original reports

Impact of the HLA Immunopeptidome on Survival of Leukemia Patients After Unrelated Donor Transplantation

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abstract

PURPOSE Immunopeptidome divergence between mismatched HLA-DP is a determinant of T-cell alloreactivity and clinical tolerability after fully HLA-A, -B, -C, -DRB1, -DQB1 matched unrelated donor hematopoietic cell transplantation (UD-HCT). Here, we tested this concept in HLA-A, -B, and -C disparities after single class I HLA-mismatched UD-HCT.

PATIENTS AND METHODS We studied 2,391 single class I HLA-mismatched and 14,426 fully HLA-matched UD-HCT performed between 2008 and 2018 for acute leukemia or myelodysplastic syndromes. Hierarchical clustering of experimentally determined peptide-binding motifs (PBM) was used as a proxy for immunopeptidome divergence of HLA-A, -B, or -C disparities, allowing us to classify 1,629/2,391 (68.1%) of the HLA-mismatched UD-HCT as PBM-matched or PBM-mismatched. Risks associated with PBM-matching status were assessed by Cox proportional hazards models, with overall survival (OS) as the primary end point.

RESULTS Relative to full matches, bidirectional or unidirectional PBM mismatches in graft-versus-host (GVH) direction (PBM-GVH mismatches, 60.7%) were associated with significantly lower OS (hazard ratio [HR], 1.48; $P < .0001$), while unidirectional PBM mismatches in host-versus-graft direction or PBM matches (PBM-GVH matches, 39.3%) were not (HR, 1.13; $P = .1017$). PBM-GVH mismatches also had significantly lower OS than PBM-GVH matches in direct comparison (HR, 1.32; $P = .0036$). The hazards for transplant-related mortality and acute and chronic graft-versus-host disease but not relapse increased stepwise from full HLA matches to single PBM-GVH matches, and single PBM-GVH mismatches. A webtool for PBM-matching of single class I HLA-mismatched donor-recipient pairs was developed.

CONCLUSION PBM-GVH mismatches inform mortality risks after single class I HLA-mismatched UD-HCT, suggesting that prospective consideration of directional PBM-matching status might improve outcome. These findings highlight immunopeptidome divergence between mismatched HLA as a driver of clinical tolerability in UD-HCT.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a powerful treatment option for patients affected by high-risk neoplastic blood disorders.[1] In this setting, alloreactive donor T cells recognizing peptides presented in the antigen-binding groove of matched or mismatched HLA molecules of the patient mediate both the beneficial graft-versus-leukemia (GVL) effect and detrimental graft-versus-host (GVH) disease (GVHD).[2] Unrelated donors (UD) fully matched with the patient for HLA-A, -B, -C, -DRB1, and -DQB1 (10 of 10 HLA-matched) are most frequently used, followed by HLA-matched or HLA-mismatched relatives or UD.[3-5] HLA-A, -B, or -C mismatches are present in more than 73% and 78% of single HLA-mismatched (9 of 10 HLA-matched) UD-HCT in studies from the United States and Europe.[6,7] In transplants performed under calcineurin inhibitor (CNI)–based GVHD prophylaxis, every mismatch at HLA-A, -B, -C or -DRB1 reduces survival probabilities by about 10%.[7-10] In 10 of 10 UD-HCT, structural similarity of mismatched HLA-DPB1 allotypes according to T-cell epitope groups was shown to define permissive mismatches associated with less mortality and less GVHD, compared with structurally dissimilar allotypes defining nonpermissive mismatches.[11-16] Mechanistically, these differences are reflective of a reduced divergence of the peptide repertoire, that is, the immunopeptidome, of permissive compared with nonpermissive HLA-DPB1 allotypes, determining in turn the size and diversity of the responding alloreactive T-cell repertoire.[17,18] Immunopeptidome divergence arises from distinct biochemical characteristics of peptides presented by polymorphic HLA allotypes, resulting in similar or differential peptide-binding motifs (PBM). Here, we hypothesized that immunopeptidome divergence, as predicted by differences in the PBM of

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

This article investigates the role of immunopeptidome divergence between single mismatched HLA-A, -B, or -C allotypes for clinical outcome of unrelated hematopoietic cell transplantation.

Knowledge Generated

Single class I HLA mismatches between donor and recipient with high immunopeptidome divergence predicted by distinct peptide-binding motif (PBM) groups present in the recipient, but not in the donor (graft-versus-host direction), are associated with inferior survival than mismatches with low immunopeptidome divergence predicted by identical PBM groups in recipient and donor.

Relevance *(C.F. Craddock)*

Prospective selection of donors without HLA class I PBM mismatches in the graft-versus-host direction may improve survival probability after HLA-disparate hematopoietic cell transplantation, which is particularly relevant for patient populations heavily dependent on mismatched donors. Validation of these data that identify HLA-restricted immunopeptidome divergence as a potentially important new driver of clinically relevant T-cell alloreactivity in an independent patient cohort will be important.*

*Relevance section written by *JCO* Associate Editor Charles F. Craddock, MD.

mismatched HLA class I alleles, might also inform clinical outcomes after 9 of 10 HLA-matched UD-HCT.

PATIENTS AND METHODS

Study Design and Population

We studied 16,817 UD-HCT reported to the Center of International Blood and Marrow Transplant Research between 2008 and 2018 in patients with acute myeloid or lymphoid leukemia, or myelodysplastic syndromes (Table 1). 14,426 (85.8%) and 2,391 (14.2%) were matched for 10 of 10 and 9 of 10 HLA-A, -B, -C, -DRB1, -DQB1 alleles, respectively, the latter with a single mismatch at HLA-A, -B, or -C. Of these, 762 (31.9%) could not be assigned a PBM group and were hence excluded, leaving 1,629 (68.1%) PBM-informative pairs for the final analysis. GVHD prophylaxis was based on CNI, with or without in vivo T-cell depletion by antithymocyte globulin or alemtuzumab. Transplants performed with post-transplant cyclophosphamide (PTCy) for GVHD prophylaxis were excluded. Transplant protocols had been approved by the review boards of the participating centers, and all patients provided written informed consent according to the Declaration of Helsinki.

HLA Typing and Matching

All pairs had second-field HLA typing (allowing for univocal determination of the peptide binding domains) of HLA-A, -B, -C, -DRB1, and -DQB1, while HLA-DPB1 typing data were available for 74.1% of them. A total of 21 PBM groups were determined for 122 HLA class I allotypes (41, 63, and 18 for HLA-A, -B, and -C, respectively), on the basis of hierarchical clustering of experimentally determined PBM (Data Supplement, online only).[19] The HLA-A, -B, and -C alleles assigned to PBM groups had a cumulative allele frequency of 90.9%, 85.4%, and 89.1%, respectively, in the study cohort. Moreover, 271 alleles not assignable to PBM groups were found in the study cohort, four of which (HLA-A*33:03, B*55:01, C*07:04, and C*16:02) had an allele frequency above 1% (Data Supplement). PBM group assignments were used as proxies for immunopeptidome divergence between informative single HLA class I mismatched allotypes in the 9 of 10 group (Fig 1). Previously described HLA supertypes, mainly on the basis of antigen binding cleft polymorphism and predicted peptide binding characteristics (Data Supplement), were defined as in the relevant references.[20,21] The PBM and supertype status of class I HLA mismatches was assigned semiautomatically via Excel worksheets with appropriately designed formulas, and manually confirmed for randomly selected pairs by expert review. Moreover, an online webtool was developed for automated PBM group assignment on the basis of second-field HLA data[22] (Data Supplement). Webtool assignments for randomly selected pairs were confirmed both manually and by Excel-based assignment.

Statistical Analysis

The primary study end point was overall survival (OS). Secondary end points included transplant-related mortality (TRM), acute GVHD (aGVHD) grades 2-4 or 3-4, chronic GVHD (cGVHD), relapse, relapse-free survival (RFS), and neutrophil and platelet engraftment. GVHD-free, relapse-free survival, defined as the time to the first occurrence of aGVHD grades 3-4, cGVHD requiring systemic treatment, relapse, or death, whichever occurs first,[23] could not be evaluated because data on the treatment of cGVHD were not available for most patients. The association between PBM mismatches and various end points were assessed

TABLE 1. Recipient, Donor, and Transplant Characteristics

Variable	Fully Matched (10 of 10), No. (%)	PBM-Matched (9 of 10), No. (%)	PBM-Mismatched (9 of 10), No. (%)	PBM Not Assigned (9 of 10), No. (%)
Recipients, No.	14,426	386	1,243	762
Diagnosis				
AML	8,249 (57)	213 (55)	727 (58)	401 (53)
ALL	2,854 (20)	89 (23)	281 (23)	201 (26)
MDS	3,323 (23)	84 (22)	235 (19)	160 (21)
Disease status at transplant				
Early	7,444 (52)	176 (46)	579 (46)	375 (49)
Intermediate	2,127 (15)	77 (20)	259 (21)	145 (19)
Advanced	4,855 (33)	133 (34)	405 (33)	242 (32)
Karnofsky index				
< 90	5,820 (40)	153 (40)	445 (36)	271 (36)
≥ 90	8,606 (60)	233 (60)	798 (64)	491 (64)
HCT comorbidity index				
0-1	5,635 (39)	173 (45)	556 (45)	355 (47)
≥ 2	8,791 (61)	213 (55)	687 (55)	407 (53)
Months from diagnosis to transplant				
Median (range)	6 (0-691)	8 (1-188)	8 (0-371)	7 (1-215)
Recipient age, years				
< 20	1,211 (8)	38 (10)	161 (13)	125 (16)
≥ 20	13,215 (92)	348 (90)	1,082 (87)	637 (84)
Median (range)	56 (0-84)	51 (1-77)	51 (1-78)	47 (1-76)
Donor age, years				
< 30	8,751 (60)	163 (42)	607 (49)	342 (45)
≥ 30	5,675 (40)	223 (58)	636 (51)	420 (55)
Median (range)	28 (17-64)	32 (19-60)	30 (18-60)	32 (18-61)
Donor/recipient sex match				
M-M	6,208 (43)	139 (36)	471 (38)	257 (34)
M-F	4,154 (29)	87 (23)	296 (24)	174 (23)
F-M	1,914 (13)	88 (23)	234 (19)	157 (21)
F-F	2,150 (15)	72 (19)	242 (19)	174 (23)
Donor/recipient CMV serostatus				
+/+	4,007 (28)	115 (30)	381 (31)	313 (41)
+/–	1,433 (10)	36 (9)	157 (13)	82 (11)
–/+	5,128 (36)	136 (35)	420 (34)	236 (31)
–/–	3,858 (27)	99 (26)	285 (23)	131 (17)
Year of transplant				
2008-2011	3,392 (24)	147 (38)	392 (32)	234 (31)
2012-2015	5,786 (40)	153 (40)	551 (44)	346 (45)
2016-2018	5,248 (36)	86 (22)	300 (24)	182 (24)
Graft type				
BM	2,777 (19)	86 (22)	274 (22)	192 (25)
PBSC	11,649 (81)	300 (78)	969 (78)	570 (75)

(continued on following page)

TABLE 1. Recipient, Donor, and Transplant Characteristics (continued)

Variable	Fully Matched (10 of 10), No. (%)	PBM-Matched (9 of 10), No. (%)	PBM-Mismatched (9 of 10), No. (%)	PBM Not Assigned (9 of 10), No. (%)
Conditioning regimen				
MAC	8,696 (60)	249 (65)	790 (64)	536 (70)
RIC	5,730 (40)	137 (35)	453 (36)	226 (30)
GVHD prophylaxis				
TAC-based	11,911 (83)	287 (74)	918 (74)	584 (77)
CSA-based	2,515 (17)	99 (26)	325 (26)	178 (23)
Use of ATG or campath				
Yes	5,446 (38)	200 (52)	665 (53)	372 (49)
No	8,980 (62)	186 (48)	578 (47)	390 (51)
HLA-DPB1 matching				
Nonpermissive	3,682 (25)	105 (27)	364 (29)	197 (26)
Permissive	5,070 (35)	112 (29)	413 (33)	256 (34)
Allele-matched	2126 (15)	31 (8)	107 (9)	72 (9)
Missing	3,548 (25)	138 (36)	359 (29)	237 (31)
PBM directionality				
Bidirectional	NA	NA	751 (60)	NA
Unidirectional GVH			238 (19)	
Unidirectional HVG			254 (21)	
Follow-up, months, median				
Median (25th-75th)	23 (7-52)	14 (4-55)	15 (5-48)	16 (5-49)

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin; BM, bone marrow; CMV, cytomegalovirus; CSA, cyclosporine A; F, female; GVH, graft-versus-host; GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; HVG, host-versus-graft; M, male; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; NA, not applicable; PBM, peptide-binding motifs; PBSC, peripheral blood stem cells; RIC, reduced intensity conditioning; TAC, tacrolimus.

using Cox proportional hazards models (Data Supplement). All clinical variables were tested for affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption were adjusted through stratification. Stepwise forward and backward model building procedures were used to select the adjusted covariates for each outcome with a threshold of $P = .05$ for both entry and retention in the model. Interactions between the PBM mismatch variables and the adjusted covariates were tested, and no directional change in effects of the main testing variables for any adjusted covariates was detected in any of the models. Center effect was adjusted in all models. For the end points relapse, TRM, and aGVHD, we also used Fine and Gray's subdistribution hazard models,[24] with death from any cause as competing event for relapse and aGVHD, and relapse as competing event for TRM. To account for multiple testing, the significance level of $P < .01$ was used for association of the main testing variable.

RESULTS

Transplant Outcomes

Univariate pointwise outcome estimates and overall *P* values for 9 of 10 versus 10 of 10 UD-HCT are presented below. The median follow-up time of patients in this cohort was 23 (7-51) months. The 3-year probability of OS was lower after 9 of 10 matched, PBM-informative UD-HCT than after 10 of 10 matched UD-HCT (42% *v* 52%, $P < .001$). OS probabilities were not significantly different between 9 of 10 UD-HCT with or without PBM information (43% *v* 42%, $P = .278$). Compared with full HLA matches, PBM-informative single class I HLA mismatches were also associated with worse 3-year probabilities of TRM (31% *v* 21%, $P < .001$) and RFS (38% *v* 47%, $P < .001$), but not of relapse (31% *v* 32%, $P = .812$). Significant differences were also observed between PBM-informative single class I HLA-mismatched and fully matched transplants for aGVHD 2-4 (51% *v* 45%, $P < .001$) and aGVHD 3-4 (25% *v* 16%, $P < .001$), but not for cGVHD (42% *v* 42%, $P = .251$).

HLA Class I PBM Status

On the basis of available PBM information,[19] PBM status could be determined for 1,629/2,391 (68.1%) informative pairs, 386/1,629 (23.7%) PBM-matched, and 1,243/1,629 (76.3%) PBM-mismatched. Manual PBM assignment and automated PBM assignment by the online webtool[22] (Data Supplement) were performed in parallel on selected pairs with concordant results (not shown). When considering directionality, 989/1,

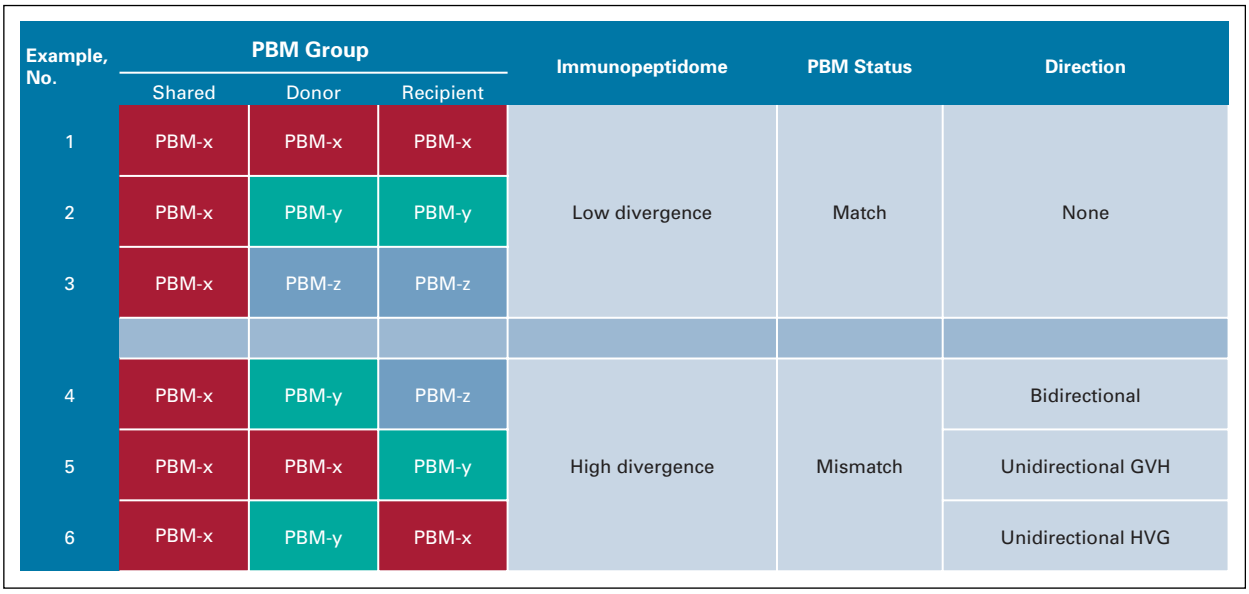

FIG 1. PBM matching strategy in single class I HLA-mismatched UD-HCT. Both the shared and the mismatched HLA-A, -B, or -C allele in the recipient and in the donor were assigned to any of 21 PBM groups, indicated here as PBM-x, PBM-y, and PBM-z (Data Supplement). If the two mismatched alleles belong to the same PBM group, the immunopeptidomes presented by the mismatched allotypes have low predicted divergence (PBM match, examples 1-3). If the two mismatched alleles belong to different PBM groups, the immunopeptidomes presented by the mismatched allotypes have high predicted divergence (PBM mismatch, examples 4-6). In these cases, directionality is mediated by the PBM group of the shared allele. If none of the two mismatched alleles belong to the same PBM group as the shared allele, the mismatch is bidirectional (example 4). If only one of the mismatched alleles in the donor or in the recipient belongs to the same PBM group as the shared allele, the mismatch is unidirectional GVH (example 5) or HVG (example 6), respectively. An online webtool for automated PBM group assignment from second field HLA data is available online.[22] GVH, graft-versus-host; HVG, host-versus-graft; PBM, peptide-binding motifs; UD-HCT, unrelated donor hematopoietic cell transplantation.

629 (60.7%) informative pairs had bidirectional or unidirectional PBM mismatches in GVH direction (PBM-GVH). The remaining 640/1,629 (39.3%) pairs were PBM-GVH matched, that is, unidirectionally PBM-mismatched in host-versus-graft (HVG) direction (PBM-HVG) or PBM-matched (Table 1). With respect to the entire cohort of single class I HLA-mismatched pairs, 989/2,391 (41.3%) were PBM-GVH mismatched, 640/2,391 (26.8%) were PBM-GVH matched, and 762/2,391 (31.9%) were noninformative.

PBM matches were more frequently HLA allele mismatched but HLA antigen matched (ie, mismatched only at the second but not at the first field), than PBM mismatches (54.9% *v* 5%). Likewise, PBM groups showed only partial (70.4%) concordance with HLA supertypes (Data Supplement).[20,21] Discordant pairs were mainly accountable to the supertype-matched group, in which 184/515 (35.7%) of pairs were PBM-mismatched. By contrast, only 55/1,114 (4.9%) of supertype-mismatched pairs were PBM matched.

PBM Mismatching and Outcome

We first investigated the associations between the presence of a PBM-mismatched or a PBM-matched HLA-A, -B, or -C allotype and outcome after 9 of 10 UD-HCT. After adjusting for significant non-HLA covariates, the PBM-mismatched group had significantly worse OS relative to the 10 of 10 matches (hazard ratio [HR], 1.38; 95% CI, 1.27 to 1.50; $P < .0001$), while this association was not statistically significant for the PBM-matched group (HR, 1.18; 95% CI, 0.99 to 1.41; $P = .0631$; Data Supplement). Nonetheless, OS was not significantly different in direct multivariate comparison between the PBM-mismatched relative to the PBM-matched group (HR, 1.16; 95% CI, 0.97 to 1.41; $P = .098$). Similarly, significantly higher multivariate hazards for all other end points except for relapse were observed relative to 10 of 10 matches both for PBM-mismatched and for PBM-matched 9 of 10 pairs, with no marked differences between the two groups (Data Supplement). There was also no difference between supertype mismatches and supertype matches (HR, 0.96; 95% CI, 0.83 to 1.12; $P = .6241$), which were both associated with significantly worse OS relative to the 10 of 10 matched group (HR, 1.35; 95% CI, 1.22 to 1.48; $P < .0001$ for supertype mismatches and HR, 1.30; 95% CI, 1.15 to 1.47; $P < .0001$ for supertype matches). In subgroup analysis, the HR of survival for the discordantly supertype-mismatched but PBM-matched pairs was similar to the 10 of 10 reference (HR, 0.98; 95% CI, 0.68 to 1.40; $P = .8907$), while it was significantly different for the supertype-matched but PBM-mismatched pairs (HR, 1.33; 95% CI, 1.11 to 1.60; $P = .0019$).

Directionality of PBM Mismatches and Outcome

To test the hypothesis that directionality has an impact for PBM mismatches, as suggested by our previous work,[25] we studied associations between bidirectional PBM-GVH mismatches, unidirectional PBM-GVH mismatches, unidirectional PBM-HVG mismatches, or PBM matches, with OS. The adjusted probabilities of 3-year OS for these four groups were 40.9%, 43.2%, 47.2%, and 47.7%, respectively, compared with 52.4% for the 10 of 10 matched group ($P < .0001$; Fig 2). Likewise, the adjusted probabilities of OS were significantly worse for bidirectional or unidirectional PBM-GVH mismatches than for the 10 of 10 reference, while those of unidirectional PBM-HVG mismatches or PBM matches were not significantly different from the reference (Fig 2).

Given the similar OS associations of the bidirectional and unidirectional PBM-GVH mismatches on the one hand, and the unidirectional PBM-HVG mismatches and PBM matches

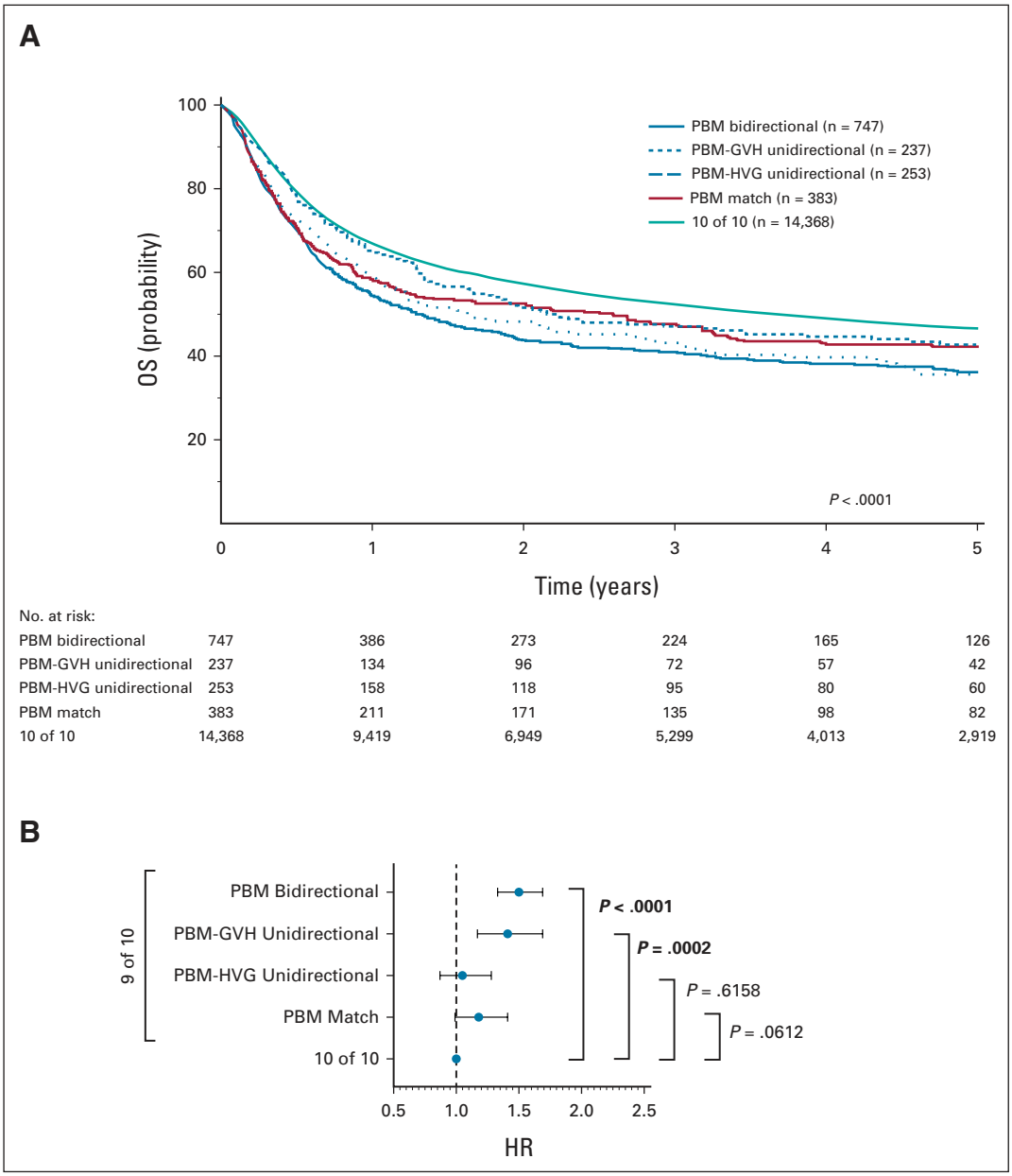

FIG 2. Associations of directional PBM mismatches with OS. (A) Adjusted KM probabilities of OS for fully HLA-matched 10 of 10 UD-HCT, or single class I HLA-mismatched 9 of 10 UD-HCT with a bidirectional PBM-GVH mismatch, a unidirectional PBM-GVH mismatch, a unidirectional PBM-HVG mismatch, or a PBM match. (B) Forest plots of adjusted HR and 95% CI (error bars) of OS, for the same groups as in A. *P* values refer to direct comparisons with the 10 of 10 reference. The overall *P* value was < .0001. Bold values indicate statistical significance. HR, hazard ratio; OS, overall survival; PBM, peptide-binding motifs; PBM-GVH, PBM mismatches in graft-versus-host direction; PBM-HVG, PBM mismatches in host-versus-graft direction; UD-HCT, unrelated donor hematopoietic cell transplantation.

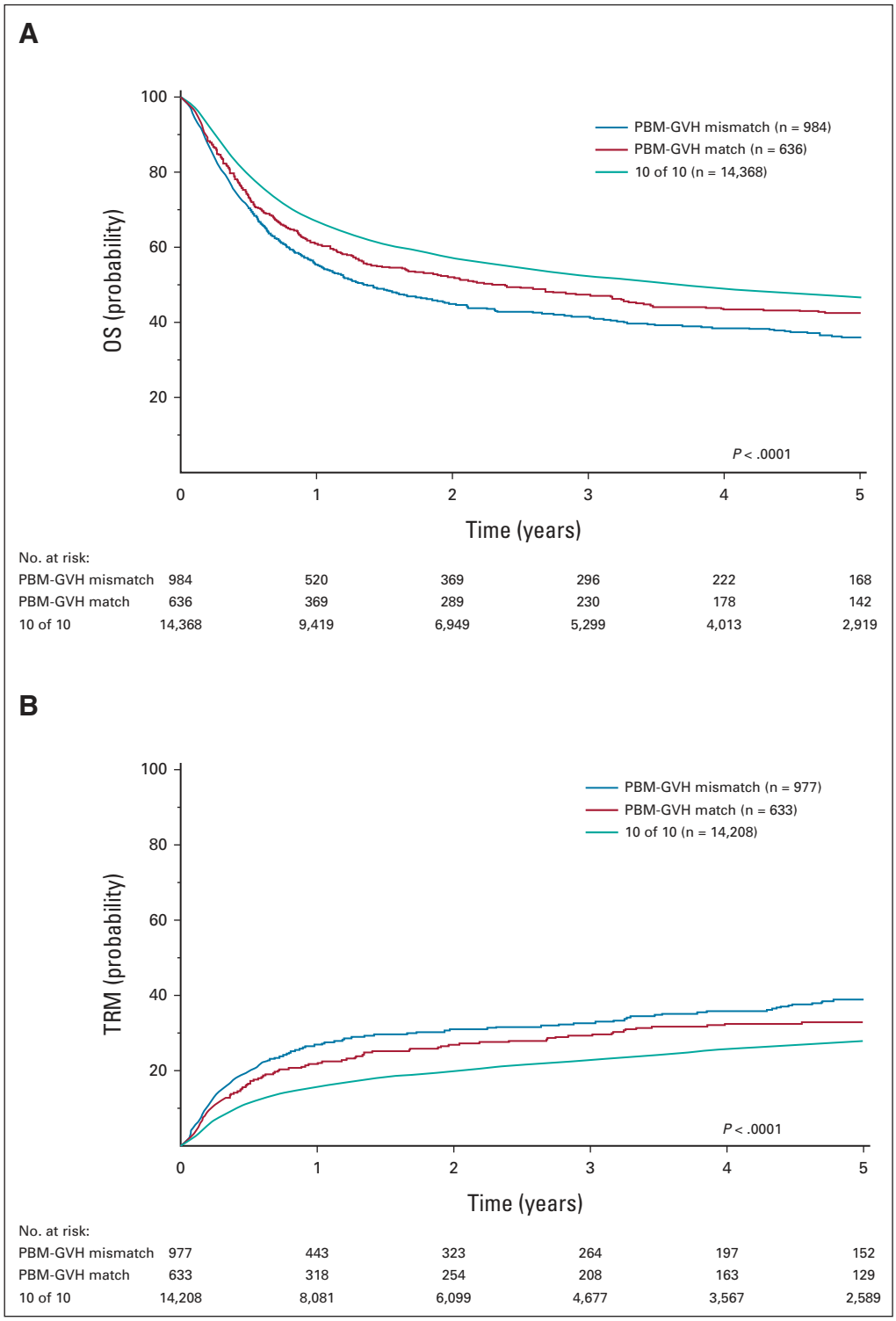

FIG 3. Association of directional PBM-GVH mismatches with OS and TRM. Data are shown for 10 of 10 UD-HCT or single class I HLA-mismatched 9 of 10 UD-HCT with a PBM-GVH mismatch (ie, a bidirectional PBM mismatch or a unidirectional PBM-GVH mismatch) or a PBM-GVH match (ie, a unidirectional PBM-HVG mismatch or a PBM match). (A) Adjusted probabilities of OS and (B) adjusted cumulative incidences of TRM. OS, overall survival; PBM, peptide-binding motifs; PBM-GVH, PBM mismatches in graft-versus-host direction; PBM-HVG, PBM mismatches in host-versus-graft direction; TRM, transplant-related mortality; UD-HCT, unrelated donor hematopoietic cell transplantation.

on the other, each of the two groups were combined to study PBM directionality of outcome associations in PBM-GVH mismatches and PBM-GVH matches. After adjustment for significant non-HLA covariates (Data Supplement), the 3-year OS probabilities were 41.4%, 47.4%, and 52.4% in the PBM-GVH mismatched, the PBM-GVH matched, and the fully matched group, respectively ($P < .0001$, Fig 3). In multivariate analysis, PBM-GVH mismatches but not PBM-GVH matches were associated with significantly worse OS, relative to the 10 of 10 reference (HR, 1.48; 95% CI, 1.33 to 1.64; $P < .0001$ and HR, 1.13; 95% CI, 0.98 to 1.30; $P = .107$, respectively; Table 2; Fig 4). Moreover, PBM-GVH mismatches had significantly worse OS than PBM-GVH matches in direct comparison (HR, 1.32; 95% CI, 1.09 to 1.59; $P = .0036$; Fig 4; Data Supplement). The adjusted univariate probabilities (Fig 3) and multivariate hazards (Table 2; Fig 4) of TRM increased stepwise from 10 of 10 matches to 9 of 10 PBM-GVH matches and 9 of 10 PBM-GVH mismatches, with markedly though not significantly different hazards between the two latter groups (Data Supplement). The associations between PBM-GVH mismatches and TRM were mostly accounted for by transplants with matched donor-recipient cytomegalovirus (CMV) serostatus (Data Supplement), resulting in a significant interaction ($P < .0001$) between PBM and CMV status for this end point. Similar stepwise increases in hazards were observed for aGVHD 2-4, aGVHD 3-4, cGVHD, and RFS, but not for relapse (Table 2; Fig 4; Data Supplement), yet without any significant interaction with CMV serostatus. Of note, the increased multivariate hazards of aGVHD 2-4 and cGVHD relative to 10 of 10 matches were significant for PBM-GVH mismatches but not for PBM-GVH matches (Table 2; Fig 4), albeit not in direct

TABLE 2. Multivariate Associations Between Directional PBM Mismatches and Clinical Outcomes

Clinical End Point	HLA Matching Status[a]	Total No. (events)	HR[b,c] (95% CI)	P[c,d] Reference	P[c,d] Overall
OS	Fully matched	14,368 (7,359)	1		**< .0001**
	PBM-GVH match	636 (376)	1.13 (0.98 to 1.30)	.1017	
	PBM-GVH mismatch	984 (635)	**1.48 (1.33 to 1.64)**	**< .0001**	
TRM[e]	Fully matched	14,208 (3,351)	1		**< .0001**
	PBM-GVH match	633 (200)	**1.22 (1.06 to 1.41)**	**.0047**	
	PBM-GVH mismatch	977 (341)	**1.56 (1.37 to 1.78)**	**< .0001**	
RFS	Fully matched	14,208 (7,981)	1		**< .0001**
	PBM-GVH match	633 (402)	**1.19 (1.05 to 1.34)**	**.0053**	
	PBM-GVH mismatch	977 (666)	**1.31 (1.19 to 1.44)**	**< .0001**	
aGVHD 2-4	Fully matched	13,733 (6,305)	1		**< .0001**
	PBM-GVH match	608 (293)	1.13 (1.02 to 1.26)	.0221	
	PBM-GVH mismatch	953 (514)	**1.29 (1.16 to 1.43)**	**< .0001**	
aGVHD 3-4	Fully matched	13,728 (2,318)	1		**< .0001**
	PBM-GVH match	608 (141)	**1.38 (1.17 to 1.63)**	**.0001**	
	PBM-GVH mismatch	953 (261)	**1.77 (1.54 to 2.04)**	**< .0001**	
cGVHD	Fully matched	14,011 (6,996)	1		**< .0001**
	PBM-GVH match	623 (298)	1.15 (1.00 to 1.32)	.0459	
	PBM-GVH mismatch	958 (448)	**1.31 (1.18 to 1.46)**	**< .0001**	
Relapse	Fully matched	14,208 (4,630)	1		0.3782
	PBM-GVH match	633 (202)	1.03 (0.88 to 1.20)	.7507	
	PBM-GVH mismatch	977 (325)	1.09 (0.97 to 1.22)	.1658	

Abbreviations: aGVHD, acute GVHD; cGVHD, chronic GVHD; HR, hazard ratio; OS, overall survival; PBM, peptide-binding motifs; PBM-GVH, peptide-binding motif mismatches in graft-versus-host direction; RFS, relapse-free survival; TRM, transplant-related mortality.

[a]Fully matched: 10 of 10; PBM-GVH match: 9 of 10 with unidirectional PBM mismatch in host-versus-graft direction, or PBM match; PBM-GVH mismatch: 9 of 10 with bidirectional or unidirectional PBM mismatch in graft-versus-host direction.

[b]Data were adjusted for significant non-HLA covariates as in the Data Supplement.

[c]Data are from Cox proportional hazards models for all end points. Fine & Gray's subdistribution hazard models were also applied to test for association of the directional PBM mismatches with cumulative incidences of TRM, aGVHD 2-4, aGVHD 3-4, and relapse while accounting for competing events, which confirmed significant associations with aGVHD 2-4, aGVHD 3-4, and TRM ($P < .0001$), but not with relapse ($P = .5014$).

[d]Statistically significant values ($P < .01$) are indicated in bold.

[e]Significant interaction was found with donor-recipient cytomegalovirus serostatus (Data Supplement).

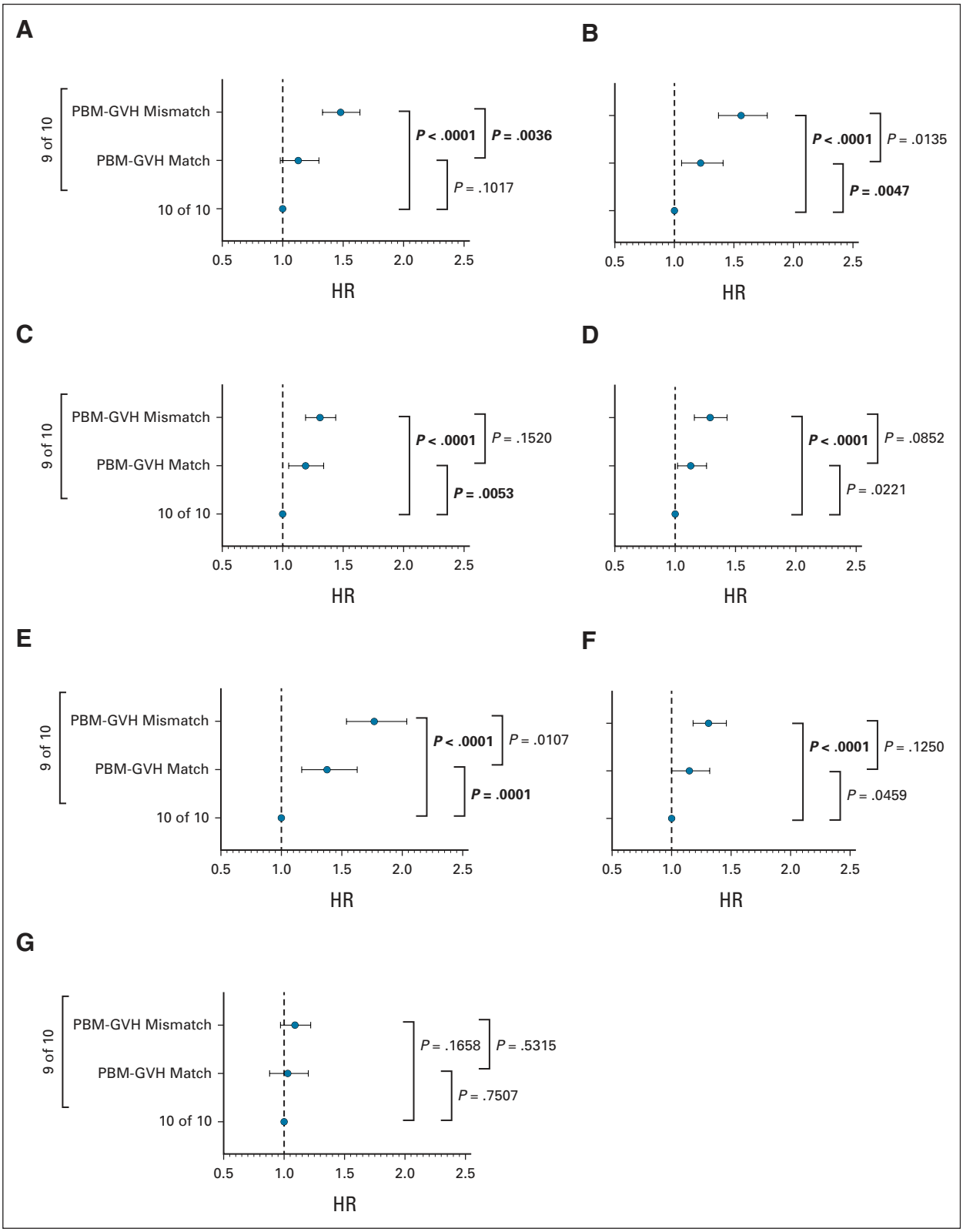

FIG 4. Adjusted hazard risks associated with directional PBM-GVH mismatches: (A) OS, (B) TRM, (C) RFS, (D) aGVHD 2-4, (E) aGVHD 3-4, (F) cGVHD, and (G) relapse. Forest plots represent the HR and 95% CI (error bars) for the indicated end points, for 9 of 10 PBM-GVH mismatches or PBM-GVH matches, relative to the 10 of 10 reference. *P* values refer to direct comparisons between the three groups as in Table 2. The overall *P* value was < .0001 for all end points except for relapse *P* = .3782. Bold values indicate statistical significance. aGVHD, acute GVHD; cGVHD, chronic GVHD; HR, hazard ratio; OS, overall survival; PBM, peptide-binding motifs; PBM-GVH, PBM mismatches in graft-versus-host direction; RFS, relapse-free survival; TRM, transplant-related mortality.

comparison between the two groups (Data Supplement). No significant associations were observed between unidirectional or bidirectional PBM-HVG mismatches and neutrophil or platelet engraftment (not shown).

DISCUSSION

HLA-mismatched UD-HCT is increasingly being offered to patients lacking a fully HLA-matched donor, who comprise up to over 80% of cases in certain ethnic minorities.[4] Inclusion of single HLA-mismatched UD into the pool decreases this fraction to < 35% in all ethnic groups. Concordantly, prospective clinical trials for HLA-mismatched UD-HCT are underway with promising results.[26] In light of these developments, the identification of clinically permissive HLA mismatches in this setting is of increasing relevance. Here, we have developed a novel and original approach to this regard, using PBM groups as surrogates of predicted immunopeptidome divergence between mismatched HLA alleles. We show that bidirectional or unidirectional PBM-GVH mismatches are associated with significantly reduced OS, compared with PBM-GVH matches and with the fully HLA-matched reference, and that this is mirrored by a stepwise increase in the risks of TRM, and aGVHD and cGVHD. Therefore, avoidance of PBM-GVH mismatched UD, facilitated by the webtool provided to this end, is likely to help improve the chances of favorable outcome.

The hypothesis that PBM mismatches might inform clinical permissiveness of HLA mismatches stems from our observations for HLA-DPB1, where the degree of immunopeptidome divergence between mismatched alleles is predictive of the magnitude and diversity of alloreactive T-cell responses in vitro,[17] and of clinical risks after fully HLA-matched UD-HCT in vivo.[14-16,27,28] The significant associations between PBM-GVH mismatches and OS observed in this study suggest that immunopeptidome divergence is of general mechanistic relevance for HLA mismatches in UD-HCT, although direct experimental evidence for HLA class I is warranted.

A potential key to the importance of PBM mismatch directionality observed in this study is the missing association of single class I HLA mismatches with relapse, compared with the fully HLA-matched group. In the absence of an apparent GVL effect attributable to PBM-GVH mismatches, their association with increased aGVHD and cGVHD is likely to be the basis for the observed impairment of survival. The reasons why PBM-GVH mismatches fail to efficiently mediate GVL are currently speculative, but include the possibility of locus- and/or allotype-specific genomic, transcriptional, or epigenetic immune escape mechanisms targeted to single class I HLA mismatches and/or the relevant peptide-processing machinery.[29-32] The observed relevance of PBM directionality is in line with previous data demonstrating an association between single allelic HLA mismatches in the GVH vector with aGVHD.[25] We show here that PBM groups can be used to leverage the relevance of GVH mismatches for informing not only GVHD but also survival, without significant impact on neutrophil engraftment. Interestingly, association of directional PBM-GVH mismatches with TRM was mainly accounted for by transplants with matched donor-recipient CMV serostatus, supporting previous evidence for an interplay between CMV and T-cell immunity,[33] which remains subject to further investigation.

An important feature likely to impact T-cell alloreactivity against mismatched HLA molecules is the strategy of GVHD prophylaxis, with PTCy emerging as a relevant alternative to CNI. Recent findings in the haploidentical setting suggest that the dampening effect of PTCy on T-cell alloreactivity might lead to better tolerability of HLA mismatches identified as excessively immunogenic in the CNI setting.[5,34] PTCy could therefore be particularly recommendable for use in 9 of 10 mismatched transplants with PBM-GVH mismatches, to limit their adverse effect. Investigation of this important issue will be warranted once a sufficient number of informative transplants will be accumulated.

Our study has limitations regarding potential bias introduced by retrospective analysis of a registry cohort, calling for validation in other cohorts and in prospective clinical trials. Nevertheless, it provides a proof of principle for the potential validity of an innovative, experimentally driven concept to identify clinically permissive class I HLA mismatches in UD-HCT. Another limitation regards incomplete coverage of PBM grouping because of missing class I HLA immunopeptidome data in public databases, underlining the importance of filling this gap by targeted experimental approaches. Nevertheless, we could identify favorable PBM-GVH match status in 26.8% of pairs in the overall cohort, a percentage likely to rise if PBM status was to be considered prospectively. Since class I HLA mismatches account for more than 70% of the 9 of 10 UD-HCT,[6,7] approximately 18.8% of patient (ie, 26.8% of 70%) candidates for such a transplant are likely to currently benefit from our approach.

A previous study reported an association between HLA-B supertypes and aGVHD but not survival after single HLA-mismatched UD-HCT.[21] Conceptually, both HLA supertypes and PBM groups rely on peptide-binding characteristics as biomarkers of immunogenicity. However, PBM groups were derived from recent experimental immunopeptidome data obtained with modern mass spectrometry approaches.[19] By contrast, HLA supertype definition included in silico predictions on the basis of structural features for many allotypes,[20,21] as well as in vitro data derived from restricted numbers of peptides. Our findings suggest that PBM mismatches might identify pairs with greater risk of mortality within the supertype-matched group, although the detailed relationship between PBM and supertypes needs to be further elucidated.

In conclusion, we describe a new strategy for improving the outcome of a sizable fraction of patients receiving 9 of 10 HLA-mismatched UD-HCT, by avoidance of donors with

PBM-GVH mismatches, along with an online webtool for its clinical implementation in donor searches. Conceptually, our findings highlight reduced peptide divergence between mismatched HLA as a driver of clinical tolerability in UD-HCT. These findings open new avenues for prospective stem-cell donor selection in HLA-mismatched transplantation, as well as for targeted immunopeptidomics-based intervention[30] to enhance immunogenicity of leukemia.

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Impact of the HLA Immunopeptidome on Survival of Patients With Leukemia After Unrelated Donor Transplantation

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