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LETTER TO HAEMATOLOGICA

Comparative evaluation of biological HLA-DPB1 mismatch models for survival and graft versus host disease prediction after unrelated donor hematopoietic cell transplantation

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Hematopoietic Cell Transplantation (HCT) from unrelated donors (UD) is a curative therapy for many hematologic malignancies. Donor-recipient allele-level matching at HLA-A, -B, -C, -DRB1 (8/8) is widely accepted to provide best results in terms of overall survival (OS), non-relapse mortality (NRM) and graft-versus-host disease (GvHD)[1]. Genotypic matching for HLA-DPB1 (DPB1) has been shown to hold limited, if any, impact on post-transplant OS, and would be challenging to adopt, due to the weak linkage disequilibrium between DPB1 and the remaining HLA class II loci. Therefore, more than 80% of 8/8-matched UD-HCT are currently DPB1-mismatched[1]. A number of studies have demonstrated that biological models can be used to identify selected, permissive DPB1 mismatch combinations, associated with lower clinical risks compared to their high-risk, non-permissive, counterparts[2-9]. Five different biological models for the assignment of DPB1 permissiveness have been identified to date, of which three are based on functional T-Cell Epitopes (TCE), while the remaining two rely on a single nucleotide polymorphism (SNP) tag for expression levels. In particular, in the *TCE3 model*, DPB1 alleles are classified into three structural groups based on variation in the peptide antigen-binding domain, which leads to functionally similar or distinct behavior in terms of T-cell alloreactivity[2-5]. The *TCE4 model* is identical to TCE3, except for the assignment of DPB1*02 to a fourth, independent group[4]. Another derivative of TCE3 is the *"delta functional distance" (ΔFD) model*, in which a FD score is assigned to every DPB1 allele based on key polymorphic aminoacids involved in anti-DPB1 alloreactivity, and differences between the FDs of patient and donor alleles defines permissive and non-permissive pairs, respectively[6,7]. The SNP models, in contrast, are based on high or low expression DPB1 alleles according to a *SNP tag* in the 3' untranslated region, in which the rs9277534 A and G variant is associated with low and high DPB1 expression, respectively[8-9]. In the *Expression model*, this SNP tag is applied to all DPB1 alleles, while in the *DP2/DP5 model*, it is applied to 19 DPB1 alleles belonging either the DP2 or the DP5 evolutionary clade. Currently, TCE3 matching is proposed by stem cell donor registries in the US and Europe, because it is the only of these models to have been validated in independent cohorts. However, a comparative evaluation of the five different biological models for DPB1 permissiveness and their association with HCT outcome has not been undertaken so far.

Here, we have filled this gap by analysing the outcome of 422 patients with available 2nd field DPB1 typing transplanted from 8/8 HLA-A, -B, -C and -DRB1 allele matched UDs in 32 centers from the Gruppo Italiano Trapianto di Midollo Osseo (GITMO), between 2012 and 2015. Of these, 43 pairs had a mismatch at HLA-DQB1, and 382 had one or two DPB1 mismatches. The availability of DPB1 typing did not introduce significant biases, since clinical outcomes were similar for the 422 transplants under analysis and those (n=522) performed in the same time-period and for which DPB1 typing was not available (Supplemental Tables 1a and 1b). Patient, donor and transplant characteristics are shown in Table 1. Transplants were performed for hematologic malignancies, with mostly myeloablative conditioning and peripheral blood as stem cell source. GvHD prophylaxis included anti-T-lymphocytic globulin (ATG) in most cases. Permissive and non-permissive mismatches were assigned by the IMGT webtool version 2.0 for TCE3, and manually for the other models, using the cut-off 1.64 or 2.665 for ΔFD as described[6,7]; for the Expression model, the rs9277534 SNP variant was predicted by DPB1 genotype[8]. Statistical methods are described in the Supplements.

The five models displayed limited overlap, and TCE4 was the most restrictive one, as in this model the fewest number of pairs (36%) were classified as permissive. For the SNP models, only donor-recipient pairs with a single unidirectional DPB1 mismatch in GvH direction could be classified, leaving 153/382 (40%) and 233/382 (61%) of pairs without classification according to the Expression or the DP2/DP5 model, respectively (Figure 1A).

Donor-recipient pairs in the permissive/low risk or non-permissive/high risk groups according to all five models were comparable concerning disease- and transplant-specific characteristics (Supplemental Tables 2-6). In univariate analysis, we confirmed previous reports[10] that DPB1 allele mismatches were not associated with any significant difference in OS, and this was reflected by a balance between significantly higher risks of aGvHD, in the presence of markedly though not significantly lower risks of relapse (Supplemental Table 7). Of all models, only TCE4 was significantly associated with superior 3-y OS and GRFS in patients transplanted from a permissive compared with a non-permissive donor, reflected by lower 3-y cumulative incidence (CI) of extensive cGvHD and NRM (Figure 1B-E). No significant associations with clinical outcomes were

found for the TCE3 or the ΔFD model (Supplemental Table 7). The Expression model and the DP2/DP5 model were both associated with a higher 100-day CI of grade≥2 aGvHD, but not with any of the other clinical endpoints (Supplemental Table 7). In multivariate analysis, TCE4 permissive pairs were independently associated with superior OS and GRFS, and with lower hazards for NRM, cGvHD and extensive cGvHD. Moreover, compared to DPB1 allele matches, permissive mismatches according to all three functional models (TCE3, TCE4 and ΔFD) had significantly lower relapse risks (Table 2). In contrast, the high-risk mismatches according to the Expression model and the DP2/DP5 model were significantly associated with grade 2-4 aGvHD, but not with NRM or OS (Table 2). Outcome associations of all clinical co-variates used in the multivariate analyses are shown in Supplemental Table 8.

Our study is the first to compare HCT outcome associations for the five major biological models of DPB1 permissiveness. The results show that the concordance on the predicted permissiveness of DPB1 mismatches among the different models is evident but far from outright, suggesting that, even if all models describe a common biological phenomenon (the alloreactivity of T-cells against incompatible DPB1 molecules), each of them may capture and emphasize only some aspects of this interaction. The outcome analyses confirm previous reports that functional DPB1 matching according to TCE is significantly associated with survival after UD-HCT[2-5], while DPB1 matching according to the SNP tag predicts the risks of aGvHD[8-9]. In this context, TCE4 is the most restrictive but appears as the best common denominator for permissiveness/low risk in all five models. In particular, the survival benefit of TCE4-permissive transplants over their non-permissive counterparts is mainly reflected by reduced NRM and cGvHD risks, providing support to the hypothesis that leveraging on permissive DPB1 mismatches might be a promising way to reduce NRM without compromising the graft-versus-malignancy effect of allogeneic HCT.

The study has several limitations. First, the number of pairs under analysis is relatively small, in particular for the two SNP tag models where DPB1 typing of up to 61% of pairs could not be classified. However, association of these two models with aGvHD risks is in agreement with previous reports from larger studies[8,9]. Second, an apparent difference with previous data from larger studies[3,5] is the lack of association with TCE3. This might reflect the stem cell source, which

was peripheral blood in 81% of our patients while bone marrow was used in at least 50% of patients from the other studies[3,5]. Moreover, 91% of our patients received in vivo T-cell depletion with ATG, while this was adopted in less than 30% of patients in other studies[3,5]. It should also be noted that TCE4 has been previously found to be associated with OS, including an analysis of non-overlapping GITMO transplants from earlier years[4], a recent study from the French Registry[11], and a large multicentre study from the International Histocompatibility Workshop[3]. Since the latter did not show a significant advantage of TCE4 over TCE3, and DPB1 permissive donors are more frequent in TCE3, TCE3 was adopted by stem cell donor registries and not further investigated in subsequent studies[5]. The greater restrictiveness of TCE4 compared to TCE3 in assigning permissiveness is due to appreciation of DPB1*02 as a separate functional group. Interestingly, HLA-DP2 is the so far only HLA-DP specificity associated with autoimmunity[12], and recent evidence suggests a similar breadth of the alloreactive T cell receptor repertoire in permissive pairs involving this allele group compared to non-permissive pairs[13], arguing in favor of a functional basis for TCE4.

In conclusion, our results highlight the relevance of refining transplant-associated risks according to the biological significance of HLA matching. In particular, they confirm the association between the SNP tag models and aGvHD, while TCE4 should be prioritized for its highest performance in predicting survival and non-relapse related events. Moreover, since most of TCE4 permissive donors are classified as low-risk for both SNP tag models, they may be the best alternative to favor positive overall outcomes. Clearly, additional and possibly prospective studies should be performed to provide more definitive evidence for the respective value of the five DPB1 models, also in view of emerging new strategies for GvHD prophylaxis, which could modulate the observed outcome associations.

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AUTHORSHIP

F.L, L.V., F.C., K.F. and F.B. designed, performed, coordinated the research, collected, analyzed, interpreted clinical data, and wrote the manuscript; F.L. performed statistical analysis. L.V. prepared the figure. N.S., V.M and A.M.G. provided immunogenetic data. All authors contributed to patient clinical care and data collection, critically reviewed the manuscript and approved the final version.

DISCLOSURES

All the authors declare no competing financial interests.

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Table 1. Patient, transplant and donor characteristics.

	Population n= 422
Median follow-up for survivors, years (range)	3.2 (0.1-6)
Patient age, years, median (range)	49 (18-70)
Patient gender, male, n (%)	244 (58%)
Type of diagnosis, n (%)	
AML	168 (40%)
ALL	63 (15%)
MDS or MPN	69 (16%)
Lymphoma and Myeloma	110 (26%)
CLL	12 (3%)
Disease status according to EBMT risk[14], n (%)	
Early	191 (45%)
Intermediate	111 (26%)
Advanced	120 (29%)
HCT-CI score[15], median (range)	1 (0-7)
Karnofsky performance status, median (range)	90% (50-100)
Donor gender, male, n (%)	306 (72%)
N° of previous pregnancies for female donors, median (range)	0 (0-6
Female donor/male recipient, n (%)	61 (14%)
Host/donor CMV serostatus, n(%)	
Pos/pos	157 (37%)
Pos/neg	166 (39%)
Neg/pos	36 (9%)
Neg/neg	53 (13%)
Missing	10 (2%)
Type of conditioning, n (%)	
MAC	271 (64%)
RIC	111 (35%)
Source of stem cells, n (%)	
PB	343 (81%)
BM	79 (19%)
ATG-based GvHD prophylaxis, n (%)	382 (91%)
GvHD prophylaxis details:	
ATG+CSA+MTX	341 (81%)
ATG+Sirolimus+MMF	26 (6%)
Other ATG-based prophylaxis	15 (4%)
CSA+MTX	24 (5%)
Other prophylaxis	16 (4%)

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasms; CLL, chronic lymphocytic leukemia; CMV, Cytomegalovirus; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; PB, peripheral blood; BM, bone marrow; ATG, anti-T-lymphocytic globulin; CSA, Cyclosporine A; MTX, Methotrexate.

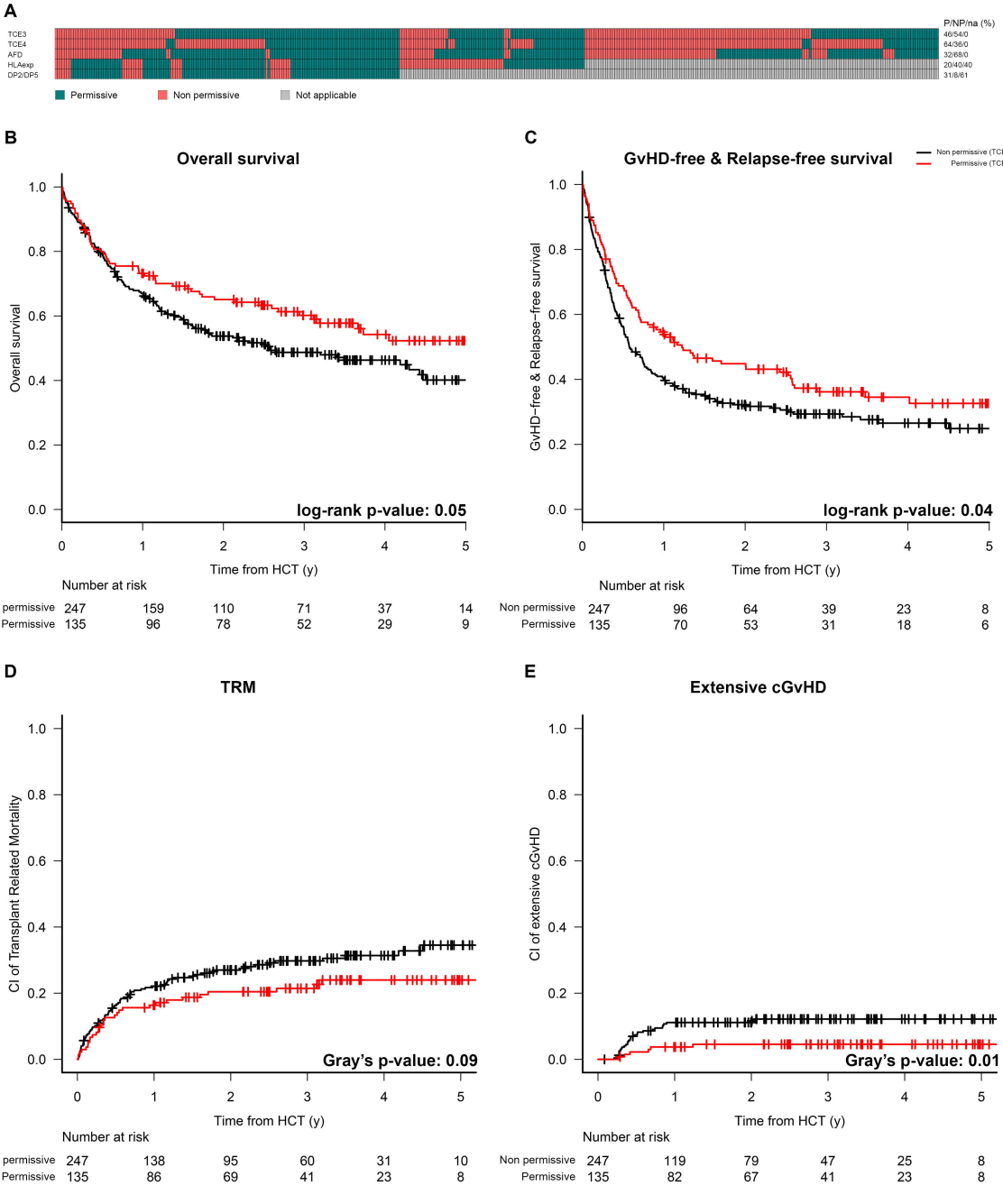
Table 2. Multivariate analysis of DPB1 mismatch models and association with HCT outcomes.

	OS	GRFS	Relapse	NRM	aGvHD≥2	aGvHD≥3	cGvHD	Ext cGvHD
	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p
TCE4[1]: NP vs P	**1.7 (1.1-2.4) 0.008**	**1.4 (1.1-1.9) 0.01**	1.1 (0.7-1.7) 0.7	**1.9 (1.1-3.2) 0.01**	1.3 (0.8-2.1) 0.2	1.5 (0.7-3.2) 0.3	**1.7 (1.1-2.6) 0.02**	**3.6 (1.4-9.5) 0.01**
Matched vs P	**2.1 (1.2-3.7) 0.01**	1.5 (0.9-2.4) 0.09	**2 (1.1-3.7) 0.03**	2 (0.9-4.8) 0.09	Not applicable[6]	Not applicable[6]	0.8 (0.3-2) 0.6	0.9 (0.1-7.5) 0.9
TCE3[2]: NP vs P	1.1 (0.8-1.6) 0.5	1.1 (0.8-1.4) 0.5	1 (0.6-1.5) 0.9	1.1 (0.7-1.8) 0.6	1.2 (0.8-1.9) 0.4	1.4 (0.6-2.9) 0.4	1.2 (0.8-1.8) 0.4	1.7 (0.8-3.6) 0.1
Matched vs P	1.6 (0.9-2.7) 0.09	1.2 (0.8-1.9) 0.3	**1.9 (1.1-3.4) 0.04**	1.4 (0.6-3) 0.4	Not applicable[6]	Not applicable[6]	0.6 (0.2-1.5) 0.3	0.5 (0.1-3.6) 0.5
ΔFD[3]: NP vs P	1 (0.7-1.5) 0.8	1 (0.7-1.4) 0.9	1.3 (0.8-2) 0.3	0.9 (0.5-1.5) 0.7	0.9 (0.5-1.4) 0.6	0.8 (0.4-1.9) 0.6	1 (0.7-1.6) 0.9	0.9 (0.4-2.1) 0.9
Matched vs P	1.5 (0.9-2.5) 0.1	1.2 (0.8-1.9) 0.4	**2 (1.1-3.6) 0.02**	1.3 (0.6-2.7) 0.5	Not applicable[6]	Not applicable[6]	0.6 (0.2-1.4) 0.2	0.3 (0.1-2.5) 0.3
Expression[4]: high vs low risk	1 (0.6-1.6) 0.9	0.8 (0.5-1.2) 0.2	0.6 (0.3-1.1) 0.1	1.1 (0.6-2.2) 0.7	**2.2 (1.1-4.2) 0.02**	1.9 (0.6-6.2) 0.3	1.2 (0.7-2.2) 0.4	1.7 (0.6-4.5) 0.3
Matched vs low risk	1.6 (0.9-2.8) 0.08	1.1 (0.7-1.7) 0.7	1.4 (0.7-2.5) 0.3	1.5 (0.7-3.4) 0.3	Not applicable[6]	Not applicable[6]	0.7 (0.3-1.7) 0.4	0.5 (0.1-3.7) 0.3
DP2/DP5[5]: high vs low risk	1.2 (0.5-2.5) 0.7	0.8 (0.5-1.6) 0.6	0.9 (0.4-2.2) 0.8	1.3 (0.5-3.7) 0.6	**3.8 (1.5-9.6) 0.006**	**6.9 (1.5-31) 0.01**	1.1 (0.4-3.1) 0.8	4.1 (1-17) 0.05
Matched vs low risk	1.8 (1-3.2) 0.05	1 (0.6-1.7) 0.9	1.6 (0.8-3.2) 0.2	1.5 (0.6-3.7) 0.3	Not applicable[6]	Not applicable[6]	0.5 (0.2-1.5) 0.2	0.6 (0.1-4.7) 0.6

[1]TCE4 NP, P and matched: N= 247, 135 and 40, respectively.
[2]TCE3 NP, P and matched: N= 174, 208 and 40, respectively.
[3]DFD NP, P and matched: N= 123, 259 and 40, respectively; cut-off 2.665.
[4]Expression high risk, low risk and matched: N= 76, 153 and 40, respectively.
[5]DP2/DP5 high risk, low risk and matched: N= 31, 118 and 40, respectively.
[6]Not applicable since no cases of aGvHD occurred in the DPB1 matched cohort.
Abbreviations are defined in the text. Co-variates in the multivariate models included patient age, disease phase, HCT-CI, female donor to male recipient, host-donor CMV serostatus, conditioning intensity, stem cell source, ATG use, HLA-matching on 5 loci, center effect.

FIGURE LEGEND

Figure 1. Comparative stratification of donor-recipient pairs according to five different biological models of DPB1 permissiveness, and outcome associations for TCE4. A) Classification of the 382 DPB1 mismatched pairs under analysis in this study, as permissive or low risk (green) or as non-permissive or high risk (red) group according to five different biological models of permissiveness, as described in the text. For the Expression and the DP2/DP5 model, only pairs with single mismatches in the GvH direction can be stratified, the others cannot be classified (grey). The model under investigation, and the relevant numbers in each category (green, red or grey) are indicated to the left and to the right of the panel, respectively. The bottom panels show Kaplan-Meier probabilities for OS (B), GRFS (C), and CI of NRM (D) and extensive cGvHD (E) stratified for functional DPB1 matching according to TCE4 model, with permissive and non-permissive groups represented by the red and black curves, respectively.


A
TCE3
TCE4
AFD
HLAexp
DP2/DP5
P/NP/na (%)
46/54/0
64/36/0
32/68/0
20/40/40
31/8/61
Permissive
Non permissive
Not applicable
B
Overall survival
Overall survival
log-rank p-value: 0.05
Time from HCT (y)
Number at risk
Non permissive 247 159 110 71 37 14
Permissive 135 96 78 52 29 9
C
GvHD-free & Relapse-free survival
Non permissive (TCE4)
Permissive (TCE4)
GvHD−free & Relapse−free survival
log-rank p-value: 0.04
Time from HCT (y)
Number at risk
Non permissive 247 96 64 39 23 8
Permissive 135 70 53 31 18 6
D
TRM
CI of Transplant Related Mortality
Gray's p-value: 0.09
Time from HCT (y)
Number at risk
Non permissive 247 138 95 60 31 10
Permissive 135 86 69 41 23 8
E
Extensive cGvHD
CI of extensive cGvHD
Gray's p-value: 0.01
Time from HCT (y)
Number at risk
Non permissive 247 119 79 47 25 8
Permissive 135 82 67 41 23 8

Clinical endpoints definitions and statistical methods.

Acute GvHD (aGvHD) and chronic GvHD (cGvHD) were defined and scored according to the Glucksberg and Seattle criteria, respectively[1-2]. NRM was defined as death from any cause while in continuous remission of the primary disease. OS was defined as the interval from HSCT to death from any cause. GRFS events were defined as grade 3–4 acute GVHD, extensive chronic GVHD, disease relapse, or death from any cause after HSCT[3]. Actuarial probabilities were determined at 3 years. Baseline characteristics among groups were compared using the Chi-square test for categorical variables, while the distribution of continuous variables was compared using the Mann-Whitney U test. The probabilities of OS and GRFS were estimated using the Kaplan-Meyer estimator and groups were compared by the log-rank test[4-5]. Cumulative incidences (CI) were estimated for GvHD, NRM and relapse to accommodate competing risks, and tests of equality across groups were performed according to Gray[6-7]. Relapse was a competing risk for NRM, death from any cause was a competing risk for relapse. Both relapse and death from any causes were competing risks for GvHD. Multivariate analysis were built to test the independent prognostic value of DPB1 mismatch permissiveness: each DPB1 mismatch model was the main effect term and was held in all steps of model building. Cox proportional hazard models were adopted for OS and GRFS[8], while Fine-Gray proportional hazard regression models for competing events were adopted for aGvHD, cGvHD, relapse and NRM. Covariates included: patient age, disease phase, HCT-CI, female donor to male recipient, host-donor CMV serostatus, conditioning intensity, stem cell source, ATG use, HLA-matching on 5 loci (HLA-A, -B, -C, -DRB1 and –DQB1), center effect (>10 HSCT performed each year Vs ≤10). Interactions between each covariate and each DPB1 mismatch model were tested and not found. In particular, no interaction was found between HLA matching on 5 loci and each model. The proportional hazard assumption was met for all variables. The type I error was fixed at 0.05. Statistical analyses were performed with R version 3.3.3 (R Development Core Team, Vienna, Austria).

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Supplemental Table 1a. Patient, donor and transplant characteristics in DPB1 typed or non-typed cases.

	Non-DPB1 typed (N=582)	**DPB1 typed (N=422)**	**p**
Median follow-up for survivors, years (range)	3.1 (0.3-6.1)	3.2 (0.3-6.2)	0.7
HSCT year, median (range)	2014 (2012-2015)	2013 (2012-2015)	0.001
Patient age, years, median (range)	51 (18-69)	49 (18-71)	0.003
Patient gender, male, n	345	245	0.7
Type of diagnosis, n			0.2
AML or ALL	308	232	
MDS or MPN	127	69	
Lymphoma or Myeloma	138	109	
CLL	9	12	
Disease status at HSCT, n			0.3
Early	293	191	
Intermediate	135	114	
Advanced	145	105	
HCT-CI score, median (range)	1 (0-7)	1 (0-7)	0.1
Karnofsky PS, median (range)	90% (40-100)	90% (50-100)	0.4
Donor age, years, median (range)	28 (18-57)	29 (18-57)	0.6
Donor gender, male, n	415	306	0.7
Female donor/male recipient, n	97	61	0.3
Host/donor CMV serostatus, n			0.3
Pos/pos	206	156	
Pos/neg	266	167	
Neg/pos	38	36	
Neg/neg	72	53	
Type of conditioning, n			0.3
MAC	390	296	
RIC	192	126	
Source of stem cells, n			0.1
PB	492	342	
BM	90	80	
ATG-based GvHD prophylaxis, n	476	350	0.6

Supplemental Table 1b. Univariate analysis for transplant outcomes in DPB1 typed or non-typed cases.[1]

	3-y OS	3-y GRFS	3-y Relapse	3-y NRM	100-d aGvHD≥2	100-d aGvHD≥3	3-y cGvHD	3-y ext cGvHD
DPB1 typing								
DPB1 typed (n=422)	52 (46-56)	32 (27-36)	30 (25-35)	27 (23-31)	21 (17-25)	7 (5-10)	28 (24-32)	9 (6-12)
Non-DPB1 typed (N=582)	56 (52-61)	35 (31-39)	34 (30-38)	23 (20-29)	22 (18-25)	7 (5-10)	27 (23-30)	9 (6-11)
p	0.15	0.45	0.08	0.08	0.60	0.85	0.68	0.80

[1]shown are mean percentages (range in parenthesis) for each outcome.

Supplemental Table 2. Patient, donor and transplant characteristics in permissive and non permissive DPB1 mismatched pairs according to the **TCE3 model**

	Permissive (N=208)	**Non-permissive (N=174)**	**p**
Median follow-up for survivors, years (range)	3.4 (0.3-6)	3.1 (0.3-6)	0.28
HSCT year, median (range)	2013 (2012-2015)	2013 (2012-2015)	0.12
Patient age, years, median (range)	48 (18-71)	50 (19-66)	0.49
Patient gender, male, n	101	117	0.72
Type of diagnosis, n			0.16
AML or ALL	121	85	
MDS or MPN	35	32	
Lymphoma and Myeloma	52	57	
Disease status at HSCT, n			0.43
Early	100	74	
Intermediate	54	45	
Advanced	54	55	
HCT-CI score, median (range)	0 (0-7)	1 (0-5)	0.97
Karnofsky PS, median (range)			0.39
Donor age, years, median (range)	27 (19-55)	30 (20-56)	0.23
Donor gender, male, n	149	126	0.87
Female donor/male recipient, n	28	29	0.38
Host/donor CMV serostatus, n	90% (60-100)	90% (50-100)	0.35
Pos/pos	71	71	
Pos/neg	81	71	
Neg/pos	19	11	
Neg/neg	31	19	
Type of conditioning, n			0.58
MAC	150	121	
RIC	58	53	
Source of stem cells, n			0.01
PB	179	132	
BM	29	42	
ATG-based GvHD prophylaxis, n	189	156	0.69

Supplemental Table 3. Patient, donor and transplant characteristics in in permissive and non permissive DPB1 mismatched pairs according to the **TCE4 model**

	Permissive (N=135)	**Non-permissive (N=247)**	**p**
Median follow-up for survivors, years (range)	3.4 (0.3-6)	3.3 (0.3-6)	0.17
HSCT year, median (range)	2014 (2012-2015)	2013 (2012-2015)	0.45
Patient age, years, median (range)	48 (18-68)	49 (19-71)	0.46
Patient gender, male, n	80	138	0.52
Type of diagnosis, n			0.86
AML or ALL	75	131	
MDS or MPN	22	45	
Lymphoma or Myeloma	38	71	
Disease status at HSCT, n			0.80
Early	62	112	
Intermediate	37	62	
Advanced	36	73	
HCT-CI score, median (range)	0 (0-7)	1 (0-5)	0.96
Karnofsky PS, median (range)	90% (70-100)	90% (50-100)	0.77
Donor age, years, median (range)	29 (19-55)	28 (19-56)	0.68
Donor gender, male, n	94	181	0.45
Female donor/male recipient, n	23	34	0.39
Host/donor CMV serostatus, n			0.35
Pos/pos	50	92	
Pos/neg	51	101	
Neg/pos	15	15	
Neg/neg	16	34	
Type of conditioning, n			0.86
MAC	95	176	
RIC	40	71	
Source of stem cells, n			0.03
PB	118	193	
BM	17	54	
ATG-based GvHD prophylaxis, n	127	230	0.13

Supplemental Table 4. Patient, donor and transplant characteristics in in permissive and non-permissive DPB1 mismatched pairs according to the **ΔFD model**[1]

	<2.665 (N=259)	**≥2.665 (N=123)**	**p**
Median follow-up for survivors, years (range)	3.5 (0.3-6)	2.9 (0.3-5)	0.12
HSCT year, median (range)	2013 (2012-2015)	2014 (2012-2015)	0.97
Patient age, years, median (range)	48 (18-71)	52 (19-66)	0.48
Patient gender, male, n	155	63	0.11
Type of diagnosis, n			0.59
AML or ALL	144	62	
MDS or MPN	45	22	
Lymphoma or Myeloma	70	39	
Disease status at HSCT, n			0.78
Early	120	54	
Intermediate	68	31	
Advanced	71	38	
HCT-CI score, median (range)	0 (0-7)	1 (0-6)	0.38
Karnofsky PS, median (range)	90% (60-100)	90% (50-100)	0.25
Donor age, years, median (range)	28 (19-56)	30 (20-52)	0.98
Donor gender, male, n	184	91	0.55
Female donor/male recipient, n	40	17	0.68
Host/donor CMV serostatus, n			0.60
Pos/pos	93	49	
Pos/neg	105	47	
Neg/pos	23	7	
Neg/neg	32	18	
Type of conditioning, n			0.59
MAC	186	85	
RIC	73	38	
Source of stem cells, n			0.15
PB	216	95	
BM	43	28	
ATG-based GvHD prophylaxis, n	233	112	0.74

[1]shown are data for cut-off 2.665; data for cut-off 1.64 were not significantly different.

Supplemental Table 5. Patient, donor and transplant characteristics in in low risk and high risk DPB1 mismatched pairs according to the **Expression model**

	Low risk (N=153)	**High risk (N=76)**	**p**
Median follow-up for survivors, years (range)	3.5 (0.3-6)	3.3 (0.3-6)	0.59
HSCT year, median (range)	2013 (2012-2015)	2013 (2012-2015)	0.31
Patient age, years, median (range)	49 (18-71)	49 (19-68)	0.98
Patient gender, male, n	93	45	0.82
Type of diagnosis, n			0.68
AML or ALL	80	44	
MDS or MPN	27	13	
Lymphoma or Myeloma	46	19	
Disease status at HSCT, n			0.94
Early	71	37	
Intermediate	39	19	
Advanced	43	20	
HCT-CI score, median (range)	1 (0-6)	0 (0-7)	0.26
Karnofsky PS, median (range)	90% (50-100)	90% (70-100)	0.49
Donor age, years, median (range)	28 (19-56)	29 (19-54)	0.19
Donor gender, male, n	105	53	0.86
Female donor/male recipient, n	28	12	0.64
Host/donor CMV serostatus, n			0.47
Pos/pos	62	24	
Pos/neg	53	33	
Neg/pos	13	5	
Neg/neg	21	12	
Type of conditioning, n			0.73
MAC	112	54	
RIC	41	22	
Source of stem cells, n			0.09
PB	129	57	
BM	24	19	
ATG-based GvHD prophylaxis, n	136	72	0.15

Supplemental Table 6. Patient, donor and transplant characteristics in low risk or high risk DPB1 mismatched pairs according to the **DP2/DP5 model**

	DP2 (N=118)	**DP5 (N=31)**	**p**
Median follow-up for survivors, years (range)	3.4 (0.3-6.2)	2.8 (0.3-6.1)	0.39
HSCT year, median (range)	2013 (2012-2015)	2013 (2012-2015)	0.74
Patient age, years, median (range)	49 (18-69)	45 (19-66)	0.16
Patient gender, male, n	74	20	0.85
Type of diagnosis, n			0.67
AML or ALL	58	18	
MDS or MPN	22	5	
Lymphoma or Myeloma	38	8	
Disease status at HSCT, n			0.83
Early	57	14	
Intermediate	28	9	
Advanced	33	8	
HCT-CI score, median (range)	1 (0-6)	1 (0-5)	0.58
Karnofsky PS, median (range)	90% (50-100)	90% (70-100)	0.98
Donor age, years, median (range)	28 (19-56)	29 (23-54)	0.09
Donor gender, male, n	84	22	0.98
Female donor/male recipient, n	20	4	0.41
Host/donor CMV serostatus, n			0.35
Pos/pos	48	8	
Pos/neg	37	14	
Neg/pos	11	2	
Neg/neg	18	6	
Type of conditioning, n			0.07
MAC	91	19	
RIC	27	12	
Source of stem cells, n			0.39
PB	96	24	
BM	22	7	
ATG-based GvHD prophylaxis, n	104	29	0.39

Supplemental Table 7. Univariate analysis for transplant outcomes and different models of DPB1 mismatch permissivity.[1]

	3-y OS	3-y GRFS	3-y Relapse	3-y NRM	100-d aGvHD≥2	100-d aGvHD≥3	3-y cGvHD	3-y ext cGvHD
DPB1 allele matching status								
Matched (n=40)	43 (26-59)	34 (20-49)	38 (23-54)	26 (13-40)	0	0	18 (8-31)	5 (1-16)
Mismatched (n=382)	53 (47-58)	32 (27-37)	29 (24-34)	27 (22-31)	23 (19-27)	8 (6-11)	29 (24-33)	9 (7-13)
p	0.81	0.68	0.16	0.81	**<0.01**	**0.05**	0.10	0.35
TCE3								
Permissive mismatch (n= 208)	53 (46-60)	33 (26-40)	30 (24-37)	26 (20-32)	22 (16-28)	7 (4-11)	27 (21-33)	6 (4-10)
Non Permissive mismatch (n=174)	52 (44-60)	30 (23-38)	27 (20-34)	28 (21-35)	24 (18-31)	9 (6-14)	31 (24-38)	13 (8-19)
p	0.50	0.35	0.64	0.43	0.48	0.57	0.31	**0.03**
TCE4								
Permissive mismatch (n=135)	60 (51-68)	36 (28-45)	30 (22-38)	21 (15-29)	21 (15-29)	7 (3-12)	26 (19-34)	4 (2-9)
Non Permissive mismatch (n=247)	49 (42-55)	29 (24-35)	28 (22-34)	30 (24-36)	24 (19-29)	9 (5-12)	30 (25-37)	12 (8-17)
p	**0.05**	**0.04**	0.96	0.09	0.56	0.58	0.3	**0.01**
ΔFD								
<2.65 (n=259)	54 (47-60)	31 (26-38)	28 (23-34)	27 (22-33)	23 (18-28)	8 (5-12)	28 (23-34)	9 (6-13)
≥2.65 (n=123)	51 (41-60)	32 (24-41)	30 (22-39)	25 (18-33)	23 (16-31)	7 (4-13)	29 (21-38)	10 (6-17)
p	0.83	0.71	0.57	0.71	0.90	0.65	0.88	0.75
Expression model								
low risk (n=153)	56 (47-63)	27 (20-35)	34 (26-42)	23 (17-30)	16 (10-22)	5 (2-9)	29 (22-36)	9 (5-14)
high risk (n=76)	57 (45-68)	39 (28-50)	23 (14-34)	26 (16-36)	32 (22-43)	9 (4-17)	34 (24-45)	12 (6-20)
p	0.95	0.23	0.23	0.81	**<0.01**	0.32	0.30	0.42
DP2/DP5 model								
low risk (n=118)	59 (49-67)	29 (21-38)	27 (19-36)	25 (17-33)	15 (9-22)	4 (2-9)	32 (24-41)	11 (6-17)
high risk (n=31)	62 (42-77)	39 (21-56)	31 (15-49)	23 (10-40)	43 (25-60)	20 (8-36)	26 (12-42)	16 (6-31)
p	0.76	0.47	0.62	0.80	**0.001**	**0.01**	0.67	0.32

[1]shown are mean percentages (range in parenthesis) for each outcome.

Supplemental Table 8. Multivariate analysis of clinical factors associated with HCT outcomes.

	OS	GRFS	Relapse	NRM	aGvHD≥2	aGvHD≥3	cGvHD	Ext cGvHD
	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p
TCE4[1]: NP Vs P	**1.7 (1.1-2.4) 0.008**	**1.4 (1.1-1.9) 0.01**	1.1 (0.7-1.7) 0.7	**1.9 (1.1-3.2) 0.01**	1.3 (0.8-2.1) 0.2	1.5 (0.7-3.2) 0.3	**1.7 (1.1-2.6) 0.02**	**3.6 (1.4-9.5) 0.01**
Matched Vs P	**2.1 (1.2-3.7) 0.01**	1.5 (0.9-2.4) 0.09	**2 (1.1-3.7) 0.03**	2 (0.9-4.8) 0.09	Not applicable[2]	Not applicable[2]	0.8 (0.3-2) 0.6	0.9 (0.1-7.5) 0.9
Disease status: Intermediate Vs Early	1.4 (0.9-2.1) 0.09	1.3 (0.9-1.8) 0.1	1.5 (0.9-2.5) 0.09	1.6 (0.9-2.7) 0.09	1.1 (0.6-1.9) 0.7	0.7 (0.3-1.7) 0.4	1.1 (0.7-1.8) 0.6	2.4 (0.9-5.5) 0.09
Advanced Vs early	**2.3 (1.6-3.3) <10^{-3}**	**1.5 (1.1-2) 0.01**	**2 (1.2-3.2) 0.005**	**1.9 (1.2-3.3) 0.01**	0.7 (0.4-1.3) 0.3	0.6 (0.2-1.6) 0.3	1 (0.6-1.7) 0.9	1.2 (0.4-3.2) 0.7
Patient age	1 (0.9-1.1) 0.2	1 (0.9-1.1) 0.2	1 (0.9-1.1) 0.3	1 (0.9-1.1) 0.2	1 (0.9-1.1) 0.4	1 (0.9-1.1) 0.9	1 (0.9-1.1) 0.1	1 (0.9-1.1) 0.9
HCTI-Score: ≥1 Vs 0	1.3 (0.9-1.9) 0.09	**1.4 (1.1-1.9) 0.008**	1.1 (0.7-1.6) 0.7	**1.6 (1.1-2.5) 0.04**	1.2 (0.7-1.9) 0.5	1.5 (0.7-3.1) 0.3	**2.2 (1.4-3.4) <10^{-3}**	**2.2 (1-5) 0.05**
Female donor to male recipient	1.1 (0.7-1.8) 0.6	1.3 (0.9-1.1) 0.2	1.1 (0.6-2.1) 0.6	1.1 (0.6-2) 0.8	0.6 (0.3-1.3) 0.2	0.8 (0.3-2.3) 0.6	1.2 (0.7-2.1) 0.4	2.4 (1.1-5.4) 0.04
CMV status: neg/neg Vs other	0.7 (0.4-1.2) 0.2	0.9 (0.6-1.4) 0.7	0.6 (0.3-1.3) 0.2	0.8 (0.4-1.7) 0.6	1.1 (0.5-2.1) 0.9	1.3 (0.5-3.6) 0.6	1 (0.5-2) 0.9	1 (0.4-2.8) 0.9
Conditioning: MAC Vs RIC	1.3 (0.9-1.9) 0.1	1.1 (0.8-1.5) 0.5	1 (0.6-1.6) 0.9	1.3 (0.8-2.1) 0.4	1.4 (0.8-2.4) 0.2	1.9 (0.7-5.1) 0.2	1.1 (0.7-1.7) 0.8	0.5 (0.2-1.1) 0.08
Stem cell source: PB Vs BM	1.1 (0.7-1.6) 0.8	1.1 (0.8-1.6) 0.6	1 (0.6-1.7) 0.9	1.1 (0.6-1.9) 0.8	1.4 (0.7-2.7) 0.3	2.4 (0.7-8.4) 0.2	0.8 (0.4-1.3) 0.3	0.6 (0.2-1.7) 0.4
ATG use	1.2 (0.5-2.7) 0.7	0.9 (0.5-1.7) 0.8	2.6 (0.6-11) 0.2	1 (0.3-2.8) 0.9	0.5 (0.2-1.4) 0.2	0.4 (0.1-1.4) 0.1	1 (0.4-2.4) 0.9	0.9 (0.2-3.2) 0.8
Overall HLA-matching: 9/10 Vs 10/10	1.8 (1-3.3) 0.06	1.1 (0.7-1.7) 0.6	2 (0.9-4.2) 0.07	1.5 (0.7-3.3) 0.3	1.4 (0.6-3) 0.5	1.2 (0.4-4.1) 0.8	0.8 (0.4-1.4) 0.4	1.5 (0.3-6.6) 0.6
Center effect: ≥10 HCT/year Vs <10	0.9 (0.7-1.3) 0.7	1 (0.7-1.3) 0.8	0.8 (0.5-1.3) 0.4	0.9 (0.5-1.4) 0.6	0.9 (0.6-1.6) 0.9	0.7 (0.3-1.5) 0.3	1.1 (0.7-1.7) 0.7	1.9 (0.8-4.6) 0.2

[1]TCE4 NP, P and matched: N= 247, 135 and 40, respectively.
[2]Not applicable since no cases of aGvHD occurred in the DPB1 matched cohort.
Shown are the data for the TCE4 functional model as main effect term and covariates as described in Statistical methods.