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Effects of mismatching for Minor Histocompatibility Antigens on clinical outcomes in HLA-matched, unrelated hematopoietic stem cell transplants:

Minor antigen mismatching in unrelated donors

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Abstract

Several studies in HLA-matched sibling hematopoietic stem cell transplantation (HSCT) have reported an association between mismatches in minor histocompatibility antigens (mHAg) and outcomes. We assessed whether single and multiple minor mHAg mismatches are associated with outcomes in 730 unrelated donor, HLA-A, B, C, DRB1, and DQB1 allele-matched hematopoietic stem cell transplants (HSCT) facilitated by the National Marrow Donor Program (NMDP) between 1996 and 2003. Patients had acute and chronic leukemia or myelodysplastic syndrome, received myeloablative conditioning regimens and calcineurin inhibitor-based graft-versus-host-disease (GvHD) prophylaxis, and most received bone marrow (85%). Donor and recipient DNA samples were genotyped for mHAg including: HA-1, HA-2, HA-3, HA-8, HB-1, $CD31^{125/563}$. Primary outcomes included grades III–IV acute GvHD and survival; secondary outcomes included chronic GvHD, engraftment, and relapse. Single disparities at HA-1, HA-2, HA-3, HA-8, and HB-1 were not significantly associated with any of the outcomes analyzed. In HLA-A2 positive individuals, single $CD31^{563}$ or multiple mHAg mismatches in the HvG vector were associated with lower risk of grades III–IV acute GVHD. Based on these data, we conclude that mHAg incompatibility at HA-1, HA-2, HA-3, HA-8, HB-1 and CD31 has no detectable effect on the outcome of HLA matched unrelated donor HSCT.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is the treatment of choice for a number of otherwise untreatable malignancies and hematologic disorders. Despite efforts to closely match recipients and donors, HSCT is limited by high rates of complications, including graft versus

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host disease, poor engraftment, and disease recurrence.[1] HLA matching reduces, but does not prevent the development of graft versus host disease. Rates of severe acute graft versus host disease (GvHD) approach 28% in HLA identical unrelated donor transplantation and 30% in HLA-matched sibling transplants.[2–6]

Disparities in minor histocompatibility antigens (mHAg) underlie the development of GvHD in HLA identical transplants.[7–9] mHAg are peptides derived from allelic variants of normal cellular proteins which, when presented by self class I or II MHC antigens, induce cellular immune responses in HLA-matched individuals lacking the same allelic variant. These protein/peptide variants most often arise due to single nucleotide polymorphisms (SNPs) or deletions. Cytotoxic T lymphocytes directed against mHAg have been isolated from recipients of HLA-matched transplants with acute GvH, and cytotoxic T cell clones from such patients have been used to identify and characterize mHAg.[10–16] While some mHAg are ubiquitously expressed (HA-3, HA-8), most have more restricted tissue expression, including HA-1, HA-2 (hematopoietic tissue), CD31 (platelets, endothelial cells), and HB-1 (B lymphoblastoid cells).[17,18] There likely exist thousands of protein variants with the potential of functioning as mHAg, although only about 2 dozen human mHAg have been identified.[19]

The role for mHAg disparities in HSCT outcomes has been supported by studies showing higher rates of acute GvH and lower survival in HLA-identical sibling transplant recipients who are mHAg disparate.[20–24] Mismatches in individual mHAg, including HA-1, HA-2, HA-8, and CD31, have been associated with increased rates of GvHD, and lower rates of leukemia recurrence observed in pairs who are disparate at HA-1 or HA-2 suggest a role for such disparities in graft versus leukemia (GvL) effects,[20,21] although this is disputed by other studies.[25] Additionally, disparities in HA-8 and CD31 were associated with decreased patient survival.[20–24] Mismatching for HA-1 in HLA-identical, HLA-A2 sibling pairs, was previously reported to be associated with higher rates of acute GvHD and a possible GvL effect.[20,21] However, investigation of the role of mHAg in transplant outcomes has been limited, due to the requirement to restrict studies to recipient/donor pairs expressing specific HLA types, as well as by the low frequencies of some mHAg alleles.[25]

In this study we investigated the effect of single and multiple disparities in autosomal mHAg on HSCT outcomes in 730 recipients of HLA-matched unrelated donor HSCTs.

MATERIALS AND METHODS

Patient Population

Recipient/donor pairs from 730 unrelated HLA-A, B, C, DRB1, and DQB1 allele-matched transplants facilitated by the National Marrow Donor Program (NMDP) were studied. HLA typing was confirmed through the NMDP's ongoing retrospective high resolution typing project as previously described (Flomenberg et al.).[26] The majority (86%) of the pairs were mismatched at HLA-DP. Transplants were performed between 1996 and 2003, and patient disease characteristics are summarized in Table 1. Eligible diagnoses included acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and myelodysplastic syndrome (MDS). Early stage disease was defined as AML and ALL in first complete remission, CML in first chronic phase, and MDS subtype refractory anemia. Intermediate stage disease was AML or ALL in second or subsequent complete remission or in first relapse, and CML in accelerated phase or second chronic phase. Advanced phase disease was AML in second or higher relapse or primary induction failure, CML in blast phase, MDS subtypes refractory anemia with excess blasts or in transformation, or MDS not otherwise classified. All patients received myeloablative conditioning regimens defined as "traditional" if single dose total body irradiation (TBI) was greater than 500 cGy, or more than 800 cGy total in fractionated doses (with or without cyclophosphamide), or cyclophosphamide

with at least 9.5 mg/kg busulfan, or "nontraditional" if conditioning included at least 9.5 mg/kg busulfan without cyclophosphamide or melphalan with a dose greater than 150 mg/m^2.

All surviving recipients included in this analysis were retrospectively contacted and provided informed consent for participation in the NMDP research program. Informed consent was waived by the NMDP Institutional Review Board for all deceased recipients. Approximately 4% of surviving patients would not provide consent for research. To adjust for the potential bias introduced by exclusion of non-consenting surviving patients, a modeling process randomly excluded appropriately the same percentage of deceased patients using a biased coin randomization with exclusion probabilities based on characteristics associated with not providing consent for use of the data in survivors.[6]

mHAg Genotyping

Recipient/donor samples were obtained from the NMDP Research Repository and included whole frozen blood, buffy coats, peripheral blood mononuclear cells, and DNA. Genotyping was performed on a panel of mHAg using a Luminex based, multiplex assay developed at the BloodCenter of Wisconsin, as described previously.[25] The mHAg panel and HLA restriction are summarized in Table 2. The mHAg panel was constructed to include well-characterized polymorphisms that have been demonstrated in previous studies to affect outcomes in HLA-matched sibling transplants. Briefly, the assay is performed in multiple steps using EraGen Biosciences's (Madison, WI) MultiCode Plx technology.[27,28] The assay is initiated with a multiplex polymerase chain reaction (PCR) amplification of target mHAg loci followed by allele-specific primer extension reactions which specifically incorporate a 3' biotin molecule. Hybridization of biotinylated extension products to EraCode-tagged Luminex™ xMAP beads is performed at room temperature and is then fluorescently labeled with streptavidin phycoerythrin (SA-PE) conjugate. Finally, the labeled xMAP beads are detected on a Luminex™ 100 instrument (Austin, TX). Genotypes are assigned based on the ratios of the relative fluorescence signals detected on paired Luminex™ beads that distinguish alternate forms of each mHAg allele. Primers used for mHAg locus-specific amplification and allele-specific extension reactions were synthesized by EraGen Biosciences.

mHAg mismatches and mismatch vectors, graft versus host (GvH) or host versus graft (HvG), or both, were assigned based on known mHAg genotypes (Table 2). With the exception of CD31 and HB-1, whose alternate alleles both encode mHAg, the antigenic peptide that comprises the mHAg for HA-1,-2,-3,-8 is encoded by only one of the two alleles.[11,14,29–31] For these latter mHAg, mismatches occurred in either the GvH or HvG direction, not both. Both $CD31^{125}$ and $CD31^{563}$ isoforms were genotyped; however, only differences at $CD31^{563}$ were analyzed due to the strong linkage between $CD31^{125}$ and $CD31^{563}$ polymorphisms.

Definitions of outcomes

The primary outcomes of the analysis were overall survival, defined as time from graft infusion (day 0) to death from any cause, and grades III–IV acute GVHD, defined by the Glucksberg scale.[32] A number of secondary endpoints were also analyzed. Failure to engraft (primary graft failure) was defined as failure to achieve an absolute neutrophil count greater than 500×10^6/L by day 28 which was maintained for three consecutive measurements. Extensive chronic GVHD was defined according to the Seattle criteria.[33] Clinical relapse of the primary disease was defined by the Center for International Blood and Marrow Transplant Research (CIBMTR) criteria.[26] Treatment-related mortality (TRM) is death in continuous complete remission of the primary disease. Disease free survival (DFS) is survival in continuous complete remission of the primary disease.

Statistical Analysis

For evaluation of mHAg matching, the cases were selected based on the previously described HLA restrictions for mHAg presentation. The sample size for each HLA restriction group, i.e. HLA-A1, A2 and B44, is noted in Table 3. For discrete factors, the number of cases and their respective percentages were calculated. Chi-Square tests were used to compare discrete factors between mHAg matched vs. 1 mismatch vs. ≥ 2 mismatches groups. For continuous factors, the median and ranges were calculated. The Kruskal-Wallis test was used to compare the continuous factors between mHAg matched vs. 1 mismatch vs. ≥ 2 mismatches groups. Probabilities for overall survival were calculated using the Kaplan-Meier estimator with variance estimated by Greenwood's formula. Comparison of survival curves was done using the log-rank test. Values for other outcomes were calculated according to cumulative incidence using a Taylor series linear approximation to estimate the variance.

Multivariate analyses were performed using the proportional hazards model to compare the mHAg matched vs. 1 mismatch vs. ≥ 2 mismatches groups with adjustment for statistically significant covariates. Due to multiple comparisons, the significance threshold was set at $p<0.01$. Potential covariates include patient age, sex, race, Karnofsky performance status, time from diagnosis to HCT, donor type, donor-recipient sex match, cytomegalovirus (CMV) serological status, type of conditioning regimen, graft source, year of transplantation, and GVHD prophylaxis regimen. Models were fit to determine which risk factors were related to a given outcome. All variables were tested for the affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption were adjusted for by stratification. Stepwise model building approach was used in developing models for the primary and secondary outcomes.

Cox regression models were used to evaluate the association between transplant outcomes versus match/mismatch at any single mHAg, mismatches at 2 mHAg versus one or no mismatches, and the directionality of the mismatch (GvH, HvG). Table 3 summarizes the sample size used for each analysis, and the power to detect both a five and ten percent difference in survival.

RESULTS

Single mHAg mismatches

A single mismatch in either direction (GvH or HvG) for HA-1, HA-2, HA-3, HA-8, and HB-1 was not significantly associated with any outcome analyzed at $p < 0.01$. Table 4 summarizes the 95% confidence intervals for the effects of single mHAg mismatches on survival, grades IIII–V acute GvHD, TRM and chronic GvHD. In no case was the relative risk significantly different than 1, at $p < 0.01$ for any mHAg, regardless of the directionality of the mismatch. Low statistical power due to small sample size shows the limited power of the analysis (Table 3).

The only significant finding occurred in HLA-A2 positive pairs where there was a significantly reduced risk of grades III–IV GvHD when pairs were mismatched for $CD31^{563}$ in the host versus graft direction (RR=0.41; CI=0.24–0.71; p=0.001). Note that a similar association was not observed for HLA-A1 (RR=0.71; CI=0.39–1.29; p=0.26) or B44 positive pairs (RR=0.95; CI=0.53–1.71; p=0.86) and comparison of outcomes of all $CD31^{563}$-mismatched recipient donor pairs without regard to HLA type showed no significant association with any outcome when compared to CD31 matched pairs (data not shown). Because previous studies indicated that donors who were heterozygous for the $CD31^{563}$ polymorphism were associated with poorer survival post-transplant, [24] we compared outcomes from transplants with 361 donors who were heterozygous for the $CD31^{563}$ SNP with 368 donors homozygous for this SNP. No

association with survival was observed (RR =0.92; 95% CI = 0.75–1.11). Likewise, no significant association was observed between a specific recipient or donor $CD31^{563}$ allele and any outcome analyzed (data not shown).

An analysis of the impact of HY antigen disparities, a proven mHAg, was also conducted on the complete dataset using sex match, i.e. female donor into male recipient, as a surrogate for HY disparity. No effect of HY mismatching was observed for any outcome in the analysis (data not shown).

Multiple mHAg Mismatches

The effect of multiple mHAg mismatches was determined by comparing outcomes for recipient/donor pairs based on the total number of mismatched mHAg for each HLA restriction (Table 5). Specific comparisons were grouped according to HLA restriction and included pairs mismatched at 2 mHAg versus 1 or no mismatches in both GvH and HvG directions.

When the effects of multiple mHAg mismatches were analyzed, a reduced risk of acute GvHD was observed among HLA-A2 positive pairs who were mismatched for 2 or more mHAg for HA-1, HA-2, HA-8 and/or $CD31^{563}$ in the HvG direction (RR=0.41; CI=0.23–0.73; p=0.003) when compared to matched pairs, perhaps reflecting the influence of $CD31^{563}$ mismatching on this group. HLA-A2 positive pairs who were mismatched for 2 or more mHAg (HA-1, HA-2, HA-8, and/or $CD31^{563}$) in the GvH direction appeared to have lower survival (RR=1.54; CI=1.09–2.18; p=0.01). Likewise, there was a suggestion that HLA-A1 positive individuals mismatched for both CD31 and HA-3 in the GvH direction had decreased survival and increased TRM compared to matched pairs (Overall survival: RR=2.01; 95% CI=1.14–3.55; p=0.02; and TRM: RR=2.28; 95% CI=1.17–4.44; p=0.02); however neither result met the significance threshold of $P<0.01$ set for the study due to multiple comparisons (Table 5). No other multiple mHAg mismatches were associated with any of the outcomes analyzed in any of the HLA-restriction groups.

DISCUSSION

This comprehensive analysis is the first to examine the role of mHAg disparities in the outcome of HLA-matched unrelated HSCT and failed to corroborate results in HLA-identical sibling transplants where single mHAg mismatches have been associated with significantly increased rates of acute GvHD (HA-1,HA-2,HA-8,CD31), decreased survival (HA-8,CD31), and lower rates of disease recurrence (HA-1,HA-2).[20–24,34–36] The present study comprised the largest number of HLA-matched recipient/donor pairs evaluated to date. Nevertheless, small subgroup size and the greater disparity in HLA and non-HLA associated polymorphic genes between unrelated donor/recipient pairs may have limited the power of our analysis.[37] Other limitations of the study include a predominance of bone marrow as a graft source and a low number of patients under the age of 20 which may restrict the relevance of these findings in peripheral blood and pediatric transplants.

We did observe that single $CD31^{563}$ mHAg mismatches in the HvG vector in HLA-A2 positive individuals were found to potentially reduce the risk of developing grades III–IV acute GVHD, although the biological mechanism remains unclear. Significant associations with outcomes were also observed for multiple mismatches in HLA-A1 positive recipient donor pairs, mismatching at HA-3 plus CD31 in the HvG direction was associated with increased survival and decreased treatment related mortality, and in HLA-A2 pairs mismatched for HA-1, HA-2, HA-8, or CD31in the HvG direction there was a significantly lower rate of acute GvHD, but may reflect the influence of CD31 mismatching. In all cases it is unclear whether the differences reflect a true biologic impact of mismatching or a random effect.

In HLA-identical sibling transplants HA-3 disparity alone had no impact on GvHD, whereas multiple studies indicated that CD31 nonidentity is a significant risk factor for overall survival and acute GvHD.[24] Any clinical risk of HA-3 mismatching is minimized by the fact that a majority of Caucasians (77%) express the HA-3 mHAg, making the likelihood of a mismatch low. It is noteworthy that in contrast to the majority of the other mHAg studied, HA-3 and CD31 are not restricted to hematopoietic cells but have a wide range of cell and tissue expression. CD31 (PECAM-1) functions as a homotypic adhesion molecule that is expressed on a variety of cells and tissues, including endothelial cells, platelets, and leukocytes. CD31 has never been directly demonstrated to be immunogenic nor function as a mHAg, as this latter property has been implied indirectly through the demonstration that recipient/donor pairs mismatched for CD31 allelic forms have higher risks of GvHD.[24] Cavanagh et al. showed that donor heterozygosity for CD31^{563} alleles was associated with decreased survival in matched sibling HSCT, a finding that suggests that any effect of CD31 polymorphisms on HSCT outcomes may instead reflect inherent functional properties of CD31 isoforms and are not due to mHAg effects.[24] However, we failed to confirm this effect in unrelated donor HSCT and further failed to observe any significant association between the presence of specific CD31 alleles in the recipient or donor and any outcome (data not shown).

Although the present study comprised the largest number of fully HLA-matched unrelated donor HSCT cases evaluated to date, statistical power to detect significant differences was low for many comparisons due in part to the relative infrequency of some mHAg alleles, low likelihood of mismatches (e.g., HA-2, HA-3), [25] and the study size limitations resulting from mHAg HLA presentation restrictions. In addition to the mHAg panel, analysis of the mHAg effects of HY disparity was also negative, potentially due to low power, with only 15% of the population at risk. Given these considerations, statistical power will remain a limitation to the characterization of mHAg disparities on unrelated HSCT outcomes. However, it should be noted that the original effects of HA-1 and HA-2 mismatching in HLA identical sibling HSCT outcomes were detected with as few as 117 HLA-A2 positive study subjects, in contrast to the present study which involved 430 HLA-A2 positive unrelated pairs.[20,22] These findings suggest that additional HLA disparities (HLA-DP) and other factors may mask the impact of mHAg disparity in unrelated donor HSCT.

The majority of the current study population (86%) was mismatched at HLA-DP, which has been recently associated with an increased risk of acute GvHD and lower relapse rates in unrelated donor HSCT.[37] The high rate of HLA-DP mismatching in our population may mask any contributions of mHAg mismatching to risk of acute GvHD in unrelated donor HSCT. By extension, the clinical impact of mHAg disparities in unrelated HSCT may be rendered moot given the likelihood that recipient/donor pairs who are selected based on allele-level matching at HLA-A, B, C, DRB1 and DQB1 are likely to be mismatched at HLA-DP. Another hypothesis to explain our findings is differences in patient immunosuppression and management, as risk for HA-1 associated GvHD may be lower in patients receiving both methotrexate and cyclosporine than in those who receive either alone.[21]

The failure of our studies to show a significant effect of mHAg disparities on outcomes in unrelated donor HSCT indicates the importance of other genetic determinants. While further studies may be warranted to verify the possible biological significance of CD31 mismatching in unrelated donor HSCT, the clinical utility of matching for mHAg is limited by the lack of significant clinical correlation with outcome and the low frequencies of many mHAg genotypes.[25,38]

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Table 1

Patient demographics

	N Eval	N (%)
Number of recipient/donor pairs		730
Number of centers		83
Age, median (range), years	729	37 (<1–65)
Age at transplant	729	
< 10 y		50 (7)
11 – 20 y		67 (9)
21 – 30 y		123 (17)
31 – 40 y		175 (24)
41 – 50 y		193 (26)
Over 50 y		121 (17)
Karnofsky prior to transplant ≥ 90	686	506 (74)
Disease at transplant	730	
AML		210 (29)
ALL		150 (21)
CML		242 (33)
MDS		128 (17)
Disease status at transplant	730	
Early		329 (45)
Intermediate		231 (32)
Advanced		119 (16)
Other		51 (7)
Graft type	730	
Bone marrow		623 (85)
PBSC		107 (15)
Donor/recipient sex match	730	
Male/Male		288 (40)
Male/Female		190 (26)
Female/Male		110 (15)
Female/Female		142 (19)
Donor/recipient CMV match	730	
Negative/Negative		275 (38)
Negative/Positive		194 (26)
Positive/Negative		101 (14)
Positive/Positive		140 (19)
Unknown		20 (3)
Donor age, median (range), years	730	35 (19–60)
Year of transplant		
1996–1999		352 (48)
2000–2003		378 (52)
Median follow-up of survivors, months		60 (10–107)

Table 2

mHAg panel

mHAg	HLA Restriction	Effect of Disparity in HLA Matched Sibling HSCT	Reference
HA-1	HLA-A2	Increased acute GvHD GVL Effect	20¯22
HA-2	HLA-A2	Increased acute GvHD GvL Effect	20,22
HA-3	HLA-A1	No effect on GvHD	20
HA-8	HLA-A2	Increased acute GvHD Decreased survival	34
HB-1	HLA-B44	Unknown	
CD31	unknown	Increased acute GVHD Decreased Survival	23,24

Table 3

Power to detect a difference in overall survival for individual and combined mHAg

mHAg HLA restriction	mHAgs mismatched in GVH vector	N (Mismatched:Matched)	Power to detect 5% increase in Overall Survival	Power to detect 10% increase in Overall Survival
HLA-A*01 (N = 327)	HA-3 and CD31	18:173	7%	13%
	HA-3 or CD31	136:173	15%	43%
	CD31	86:123	11%	31%
	HA-3	42:249	9%	22%
HLA-A*02 (N = 430)	HA-1,HA-2, HA-8 and/or CD31	90:161	12%	34%
	HA-1,HA-2, HA-8 or CD31	179:161	16%	48%
	CD31	96:164	12%	35%
	HA-1	90:228	13%	37%
	HA-2	19:397	7%	13%
	HA-8	95:269	14%	39%
HLA-B*44 (N = 257)	HB-1 and CD31	26:120	7%	15%
	HB-1 or CD31	111:120	12%	34%
	CD31	52:106	9%	22%
	HB-1	61:129	10%	25%
No known restriction (N = 730)	CD31	174:280	19%	56%

Table 4

Multivariate results: Single mHAg Mismatches

HLA restriction/mHAg/Vector			Survival			Acute GVHD III–IV			TRM			cGVHD		
			RR	95%CI	P Value	RR	95%CI	P Value	RR	95%CI	P Value	RR	95%CI	P Value
A1	CD31	GvH MM vs. Matched	0.95	0.65–1.40	0.81	0.59	0.33–1.06	0.08	1.15	0.74–1.78	0.53	0.89	0.57–1.38	0.60
		HvG MM vs. Matched	0.89	0.60–1.32	0.56	0.71	0.39–1.29	0.26	0.94	0.58–1.52	0.81	1.28	0.84–1.94	0.25
	HA-3	GvH MM vs. Matched	1.46	0.95–2.25	0.08	0.79	0.40–1.58	0.51	1.37	0.83–2.28	0.22	1.22	0.72–2.08	0.45
		HvG MM vs. Matched	1.53	0.98–2.40	0.06	1.07	0.52–2.19	0.86	1.33	0.77–2.31	0.30	1.12	0.67–1.89	0.67
A2	CD31	GvH MM vs. Matched	0.92	0.65–1.29	0.63	0.88	0.57–1.36	0.57	1.03	0.69–1.53	0.89	0.87	0.59–1.28	0.48
		HvG MM vs. Matched	0.65	0.46–0.92	0.02	0.41	0.24–0.71	0.001	0.65	0.42–0.99	0.04	0.88	0.61–1.25	0.47
	HA-1	GvH MM vs. Matched	1.27	0.92–1.74	0.14	1.05	0.67–1.63	0.83	1.13	0.76–1.67	0.55	0.89	0.61–1.29	0.54
		HvG MM vs. Matched	1.03	0.76–1.41	0.85	0.78	0.50–1.21	0.27	1.09	0.76–1.57	0.65	0.83	0.59–1.17	0.28
	HA-2	GvH MM vs. Matched	1.14	0.63–2.04	0.67	1.18	0.55–2.57	0.67	1.55	0.81–2.96	0.18	1.26	0.65–2.45	0.49
		HvG MM vs. Matched	1.09	0.52–2.28	0.81	0.45	0.11–1.84	0.27	1.16	0.50–2.68	0.73	0.91	0.42–1.97	0.81
	HA-8	GvH MM vs. Matched	1.19	0.86–1.63	0.30	0.93	0.60–1.43	0.73	1.00	0.68–1.47	0.99	0.96	0.68–1.36	0.82
		HvG MM vs. Matched	1.18	0.82–1.71	0.37	0.70	0.40–1.22	0.21	1.04	0.67–1.61	0.86	0.91	0.61–1.35	0.63
B44	CD31	GvH MM vs. Matched	0.87	0.55–1.36	0.54	1.24	0.67–2.28	0.49	1.09	0.64–1.85	0.45	0.79	0.50–1.27	0.33
		HvG MM vs. Matched	1.02	0.66–1.57	0.94	0.95	0.53–1.71	0.86	0.80	0.46–1.42	0.75	0.70	0.44–1.09	0.11

HLA restriction/mHAg/Vector		Survival			Acute GVHD III–IV			TRM			cGVHD		
		RR	95%CI	P Value	RR	95%CI	P Value	RR	95%CI	P Value	RR	95%CI	P Value
HB–1	GvH MM vs. Matched	1.00	0.66–1.52	0.99	1.35	0.76–2.40	0.30	0.99	0.59–1.66	0.96	0.77	0.49–1.22	0.27
	HvG MM vs. Matched	0.91	0.57–1.46	0.70	0.96	0.53–1.71	0.91	0.97	0.55–1.73	0.92	0.90	0.56–1.42	0.64

Table 5

Multivariate results: Multiple mHAg mismatches

		Survival			Acute GVHD III–IV			TRM			cGVHD		
		RR	**95%CI**	**P Value**	**RR**	**95%CI**	**P Value**	**RR**	**95%CI**	**P Value**	**RR**	**95%CI**	**P Value**
A1	HA-3 or CD31 GvH MM vs. Matched	0.85	0.61–1.17	0.31	0.78	0.49–1.23	0.28	0.92	0.63–1.34	0.68	1.01	0.71–1.42	0.98
	HA-3 and CD31 GvH MM vs. Matched	2.01	1.14–3.55	0.02	0.76	0.27–2.16	0.61	2.28	1.17–4.44	0.02	0.69	0.25–1.91	0.48
	HA-3 or CD31 HvG MM vs. Matched	0.93	0.67–1.27	0.63	0.95	0.59–1.53	0.85	0.84	0.57–1.24	0.39	1.31	0.93–1.84	0.13
	HA-3 and CD31 HvG MM vs. Matched	1.76	0.97–3.19	0.06	1.50	0.58–3.83	0.40	1.59	0.79–3.23	0.20	1.09	0.47–2.54	0.84
A2	HA-1, HA-2, HA-8 or CD31 GvH MM vs. Matched	1.15	0.86–1.53	0.35	1.16	0.77–1.74	0.47	1.05	0.74–1.48	0.81	1.25	0.91–1.71	0.17
	HA-1, HA-2, HA-8 and/or CD31 GvH MM vs. Matched	1.54	1.09–2.18	0.01	1.22	0.76–1.95	0.41	1.52	1.02–2.26	0.04	0.87	0.59–1.29	0.50
	HA-1, HA-2, HA-8 or CD31 HvG MM vs. Matched	0.85	0.64–1.13	0.25	0.69	0.48–1.00	0.05	0.89	0.63–1.25	0.50	1.09	0.80–1.48	0.59

		Survival			Acute GVHD III–IV			TRM			cGVHD		
		RR	95%CI	P Value	RR	95%CI	P Value	RR	95%CI	P Value	RR	95%CI	P Value
	HA-1, HA-2, HA-8 and/or CD31M HvG MM vs. Matched	0.90	0.63–1.30	0.58	0.41	0.23–0.73	0.003	0.93	0.60–1.44	0.76	0.72	0.47–1.10	0.13
B44	HB-1 or CD31 GvH MM vs. Matched	1.19	0.83–1.70	0.35	0.90	0.54–1.50	0.68	1.31	0.84–2.04	0.24	0.60	0.41–0.89	0.0114
	HB-1 and CD31 GvH MM vs. Matched	0.82	0.44–1.55	0.54	1.55	0.76–3.16	0.23	1.08	0.52–2.22	0.84	1.05	0.61–1.83	0.85
	HB-1 or CD31 HvG MM vs. Matched	0.95	0.66–1.35	0.77	0.89	0.54–1.45	0.63	0.81	0.53–1.26	0.35	0.96	0.67–1.38	0.84
	HB-1and CD31 HvG MM vs. Matched	1.28	0.68–2.41	0.45	0.62	0.24–1.60	0.33	1.11	0.51–2.39	0.79	0.57	0.27–1.20	0.14