



Clinical Research

Refinement of the Definition of Permissible HLA-DPB1 Mismatches with Predicted Indirectly ReCognizable HLA-DPB1 Epitopes



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Hematopoietic stem cell transplantation with HLA-DPB1–mismatched donors leads to an increased risk of acute graft-versus-host disease (GVHD). Studies have indicated a prognostic value for classifying HLA-DPB1 mismatches based on T cell–epitope (TCE) groups. The aim of this study was to determine the contribution of indirect recognition of HLA-DP–derived epitopes, as determined with the Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE) method. We therefore conducted a retrospective single-center analysis on 80 patients transplanted with a 10/10 matched unrelated donor that was HLA-DPB1 mismatched. HLA-DPB1 mismatches that were classified as GVH nonpermissive by the TCE algorithm correlated to higher numbers of HLA class I as well as HLA class II presented PIRCHE (PIRCHE-I and -II) compared with permissive or host-versus-graft nonpermissive mismatches. Patients with acute GVHD grades II to IV presented significantly higher numbers of PIRCHE-I compared with patients without acute GVHD ($P < .05$). Patients were divided into 2 groups based on the presence or absence of PIRCHE. Patients with PIRCHE-I or -II have an increased hazard of acute GVHD when compared with patients without PIRCHE-I or -II (hazard ratio [HR], 3.19; 95% confidence interval [CI], 1.10 to 9.19; $P < .05$; and HR, 4.07; 95% CI, .97 to 17.19; $P = .06$, respectively). Patients classified as having an HLA-DPB1 permissive mismatch by the TCE model had an increased risk of acute GVHD when comparing presence of PIRCHE-I with absence of PIRCHE-I (HR, 2.96; 95% CI, .84 to 10.39; $P = .09$). We therefore conclude that the data presented in this study describe an attractive and feasible possibility to better select permissible HLA-DPB1 mismatches by including both a direct and an indirect recognition model.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) with a matched unrelated donor that is mismatched for HLA-DPB1 leads to an increased risk of acute graft-versus-host disease (GVHD) [1–6]. It is well established that certain HLA-DP mismatches more frequently lead to this effect of T cell–related alloreactivity than others [2,5,6]. HLA mismatch-induced T cell alloreactivity can be evoked via 2 routes of mismatched antigen recognition: direct and indirect recognition. Direct recognition occurs when donor T cells recognize an intact mismatched HLA molecule that is present on the cell surface of a recipient's cell. These directly recognizing T cells are likely

viral peptide-specific memory T cells showing cross-reactivity toward allogeneic HLA, due to molecular mimicry [7].

Direct recognition of HLA-DP mismatches can be predicted with the T cell–epitope (TCE) model, developed by Fleischhauer et al. [2,5]. This TCE model is based on cross-reactivity patterns of alloreactive T cells isolated from a patient that had rejected his graft [2]. In this model, HLA-DPB1 alleles of donor and recipient are divided into 3 (or 4) groups, either predicted to have high, intermediate, or low immunogenic potential. HLA-DPB1 mismatches are subsequently classified as permissive or nonpermissive based on the concept of thymic education of T cells. Mismatches are defined as permissive if they belong to the same immunogenicity group and as nonpermissive if they belong to groups with different immunogenicity. The direction of nonpermissiveness is based on the direction of immunogenicity: when the recipient has a higher TCE-assigned immunogenicity than the donor, mismatches are designated as GVH nonpermissive, whereas

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when the recipient has a lower TCE-assigned immunogenicity than the donor, the mismatch is designated as host-versus-graft (HVG) nonpermissive. Several studies have shown that HLA-DPB1 mismatches classified as nonpermissive by the TCE model are associated with both an increased risk of acute GVHD [2,5,6] and an increased risk of overall mortality [5,6].

HLA molecules can also be recognized indirectly. Indirect recognition has not been included in the TCE model. During indirect recognition, donor T cells recognize polymorphic peptides derived from the mismatched HLA molecule presented by an HLA molecule that is shared between donor and recipient. T cells specific for such polymorphic HLA-derived peptides have been found frequently during graft failure after solid organ transplantation [8–12]. Indirect recognition can be predicted *in silico* as well: Our group previously developed a model that predicts the numbers of peptides derived from mismatched HLA alleles that can be presented by shared HLA. These peptides have been designated as Predicted Indirectly Recognizable HLA Epitopes (PIRCHE) [13–15]. Increasing numbers of PIRCHE are correlated to an increased risk of alloreactivity, as reflected by increased probabilities of acute GVHD after HSCT [15] and by the development of donor-specific antibodies after kidney transplantation [13].

We hypothesize that nonpermissibility of HLA-DPB1 mismatches can, next to the TCE model, be explained by indirect recognition of the HLA-DPB1–mismatched alleles. To investigate whether PIRCHE can indeed provide an additive explanation for nonpermissibility of certain HLA-DPB1 mismatch combinations, the number of HLA-DPB1–derived PIRCHE was determined in patients transplanted with an HLA-A, -B, -C, -DRB1, -DQB1 (10/10)-matched, HLA-DPB1–mismatched unrelated donor. The numbers of PIRCHE were studied for their correlation with permissiveness as determined by the TCE model and to clinical measures of alloreactivity (acute and chronic GVHD, relapse/progression of the original disease, and transplant-related mortality [TRM]).

METHODS

Patients

All adult patients receiving a peripheral blood stem cell transplantation from a 10/10 (matched for HLA-A, -B, -C, -DRB1, -DQB1 on a 4-digit level) matched unrelated donor after nonmyeloablative conditioning between 2007 and 2012 at the University Medical Center Utrecht were included in this analysis (Supplementary Tables 1, 2, and 3). Nonmyeloablative conditioning consisted of total body irradiation of 2 Gy for 1 day, antithymocyte globulin (Genzyme, Cambridge, MA) 2 mg/kg/day for 4 days, and fludarabine 30 mg/m²/day for 3 days. Patients received an unmanipulated peripheral blood stem cell graft. Immunosuppressive therapy consisted of cyclosporine A 4.5 mg/kg twice daily until day 120, which was then tapered by a 10% dose reduction per week in the absence of GVHD. Cyclosporine A was combined with mycophenolate mofetil 15 mg/kg, 3 times a day until day 84; if there was no GVHD, this regimen was tapered and stopped in 2 weeks. All patients received antibiotic prophylaxis, including co-trimoxazole 480 mg twice a day and valacyclovir 500 mg twice a day, as previously reported [16]. Mean donor age was 36.5 years (range, 22 to 45), and disease stage before HSCT was complete remission in 32 patients (40%), partial remission in 32 patients (40%), progressive in 2 (3%), and not available for 14 patients (18%).

HLA Typing

Sequencing-based high-resolution HLA typing was performed before HSCT for all patients and donors for the HLA-A, -B, -C, -DRB1, and -DQB1 loci by PCR and sequencing using the SBTexcellerator HLA-A, -B, -C, -DRB1, and -DQB1 kits (GenDx, Utrecht, the Netherlands). Purified sequencing products were electrophoresed using a 3730 DNA analyzer (Applied Biosystems, Foster City, CA), and sequences were analyzed using SBTengine software (GenDx). All protocols were executed according to manufacturers' guidelines. All allele and genotype ambiguities at the 4-digit level were resolved, and all null alleles were excluded.

HLA-DPB1 typing was performed retrospectively using sequencing-based typing (for the primers used see Supplementary Table 4). Homozygotic typings were confirmed with sequence-specific oligonucleotide technologies (One Lambda, Canoga Park, CA). After HLA-DPB1 typing, ambiguities could not be resolved for 18 individuals (11%). For all these unresolved ambiguities, the locations of the polymorphic residues were analyzed. All polymorphisms were located outside positions affecting the immunogenicity as predicted by the TCE model and were more than 9 amino acids apart. Consequently, all allelic combinations possible with these ambiguities led to identical TCE and PIRCHE assignment. We therefore included these pairs using their ambiguous HLA-DPB1 typings.

TCE Classification

HLA-DPB1 mismatches were classified as either permissive or nonpermissive in the GVH or HVG direction, as described previously [2,5,6,17]. To this end, HLA-DPB1 typing data were entered in the online tool available for determining TCE groups (<http://www.ebi.ac.uk/ipd/imgt/hla/dpb.html> [accessed January 2013]), which uses the TCE 3-group model. For 2 donor–recipient combinations (3%), permissiveness could not be determined with this tool, because one of their HLA-DPB1 alleles was not included in the TCE model (HLA-DPB1*35:01 and DPB1*36:01).

PIRCHE Determination

PIRCHE were identified for each donor–recipient pair as described previously [14,15]. In short, for HLA class I presented PIRCHE (PIRCHE-I), processing by the proteasome and transportation via the TAP channel of the amino acid sequences of all donor and recipient HLA molecules (sequences as defined by international immunogenetics information system (IMGT): <ftp://ftp.ebi.ac.uk/pub/databases/ipd/imgt/hla> [accessed December 2012]) was predicted using NetChop C-term 3.0 [18,19]. Predicted processed nonameric peptides were tested for their binding capacity to the HLA class I molecules using NetMHCpan 2.4 [20, 21]. Peptides with IC₅₀ binding values ≤ 500 nM were accepted as relevant binders [22]. For HLA class II presented PIRCHE (PIRCHE-II), the nonameric binding cores of potential HLA-DRB1 binders (15-mers) were predicted with NetMHCIIpan 2.0 [23–25] considering IC₅₀ binding values ≤ 1000 nM as being relevant [26]. For each donor–recipient pair, only unique recipient-specific peptide–HLA complexes were counted as PIRCHE.

Statistical Analyses

The primary clinical endpoint tested was incidence of acute GVHD grades II to IV [27]. Secondary clinical endpoints were extensive or limited chronic GVHD [16], relapse/progression of the primary malignant disease (for patients transplanted for a malignant disease only, $n = 76$ (as defined by the HOVON study group [28–32])), TRM, and overall survival.

Differences in distribution of PIRCHE in the TCE-assigned permissive groups were tested with Mann–Whitney U tests comparing the 3 TCE groups (HVG nonpermissive, GVH nonpermissive, and permissive) with each other. These tests were also performed to compare the distribution of PIRCHE among clinical outcomes.

Cumulative incidence curves were constructed for primary and secondary clinical endpoints. Relapse and nonrelapse mortality were regarded as competing risks for acute GVHD, mortality related to relapse was regarded as a competing risk for chronic GVHD, TRM was regarded as a competing risk for relapse, and mortality related to relapse and mortality due to other causes were regarded as competing risks for TRM. Differences in cumulative incidences were tested with the Gray test.

The time-dependent association of TCE-assigned nonpermissiveness and numbers of PIRCHE with development of primary and secondary endpoints was tested with Cox regression analyses. Models were adjusted for (when relevant) age of the recipient at transplantation, primary disease, cytomegalovirus status, development of acute GVHD grades II to IV for chronic GVHD, and development of acute and chronic GVHD for relapse. Backward selection was used to determine which variables should be included in the model; the likelihood ratio test was used to select the relevant variables ($P < .10$). Variables were also included when they were associated with the variable of interest (either TCE classification or PIRCHE), as tested with ANOVA for the continuous variable age and chi-square for categorical variables: primary disease, gender, cytomegalovirus status, and Epstein–Barr virus status (serostatus of both recipient and donor and the match status between them).

Statistical procedures were performed with SPSS statistics software, version 20.0 (SPSS Inc, Chicago, IL), and competing risk analyses were performed with R version 3.0.0 (The R Foundation for Statistical Computing, Vienna, Austria). $P < .05$ was considered to be statistically significant.

RESULTS

Within our cohort of 88 patients transplanted with 10/10-matched unrelated donors within a uniform reduced-intensity regimen [16], 8 recipients (9%) were transplanted with an HLA-DPB1 matched donor. Of the 80 mismatched pairs, 2 pairs (3%) could not be analyzed with the classical direct recognition (TCE) model because for 1 of the alleles immunogenicity could not be predicted. Of the remaining 78 pairs (97%) with a TCE designation, 54 (69%) were classified as permissive, 12 (15%) as GVH nonpermissive, and 12 (15%) as HVG nonpermissive (Supplementary Table 1). To assess the impact of indirect recognition, the numbers of PIRCHE were determined as described in Methods. Recipients presented a median of 1 PIRCHE-I (range, 0 to 7) and 3.5 PIRCHE-II (range, 0 to 20).

To correlate the presence of PIRCHE with clinical outcome, donor–recipient pairs were divided into 2 PIRCHE groups, according to the absence or presence of PIRCHE. Baseline characteristics were evenly distributed among the TCE groups and the absence or presence of PIRCHE, apart from age at HSCT (patients with GVH nonpermissive mismatches were younger than those with HVG nonpermissive or permissive mismatches; Supplementary Tables 1, 2, and 3). Models testing TCE-assigned permissiveness were therefore corrected for age at HSCT.

Correlation of the TCE Model with Acute GVHD

The correlation between direct recognition as predicted with the TCE groups and acute GVHD was investigated with univariate cumulative incidence analyses and in multivariate Cox regression models. TCE permissive mismatches displayed the lowest incidence of acute GVHD, although the cumulative incidences were not significantly different among the 3 TCE groups and not when comparing permissive with GVH and HVG nonpermissive combined (Figure 1). In multivariate analyses, which were corrected for age of the recipient, TCE groups were not significantly associated with acute GVHD (Table 1).

Correlation of the PIRCHE Model with Acute GVHD

The influence of indirect recognition on acute GVHD was assessed by correlating numbers of PIRCHE to the incidence of acute GVHD grades II to IV. Recipients with acute GVHD grades II to IV presented significantly higher numbers of PIRCHE-I ($P = .05$) but not of PIRCHE-II (Figure 2A,B). When analyzing the presence of PIRCHE, PIRCHE-I and -II were significantly associated with increased incidences of acute GVHD compared with recipients without PIRCHE ($P = .01$ and $P = .04$, respectively; Figure 2C,D). In Cox regression analyses, the presence of PIRCHE-I was associated with an increased hazard of acute GVHD compared with absence (hazard ratio [HR], 3.19; 95% confidence interval [CI], 1.10 to 9.19; $P = .03$). For PIRCHE-II there was a similar trend (HR, 4.07; 95% CI, .97 to 17.19; $P = .06$; Table 1). Thus, the presence of PIRCHE is correlated with increased acute GVHD risks.

Comparing the TCE and PIRCHE Models

To test whether the sensitivity of the direct recognition (TCE) and indirect recognition (PIRCHE) models differ, receiver operator characteristic curves were constructed and the areas under the curve (AUC) calculated as a measure of predictive capacity. Only PIRCHE-I significantly predicted acute GVHD (TCE AUC, .586, $P = .21$; PIRCHE-I AUC, .641, $P = .04$; PIRCHE-II AUC, .580, $P = .25$).

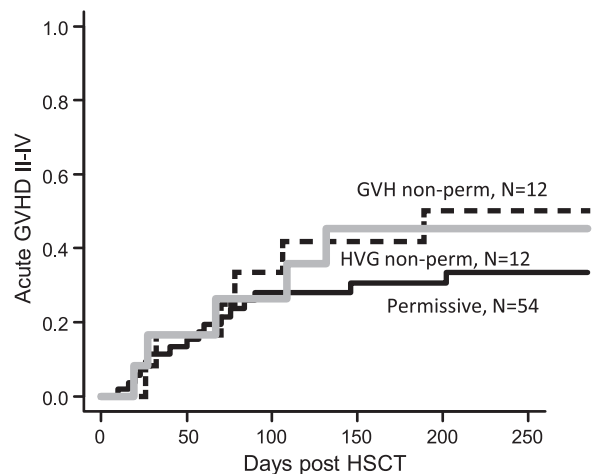


Figure 1. Cumulative acute GVHD incidence curves for the 3 TCE classifications. Although HVG (gray line) and GVH (dashed line) nonpermissive mismatches have a higher incidence of acute GVHD than permissive (black solid line) mismatches, this was not significantly different. Non-perm indicates nonpermissive.

To investigate the relationship between the TCE and PIRCHE model, the correlation between the TCE groups and numbers of PIRCHE was analyzed. Pairs with TCE-predicted GVH nonpermissive mismatches had higher numbers of PIRCHE-I when compared with both permissive or HVG nonpermissive mismatches ($P < .01$). There was a nonsignificant increase in numbers of PIRCHE-II when comparing GVH nonpermissive with permissive mismatches; however, PIRCHE-II was significantly higher in the GVH nonpermissive group when compared with HVG nonpermissive ($P < .01$) and significantly lower in the HVG nonpermissive compared with the permissive group ($P = .03$). When analyzing the presence of PIRCHE, the TCE permissive group contained 22 pairs (41%) without PIRCHE-I, 12 pairs (22%) without PIRCHE-II, and 32 (59%) and 42 pairs (78%) with PIRCHE-I or PIRCHE-II, respectively. The TCE and PIRCHE classification are thus correlated, although the PIRCHE model designates mismatches as nonpermissible when they are permissive according to the TCE model.

To study whether the PIRCHE model can further define HLA-DP mismatches, the correlation of PIRCHE with acute GVHD within the TCE permissive mismatches was studied.

Table 1
Association of the TCE and PIRCHE Models with Acute GVHD

Variable	HR	95% CI	P
TCE model			
GVH nonpermissive*	1.82	.68–4.87	NS
HVG nonpermissive*	1.37	.50–3.76	NS
PIRCHE-I			
PIRCHE-I present	3.19	1.10–9.19	<.05
PIRCHE-II			
PIRCHE-II present	4.07	.97–17.19	NS
PIRCHE-I within TCE permissive			
PIRCHE-I present	2.96	.84–10.39	NS

Hazard ratios of the different TCE and PIRCHE groups as determined with Cox regression analyses. Presence of PIRCHE-I was significantly associated with an increased hazard of acute GVHD compared with absence of PIRCHE-I. There was a trend for an increased hazard of acute GVHD for presence compared with absence of PIRCHE-II. Within the TCE permissive group, presence of PIRCHE-I was associated with an increased hazard of acute GVHD compared with absence PIRCHE-I. NS indicates not significant.

* Reference: permissive mismatches.

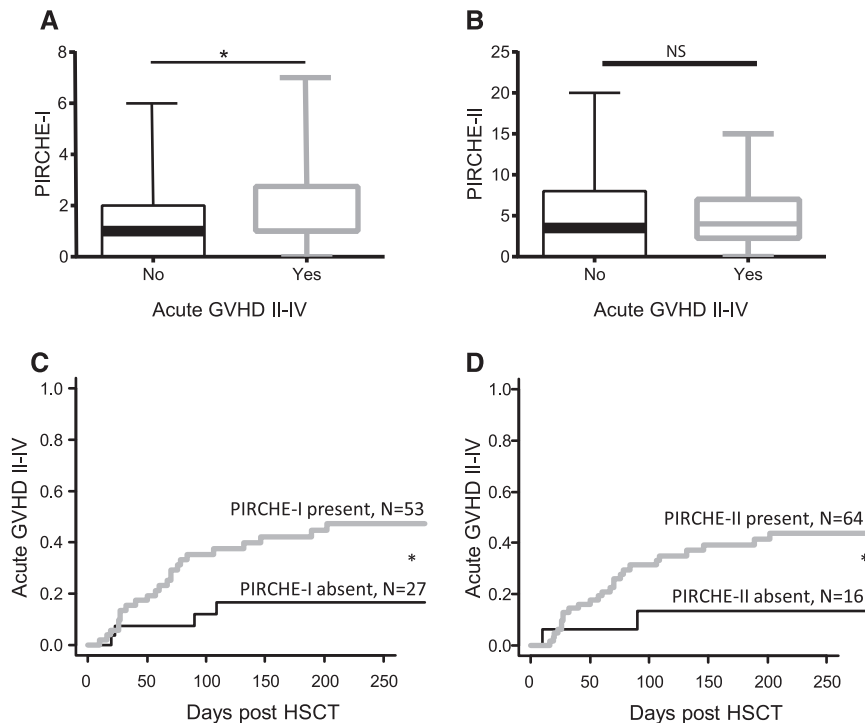


Figure 2. Boxplots showing the correlation between numbers of PIRCHE-I and acute GVHD (A) and the correlation between numbers of PIRCHE-II and acute GVHD (B). Boxes represent the 25th to 75th percentile, the horizontal lines the median, and the whiskers all PIRCHE values from minimum to maximum. (C) Cumulative acute GVHD incidence curves for the absence and presence of PIRCHE-I. (D) Cumulative acute GVHD incidence curves for the absence and presence of PIRCHE-II. Recipients developing acute GVHD presented significantly higher numbers of PIRCHE-I but not of PIRCHE-II. Cumulative incidence of acute GVHD was significantly increased for recipients presenting PIRCHE-I (gray line), compared with those without PIRCHE-I (solid line), similar for PIRCHE-II. * $P < .05$.

Within the TCE permissive mismatches, recipients without PIRCHE-I had a lower risk of acute GVHD when compared with recipients with PIRCHE-I (HR, 2.96; 95% CI, .84 to 10.39; $P = .09$; Figure 3, Table 1). This effect was not observed for PIRCHE-II. Of the 16 recipients (30%) who developed acute GVHD despite the permissive TCE classification, 13 (81%) have PIRCHE-I and are thus more adequately classified by PIRCHE-I. A similar strategy could not be performed for the TCE nonpermissive group, because all recipients with GVH nonpermissive mismatches have PIRCHE-I and -II, and there were few patients without PIRCHE-I or -II in the HVG nonpermissive group (5 and 4, respectively).

Secondary Endpoints

The TCE and PIRCHE models were studied for their correlation with other clinical outcomes. Neither TCE nonpermissiveness nor PIRCHE were associated with an increased hazard of chronic GVHD in multivariate models corrected for cytomegalovirus, recipient age, and acute GVHD development (data not shown). Neither the TCE classification nor the presence of PIRCHE were significantly associated with TRM in our cohort in multivariate models that were corrected for the primary disease group and age of the recipient (data not shown). In multivariate models, corrected for age of the recipient and primary disease, neither TCE nonpermissiveness nor the presence of PIRCHE was associated with overall survival (data not shown).

The TCE model correlates to relapse; HVG nonpermissive mismatches lead to a significantly increased incidence of relapse when compared with GVH nonpermissive mismatches or permissive mismatches ($P = .03$ and $P < .001$, respectively). In multivariate analyses, which were corrected for the development of chronic GVHD and age of the

recipient, HVG nonpermissive mismatches were clearly associated with an increased hazard of relapse when compared with permissive mismatches (HR, 3.71; 95% CI, 1.50 to 9.21; $P < .01$). PIRCHE were not correlated to relapse, neither in univariate nor in multivariate analyses. To summarize, TCE-assigned HVG nonpermissive mismatches are correlated with an increased relapse risk, whereas PIRCHE do not correlate with other endpoints than acute GVHD.

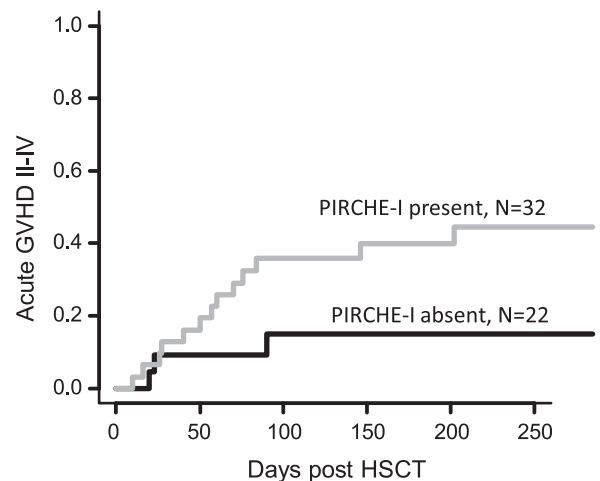


Figure 3. Cumulative acute GVHD incidence curves for the absence and presence of PIRCHE-I within the TCE permissive group. Within the TCE permissive group, recipients presenting PIRCHE-I (gray line) have an increased probability ($P = .06$) of acute GVHD compared with those without PIRCHE-I (solid line).

DISCUSSION

HLA-DP mismatches are correlated to alloreactivity after HSCT [1-6,33-36]. It is recognized that directly recognizable HLA-DPB1 mismatches, as predicted with the TCE model, correlate with inferior outcomes after HSCT [2,5,6,33]. The present study was initiated to investigate whether indirectly recognizable HLA-DPB1 mismatches predict nonpermissible mismatches, next to the TCE model. In our local cohort of 10/10-matched unrelated stem cell transplants, predicted indirectly recognizable HLA-DPB1 mismatches, as determined via the PIRCHE concept, indeed correlate with acute GVHD. Moreover, our observations indicate that the PIRCHE model is able to further define HLA-DP permissibility within the TCE permissive group.

Despite a clear association between the TCE and PIRCHE predictions, the PIRCHE model appears to more adequately classify HLA-DPB1 mismatches as permissible and non-permissible *in vivo*. These PIRCHE-predicted improvements are likely explained by the lengths of the polymorphic parts of the HLA-DPB1 alleles that are considered in both models. The TCE model is based on polymorphisms within the peptide binding groove and T cell receptor contact regions only: the 6 hypervariable regions of the alpha-1 domain encoded by exon 2 [2]. However, PIRCHE can also be derived from polymorphic regions outside the peptide binding groove. Inclusion of these polymorphic regions apparently enhances the predictive capacity of the PIRCHE model when compared with the TCE model. Inclusion of polymorphisms outside exon 2 in an indirect model for antigen recognition is justified by peptide elution data; DP-derived peptides encoded by exon 1 have been frequently eluted from HLA (SYFPEITHI database [<http://www.syfpeithi.de/bin/MHCServer.dll/FindYourMotif.htm>], accessed May 2014). Because the PIRCHE model considers a greater part of the HLA-DP alleles, it is a more stringent model, consequently predicting clinically relevant nonpermissibility at a higher frequency.

Investigating the interaction between the classical direct recognition (TCE) model and our here newly proposed indirect recognition (PIRCHE) model is of interest, because the 2 models theoretically aim to predict 2 different routes of DP-antigen recognition. One could speculate that these 2 immune responses are complementary or even enhance each other *in vivo*. Hence, additive or even synergistic effects may be expected. However, because of the strong correlation between the TCE and PIRCHE model in this study and the small cohort size, interactions between the 2 models are difficult to test. Observations in the current study may even implicate that the PIRCHE model provides an explanation for the previous findings of the TCE model, because all patients with a GVH nonpermissive TCE mismatch had PIRCHE-I and -II. Future studies conducted on larger patient cohorts allow studying the interactions between nonpermissible mismatches according to the TCE model and presence of PIRCHE and will elucidate what the predictive capacity of the combination of these 2 models is or even if they truly predict independently.

Both the TCE and PIRCHE model have some practical constraints. The TCE model is restricted by the fact that cellular recognition patterns have not been determined for all HLA-DP alleles. In our cohort, we could therefore not predict permissiveness in 2 cases. To resolve this problem, it is necessary to expand the immunogenicity predictions to not yet tested HLA-DPB1 alleles, possibly *in silico*. The PIRCHE model is limited by the requirement of full exon 1 to 6 sequences, because PIRCHE can also be derived from regions outside exon 2. In the present study, the amino acid

sequences of 4 HLA-DPB1 alleles (HLA-DPB1*20:01, 33:01, 36:01, 138:01) present in 10 individuals (6%) could only partly be included in the PIRCHE model. We therefore may have over- or underestimated the numbers of PIRCHE for these pairs. Our study underlines the need for submission of complete exon 1 to 6 sequences to the IMGT database to prevent these gaps in the sequence knowledge. Such data will allow more concise studying of the role of PIRCHE in clinical outcomes.

Furthermore, the current study only predicts nonameric PIRCHE-I. Although HLA class I molecules can bind peptides of other lengths, the current HLA binding predictors are most reliable for nonameric peptides. Future improvements on binding predictions for non-nonameric peptides may facilitate studies on the effect of these non-nonameric PIRCHE-I.

The current study excludes HLA-DPA1-derived PIRCHE. It is highly likely that many of the HLA-DPB1-mismatched pairs have an additional HLA-DPA1 mismatch, which could lead to potential PIRCHE. Future studies should also include analyzing the effect of HLA-DPA1-derived PIRCHE, which may lead to further improvement of the predictive potential.

Mismatching for HLA-DP may lead to increased graft-versus-leukemia effects, because HLA-DP is preferentially expressed on the hematopoietic cell lineage [37]. Theoretically, high numbers of DP-derived PIRCHE as well as TCE nonpermissible mismatches may lead to such an increased antitumor effect. In the present study, we did not find a reduced relapse risk in patients with an increased chance of alloreactivity targeting the HLA-DP mismatch (ie, TCE GVH nonpermissive or high PIRCHE). In that perspective, it is noteworthy that HLA-DP is expressed on the vast majority of leukemic cells, but with considerable variety. For example, HLA-DP expression is lower on AML than on B-ALL or B-CLL cells [37]. Most acute leukemia patients in our study (82%) suffered from acute myelogenous leukemia, a low HLA-DP expressing leukemia. Research in specific disease subgroups is warranted to analyze the antitumor effect of TCE nonpermissiveness and of high numbers of DP-derived PIRCHE to correct for differences in HLA-DP expression levels.

To conclude, our data show a correlation between indirectly recognizable HLA-DP mismatches, as determined with the PIRCHE model, and acute GVHD. These data might be useful to complement currently used models for direct recognition to provide a superior donor selection. Extended studies on large cohorts are essential to refine the integration of these 2 models in donor-selection procedures, both with respect to acute GVHD and graft-versus-leukemia effects, and longer term effects like chronic GVHD and TRM.

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Financial disclosure: E.S. is mentioned as an inventor on the patent titled "Method for prediction of an immune response against mismatched human leukocyte antigens"; number: WO 2014072467 A1.

Conflict of interest statement: The University Medical Center Utrecht has filed a patent application on the prediction of an alloimmune response against mismatched HLA.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.bbmt.2014.06.026>.

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