Risk-associations between HLA-DPB1 T cell epitope matching and outcome of unrelated hematopoietic cell transplantation are independent from HLA-DPA1

Katharina Fleischhauer[1,2], **Marcelo A. Fernandez-Viña**[3], **Tao Wang**[4], **Michael Haagenson**[5], **Minoo Battiwalla**[6], **Lee Ann Baxter-Lowe**[6,7], **Fabio Ciceri**[8], **Jason Dehn**[9], **James Gajewski**[10], **Gregory A. Hale**[10], **Martin BA Heemskerk**[21], **Susana R. Marino**[13], **Philip L. McCarthy**[3,4], **David Miklos**[15], **Machteld Oudshoorn**[16], **Marilyn S. Pollack**[17], **Vijay Reddy**[18], **David Senitzer**[19], **Bronwen E. Shaw**[20], **Edmund K. Waller**[21], **Stephanie J. Lee**[22], and **Stephen R. Spellman**[5]

[1]Institute of Experimental Cellular Therapy, Essen University Hospital, Essen, Germany [2]Unit of Molecular and Functional Immunogenetics, San Raffaele Scientific Institute, Milan, Italy [3]Histocompatibility, Immunogenetics, and Disease Profiling Laboratory, Stanford University Medical School, Stanford, CA, USA [4]Division of Biostatistics, Medical College of Wisconsin, Milwaukee, WI, USA [5]Center for International Blood and Marrow Transplant Research, Minneapolis, MN, USA [6]National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA [7]Immunogentics and Transplantation Lab, University of California San Francisco Medical Center, San Francisco, CA, USA [8]Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milan, Italy [9]National Marrow Donor Program, Minneapolis, MN, USA [10]Oregon Health and Science University, Portland, OR, USA [11]Pediatric Hematology/Oncology/BMT, All Children's Hospital, St Petersburg, FL, USA [12]Dutch Transplant Foundation, Leiden, Netherlands [13]Department of Pathology, University of Chicago Hospitals, Chicago, IL, USA [14]Blood and Marrow Transplant, Roswell Park Cancer Institute, Buffalo, NY, USA [15]Department of Medicine/Bone Marrow Transplant, Stanford Hospitals and Clinics, Stanford, CA, USA [16]Europdonor Foundation, Leiden University Medical Center, Leiden, Netherlands [17]Department of Pathology, University of Texas Health Care System, Veterans

Correspondence to: Katharina Fleischhauer, MD, Institute for Experimental Cellular Therapy, Essen University Hospital, Hufelandstrasse 55, 45122 Essen, Germany, katharina.fleischhauer@uk-essen.de, Tel: +49 201 723 4582 Fax: +49 201 723 4546, Unit of Molecular and Functional Immunogenetics, San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy, fleischhauer.katharina@hsr.it, Tel : +39 02 2643 4341 Fax : +39 02 2643 6198.

The online version of the article contains a data supplement.

Health Care System [18]Department of Hemaotology/Oncology, University of Florida, Gainesville, FL, USA [19]Hematology/Hematopoietic Cell Transplant, City of Hope National Medical Center, Duarte, CA, USA [209]Anthony Nolan Research Institute, London, UK [21]Bone Marrow and Stem Cell Transplant Center, Emory University Hospital, Atlanta, GA, USA [22]Clinical Transplant Research, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Abstract

HLA-DP antigens are beta-alpha heterodimers encoded by polymorphic HLA-DPB1 and -DPA1 alleles, respectively, in strong linkage disequilibrium (LD) with each other. Non-permissive unrelated donor (UD)-recipient HLA-DPB1 mismatches across three different T cell epitope (TCE) groups are associated with increased mortality after hematopoietic cell transplantation (HCT), but the role of HLA-DPA1 is unclear. We studied 1281 onco-hematologic patients after 10/10 HLA-matched UD-HCT facilitated by the National Marrow Donor Program. Non-permissive mismatches defined solely by HLA-DPB1 TCE groups were associated with significantly higher risks of treatment-related mortality compared to permissive mismatches (HR 1.30, CI 1.06–1.53; p=0.009) or allele matches. Moreover, non-permissive HLA-DPB1 TCE group mismatches in the graft versus host (GvH) direction significantly decreased the risk of relapse compared to permissive mismatches (HR 0.55, CI 0.37–0.80; p=0.002) or allele matches. Splitting each group into HLA-DPA1*02:01 positive or negative, in frequent LD with HLA-DPB1 alleles from two of the three TCE groups, or into HLA-DPA1 matched or mismatched, did not significantly alter the observed risk associations. Our findings suggest that the effects of clinically non-permissive HLA-DPB1 TCE group mismatches are independent of HLA-DPA1, and that selection of donors with non-permissive DPB1 TCE mismatches in GvH direction might provide some protection from disease recurrence.

Keywords

Non-permissive mismatch; HLA-DPB1 T cell epitope matching; Unrelated HCT; relapse; transplant related mortality; HLA-DPA1

Introduction

HLA-DP antigens are heterodimers formed by a polymorphic alpha and a polymorphic beta chain encoded by the HLA-DPA1 and -DPB1 loci in the class II region of the major histocompatibility complex (MHC), respectively. With 248 currently described alleles, the HLA-DPB1 locus displays considerably higher polymorphism than the HLA-DPA1 locus, for which only 37 different alleles have been reported so far. Of these, only 12 HLA-DPB1 and 3 -DPA1 alleles are found with a frequency of over 1% in Caucasians.[1] The effective HLA-DP antigen polymorphism is further limited by tight linkage disequilibrium (LD) between HLA-DPA1 and -DPB1, with most HLA-DPB1 alleles found in *cis* with either HLA-DPA1*01:03 or -DPA1*02:01. However, potential heterodimer pairing between *trans*-encoded alpha and beta chains can increase phenotypic variability, depending on the status of heterozygosity.

In unrelated hematopoietic stem cell transplantation (HCT), donor-recipient allelic HLA-DPB1 disparity occurs in approximately 80% of pairs matched for 10/10 of the non-DP HLA loci (HLA-A, -B, -C, -DRB1 and –DQB1), due to a lack of consideration in donor selection and low LD between HLA-DP and the other HLA class II loci. Donor T cell alloreactivity against allelic HLA-DPB1 mismatches in the recipient is a significant protective factor against leukemia relapse,[2–3] however, overall mortality (OM) is not improved due to concomitantly increased acute graft versus host disease (aGvHD).[4–5] An innovative approach to defining "non-permissive", i.e. clinically poorly tolerated HLA-DPB1 mismatches on the basis of alloreactive T cell epitope (TCE) groups, was recently shown to identify subgroups of patients with significant risks of aGvHD and transplant-related mortality.[6–8] In this model, HLA-DPB1 alleles are classified into three distinct TCE groups on the basis of T cell crossreactivity patterns. The discrimination of permissive and non-permissive HLA-DPB1 mismatches is made based on whether the donor and recipient alleles are from the same (permissive) or different (non-permissive) TCE groups. The model is based on the rationale that the alloreactive T cell repertoire is shaped by thymic selection, leading to central deletion of clones reactive to self-epitopes. TCE group matching therefore considers not only the mismatch itself, but also the second DPB1 allele carried by patient or donor, which may contribute to the final repertoire of alloreactive T cells. A potential limitation of this model is that there may be differences in the magnitude of the functional effect of mismatches involving TCE groups 1 and 2 compared to 1 and 3, or 2 and 3, all considered as non-permissive. Such subtle differences are difficult to investigate due to statistical power limitations arising from the numerous subgroups involved in such an analysis. A web-based tool for application of the algorithm in unrelated donor searches has recently been made available through the IMGT-HLA database (http://www.ebi.ac.uk/ipd/imgt/hla/dpb.html).[9]

An unanswered and clinically relevant question is whether or not HLA-DPA1 polymorphism contributes to the immunogenicity of non-permissive HLA-DPB1 mismatches. Interestingly, most HLA-DPB1 alleles belonging to two of the three distinct TCE groups, the highest-risk group containing HLA-DPB1*09:01, *10:01, *14:01, *17:01 and 45:01, that are in LD with HLA-DPA1*02:01, while most frequent alleles from the third, lowest-risk TCE group including HLA-DPB1*02:01, *04:01 and *04:02 that are in LD with HLA-DPA1*01:03. This raises the possibility that the presence of HLA-DPA1*02:01, encoded either in *cis* or in *trans* with a non-permissive HLA-DPB1 TCE high-risk group mismatch, might be necessary for the formation of the relevant allo-epitope. Alternatively, donor-recipient allelic HLA-DPA1 disparity recognized by a TCR expressed on donor T-cells might be required to reveal the immunogenicity of non-permissive HLA-DPB1 TCE group mismatches. In the present study, we have tested both hypotheses by retrospective clinical analysis of 1281 10/10 HLA-matched unrelated HCTs facilitated through the National Marrow Donor Program (NMDP).

Methods

Data source

The Center for International Blood and Marrow Transplant Research (CIBMTR) is a research affiliation of the International Bone Marrow Transplant Registry (IBMTR), Autologous Blood and Marrow Transplant Registry (ABMTR) and the NMDP established in 2004 that comprises a voluntary working group of more than 450 transplantation centers worldwide that contribute detailed data on consecutive allogeneic and autologous HCT to a Statistical Center at the Medical College of Wisconsin in Milwaukee and the NMDP Coordinating Center in Minneapolis. Participating centers are required to report all transplants consecutively; compliance is monitored by on-site audits. Patients are followed longitudinally. Computerized checks for discrepancies, physicians' review of submitted data and on-site audits of participating centers ensure data quality. Observational studies conducted by the CIBMTR are performed in compliance with all applicable federal regulations pertaining to the protection of human research participants. Protected Health Information used in the performance of such research is collected and maintained in CIBMTR's capacity as a Public Health Authority under the HIPAA Privacy Rule.

Study population

The study included 1281 patients who received a first marrow or peripheral blood stem cell unrelated donor transplantation between 1988 and 2003, for the treatment of hematologic malignancies. The clinical and immunogenetic characteristics of the patients and transplants are detailed in Table 1. The cohort included all those patients and donors for whom complete HLA typing data, including HLA-DPA1 and DPB1, were available through the CIBMTR.

Surviving patients who did not provide signed, informed consent to allow analysis of their clinical data or HLA typing of stored NMDP Research Repository samples were excluded. All surviving recipients included in this analysis were retrospectively contacted and provided informed consent for participation in the NMDP research program. To adjust for the potential bias introduced by exclusion of non-consenting surviving patients, a modeling process randomly excluded the same percentage of deceased patients using a biased coin randomization with exclusion probabilities based on characteristics associated with not providing consent for use of the data in survivors.[10] This procedure is standard for CIBMTR analyses to avoid bias from the retrospective consent process.

Only patients with high resolution typing of HLA-A, B, C, DRB1, DQB1, DPA1 and DPB1, verified using appropriate DNA-based methods as previously described,[11] were included in the study. All cases were high resolution matched for HLA-A, B, C, DRB1 and DQB1 (10/10 allele matched). Cases were divided by HLA-DPB1 type into three TCE categories: HLA-DPB1 matched, permissively HLA-DPB1 mismatched (TCE matched) and non-permissively HLA-DPB1 mismatched (TCE mismatched), according to the three-group model (TCE3) as previously described.[6] HLA-DPA1 was classified by the presence or absence of HLA-DPA1*02:01, or as matched and mismatched at the allele level (Figure 1).

Clinical Outcomes

The primary outcomes were overall survival, transplant related mortality, relapse and acute GVHD. Overall survival (OS) considered death from any cause as the event and surviving patients were censored at the date of last contact. Treatment-related mortality (TRM) was defined as death in continuous remission of the primary disease with relapse as a competing risk. Relapse was defined as disease recurrence with death in continuous complete remission as a competing risk. Acute GVHD was assigned using the IBMTR consensus criteria with death as a competing risk.

Statistical methods

To compare pre-transplant characteristics for discrete factors, the number of cases and their respective percentages were calculated and chi-square tests were performed to compare the three histocompatibility groups; HLA-DPB1 matched, permissive mismatched and non-permissive mismatched. For continuous factors, the medians and ranges were calculated and the Kruskal-Wallis test was used to analyze differences between the groups. Probabilities of overall survival were calculated using the Kaplan-Meier estimator. Estimated cumulative incidence was used to describe the probabilities for events with competing risks. These included GVHD, relapse and TRM. Comparisons of survival curves were done with the log-rank test.

Multivariate analyses were performed using the Cox proportional hazards model, evaluating each HLA-DP matching algorithm (Figure 1) 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 adjusted through stratification. A stepwise model building procedure was used to select risk factors for each outcome with a threshold of p 0.05 for entering into the models. Due to multiple comparisons, p <0.01 was used to determine statistical significance for the main effect. All analyses were performed using SAS version 9.2 (SAS Institute, Inc).

Results

Study Population and Outcomes

A total of 1281 recipients of fully HLA-A, B, C, DRB1, DQB1, DPA1, DPB1 high resolution typed UD-HCTs were included in the analysis. The HLA-DPA1 and –DPB1 alleles found in this study population are listed in supplementary Table 1. All pairs were matched for HLA-A, B, C, DRB1 and DQB1 alleles (10/10), and 239 of them (19%) were also HLA-DPB1 allele matched (12/12), while the remaining 1042 (81%) pairs were HLA-DPB1 allele mismatched. In the latter group, permissive and non-permissive mismatches were determined using the three-group TCE3 algorithm previously described,[6] resulting in 585 permissively mismatched and 457 non-permissively mismatched cases (Table 1). Of the latter, 226 and 231 were mismatched in the Graft versus Host (GvH) or Host versus Graft (HvG) direction, respectively. HLA-DPA1 allele mismatches were present in 512 (40%) of the 1281 pairs overall, and in 502 (48.2%) of the 1042 HLA-DPB1 allele mismatched pairs. The HLA-DPB1 allele matched cohort included more CML cases (43%) and fewer ALL

cases (16%) than the permissive (32%-CML, 23%-ALL) and non-permissive groups (33%-CML, 23% ALL), and the groups differed slightly for GVHD prophylaxis regimens. All other characteristics were well balanced across the groups (Table 1).

Over 90% of the patients under study were of Caucasian origin and 87% received bone marrow as the stem cell source. 1159 (90%) of the transplants were part of the International Histocompatibility Working Group (IHWG) cohort previously analyzed for the effects of HLA-DPB1 TCE matching in unrelated HCT,[6] while 122 (10%) were new cases.

The overall incidence of the four major clinical endpoints of this study were OM 63% at 5 years, TRM 42% at 5 years, severe grade C-D aGvHD 32% at 100 days, and relapse 25% at 5 years with a median follow-up time of 102 months.

Algorithms for the role of HLA-DPA1 in non-permissive mismatches

In order to investigate the role of HLA-DPA1 in associations between clinical risks and non-permissive HLA-DPB1 TCE group mismatches, two different algorithms were analyzed separately. The first, designated DPB1 TCE3/DPA1*02:01, was evaluated due to the observed strong LD between alleles from 2 of the 3 HLA-DPB1 TCE groups and HLA-DPA1*02:01,[1] and predicts the presence of HLA-DPA1*02:01 in the patient, the donor or both as a necessary requirement for the assignment of a non-permissive HLA-DPB1 mismatch (Figure 1). The second, designated DPB1 TCE3/DPA1 allele, is based on the hypothesis that allelic mismatches for HLA-DPA1 are a necessary requirement for a HLA-DPB1 TCE3 group mismatch to be classified as non-permissive (Figure 1). In the two algorithms, the number of permissive/non-permissive mismatched cases was 768/274 and 804/238, respectively (Table 1).

Clinical risk associations of non-permissive HLA-DPB1 TCE group mismatches

We first investigated the associations between clinical endpoints of UD-HCT and non-permissive HLA-DPB1 TCE3 group mismatches as previously described[6], i.e. without consideration of HLA-DPA1 (Figure 1). Consistent with our previous results, we found a significant correlation between non-permissive HLA-DPB1 mismatches in either the GvH or HvG direction and TRM (Table 2 and Figure 2A). Increased risks were also observed for OM and severe aGvHD, with HR similar to those observed in our previous report[6], although these were not statistically significant according to the definitions from the present study (Table 2 and Figure 2B, C). Also in line with previous observations, HLA-DPB1 allele matched transplants were associated with an increased risk of relapse compared to permissive or non-permissive transplants in univariate analysis (Figure 2D), but did not reach statistical significance when adjusted for significant co-variates (Table 2).

The risk stratifications for the endpoints TRM and aGvHD were evenly distributed between non-permissive mismatches in the GvH or HvG direction (Table 2) which, again, is in line with previous results. Interestingly however, the risks of relapse were significantly lower after transplantation from a donor non-permissively mismatched in the GvH but not in the HvG direction, compared to transplants from a permissively mismatched donor (Table 2 and Figure 2E). However, we did not find a significantly lower risk of OM associated with non-permissive GvH compared to HvG mismatches (Table 2).

Similar results were obtained for the four-group model of HLA-DPB1 TCE4 group matching previously described (data not shown).[8]

Role of HLA-DPA1 in non-permissive HLA-DP TCE group mismatches

In order to investigate the impact of HLA-DPA1 polymorphism on non-permissive HLA-DP mismatches, we further stratified HLA-DPB1 allele matched and permissively or non-permissively mismatched groups according to two algorithms of HLA-DPA1 matching status as described above (Figure 1). Neither the presence or absence of HLA-DPA1*02 in the patient or the donor, nor the presence or absence of HLA-DPA1 allele mismatches between patient and donor, when added as a requirement in addition to HLA-DPB1 TCE3 group disparity in the definition of non-permissive HLA-DP mismatches, had a significant impact on the risks of TRM (Figure 3A, B) or any of the other clinical endpoints (Tables 3 and 4). Similar results were obtained for the four-group model of HLA-DPB1 TCE4 group matching previously described (data not shown).[8] Interestingly, when the HLA-DPB1 allele mismatched group was considered as a whole (i.e. HLA-DPB1 permissive and non-permissive combined), the presence of an allelic HLA-DPA1 mismatch was associated with a reduced relapse risk which was significant in univariate analysis (p= 0.02) but not after adjustment for disease type and status at transplantation (HR 0.73; 95%CI 5.56–0.94, p=0.016). This trend was similar in the HLA-DPB1 permissive (HR 0.74; 95%CI 0.54–1.04; p=0.08; Table 4) and in the non-permissive group (HR 0.74; 95%CI 0.49–1.13; p=0.16).

Discussion

The present study is the first to address the influence of HLA-DPA1 matching in the context of the HLA-DPB1 TCE group model. The results show that the presence of HLA-DPA1*02:01 does not appear to be necessary for the formation of immunogenic, clinically non-permissive HLA-DPB1 TCE group mismatches. Likewise, allelic donor-recipient HLA-DPA1 disparity does not seem to be required for that purpose. The HLA-DP alpha chains encoded by HLA-DPA1*01:03 and DPA1*02:01 differ by three amino acids at positions 31, 50 and 83 in the peptide antigen binding groove which potentially could have an effect on peptide presentation and/or T cell receptor recognition. Accordingly, we found that a number of T cells alloreactive to HLA-DPB1 encoded antigens display a preference for HLA-DPB1 heterodimers with either HLA-DPA1*01:03 or DPA1*02:01, as shown by transfection experiments of the relevant DPB1 coding sequence into HLA-DPA1 homozygous B lymphoblastoid cell lines[12]. In line with this experimental evidence, we found that allelic HLA-DPA1 mismatches appeared to increase the well-known protective effect of HLA-DPB1 disparity on relapse. This was shown by a suggestive, though not statistically significant trend in multivariate analysis, towards reduced hazards of relapse in the presence of combined HLA-DPB1-DPA1 allelic mismatches, compared to HLA-DPB1 mismatches but –DPA1 matches. This effect was similar in the HLA-DPB1 permissive and non-permissive transplants after separate analysis, suggesting that the relevant mismatch is indeed at the HLA-DPA1 level. This association may have gone undetected in previous studies which compared risk-associations between HLA-DPA1 matched or mismatched transplants regardless of the HLA-DPB1 matching status[13–18], due to the confounding effect of concomitant HLA-DPB1 mismatches in these analyses. Based on these findings, HLA-

DPA1 typing in unrelated stem cell donor searches might be considered for patients at high risk of relapse, a notion that is in accordance also with recommendations from a previous report.[18]

The results from this study confirm our previous findings regarding the associations of permissive or non-permissive HLA-DPB1 TCE mismatches with clinical outcome of HCT.[6] In fact, the HR for all clinical endpoints – OM, TRM, aGvHD and Relapse – observed here are similar to those observed in the IHWG transplant cohort, although not all were statistically significant according to the statistical definitions of the present study, likely reflecting lower statistical power. It should be noted that 90.5% of the patients analyzed here overlap with the IHWG cohort that included a large number of transplants coordinated by the Japanese Marrow Donor Program. Interestingly, the reduced risk of leukemia relapse associated with non-permissive HLA-DPB1 TCE group mismatches was more marked in the present study (HR 0.55; p=0.002) than in the IHWG report (HR 0.80; p=0.02)[6] and highly significant, possibly reflecting greater disease type homogeneity for AML and MDS patients. This observation could be relevant for the selection of stem cell donors for high risk AML patients who might benefit from GvL mediated by T cells alloreactive to non-permissive DPB1 TCE mismatches, in particular if in correlation with allelic HLA-DPA1 disparity. Additional prospective analyses of independent transplant cohorts are warranted to better investigate this important point.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

References

1. Hollenbach JA, Madbouly A, Gragert L, et al. A combined DPA1-DPB1 amino acid epitope is the primary unit of selection on the HLA-DP heterodimer. Immunogenetics. 2012; 64(8):559–569. [PubMed: 22526601]

2. Rutten CE, van Luxemburg-Heijs SA, Griffioen M, et al. HLA-DP as specific target for cellular immunotherapy in HLA class II-expressing B-cell leukemia. Leukemia. 2008; 22(7):1387–1394. [PubMed: 18418406]

3. Rutten CE, van Luxemburg-Heijs SA, Halkes CJ, et al. Patient HLA-DP-specific CD4+ T cells from HLA-DPB1-mismatched donor lymphocyte infusion can induce graft-versus-leukemia reactivity in the presence or absence of graft-versus-host disease. Biol Blood Marrow Transplant. 2013; 19(1): 40–48. [PubMed: 22871556]

4. Shaw BE, Gooley TA, Malkki M, et al. The importance of HLA-DPB1 in unrelated donor hematopoietic cell transplantation. Blood. 2007; 110(13):4560–4566. [PubMed: 17726164]

5. Stevanovic S, van Bergen CA, van Luxemburg-Heijs SA, et al. HLA-class II upregulation during viral infection leads to HLA-DP directed Graft-versus-Host Disease after CD4+ donor lymphocyte infusion. Blood. 2013; 122(11):1963–1973. [PubMed: 23777765]

6. Fleischhauer K, Shaw BE, Gooley T, et al. Effect of T-cell-epitope matching at HLA-DPB1 in recipients of unrelated donor haemopoietic-cell transplantation: a retrospective study. Lancet Oncol. 2012; 13(4):366–374. [PubMed: 22340965]

7. Zino E, Frumento G, Marktel S, et al. A T cell epitope encoded by a subset of HLA-DPB1 alleles determines non-permissive mismatches for hematological stem cell transplantation. Blood. 2004; 103(4):1417–1424. [PubMed: 14576061]

8. Crocchiolo R, Zino E, Vago L, et al. Non-Permissive HLA-DPB1 Disparity is a Significant Independent Risk Factor for Mortality after Unrelated Hematopoietic Stem Cell Transplantation. Blood. 2009; 114(7):1437–1444. [PubMed: 19515726]

9. Shaw BE, Robinson J, Fleischhauer K, Madrigal JA, Marsh SG. Translating the HLA-DPB1 T-cell epitope-matching algorithm into clinical practice. Bone Marrow Transplant. 2013; 48(12):1510–1522. [PubMed: 23811815]

10. Farag SS, Bacigalupo A, Eapen M, et al. The effect of KIR ligand incompatibility on the outcome of unrelated donor transplantation: a report from the center for international blood and marrow transplant research, the European blood and marrow transplant registry, and the Dutch registry. Biol Blood Marrow Transplant. 2006; 12(8):876–884. [PubMed: 16864058]

11. Spellman S, Setterholm M, Maiers M, et al. Advances in the selection of HLA-compatible donors: refinements in HLA typing and matching over the first 20 years of the National Marrow Donor Program Registry. Biol Blood Marrow Transplant. 2008; 14(9Suppl):37–44. [PubMed: 18721779]

12. Lauterbach N, Crivello P, Wieten L, et al. Allorecognition of HLA-DP by CD4+ T cells is affected by polymorphism in its alpha chain. Mol Immunol. 2014; 59(1):19–29. [PubMed: 24462895]

13. Varney MD, Lester S, McCluskey J, Gao X, Tait BD. Matching for HLA DPA1 and DPB1 alleles in unrelated bone marrow transplantation. Hum Immunol. 1999; 60(6):532–538. [PubMed: 10408803]

14. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. N Engl J Med. 1998; 339:1177–1185. [PubMed: 9780337]

15. Petersdorf EW, Kollman C, Hurley CK. Effect of HLA class II gene disparity on clinical outcome in unrelated donor hematopoietic cell transplantation for chronic myeloid leukemia: the US National Marrow Donor Program Experience. Blood. 2001; 98(10):2922–2929. [PubMed: 11698272]

16. Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007; 110(13):4576–4583. [PubMed: 17785583]

17. Schaffer M, Aldener-Cannavá A, Remberger M, Ringdén O, Olerup O. Roles of HLA-B, HLA-C and HLA-DPA1 incompatibilities in the outcome of unrelated stem-cell transplantation. Tissue Antigens. 2003; 62(3):243–250. [PubMed: 12956878]

18. Fernández-Viña MA, Klein JP, Haagenson M, et al. Multiple mismatches at the low expression HLA loci DP, DQ, and DRB3/4/5 associate with adverse outcomes in hematopoietic stem cell transplantation. Blood. 2013; 121(22):4603–4610. [PubMed: 23596045]

| Algorithm	DPA1*02:01		DPA1 Allele	
	Present[a]	Absent[a]	Matched	Mismatched
DPB1 TCE3[b]				
Permissive HLA-DPB1 mismatch	Not considered	Not considered	Not considered	Not considered
Non-Permissive HLA-DPB1 mismatch	Not considered	Not considered	Not considered	Not considered
*DPB1 TCE3 / DPA1*02*				
Permissive HLA-DPB1 mismatch	*Permissive*	*Permissive*	*Not considered*	*Not considered*
Non-Permissive HLA-DPB1 mismatch	Non-Permissive	Permissive	Not considered	Not considered
DPB1 TCE3 / DPA1 allele				
Permissive HLA-DPB1 mismatch	Not considered	Not considered	Permissive	Permissive
Non-Permissive HLA-DPB1 mismatch	Not considered	Not considered	Permissive	Non-Permissive

[a] in either the patient or the donor, or both

[b] according to the three-group TCE3 algorithm previously described[6]

Figure 1. Algorithms for permissive or non-permissive HLA-DP TCE group mismatches used in this study

10/10 HLA allele matched donor-recipient pairs were classified according to three different algorithms: 1) DPB1 TCE3 considers as non-permissive only those pairs in whom the HLA-DPB1 allele mismatch involves a TCE3 group mismatches, regardless of the HLA-DPA1 matching status, as previously described.[6] 2) DPB1 TCE3/DPA1*02:01 considers as non-permissive only those pairs in whom the HLA-DPB1 allele mismatch involves a TCE3 group mismatch, and in whom either the patient or the donor, or both, carry at least one DPA1*02:01 allele. 3) DPB1 TCE3/DPA1 allele considers as non-permissive only those pairs in whom the HLA-DPB1 allele mismatch involves a TCE3 group mismatch, and in whom a donor-recipient HLA-DPA1 allele mismatch is also present.

A.
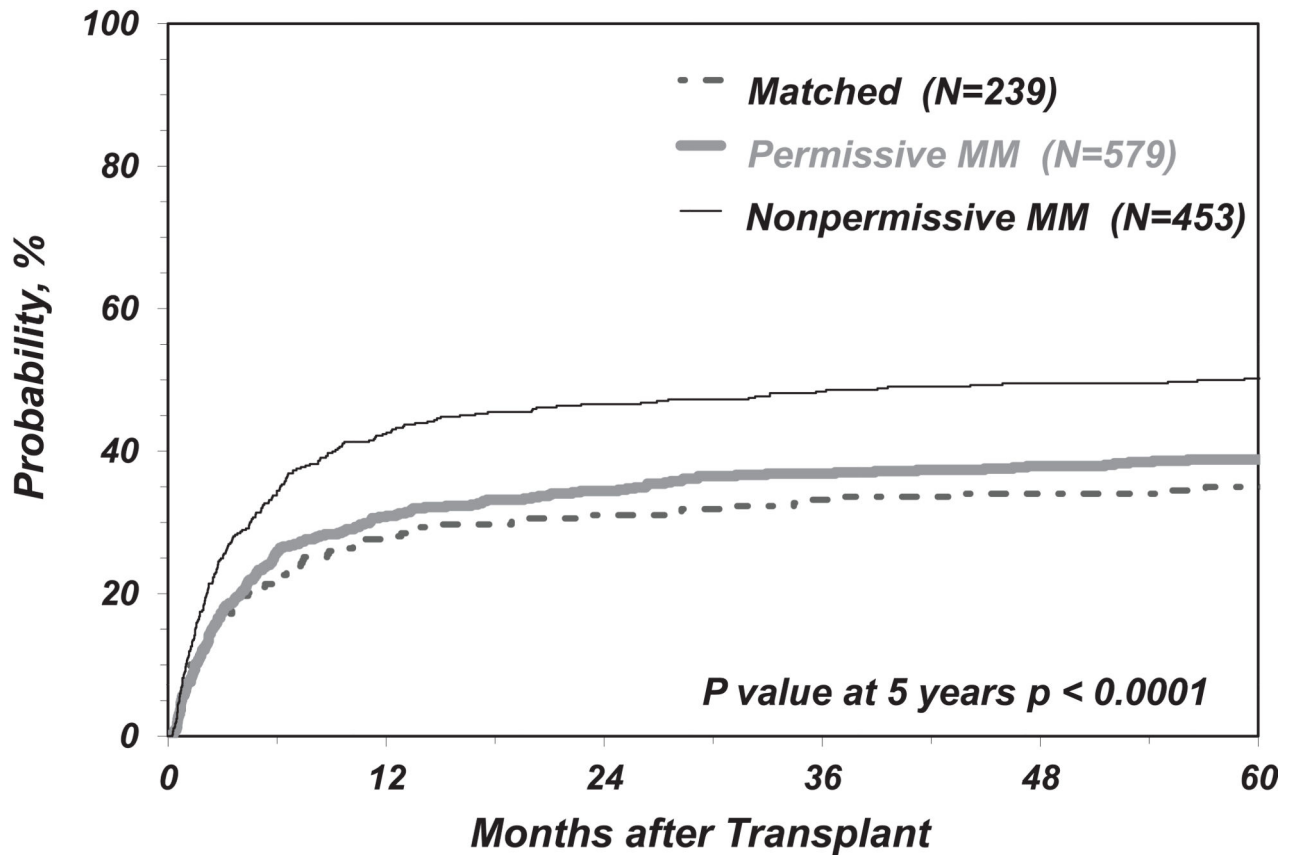
B.

C.
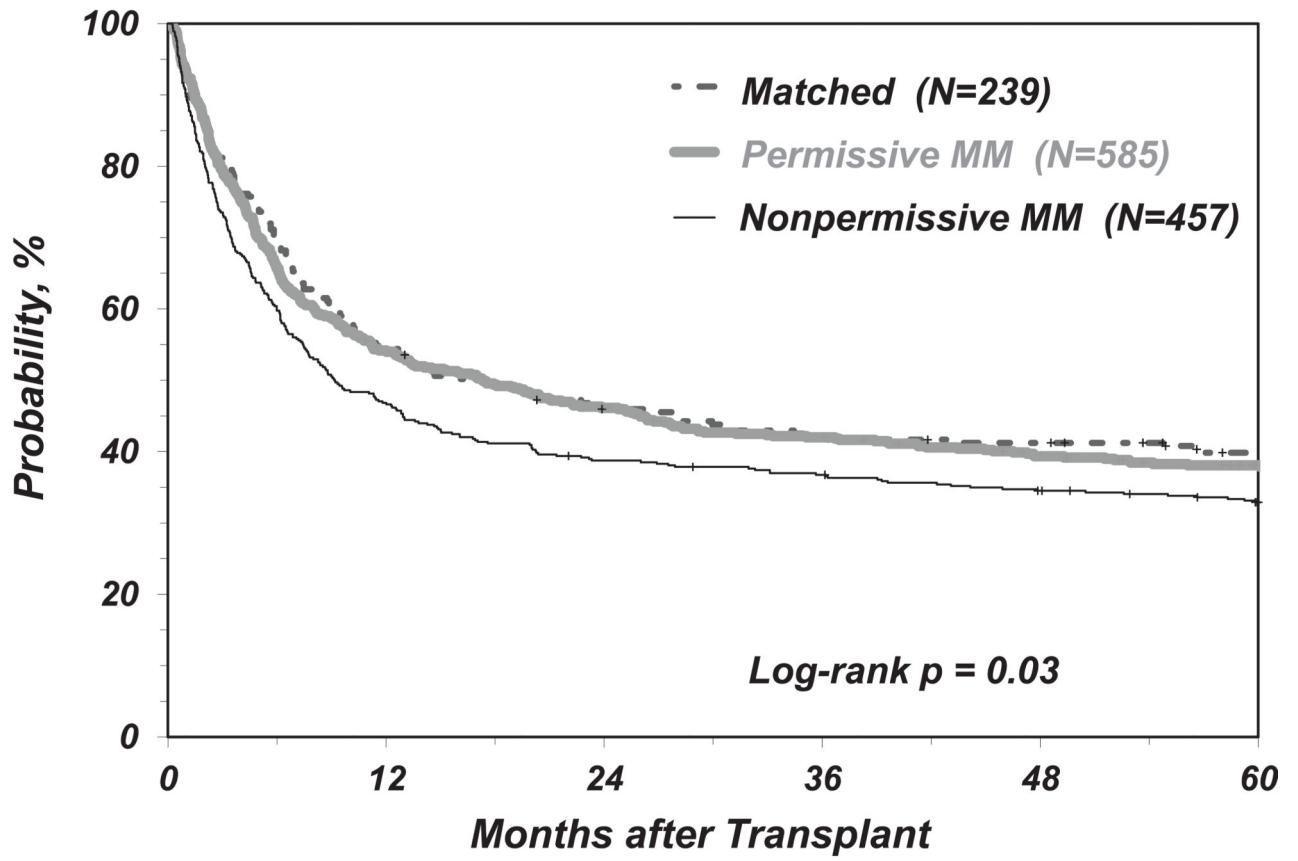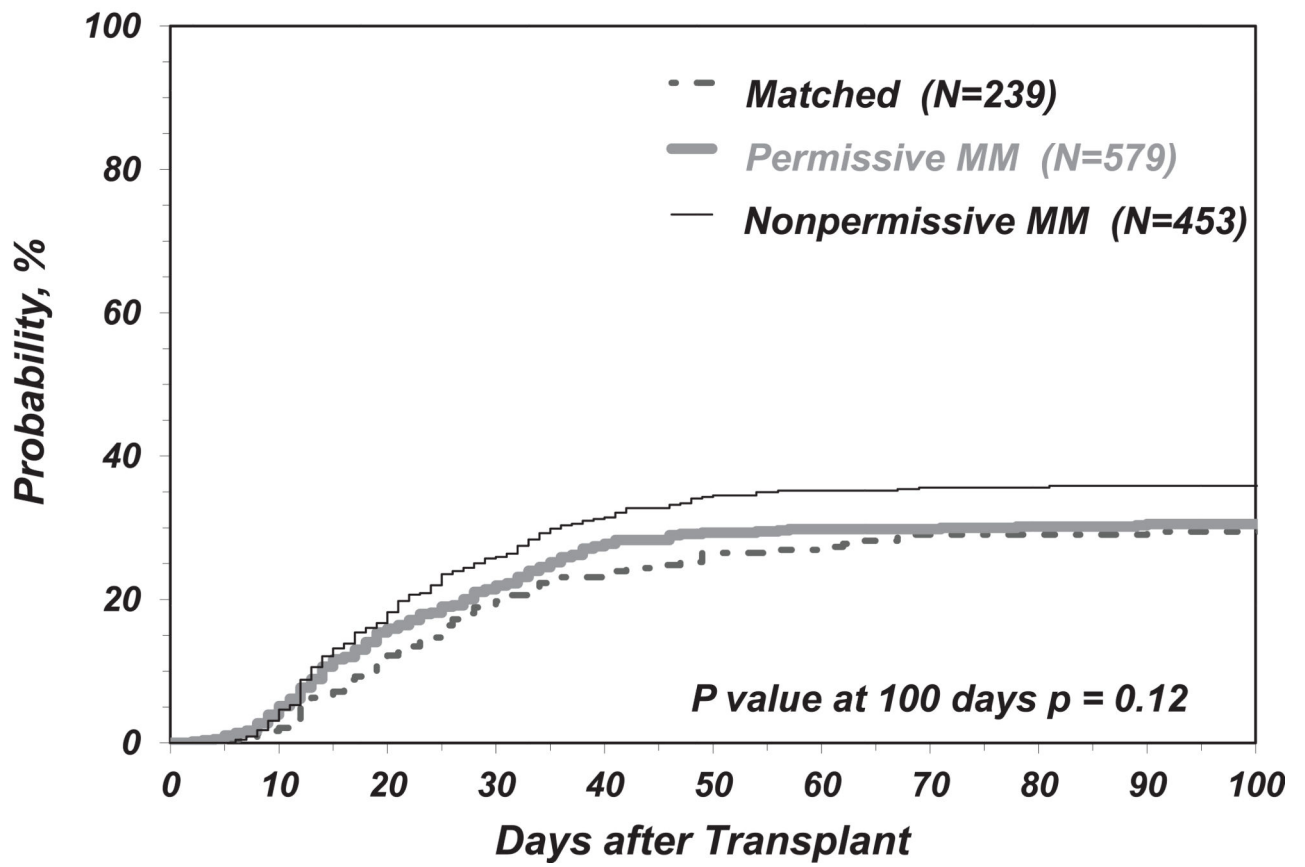
D.
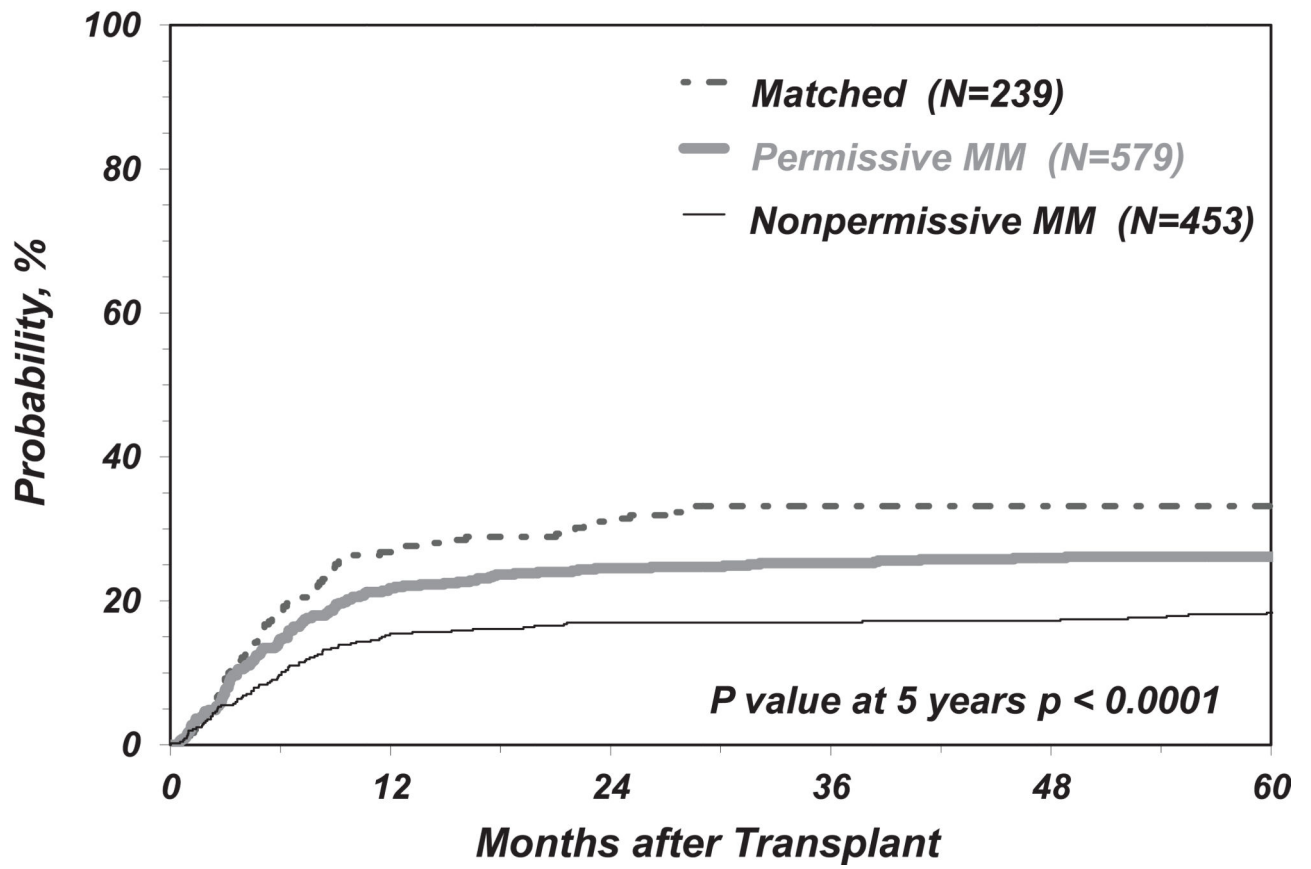
E.
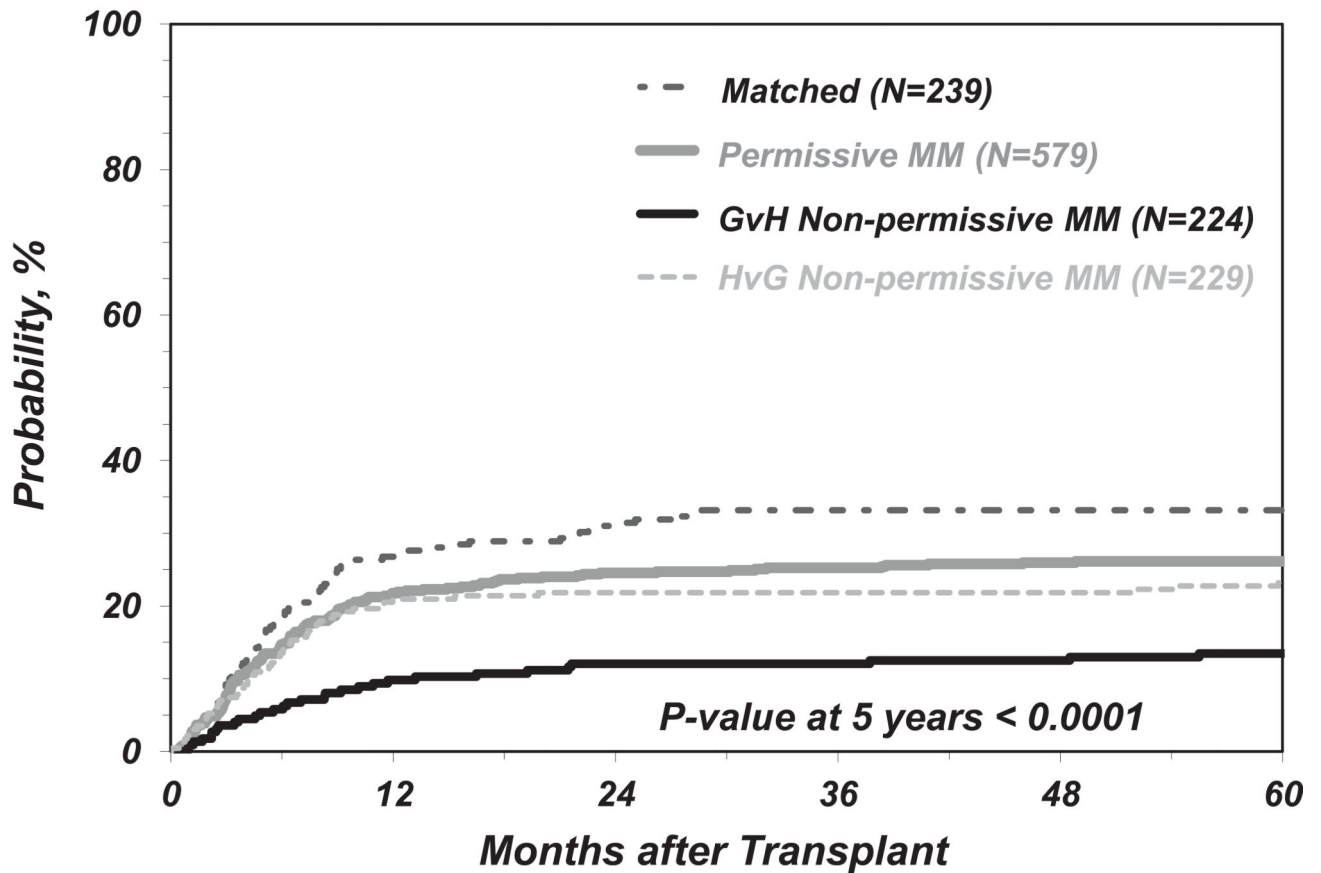
Figure 2. Risk-associations between HLA-DPB1 TCE group mismatches and outcome of UD-HCT

Shown are the cumulative incidence curves of TRM (A), KM curves of OS (B), cumulative incidence curves of grade 3–4 GvHD (C) and cumulative incidence curves of Relapse (D, E) in patients matched for 10/10 of the non-HLA-DP alleles and matched for both HLA-DPB1 alleles ("Matched"), HLA-DPB1 allele mismatched but TCE group matched ("Permissive"), HLA-DPB1 allele mismatched and TCE group mismatched ("Non-Permissive") (A-D), or HLA-DPB1 allele mismatched and TCE group mismatched in the GvH ("Non-Permissive GvH") or HvG ("Non-Permissive HvG") direction (E).

A.
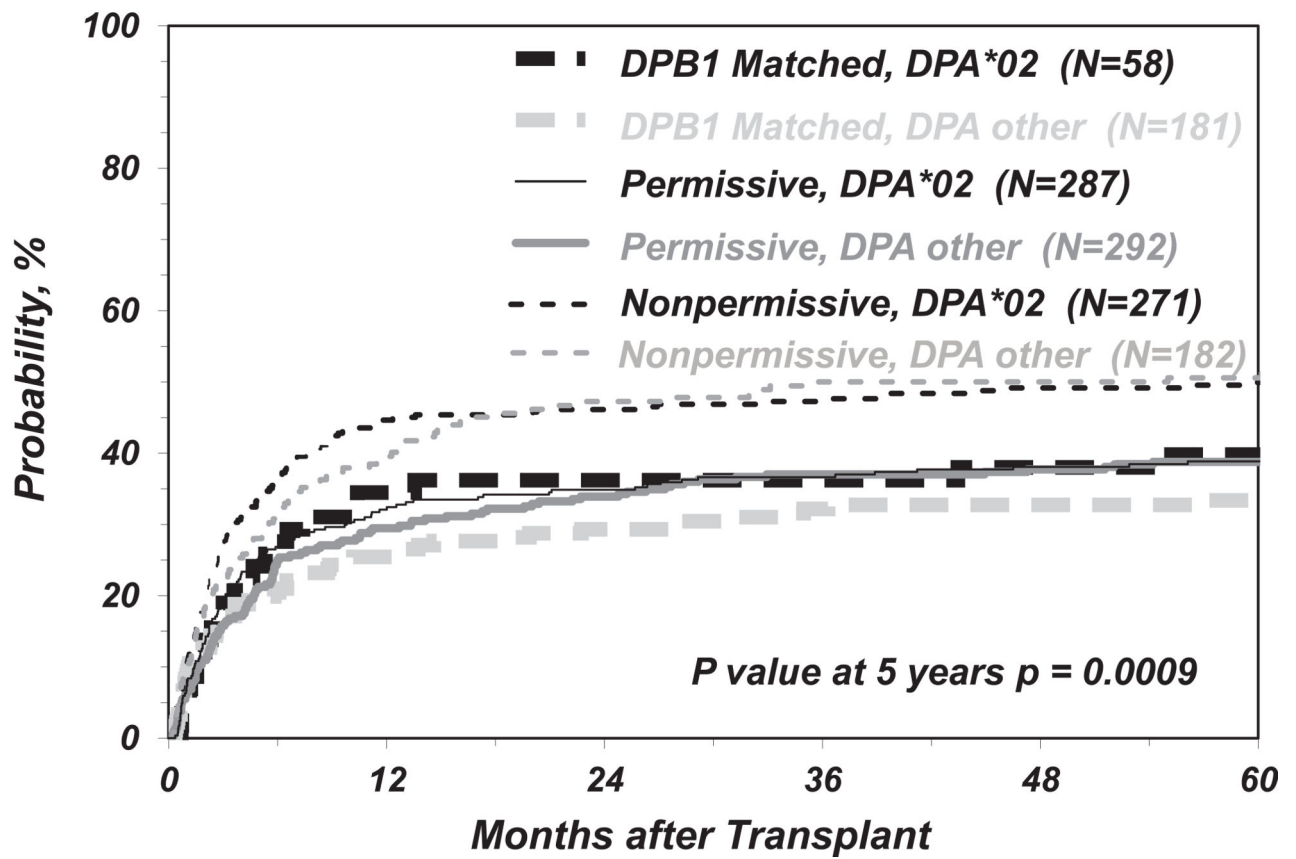
B.
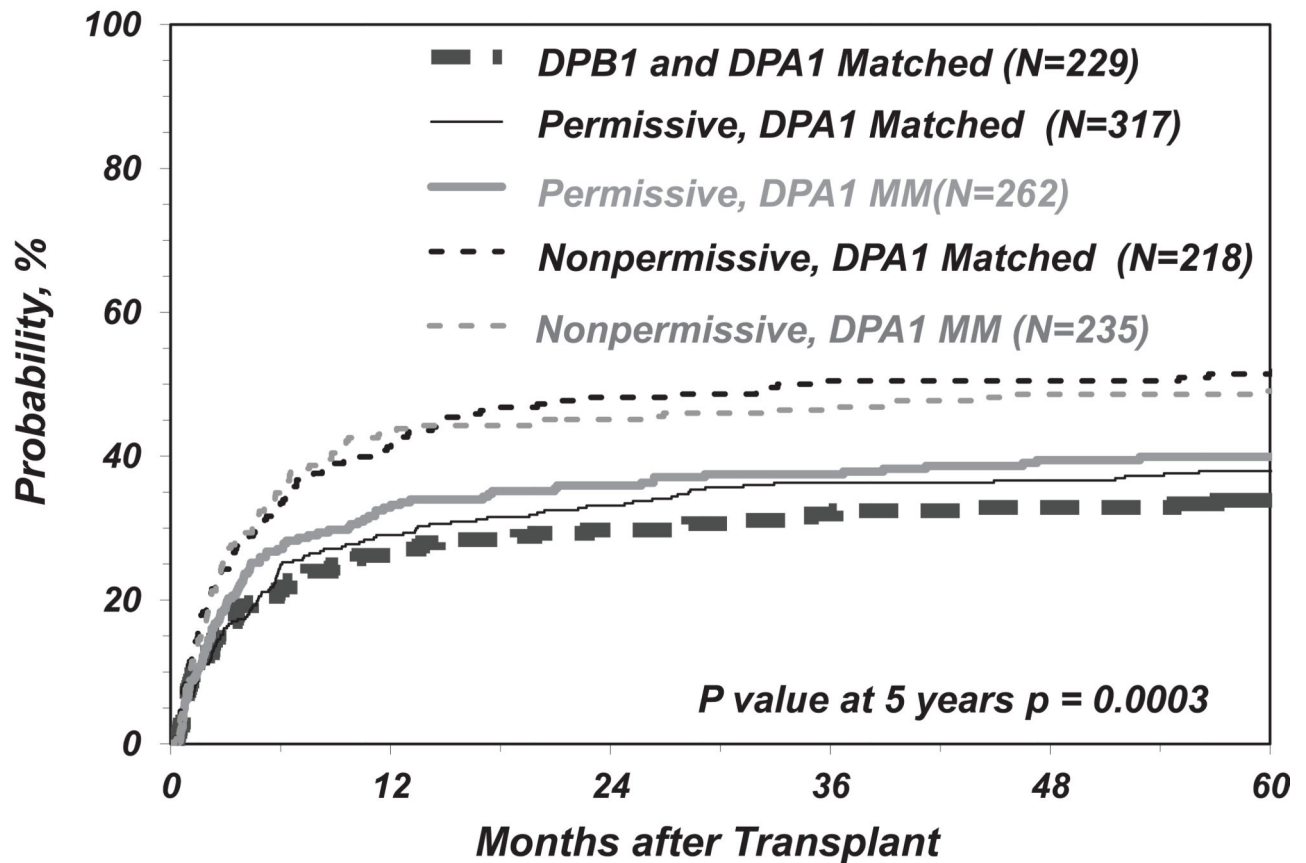
Figure 3. Role of HLA-DPA1 matching status in risk-associations between HLA-DPB1 TCE group mismatches and TRM after UD-HCT

Shown are the cumulative incidence curves of TRM in HLA-DPB1 allele matched, permissively or non-permissively mismatched transplants in the presence or absence of HLA-DPA1*02 in either the patient or the donor, or both (A), or in the presence or absence of HLA-DPA1 allele mismatching (B).

Table 1

Patient and Transplant characteristics.

| Variable	Full matches N (%)	TCE Permissive mismatches N (%)	TCE Non-permissive mismatches N (%)	P-value[a]
Number of Patients	239	585	457	
Number of Centers	54	84	82	
Median Recipient Age	36 (1–61)	36 (<1–65)	36 (<1–63)	0.31
Recipient Age				0.57
0–9	17 (7)	46 (8)	36 (8)	
10–19	17 (7)	62 (11)	45 (10)	
20–29	39 (16)	106 (18)	78 (17)	
30–39	61 (26)	135 (23)	117 (26)	
40–49	62 (26)	152 (26)	128 (28)	
50+	43 (18)	84 (14)	53 (12)	
Recipient Race/Ethnicity				0.25
Caucasian	217 (91)	551 (94)	431 (94)	
African American	3 (1)	9 (2)	6 (1)	
Asian/Pac. Islander	4 (2)	5 (1)	2 (<1)	
Hispanic	12 (5)	19 (3)	14 (3)	
Native American	0	1 (<1)	0	
Other/Multiple/Unknown	3 (1)	0	4 (1)	
Sex				0.79
Male	136 (57)	331 (57)	268 (59)	
Female	103 (43)	254 (43)	189 (41)	
Karnofsky Score				0.58
< 90	67 (28)	134 (23)	119 (26)	
>= 90	162 (68)	426 (73)	320 (70)	
Missing	10 (4)	25 (4)	18 (4)	
Disease				0.03
AML	66 (28)	153 (26)	133 (29)	
ALL	39 (16)	137 (23)	105 (23)	
CML	102 (43)	188 (32)	153 (33)	
MDS	32 (13)	107 (18)	66 (14)	
Disease Status at Tx[b]				0.09
Early	167 (70)	407 (70)	319 (70)	
Intermediate	11 (5)	34 (6)	22 (5)	
Advanced	61 (26)	132 (23)	114 (25)	
Other	0	12 (2)	2 (<1)	
HLA-DPB1 Matching				< 0.0001
Two Mismatches	0	126 (22)	234 (51)	
One Mismatch	0	459 (78)	223 (49)	

Variable	Full matches	TCE Permissive mismatches	TCE Non-permissive mismatches	P-value[a]
	N (%)	N (%)	N (%)	
Fully Matched	239 (100)	0	0	
*HLA-DPA1*0201*				< 0.0001
Absent	181 (76)	295 (50)	183 (40)	
Present	58 (24)	290 (50)	274 (60)	
HLA-DPA1 allele				< 0.0001
Matched	229 (96)	321 (55)	219 (48)	
Mismatched	10 (4)	264 (45)	238 (52)	
Graft Type				0.08
Bone Marrow	206 (86)	513 (88)	417 (91)	
PBSC	33 (14)	72 (12)	40 (9)	
GVHD Prophylaxis				0.004
FK506 + (MTX, MMF or Steroids) ± Other	70 (29)	147 (25)	132 (29)	
FK506 ± Other	2 (1)	2 (<1)	1 (<1)	
CSA + MTX ± Other	155 (65)	416 (71)	294 (64)	
CSA ± Other (No MTX)	8 (3)	17 (3)	25 (5)	
MTX ± Other (No CSA)	0	1 (<1)	5 (1)	
Other	4 (2)	2 (<1)	0	
In vivo T-cell depletion				0.49
Yes	31 (13)	60 (10)	54 (12)	
No	208 (87)	525 (90)	403 (88)	
Sex Match				0.11
Male -> Male	88 (37)	233 (40)	200 (44)	
Male -> Female	60 (25)	127 (22)	115 (25)	
Female -> Male	48 (20)	98 (17)	68 (15)	
Female -> Female	43 (18)	127 (22)	74 (16)	
CMV Match				0.92
Negative/Negative	81 (34)	208 (36)	167 (37)	
Negative/Positive	64 (27)	159 (27)	128 (28)	
Positive/Negative	40 (17)	98 (17)	65 (14)	
Positive/Positive	48 (20)	103 (18)	88 (19)	
Unknown	6 (3)	17 (3)	9 (2)	
Median Donor Age	35 (18–59)	36 (18–60)	35 (19–57)	0.10
Donor Age				0.45
18–29	69 (29)	154 (26)	133 (29)	
30–39	88 (37)	228 (39)	192 (42)	
40–49	64 (27)	167 (29)	105 (23)	
50+	18 (8)	36 (6)	27 (6)	
Conditioning Regimen				0.87
Cy + TBI	181 (76)	421 (72)	329 (72)	
Bu + Cy	39 (16)	124 (21)	96 (21)	

Variable	Full matches N (%)	TCE Permissive mismatches N (%)	TCE Non-permissive mismatches N (%)	P-value[a]
TBI 500 single dose or 800 fractionated	10 (4)	22 (4)	19 (4)	
Melphalan > 150 mg/m^2	5 (2)	9 (2)	5 (1)	
Bu > 9 mg/kg	4 (2)	9 (2)	8 (2)	
Year of Transplant				0.61
1988–1991	8 (3)	23 (4)	15 (3)	
1992–1994	41 (17)	72 (12)	63 (14)	
1995–1997	49 (21)	146 (25)	116 (25)	
1998–2000	85 (36)	189 (32)	151 (33)	
2001–2003	56 (23)	155 (26)	112 (25)	
Median follow-up of survivors, months	99 (13 – 200)	106 (10 – 245)	102 (22 – 196)	0.52[b]

Abbreviations:

CsA = Cyclosporine; CMV = Cytomegalovirus; FK506 = Tacrolimus; HLA = Human leukocyte antigens; MMF = Mycophenolate mofetil; MTX = Methotrexate; tx = transplant.

[a]Log-rank p-value.

[b]Early stage disease was defined as AML or ALL in first complete remission, CML in first chronic phase, and MDS subtype refractory anemia. Intermediate stage disease was defined as AML or ALL in second or subsequent complete remission, and CML in accelerated phase or second chronic phase. Advanced phase disease was defined as AML in first or higher relapse or primary induction failure, CML in blast phase, MDS subtypes refractory anemia with excess blasts or in transformation.

Table 2

Multivariate regression models for association between non-permissive DPB1 TCE3 mismatches and clinical outcome.

	Permissive HLA-DPB1 mismatch (N = 585)	HLA-DPB1 match (N = 239)		Non-Permissive HLA-DPB1 mismatch (N = 457)		Non-Permissive HLA-DPB1 GvH (N=226)		Non-Permissive HLA-DPB1 HvG (N=231)	
	Ref.	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Overall mortality[a]	1	0.94 (0.78–1.14)	0.56	1.16 (0.99–1.35)	0.05	1.11 (0.92–1.35)	0.27	1.20 (1.00–1.44)	0.05
Treatment-related mortality[b]	1	0.86 (0.68–1.11)	0.03	1.3 (1.06–1.53)	0.009	1.29 (1.03–1.60)	0.03	1.27 (1.01–1.58)	0.04
Relapse[c]	1	1.31 (0.99–1.73)	0.05	0.80 (0.62–1.05)	0.1	0.55 (0.37–0.80)	0.002	1.09 (0.8–1.48)	0.6
aGVHD C-D[d]	1	0.96 (0.73–1.26)	0.77	1.27 (1.02–1.57)	0.03	1.34 (1.03–1.74)	0.03	1.20 (0.92–1.56)	0.17

[a] adjusted for disease, disease status, GVHD prophylaxis, use of in vivo T cell depletion, preparative regimen, and recipient age at transplant.

[b] adjusted for disease status, GVHD prophylaxis, use of in vivo T cell depletion, preparative regimen and recipient age at transplant.

[c] adjusted for disease and disease status.

[d] adjusted for disease, disease status, graft type and year of transplant.

Table 3

Multivariate regression models for association between non-permissive DPB1 TCE3 / DPA1*02:01 mismatches and clinical outcome.

	Permissive HLA-DPB1 mismatch + HLA-DPA1*02:01 (N = 290)	HLA-DPB1 match + HLA-DPA1*02:01 (N = 58)		Non-Permissive HLA-DPB1 mismatch + HLA-DPA1*02:01 (N = 274)	
	Ref.	HR (95% CI)	p-value	HR (95% CI)	p-value
Overall mortality[a]	1	0.88 (0.74–1.06)	0.18	0.98 (0.81–1.19)	0.83
Treatment-related mortality[b]	1	0.78 (0.61–0.98)	0.03	1.02 (0.81–1.27)	0.89
Relapse[c]	1	1.40 (1.05–1.78)	0.02	0.75 (0.52–1.08)	0.12
aGVHD C–D[d]	1	0.91 (0.69–1.18)	0.48	1.29 (0.99–1.66)	0.05

[a] adjusted for disease, disease status, GVHD prophylaxis, use of in vivo T cell depletion, preparative regimen, and recipient age at transplant.

[b] adjusted for disease status, GVHD prophylaxis, use of in vivo T cell depletion, preparative regimen and recipient age at transplant.

[c] adjusted for disease and disease status.

[d] adjusted for disease, disease status, graft type and year of transplant.

Table 4

Multivariate regression models for association between non-permissive DPB1 TCE 3/ DPA1 allele mismatches and clinical outcome.

	Permissive HLA-DPB1 mismatch + DPA1 match (N = 321)		Permissive HLA-DPB1 mismatch + DPA1 mismatch (N = 264)		HLA-DPB1 match + HLA-DPA1 match (N = 229)		HLA-DPB1 match + HLA-DPA1 mismatch (N=10)		Non-Permissive HLA-DPB1 mismatch + HLA-DPA1 match (N = 219)		Non-Permissive HLA-DPB1 mismatch + HLA-DPA1 mismatch (N = 238)	
	Ref.		HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Overall mortality[a]	1		0.92 (0.75–1.13)	0.42	0.87 (0.71–1.08)	0.22	2.31 (1.22–4.41)	0.011	1.22 (0.99–1.50)	0.06	1.03 (0.84–1.27)	0.76
Treatment-related mortality[b]	1		1.01 (0.78–1.31)	0.93	0.84 (0.63–1.10)	0.2	1.97 (0.86–4.50)	0.11	1.39 (1.08–1.78)	0.01	1.19 (0.93–1.53)	0.16
Relapse[c]	1		0.74 (0.54–1.04)	0.08	1.13 (0.83–1.54)	0.44	2.70 (0.981–7.39)	0.05	0.84 (0.59–1.19)	0.33	0.62 (0.43–0.89)	0.01
aGVHD C-D[d]	1		1.11 (0.83–1.49)	0.49	1.00 (0.74–1.37)	0.98	1.02 (0.32–3.21)	0.98	1.22 (0.90–1.66)	0.19	1.42 (1.06–1.89)	0.02

[a] adjusted for disease, disease status, GVHD prophylaxis, use of in vivo T cell depletion, preparative regimen, and recipient age at transplant.

[b] adjusted for disease status, GVHD prophylaxis, use of in vivo T cell depletion, preparative regimen and recipient age at transplant.

[c] adjusted for disease and disease status.

[d] adjusted for disease, disease status, graft type and year of transplant.