Refined HLA-DPB1 mismatch with molecular algorithms predicts outcomes in hematopoietic stem cell transplantation

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SUPPLEMENTAL MATERIAL

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Supplemental Material 1. Outcome definitions and statistical analysis

The primary outcome was acute GVHD (aGVHD) and secondary outcomes were overall survival (OS), progression-free survival (PFS), relapse, non-relapse mortality (NRM), and neutrophil engraftment. All outcomes were analyzed from the time of stem cell infusion to the date of the event or censored at 60 days after HSCT (for engraftment) or at last follow-up (for all other outcomes).

The event for OS was death from any cause, and either death or relapse were considered an event for PFS. The cumulative incidence of relapse and NRM were evaluated by the competing risks method, where death was the competing risk for relapse and relapse was the competing risk for NRM. Diagnosis and clinical grading of aGVHD were based on established criteria.[1] Death without aGVHD and relapse before or without aGVHD were competing events for aGVHD. Neutrophil engraftment was defined as the first date of absolute neutrophil count $\geq 0.5\times10^9$/L for 3 consecutive days, confirmed by the presence of donor chimerism.[2] Patients who died within 60 days after HSCT without neutrophil engraftment were considered to have a competing event. Patients who experienced engraftment later than 28 days after HSCT (delayed engraftment) were considered to have experienced engraftment. Patients who did not experience an event were censored at 60 days.

Patient and HSCT characteristics were summarized using descriptive statistics. Categorical variables were reported as numbers and percentages, and medians and ranges were used for continuous variables. Comparisons between the 4 HLA-DPB1 matching groups were performed using a chi-square or Fisher exact test for categorical variables and Kruskal–Wallis equality-of-populations rank test for continuous variables.

Univariate and multivariable Cox proportional hazards regression was used to determine the impact of baseline characteristics, PS, ME, and HLA-DPB1 matching on survival outcomes, and univariate and multivariable sub-distributional hazards regression was used to analyze cumulative incidence outcomes, including relapse, NRM, aGVHD, and engraftment. All regression models were tested for proportional hazards assumption and interaction terms. Each PS, ME, and HLA-DPB1 match group with a P value <0.1 in the univariate analysis was analyzed in separate multivariable regression models adjusted for significant baseline characteristics (age, sex, donor age, donor-recipient sex match, donor-recipient CMV serostatus, Disease Risk Index (DRI), Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI), conditioning regimen intensity, stem cell source, GVHD regimen, and year of HSCT). PS and ME were analyzed as both continuous variables and categorical variables (low vs. high), and they were analyzed only as categorical variables in multivariable analyses. To determine the optimal cutoff for low vs. high PS and ME groups, the concordance probabilities of PS and ME for aGVHD prediction were tested at the 25th, 50th, and 75th percentile cutoffs. The cutoffs at the 50th percentile were selected for the analysis to maximize the concordance probability for the prediction clinically significant aGVHD.

The discrimination power ((the model's ability to distinguish between patients who did and did not develop the outcome of interest) of the TCE, ME and PS model on aGVHD were compared by the Harrell's C-concordance index (C-index). A C-index of 0.50 indicates a model that does not discriminate better than chance alone and a C-index of 1.00 indicates perfect discrimination.

Finally, we also performed a time to event decision-curve analysis to assess the clinical net benefit of all models in making a decision regarding patient selection for GVHD regimen modification in comparison with "universal GVHD regimen modification" (treat/modify all) and "no GVHD modification" (treat/modify none) strategy. The results were presented as decision curves which Y-axis represents the clinical net

benefit (positive values) or risk (negative values) of using model-guided GVHD regimen modification in comparison with no GVHD regimen modification (clinical net benefit =0) whereas the X-axis represents threshold probabilities of aGVHD grade 2-4 at 100 days post-transplant.

The analyses were performed using the complete-case method without data imputation. All statistical calculations were carried out using STATA 13.1 (Stata Corp., College Station TX, USA). P values <0.05 were considered significant in multivariable analysis. All tests were two-sided. No adjustments were made for multiple comparisons to avoid expansion of type 2 errors or false exclusion of transplant risk related to molecular HLA disparity.

Supplemental Table 1. Cross-tabulation between the number of patients in the PS/ME and the TCE models

	Whole cohort	DP permissive mismatch, N (%)	DP GVH non-permissive mismatch, N (%)	DP HVG non-permissive mismatch, N (%)
GVH DP ME				
• **Cutoff value**	4	5	9	5
• **Low**	760 (50.2)	364 (55.9)	161 (59.9)	141 (61.8)
• **High**	754 (49.8)	287 (44.1)	108 (40.2)	87 (38.2)
GVH PS-I				
• **Cutoff value**	0	1	3	1
• **Low**	776 (51.3)	429 (65.9)	173 (64.3)	139 (61.0)
• **High**	738 (48.8)	222 (34.1)	96 (35.7)	89 (39.0)
GVH PS-II				
• **Cutoff value**	2	3	8	2
• **Low**	286 (43.9)	336 (51.6)	142 (52.8)	120 (52.6)
• **High**	365 (56.1)	315 (48.4)	127 (47.2)	108 (47.4)
HVG DP ME				
• **Cutoff value**	4	5	5	9
• **Low**	817 (54.0)	362 (55.6)	169 (62.8)	123 (54.0)
• **High**	697 (46.0)	289 (44.4)	100 (37.2)	105 (46.1)
HVG PS-I				
• **Cutoff value**	0	1	0	3
• **Low**	804 (53.1)	418 (64.2)	136 (50.6)	137 (60.1)
• **High**	710 (46.9)	233 (35.8)	133 (49.4)	91 (39.9)
HVG PS-II				
• **Cutoff value**	1	3	1	8
• **Low**	760 (50.2)	352 (54.1)	139 (51.7)	117 (51.3)

• **High**	754 (49.8)	299 (45.9)	130 (48.3)	111 (48.7)

Note: Low ME and PS is </= median, High ME and PS is > median

Supplemental Table 2. Cross-tabulation between the number of patients in the PS and ME models in the whole cohort

		GVH DP ME		HVG DP ME	
		Low	High	Low	High
GVH PS-I	Low	652 (82.3)	124 (17.2)	475 (58.1)	301 (43.2)
	High	140 (17.7)	598 (82.8)	342 (41.9)	396 (56.8)
GVH PS-II	Low	707 (89.3)	95 (13.2)	472 (57.8)	330 (47.4)
	High	85 (10.7)	627 (86.8)	345 (42.2)	367 (52.6)
HVG PS-I	Low	464 (58.6)	340 (47.1)	692 (84.7)	112 (16.1)
	High	328 (41.4)	382 (52.9)	125 (15.3)	585 (83.9)
HVG PS-II	Low	447 (56.4)	313 (43.4)	695 (85.1)	65 (9.3)
	High	345 (43.6)	409 (56.6)	122 (14.9)	632 (90.7)

Note: Low ME and PS is </= median, High ME and PS is > median

Supplemental Table 3. Univariate analysis of the impact of baseline characteristics, PS-I, PS-II, and ME on overall survival in patients who underwent HSCT from unrelated donors (n=1514)

Variable	**HR**	**95% CI**	**P**
Age*			
Continuous	1.01	1.00-1.01	0.012
<50 years	Ref		
≥50 years	1.20	1.03-1.40	0.021
Sex			
Female	Ref		
Male	1.06	0.92-1.22	0.417
Donor Age			
Continuous	1.01	1.01-1.02	<0.001
≤40 years	Ref		
>40 years	1.25	1.06-1.48	0.007
Donor-recipient sex match			
Female to female	Ref		
Female to male	1.45	1.22-1.91	0.007
Male to female	1.20	0.94-1.54	0.145
Male to male	1.14	0.90-1.44	0.275
ABO match			
Match	Ref		
Minor mismatch	1.11	0.93-1.32	0.249
Major mismatch	1.05	0.88-1.26	0.550

Bidirectional mismatch	1.42	1.10-1.84	0.008
Donor-recipient CMV serostatus			
NR-NR	Ref		
NR-R	1.22	0.98-1.53	0.073
R-NR	0.87	0.62-1.24	0.451
R-R	1.13	0.89-1.43	0.307
DRI			
Low	Ref		
Intermediate	1.21	0.95-1.53	0.117
High	2.11	1.67-2.66	<0.001
Very high	3.14	2.39-4.11	<0.001
HCT-CI			
Continuous	1.14	1.10-1.17	<0.001
<3	Ref		
≥3	1.75	1.52-2.02	<0.001
HSCT protocol			
Clinical trial protocol	Ref		
Standard of care	1.22	1.06-1.40	0.006
Conditioning regimen intensity			
MA	Ref		
RIC/NMA	0.96	0.83-1.11	0.581
Stem cell source			
PB	Ref		
BM	1.03	0.90-1.19	0.675
GVHD regimen			
Others	Ref		
Tacrolimus/methotrexate	1.01	0.83-1.23	0.939
Year of HSCT			
2005-2009	Ref		
2010-2013	0.88	0.74-1.04	0.135
2014-2018	0.77	0.64-0.92	0.003
GVH DP ME			
Continuous	1.01	0.99-1.02	0.389
Low	Ref		
High	1.04	0.91-1.20	0.535
GVH PS-I			
Continuous	1.02	0.99-1.06	0.187
Low	Ref		
High	1.07	0.93-1.23	0.324
GVH PS-II			
Continuous	1.01	0.99-1.02	0.246
Low	Ref		
High	1.06	0.93-1.23	0.334
HVG DP ME			
Continuous	1.01	0.99-1.02	0.171
Low	Ref		

High	1.12	0.98-1.29	0.093
HVG PS-I			
Continuous	1.02	0.99-1.05	0.208
Low	Ref		
High	1.18	1.03-1.35	0.018
HVG PS-II			
Continuous	1.00	0.99-1.02	0.766
Low	Ref		
High	1.09	0.95-1.25	0.200
GVH-HVG PS-I combination			
Low-Low	Ref		
Low-High	1.23	1.01-1.50	0.037
High-Low	1.09	0.90-1.33	0.387
High-High	1.21	1.02-1.45	0.033
GVH-HVG PS-II combination			
Low-Low	Ref		
Low-High	1.10	0.90-1.33	0.355
High-Low	1.07	0.87-1.31	0.524
High-High	1.14	0.96-1.37	0.143
GVH-HVG DP ME combination			
Low-Low	Ref		
Low-High	1.07	0.89-1.30	0.468
High-Low	0.99	0.82-1.20	0.938
High-High	1.16	0.96-1.40	0.108
HLA-DPB1 matching			
Match	Ref		
Permissive mismatch	1.06	0.88-1.26	0.538
GVH nonpermissive mismatch	1.20	0.97-1.49	0.091
HVG nonpermissive mismatch	1.11	0.89-1.39	0.359

Note: PS and ME were categorized into low and high groups using the median as a cutoff point. All models were tested for proportional hazards assumption using the scaled Schoenfeld residuals test.

Abbreviations: PS-I, Predicted Indirectly Recognizable HLA Epitopes score I; PS-II, Predicted Indirectly Recognizable HLA Epitopes score II; DP ME, HLA-DPB1 mismatched eplets; HSCT, hematopoietic stem cell transplantation; HR, hazard ratio; CI, confidence interval; CMV, cytomegalovirus; NR, nonreactive; R, reactive; DRI, Disease Risk Index; HCT-CI, Hematopoietic Cell Transplant-Comorbidity Index; MA, myeloablative; RIC, reduced-intensity conditioning; NMA, nonmyeloablative; PB, peripheral blood; BM, bone marrow; GVHD, graft-versus-host disease; GVH, graft versus host; HVG, host versus graft.

*Age had a significant time-varying effect and violated the proportional hazards assumption; therefore, the impact of age on outcome was adjusted by interaction term between age and follow-up time (time-varying covariate). The final model did not violate the proportional hazards assumption.

Supplemental Table 4. Univariate analysis of the impact of baseline characteristics, PS-I, PS-II, and ME on progression-free survival in patients who underwent HSCT from unrelated donors (n=1514)

Variable	**HR**	**95% CI**	**P**
Age*			
Continuous	1.00	0.99-1.01	0.062

<50	Ref		
≥50	1.11	0.96-1.28	0.162
Sex			
Female	Ref		
Male	1.06	0.92-1.21	0.432
Donor Age			
Continuous	1.01	1.01-1.02	<0.001
≤40 years	Ref		
>40 years	1.26	1.07-1.48	0.004
Donor-recipient sex match			
Female to female	Ref		
Female to male	1.36	1.05-1.76	0.020
Male to female	1.18	0.94-1.50	0.156
Male to male	1.14	0.91-1.42	0.246
ABO match			
Match	Ref		
Minor mismatch	1.11	0.94-1.30	0.222
Major mismatch	1.01	0.85-1.20	0.909
Bidirectional mismatch	1.47	1.15-1.88	0.002
Donor-recipient CMV serostatus			
NR-NR	Ref		
NR-R	1.13	0.92-1.39	0.248
R-NR	0.89	0.64-1.23	0.472
R-R	1.03	0.82-1.28	0.822
DRI			
Low	Ref		
Intermediate	1.18	0.94-1.47	0.149
High	1.94	1.56-2.41	<0.001
Very high	3.03	2.35-3.90	<0.001
HCT-CI			
Continuous	1.10	1.07-1.13	<0.001
<3	Ref		
≥3	1.50	1.32-1.72	<0.001
HSCT protocol			
Clinical trial protocol	Ref		
Standard of care	1.19	1.04-1.37	0.010
Conditioning regimen intensity			
MA	Ref		
RIC/NMA	1.05	0.92-1.21	0.426
Stem cell source			
PB	Ref		
BM	1.05	0.92-1.20	0.439
GVHD regimen			
Others	Ref		
Tacrolimus/methotrexate	0.99	0.83-1.20	0.973
Year of HSCT			

2005-2009	Ref		
2010-2013	0.86	0.73-1.02	0.079
2014-2018	0.75	0.64-0.89	0.001
GVH DP ME			
Continuous	0.99	0.98-1.01	0.798
Low	Ref		
High	0.99	0.87-1.13	0.924
GVH PS-I			
Continuous	1.00	0.97-1.04	0.803
Low	Ref		
High	1.03	0.90-1.17	0.710
GVH PS-II			
Continuous	1.00	0.97-1.04	0.803
Low	Ref		
High	1.01	0.89-1.15	0.844
HVG DP ME			
Continuous	1.01	0.99-1.02	0.173
Low	Ref		
High	1.14	1.00-1.30	0.045
HVG PS-I			
Continuous	1.01	0.98-1.04	0.452
Low	Ref		
High	1.17	1.03-1.33	0.018
HVG PS-II			
Continuous	1.00	0.99-1.02	0.642
Low	Ref		
High	1.09	0.9601.24	0.184
GVH-HVG PS-I combination			
Low-Low	Ref		
Low-High	1.26	1.04-1.52	0.016
High-Low	1.06	0.88-1.28	0.530
High-High	1.16	0.98-1.27	0.085
GVH-HVG PS-II combination			
Low-Low	Ref		
Low-High	1.12	0.93-1.34	0.237
High-Low	1.02	0.84-1.24	0.830
High-High	1.09	0.92-1.29	0.320
GVH-HVG DP ME combination			
Low-Low	Ref		
Low-High	1.09	0.91-1.31	0.344
High-Low	0.94	0.78-1.13	0.498
High-High	1.13	0.95-1.35	0.177
HLA DP matching			
Match	Ref		
Permissive mismatch	1.04	0.88-1.23	0.627
GVH nonpermissive mismatch	1.09	0.89-1.34	0.393

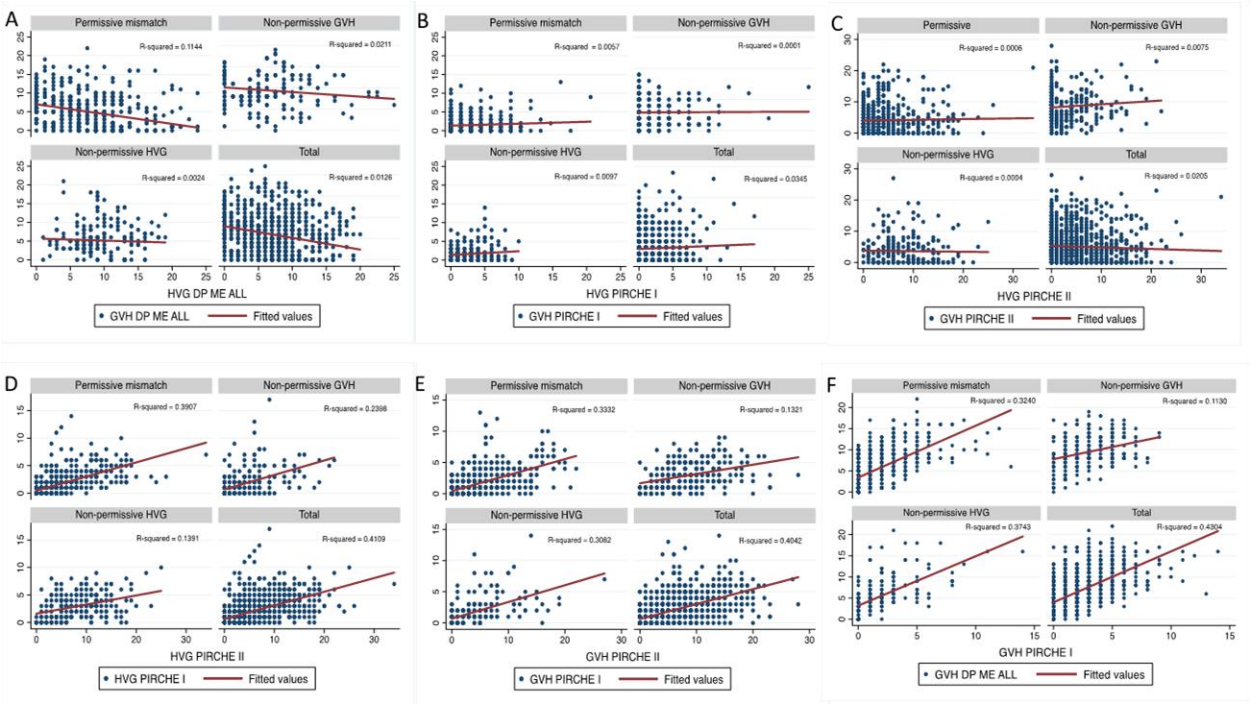
HVG nonpermissive mismatch	1.06	0.85-1.31	0.614

Note: PS and ME were categorized into low and high groups using the median as a cutoff point. All models were tested for proportional hazards assumption using the scaled Schoenfeld residuals test.

Abbreviations: PS-I, Predicted Indirectly Recognizable HLA Epitopes score I; PS-II, Predicted Indirectly Recognizable HLA Epitopes score II; DP ME, HLA-DPB1 mismatched eplets; HSCT, hematopoietic stem cell transplantation; HR, hazard ratio; CI, confidence interval; CMV, cytomegalovirus; NR, nonreactive; R, reactive; DRI, Disease Risk Index; HCT-CI, Hematopoietic Cell Transplant-Comorbidity Index; MA, myeloablative; RIC, reduced-intensity conditioning; NMA, nonmyeloablative; PB, peripheral blood; BM, bone marrow; GVHD, graft-versus-host disease; GVH, graft versus host; HVG, host versus graft.

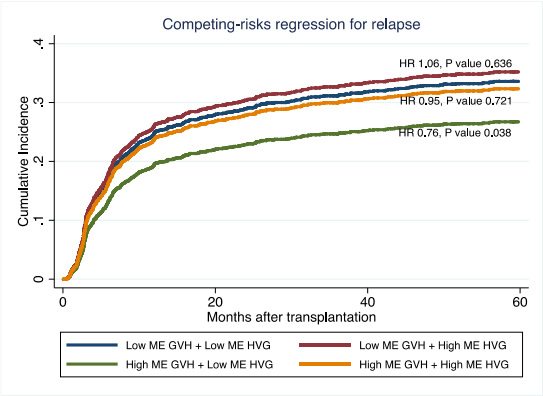
*Age had a significant time-varying effect and violated the proportional hazards assumption; therefore, the impact of age on outcome was adjusted by interaction term between age and follow-up time (time-varying covariate). The final model did not violate the proportional hazards assumption.

Supplemental Figure 1. Scatterplots showing correlations between the graft versus host (GVH) and host versus graft (HVG) direction for the number of mismatched eplets (ME) and Predicted Indirectly Recognizable HLA Epitopes (PIRCHE) scores (PS). (A) GVH and HVG ME. (B) GVH and HVG PS-I. (C) GVH and HVG PS-II. (D) HVG PS-I and HVG PS-II. (E) GVH PS-I and GVH PS-II. (F) GVH ME and GVH PS-I.



Supplemental Figure 2. (A) Adjusted cumulative incidence of acute GVHD grade 2-4, (B) adjusted cumulative incidence of relapse in the whole cohort stratified by the combination of ME and PS in the graft versus host (GVH) and host versus graft (HVG) direction. PS and ME were categorized into low and high groups using the median as a cutoff point.

A



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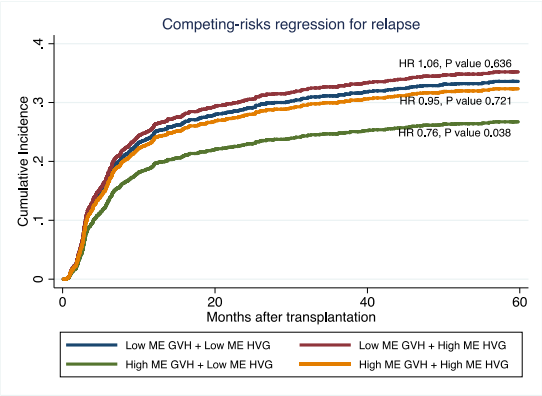
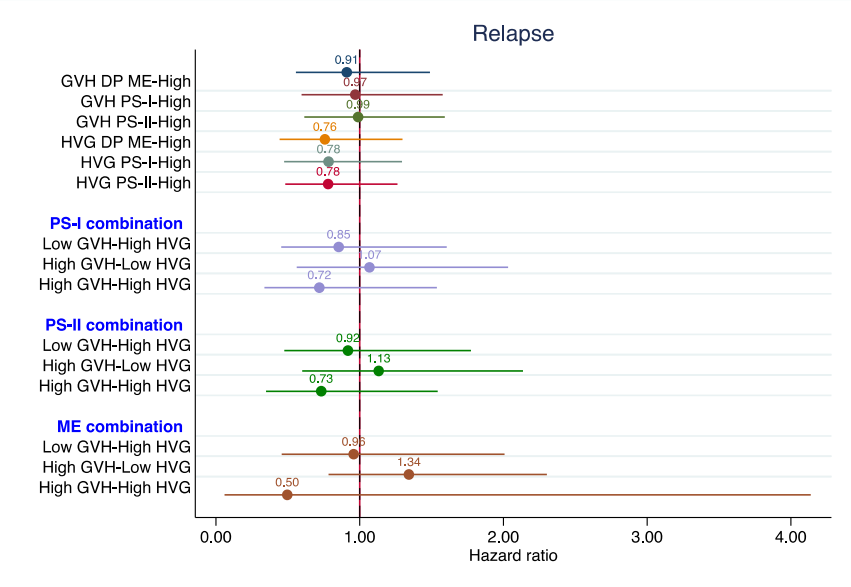
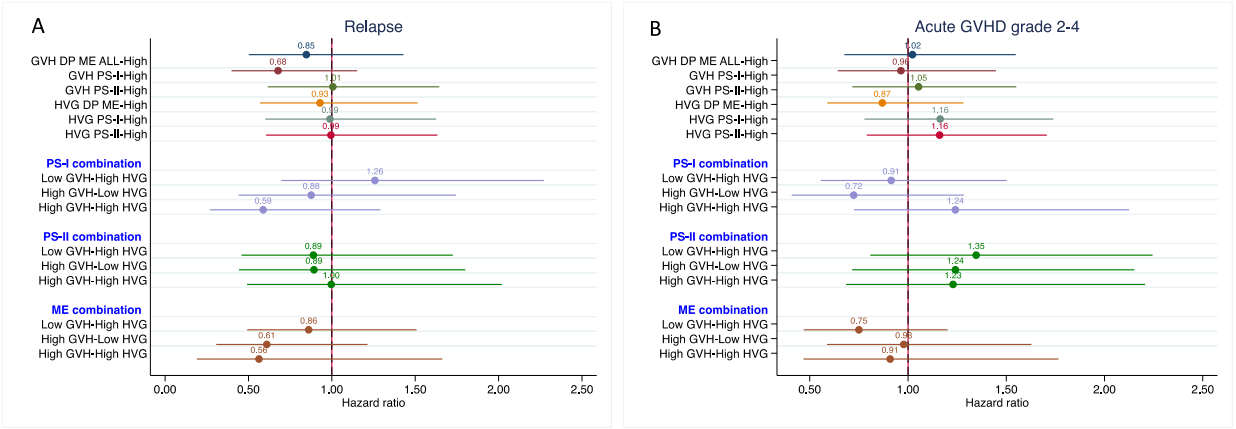


Supplemental Figure 3. Forest plots showing results from the multivariable analyses of the impact of molecular mismatch scores (number of mismatched eplets [ME], Predicted Indirectly Recognizable HLA Epitope score [PS]-I, and PS-II) on relapse in patients with HLA-DPB1 nonpermissive mismatch in the graft versus host (GVH) direction, stratified by ME GVH and host versus graft (HVG) combinations. Dots and bars in the forest plots represent adjusted hazard ratios and 95% confidence intervals. PS and ME were categorized into low and high groups using the median as a cutoff point.



Supplemental Figure 4. Forest plots showing results from the multivariable analyses of the impact of molecular mismatch scores (mismatched eplet [ME], Predicted Indirectly Recognizable HLA Epitope score [PS]-I, and PS-II) on outcomes in patients with HLA-DPB1 nonpermissive mismatch in the host versus graft (HVG) direction. (A) Relapse. (B) Acute graft-versus-host disease (GVHD) grade 2-4. Dots and bars in the forest plots represent adjusted hazard ratios and 95% confidence intervals. PS and ME were categorized into low and high groups using the median as a cutoff point.



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