Non-Permissive HLA-DPB1 Disparity is a Significant Independent Risk Factor for Mortality after Unrelated Hematopoietic Stem Cell Transplantation

Running Title: Nonpermissive HLA-DPB1 disparity in unrelated HSCT

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Section TRANSPLANTATION

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ABSTRACT

The importance of donor-recipient human leukocyte antigen (HLA)-DPB1 matching for the clinical outcome of unrelated hematopoietic stem cell transplantation (HSCT) is controversial. We have previously described an algorithm for non-permissive HLA-DPB1 disparities involving HLA-DPB1*0901,*1001,*1701,*0301,*1401,*4501, based on T cell alloreactivity patterns. By revisiting the immunogenicity of HLA-DPB1*02, a modified algorithm was developed and retrospectively tested in 621 unrelated HSCT facilitated through the Italian Registry for onco-hematologic adult patients. The modified algorithm proved to be markedly more predictive of outcome than the original one, with significantly higher Kaplan Meier probabilities of 2-year survival in permissive as compared to non-permissive transplants (55% versus 39%, p=0.005). This was due to increased adjusted hazards of non-relapse mortality (HR =1.74; CI 1.19-2.53; p=0.004) but not of relapse (HR =1.02; CI 0.73-1.42; p=0.92). Taking 10/10 allele matched permissive pairs as reference, the increase in the hazards of overall mortality by non-permissive HLA-DPB1 disparity was similar in 10/10 (HR=2.12; CI 1.23-3.64; p=0.006) and 9/10 allele matched transplants (HR=2.21; CI 1.28-3.80; p=0.004), both in early and in advanced stage disease. These data call for revisiting current HLA matching strategies for unrelated HSCT, suggesting that searches should be directed up-front towards identification of HLA-DPB1 permissive, 10/10 or 9/10 matched donors.
INTRODUCTION

Human Leukocyte Antigen (HLA)-DP was first described in 1980 as a distinct group of HLA class II antigens eliciting variable T cell responses in mixed lymphocyte reactions (MLR)\(^1\). Since then, increasing evidence has accumulated to show that HLA-DP molecules function as \textit{bona fide} restriction elements for viral and tumor antigen-specific T cells\(^2\textsuperscript{-4}\) and can elicit both humoral and cellular alloresponses relevant in clinical transplantation\(^5\textsuperscript{-7}\). The HLA-DP antigens are αβ heterodimers encoded by the genes of the DPA1 locus, which displays limited polymorphism, and of the highly polymorphic DPB1 locus, with 132 alleles coding for 116 different proteins described to date (http://www.ebi.ac.uk/imgt/hla). Due to weak linkage disequilibrium with other HLA class II loci\(^8\), unrelated hematopoietic stem cell transplantation (HSCT) is generally performed across allelic HLA-DPB1 mismatches. The definition of non-permissive mismatches for this locus has therefore important practical implications. Based on cross-reactive T cell alloreactivity patterns, our group has previously described an algorithm for non-permissive HLA-DPB1 mismatches, which were shown by us and subsequently by others to be significantly associated with transplant related (TRM) but not with overall mortality (OM)\(^9\textsuperscript{-11}\).

In the present study, we have modified this algorithm by integrating our functional data with those reported by others\(^12\textsuperscript{-14}\), and tested its clinical predictive value by retrospective analysis of 621 unrelated HSCT facilitated through the Italian Bone Marrow Donor Registry (IBMDR). The results provide compelling evidence that the clinical outcome of unrelated HSCT can be significantly improved by consideration of non-permissive HLA-DPB1 disparity in donor selection.
METHODS

Patients

A total of 621 adult oncohematologic patients (over 18 years) received an unrelated donor (UD) HSCT in Italy between 1999 and 2006, after informed consent was obtained in accordance with the Declaration of Helsinki for protocols approved by the San Raffaele Hospital ethical committee. Eligible diagnoses are listed in Table 1. Clinical data were obtained from Gruppo Italiano Trapianto di Midollo Osseo, CSE e terapia cellulare (GITMO) registry, while the HLA typing results were collected through the IBMDR\textsuperscript{15}. According to disease status at HSCT, patients were categorized into 3 disease groups: CML in first chronic phase (CML-CP1; n=31), acute leukemia in first complete remission (AL-CR1; n=127), and all other diseases including advanced stage leukemia (ADV; n=463). HSCT conditioning regimens were either myeloablative (MA) or reduced intensity (RIC), according to local or cooperative protocols (Table 1). GvHD prophylaxis was mostly performed with cyclosporine and methotrexate.

HLA typing

All 621 pairs were typed for HLA-A, B, C, DRB1, DQB1, DPB1, and 616, 537 and 277 pairs also for DRB3/4/5, DQA1 and DPA1, respectively, by standard methods including sequence-specific oligonucleotide probing (SSO), sequence-specific priming (SSP) and/or sequence based typing (SBT). For all loci tested, typing was performed to the 4-digit level, according to the quality standards of the European Federation of Immunogenetics (EFI) which foresee resolution of all alleles differing for exons 2 and 3 for HLA class I, and exon 2 for HLA class II, as well as all Null alleles (http://www.efiweb.eu/index.php?id=102).
Clinical endpoint definitions

Overall survival (OS), non-relapse mortality (NRM), graft failure and Relapse Incidence (RI) were defined according to EBMT criteria (http://www.ebmt.org/). Grading of aGvHD was performed according to current criteria\textsuperscript{16}.

Statistical analysis

Continuous variables were expressed as median (range) while categorical ones were expressed as proportions. Chi-square and Mann-Whitney U-tests were used for comparisons between major clinical parameters in permissive and non-permissive pairs for categorical and continuous variables, respectively, and did not reveal any significant differences between the two groups. Probabilities of OS with respective 95% confidence interval (CI) were calculated using the Kaplan-Meier estimator\textsuperscript{17} and survival curves were compared using the log-rank test\textsuperscript{18}. Univariate regression analysis was used to test association between HLA or non-HLA variables and OM, NRM, RI (Cox regression)\textsuperscript{19}, aGvHD and graft failure (logistic regression). For numerical reasons, in uni- and multivariate analyses, pairs with ≥ 2 HLA mismatches at loci other than HLA-DPB1 were grouped together. Variables with a p<0.20 were included in the multivariate analysis and only variables with a p<0.05 were retained in the final multivariate model. Non-HLA factors included donor gender, age (continuous variable) and CMV status; patient gender, age (continuous variable), CMV status; year of transplantation (continuous variable), use of anti-thymocyte globulin (ATG) as GvHD prophylaxis, disease group (CML-CP1 versus AL-CR1 versus ADV), stem cell source (peripheral blood versus bone marrow), conditioning regimen (MA versus RIC), use of total body irradiation (TBI). HLA-DPB1 permissiveness was tested for interaction with the number of HLA mismatches and the term of interaction was 0.03 for both OM and NRM (0-1 versus >1 HLA mismatch). No significant interactions resulted with regards to the other clinical endpoints.
RESULTS

A modified algorithm for non-permissive HLA-DPB1 mismatches

We have previously described an algorithm for non-permissive HLA-DPB1 disparities, on the basis of cross-reactive patterns by alloreactive T cells involved in HSCT rejection targeted to HLA-DPB1*0901\(^{11}\). This algorithm foresees group-specific rather than allele-specific HLA-DPB1 matching, dividing HLA-DPB1 alleles into 3 groups with high (HLA-DPB1*0901,*1001,*1701), intermediate (HLA-DPB1 *0301,*1401,*4501) or low (most other HLA-DPB1 alleles) immunogenicity\(^{11,20}\), presumably on the basis of a shared alloreactive T cell epitope (TCE). The modality of classification of donor-recipient pairs as permissive or non-permissive according to this 3-group algorithm, henceforward referred to as TCE3, is described in Figure 1. By retrospective analysis of unrelated HSCT stratified according to TCE3, we and others showed an association of non-permissive HLA-DPB1 mismatches with TRM, grade 2-4 aGvHD and graft rejection, in 10/10 matched pairs\(^ {9-11,21}\).

The patient from whom the T cell clones tested to define the TCE3 were derived, shared HLA-DPB1*0201 with her stem cell donor\(^ {22}\). Due to negative selection of potentially self-reactive T cells from the patient’s repertoire, the T cell clones used for establishing the algorithm were not informative for HLA-DPB1*02, which might encode a second, distinct immunogenic TCE. The existence of such an epitope is indeed suggested by previous reports showing that HLA-DPB1*0201 elicits T cell responses in classical MLR, although apparently to a lower extent as compared to the antigens encoded by the immunogenic alleles from the 3-group algorithm\(^ {13,14,23}\). On the basis of these observations, we designed a 4-group algorithm including HLA-DPB1*02 as a separate group with immunogenicity lower than that of group 2 alleles but higher than that of the low immunogenic alleles. This algorithm is henceforward referred to as TCE4, and is described in Figure 1.
HLA Matching of Patients and Donors

Of the 621 pairs studied, 41 were 4-digit matched for all 12 HLA alleles including DPB1 (12/12), while 43 were identical for both HLA-DPB1 alleles but presented one or more mismatches at other HLA loci, for a total of 84 DPB1 matched pairs. The remaining 537 pairs presented at least one allelic mismatch at HLA-DPB1, with zero (10/10; n=201), one (9/10; n=199) or more than one (≤8/10; n=137) allelic or antigenic mismatches at the other loci. 616 pairs were also typed for HLA-DRB3/4/5; of these, only 66 pairs (10.7%) had a mismatch for at least one of these three loci while the remaining 550 pairs (89.3%) were matched (Table 1). 537 and 277 pairs were also typed for HLA-DQA1 and DPA1, respectively. Only 21/537 pairs (3.9%) were DQA1 mismatched, while 131/277 pairs (47.3%) presented mismatches at DPA1 (Table 1).

On the basis of HLA-DPB1 frequencies in the Caucasian population, it can be predicted that permissive HLA-DPB1 mismatches according to TCE3 or TCE4 are present in approximately 55% and 30% of pairs, respectively. This was confirmed in our cohort of 537 HLA-DPB1 allele mismatched pairs. When classified according to TCE3, 287 (53.4%) scored as permissive, and 145 (27.0%) or 105 (19.6%) as non-permissive in graft versus host (GvH) or host versus graft (HvG) direction, respectively. When classified according to TCE4, 158/537 (29.4%) scored as permissive, and 219/537 (40.8%) or 160/537 (29.7%) as non-permissive in graft versus host (GvH) or host versus graft (HvG) direction, respectively (Table 1).

Clinical Outcome

Kaplan Meier Estimates of Survival. The observed probability of survival at 2 years in the total cohort was 44% (273 of 621 patients). In line with previous reports, observed survival probabilities in these 621 patients were not markedly different after HLA-DPB1 allele matched (43%) versus mismatched (44%) transplants. Also when 242 transplants fully matched for HLA-A,B,C,DRB1,DQB1 alleles were considered separately, allelic DPB1 mismatches did not have a
significant impact on the probability of OS (95% CI) at 2 years which was 55% (39-70) and 47% (39-54) for DPB1 allele matched (12/12, n=41) or mismatched (10/10, n=201) transplants, respectively (p=0.84; Figure 2A). When pairs with allelic DPB1 disparities were further subdivided into those with permissive (n=287) or non-permissive GvH (n=145) or HvG (n=105) disparities according to TCE3, there was a trend for worse survival in the non-permissive pairs which however was not statistically significant (47% (41-53) in the permissive versus 38% (30-46) in the non-permissive GvH and 41% (31-51) in the non-permissive HvG group; p=0.23; Figure 2B, left panel). This is in line with previous observations made by us and others which failed to document a significant association between non-permissive DPB1 disparities according to TCE3, and OS in global cohorts of patients with varying degrees of matching for the other loci\textsuperscript{9-11}. TCE3-permissive pairs could be further subdivided into those classified as permissive also according to TCE4 (n=158), and those classified as non-permissive in GvH (n=74) or HvG (n=55) direction according to TCE4. Interestingly, the 2 year OS probabilities associated with these non-permissive TCE4 disparities were 40% (28-52) for the GvH and 36% (23-49) for the HvG group, significantly lower as compared to the permissive TCE4 disparities (55% (46-63); p=0.008; Figure 2B, middle panel). This translated into a significant impact of non-permissive HLA-DPB1 TCE4 disparities on OS in the total cohort, with 2 year survival probabilities of 55% (46-63), 39% (32-45) and 40% (32-47) in the permissive (n=155), GvH (n=219) or HvG (n=160) group, respectively (p=0.005; Figure 2B right panel). Given the similar impact of non-permissive GvH and HvG mismatches on survival (p=0.94 in the heterogeneity test) and all other clinical endpoints studied (data not shown), data for the two groups from here on are shown together.

The impact of non-permissive HLA-DPB1 mismatches on OS was also analyzed in the subgroups matched for 10/10, 9/10 or \(\leq 8/10\) of the HLA-A,B,C,DRB1,DQB1 alleles. Concordant with our previous findings\textsuperscript{11}, non-permissive DPB1 disparities defined according to TCE3 were significantly predictive of survival in the 10/10 matched pairs, with 2 year survival probabilities of 53% (43-63)
versus 40% (29-51) (p=0.03; Figure 3A, left panel). Also in these pairs, however, further
classification of the TCE3-permissive mismatches into TCE4-permissive or TCE4-non-permissive,
demonstrated that the latter had a significantly lower probability of survival as compared to the
former (65% (52-78) versus 39% (24-54); p=0.02; Figure 3A, middle panel). Note that the survival
probabilities associated with non-permissive mismatches classified as such in TCE4 but not in
TCE3 were very similar to those associated with non-permissive TCE3 disparities (40% versus
39%). In line with this, in the 10/10 matched pairs overall, non-permissive HLA-DPB1 disparities
according to TCE4 were highly predictive of OS, with 2-year survival probabilities of 65% (53-78)
versus 40% (31-48) in TCE4-permissive pairs (p=0.003; Figure 3A, right panel). Importantly,
TCE3 was not predictive of OS in 9/10 matched pairs (48% (38-58) versus 39% (29-49) in the
TCE3 permissive and non-permissive pairs; p=0.42; Figure 3B, left panel). Again, pairs classified
as TCE3-permissive could be further subdivided into TCE4-permissive or TCE4-non-permissive,
and the latter had a significantly lower probability of survival as compared to the former (55% (40-
70) versus 41% (27-55); p=0.016; Figure 3B, middle panel). As a result, TCE4 was significantly
predictive of OS also in the 9/10 matched pairs, with 2-year survival probabilities of 55% (40-70)
versus 39% (31-48; p=0.02) in TCE4-permissive versus non-permissive pairs (Figure 3B, right
panel). In contrast, the effect of non-permissive HLA-DPB1 mismatches was abrogated by the
presence of 2 or more mismatches at other HLA loci, both for TCE3 and TCE4 (37% (31-43)
versus 39% (26-52); p=0.72 for TCE3 and 40% (25-55) versus 37% (27-47); p=0.61 for TCE4;
Figure 3C, left and right panel). Importantly, the survival estimates in patients transplanted from
10/10 or 9/10 matched donors with TCE4-non-permissive HLA-DPB1 mismatches were similar to
that of patients transplanted from ≤8/10 matched donors overall (40% versus 38%; p=0.64).
The survival advantage mediated by HLA-DPB1 TCE4-permissiveness in 10/10 or 9/10 matched
transplants was observed not only in early disease stage patients (85% versus 48%; p=0.004) but
also, although less markedly, in advanced disease stage patients (47% and 35%; p=0.02) (Figure 4).
Cox and Logistic regression analysis of OM, NRM, Graft Failure, aGvHD and RI. The survival advantage mediated by the presence of permissive rather than non-permissive HLA-DPB1 TCE4 mismatches was also observed in unadjusted as well as adjusted Cox regression models of OM. Non-permissive HLA-DPB1 TCE4 disparity was found to be a significant risk factor for OM (HR =1.50; CI 1.13-2.01; p=0.005), independently from other significant non-HLA variables including donor and patient gender, patient age, conditioning regimen (MA or RIC), stem cell source and disease group (CML-CP1, AL-CR1, or ADV). In line with previous reports\textsuperscript{26,27}, the presence or absence of additional mismatches at HLA-DRB3/4/5 (HR=0.97; CI 0.69-1.40; p=0.88), DQA1 (HR=1.03; CI 0.58-1.84; p=0.91) and DPA1 (HR=1.20; CI 0.88-1.63; p=0.25) did not have a significant impact on OM, neither in patients overall, nor in the subgroups of patients scored as TCE4-permissive or non-permissive (data not shown). Taking 10/10 allele matched, HLA-DPB1 TCE4-permissive transplants as reference, the adjusted hazards of OM were significantly increased by the presence of TCE4-non-permissive disparities in 10/10 and 9/10 allele matched transplants (Table 2).

The increase in mortality risk was due to a significant increase in NRM in adjusted models associated with non-permissive TCE4 disparities (HR =1.74; CI 1.19-2.53; p=0.004 overall, and Table 2). In the total cohort, there was also a significant increase in the adjusted hazards of aGvHD 3-4 in the TCE4-non-permissive GvH as compared to the other groups (HR =1.89; CI 1.12-3.21; p=0.02), which was however dependent on allelic mismatches at other loci, since it was not evident in the separate analysis of the 10/10 or 9/10 allele-matched subgroups (Table 2). Also the hazards of relapse were not significantly different between the TCE4-non-permissive GvH as compared to the combined TCE4-permissive and HvG groups (HR =1.02; CI 0.73-1.42; p=0.92 overall and Table 2). These findings suggest that non-permissive TCE4 disparities, different from allelic HLA-DPB1 mismatches, do not significantly increase the risk of GvHD and do not enhance graft versus leukemia (GvL) activity. There was a trend for increased incidence of graft failure in non-
DISCUSSION

Following current national and international guidelines, unrelated HSC donor-recipient searches are primarily based on 4-digit typing for HLA-A, B, C and DRB1 alleles, because matching for these alleles has been shown to significantly improve clinical outcome in terms of OS, NRM and aGvHD (http://www.marrow.org, http://www.ibmdr.galliera.it, and refs. 28-30). Donor-recipient pairs high resolution matched for these 4 loci (8/8), in most instances are also matched for HLA-DQB1 (10/10), due to strong linkage disequilibrium between DRB1 and DQB1 alleles8. In contrast, HLA-DPB1 displays weak linkage disequilibrium with the other class II loci8, and therefore only approximately 15% of 10/10 matched pairs are also 4-digit allele matched for HLA-DPB1 (12/12)31. It has recently been shown that matching for HLA-DPB1 is a double-edged sword, since it significantly reduces NRM and aGvHD, but on the other hand increases the hazards of disease relapse, ultimately resulting in no significant advantage in OS24,25. Our previous data have shown that in 10/10 matched pairs, group-specific rather than allele-specific HLA-DPB1 matching on the basis of T cell alloreactivity patterns defining the 3-group algorithm TCE3, is significantly predictive of survival11. This finding was confirmed in the present study. However, we also demonstrate that the TCE3 misclassifies approximately 50% of the permissive pairs involving HLA-DPB1*02, since these have a probability of survival as low as those classified as TCE3-non-permissive (Figures 2 and 3). As a result, the modified 4-group algorithm TCE4 we developed in the present study is markedly more predictive of OS than TCE3, both in 10/10 and in 9/10 matched pairs (Figure 3) and in the total cohort of all 537 informative patients (Figure 2). Our data demonstrate in fact that survival probabilities can be significantly increased by selecting donors with TCE4-permissive HLA-DPB1 disparities. Importantly, this advantage was observed not only
in patients transplanted with acute leukemia in first complete remission, in whom the impact of donor-recipient HLA matching status is known to be most pronounced, but also in patients with advanced disease at transplantation (Figure 4). On the basis of these data, we suggest that HLA-DPB1 TCE4 disparity should be characterized up-front in unrelated donor searches, in order to prospectively direct selection of potentially 10/10 or 9/10 matched donors towards those presenting TCE4-permissive HLA-DPB1 mismatches.

While the observation that survival probabilities after unrelated HSCT can be significantly improved by “intelligent” donor selection based on avoidance of TCE4-non-permissive HLA-DPB1 disparity is good news for patients, the bad news is that approximately 70% of HLA-DPB1 allelic mismatches found in Caucasian or Japanese donor-recipient pairs are predicted to score as TCE4-non-permissive. This is due to the fact that HLA-DPB1*02, classified as immunogenic in TCE4 but not in TCE3, has an allelic frequency of 20% in Caucasians and Japanese. Based on this, the overall predicted probability for a given donor-recipient pair to be TCE4-permissively HLA-DPB1 mismatched is 26%, regardless of matching status at the other HLA loci. However, extension of the search to include both the 10/10 and the 9/10 allele matched pool, which in our cohort was present for 400/537 (74%) or transplants, raises the chances of identifying a TCE4-permissive donor. In fact, in our retrospective study of randomly selected donors with regards to HLA-DPB1, 115/537 (21.4%) of transplants were performed from 10/10 or 9/10 matched, HLA-DPB1 TCE4-permissive donors (Table 1). This number might increase if HLA-DPB1 TCE4-permissiveness were to be included prospectively into the algorithms for donor selection.

It is interesting to note that, different from allelic HLA-DPB1 mismatches, TCE4-non-permissive DPB1 disparities did not further enhance GvHD or GvL in our patients (Table 2). This suggests that NRM and GvHD/GvL may be governed by distinct immunological mechanisms, an observation that deserves further investigation since it might have potential impacts on how to exploit HLA-DPB1 disparity for the prevention of disease relapse after unrelated HSCT.
The molecular nature of the epitope target of preferential T cell alloreactivity directed against HLA-DPB1 is as yet largely elusive. The patient from whom the HLA-DPB1-specific T cells used to originally define non-permissive disparities were derived, was not informative for HLA-DPB1*02 since DPB1*0201 was shared between the patient and her stem cell donor and T cells specific for the antigen encoded by this allele are likely to have been deleted from the patient’s repertoire. Consequently, the shared epitope recognized by cross-reactive alloreactive T cells on HLA-DP antigens from group 1 and 2 alleles can be predicted to be structurally different from the epitope recognized by HLA-DPB1*02-specific T cells. Interestingly, group 1 and 2 alleles share most amino acid residues in regions A and F of the HLA-DP beta chain, and are in linkage disequilibrium with DPA1*02. In contrast, HLA-DPB1*02 has markedly different amino acid sequences in regions A and F, and is found in linkage disequilibrium with DPA1*01, further supporting the notion that the relevant T cell epitope encoded by this allele is substantially different from the other. The role of the DPα chain, encoded in cis or in trans, in formation of the relevant epitopes remains to be investigated. The HLA-DPA1 matching status in this study was not significantly predictive of survival; however, DPA1 typing was available for only 51% of the pairs. Moreover, the role of defined DPA1-DPB1 combinations remains to be investigated in larger transplant cohorts.

Our data demonstrate that the approach used here to define matching algorithms on the basis of T cell alloreactivity is a powerful strategy for the characterization of non-permissive mismatches in unrelated HSCT. This objective is subject of rising interest in the field, since it has become increasingly difficult to find allele-level matched UDs due to the impressive degree of polymorphism unraveled by increasingly sophisticated HLA typing techniques. Recently, different authors have attempted to define non-permissive disparities by structural comparison of amino acid sequences encoded by mismatched alleles. The results of such approaches are dependent on the availability of high-powered statistical analysis, given the extreme complexity of this type of
comparison. In addition, structural approaches are likely to miss immunogenic epitopes dependent on conformational mismatches as well as peptide-dependent epitopes, which have been shown to be relevant for T cell alloreactivity, and are therefore more problematic than similar strategies used to define permissive mismatches for humoral alloreactivity in solid organ transplantation\textsuperscript{35}. The use of alloreactive T cell cross-reactivity patterns circumvents these problems and might in the future be applied also for defining non-permissive mismatches at other HLA loci.

Taken together, our data demonstrate that group-specific HLA-DPB1 matching according to TCE4 is markedly more predictive for the clinical outcome of unrelated HSCT than the original TCE3, showing a significant association with NRM and OS in 10/10 and 9/10 matched transplants. These findings call for revisiting current concepts of unrelated donor-recipient matching, suggesting that UD searches should be directed up-front towards identification of a 10/10 or 9/10 matched donor presenting TCE4-permissive HLA-DPB1 disparities. By using this strategy, patients both in early and in advanced stage disease could potentially be offered a significantly improved chance of survival after transplantation.
ACKNOWLEDGEMENTS

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AUTHORSHIP

Contribution: R.C. designed the study, collected data, and performed statistical analyses; E.Z and J.M. developed the algorithm for non-permissive HLA-DPB1 disparities; L.V. prepared the figures and critically reviewed the manuscript; R.O. and B.B. collected clinical data; T.L., R.F., G.B., and A.Bo provided advice and participated in general discussion; S.P. and N.S. collected immunogenetics data; M.P.S. supervised statistical analyses; L.G. and V.M. performed HLA typing; F.C. and A.Ba. counseled on study design and participated in critical discussion; K.F. supervised the study and wrote the manuscript.

Conflict-of-interest disclosure: We declare that a patent application describing the results of the manuscript has been filed on behalf of the San Raffaele Scientific Institute and that the patent application has not been published yet.
REFERENCES


TABLE 1. Patient, donor and transplant characteristics.

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TABLE 2. Cox Regression models for OM, NRM and relapse and logistic regression models for graft failure and aGvHD.

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<td>aGvHD 2-4†</td>
<td>aGvHD 3-4†</td>
<td>Relapse†</td>
<td>OM*</td>
<td>NRM*</td>
<td>Graft failure</td>
<td>aGvHD 2-4†</td>
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<td>HLA-DPB1 Non-Permissive</td>
<td>(1.26-3.30; p=0.003)</td>
<td>(1.58-7.01; p=0.002)</td>
<td>(0.74-45.4; p=0.09)</td>
<td>(0.52-2.11; p=0.88)</td>
<td>(0.49-6.74; p=0.37)</td>
<td>(0.69-2.16; p=0.49)</td>
<td>(1.23-3.64; p=0.006)</td>
<td>(1.45-8.02; p=0.005)</td>
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<td>HLA-DPB1 Permissive</td>
<td>(0.72-2.40; p=0.36)</td>
<td>(1.06-5.80; p=0.04)</td>
<td>(0.19-25.1; p=0.52)</td>
<td>(0.81-4.12; p=0.14)</td>
<td>(1.31-19.2; p=0.02)</td>
<td>(0.61-2.40; p=0.58)</td>
<td>(0.71-2.69; p=0.33)</td>
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<td>9/10 allele matched</td>
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<td>HLA-DPB1 Non-Permissive</td>
<td>(1.42-3.69; p=0.001)</td>
<td>(2.01-8.72; p=0.0001)</td>
<td>(0.88-52.9; p=0.06)</td>
<td>(0.95-3.65; p=0.07)</td>
<td>(1.21-14.4; p=0.02)</td>
<td>(0.70-2.22; p=0.44)</td>
<td>(1.28-3.80; p=0.004)</td>
<td>(1.58-8.61; p=0.002)</td>
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<td>≤8/10 allele matched</td>
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<td>HLA-DPB1 Irrespective</td>
<td>(1.43-3.71; p=0.001)</td>
<td>(1.88-8.23; p=0.0001)</td>
<td>(0.76-47.0; p=0.00)</td>
<td>(0.90-3.53; p=0.09)</td>
<td>(0.67-6.68; p=0.18)</td>
<td>(0.74-2.31; p=0.36)</td>
<td>(1.18-3.54; p=0.01)</td>
<td>(1.30-7.19; p=0.01)</td>
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</table>

* TCE4-non-permissive mismatches were considered in the combined group of GvH and HvG direction, and confronted with permissive mismatches. Numbers were for 10/10 allele matched pairs: n=140 non-permissive, n=61 permissive; for 9/10 allele matched pairs: n=145 non-permissive, n=54 permissive; for ≤8/10 allele matched pairs: n=137.
† TCE4-non-permissive mismatches were considered only in the GvH direction, and confronted with the combined group of TCE4-permissive mismatches and TCE4-non-permissive mismatches in HvG direction. Numbers were for 10/10 allele matched pairs: n=72 non-permissive, n=129 permissive; for 9/10 allele matched pairs: n=85 non-permissive, n=114 permissive; for ≤8/10 allele matched pairs: n=137.

‡ N.A.: not applicable since the number of events in each subgroup was too limited to allow statistically meaningful analysis.

¶ N.A.: not applicable since the p-value in univariate models was >0.2.

Multivariate models include gender, age and CMV status of donor and patient, year of transplantation, use of ATG, disease group, stem cell source, conditioning regimen and use of TBI.
**FIGURE LEGENDS**

**Figure 1:** An algorithm for non-permissive HLA-DPB1 disparities according to TCE3 or TCE4. A) HLA-DPB1 alleles were classified into 3 groups (TCE3), or 4 groups (TCE4), on the basis of T cell alloreactivity. TCE3 Group 1 and TCE4 Group 1: Alleles encoding antigens recognized by all T cell clones studied by Zino et al\textsuperscript{11}. TCE3 Group 2 and TCE4 Group 2: Alleles encoding antigens recognized by some but not all T cell clones studied by Zino et al\textsuperscript{11}. TCE 3 Group 3: Alleles encoding antigens recognized by none of the T cell clones studied by Zino et al\textsuperscript{11}. “Others” refer to all alleles that can be classified according to the algorithm of Zino et al\textsuperscript{20}. TCE4 Group 3: DPB1\textsuperscript{*02}, encoding antigens eliciting intermediate levels of MLR reactivity\textsuperscript{13,14,23}. TCE4 Group 4: All alleles from TCE3 Group 3 except for DPB1\textsuperscript{*02} B) The 3 or 4 groups of HLA-DPB1 alleles can be present in different combinations in diploid cells. Numbers indicate the group of the first (before the slash) and the second (after the slash) HLA-DPB1 allele of donor or recipient. Classification of HLA-DPB1 group disparities as permissive or non-permissive in GvH or HvG direction is indicated for all possible combinations. Note that all non-permissive TCE3 disparities are also TCE4-non-permissive (grey boxes). In contrast, only a part of the TCE3-permissive disparities are permissive also according to TCE4 (white boxes), while the remaining TCE3-permissive disparities score as non-permissive in TCE4 (striped boxes).

**Figure 2:** Impact of allelic or allele-group HLA-DPB1 disparities on OS after unrelated HSCT. Shown are Kaplan Meier estimates of survival. A) HLA-A,B,C,DRB1,DQB1 matched transplants (n=242), stratified according to the presence (10/10; solid line) or absence (12/12; dotted line) of allelic DPB1 mismatches. B) Left
panel: All HLA-DPB1 mismatched transplants (n=537), stratified according to the presence of TCE3-permissive (solid line) or TCE3-non-permissive HvG (dotted line) or GvH (dash-dot line) mismatches. Middle panel: TCE3-permissive transplants (n=287), subdivided into those permissive also according to TCE4 (solid line), or those TCE4-non-permissive in HvG (dotted line) or GvH (dash-dot line). Right panel: All HLA-DPB1 mismatched transplants (n=537), stratified according to the presence of TCE4-permissive (solid line) or TCE4-non-permissive HvG (dotted line) or GvH (dash-dot line) mismatches.

Figure 3: Predictive value of TCE3 and TCE4 for OS after unrelated HSCT, stratified according to matching status at other HLA loci. Shown are Kaplan Meier estimates of survival after HLA-DPB1 allele mismatched transplants, matched for 10/10 (panel A; n=201), 9/10 (panel B; n=199) or ≤8/10 (panel C; n=137) of the alleles at HLA loci A,B,C,DRB1,DQB1. Left panels: Transplants were stratified according to the presence of TCE3-permissive (solid lines) or non-permissive (dashed lines) HLA-DPB1 mismatches. Middle panels: TCE3-permissive transplants were further subdivided into those permissive also according to TCE4 (solid lines) and those non-permissive according to TCE4 (dashed lines). Right panels: Transplants were stratified according to the presence of TCE4-permissive (solid lines) or TCE4-non-permissive (dashed lines) HLA-DPB1 mismatches.

Figure 4: Association of non-permissive HLA-DPB1 disparities according to TCE4 with increased mortality is seen both in early and in advanced disease. Kaplan Meier estimates of survival after 10/10 or 9/10 allele matched unrelated HSCT for early-stage acute leukemia (AL-CR1, n=81; left panel) or advanced disease (ADV, n=296; right
panel). Transplants were divided into TCE4-permissive (solid lines) or TCE4-non-permissive HLA-DPB1 disparities (dashed lines).
### Figure 1

#### A

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#### B

**RECIPIENT DPB1 GROUP**

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<td>1/3</td>
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</table>

- **Permissive**
- **Non-permissive HvG**
- **Non-permissive GvH**

- 🟢 Permissive in TCE3 and TCE4
- ⚫ Permissive in TCE3, but not in TCE4
- ⭕ Non-permissive in TCE3 and TCE4
Figure 2

A

B

Survival Probability vs. Follow-up Months

TCE3

TCE3-PERMISSIVE

Survival Probability vs. Follow-up Months

TCE4

Survival Probability vs. Follow-up Months
Figure 3

10/10 ALLELE-MATCHED

- **TCE3**
  - TCE3-permissive (n=110)
  - TCE3-non-permissive (n=91)
  - p=0.03

- **TCE3-PERMISSIVE**
  - TCE3-permissive (n=54)
  - TCE3-non-permissive (n=50)
  - p=0.016

- **TCE4**
  - TCE4-permissive (n=61)
  - TCE4-non-permissive (n=49)
  - p=0.003

Follow-up Months

9/10 ALLELE-MATCHED

- **TCE3**
  - TCE3-permissive (n=104)
  - TCE3-non-permissive (n=95)
  - p=0.42

- **TCE3-PERMISSIVE**
  - TCE3-permissive (n=54)
  - TCE3-non-permissive (n=50)
  - p=0.016

- **TCE4**
  - TCE4-permissive (n=54)
  - TCE4-non-permissive (n=43)
  - p=0.02

Follow-up Months

≤8/10 ALLELE-MATCHED

- **TCE3**
  - TCE3-permissive (n=72)
  - TCE3-non-permissive (n=65)
  - p=0.72

- **TCE3-PERMISSIVE**
  - TCE3-permissive (n=43)
  - TCE3-non-permissive (n=29)
  - p=0.61

- **TCE4**
  - TCE4-permissive (n=43)
  - TCE4-non-permissive (n=94)
  - p=0.88

Follow-up Months
Figure 4

EARLY DISEASE

Survival Probability

Follow-up Months

TCE4-permissive (n=27)

TCE4-non-permissive (n=54)

p=0.004

ADVANCED DISEASE

TCE4-permissive (n=79)

TCE4-non-permissive (n=217)

p=0.02
Non-permissive HLA-DPB1 disparity is a significant independent risk factor for mortality after unrelated hematopoietic stem cell transplantation

Roberto Crocchiolo, Elisabetta Zino, Luca Vago, Rosi Oneto, Barbara Bruno, Simona Pollichieni, Nicoletta Sacchi, Maria Pia Sormani, Jessica Marcon, Teresa Lamparelli, Renato Fanin, Lucia Garbarino, Valeria Miotti, Giuseppe Bandini, Alberto Bosi, Fabio Ciceri, Andrea Bacigalupo and Katharina Fleischhauer