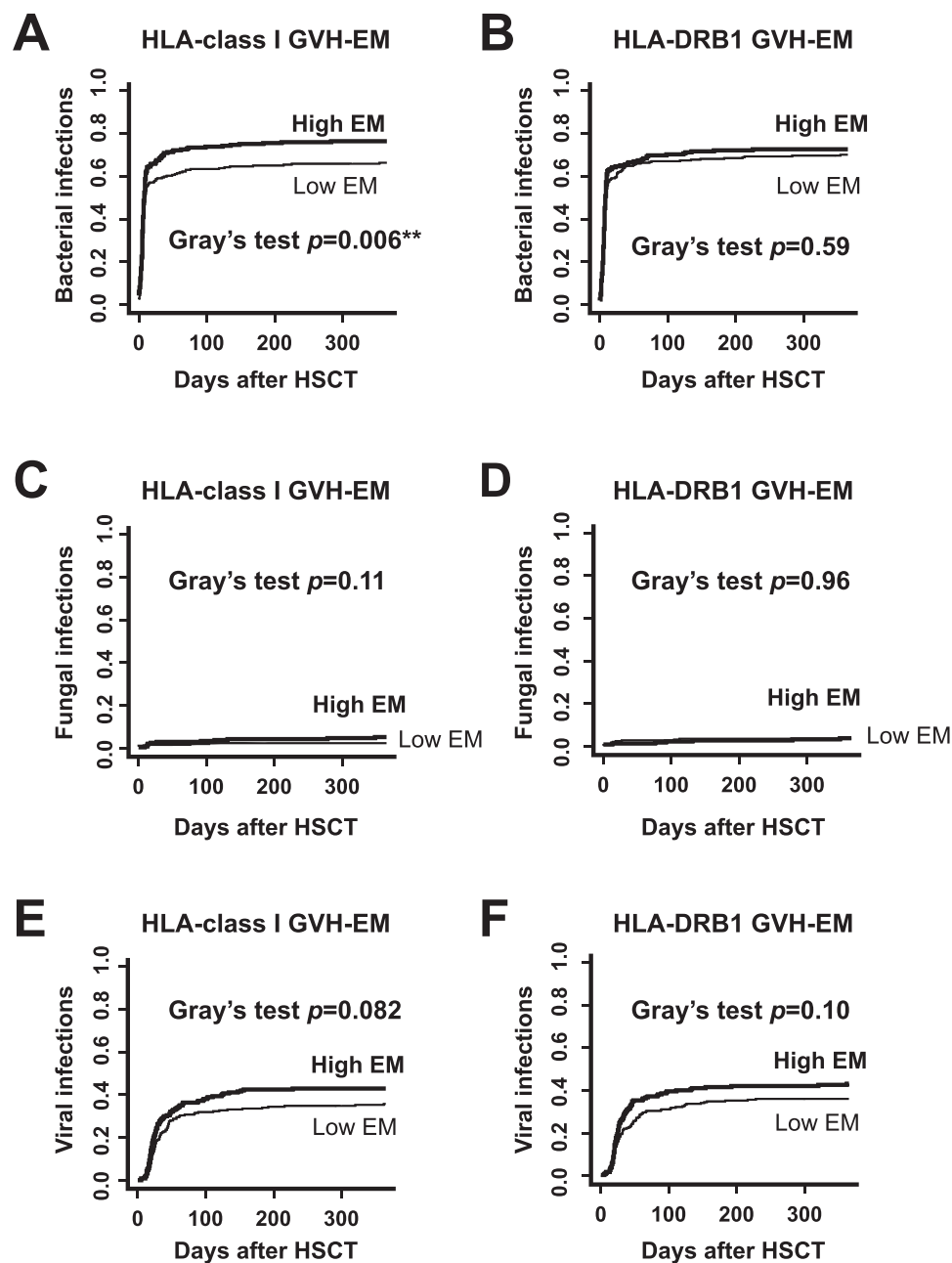


Figure 6. Infection incidence rates according to GVH-EM. Comparisons of the incidence rates of infections in the whole cohort according to HLA class I and HLA-DRB1 GVH-EM: (A,B) bacterial infections; (C,D) fungal infections; (E,F) viral infections.

>.1% in the Japanese population [25], errors may still occur at the allele level, which potentially can affect EM estimation.

In conclusion, high HLA-DRB1 GVH-EM may lead to potent GVT effects and a favorable prognosis following CBT, whereas high HLA class I GVH-EM should be avoided to reduce the likelihood of infections and NRM following CBT. This approach may facilitate appropriate unit selection and improve the overall prognosis of patients with hematologic malignancies who undergo CBT.

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Authorship statement: M.M-F. designed the study, reviewed and analyzed data, and wrote the manuscript; T.S. conceived the primary hypothesis, designed the study, reviewed the data, and wrote the manuscript; T. Iemura, Y.A., J.K., K. Yamashita, T. Kondo, and A.T-K. interpreted data and revised the manuscript; K.O., Y.U., O.Y., N. Anzai, T.M., T. Ishikawa, Y. Otsuka, A.Y., N. Yuhji, K.I., A.O., M.I., Y. Okamoto, T. Kitano, T. Ikeda, S.K., T.A., K. Yago, M.W., M.N., M.T., T.T., R.Y., N. Arima, N. Yoshinaga, and M.H. contributed to data collection and provided fundamental critiques on the manuscript.

Data availability: Raw data were created by KSCTG. The data that support the findings of this study are available on request from the corresponding author.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.tct.2023.03.002.

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Supplementary Figure Legends

Supplementary Figure 1. Distribution of EM by disease stage

Histograms of EM in patients receiving CBT with standard and advanced stage disease for (A) HLA-class I GVH-EM, (B) HLA-DRB1 GVH-EM, (C) HLA-class I HVG-EM, and (D) HLA-DRB1 HVG-EM. P-values of Mann–Whitney U test between patients receiving CBT with standard and advanced stage disease are shown.

Supplementary Figure 2. Outcomes depending on HLA-DRB1 GVH-EM in patients with AML/MDS transplanted in the standard stage

(A) overall survival, (B) disease-free survival, (C) relapse, and (D) non-relapse mortality according to HLA-DRB1 GVH-EM within patients with AML/MDS transplanted in the standard stage (n = 197) are shown.

Supplementary Figure 3. Outcomes depending on HLA-DRB1 47F epitope mismatch

Comparisons of (A) disease-free survival, (B) relapse and (C) non-relapse mortality between standard stage patients who received HLA-DRB1 47F epitope mismatched CBT (47F+) and those who received HLA-DRB1 47F epitope matched CBT (47F–).

Supplementary Table 1. Univariate analysis of clinical factors in the whole cohort

		DFS		Relapse		NRM	
		HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Patient age (years)	16–55	1.00 (ref)		1.00 (ref)		1.00 (ref)	
	56–	1.23 (0.95–1.59)	0.12	0.78 (0.54–1.14)	0.20	1.72 (1.19–2.48)	0.004*
Patient sex	Female			1.00 (ref)		1.00 (ref)	
	Male	1.33 (1.02–1.75)	0.039*	1.14 (0.78–1.68)	0.49	1.39 (0.94–2.04)	0.095
HCT-CI	0			1.00 (ref)		1.00 (ref)	
	≥ 1	1.79 (1.37–2.33)	<0.001*	1.25 (0.85–1.82)	0.26	1.98 (1.37–2.85)	<0.001*
Diagnosis	AML/MDS						
	Others	1.12 (0.86–1.47)	0.39	1.02 (0.7–1.5)	0.91	1.18 (0.82–1.71)	0.37
Disease stage	Standard						
	Advanced	1.90 (1.47–2.46)	<0.001*	1.73 (1.20–2.50)	0.003*	1.64 (1.14–2.35)	0.008*
TNC, $\times 10^7/\text{kg}$	≥ 2.5			1.00 (ref)		1.00 (ref)	
	< 2.5	0.83 (0.62–1.11)	0.20	0.86 (0.57–1.30)	0.48	0.85 (0.57–1.28)	0.45
Sex mismatch	Matched						
	M to F	0.85 (0.59–1.24)	0.40	0.66 (0.38–1.14)	0.13	1.14 (0.69–1.88)	0.61
	F to M	1.05 (0.77–1.44)	0.75	0.75 (0.47–1.20)	0.23	1.36 (0.88–2.09)	0.16
ABO mismatch	Matched						
	Minor mis	1.21 (0.86–1.69)	0.27	1.68 (1.02–2.78)	0.042*	0.81 (0.5–1.29)	0.37
	Major mis	1.09 (0.77–1.53)	0.64	1.51 (0.91–2.51)	0.11	0.82 (0.52–1.30)	0.40

	Both	1.00 (0.66– 1.51)	0.99		1.62 (0.93– 2.83)	0.091	0.63 (0.34– 1.17)	0.14
GVHD prophylaxis	CNI+MMF							
	CNI+MTX	0.88 (0.66– 1.18)	0.39		0.96 (0.63– 1.47)	0.87	0.87 (0.58– 1.3)	0.50
Conditioning regimen	MAC							
	RIC	0.97 (0.74– 1.26)	0.81		0.85 (0.58– 1.25)	0.41	1.14 (0.79– 1.63)	0.48
Year of HSCT	2000–2014							
	2015–	0.96 (0.73– 1.25)	0.75		0.70 (0.48– 1.02)	0.060	1.19 (0.83– 1.71)	0.35

Abbreviations: DFS, disease-free survival; NRM, non-relapse mortality; HR, hazard ratio; CI, confidence interval; HCT-CI, hematopoietic cell transplant-comorbidity index; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; TNC, total nucleated cell count; CNI, calcineurin inhibitor; MTX, methotrexate; MMF, mycophenolate mofetil; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation.

Supplementary Table 2. Univariate analysis of EM at HLA-A/B/C in the GVH direction

	All patients (N = 492)			Standard stage (N = 307)			Advanced stage (N = 185)	
	HR (95% CI)	p		HR (95% CI)	p		HR (95% CI)	p
HLA-A GVH-EM								

(Median 0, range 0–9)						
OS	1.06 (0.79–1.43)	0.68	0.90 (0.58–1.39)	0.63	1.15 (0.77–1.72)	0.48
DFS	1.01 (0.76–1.34)	0.94	0.90 (0.6–1.35)	0.61	1.03 (0.70–1.52)	0.86
Relapse	0.90 (0.60–1.35)	0.62	1.17 (0.67–2.03)	0.58	0.63 (0.34–1.15)	0.13
NRM	1.10 (0.75–1.62)	0.62	0.70 (0.38–1.29)	0.26	1.53 (0.91–2.58)	0.11
aGVHD, II–IV	1.08 (0.80–1.44)	0.63	1.26 (0.87–1.82)	0.22	0.84 (0.51–1.36)	0.47
aGVHD, III–IV	1.47 (0.89–2.42)	0.13	1.83 (0.95–3.51)	0.070	1.06 (0.49–2.29)	0.88
cGVHD	1.20 (0.83–1.74)	0.34	1.37 (0.86–2.20)	0.19	0.99 (0.54–1.81)	0.98
HLA-B GVH-EM (Median 1, range 0–7)						
OS	1.13 (0.86–1.49)	0.39	1.18 (0.80–1.75)	0.40	1.14 (0.77–1.71)	0.51
DFS	1.08 (0.83–1.41)	0.56	1.04 (0.72–1.51)	0.82	1.18 (0.81–1.73)	0.39
Relapse	0.69 (0.46–1.02)	0.065	0.78 (0.45–1.35)	0.37	0.60 (0.33–1.07)	0.083
NRM	1.54 (1.07–2.22)	0.019*	1.32 (0.80–2.19)	0.28	1.94 (1.16–3.24)	0.012*
aGVHD, II–IV	1.14 (0.86–1.50)	0.36	1.09 (0.76–1.55)	0.65	1.22 (0.78–1.89)	0.39
aGVHD, III–IV	1.01 (0.62–1.66)	0.96	0.85 (0.43–1.67)	0.64	1.28 (0.61–2.68)	0.52
cGVHD	0.90 (0.63–	0.58	0.92 (0.58–1.45)	0.72	0.87 (0.47–1.61)	0.65

	1.30)					
HLA-C GVH-EM (Median 1, range 0–7)						
OS	1.04 (0.78–1.37)	0.80	0.96 (0.65–1.43)	0.85	1.33 (0.89–1.99)	0.16
DFS	0.99 (0.75–1.29)	0.93	0.87 (0.60–1.27)	0.47	1.31 (0.89–1.93)	0.17
Relapse	0.77 (0.52–1.15)	0.20	0.77 (0.45–1.33)	0.35	0.81 (0.46–1.44)	0.48
NRM	1.25 (0.87–1.81)	0.23	1.02 (0.61–1.71)	0.94	1.77 (1.05–2.99)	0.032*
aGVHD, II–IV	1.07 (0.81–1.42)	0.63	1.24 (0.87–1.76)	0.24	0.82 (0.50–1.33)	0.42
aGVHD, III–IV	1.24 (0.76–2.03)	0.39	1.21 (0.63–2.30)	0.57	1.33 (0.63–2.82)	0.46
cGVHD	0.94 (0.66–1.36)	0.76	1.03 (0.66–1.60)	0.91	0.79 (0.42–1.50)	0.47

Abbreviations: HR, hazard ratio; CI, confidence interval; EM, epitope mismatch; OS, overall survival; DFS, disease-free survival; NRM, non-relapse mortality; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

Supplementary Table 3. Patient characteristics in the lower and higher HLA-DRB1 GVH-EM groups

Variable		HLA-DRB1 GVH-EM		p
		Low (N = 277)	High (N = 215)	
Patient age, years	Median (range)	56 (16–77)	54 (17–72)	0.79

	16–55	134	48.4	119	55.3	0.15
	≥ 56	143	51.6	96	44.7	
Patient sex	Female	103	37.2	91	42.3	0.28
	Male	174	62.8	124	57.7	
HCT-CI	0	165	59.6	139	64.7	0.17
	≥ 1	99	35.7	72	33.5	
Diagnosis	AML/MDS	183	66.1	133	61.9	0.38
	Others (ALL, ML and CML/MPN)	94	33.9	82	38.1	
Disease stage	Standard	174	62.8	133	61.9	0.90
	Advanced	103	37.2	82	38.1	
TNC, × 10 ⁷ /kg	< 2.5	92	33.2	63	29.3	0.54
	≥ 2.5	167	60.3	134	62.3	
Sex mismatch	Matched	118	42.6	95	44.2	0.37
	Male to Female	46	16.6	46	21.4	
	Female to Male	82	29.6	51	23.7	
ABO mismatch	Matched	94	33.9	67	31.2	0.58
	Minor mismatch	68	24.5	65	30.2	
	Major mismatch	74	26.7	48	22.3	
	Both	40	14.4	34	15.8	
GVHD prophylaxis	CNI+MMF	162	58.5	129	60.0	0.22
	CNI+MTX	83	30.0	71	33.0	
Conditioning regimen	MAC	170	61.4	130	60.5	0.91
	RIC	107	38.6	85	39.5	
Year of HSCT	2000–2014	145	52.3	102	47.4	0.32
	2015–	132	47.7	113	52.6	
Follow-up period, months	Median (range)	33 (3–164)		35 (2–134)		0.84

Abbreviations: HCT-CI, hematopoietic cell transplant-comorbidity index; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; CML, chronic myelogenous leukemia; MPN, myeloproliferative neoplasia; TNC, total nucleated cell count; GVHD, graft-versus-host disease; CNI, calcineurin inhibitor; MTX, methotrexate; MMF, mycophenolate mofetil;

MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; HSCT, hematopoietic stem cell transplantation.

Supplementary Table 4. Multivariate analysis of EM in the GVH direction as a continuous variable

	All patients (N = 492)		Standard stage (N = 307)		Advanced stage (N = 185)	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
HLA-class I GVH-EM						
OS	1.05 (1.00– 1.10)	0.063	1.01 (0.94– 1.08)	0.84	1.10 (1.02– 1.18)	0.009*
DFS	1.02 (0.97– 1.07)	0.43	0.98 (0.92– 1.05)	0.58	1.07 (1.00– 1.15)	0.042*
Relapse	0.95 (0.89– 1.02)	0.18	0.99 (0.89– 1.10)	0.91	0.92 (0.82– 1.02)	0.12
NRM	1.07 (1.01– 1.14)	0.021*	0.99 (0.91– 1.08)	0.83	1.18 (1.06– 1.30)	0.001*
HLA-DRB1 GVH-EM						
OS	1.00 (0.92– 1.08)	0.98	0.93 (0.83– 1.04)	0.20	1.08 (0.96– 1.21)	0.18
DFS	0.99 (0.92– 1.07)	0.84	0.91 (0.82– 1.02)	0.098	1.07 (0.96– 1.20)	0.22
Relapse	0.98 (0.87– 1.10)	0.70	0.85 (0.72– 1.01)	0.073	1.09 (0.93– 1.28)	0.30

NRM	1.00 (0.90– 1.11)	0.99		1.00 (0.85– 1.16)	0.97		0.99 (0.84– 1.16)	0.87
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The univariate analysis of other covariates was given in Supplementary Table 1.

Abbreviations are shown in Supplementary Table 2.

Supplementary Table 5. The multivariate analysis of HLA-DRB1 EM in the GVH direction in subgroups stratified by disease stage and underlying disease

	AML/MDS standard stage, N = 197		AML/MDS advanced stage, N = 119		Other diseases advanced stage, N = 110		Other diseases advanced stage, N = 66	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
OS	0.47 (0.26- 0.83)	0.010*	1.11 (0.67- 1.84)	0.69	1.14 (0.60- 2.19)	0.69	1.04 (0.48- 2.23)	0.92
DFS	0.42 (0.24- 0.73)	0.002*	1.10 (0.68- 1.78)	0.71	1.04 (0.57- 1.91)	0.89	1.05 (0.49- 2.24)	0.90
Relapse	0.22 (0.08- 0.63)	0.004*	1.17 (0.54- 2.53)	0.69	1.07 (0.42- 2.70)	0.89	1.25 (0.40- 3.92)	0.70
NRM	0.94 (0.48- 1.82)	0.85	0.88 (0.46- 1.68)	0.71	0.91 (0.42- 1.99)	0.82	0.76 (0.22- 2.67)	0.67

The univariate analysis of other covariates was given in Supplementary Table 1.

Abbreviations are shown in Supplementary Tables 2 and 3.

Supplementary Table 6. The univariate analysis of single HLA-DRB1 epitope mismatch in the GVH direction

Epitope	Frequency (%)	DFS		Relapse		NRM	
		HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
4Q	8.5	0.84 (0.41-1.72)	0.63	0.85 (0.3-2.43)	0.77	0.82 (0.29-2.31)	0.71
11STS	11.4	0.58 (0.28-1.19)	0.14	0.45 (0.14-1.4)	0.17	0.77 (0.3-1.94)	0.58
13FE	8.5	0.82 (0.40-1.68)	0.59	0.61 (0.18-2.01)	0.41	1.05 (0.42-2.64)	0.91
16Y	11.7	0.85 (0.49-1.49)	0.58	0.91 (0.41-1.98)	0.80	0.88 (0.41-1.90)	0.74
37YV	11.4	1.80 (1.11-2.92)	0.016	1.61 (0.82-3.19)	0.17	1.62 (0.81-3.23)	0.17
47F	10.7	0.46 (0.22-0.99)	0.048	0.27 (0.06-1.09)	0.066	0.74 (0.29-1.88)	0.53
57S	11.4	1.25 (0.74-2.13)	0.40	1.40 (0.68-2.89)	0.36	1.00 (0.45-2.22)	1.00
57V	9.8	0.88 (0.46-1.69)	0.71	0.67 (0.24-1.91)	0.46	1.07 (0.45-2.58)	0.87
67LQ	8.1	1.07 (0.56-2.04)	0.85	0.83 (0.29-2.35)	0.73	1.25 (0.53-2.93)	0.61
70D	12.1	1.02 (0.59-1.76)	0.94	0.80 (0.34-1.84)	0.59	1.27 (0.64-2.53)	0.50
70R	13.7	0.69 (0.38-1.26)	0.23	0.74 (0.32-1.75)	0.49	0.70 (0.30-1.63)	0.40
96H	9.4	0.74 (0.38-1.46)	0.39	0.49 (0.15-1.58)	0.23	1.05 (0.46-2.41)	0.91
96Y	5.9	1.44 (0.73-2.84)	0.30	0.84 (0.26-2.80)	0.78	1.86 (0.80-4.30)	0.15
98E	7.5	1.02 (0.50-2.09)	0.96	0.71 (0.22-2.31)	0.56	1.28 (0.50-3.25)	0.60
104A	7.5	1.02 (0.50-	0.96	0.71 (0.22-2.31)	0.56	1.28 (0.50-	0.60

		2.09)				3.25)	
142M	6.8	0.53 (0.22- 1.29)	0.16	0.43 (0.11- 1.77)	0.24	0.68 (0.21- 2.28)	0.53

In view of multiple testing, p-values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) control at 0.05 level. Abbreviations are shown in Supplementary Table 2.

Supplementary Table 7. Causes of non-relapse mortality according to HLA-class I

GVH-EM in the whole cohort

	HLA-class I GVH-EM				p
	Low ($\leq$ median) (N = 254)		High ($>$ median) (N = 238)		
	N	%	N	%	
Infection	10	3.9	18	7.6	0.12
GVHD	1	0.4	9	3.8	0.019*
Acute	1	0.4	6	2.5	
Chronic	0	0.0	3	1.3	
ARDS	2	0.8	2	0.8	1.00
IP	5	2.0	2	0.8	0.50
Hemorrhage	1	0.4	3	1.3	0.57
TMA/VOD	7	2.8	7	2.9	1.00
Organ failure	12	4.7	12	5	1.00
Secondary malignancy	1	0.4	0	0.0	1.00
Others	14	5.5	11	4.6	0.81
Total	53	20.9	64	26.9	0.14

Abbreviations: EM, epitope mismatch; GVHD, graft-versus-host disease; ARDS, acute respiratory distress syndrome; IP, interstitial pneumonia; TMA, thrombotic microangiopathy; VOD, veno-occlusive disease.

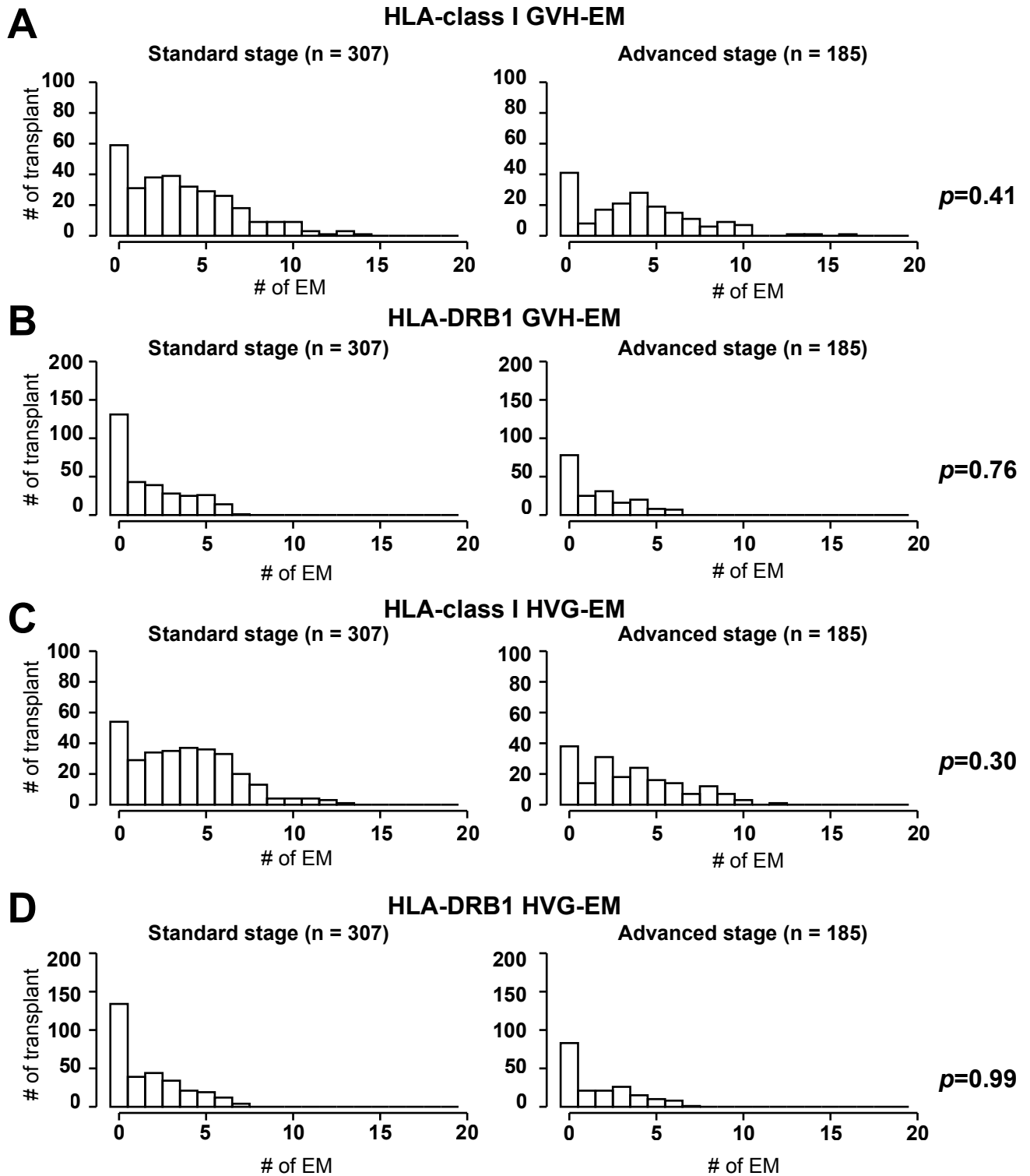
Supplementary Table 8. Univariate analysis of EM in the HVG direction

	All patients (N = 492)		Standard stage (N = 307)		Advanced stage (N = 185)	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
HLA-DRB1 HVG-EM						
OS	0.81 (0.61– 1.06)	0.13	0.77 (0.52– 1.15)	0.20	0.80 (0.54– 1.19)	0.27
DFS	0.88 (0.68– 1.14)	0.33	0.89 (0.62– 1.28)	0.53	0.84 (0.58– 1.22)	0.36
Relapse	0.97 (0.67– 1.41)	0.88	0.98 (0.58– 1.63)	0.93	0.97 (0.58– 1.65)	0.92
NRM	0.83 (0.57– 1.20)	0.33	0.82 (0.49– 1.38)	0.46	0.82 (0.49– 1.38)	0.45
HLA-A HVG- EM (Median 0, range 0–10)						
OS	1.01 (0.75– 1.35)	0.97	1.06 (0.70– 1.6)	0.79	0.88 (0.59– 1.33)	0.56
DFS	1.04 (0.79– 1.37)	0.77	1.08 (0.73– 1.59)	0.70	0.92 (0.62– 1.35)	0.67
Relapse	0.94 (0.63– 1.39)	0.75	1.17 (0.68– 2.00)	0.58	0.69 (0.39– 1.22)	0.20
NRM	1.18 (0.81– 1.71)	0.39	1.02 (0.59– 1.76)	0.94	1.29 (0.77– 2.18)	0.33
HLA-B HVG- EM (Median 1, range 0–7)						
OS	1.29 (0.97– 1.70)	0.076	1.56 (1.06– 2.29)	0.023*	1.15 (0.76– 1.74)	0.52
DFS	1.20 (0.91– 1.57)	0.192	1.45 (1.01– 2.10)	0.046*	1.04 (0.69– 1.56)	0.84
Relapse	1.02 (0.69– 1.49)	0.94	1.15 (0.68– 1.95)	0.59	0.95 (0.53– 1.70)	0.87
NRM	1.25 (0.86– 1.81)	0.25	1.55 (0.93– 2.57)	0.092	1.04 (0.59– 1.84)	0.89
HLA-C HVG-						

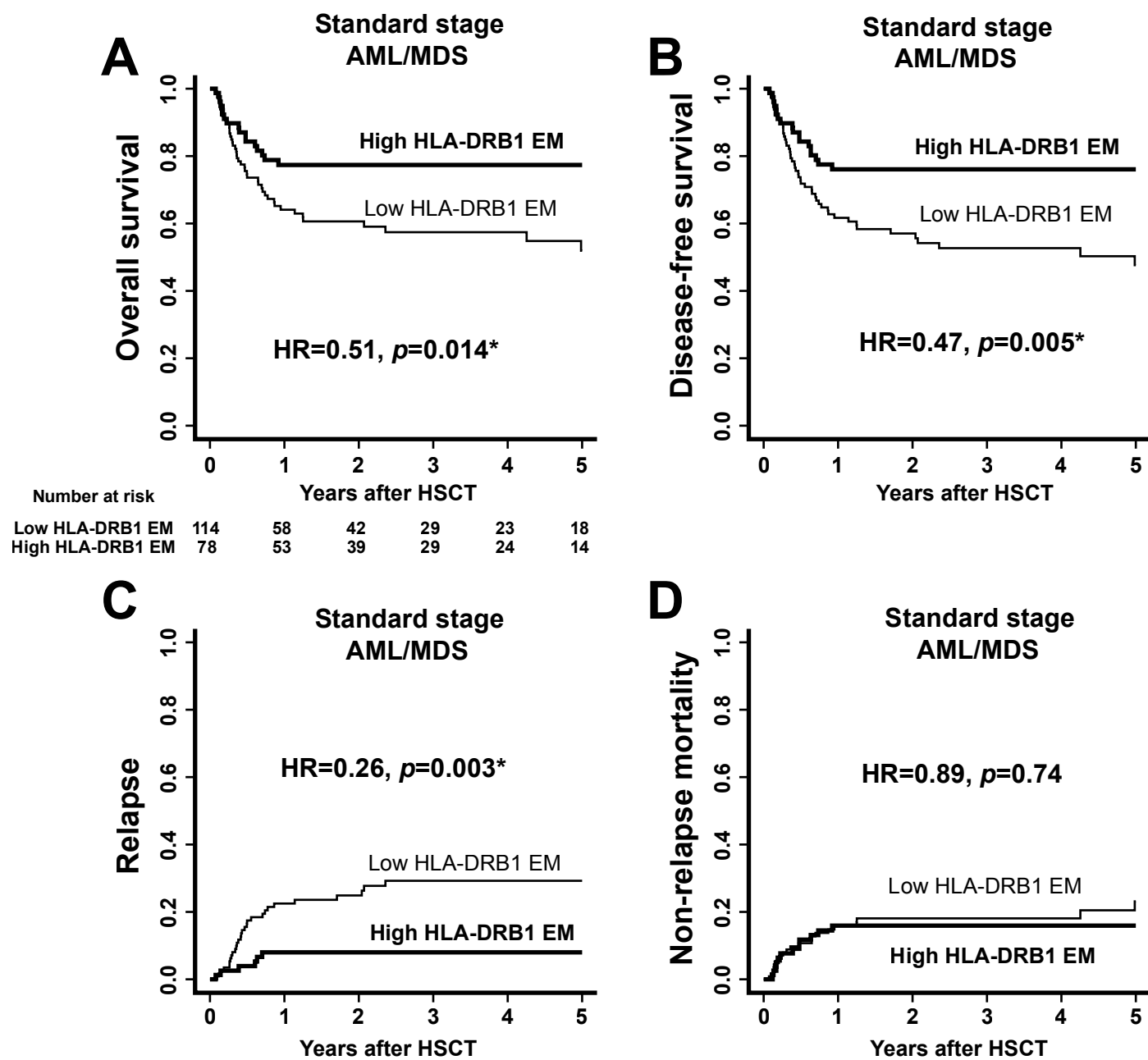
EM (Median 0, range 0–5)						
OS	0.92 (0.71– 1.21)	0.57	0.97 (0.66– 1.43)	0.89	1.00 (0.68– 1.47)	0.99
DFS	0.89 (0.68– 1.15)	0.36	0.88 (0.61– 1.26)	0.48	1.03 (0.71– 1.50)	0.87
Relapse	0.86 (0.59– 1.24)	0.41	0.89 (0.54– 1.49)	0.67	0.92 (0.54– 1.57)	0.77
NRM	0.95 (0.66– 1.36)	0.78	0.86 (0.52– 1.43)	0.57	1.20 (0.72– 2.00)	0.50
HLA-class I HVG-EM						
OS	1.12 (0.85– 1.47)	0.42	1.27 (0.86– 1.86)	0.22	1.04 (0.71– 1.53)	0.84
DFS	1.06 (0.82– 1.37)	0.65	1.29 (0.89– 1.85)	0.18	0.90 (0.62– 1.30)	0.57
Relapse	0.88 (0.61– 1.27)	0.49	1.22 (0.73– 2.04)	0.45	0.64 (0.37– 1.10)	0.11
NRM	1.25 (0.87– 1.79)	0.23	1.25 (0.76– 2.08)	0.38	1.33 (0.79– 2.22)	0.28

Abbreviations are shown in Supplementary Table 2.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

