High-risk HLA alleles for severe acute graft-*versus*-host disease and mortality in unrelated donor bone marrow transplantation

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Supplemental methods

Statistical method

The cumulative incidence of acute GVHD was assessed by a method described elsewhere to eliminate the effect of competing risk.[1] A competing event regarding acute GVHD was defined as death without acute GVHD. Overall survival was calculated using the Kaplan-Meier method. Multivariable competing risk regression analyses[2] were conducted to evaluate the impact of acute GVHD and transplant-related mortality, and confounders considered were sex (donor-recipient pair), patient age (linear), donor age (linear), disease, risk of leukemia relapse (standard and high), GVHD prophylaxis (cyclosporine-based regimen, tacrolimus-based regimen, and the other regimen without cyclosporine or tacrolimus), and conditioning regimen (reduced-intensity conditioning and myeloablative conditioning). Conditioning regimens were classified as myeloablative when total-body irradiation was >8 Gy, oral busulfan was ≥9 mg/kg, intravenous busulfan was ≥7.2 mg/kg, or melphalan was >140 mg/m^2, in accordance with the report by Giralt et al.[3] We also included separate variables for HLA-A,-C,-B,-DRB1,-DQB1 and -DPB1 allele mismatches in the GVH direction in the multivariate analysis. A competing event regarding acute GVHD was defined as death without acute GVHD. For transplant-related mortality, relapse was the competing event. An adjusted comparison of the groups with regard to overall survival was performed using Cox's proportional-hazards regression model.[4] Missing events were treated as an unknown group. All analyses were conducted using STATA version 13 (StataCorp., College Station, TX).

Reference: Supplemental methods

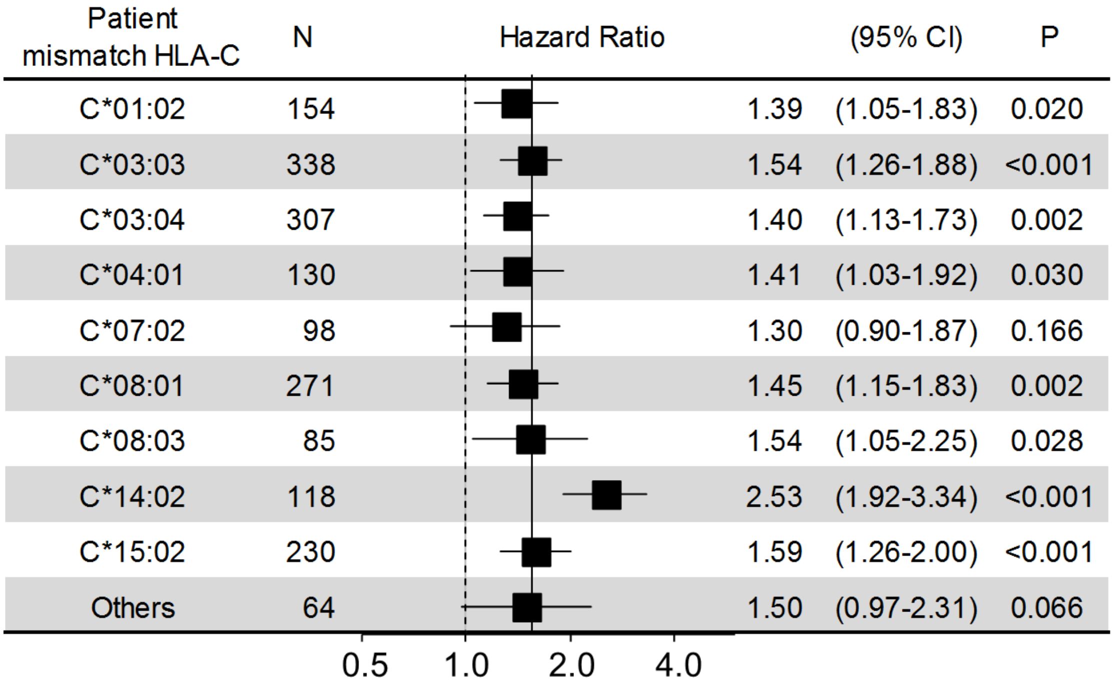
1. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18(6):695-706.
2. Fine JP GR. A proportilnal hazards model for subdistribution of a competing risk. J Am Stat Assoc. 1999;94(456-509.
3. Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. Biol Blood Marrow Transplant. 2009;15(3):367-369.
4. Cox D. Regression model and life tables. J R Stat Soc B. 1972;34(2):187-200.

Supplemental Table 1. Effect of HLA alleles with a frequency >5% on grades III-IV acute GVHD

	Patient				Donor			
HLA allele	N	HR	(95% CI)	P	N	HR	(95% CI)	P
HLA-A								
A*24:02	4827	1.06	(0.93-1.20)	0.3824	4836	1.05	(0.92-1.19)	0.4716
A*21:01	1306	1.07	(0.93-1.24)	0.3422	1341	1.02	(0.88-1.17)	0.8344
A*33:03	1094	0.82	(0.69-0.98)	0.0262	1094	0.82	(0.69-0.98)	0.0262
A*11:01	1073	0.94	(0.79-1.11)	0.4524	1078	0.93	(0.78-1.09)	0.3617
A*02:06	1076	0.96	(0.82-1.13)	0.6193	1058	1.08	(0.93-1.26)	0.3298
A*31:01	962	1.09	(0.92-1.28)	0.3217	962	1.09	(0.92-1.28)	0.3217
A*26:01	907	1.01	(0.86-1.19)	0.9044	915	0.91	(0.77-1.08)	0.2964
HLA-B								
B*52:01	2014	1.05	(0.92-1.20)	0.4751	2014	1.05	(0.92-1.20)	0.4751
B*54:01	1120	0.96	(0.81-1.14)	0.6462	1122	0.96	(0.81-1.13)	0.6267
B*40:02	1077	0.97	(0.82-1.13)	0.6756	1102	0.99	(0.85-1.16)	0.9323
B*51:01	1058	1.37	(1.19-1.59)	<0.0001	1079	1.35	(1.17-1.56)	<0.0001
B*35:01	1064	0.86	(0.73-1.01)	0.0678	1064	0.85	(0.72-1.00)	0.0530
B*44:03	1041	0.79	(0.66-0.94)	0.0088	1041	0.80	(0.67-0.96)	0.0174
B*07:02	1002	1.09	(0.92-1.29)	0.3301	1002	1.09	(0.92-1.29)	0.3301
B*15:01	988	0.90	(0.76-1.06)	0.2142	995	0.90	(0.76-1.06)	0.2180
HLA-C								
C*01:02	2194	1.00	(0.88-1.13)	0.9893	2208	1.09	(0.96-1.23)	0.1860
C*12:02	2020	1.05	(0.91-1.20)	0.5030	2013	1.03	(0.90-1.18)	0.6252
C*07:02	1644	1.07	(0.93-1.23)	0.3309	1647	1.07	(0.93-1.23)	0.3226
C*03:03	1576	0.85	(0.74-0.98)	0.0242	1604	0.83	(0.72-0.96)	0.0123
C*03:04	1496	1.08	(0.94-1.23)	0.2999	1544	1.09	(0.95-1.24)	0.2334
C*14:03	1041	0.79	(0.66-0.94)	0.0088	1040	0.80	(0.67-0.96)	0.0138
C*08:01	901	0.98	(0.83-1.16)	0.8447	905	0.97	(0.82-1.14)	0.6863
C*14:02	860	1.35	(1.15-1.58)	0.0002	868	1.11	(0.94-1.31)	0.2121
HLA-DRB1								
DRB1*15:02	1928	1.00	(0.87-1.14)	0.9681	1935	1.00	(0.87-1.14)	0.9448
DRB1*04:05	1912	0.97	(0.85-1.10)	0.6371	1922	0.98	(0.86-1.12)	0.7772

DRB1*09:01	1861	0.98	(0.86-1.12)	0.7686	1861	0.99	(0.87-1.13)	0.8866
DRB1*08:03	1039	1.15	(0.98-1.35)	0.0841	1057	1.09	(0.93-1.28)	0.2819
DRB1*01:01	1001	1.07	(0.90-1.27)	0.4720	1001	1.06	(0.89-1.26)	0.4933
DRB1*13:02	979	0.82	(0.68-0.98)	0.0337	982	0.84	(0.70-1.01)	0.0577
DRB1*15:01	938	1.05	(0.89-1.24)	0.5403	948	1.05	(0.90-1.24)	0.5254
HLA-DQB1								
DQB1*06:01	2789	1.12	(0.99-1.26)	0.0728	2800	1.08	(0.96-1.22)	0.1931
DQB1*03:03	1954	1.01	(0.88-1.15)	0.9248	1940	0.98	(0.86-1.11)	0.7276
DQB1*04:01	1887	0.96	(0.84-1.09)	0.5186	1895	0.98	(0.86-1.11)	0.7230
DQB1*03:02	1176	0.98	(0.84-1.14)	0.7794	1174	0.90	(0.76-1.05)	0.1782
DQB1*03:01	1115	1.03	(0.88-1.20)	0.7074	1086	1.14	(0.98-1.32)	0.0984
DQB1*05:01	1051	1.05	(0.89-1.25)	0.5613	1055	1.05	(0.88-1.24)	0.6024
DQB1*06:04	933	0.81	(0.67-0.98)	0.0297	935	0.82	(0.68-0.99)	0.0423
DQB1*06:02	896	1.05	(0.89-1.24)	0.5736	913	1.05	(0.89-1.24)	0.5262
HLA-DPB1								
DPB1*05:01	4238	1.05	(0.93-1.18)	0.4351	4134	1.14	(1.01-1.29)	0.0342
DPB1*02:01	2689	1.05	(0.93-1.18)	0.4560	2813	0.92	(0.82-1.04)	0.1887
DPB1*09:01	1696	0.98	(0.85-1.13)	0.8058	1696	0.95	(0.82-1.09)	0.4468
DPB1*04:02	1379	1.09	(0.95-1.27)	0.2244	1364	0.96	(0.83-1.12)	0.6430
DPB1*04:01	746	0.86	(0.70-1.06)	0.1504	711	0.83	(0.67-1.03)	0.0885

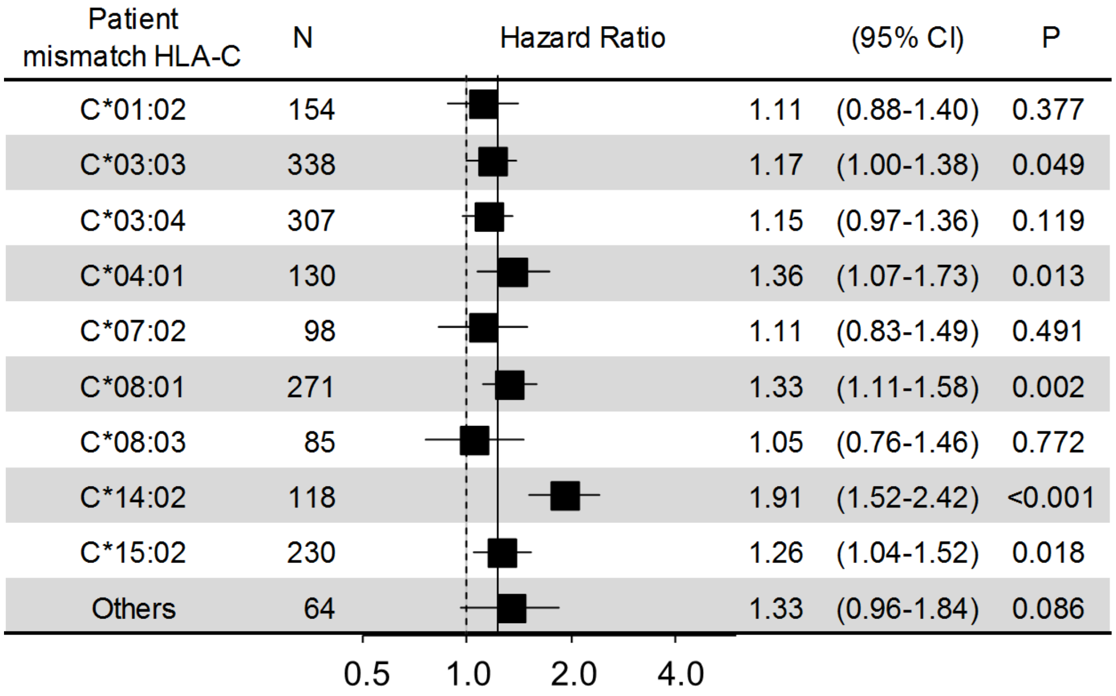
N: number of HLA allele-positive patients among 6967 patients; HR: hazard ratio indicates comparison of specific HLA allele-positive to -negative patients, adjusted for clinical factors and HLA allele matching as listed in Table 1.



Patient mismatch HLA-C	N	Hazard Ratio	(95% CI)	P
C*01:02	154	1.39	(1.05-1.83)	0.020
C*03:03	338	1.54	(1.26-1.88)	<0.001
C*03:04	307	1.40	(1.13-1.73)	0.002
C*04:01	130	1.41	(1.03-1.92)	0.030
C*07:02	98	1.30	(0.90-1.87)	0.166
C*08:01	271	1.45	(1.15-1.83)	0.002
C*08:03	85	1.54	(1.05-2.25)	0.028
C*14:02	118	2.53	(1.92-3.34)	<0.001
C*15:02	230	1.59	(1.26-2.00)	<0.001
Others	64	1.50	(0.97-2.31)	0.066

Supplemental Figure 1: Impact of patient mismatched HLA-C allele on transplant-related mortality

Results of multivariable competing risk regression analysis for the effect of patient mismatched HLA-C allele on transplant-related mortality. The hazard ratio (HR) of each mismatched HLA-C allele was estimated by comparison to HLA-C match (n=4825). The solid vertical line at 1.56 indicates the HR of overall HLA-C mismatch in the GVH direction. ‘Others’ includes patient mismatched HLA-C alleles with fewer than 20 patients, as follows: C*01:03, C*03:14, C*03:23, C*05:01, C*07:04, C*12:02, C*12:03 and HLA-C*14:03.



Patient mismatch HLA-C	N	Hazard Ratio	(95% CI)	P
C*01:02	154	1.11	(0.88-1.40)	0.377
C*03:03	338	1.17	(1.00-1.38)	0.049
C*03:04	307	1.15	(0.97-1.36)	0.119
C*04:01	130	1.36	(1.07-1.73)	0.013
C*07:02	98	1.11	(0.83-1.49)	0.491
C*08:01	271	1.33	(1.11-1.58)	0.002
C*08:03	85	1.05	(0.76-1.46)	0.772
C*14:02	118	1.91	(1.52-2.42)	<0.001
C*15:02	230	1.26	(1.04-1.52)	0.018
Others	64	1.33	(0.96-1.84)	0.086

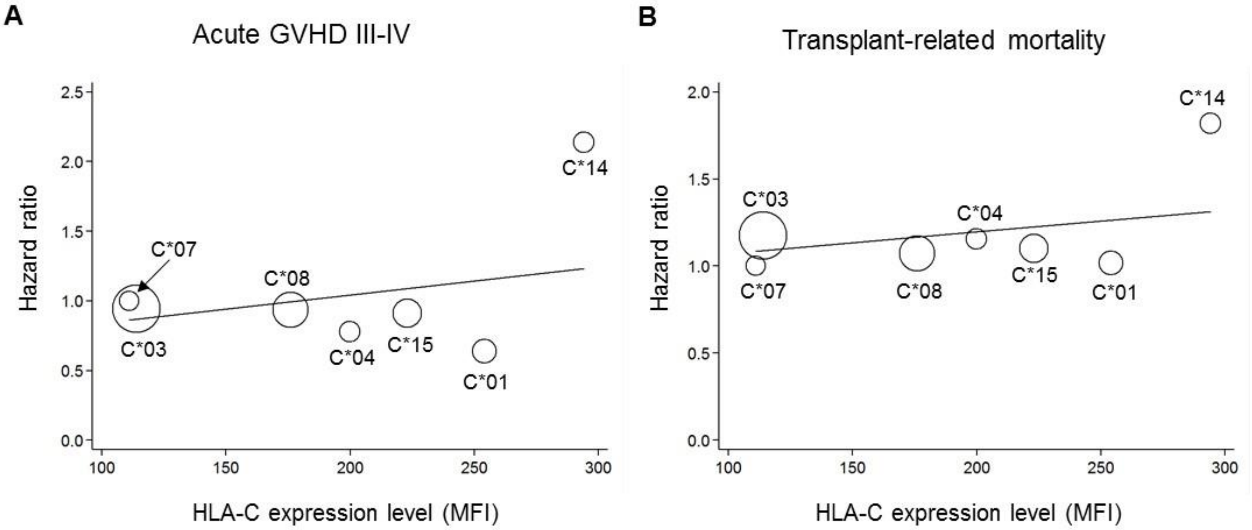
Supplemental Figure 2: Impact of patient mismatched HLA-C allele on overall mortality
Results of multivariable competing risk regression analysis for the effect of patient mismatched HLA-C allele on overall mortality. The hazard ratio (HR) of each mismatched HLA-C allele was estimated by comparison to HLA-C match (n=4825). The solid vertical line at 1.22 indicates the HR of overall HLA-C mismatch in the GVH direction. Others contain patient mismatched HLA-C alleles with fewer than 20 patients, as follows: C*01:03, C*03:14, C*03:23, C*05:01, C*07:04, C*12:02, C*12:03 and HLA-C*14:03.

Supplemental Table 2: Risk of acute GVHD and treatment-related mortality with patient mismatched HLA-C allotype

		Grades III-IV acute GVHD			Transplant-related mortality		
mismatch allotype	N	HR	(95% CI)	P	HR	(95% CI)	P
C*07	114	1.00			1.00		
C*01	167	0.64	(0.38-1.08)	0.092	1.02	(0.64-1.61)	0.939
C*03	647	0.95	(0.63-1.43)	0.790	1.18	(0.81-1.71)	0.402
C*04	130	0.78	(0.43-1.41)	0.413	1.16	(0.71-1.89)	0.566
C*08	356	0.93	(0.59-1.47)	0.770	1.07	(0.71-1.62)	0.739
C*14	126	2.14	(1.27-3.60)	0.004	1.82	(1.15-2.86)	0.010
C*15	230	0.91	(0.54-1.54)	0.733	1.10	(0.73-1.66)	0.652

Risks of grades III-IV acute GVHD and treatment-related mortality of each patient's mismatched HLA-C allotype were compared to the risk of HLA-C*07 using a multivariable competing risk regression model.

N: number of patients; HR: hazard ratio, adjusted for clinical factors and HLA allele matching as listed in Table 1; CI: confidence interval.



Supplemental Figure 3: Association between expression level of patient mismatched HLA-C allotype and transplant outcome.

Hazard ratios (HRs) of (A) grades III-IV acute GVHD and (B) transplant-related mortality of each patient's mismatched HLA-C allotype are shown on the y-axis. The expression level of each HLA-C allotype is shown on the x-axis. HRs of grades III-IV acute GVHD and treatment-related mortality of each patient's mismatched HLA-C allotype were compared to HLA-C*07 using a multivariable competing risk regression model. The size of each circle is proportional to the number of patients with the indicated mismatched allotype. The expression level of each HLA-C allotype was defined as its median fluorescence intensity (MFI) as described previously.[1, 2] The expression level of a patient's mismatched HLA-C allotype was modeled as a continuous linear variable in multivariable analysis, and p-values are indicated.

Reference: Supplemental Figure 3

1. Apps R, Qi Y, Carlson JM, et al. Influence of HLA-C expression level on HIV control. Science. 2013;340(6128):87-91.
2. Petersdorf EW, Gooley TA, Malkki M, et al. HLA-C expression levels define permissible mismatches in hematopoietic cell transplantation. Blood. 2014;124(26):3996-4003.

Supplemental Table 3: Impact of donor-patient HLA-C mismatch combination on grades III-IV acute GVHD

Mismatch combination donor-patient	N	HR	(95% CI)	P
C locus match	4825	1.00		NA
C*03:03-C*03:04	103	0.73	(0.41-1.30)	0.291
C*08:01-C*03:03	95	1.80	(1.18-2.75)	0.007
C*03:03-C*08:01	95	1.15	(0.69-1.93)	0.586
C*03:04-C*03:03	89	0.96	(0.55-1.66)	0.876
C*03:04-C*08:01	71	1.71	(0.98-2.99)	0.061
C*03:04-C*15:02	70	1.75	(1.09-2.79)	0.020
C*14:02-C*15:02	68	1.41	(0.82-2.42)	0.218
C*03:03-C*04:01	58	1.35	(0.74-2.45)	0.333
C*04:01-C*03:03	56	1.34	(0.76-2.36)	0.318
C*03:03-C*01:02	56	1.46	(0.81-2.61)	0.205
C*08:01-C*03:04	52	1.14	(0.63-2.05)	0.672
C*15:02-C*14:02	51	4.39	(2.98-6.45)	<0.001
C*08:01-C*15:02	44	0.78	(0.31-1.95)	0.594
C*07:02-C*03:04	43	2.13	(1.21-3.72)	0.008
C*01:02-C*03:03	41	2.16	(1.14-4.09)	0.019
C*15:02-C*03:04	35	2.76	(1.64-4.63)	<0.001
C*03:04-C*14:02	35	2.66	(1.43-4.93)	0.002
C*03:04-C*07:02	34	1.89	(0.90-3.94)	0.091
C*08:01-C*08:03	34	0.85	(0.31-2.37)	0.757
C*01:02-C*08:03	34	2.04	(1.00-4.18)	0.051
C*08:03-C*08:01	33	2.30	(1.22-4.31)	0.010
C*03:03-C*07:02	28	0.85	(0.33-2.21)	0.737
C*07:02-C*03:03	27	2.46	(1.30-4.66)	0.006
C*03:03-C*15:02	26	2.82	(1.50-5.30)	0.001
C*14:02-C*03:04	26	2.52	(1.24-5.13)	0.011
C*03:04-C*01:02	25	1.32	(0.56-3.09)	0.527
C*15:02-C*08:01	24	1.90	(0.82-4.40)	0.134
C*03:04-C*04:01	22	2.67	(1.34-5.31)	0.005

C*01:02-C*14:02	21	4.01	(2.19-7.35)	<0.001
C*01:02-C*04:01	19	0.88	(0.27-2.87)	0.829
C*01:02-C*03:04	18	2.72	(1.09-6.80)	0.032
C*04:01-C*03:04	18	3.64	(1.66-7.99)	0.001
C*14:02-C*01:02	17	0.81	(0.19-3.41)	0.769
Others	327	1.45	(1.12-1.89)	0.005

Mismatch combinations are as follows: C*03:03-C*03:04 mismatch combination meant that the donor has HLA-C*03:03, the patient has HLA-C*03:04 and another HLA-C alleles of the donor and patient are identical. Each mismatch pair in HLA-C was compared with the HLA-C allele match.

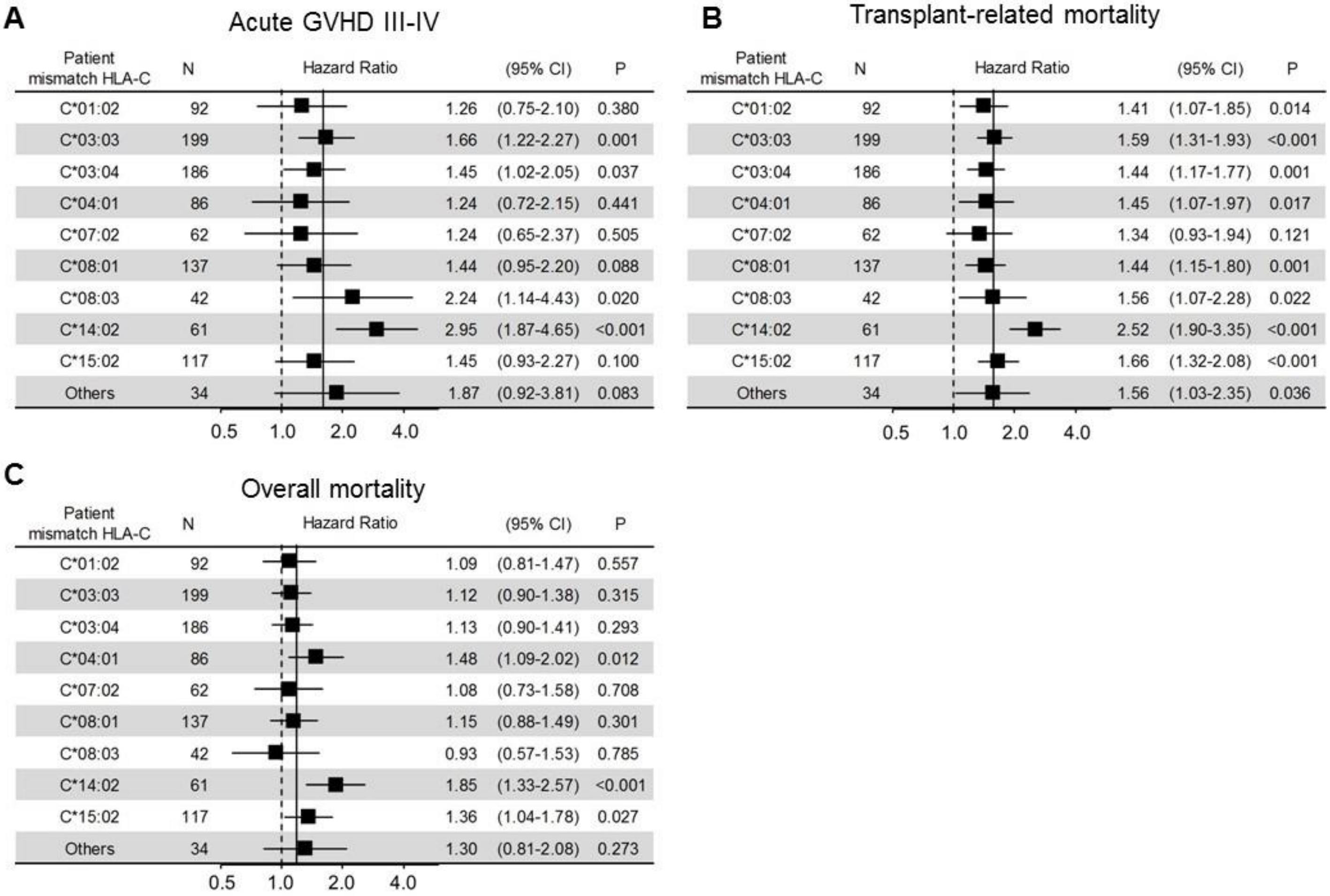
N: number of pairs; HR: hazard ratio, adjusted for clinical factors and HLA allele matching as listed in Table 1; CI: confidence interval; NA: not applicable.

Supplemental Table 4: Effect of HLA-C*14:02, -B*51:01 and their haplotypes on acute GVHD and mortality in patients transplanted from 2002 through 2010

HLA allele / haplorypte	N.	(%)	acute GVHD grades III-IV			Overall mortality		
			HR	(95% CI)	P	HR	(95% CI)	P
Patient								
B*51:01	636	(15)	1.38	(1.13-1.69)	0.002	1.17	(1.03-1.32)	0.013
C*14:02	519	(12)	1.36	(1.09-1.69)	0.006	1.17	(1.02-1.33)	0.023
C*14:02 - B*51:01	510	(12)	1.35	(1.09-1.69)	0.007	1.18	(1.03-1.34)	0.018
Donor								
B*51:01	647	(16)	1.38	(1.13-1.69)	0.002	1.13	(1.00-1.28)	0.052
C*14:02	532	(13)	1.16	(0.93-1.47)	0.194	1.08	(0.94-1.23)	0.276
C*14:02 - B*51:01	524	(13)	1.17	(0.93-1.48)	0.178	1.06	(0.93-1.22)	0.373

The effect of patient HLA-C*14:02 and HLA-C*51:01 and their haplotypes on grades III-IV acute GVHD and overall mortality in patients transplanted from 2002 through 2010 (n=4174) were analyzed.

N: number of HLA allele-positive donors or patients; HR: hazard ratio indicates comparison of the specific HLA allele/haplotype-positive group to the -negative group, adjusted for clinical factors and HLA allele matching as listed in Table 1; CI: confidence interval.



Acute GVHD III-IV

Patient mismatch HLA-C	N	Hazard Ratio	(95% CI)	P
C*01:02	92	1.26	(0.75-2.10)	0.380
C*03:03	199	1.66	(1.22-2.27)	0.001
C*03:04	186	1.45	(1.02-2.05)	0.037
C*04:01	86	1.24	(0.72-2.15)	0.441
C*07:02	62	1.24	(0.65-2.37)	0.505
C*08:01	137	1.44	(0.95-2.20)	0.088
C*08:03	42	2.24	(1.14-4.43)	0.020
C*14:02	61	2.95	(1.87-4.65)	<0.001
C*15:02	117	1.45	(0.93-2.27)	0.100
Others	34	1.87	(0.92-3.81)	0.083

Transplant-related mortality

Patient mismatch HLA-C	N	Hazard Ratio	(95% CI)	P
C*01:02	92	1.41	(1.07-1.85)	0.014
C*03:03	199	1.59	(1.31-1.93)	<0.001
C*03:04	186	1.44	(1.17-1.77)	0.001
C*04:01	86	1.45	(1.07-1.97)	0.017
C*07:02	62	1.34	(0.93-1.94)	0.121
C*08:01	137	1.44	(1.15-1.80)	0.001
C*08:03	42	1.56	(1.07-2.28)	0.022
C*14:02	61	2.52	(1.90-3.35)	<0.001
C*15:02	117	1.66	(1.32-2.08)	<0.001
Others	34	1.56	(1.03-2.35)	0.036

Overall mortality

Patient mismatch HLA-C	N	Hazard Ratio	(95% CI)	P
C*01:02	92	1.09	(0.81-1.47)	0.557
C*03:03	199	1.12	(0.90-1.38)	0.315
C*03:04	186	1.13	(0.90-1.41)	0.293
C*04:01	86	1.48	(1.09-2.02)	0.012
C*07:02	62	1.08	(0.73-1.58)	0.708
C*08:01	137	1.15	(0.88-1.49)	0.301
C*08:03	42	0.93	(0.57-1.53)	0.785
C*14:02	61	1.85	(1.33-2.57)	<0.001
C*15:02	117	1.36	(1.04-1.78)	0.027
Others	34	1.30	(0.81-2.08)	0.273

Supplemental Figure 4: Impact of patient mismatched HLA-C allele on clinical outcomes in patients transplanted from 2002 through 2010

Results of multivariable competing risk regression analysis for the effect of patient mismatched HLA-C allele on grades III-IV acute GVHD (A), transplant-related mortality (B), and overall mortality (C). The hazard ratio (HR) of each mismatched HLA-C allele was estimated by comparison to HLA-C match (n=2963). The solid vertical line at 1.60 (A), 1.56 (B), and 1.18 (C) indicates the HR of overall HLA-C mismatch in the GVH direction. Others contain patient mismatched HLA-C alleles with fewer than 20 patients, as follows: C*01:03, C*03:14, C*03:23, C*05:01, C*07:04, C*12:02, C*12:03 and HLA-C*14:03.