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HLA-DPB1 matching in unrelated hematopoietic stem cell transplantation program contributes to a higher incidence of disease relapse

Marija Burek Kamenaric[1], Marija Maskalan[1], Zorana Grubic[1], Mirta Mikulić[2], Ranka Serventi Seiwerth[2], Nadira Duraković[2], Radovan Vrhovac[2], Katarina Stingl Jankovic[1], Renata Zunec[1]

[1]Tissue Typing Centre, Clinical Department for Transfusion Medicine and Transplantation Biology, University Hospital Center Zagreb, Zagreb, Croatia; [2]Department of Hematology, Internal Clinic, University Hospital Center Zagreb, Zagreb, Croatia

mburek@kbc-zagreb.hr; marija.maskalan@kbc-zagreb.hr; zgrubic@kbc-zagreb.hr; mirmikulic@gmail.com; serventi_seiwerth@hotmail.com; nadira.durakovic@kbc-zagreb.hr; rvrhovac@mef.hr; katarinastingl@gmail.com; rzunec@kbc-zagreb.hr

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Corresponding author:
Marija Burek Kamenaric
Tissue Typing Centre, Clinical Department for Transfusion Medicine and Transplantation Biology, University Hospital Center Zagreb, Kispaticeva 12, Zagreb, Croatia, HR-10000
Tel: +385 1 23 67 287
Fax: +385 1 23 67 337
E-mail: mburek@kbc-zagreb.hr

ABSTRACT

The impact of patient/donor matching for HLA-A, -B, -C, -DRB1 and -DQB1 genes in hematopoietic stem cell transplantation (HSCT) is well-recognized, but typing for additional genes, such as HLA-DPB1, is still controversial. Based on defined T-cell epitope (TCE) groups, all HLA-DPB1 mismatches can be classified as permissive or non-permissive. In this retrospective study we analysed 82 patient/matched unrelated donor (MUD) pairs who underwent HSCT, and explored the impact of HLA-DPB1 matches, permissive and non-permissive mismatches on transplantation outcomes. Patient/MUD pairs matched for HLA-DPB1 alleles in univariate analysis were associated with a significantly higher incidence of disease relapse compared to pairs who were permissive/non-permissive HLA-DPB1 mismatched according to the TCE3 and TCE4 algorithms ($P = 0.025$ and $P = 0.026$, respectively), although the significance was lost in multivariate analysis. The analysis did not reveal any significant influence of HLA-DPB1 alleles on overall survival (OS), non-relapse mortality (NRM) or graft-versus-host disease (GVHD) incidence. In conclusion, our study presents evidence that HLA-DPB1 matching influenced the relapse rate in patients after HSCT so the HLA-DPB1 alleles should be implemented in the MUD search algorithm as a transplantation determinant.

Keywords: HLA-DPB1, permissive mismatch, non-permissive mismatch, TCE3 algorithm, TCE4 algorithm, haematopoietic stem cell transplantation

Introduction

The huge polymorphism of the classical human leukocyte antigen (HLA) genes and the recognition of HLA incompatibilities by the immune system represent a major barrier to allogeneic hematopoietic stem cell transplantation (HSCT) [1]. To lower risks of acute graft-versus-host disease (GVHD) and mortality after HSCT, high resolution typing and matching for HLA-A, -B, -C, -DRB1, and -DQB1 alleles (10/10 match) between unrelated donor and patient still remains the gold standard, while the importance of HLA-DPB1 gene remains uncertain and pre-transplantation typing is not routinely performed. Because of weak linkage disequilibrium between HLA-DPB1 alleles and other HLA class II alleles [2], HSCT is generally performed across HLA-DPB1 allelic mismatches.

The HLA-DBP1 gene is located in the HLA class II region of the chromosome 6p21.3, lying centromeric to the other HLA class II loci. It is highly polymorphic and in the terms of structure and function HLA-DPB1 resembles other HLA class II molecules - they are cell-surface heterodimers, consisting of an alpha and beta chain, that function as receptors for processed peptides derived predominantly from membrane and extracellular proteins, which they present to CD4 T lymphocytes initiating an immune response [3]. The common assumption is that HLA-DP molecules are less important in the immune response than HLA-DR or HLA-DQ molecules, because of a ~10-fold lower cell surface expression [4]. However, the HLA-DPB1 gene encodes fully functional molecules with specific responses reported in a number of settings.

In the performed *in vitro* assays set up to detect anti-HLA-DP alloreactive T cells, the observed T-cell reactivity patterns suggest the expression of a shared T-cell epitope (TCE) encoded by the HLA-DPB1 alleles [5]. The HLA-DPB1 alleles were thus classified according to their predicted immunogenicity in to high immunogenic group 1 (HLA-DPB1*09:01, *10:01, *17:01), intermediately immunogenic group 2 (HLA-DPB1 *03:01, *14:01, *45:01), and poorly immunogenic group 3 (most other HLA-DPB1 alleles). On the basis of a shared alloreactive T-cell epitope, the TCE3 algorithm was proposed for use in unrelated donor selection identifying permissive or non-permissive HLA-DPB1 mismatches. Later, a modified 4-group algorithm (TCE4), including the HLA-DPB1*02 gene as a separate group with immunogenicity lower than that of group 2 alleles but higher than that of the low immunogenic alleles in group 3, was designed [6]. The latest classification into three TCE immunogenic groups, including 390 HLA-DPB1 alleles, was reported by Crivello et al. [7].

To date, the biological role of HLA-DP in HSCT still remains controversial. Retrospective studies analysing HLA-DPB1- matching status between matched unrelated donors (MUDs) and recipients at the allelic level, or classified as permissive/non-permissive mismatches were performed [5, 6, 8-19]. The given results are heterogeneous and an overview of results from literature is summarized in Table 1. However, analysis suggests that HLA-DPB1 classification according to T-cell epitope grouping can identify permissive and non-permissive unrelated recipient-donor combinations relevant to GVHD occurrence, mortality and disease relapse rate after HSCT.

In an effort to contribute to the clarification of the role of HLA-DPB1 in HSCT, we performed a retrospective single centre analysis of the impact of permissive/non-permissive HLA-DPB1 disparities on the overall survival (OS), non-relapse mortality (NRM), GvHD occurrence and disease relapse rate among 82 patients who underwent HSCT from MUD. The validation of these findings could support the utility of including HLA-DPB1 alleles in pre-transplantation typing to further improve MUD selection.

2. Materials and methods

2.1. Study population

The study included 82 patients with hematological malignancies who had received a hematopoietic stem cell transplant from a 10/10 (HLA-A, -B, -C, -DRB1, -DQB1) MUD in the period of 2010 – 2015 at the University Hospital Centre Zagreb, Department for Internal Medicine, Division of Hematology. Namely, the University Hospital Centre Zagreb is the only hospital in Croatia where unrelated HSCT is performed and the patients originated from different areas of Croatia. The included patients were adults (N=67) or children (N=15) with a broad range of diseases that were an indication for HSCT: acute myelogenous leukaemia (AML, N=37), acute lymphoblastic leukaemia (ALL, N=17), myelodysplastic syndrome (MDS, N=6), chronic myelogenous leukaemia (CML, N=3), non-Hodgkin lymphoma (NHL, N=4), Hodgkin lymphoma (HL, N=3), myelofibrosis (MF, N=2), severe combined immunodeficiency (SCID, N=3) and other malignancies (N=7). The majority of the patients were treated with a reduced-intensity conditioning (RIC) regimen mainly based on fludarabine in a dose of 30 mg/m2 daily over 4-6 days, IV busulfan 3.2 mg/kg daily over 2-3 days, and anti–thymocyte globulin (ATG) in a total dose of 5 mg/kg infused over 2 days. Other patients were treated with a myeloablative conditioning regime (MAC) receiving IV busulfan in a daily dose of 3.2 mg/kg over 4 days, 60 mg/kg of cyclophosphamide over 2 days and ATG in total dose of 1.5 mg/kg over 11 hours. GvHD prophylaxis was performed with cyclosporine (CsA) and mycophenolate mofetil (MMF). Patients received bone marrow (BM) grafts or peripheral blood stem cell (PBSC) grafts, mobilized from donors with granulocyte-colony-stimulating factor (GCSF) (filgrastime, 10 μg/kg per day). No manipulation of the graft, such as T-cell depletion, was performed in any of the cases. Characteristics of the patients and their MUDs are given in Table 2.

2.2. HLA typing

All patient-MUD pairs were HLA typed at the allelic level for HLA-A, -B, -C, -DRB1 and -DQB1 using the standard polymerase chain reaction-sequence specific priming (PCR-SSP) protocol for Olerup SSP® typing kits (Olerup GmbH, Vienna, Austria) or by PCR-sequence specific oligonucleotide probing (PCR-SSOP) method, using the commercially available Immucor *Lifecodes HLA-SSO typing kit* (Immucor Transplant Diagnostics, Inc, Stamford, USA) [20, 21]. For the purpose of this study, the retrograde allelic HLA-DPB1 typing of patients and their MUDs was performed using the Luminex technology (Immucor) in the combination with PCR-SSP method (Olerup). The combination of alleles that could not

be discriminated (HLA-DPB1*03:01/104:01 and DPB1*04:02/105:01) was designated by case ‘P’ and the common alleles were used for alternative genotypes.

2.3. Study design

The first step was to determine whether the patient and their MUD were HLA-DPB1-matched or mismatched (MM) and HLA-DPB1 alleles were assigned to corresponding immunogenic T-cell-epitope groups (group 1, 2, 3 and/or 4). Furthermore, all patient-MUD pairs (N=75) who were a single or a double HLA-DPB1 allele MM were classified as permissive (T-cell-epitope group matched) or non-permissive (T-cell-epitope group MM) according to TCE3 [5, 7] and TCE4 [6] algorithm. The patient-MUD pairs were defined as HLA-DPB1 permissive mismatched if both the patient's and the donor's HLA-DPB1 alleles were from the same immunogenic group (1/1, 2/2, 3/3 and/or 4/4) or they carried at least one allele from the high immunogenic group 1 (1/2, 1/3 and/or 1/4). Also, pairs were permissive mismatched if neither the patient nor donor had a group 1 allele but carried at least one immunogenic group 2 allele (2/3 and/or 2/4). Additionally, in TCE4 classification a part of the TCE3-permissive disparities (3/3) becomes non-permissive, and only patient-MUD pairs, both carrying at least one immunogenic group 3 allele (3/4 vs 4/4), were permissive mismatched. All non-permissive TCE3 disparities were also TCE4 non-permissive. For confirmation, the classification of all patient-MUD pairs was also performed using an online calculator, the DPB1 T-Cell Epitope Algorithm v2.0 (http://www.ebi.ac.uk). Patient-MUD pairs without HLA-DPB1 allelic MMs (N=7) were not included in permissive HLA-DPB1 disparity. Since the chance of matching for HLA-DPB1 was the same whether the other HLA loci were matched or not [10], we analysed HLA-DPB1 matching as an individual risk factor for transplantation outcomes. Clinical data were collected from the transplantation centre and the effect of HLA-DPB1 match and HLA-DPB1 permissive/non-permissive MMs on HSCT outcome were estimated. The OS, NRM, GvHD incidence and disease relapse rate were the main research endpoints analysed. The starting point for time-to-event analysis was “date of transplantation”. The OS rate was defined as the time to death from any cause. Surviving patients were censored at the time of last follow-up. NRM was defined as all causes of death without evidence of initial disease. Acute and chronic GvHD were diagnosed according to the standard criteria [22, 23].

2.4. Statistical analysis

The observed HLA-DPB1 allele frequencies in the research group were calculated by direct counting. The 2-year probabilities of OS were analysed using Kaplan-Meier methods evaluating the influence of the HLA-DPB1 matches/mismatches on specified transplantation endpoint. Cumulative incidence was used to estimate the disease relapse rate, NRM and probability of GvHD occurrence after HSCT. Death without relapse was regarded as a competing risk for relapse, and relapse as a competing risk for non-relapse mortality. The two-sided P values were obtained from the log-rank test and were set to $P \leq 0.05$. Logistic regression analysis was performed to explore the effect of major clinical variables (patient age at transplantation, patient/MUD gender, source of stem cells, conditioning regimen) with HSCT outcome. The likelihood ratio and significance values are presented as Odds Ratio (OR) with a 95% Confidence Interval (CI) and the *P*-value for each variable. All statistical analyses were performed using XLSTAT-Biomed solution software, version 2017.3.

3. Results

3.1. HLA-DPB1 allele polymorphism

Among the 82 patient-MUD pairs in this study, only 22 of the 894 HLA-DPB1 alleles known to date (IMGT/HLA database v.3.29) [24] were detected. The most frequent HLA-DPB1 alleles were HLA-DPB1*04:01 (37.19%), followed by DPB1*04:02P (17.68%), DPB1*02:01 (12.19%), DPB1*03:01P (9.14%) and DPB1*01:01 (6.09%). The remaining 17 alleles had a frequency of less than 5.0%. In the patient group, 60 (73.17%) samples were HLA-DPB1 heterozygous and 22 (26.83%) samples were homozygous. A similar distribution of HLA-DPB1 heterozigosity (59/82) and homozigosity (23/82) was detected among the MUDs.

3.2. HLA-DPB1 matching status of patients and their MUDs

Out of the 82 patient-MUD pairs studied, only 7 pairs shared a complete identity for all 12 HLA alleles (12/12). Consequently, the rate of HLA-DPB1 allele match between patients and MUDs is 8.53%. The remaining 75 pairs had at least one (50/75) or both (25/75) allelic MM at HLA-DPB1. According to the TCE3 algorithm, permissive MMs were present in 38 (50.66%) patient-MUD pairs and non-permissive HLA-DPB1 MMs were detected in 37 (49.34%) pairs (12 HvG; 25 GvH direction). While considering HLA-DPB1*02 as a separate immunogenic group according to the TCE4 algorithm, 24 (32.0%) patient-MUD pairs were permissive mismatched, while non-permissive HLA-DPB1 MMs were detected in 51 (68.0%) pairs (18 HvG; 33 GvH direction).

3.3. Clinical outcome

Overall survival. The estimated 2-year probability of OS in the whole group was 53.65%. There was no significant difference in OS between those recipient-MUD pairs who were matched and those who were (TCE3 and TCE4) permissive/non-permissive mismatched for HLA-DPB1 alleles, (TCE3: OR = 0.58 [CI, 0.32–7.72], P = 0.41; TCE4: OR = 3.42 [CI, 0.61–19.39], P = 0.58), although there is the tendency of worse OS for HLA-DPB1-matched recipient-MUD pairs (Figure 1a and 2a). Logistic regression analysis showed that none of the analysed baseline characteristics regarding age, gender, conditioning regime, source of stem cells and HLA compatibility have a significant influence on OS (Table 3).

Non-relapse mortality. The 2-year probability of NRM was 26.83%. According to the univariate analysis the impact of HLA-DPB1 matched and TCE3 or TCE4 permissive/non-permissive HLA-DPB1 mismatched recipient-MUD pairs had no deleterious impact on NRM

(TCE3: OR = 0.30 [CI, 0.03–2.65], P = 0.91; TCE4: OR = 1.22 [CI, 0.11–13.97], P = 0.82) (Figure 1b and 2b). The only factor showing a statistically significant (P=0.04) influence on NRM in logistic regression analysis were the patient's age <18 (Table 3) while other factors were not significantly associated.

Graft versus host disease. The overall incidence of GvHD was 37.81%. Among 31 GvHD positive patients, 23 were determined as having acute GvHD while 8 patients revealed chronic GvHD. The univariate analysis of clinical data from the HLA-DPB1-matched and the HLA-DPB1 (TCE3 and TCE4) permissive/non-permissive mismatched groups revealed no significant difference of GvHD incidence (TCE3: OR = 0.55 [CI, 0.09–3.12], P = 0.54; TCE4: OR = 0.85 [CI, 0.13–5.36], P = 0.90) (Figure 1c and 2c). Patient, donor and graft variables were not significantly associated with GvHD occurrence in logistic regression analysis (Table 3)

Relapse. Among our group of analysed patients, 19 relapsed. The HLA-DPB1 allele matched recipient-MUD pairs were associated with significant increase in disease relapse compared with both permissive and non-permissive HLA-DPB1 allele MMs, according to both the TCE3 (OR = 4.72 [CI, 0.91–24.36], P = 0.025) and the TCE4 (OR = 7.02 [CI, 1.12–44.01], P = 0.026) algorithms (Figure 1d and 2d). There was no statistically significant difference in relapse rate observed between permissive and non-permissive cases, although there is clearly visible separation between groups on the graph pointing smaller relapse rate among HLA-DPB1 non-permissive MM group of patients. Logistic regression analysis of patient, donor and graft variables which might be associated with relapse incidence was performed in regards to HLA-DPB1 level matching, and none of the analysed characteristics were found to have a significant influence on the researched endpoint (Table 3).

4. Discussion

The results of this study pointed out several findings about HLA-DPB1 allele distribution, HLA-DPB1 matching rate among patients and their MUDs as well as the role of HLA-DPB1 alleles in HSCT. The most frequent HLA-DPB1 alleles found in our study group (HLA-DPB1*04:01, -DPB1*04:02P, -DPB1*03:01P, -DPB1*02:01, -DPB1*01:01,) are in concordance with the frequencies of HLA-DPB1 alleles in Croatian population [25]. It is interesting to note that the most frequent allele among our patients, HLA-DPB1*04:01, is also the most frequent allele in the rest of the Europe and North America but its frequency lowers as we go towards south. At the same time, the frequency of DPB1*04:02 increases and it is the most frequent allele in Middle and South America. In Asia, the frequency of HLA-DPB1*04:01 is high in the east and as we move to the west its frequency decreases, and the frequency of HLA-DPB1*02:01 becomes predominant [26]. Also, an important observation is that these four alleles are present in 82.31% of individuals of the researched group. According to Sidney et al., a panel of five HLA-DPB1 molecules (DPB1*01:01, DPB1*02:01, DPB1*04:01, DPB1*04:02, and DPB1*05:01) is encountered with an average phenotypic frequency of >15% across the seven main populations (Australia, Europe, North America, Oceania, South America, Southeast Asia, and sub-Saharan Africa) covering ~92% of the average population at the HLA-DPB1 locus [27].

The frequency of HLA-DPB1 alleles was approximately equal in both donors and patients; however, only 7/82 patient/MUD pairs shared a complete HLA-DPB1 compatibility. Since none of the transplanted pairs had HLA–DPB1 typing performed before transplantation, this was an unexpected finding. However, the low percentage of HLA-DPB1 matched patient/MUD pairs is comparable to the rate observed in the IHWS [11], French [12], Austrian [13] and Swiss [15] studies. According to our results and data from the above mentioned studies, mismatching at the HLA-DPB1 alleles is very frequent in patient/ MUD pairs (up to ~91%). The mismatching rate of HLA-DPB1 alleles in HLA identical siblings is around 5% [28, 29]. This is a consequence of the low linkage disequilibrium between HLA-DPB1 and HLA-DR/-DQ loci as well as the high HLA-DPB1 polymorphism and thus the probability of finding a 10/10 MUD also matched for HLA-DPB1 alleles is very low. Therefore, to overcome the difficulty of finding a HLA-DPB1 MUD, the classification of HLA-DPB1 alleles according to their immunogenicity as permissive or non-permissive has a benefit for increasing the number of acceptable donors [5, 6]. Tram et al., recently performed a study about the likelihood of identifying a HLA-DPB1 permissive MUD for patients with

10/10 matched donors in the Be The Match Registry and concluded that a young HLA-DPB1 permissive MUD is at the start possible for 59% of the patients carrying TCE group 3 alleles and improves to 70% after additional DPB1 typing of 4 donors [30]. The ability to find a HLA-DPB1 permissive match for each TCE group is similar to the frequency of expression of these alleles in given populations.

The data from different studies presented in Table1 mostly suggest that HLA-DPB1 allele MMs may be tolerated, or are even beneficial in HSCT. Fleischhauer et al., observed in their study that being matched or mismatched for HLA-DPB1 alleles according to TCE groups provides a better prediction of transplant outcomes than does consideration of HLA-DPB1 allele level matching alone [14]. Our study supports findings that HLA-DPB1 mismatching reduces the risk of disease relapse compared with HLA-DPB1 allele-matched cases. The same observation was reported in a few other studies [10, 11, 17]. On the other hand, the association of HLA-DPB1 MMs with theoretically expected higher GvHD occurrence was not proven in this research (no effect on GvHD). The possible explanation of the beneficial effect of HLA-DPB1 MMs regarding disease relapse rate is the knowledge that the vast majority of leukemic cells expressed variable levels of DPB1 antigens on the cells surface and could be killed by DPB1-specific cytotoxic T cells, inducing a graft versus leukaemia effect (GvL) [31, 32]. In this way, allospecific T cells, which are responsible for GvHD, might also be directly responsible for the anti-leukemic effect with HLA-DPB1 as a specific GvL target. Rutten et al., demonstrated that HLA-DP-specific T cells were found in patients with beneficial clinical responses in both presence and absence of GvHD and the conclusion was that the balance between GvHD and GvL reactivity in each individual is also determined with the local environment and the induction of other immune responses [33].

In the current study, no significant differences between HLA-DPB1 permissive and non-permissive MMs on HSCT outcome were observed and the TCE3-group algorithm compared to the TCE4-group algorithm showed similar associations with all investigated clinical endpoints. These results are not in agreement with the data reported in three different studies [5, 6, 14] with better prediction of HSCT outcome with permissive DPB1 MMs compared to non-permissive MMs. These differences are possibly associated with the transplant characteristics of each investigated group such as the intensity of the conditioning regimen, stem cell source and GvHD prophylaxis. In the mentioned studies, the conditioning regimen was mostly MAC while in our study group, around half of the patients (54.87%) were treated with RIC using ATG which is known to decrease the risk of GvHD after allogeneic HSCT [34], and because of that it maybe influences or even overcomes the HLA-

DPB1 MM effect. On the other hand, the BM was the main stem cell source in those studies compared with our study group whereas PBSC were used in the majority of patients (71.95%) and it is known that the use of PBSC compared to BM is associated with increased risk of GvHD [35]. The multivariate analysis did not point to any of these two factors as significant for the HSCT outcome in our group of patients. However, it is possible that those two factors in combination with the reactivity of the HLA-DPB1 allospecific T cells influence the balance between GvHD and GvL. According to data presented in the study by Fleischhauer et al., allogeneic PBSC rejection was mediated by CD4+ T-cells recognizing a HLA-DPB1*09:01 alloantigen (high immunogenic group 1) from the patient's blood [36]. So, it is possible that the strength of HLA-DPB1 immunogenicity directs the HSCT outcome toward GvL or toward GvHD. In that case, a classification of HLA-DPB1 alleles as permissive or non-permissive should be applied in the selection of MUDs to improve HSCT outcome.

In conclusion, patient/MUD pairs matched for HLA-DPB1 were associated with a significantly higher incidence of disease relapse compared to pairs who were permissive or non-permissive HLA-DPB1 mismatched. Our study should be helpful in our transplant centre practice regarding the impact of HLA-DPB1 matching since it is clear that HLA-DPB1 alleles should be treated as transplantation determinant. These results suggest that HLA-DPB1 matching is not preferable for patient/MUD pairs, but this should be confirmed in a larger and more homogeneous patient cohort to help further assess the influence of HLA-DPB1 alleles on outcomes after HSCT.

Conflict of interest:
The authors have declared no conflicting interests.

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Table 1. A literature overview of the HLA-DPB1 allele associations with the unrelated hematopoietic stem cell transplantation outcomes.

Studie	**No of patients**	**OS**	**GvHD**	**NRM/TRM**	**RELAPSE**
Petersdorf EW et al. 2001, [8]	205	HLA-DPB1 MM - no effect	Two HLA-DPB1 MM - increased risk of GvHD	/	/
Loiseau P et al. 2002, [9]	57	Two HLA-DPB1 MM - poorer survival	Two HLA-DPB1 MM - increased GvHD	/	HLA-DPB1 MM - no effect
Zino E et al. 2004, [5]	118	/	Non-p HLA-DPB1 MM – increased aGvHD	Non-p HLA-DPB1 MM - increased TRM	Non-p HLA-DPB1 MM – no effect
Shaw BE et al. 2006, [10]	423	HLA-DPB1 match - worse OS	/	/	HLA-DPB1 match - higher relapse rate
Shaw BE et al. 2007, [11]	5929	HLA-DPB1 MM – no effect	HLA-DPB1 MM - increased risk of aGvHD	HLA-DPB1 MM - higher TRM	HLA-DPB1 MM - decreased relapse
Loiseau P et al. 2007, [12]	334	HLA-DPB1 MM - no effect	HLA-DPB1 MM - no effect	/	HLA-DPB1 MM - no effect
Ludajic K et al. 2008, [13]	161	HLA-DPB1 MM - worse OS	HLA-DPB1 MM – increased GvHD	HLA-DPB1 MM - higher TRM	HLA-DPB1 MM - no effect
Crocchiolo R et al. 2009, [6]	621	Non-p HLA-DPB1 MM – lower OS	Non-p HLA-DPB1 MM - increased GvHD	Non-p HLA-DPB1 MM – increased NRM	Non-p HLA-DPB1 MM - no effect
Fleischhauer K et al. 2012, [14]	8539	/	Non-p HLA-DPB1 MM - increased aGVHD	Non-p HLA-DPB1 MM – increased NRM	Non-p HLA-DPB1 MM – no effect
Bettens F et al. 2012, [15]	246	HLA-DPB1 match - beneficial effect for OS	HLA-DPB1 MM – increased GvHD	/	/
Touzeau C et al. 2012, [16]	141	Non-p HLA-DPB1 MM – no effect	Non-p HLA-DPB1 MM – no effect	/	Non-p HLA-DPB1 MM – no effect
Pidala J et al. 2014, [17]	8003	/	HLA-DPB1 MM – increased GvHD	HLA-DPB1 MM – increased TRM	HLA-DPB1 MM – decreased relapse
Gagne K et al. 2015, [18]	1342	HLA-DPB1 MM – no effect	HLA-DPB1 MM – increased GvHD	HLA-DPB1 MM – no effect	Non-p HLA-DPB1 MM – increased relapse
Moyer AM et al. 2017, [19]	153	HLA-DPB1 MM – no effect	HLA-DPB1 MM – increased cGvHD; Non-p HLA-DPB1 MM - increased aGVHD	HLA-DPB1 MM – no effect	HLA-DPB1 MM – no effect

Legend: GvHD = graft versus host disease (a=acute; c=chronic); HLA = human leukocyte antigen; MM = mismatch; No =Number; Non-p = non-permissive; NRM = non-relapse mortality; OS = overall survival; TRM = transplant relate mortality; / = not investigated in the study

Table 2. Patients and matched unrelated donors characteristics and hematopoietic stem cell transplantation variables.

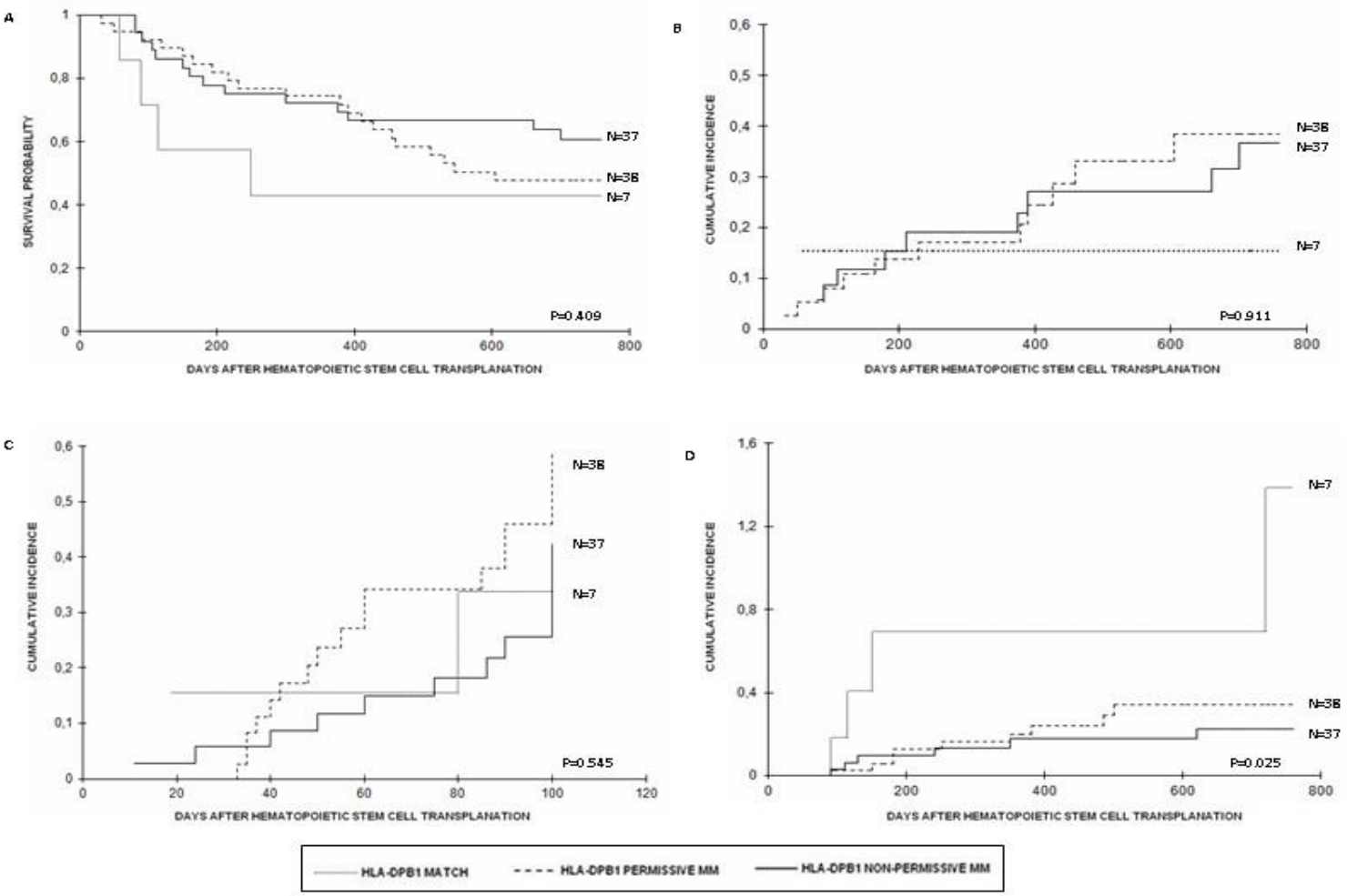
Patient and donor characteristics	**n**	**%**
Number of patient/MUD pairs	82	
Patients age: year, median (range) 40 (1-62)		
Donor age: year, median (range) 34 (18-58)		
Gender - patient/donor:		
Female-female	17	20.73
Female-male	11	13.41
Male-female	24	29.27
Male-male	30	36.59
Diagnosis:		
Acute myelogenous leukaemia (AML)	37	45.1
Acute lymphoblastic leukaemia (ALL)	17	20.7
Myelodysplastic syndrome (MDS)	6	7.3
Chronic myelogenous leukaemia (CML)	3	3.7
Non-Hodgkin lymphoma (NHL)	4	4.9
Hodgkin lymphoma (HL)	3	3.7
Myelofibrosis	2	2.4
Severe combined immunodeficiency (SCID)	3	3.7
Other	7	8.5
Conditioning regimen:		
Myeloablative (MAC)	37	45.12
Reduced intensity (RIC)	45	54.88
Stem cell source:		
Bone marrow (BM)	23	28.05
Peripheral blood (PBSC)	59	71.95
Number of HLA-DPB1 mismatches:		
0	7	8.53
1	50	47.56
2	25	43.91

Legend: HLA = human leukocyte antigen; MUD = matched unrelated donor; n = number;

Table 3. The logistic regression analysis of different risk factors associated with overall survival, non-relapse mortality, graft versus host disease occurrence and relapse incidence in patients with hematological malignancies who underwent HSCT from unrelated donor (N=82).

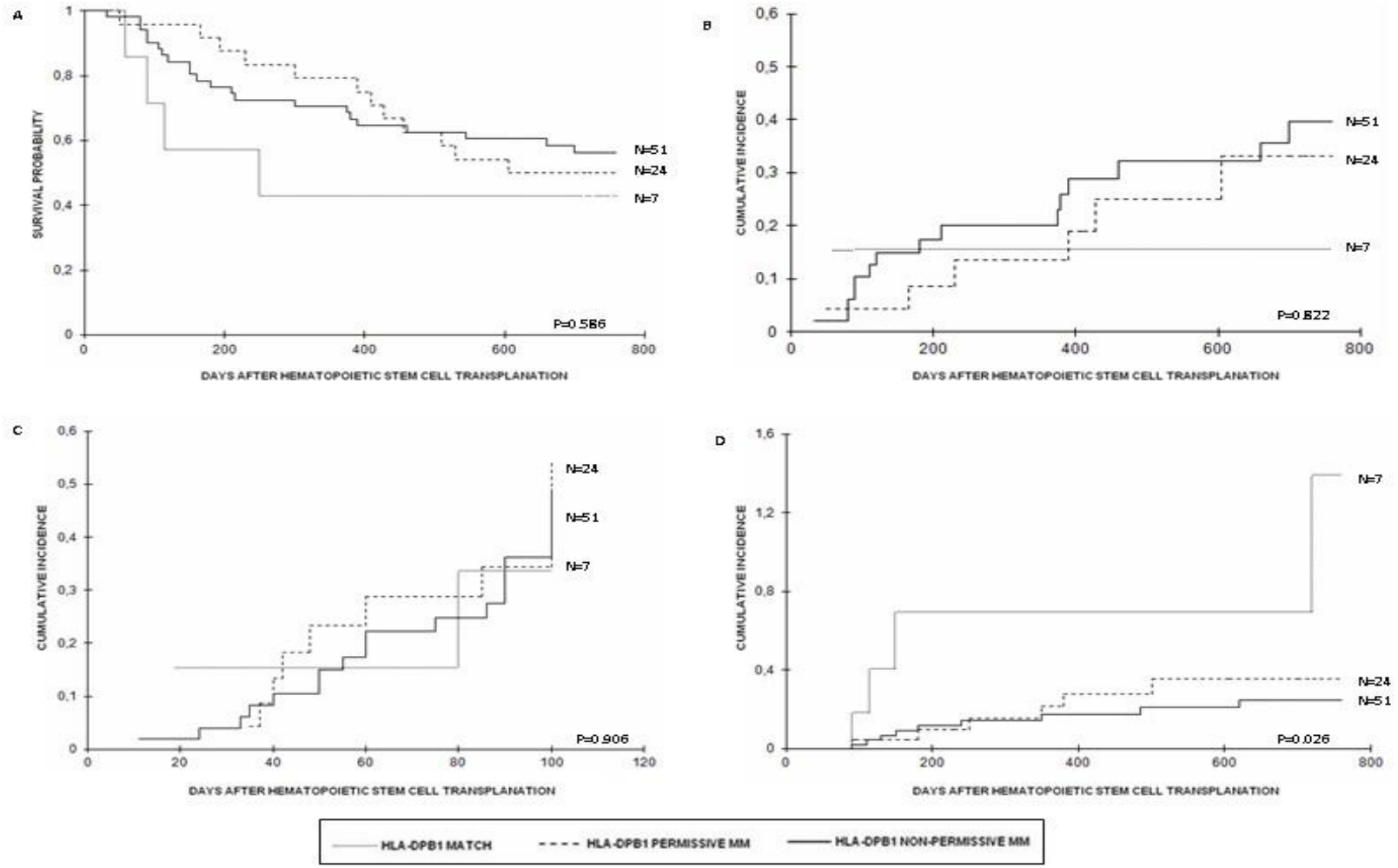
	OS		**NRM**		**GvHD**		**RELAPSE**	
Variable	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P
Patient characteristics:								
Age	2.04 [0.50-8.29]	0.32	7.99 [0.71-90.04]	**0.04**	1.18 [0.28-5.10]	0.81	0.29 [0.05-2.01]	0.22
Gender	0.52 [0.15-1.73]	0.29	1.45 [0.33-6.37]	0.62	2.24 [0.67-7.51]	0.19	0.67 [0.17-2.63]	0.57
Conditioning regimen	0.71 [0.22-2.27]	0.58	1.36 [0.32-5.80]	0.67	1.83 [0.57-5.93]	0.31	0.54 [0.13-2.19]	0.40
Graft type	1.23 [0.36-4.20]	0.74	0.67 [0.14-3.07]	0.61	1.19 [0.33-4.27]	0.78	4.04 [0.76-21.23]	0.10
Diagnosis - myeloid	0.69 [0.15-7.43]	0.79	0.58 [0.25-1.93]	0.24	2.06 [0.45-7.52]	0.82	0.21 [0.14-1.94]	0.18
Diagnosis - lymphoid	1.02 [0.08- 6.12]	0.62	1.37 [0.34-7.70]	0.59	0.27 [0.13-2.01]	0.66	2.21 [0.79-8.23]	0.23
Patient-donor HLA-DPB1 disparity:								
Number of HLA-DPB1 mismatches	0.29 [0.04-2.55]	0.27	3.16 [0.23-41.95]	0.38	2.06 [0.28-14.72]	0.47	0.26 [0.03-3.67]	0.19
TCE3 permissive/non permissive disparity	0.58 [0.15-2.23]	0.43	1.94 [0.10-36.24]	0.65	0.60 [0.16-2.25]	0.45	1.07 [0.23-5.72]	0.92
TCE4 permissive/non permissive disparity	1.68 [0.39-7.21]	0.48	1.62 [0.34-7.78]	0.54	1.12 [0.26-4.67]	0.88	0.66 [0.11-3.72]	0.63

Legend: GvHD = graft versus host disease; HLA = human leukocyte antigen; NRM = non-relapse mortality; OS = overall survival; TCE3 = three-group T-cell epitope; TCE4 = four-group T-cell epitope



Legend: HLA = human leukocyte antigen; MM = mismatch

Figure 1. Association of HLA-DPB1-match and HLA-DPB1 permissive/non-permissive mismatch on HSCT outcomes in patient/matched unrelated donor pairs according to three-group T-cell epitope (TCE3) algorithm: A) overall survival; B) non-relapse mortality; C) graft versus host disease; D) relapse rate.



Legend: HLA = human leukocyte antigen; MM = mismatch

Figure 2. Association of HLA-DPB1-match and HLA-DPB1 permissive/non-permissive mismatch on HSCT outcomes in patient/matched unrelated donor pairs according to four-group T-cell epitope (TCE4) algorithm: A) overall survival; B) non-relapse mortality; C) graft versus host disease; D) relapse rate.