

# In silico prediction of nonpermissive HLA-DPB1 mismatches in unrelated HCT by functional distance

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## Key Points

- Nonpermissive T-cell epitope group mismatches can be predicted in silico for any HLA-DPB1 allele by functional distance scores.
- In silico-predicted nonpermissive DPB1 mismatches are associated with mortality and GVHD after 8/8 matched HCT.

In silico prediction of high-risk donor–recipient HLA mismatches after unrelated donor (UD) hematopoietic cell transplantation (HCT) is an attractive, yet elusive, objective. Nonpermissive T-cell epitope (TCE) group mismatches were defined by alloreactive T-cell cross-reactivity for 52/80 HLA-DPB1 alleles (TCE-X). More recently, a numerical functional distance (FD) scoring system for in silico prediction of TCE groups based on the median impact of exon 2–encoded amino acid polymorphism on T-cell alloreactivity was developed for all DPB1 alleles (TCE-FD), including the 28/80 common alleles not assigned by TCE-X. We compared clinical outcome associations of nonpermissive DPB1 mismatches defined by TCE-X or TCE-FD in 8/8 HLA-matched UD-HCT for acute leukemia, myelodysplastic syndrome, and chronic myelogenous leukemia between 1999 and 2011 (N = 2730). Concordance between the 2 models was 92.3%, with most differences arising from DPB1\*06:01 and DPB1\*19:01 being differently assigned by TCE-X and TCE-FD. In both models, nonpermissive mismatches were associated with reduced overall survival (hazard ratio [HR], 1.15,  $P < .006$  and HR, 1.12,  $P < .03$ ), increased transplant-related mortality (HR, 1.31,  $P < .001$  and HR, 1.26,  $P < .001$ ) as well as acute (HR, 1.16,  $P < .02$  and HR, 1.22,  $P < .001$ ) and chronic (HR, 1.20,  $P < .003$  and HR, 1.22,  $P < .001$ ) graft-versus-host disease (GVHD). We show that in silico prediction of nonpermissive DPB1 mismatches significantly associated with major transplant outcomes is feasible for any DPB1 allele with known exon 2 sequence based on experimentally elaborated FD scores. This proof-of-principle observation opens new avenues for developing HLA risk-prediction models in HCT and has practical implications for UD searches.

## Introduction

Mismatches in the human leukocyte antigen (HLA) genes between donors and recipients mediate alloreactivity in hematopoietic cell transplantation (HCT) and contribute to the detrimental graft-versus-host disease (GVHD) and the beneficial graft-versus-leukemia effect.<sup>1,2</sup> The search for mismatches that can be better tolerated than others, tilting the GVHD–graft-versus-leukemia balance toward the reduction in relapse rates with lower risks for transplant-related mortality (TRM), has been an intense area of research in HCT.<sup>3</sup> However, our understanding of how to control and intelligently use alloreactivity is still incomplete, restricting its application in the clinical setting.

The exponential rate of discovery of new HLA alleles brought about by the advent of next-generation sequencing–based typing<sup>4</sup> makes the *in vitro* functional testing of alloreactivity for all alleles unfeasible and underscores the need for functionally based *in silico* prediction models of nonpermissive mismatches applicable to all alleles. Considerable effort has been invested in developing algorithms able to predict high-risk mismatches in HCT. These models include predictions based on structural similarity of the mismatched HLAs,<sup>5–9</sup> mismatched key amino acid residues,<sup>10,11</sup> individual high- or low-risk mismatch combinations,<sup>12–17</sup> and predicted indirectly recognized HLA epitopes.<sup>18,19</sup> However, clinical outcome associations observed for these various predictive algorithms have emerged from mostly limited-sized retrospective studies that were not validated in independent cohorts in many instances,<sup>20–24</sup> potentially exposing a lack of experimental evidence supporting the underlying hypotheses.

Successful translation of an experimentally proven hypothesis about T-cell alloreactivity into a clinically proven algorithm of nonpermissive mismatches has been achieved for the HLA-DPB1 (DPB1) locus.<sup>25</sup> Functional assessment of alloreactive DPB1-specific T-cell cross-reactivity patterns led to the classification of 72 DPB1 alleles into 3 T-cell epitope (TCE) groups,<sup>26</sup> and alloreactivity levels across TCE groups were shown to be higher than those between alleles within the same group.<sup>27</sup> This differential alloreactivity was further dissected by pinpointing the weight of specific amino acid changes in the DPB1 peptide-binding groove responsible for these alloreactivity patterns.<sup>28</sup> Moreover, poorly tolerated “nonpermissive” DPB1 mismatches have been associated with decreased survival rates in patients after HCT, while the remaining “permissive” DPB1 mismatches were associated with survival rates similar to those in patients receiving DPB1 allele matches,<sup>29,30</sup> with the added value of reduced relapse risk for hematological malignancies. Despite these successes, the original TCE algorithm (TCE-X)<sup>26</sup> was applicable only to those alleles for which functional evidence of cross-reactivity patterns had been obtained, and could not be extended to the remaining 903 DPB1 alleles identified to date.<sup>4</sup> To address this limitation, we refined the TCE-X model by using the measurement of functional distance (FD) based on the median impact of amino acid polymorphism in DPB1’s peptide-binding groove to define the TCE groups, and extending it *in silico* to all known alleles (TCE-FD).<sup>28</sup> In the present study, we sought to clinically validate the *in silico* assignment of TCE matching by TCE-FD and compare its performance with TCE-X in a large cohort of otherwise HLA-matched HCT from unrelated donors (UDs), providing a proof of principle that *in silico*–defined permissiveness of HLA mismatches can inform risk after HCT.

## Materials and methods

### Study population and clinical data

The study included 2730 patients diagnosed with acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia (CML), or myelodysplastic syndrome (MDS) who underwent a first myeloablative bone marrow or peripheral blood stem cell transplantation from a UD between 1999 and 2011.<sup>30,31</sup> Details of the demographic, immunogenetic and clinical characteristics of patients and transplants are provided in Table 1. The clinical data were collected and stored by the Center for International

Blood and Marrow Transplant Research, and all participants gave informed consent in agreement with the principles of the Declaration of Helsinki. The study was approved by the National Marrow Donor Program Institutional Review Board in conformity with the federal regulation regarding the protection of human research participants.

### HLA typing and DPB1 matching

Only patient/donor pairs with second field resolution typing at the HLA loci A, B, C, DRB1, DQB1, and DPB1 were included. Patients and donors were 8/8 matched for HLA-A, -B, -C, and -DRB1 but were mismatched for DPB1. Pairs with mismatches at other major HLA loci were not included to avoid a confounding effect on clinical outcome. For definition of permissive and nonpermissive DPB1 mismatches, the TCE algorithm was applied as previously described.<sup>26</sup> Briefly, DPB1 alleles were classified into 3 TCE groups according to the original TCE-X or the new TCE-FD model, as described in “Results” (supplemental Table 1). Nonpermissive and permissive DPB1 mismatches were subsequently defined according to the TCE3 model,<sup>26,29</sup> using the online DPB1 TCE Web tool from the IMGT/HLA database<sup>4</sup> (<https://www.ebi.ac.uk/ipd/imgt/hla/dpb.html>) version 1 and version 2 for TCE-X and TCE-FD, respectively. No novel DPB1 alleles were identified in this cohort at this level of resolution.

### Clinical outcomes

The primary study end point was overall survival (OS); secondary study end points were disease-free survival (DFS), TRM, relapse incidence (RI), chronic GVHD (cGVHD) and acute GVHD (aGVHD), defined as previously described.<sup>30</sup>

### Statistical analysis

For evaluation of clinical results, the Kruskal-Wallis and  $\chi^2$  tests were used to analyze differences for discrete or continuous factors, respectively, between the 2 different HLA-DPB1 matching groups (mismatched permissive or mismatched nonpermissive). Kaplan-Meier probabilities were calculated for OS and DFS. Estimated cumulative incidence was calculated for events with competing risks (ie, TRM, RI, and aGVHD). Comparisons of survival curves and cumulative incidence rates were done with the log-rank test and the Gray test, respectively. Multivariable regression analyses using the Cox proportional hazards model were generated for each HLA-DP matching algorithm independently as the main effect. Models were fit to determine which risk factors were related to a given outcome. All variables were tested for affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption were added as time-dependent covariates. A forward stepwise model-building procedure was used to select risk factors for each outcome with a threshold of  $P \leq .01$  for entering the models. Due to multiple comparisons,  $P < .01$  was used to determine statistical significance for the main effect. All analyses were performed using SAS version 9.4 (SAS Institute, Inc.).

## Results

### Experimental and *in silico* prediction of nonpermissive DPB1 TCE group mismatches

The original classification of DPB1 alleles into 3 structurally different TCE groups (TCE-X) was based on the ability of T cells obtained

**Table 1. Patient and transplant characteristics**

	TCE-X		TCE-FD	
	Nonpermissive (n = 1166)	Permissive (n = 1564)	Nonpermissive (n = 1279)	Permissive (n = 1451)
<b>Recipient age, n (%), y</b>				
<19	213 (18)	305 (19)	250 (19)	268 (18)
20-59	901 (77)	1167 (75)	970 (76)	1098 (76)
60+	52 (5)	92 (6)	59 (5)	85 (6)
Median (range), y	39 (<1 to 66)	38 (<1 to 66)	38 (<1 to 66)	39 (<1 to 66)
<b>Recipient sex, n (%)</b>				
Male	649 (56)	869 (56)	701 (55)	817 (56)
Female	517 (44)	695 (44)	578 (45)	634 (44)
<b>Recipient race, n (%)</b>				
White	1088 (93)	1435 (92)	1191 (93)	1332 (92)
African American	27 (2)	48 (3)	29 (2)	46 (3)
Asian	15 (1)	23 (1)	15 (1)	23 (2)
Pacific Islander	21 (2)	31 (2)	24 (2)	28 (2)
Native American	2 (<1)	5 (<1)	3 (<1)	4 (<1)
No data	13 (1)	22 (1)	17 (1)	18 (1)
<b>Karnofsky score prior to HCT, n (%)</b>				
<90	290 (25)	374 (24)	318 (25)	346 (24)
≥90	758 (65)	1043 (67)	836 (65)	965 (66)
No data	118 (10)	147 (9)	125 (10)	140 (10)
<b>Disease at HCT, n (%)</b>				
AML	585 (50)	731 (47)	623 (49)	693 (48)
ALL	308 (26)	417 (26)	344 (27)	381 (26)
CML	166 (14)	245 (16)	193 (15)	218 (15)
MDS	107 (10)	171 (11)	119 (9)	159 (11)
<b>Disease status at HCT, n (%)</b>				
Early	519 (45)	703 (45)	582 (46)	640 (44)
Intermediate	349 (30)	417 (27)	374 (29)	392 (27)
Advanced	298 (25)	444 (28)	323 (25)	419 (29)
<b>Graft type, n (%)</b>				
Bone marrow	572 (49)	753 (48)	626 (49)	699 (48)
Peripheral blood	594 (51)	811 (52)	653 (51)	752 (52)
<b>Donor age, n (%), y</b>				
18-32	567 (49)	741 (47)	622 (49)	686 (47)
33+	576 (49)	790 (51)	632 (49)	734 (51)
No data	23 (2)	33 (2)	25 (2)	31 (2)
Median age (range), y	33 (18-56)	33 (18-57)	33 (18-57)	34 (18-57)
<b>Donor/recipient sex match, n (%)</b>				
Male/male	488 (42)	601 (38)	523 (41)	566 (39)
Male/female	327 (28)	423 (27)	361 (28)	389 (27)
Female/male	161 (14)	268 (17)	178 (14)	251 (17)
Female/female	190 (16)	271 (17)	217 (17)	244 (17)
No data	0	1 (<1)	0	1 (<1)
<b>Donor/recipient CMV match, n (%)</b>				
-/-	381 (33)	504 (32)	429 (34)	456 (31)

Only DPB1 - mismatched pairs that could be classified as nonpermissive or permissive by the TCE-X and the TCE-FD model are shown. GVHD prophylaxis was based on methotrexate, with or without cyclosporine A or other, or mycophenolate mofetil, cyclosporine A, or FK506 alone or with other.

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin.

**Table 1. (continued)**

	TCE-X		TCE-FD	
	Nonpermissive (n = 1166)	Permissive (n = 1564)	Nonpermissive (n = 1279)	Permissive (n = 1451)
-/+	388 (33)	549 (35)	426 (33)	511 (35)
+/-	145 (12)	178 (12)	159 (12)	164 (11)
+/+	232 (20)	311 (20)	246 (19)	297 (21)
No data	20 (2)	22 (1)	19 (2)	23 (2)
<b>Donor/recipient ABO match, n (%)</b>				
Matched	450 (39)	646 (41)	509 (40)	587 (40)
Mismatched	641 (55)	809 (51)	689 (53)	761 (52)
No data	75 (6)	109 (8)	81 (7)	103 (8)
<b>Total body irradiation, n (%)</b>				
No	505 (43)	652 (42)	546 (43)	611 (42)
Yes	649 (56)	893 (57)	720 (56)	822 (57)
No data	12 (1)	19 (1)	13 (1)	18 (1)
<b>In vivo T-cell depletion (ATG or alemtuzumab), n (%)</b>				
No	845 (72)	1169 (75)	919 (72)	1095 (75)
Yes	321 (28)	395 (25)	360 (28)	356 (25)
<b>DQB1 matching, n (%)</b>				
Mismatch	83 (7)	143 (9)	99 (8)	127 (9)
Match	1075 (92)	1402 (90)	1170 (91)	1307 (90)
No data	8 (<1)	19 (1)	10 (<1)	17 (1)
<b>Year of HCT, n (%)</b>				
1999-2006	754 (65)	988 (63)	823 (64)	919 (63)
2007-2011	412 (35)	576 (37)	456 (36)	532 (37)
Median follow-up of survivors (range), mo	61 (3-151)	62 (3-150)	61 (3-151)	62 (3-150)

Only DPB1\* mismatched pairs that could be classified as nonpermissive or permissive by the TCE-X and the TCE-FD model are shown. GVHD prophylaxis was based on methotrexate, with or without cyclosporine A or other, or mycophenolate mofetil, cyclosporine A, or FK506 alone or with other.  
 ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin.

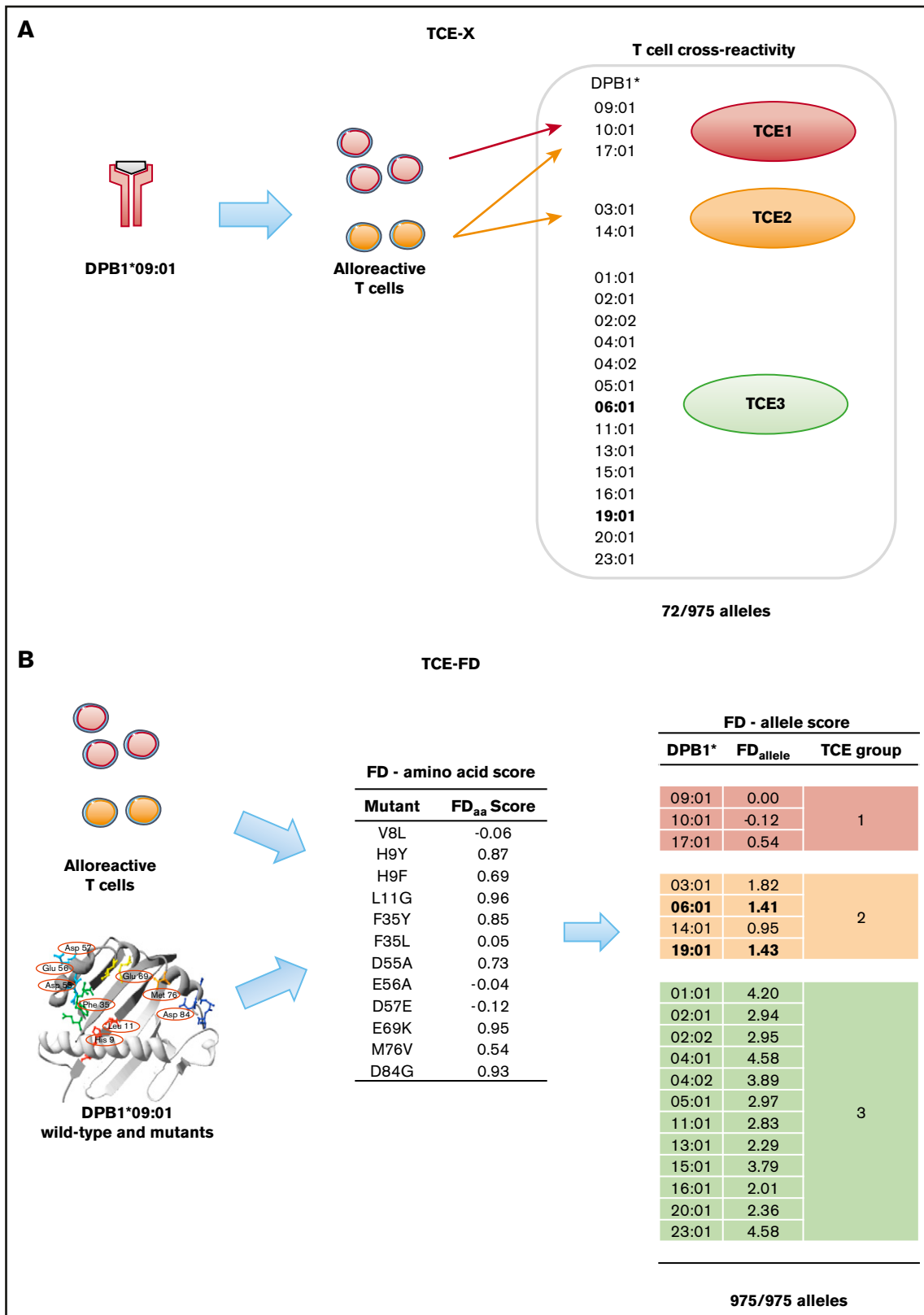
from a patient with allograft rejection post-HCT alloreactive to DPB1\*09:01 to cross-recognize other DPB1 alleles encoding similar epitopes (Figure 1A). This initial classification was limited to a set of 72 DPB1 alleles for which B lymphoblastoid cell lines were available for direct in vitro testing<sup>26,32</sup> and includes only 52/80 DPB1 alleles that have been described to be common and well-documented (CWD) in catalogs from the American Society of Histocompatibility and Immunogenetics and the European Federation for Immunogenetics<sup>33,34</sup> (supplemental Table 1).

In view of this and of the ever-increasing number of known DPB1 alleles (975 known variants to date),<sup>4</sup> we developed an alternative model for DPB1 TCE group assignment by in silico prediction based on experimentally defined FD for each DPB1 allele (TCE-FD model). Briefly,  $FD_{aa}$  scores for 12 key polymorphic amino acid residues encoded by exon 2 of DPB1\*09:01 were determined as the median impact of naturally occurring substitutions at each of these residues on allorecognition of wild-type DPB1\*09:01 by alloreactive T cells (Figure 1B). This allowed us to calculate the corresponding  $FD_{allele}$  score as the sum of  $FD_{aa}$  scores encoded by each allele's exon 2 sequence. We found that  $FD_{allele}$  score ranges faithfully reflected the TCE groups, with  $FD_{allele} < 0.6$  for TCE group 1,

$0.6 < FD_{allele} < 2.0$  for TCE group 2, and  $FD_{allele} > 2.0$  for TCE group 3.<sup>28</sup> Only 2 alleles fell short of this definition, namely DPB1\*06:01 and DPB1\*19:01, which had been assigned to TCE group 3 by TCE-X despite  $FD_{allele}$  scores of 1.41 and 1.43, respectively. Further functional testing with a larger panel of T-cell effectors confirmed their classification into TCE group 2.<sup>28</sup> Because all known DPB1 alleles have at least complete exon 2 sequence available, the TCE-FD model allows for in silico prediction of TCE assignment for all alleles at this locus. To confirm the clinical usefulness of this model, we tested its influence on HCT outcome, as explained below.

### Study population and outcomes

The study cohort included 2730 patients with acute leukemia, CML, or MDS who received an 8/8 HLA-matched DPB1-mismatched UD HCT that could be classified as nonpermissive or permissive by the TCE-X and the TCE-FD model. Overall outcomes for the investigated clinical end points were OS 41%, DFS 37%, TRM 32%, RI 30%, severe (grades III-IV) aGVHD 19% at 100 days, and cGVHD 50% at 5 years. Median follow-up time was 61 months.



**Figure 1. TCE group classification of DPB1 alleles by TCE-X or TCE-FD.** (A) TCE classification of DPB1 alleles based on in vitro cross-reactivity patterns of alloreactive T cells nominally directed against DPB1\*09:01.<sup>26</sup> Alleles that were reproducibly cross-recognized by a panel of 5 DPB1\*09:01-specific alloreactive T-cell effectors were grouped together as TCE group 1 (red), whereas DPB1 alleles recognized by only a part or none of these T cells were classified into TCE groups 2 (orange) and 3 (green), respectively.<sup>26</sup> In total, 72 of the 975 DPB1 alleles known to date were experimentally tested for TCE-X (see Supplemental Table 1 for a comprehensive list of TCE group

## Prediction of nonpermissive or permissive DPB1 mismatches by TCE-X or TCE-FD

In 2697 of 2730 pairs, the DPB1 alleles of patient and donors were included in the 72 alleles assignable by TCE-X. Only in 33 of 2730 pairs (1.2%) was  $\geq 1$  DPB1 allele of patient and/or donor not part of these 72 alleles. These included 9 alleles: DPB1\*18:01 ( $n = 16$ ), DPB1\*26:01 ( $n = 5$ ), DPB1\*27:01 ( $n = 4$ ), DPB1\*35:01 ( $n = 3$ ), DPB1\*85:01 ( $n = 2$ ), and DPB1\*21:01, \*29:01, \*30:01, and \*130:01 ( $n = 1$  each) (supplemental Table 1). In the TCE-X classification, these 9 alleles were assigned to TCE group 3 by default, whereas 4 of them were classified as TCE group 1 or 2 by TCE-FD (supplemental Table 1). This resulted in discordant assignment as permissive by TCE-X and nonpermissive by TCE-FD in only 5 cases. An additional 205 discordances in the same ( $N = 156$ ) or the opposite ( $N = 49$ ) direction were all due to the presence of DPB1\*06:01 and/or \*19:01, which were assigned to TCE groups 2 and 3 by TCE-FD and TCE-X, respectively (Table 2). Overall, 1118 and 1402 pairs were concordantly assigned as nonpermissive or permissive, respectively, by TCE-X and TCE-FD, for an overall concordance of 92.3%. TCE-X had slightly more permissive pairs (1564/2730, 57.3%) compared with TCE-FD (1451/2730, 53.2%) (Table 2).

## Clinical risk associations of nonpermissive DPB1 mismatches by TCE-X or TCE-FD

As previously reported,<sup>26,29-31,35,36</sup> the cumulative incidence of TRM was significantly lower for HCT from nonpermissively DPB1-mismatched UDs according to TCE-X compared with permissively mismatched HCT (Figure 2A). The same was observed when nonpermissive and permissive DPB1 mismatches were defined according to the TCE-FD model (Figure 2B). The association of nonpermissive DPB1 mismatches according to the 2 models with all major outcome end points was analyzed using multivariable Cox regression models adjusted for the main clinical variables (Table 3). The significant association with TRM was confirmed for TCE-X and TCE-FD (hazard ratio [HR], 1.31; 95% confidence interval [CI], 1.14-1.50,  $P < .001$  for TCE-X and HR, 1.26; 95% CI, 1.1-1.44,  $P < .001$  for TCE-FD). In line with this, the probability of OS was lower for nonpermissive DPB1 mismatches defined by TCE-X and TCE-FD, although this was significant for TCE-X (HR, 1.15; 95% CI, 1.04-1.27,  $P < .006$ ) but not for TCE-FD (HR, 1.12; 95% CI, 1.01-1.23,  $P < .03$ ) (Table 3). Interestingly, in this cohort, nonpermissive DPB1 mismatches by TCE-X and TCE-FD were also associated with increased risks for aGVHD II-IV (HR, 1.16; 95% CI, 1.03-1.30;  $P < .02$  for TCE-X and HR, 1.22; 95% CI, 1.09-1.37;  $P < .001$  for TCE-FD) and cGVHD (HR, 1.20; 95% CI, 1.07-1.34;

$P < .003$  for TCE-X and HR, 1.22; 95% CI, 1.09-1.36;  $P < .001$  for TCE-FD) (Table 3).

Because TCE-X and TCE-FD differ mainly in the assignment of DPB1\*06:01 and 19:01 to different TCE groups, we analyzed multivariable outcome associations of the discordant groups separately, using the concordant permissive pairs as reference (supplemental Table 2). The HRs obtained for the 2 discordant subgroups mirrored the above-described findings for the overall cohort. However, probably due to the low number of discordant pairs, these associations were not significant for any of the end points studied with the exception of aGVHD II-IV, for which pairs considered permissive by TCE-X but deemed nonpermissive by TCE-FD showed significantly increased risks compared with the concordant permissive pairs (HR, 1.53; 95% CI 1.22-1.93;  $P < .001$ ) (supplemental Table 2).

We also tested an additional approach of identifying high-risk DPB1 mismatches based on the numerical difference in FD scores of DPB1 alleles between patients and donors, designated  $\delta$  Functional Distance or dFD, as previously described.<sup>37</sup> The cutoff threshold for high or low-risk dFD scores was set at 2.665 in our previous study.<sup>37</sup> Here, we tested this cutoff and additionally the value 1.64, which is the mean of dFD scores observed in the present cohort (range, 0.00-9.40). As shown in supplemental Table 3, above-threshold dFD scores were significantly associated with TRM for both cutoff values in multivariable models. Moreover, dFD scores above the cutoff 1.64 were also significantly associated with OS and aGVHD II-IV, similar to TCE-defined nonpermissive mismatches. Based on these data, the dFD model is not superior to the TCE model in defining high-risk DPB1 mismatches in UD HCT.

## Discussion

In this study we have performed a clinical validation of in silico-predicted nonpermissive DPB1 mismatches via extension of the refined TCE matching algorithm (TCE-FD) to all known DPB1 alleles. Matching concordance between the 2 TCE models was 92.3%, with most differences arising from alleles DPB1\*06:01 and DPB1\*19:01 having different TCE group assignments in TCE-X and TCE-FD. By studying the impact of DPB1 mismatches in a large cohort of otherwise HLA-matched HCT from UDs and comparing it with the previous limited algorithm (TCE-X), we show that in silico-predicted nonpermissive TCE-FD mismatches result in reduced OS and increased TRM, as well as high risks for aGVHD and cGVHD. The hazards for all clinical end points were very similar for the 2 models, likely reflecting the fact that the most common

**Figure 1. (continued)** assignment for these alleles). These 72 DPB1 alleles classifiable by TCE-X represent only 7.4% of all known DPB1 alleles and account for only 65% of the CWD alleles for this locus.<sup>33,34</sup> Shown here are 19 DPB1 alleles reported with a frequency  $\geq 0.5\%$  in a large population of European descent,<sup>58</sup> which together make up 98% of the total DPB1 allele frequency. Two alleles (DPB1\*06:01 and \*19:01) that changed classification from TCE3 in TCE-X to TCE2 in TCE-FD are indicated in bold. (B) TCE classification of any known DPB1 allele based on in silico prediction by FD scores. The latter were experimentally obtained by generating a panel of 12 site-directed single amino acid mutants of DPB1\*09:01 and assessing the median strength of recognition for each mutant by alloreactive T cells relative to wild-type DPB1\*09:01.<sup>28</sup> Based on this, the FD amino acid (FD<sub>aa</sub>) scores were calculated for each mutant, with high scores representing a high functional impact and low scores representing a low functional impact of the mutation. For each DPB1 allele, the sum of FD<sub>aa</sub> scores of each of the 12 polymorphic residues analyzed was used to calculate the FD<sub>allele</sub> score. Experimental TCE classification by TCE-X was found to reflect the range of FD<sub>allele</sub> scores as follows: TCE1, FD<sub>allele</sub> < 0.6; TCE2, 0.6 < FD<sub>allele</sub> < 2.0; and TCE3, FD<sub>allele</sub> > 2.0.<sup>28</sup> TCE classification of the 19 DPB1 from panel A is indicated with TCE1, TCE2, and TCE3 in red, orange, and green, respectively. Two alleles (DPB1\*06:01 and \*19:01) that changed classification from TCE3 in TCE-X to TCE2 in TCE-FD are indicated in bold.



**Table 2. Cross-tabulation between TCE-X and TCE-FD**

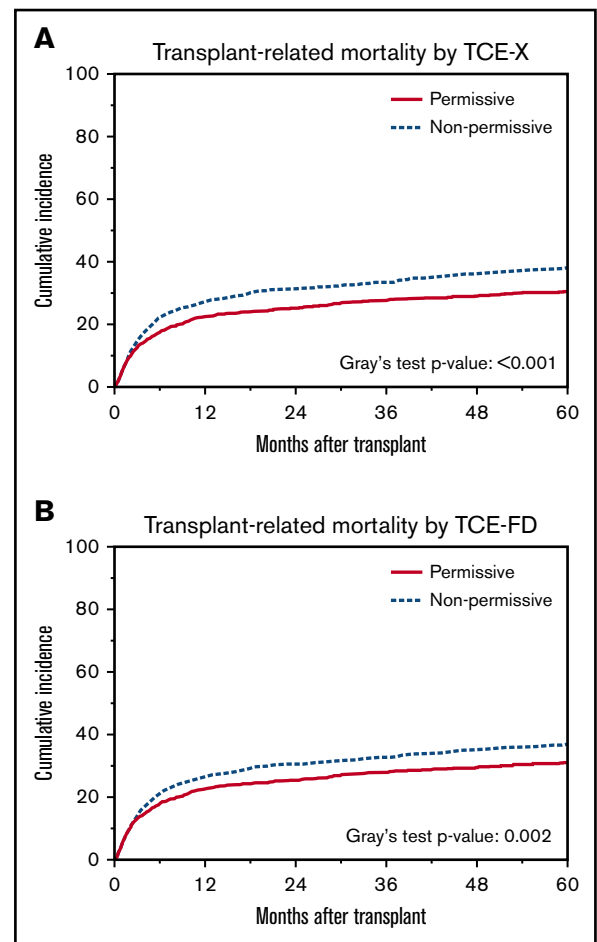
TCE-FD	TCE-X		Total, N (%)
	Nonpermissive, n (%)	Permissive, n (%)	
Nonpermissive	1118 (40.9)	161 (5.9)	1279 (46.8)
Permissive	49 (1.9)	1402 (51.3)	1451 (53.2)
Total	1166 (42.8)	1564 (57.2)	2730 (100)

Percentages refer to the total number of 2730 8/8 HLA-matched DPB1-mismatched donor–recipient pairs analyzed in this study. The 210 discordant assignments as TCE-X permissive and TCE-FD nonpermissive (n = 161) or vice versa (n = 49) were due to the presence of DPB1\*06:01 and/or 19:01, which were classified as TCE group 2 and 3 by TCE-FD and TCE-X, respectively (n = 205) or to the presence of a DPB1 allele that was classified as TCE group 1 or 2 by TCE-FD but was attributed to TCE group 3 by TCE-X because of missing data (see Supplemental Table 1) (n = 5).

alleles in European populations share the same TCE classification in both. In line with this, analysis of 210 pairs with discordant assignments did not reveal any superiority of one of the models in predicting outcome. Therefore, the differential TCE group assignment of DPB1\*06:01 and \*19:01 appears not to have a strong impact on the predictive value of the model, as also shown by subgroup analyses of discordant pairs. On the other hand, the applicability of TCE-FD to all current and future DPB1 alleles is a major strength that renders it overall superior to TCE-X.

Several attempts to translate *in vitro* functional evidence of differential alloreactivity<sup>38,39</sup> or *in silico*–predicted allorecognition patterns<sup>5,6,40</sup> into clinically relevant HLA-matching algorithms have been carried out to inform risk assessment in HCT. Nevertheless, *in silico* immunogenicity predictions, as well as functional *in vitro* data, do not always correlate with each other or adequately inform clinical risk prediction, as previously shown by other investigators.<sup>20-24,41,42</sup> In addition, approaches relying on predicted binding of peptides to HLA molecules are limited by the accuracy of these predictive tools and their algorithms.<sup>43</sup> More successful strategies have been developed for transplantation of solid organs, for which prediction of the recognition of antibody-accessible epitopes present in donor HLAs and absent in the recipient's HLAs<sup>44,45</sup> is used to avoid humoral responses against the graft.<sup>7,46,47</sup> Of note, however, the same algorithm failed to predict outcome in the HCT setting.<sup>48</sup>

With our approach based on the experimentally determined FD that arises from the specific weight of amino acid differences in DPB1's peptide-binding groove between different alleles,<sup>28</sup> we were able to extend the TCE algorithm to all 975 DPB1 alleles described to date,<sup>4</sup> including 80 DPB1 alleles reported as CWD in the American Society of Histocompatibility Immunogenetics or the European Federation for Immunogenetics catalog,<sup>33,34</sup> without the need for further *in vitro* testing. Importantly, although other approaches have tried to correlate the impact of amino acid substitutions at different positions of the HLA peptide-binding groove with alloreactivity patterns and HCT outcome,<sup>10-13,49</sup> ours does not consider all substitutions equally; rather, it draws on direct experimental evidence on the differential impact of different types of amino acid substitutions at key positions. A further strength of our approach is that TCE grouping can now be applied prospectively to any new DPB1 allele discovered for which its exon 2 sequence is known. In the relatively ethnically conserved cohort under analysis in this study, DPB1 alleles assignable by TCE-FD, but not by TCE-X,



**Figure 2. Cumulative incidence of TRM by nonpermissive or permissive DPB1 mismatches according to TCE-X or TCE-FD.** Shown are probabilities of TRM for transplants stratified according to DPB1 nonpermissive or permissive mismatches by TCE-X (A) or TCE-FD (B). The numbers in each group were n = 1166, 1564, 1279, and 1451 for TCE-X nonpermissive, TCE-X permissive, TCE-FD nonpermissive, and TCE-FD permissive pairs, respectively.

were present in only 1.2% of pairs. However, the switch to next-generation sequencing–based HLA typing methods has significantly accelerated the discovery of new alleles in recent years,<sup>50,51</sup> with 378 of the current 975 DPB1 alleles reported in the last 2 years alone.<sup>4</sup> Moreover, HCT is also becoming increasingly feasible in emerging countries,<sup>52</sup> further contributing to the likelihood of new alleles being discovered in new ethnic groups. Based on all of this, the usefulness of *in silico* prediction by TCE-FD is likely to increase over time. The TCE-X and TCE-FD matching algorithms are freely available through the IMGT/HLA database ([www.ebi.ac.uk/ipd/imgt/hla](http://www.ebi.ac.uk/ipd/imgt/hla)).<sup>53</sup> TCE-FD has also been incorporated into UD search tools provided to clinicians by stem cell donor registries in the United States (HapLogic; <https://bethematch.org>) and Germany (Optimatch; [www.zkrd.de](http://www.zkrd.de)).<sup>54,55</sup>

In addition to the associations with mortality reported previously, both algorithms were also able to detect an association with acute and chronic GVHD in this cohort. This finding is concordant with a recent report underlining the relevant effect of DPB1 mismatching on GVHD risks in patients transplanted with UDs compared with sibling donors.<sup>56</sup> In that same study, permissive and nonpermissive

**Table 3. Multivariable regression models for association between nonpermissive DPB1 mismatches and clinical outcome**

	TCE-X nonpermissive (n = 1166)		TCE-FD nonpermissive (n = 1279)	
	HR (95% CI)	P	HR (95% CI)	P
OS	1.15 (1.04-1.27)	.005	1.12 (1.01-1.23)	.028
DFS	1.11 (1.00-1.22)	.044	1.07 (0.97-1.18)	.159
TRM	1.231 (1.14-1.50)	<.001	1.26 (1.1-1.44)	<.001
Relapse	0.93 (0.81-1.07)	.307	0.91 (0.79-1.05)	.194
cGVHD	1.20 (1.07-1.34)	.002	1.22 (1.09-1.36)	<.001
aGVHD II-IV	1.16 (1.03-1.30)	.014	1.22 (1.09-1.37)	<.001
aGVHD III-IV	1.08 (0.90-1.29)	.428	1.03 (0.86-1.24)	.759

Data are shown using permissive mismatches as reference (n = 1564 for TCE-X and n = 1451 for TCE-FD).

mismatching for DPB1 significantly decreased the risk for relapse or disease progression after UD HCT, whereas permissive DPB1 mismatches were associated with similar incidences of GVHD-related outcomes compared with sibling donors. These findings support again the concept of feasible<sup>57</sup> intelligent DPB1 mismatching for specific leukemia immunotherapy in the context of allogeneic HCT, as proposed also by other investigators.<sup>58-61</sup>

In a previous single-center study, we found significant associations between the numerical difference in FD (dFD) and HCT outcome,<sup>37</sup> which appeared to be even stronger compared with nonpermissive TCE mismatches. Although we did find significant associations of above-threshold dFD scores with OS, TRM, and aGVHD, when the mean dFD value was used as cutoff in the present study, these associations were not stronger than those observed with the TCE model of nonpermissive mismatches. These data suggest that dFD could be a surrogate for TCE, which remains superior because it has been most widely tested clinically and does not present the difficulty of defining a generally applicable cutoff value associated with the dFD model.

The TCE model of nonpermissive mismatches still has some limitations. In particular, all evidence for FD between DPB1 alleles is based on single-position site-directed mutagenesis of only 1 allele: DPB1\*09:01. Moreover, our quantification of FD assumes an additive effect of the different amino acid substitutions appearing naturally together in other alleles. Future experiments examining FD derived from mutagenesis of other DPB1 alleles representative of all 3 TCE groups and studying the joint effect of several mutations in the molecule's peptide-binding groove are warranted to further refine our *in silico* predictions. In addition, direct characterization of peptide repertoires and their overlap between TCE groups and alleles within each group are also being undertaken to complement the definition of FD. Our findings should also be revalidated in additional clinical cohorts, in particular in large cohorts homogeneously receiving *in vivo* T-cell depletion by antithymocyte globulin or posttransplant cyclophosphamide. Interestingly, a recent report on a single-center study showed the validity of the TCE-FD algorithm in a cohort of patients treated with *in vivo* T-cell depletion by antithymocyte globulin,<sup>62</sup> suggesting a predictive value of our new approach in that setting.

Recent reports have suggested an effect of differential 3' untranslated region-controlled expression levels on permissiveness of

DPB1 mismatches in the context of HCT.<sup>63,64</sup> Moreover, another model of analysis of DPB1 mismatches developed for Japanese HCT recipients and based on the evolutionary relationship between DPB1 alleles defined by the region between exon 3 and the 3'UTR has also provided evidence of increased risks for aGVHD in transplant pairs with mismatches across the 2 evolutionary allele groups: DP2 and DP5.<sup>65</sup> The TCE model and these 2 models correlate to some extent, and their mode of interaction is currently under investigation by us and other investigators.<sup>65-67</sup>

In conclusion, in this study we have demonstrated the feasibility and clinical relevance of *in silico* assignment of nonpermissive DPB1 mismatches conferring higher risks for complications after HCT. Nonpermissive TCE group mismatches can now be predicted *in silico* for any DPB1 allele by FD scores. This successful proof-of-principle experience of translation of direct FD to *in silico* prediction of clinically detrimental HLA mismatches in HCT should open new potential avenues for future development of risk prediction models including other loci of the HLA system.

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## Authorship

Contribution: P.C., K.F., S.R.S., and S.J.L. designed the study; K.W.A. and H.-L.W. performed statistical analyses; E.A.-B., P.C., and K.F. drafted the manuscript; and all authors participated in manuscript writing and review and provided final approval of the manuscript.

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