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Role of HLA-B Exon 1 in Graft-versus-Host Disease After Unrelated Donor Transplantation: A Retrospective Cohort Study

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Summary

Background—The success of unrelated hematopoietic-cell transplantation (HCT) is limited by graft-versus-host disease (GVHD). A sequence dimorphism in exon one of *HLA-B* gives rise to leader peptides containing methionine (M) or threonine (T) which differentially influence NK and T-cell alloresponses. The main aim of the study was to evaluate the role of the leader dimorphism in GVHD after HLA-B-mismatched unrelated HCT.

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Contributors

EWP and MC designed the study. All authors assembled the data. COH, MJM, PS and TG performed statistical analysis. EWP drafted the manuscript. All authors critically reviewed and edited the manuscript and approved the final version.

Data Sharing

This study is not a clinical trial.

Methods—The impact of HLA-A,B,C,DRB1,DQB1 mismatching was defined in a retrospective cohort study of 33,982 unrelated transplants performed between 01/01/1988 and 31/12/2016. Risks of GVHD associated with M- and T-leaders were determined using multivariate regression models in 17,100 HLA-matched and 1,457 single HLA-B-mismatched transplants. Leader frequencies were defined in 2,004,742 BeTheMatch® registry donors.

Findings—Median follow-up was 1841 days (interquartile range, 2054 days). Mortality and GVHD increased with increasing numbers of HLA mismatches. A single HLA-B mismatch increased grades III-IV acute GVHD (odds ratio 1·89, 95% CI 1·53–2·33, p<0·0001). Acute GVHD risk was higher with leader-mismatching compared to leader-matching (odds ratio 1·73, 95% CI 1·02–2·94, p=0·042 for grades II-IV) and with an M-leader shared allotype compared to a T-leader shared allotype (odds ratio 1·98, 95% CI 1·39–2·81, p=0·00014 for grades III-IV). The preferred HLA-B-mismatched donor is leader-matched and shares a T-leader allotype. The majority of the two million registry donors have TT or MT genotype.

Interpretation—The HLA-B leader informs GVHD risk after HLA-B-mismatched unrelated HCT and differentiates high-risk HLA-B mismatches from those with lower risk. A new paradigm for GVHD considers the leader of the matched allotype to be as important as the leader of the mismatched allotype. Prospective identification of leader-matched donors is feasible for the vast majority of patients in need of a transplant, and may lower GVHD and increase availability of HCT as curative therapy.

Keywords

unrelated donor; hematopoietic-cell transplantation; HLA-B leader; graft-versus-host disease

Introduction

Advances in clinical practice have improved outcome after unrelated donor HCT.[1] The mainstay of donor selection is matching of the HLA peptide-binding region to lower the risk of GVHD.[2] When HLA-matched donors are unavailable, mismatching between alleles of the same serological specificity (allele-mismatch) lowers GVHD risk compared to mismatching between serologically-different allotypes (antigen-mismatch) for some but not all HLA genes.[3,4]

HLA-B is the most polymorphic protein-encoding locus in the human genome.[5] Clinically relevant variation may include epitopes of the expressed HLA-B molecule as well as leader peptides.[6–10] Not currently considered in clinical practice are the HLA class I leader peptides which are preferentially bound by HLA-E, the ligand for CD94/NKG2 natural killer (NK) receptors.[11–13] The rs1050458C/T dimorphism at position −21 of exon one of *HLA-B* gives rise to leader peptides with either methionine (M) or threonine (T) at the second residue of the processed leader peptide, whereas virtually all HLA-A and HLA-C leaders have methionine.[5] M- and T-leaders differentially influence HLA-E expression and the strength of inhibitory and activating NK and T-cell responses,[9–13] particularly those involved in HIV control.[8,10]

Further titration of the immune response is contributed by *HLA-E*, *HLA-A* and *HLA-C*.[8–10, 14] The two major alleles E*01:01 and E*01:03 have nearly identical structure, occur at similar frequencies worldwide, but are differentially expressed.[15,16] HLA-C contributes C1 and C2 ligands for KIR2DL receptors. HLA-A expression levels vary in an allele-dependent manner,[17] where higher HLA-A expression contributes more leader peptide, enhancing HLA-E stability and expression.[10] The complexity of allorecognition is compounded by the physical linkage of *HLA-E, C, A* and *B* on the same haplotype. Positive linkage disequilibrium (LD) favors the association of certain M- and T-leader allotypes with certain KIR ligands or HLA-E and HLA-A alleles, tipping the immune response to higher or lower degrees of NK and T-cell responses.[9] The strong positive LD across the HLA region has hampered the study of the HLA-B leader dimorphism in health and disease.[8]

We tested a series of hypotheses to define the clinical significance of the HLA-B leader dimorphism in HLA-B-mismatched unrelated HCT. A paradigm inclusive of clinically relevant *HLA-B* variation may help to increase the availability and safety of HLA-mismatched transplantation.

Methods

Study design and data sources

Outcomes were assessed in 33,982 patients transplanted between 1988 and 2016 from unrelated donors for the treatment of a blood disorder, whose data were contributed by members of the International Histocompatibility Working Group (IHWG) in HCT (appendix tables 1 and 2 pp 6–11). All transplants have complete HLA typing and clinical data and were included in the analysis; there were no exclusion criteria. Of the 33,982 patients, 1,457 were transplanted from donors with one HLA-B mismatch. HLA-B leader frequencies were defined in 2,004,742 BeTheMatch® registry donors using the HLA-B tissue type of each donor and alignment against reference sequences as described in the appendix p 2.[18]

Procedures

HLA typing and definition of HLA-B leaders, Bw4/Bw6, C1/C2 and allele/antigen mismatches are described in the appendix p 2.[5,19,20] Average HLA-A and HLA-C expression was calculated from established values.[10,21] The strong positive LD across the HLA region motivated the use of haplotypes that differ for an M- or a T-leader but have the same HLA-Bw, HLA-E or KIR ligand, to isolate the effect of the leader on GVHD. To this end, haplotypes defined by HLA-B leader-Bw, leader-KIR and leader-HLA-E, were determined for HLA-B-mismatched transplants who were homozygous at one or both markers of interest.

Full *HLA-B* coding gene sequences for 92 alleles from patient-donor transplant pairs with two or more occurrences in the study population were used to define two lineages corresponding to M- and T-leaders (appendix p 3). Neighbor-joining tree and genetic distance methods are described in the appendix p 3.[22,23]

Protocols were approved by the institutional review boards of the National Institutes of Health Office for Human Research Protections and each participating IHWG center. The

funding agencies had no role in study design, data collection and analysis, the decision to submit the manuscript for publication, or the preparation of the manuscript.

Statistical analysis

We examined the association of HLA mismatches and HLA-B leader genotype with acute GVHD (grades II-IV and III-IV), chronic GVHD, relapse, death not preceded by relapse, disease-free survival and overall mortality. HLA-B mismatches had uniformly high risks among single mismatches, and therefore hypotheses focused on single HLA-B-mismatched HCT. Logistic regression was used to assess associations with acute GVHD. For all other endpoints, Cox regression models were fit to compare the hazards of failure between appropriate group, and patients who did not fail by last contact were censored at last contact. s. Regression models were adjusted for patient age, donor age, source of cells, disease status, T-cell depletion, transplant type, use of total body irradiation, patient sex, donor sex, cytomegalovirus serologic status, patient race, donor race, HLA-DPB1 match status, average HLA-A and HLA-C expression, HLA-E genotype, distance and year of transplantation.[24,25] Various interactions, as detailed in Results, were examined by including appropriate factors in these regression models. Covariates with missing data were included in models by creating an additional category to reflect the missing value of the appropriate covariate. If outcome data were missing for a particular patient, such a patient was excluded from the appropriate regression analysis. Two-sided p values from Cox regression models were obtained from the Wald test. Several comparisons were made, all focused on refinements of the concept of leader-matching *vs.* leader-mismatching. The outcomes examined are highly correlated, minimizing the impact of multiple comparisons that result from the various outcomes. For this reason, no adjustments were made to the p values associated with the fitted regression models.

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Risks increased with increasing numbers of HLA-A,B,C,DRB1,DQB1 mismatches (appendix table 4 pp 14–15). Among single HLA mismatches, HLA-B mismatches had the lowest disease-free survival and highest acute GVHD, and therefore hypotheses focused on HLA-B mismatches and specifically the role of HLA-B leaders in outcome. All leader models adjusted for HLA-E genotype because patient E*01:03-homozygosity was associated with suggestively lower disease-free survival compared to E*01:01-homozygosity (appendix table 4 pp 14–15).

M- and T-leaders define distinct lineages of HLA-B allotypes, with minor exceptions (figure 1). Since both HLA-B allotypes are co-expressed and contribute to immune responses, we evaluated the role of patient and donor leader genotype in outcome (figure 2A).

The leader genotype is defined by an individual's two HLA-B allotypes (figure 2A). Compared to absence of an HLA-B M-leader, presence of any M-leader in either patients or donors increased grades III-IV GVHD after HLA-B-mismatched but not HLA-matched transplantation (table 1; appendix table 5 p 16).

Consistent with other populations,[9,10] M-leader allotypes were observed more often with Bw6, C1, low-expression HLA-C allotypes, and HLA-E*01:01; T-leader allotypes occurred with all markers (appendix table 6 p 17). We therefore sought to isolate HLA-B leader effects as much as possible from those contributed by haplotype-linked variants by studying pairwise haplotypes that differed for leaders, but that shared HLA-Bw, HLA-E allele, or KIR ligands (table 1). M-leaders increased GVHD risk in patients with M-Bw6/T-Bw6 haplotypes relative to T-Bw6/T-Bw6 haplotypes (odds ratio 2·11, 95% CI 1·27–3·49, p=0·0037); M-E*01:01/M-E*01:01 relative to T-E*01:01/T-E*01:01 (odds ratio 3·81, 95% CI 1·18–12·28, p=0·025), and M-C1/T-C1 relative to T-C1/T-C1 (odds ratio 2·16, 95% CI 1·12–4·14, p=0·021). Similar associations were observed for donor haplotypes (table 1). These data suggest that the increased GVHD observed with M-leader allotypes is not explained by HLA-Bw, HLA-E*01:01, or KIR ligands. The limited numbers of transplants with M-E*01:03, M-Bw4, and M-C2 haplotypes precluded meaningful analysis.

In single HLA-B-mismatched transplantation, the mismatched HLA-B allotypes are non-shared, and the matched HLA-B allotype is shared (figure 2B). Since MM and MT patient and donor genotypes were associated with higher GVHD risk than TT genotypes, we tested the hypothesis that the leaders of the non-shared and shared allotypes both provide information on outcome.

We first focused on the non-shared HLA-B allotypes and whether the patient and donor had the same leader (both M-leader or both T-leader; "leader-matched") or different leaders (one M-leader and one T-leader; "leader-mismatched") (figure 2C, comparison C1). Compared to leader-matched transplants, leader-mismatched transplants had higher risk of grades II-IV acute GVHD (odds ratio 1·73, 95% CI 1·02–2·94, p=0·042) (table 2). These results suggest that the "leader match status" of non-shared HLA-B allotypes provides information on GVHD risk.

Non-shared allotypes can also be described by the genetic distance between them (figure 1). For every 1% increase in nucleotide divergence, grades II-IV GVHD increased by 1·28 (95% CI 1·15–1·43, p<0·0001) and grades III-IV by 1·19 (95% CI 1·06–1·33, p=0·0036). Not surprisingly, the genetic distance among leader-matches was, on average, lower than that among leader-mismatches (appendix figure 1 p 4). Adjustment for distance, therefore, negated the risk of leader-mismatching relative to leader-matching (table 2).

Since leader genotype suggests a contribution to GVHD from both haplotypes, we next investigated the role of the leader of the shared allotype by comparing transplants whose shared allotype had an M-leader to transplants whose shared allotype had a T-leader regardless of the leader match status of the non-shared allotypes (figure 2C, comparison C2). Severe GVHD was increased in transplants whose shared allotype had an M-leader relative to those with a T-leader (odds ratio 1·98, 95% CI 1·39–2·81, p=0·00014; table 2).

Assessment of leader match status of the non-shared HLA-B allotypes and of the leader of the shared allotype described above assumes each effect is the same across all categories of the other. A statistical test of interaction between leader match status and the specific leader (M or T) of the shared allotype in fact yields p=0·081 for grades II-IV and p=0·061 for grades III-IV GVHD, suggesting differential effects of one across the various categories of the other. When the leader match status is combined with the leader of the shared allotype, four transplant groups are defined (figure 2C, comparison C3), the detrimental effect of a shared M-leader appears to be confined to transplants whose non-shared HLA-B allotypes are leader-matched (odds ratio 2·15, 95% CI 1·50– 3·10, p<0·0001 for leader-matched *vs.* odds ratio, 0·63 [0·77/1·23] for leader-mismatched transplants; table 2). These results suggest that the impact of leader match status of the non-shared allotypes on GVHD must be viewed in concert with the specific leader of the shared allotype, and *vice versa*. Whereas the leader match status and leader of the shared allotype influenced GVHD, there was no suggestion of an association with relapse. Compared to leader-matched transplants with a shared T-leader allotype, leader-matched/share M, leader-mismatched/share T and leader-mismatched/share M transplants had similar risk of relapse (hazard ratio 0·99, 95% CI 0·73–1·33, p=0·92; hazard ratio 0·97, 95% CI 0·53 –1·77, p=0·91; hazard ratio 1·82, 95% CI 0·83–4·01, p=0·14, respectively). The lack of correlation with relapse suggests that the underlying mechanisms that involve the leader in GVHD may be independent of pathways involved in relapse.

Non-shared HLA-B allotypes may be characterized by their specific leaders: M patient/M donor; T patient/T donor; M patient/T donor; T patient/M donor. Together with the leader of the shared allotype, eight transplant groups are defined (leader of the patient's non-shared allotype, the donor's non-shared allotype, and the shared allotype, respectively): MMM, TTM, MMT, TTT, MTM, TMM, MTT, TMT (figure 2C, comparison C4; figure 2D, table 2). After consideration of the leader match status of the non-shared HLA-B allotype and specific leader (M *vs.* T) of the shared allotype, further consideration of the specific leaders of the non-shared allotypes did not significantly contribute to GVHD risk (p=0·66), although the numbers of MTM and TMM transplants are limited. These results suggest that knowledge of the specific leaders of the non-shared allotypes does not significantly enhance information on GVHD risk beyond that already defined by the leader match status and the leader of the shared allotype.

HIV disease progression depends on HLA-E expression as determined by HLA-A expression level, and the HLA-B leader dimorphism.[10] In HLA-B-mismatched HCT, the association of mean HLA-A expression with GVHD was not different between patient MM, MT and TT genotypes (interaction p=1·00 grades II-IV; p=0·54 grades III-IV), donor MM, MT, TT genotypes (interaction p=0·75 grades II-IV; p=0·44 grades III-IV) or the leader of the shared allotype (interaction p=0·44 grades II-IV; p=0·18 grades III-IV). Furthermore, mean HLA-A expression was not associated with grade II-IV (odds ratio 0·87, 95% CI 0·62–1·23, p=0·44) or grade III-IV GVHD (odds ratio 1·11, 95% CI 0·75–1·65, p=0·61), suggesting that the mechanisms involved in GVHD may be different than those governing HIV progression, particularly the relevance of HLA-E expression as a specific mechanism underlying leader-associated GVHD risks.

Most HLA-B allele-mismatches are leader-matched, but antigen-mismatches may be either leader-matched or leader-mismatched. Compared to allele-mismatched transplants, leader-matched antigen-mismatched transplants had higher grades II-IV acute GVHD (odds ratio 1·57, 95% CI 1·15–2·15, p=0·0044), and leader-mismatched antigen-mismatched transplants even higher risk (odds ratio 2·22, 95% CI 1·27–3·88, p=0·0051), with similar trends for grades III-IV. The effect of MM, MT or TT leader genotypes did not depend on whether the HLA-B mismatch was allele or antigen (interaction p=0·28 for patients, p=0·75 for donors). These results suggest that leader match status differentiates high-risk (odds ratio 1·41 [2·22/1·57]) from low-risk HLA-B antigen-mismatches.

When available, an HLA-A,B,C,DRB1,DQB1-matched donor, regardless of patient leader genotype, remains the donor of choice (table 3). When HLA-matched donors are unavailable, choices for single HLA-B-mismatched donors depend on the patient's leader genotype (table 3). Patients benefit from leader-matching, particularly MT and TT patients; the small number of leader-mismatched transplants limits comparison for MM patients. MT patients have two leader-matched (MT) donors, one who is matched for the M-leader allotype and mismatched for the T-leader allotype (TTM), and the other mismatched for the M-leader allotype and matched for the T-leader allotype (MMT). Compared to MMT transplants, TTM transplants had higher risk of severe acute GVHD (odds ratio 1·99, 95% CI 1·10–3·59, p=0·022; table 3). These data suggest that MMT transplantation is preferred over TTM transplantation (appendix figure 2 p 5). When two or more donors are available for MMT pairings, allele-mismatched donors are preferable to antigen-mismatched donors.

Patients with the TT leader genotype have two donor choices: leader-matched TT donors (TTT) and leader-mismatched MT donors (TMT). Chronic GVHD risk was higher and acute GVHD suggestively higher with TMT compared to TTT transplantation (odds ratio 1·96, 95% CI 1·00–3·84, p=0·051 for chronic GVHD; table 3). These data suggest that when TT patients have donor choices, TTT is preferred over TMT (appendix figure 2 p 5).

The findings suggest that the leader informs prospective donor selection for MT and TT patients who comprise 94·6% (1,378/1,457) of all single HLA-B-mismatched transplants and 91·7% (31,162/33,982) of the 33,982 transplants in the study population. To understand the pool of potential donors, we evaluated the distribution of leader genotypes among 2,004,742 current US registry donors. The donor leader genotype frequencies were similar to those of the 33,982 patients: 8.4% (167,803/2,004,742) MM, 39.4% (790,335/2,004,742) MT and 52.2% (1,046,604/2,004,742) TT. Leader genotype frequencies among donors of African-, Asian-, Caucasian-, Hispanic- and Native-American ancestry were: 57% (110,536/194,447), 74% (197,978/268,599), 46% (538,702/1,165,958), 53% (192,690/363,051) and 53% (6,698/12,687) TT, respectively; 37% (72,032/194,447), 24% (64,393/268,599), 43% (506,349/1,165,958), 39% (142,594/363,051) and 39% (4,967/12,687) MT, respectively; 6%,(11,879/194,447), 2% (6,228/268,599), 10% (120,907/1,165,958), 8% (27,767/363,051) and 8% (1,022/12,687) MM, respectively. These frequencies strongly suggest that the vast majority of MT and TT patients who require unrelated transplantation have donor choices.

Discussion

An unmet need in HCT remains a strategy to guide the selection of mismatched donors when matched donors are unavailable. The need is particularly acute for HLA-B because of its extreme polymorphism within the peptide-binding region. We uncovered a role for the HLA-B leader in outcome after HLA-B-mismatched HCT. The leader discloses that HLA-B mismatches are not equally deleterious; furthermore, the shared and the mismatched allotypes all contribute to outcome. GVHD risk is increased when the non-shared HLA-B allotypes have different leaders, and when the shared HLA-B contributes an M-leader. When HLA-matched donors are unavailable, the leader may be useful in identifying high-risk HLA-B mismatches that should be avoided.

Traditional concepts posit that risks are conferred by the HLA mismatch itself and current practice places emphasis on the donor's HLA mismatch exclusively. In the current study, transplants with one HLA-B mismatch provided a model for evaluating the clinical significance of both non-shared and shared allotypes. Comparison of haplotypes that differed for the leaders but not haplotype-linked HLA-E, KIR ligands, and HLA-Bw removed the potential contribution by these variables. Dissection of effects of non-shared allotypes from those of the shared allotype suggests that GVHD is a complex clinical phenotype related to the biology of leaders and to genetic distance. That both HLA haplotypes contribute to the immune response has been shown in HLA-associated diseases,[10,21] but the findings are novel in transplantation where the focus has traditionally been the donor HLA mismatch.[2] Notably, MT patients present an entirely new approach for reducing GVHD risk because they encode one M-leader and one T-leader allotype, and GVHD risk depends on whether matching the donor should be attempted for the M-leader or the T-leader allotype. When matched donors are unavailable, MT patients benefit not only from leader-matching but also from sharing a T-leader allotype with their donor. Furthermore, patients benefit when the non-shared leader-matched allotypes are allele- rather than antigen-mismatched. For the over 90% (1,378/1,457) of patients in need of a transplant (MT and TT), the frequency of donors in the current US registry suggests that consideration of leader lineage is feasible in clinical practice.

The use of phylogeny to group HLA-B allotypes into two functional lineages according to a polymorphism that is not currently tested in clinical practice, provides a novel approach for identifying combinations of mismatched allotypes that are better tolerated (mismatching between allotypes from the same leader lineage, "leader-matched") than other combinations (mismatching between allotypes from different leader lineages, "leader-mismatched"). The specific allotypes within each leader lineage would not otherwise have been predicted had sequence features within the peptide-binding region been used as the basis for comparing pairwise allotypes. In this way, the delineation of HLA-B allotypes by their leader lineage may enhance patient care and further understanding of the immunobiology of GVHD.

A limitation of the current study is that it was not designed to test specific mechanisms through which the HLA-B leader influences GVHD, and they remain to be elucidated in the future with functional models. Nonetheless, the association of distance with GVHD supports T-cell recognition of the peptide-binding region. The contribution of the shared allotype to

GVHD implicates a feature of M-lineage allotypes, either the HLA-B leader itself or a proxy in positive LD with the leader dimorphism. Although the lack of correlation between HLA-A expression, GVHD and leader genotype makes HLA-E expression a less-attractive candidate,[10] the differential effect of HLA-E*01:03 on outcome still leaves open the possibility of cytotoxic T-cell with or without CD94/NKG2 recognition of HLA-E variation. HLA-E*01:01 and E*01:03 were the dominant alleles in the transplantation cohort, similar to other populations.[9,26] A larger transplant experience will be required to address the significance of rare HLA-E variation, the role of regulatory regions,[27] HLA-G,[28] receptor diversity and affinity,[29] and to examine the leader effect in other mismatched populations including two-locus mismatched unrelated donor, haploidentical and cord blood transplants, and with different immunosuppression regimens including post-transplant cyclophosphamide. We did not observe an association between leaders and relapse. Whether the underlying leukemia could alter HLA expression remains to be defined. The relative contribution of NK and T-cell pathways to GVHD remains an important question, information which may clarify a role for pharmacologic HLA-E/NKG2A blockade if HLA-E/NKG2A are confirmed to be target molecules.[30]

In conclusion, HLA-B serves as a cornerstone of the immune response, playing a key role in the development of GVHD. The HLA-B leader is a sequence feature outside of the peptide-binding region that is not currently considered in the support of patients undergoing hematopoietic-cell transplantation. Phylogeny informs HLA-B mismatch combinations which associate with a hierarchy of GVHD risks depending on the leader lineage of the matched and mismatched allotypes. Consideration of the HLA-B leader may enhance donor selection when matched donors are unavailable. Most patients in need of a transplant have the MT or TT leader genotype, for whom donors who are leader-matched and share a T-leader allotype are preferred. The frequencies of leader genotypes of US registry donors suggest that future patients have donor options. The high risk of GVHD associated with M-leader allotypes supports consideration of better immunosuppressive regimens for patients with MM and MT genotypes, which is readily defined by the patient's HLA-B tissue type.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in context

Evidence before this study

Complete and precise HLA matching of unrelated donors is used to lower the risks after hematopoietic-cell transplantation; however, many patients lack compatible donors, particularly patients of non-Caucasian background. Although transplantation from donors with one HLA mismatch can offer lifesaving therapy, severe acute graft-versus-host disease (GVHD) limits the broad utility of HLA-mismatched transplantation. The features that define risky HLA mismatches remains an important research question.

The mature HLA class I molecule that is expressed on the cell surface is encoded by exons 2–7. Sequence variation in exons 2, 3 and 4 provide the basis for the tissue type of the class I molecule, or “allotype”. Exon 1 of class I genes encodes a separate leader peptide which is not a structural moiety of the mature class I molecule, but can be bound and presented by class I, notably HLA-E. HLA-A and C leader sequences are largely invariant, and encode M at the −21 position (rs1050458); however, HLA-B leader sequences encode M or T at the −21 position, which differentially effect T- and NK cell immune responses. We hypothesized that the dimorphic HLA-B leader provides information on risks of GVHD associated with HLA-B mismatching after unrelated donor transplantation. During the design and conduct of the study between 20/01/2017 and 11/03/2019, we searched PubMed for articles published on the clinical significance of the HLA-B leader in hematopoietic-cell transplantation. The search terms were “unrelated donor”, “hematopoietic-cell transplantation”, “HLA-B leader”, “graft-versus-host disease”. No reports matched these terms. Several studies have investigated the influence of the dimorphic HLA-B leader on the course of HIV infection.

Added value of this study

When HLA-matched donors are not available, clinicians lack data to prioritize the selection of HLA mismatched donors. Given the extreme polymorphism of HLA-B, and the high risks of GVHD associated with HLA-B mismatching, we sought to evaluate the role of the HLA-B leader dimorphism in GVHD. If the leader dimorphism can differentiate high-risk HLA-B mismatches from those with lower risks, then these data could be helpful for avoiding deleterious HLA-B mismatches. To test the hypothesis that the HLA-B leader has clinical relevance in unrelated donor transplantation, we identified a cohort of patients who received a transplant from a donor who was mismatched for only one HLA-B allotype and matched for the second HLA-B allotype (“single mismatch”). We identified two phylogenetically distinct lineages of HLA-B allotypes based on their M- or T-leader. Each leader-defined lineage comprises HLA-B allotypes that differ from one another within their peptide-binding regions, yet are associated with the same leader peptide, either T-leader or M-leader. Each HLA-B allotype in each patient and donor was defined according to their associated leader. In this way, the leader of the patient’s mismatched allotype and of the donor’s mismatched allotype (“non-shared” allotypes) could be the same (“leader-matched”) or different (“leader-mismatched”). The second matched allotype (“shared” allotype) could be associated with a T-leader or an M-leader. Severe GVHD was significantly higher with leader-mismatching compared to leader-

matching, and when the shared HLA-B allotype had an M-leader compared to a T-leader. Over 90% (1,378/1,457) of patients encode MT or TT leader genotypes, for whom a hierarchy of risks are evident and depend on the presence of leader (mis)matching and the leader of the shared allotype. These findings provide new knowledge in the field on risky HLA-B mismatch combinations, and advance understanding of factors associated with GVHD. The data from the current study add further value to existing HLA-B frequencies. Notably the frequencies of HLA-B leader genotypes among two million registry donors strongly suggest that patients in need of a transplant have choices for leader-matched donors.

Implications of all the available evidence

The current study demonstrates that transplantation from HLA-B-mismatched donors may be optimized through a new paradigm based on the leader lineage of both the matched and mismatched allotypes. For the vast majority of patients in need of a transplant, the HLA-B leader may be used to avoid risky donor HLA-B mismatches, and to identify high-risk patients who may benefit from better immunosuppressive regimens.

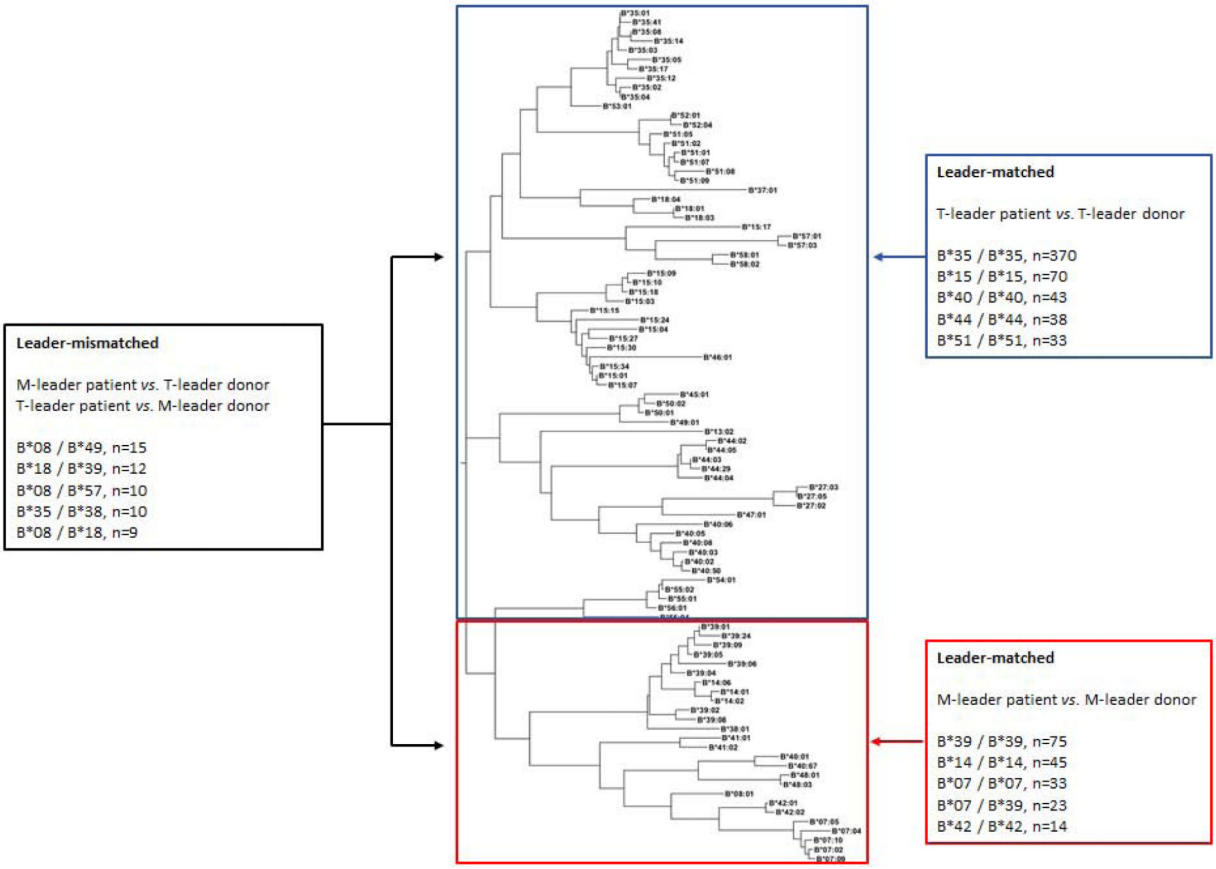

Figure 1: HLA-B phylogeny

The five most frequently observed HLA-B mismatches in leader-matched (blue and red boxes) and leader-mismatched (black box) transplants are shown.

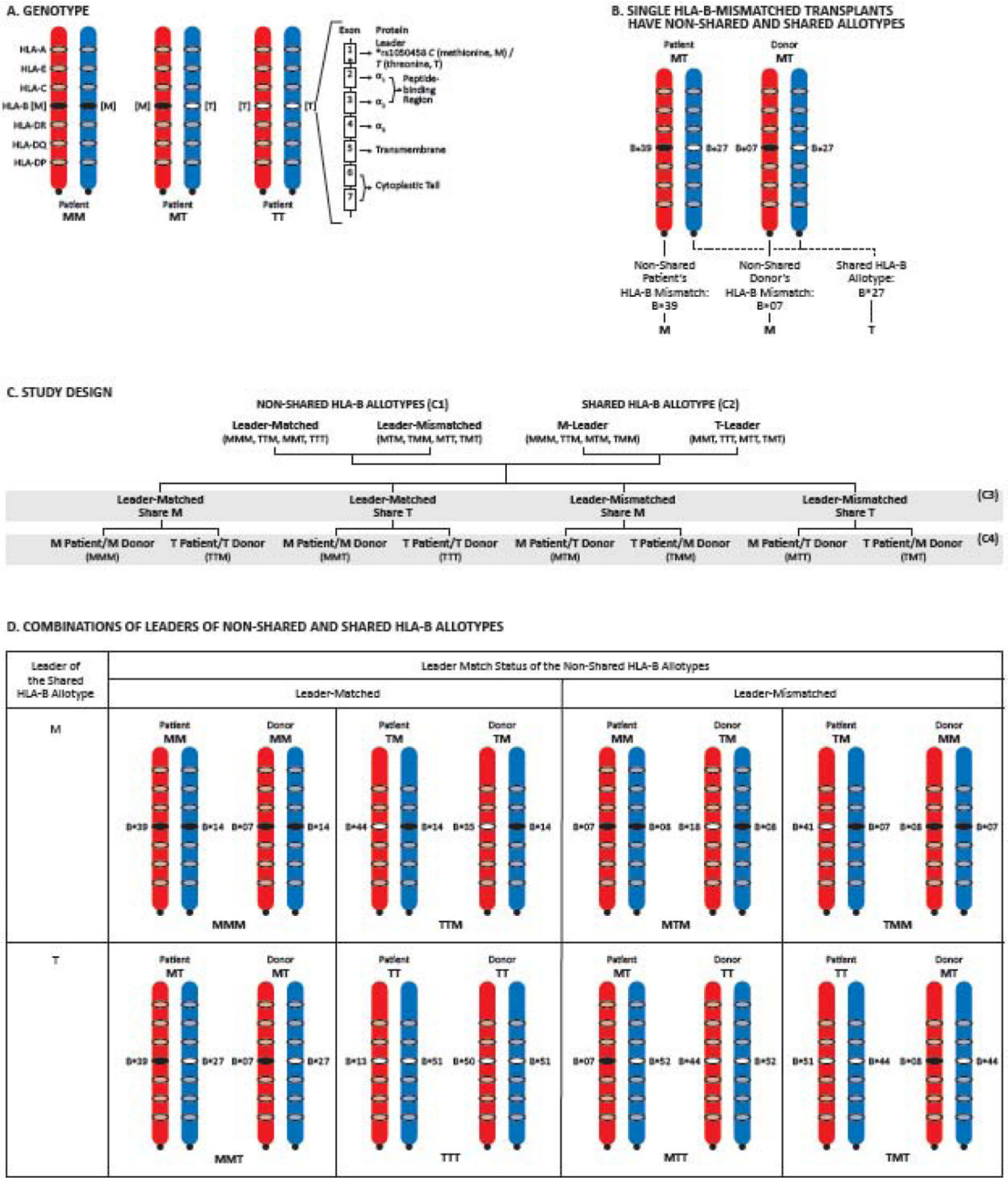

Figure 2: HLA-B leaders in HLA-B-mismatched unrelated donor transplantation

(A) The rs1050458C/T dimorphism at position −21 of exon one gives rise to leader peptides with methionine (M; black circle) or threonine (T; open circle) at the second residue of the processed leader peptide. (B) Among single HLA-B-mismatched transplants, the leaders from the patient's non-shared allotype, the donor's non-shared allotype, and the shared allotype are described by a three-letter nomenclature. An MMT transplant is illustrated. (C) Four-group (C3) and eight-group (C4) comparisons. (D) The eight-group model.

Table 1:

Risks associated with the HLA-B leader among transplants with a single HLA-B mismatch: genotype and haplotype models

			Grades II-IV acute GVHD			Grades III-IV acute GVHD		
			Patients with endpoint/ evaluabl patients*	Odds Ratio (95% CI)	p value	Patients with endpoint/ evaluable patients*	Odds ratio (95% CI)	p value
Leader genotype	Patient	TT	264/503	1·0	··	111/503	1·0	··
		MT	198/371	1·11 (0·82–1·52)	0·50	114/366	1·64 (1·15–2·32)	0·0057
		MM	32/55	1·51 (0·78–2·94)	0·22	22/55	2·40 (1·20–4·78)	0·013
	Donor	TT	250/489	1·0	··	104/489	1·0	··
		MT	210/377	1·29 (0·94–1·76)	0·11	121/373	1·82 (1·28–2·59)	0·00094
		MM	34/63	1·27 (0·68–2·39)	0·45	22/62	2·06 (1·04–4·07)	0·038
Leader-Bw haplotype	Patient	T-Bw6/T-Bw6	81/167	1·0	··	31/167	1·0	··
		M-Bw6/T-Bw6	110/210	1·17 (0·77–1·78)	0·46	65/207	2·11 (1·27–3·49)	0·0037
	Donor	T-Bw6/T-Bw6	74/152	1·0	··	26/152	1·0	··
		M-Bw6/T-Bw6	116/214	1·15 (0·75–1·78)	0·52	65/210	2·23 (1·32–3·79)	0·0029
Leader-HLA-E haplotype	Patient	T-E*01:01/T-E*01:01	41/77	1·0	··	19/77	1·0	··
		M-E*01:01/M-E*01:01	10/19	1·41 (0·46–4·35)	0·55	10/19	3·81 (1·18–12·28)	0·025
	Donor	T-E*01:01/T-E*01:01	31/69	1·0	··	12/69	1·0	··
		M-E*01:01/M-E*01:01	11/23	0·92 (0·33–2·56)	0·88	9/22	3·15 (1·04–9·55)	0·043
Leader-KIR haplotype	Patient	T-C1/T-C1	55/91	1·0	··	18/91	1·0	··
		M-C1/T-C1	87/147	0·90 (0·51–1·57)	0·70	47/147	2·16 (1·12–4·14)	0·021
	Donor	T-C1/T-C1	47/85	1·0	··	15/85	1·0	··
		M-C/ T-C1	91/144	1·42 (0·80–2·52)	0·23	48/144	2·87 (1·43–5·76)	0·0031

Leader genotype models adjusted the year of transplantation, transplant type, disease status, cytomegalovirus serologic status, patient age, donor age, source of cells, patient sex, donor sex, total body irradiation, T-cell depletion, HLA-DPB1 mismatching, donor race, patient race, mean HLA-A and HLA-C expression, HLA-E and distance.

Leader- Bw haplotype and Leader-KIR haplotype models adjusted for the year of transplantation, disease status, patient age, and distance. Leader-HLA-E models adjusted for the year of transplantation, patient age, and distance.

*Numbers are provided for patients who achieved the endpoint among the total number of evaluable patients.

Table 2:

GVHD risks associated with the HLA-B leader in HLA-B-mismatched transplantation

		Grades II-IV acute GVHD			Grades III-IV acute GVHD		
		Patients with endpoint/ evaluable patients‡	Odds ratio (95% CI)	p value	Patients with endpoint/ evaluable patients‡	Odds ratio (95% CI)	p value
Leader match status of the non-shared HLA-B allotypes	Same (leader-matched) *	448/855	1·0	..	226/851	1·0	..
	Different (leader-mismatched) *	46/74	1·73 (1·02–2·94)	0·042	21/73	1·31 (0·73–2·34)	0·36
Leader of the shared HLA-B allotype	T-leader	330/636	1·0	..	144/635	1·0	..
	M-leader	164/293	1·23 (0·90–1·70)	0·20	103/289	1·98 (1·39–2·81)	p=0·00014
Leader match status and leader of the shared allotype together	Leader-matched/T-leader shared allotype	294/582	1·0	..	127/581	1·0	..
	Leader-matched/M-leader shared allotype	154/273	1·33 (0·95–1·85)	0·093	99/270	2·15 (1·50–3·10)	p<0·0001
	Leader-mismatched/T-leader shared allotype	36/54	1·23 (0·61–2·49)	0·56	17/54	1·23 (0·58–2·61)	0·58
	Leader-mismatched/M-leader shared allotype †	10/20	0·59 (0·21–1·62)	0·30	4/19	0·77 (0·23–2·63)	0·68
Leaders of the non-shared and the shared allotypes together	Non-shared patient T-leader/ non-shared donor T-leader/shared T-leader (TTT)	239/469	1·0	..	99/469	1·0	..
	Non-shared patient M-leader/non-shared donor M-leader/shared T-leader (MMT)	55/113	1·09 (0·69–1·75)	0·70	28/112	1·23 (0·72–2·12)	0·45
	Non-shared patient M-leader/non-shared donor M-leader/shared M-leader (MMM)	28/49	1·66 (0·82–3·34)	0·16	20/49	2·66 (1·27–5·57)	0·0097
	Non-shared patient T-leader/non-shared donor T-leader/shared M-leader (TTM)	126/224	1·32 (0·92–1·90)	0·13	79/221	2·22 (1·49–3·33)	0·00010
	Non-shared patient M-leader/non-shared donor T-leader/shared M-leader (MTM)	4/6	1·12 (0·19–6·79)	0·90	2/6	1·52 (0·24–9·67)	0·65
	Non-shared patient T-leader/non-shared donor M-leader/shared M-leader (TMM)	6/14	0·46 (0·14–1·59)	0·22	2/13	0·56 (0·11–2·90)	0·49
	Non-shared patient M-leader/non-shared donor T-leader/shared T-leader (MTT)	11/20	0·74 (0·27–2·01)	0·56	5/20	0·89 (0·28–2·87)	0·85
	Non-shared patient T-leader/non-shared donor M-leader/shared T-leader (TMT)	25/34	1·80 (0·73–4·43)	0·20	12/34	1·55 (0·63–3·79)	0·34

Models that examine the leader of the shared allotype among leader-matched transplants adjusted for the year of transplantation, transplant type, disease status, cytomegalovirus serologic status, patient age, donor age, source of cells, patient sex, donor sex, total body irradiation, T-cell depletion, HLA-DPB1 mismatching, donor race, patient race, mean HLA-A and HLA-C expression, HLA-E and distance.

[*] After adjustment for distance, the odds ratio for grades II-IV was 0·91 (95% CI 0·49–1·69, p=0·77) and the odds ratio for grades III-IV was 0·84 (95% CI 0·43–1·64, p=0·61) for leader-mismatching relative to leader-matching.

[†] The effect of a leader-mismatch/share M relative to a leader-mismatch/share T is 0·47 (0·59/1·23) for grades II-IV and 0·63 (0·77/1·23) for grades III-IV acute GVHD.

[≠] Numbers are provided for patients who achieved the endpoint among the total number of evaluable patients.

Table 3:

Multivariate models for donor choices based on patient leader genotype

	Donor	Number of transplants	HLA-B-mismatched donor compared to an HLA-matched donor*			Relative preference among HLA-B-mismatched donors when HLA-matched donors are unavailable†		
			Grades II-IV acute GVHD	Grades III-IV acute GVHD	Chronic GVHD	Grades II-IV acute GVHD	Grades III-IV acute GVHD	Chronic GVHD
			Odds ratio (95% CI), p value	Odds ratio (95% CI), p value	Odds ratio (95% CI), p value	Odds ratio (95% CI), p value	Odds ratio (95% CI), p value	Odds ratio (95% CI), p value
Patient MM leader genotype	HLA 10/10	1,865	1·0	1·0	1·0	Not applicable	Not applicable	Not applicable
	HLA-B-mismatched MM (leader-matched; MMM)	68	1·72 (0·91–3·26), p=0·095	3·42 (1·76–6·65), P=0·00029	0·92 (0·59–1·42), p=0·70	1·0	1·0	1·0
	HLA-B-mismatched MT (leader-mismatched; MTM)	11	2·89 (0·49–17·21), p=0·24	3·07 (0·49–19·04), p=0·23	0·99 (0·35–2·80), p=0·98	0·55 (0·03–10·47), p=0·69	0·09 (0·004–2·08), p=0·13	0·94 (0·14–6·37), p=0·95
Patient TT leader genotype	HLA 10/10	7,343	1·0	1·0	1·0	NA	NA	NA
	HLA-B-mismatched TT (leader-matched; TTT)	730	1·39 (1·13–1·69), p=0·0014	1·21 (0·95–1·54), p=0·13	1·02 (0·89–1·18), p=0·74	1·0	1·0	1·0
	HLA-B- mismatched MT (leader-mismatched; TMT)	73	4·05 (1·84–8·89), P=0·00049	2·79 (1·33–5·84), p=0·0065	1·34 (0·79–2·26), p=0·28	2·27 (0·87–5·92), p=0·093	1·62 (0·61–4·31), p=0·33	1·96 (1·00–3·84), p=0·051
Patient MT leader genotype	HLA 10/10	6,867	1·0	1·0	1·0	NA	NA	NA
	HLA-B-mismatched MT (M-leader non-shared allotype/T-leader shared allotype; MMT)	160	1·11 (0·75–1·63), p=0·60	1·57 (1·00–2·45), p=0·049	0·86 (0·64–1·15), p=0·30	1·0	1·0	1·0
	HLA-B-mismatched MT (T-leader non-shared allotype/M-leader shared allotype; TTM)	338	1·52 (1·15–2·02), p=0·0033	2·80 (2·08–3·77), p<0·0001	0·89 (0·72–1·10), p=0·28	1·32 (0·80–2·20), p=0·28	1·99 (1·10–3·59), p=0·022	0·89 (0·60–1·33), p=0·58
	HLA-B-mismatched MM (leader-mismatched; TMM)	26	0·91 (0·31–2·67), p=0·86	0·79 (0·17–3·64), p=0·76	0·74 (0·33–1·67), p=0·47	0·79 (0·23–2·71), p=0·70	0·61 (0·11–3·51), p=0·58	0·61 (0·22–1·70), p=0·34
	HLA-B-mismatched TT (leader-mismatched; MTT)	51	1·69 (0·68–4·21), p=0·26	1·82 (0·64–5·18), p=0·26	1·18 (0·65–2·16), p=0·58	1·41 (0·49–4·08), p=0·53	1·23 (0·35–4·36), p=0·75)	1·97 (0·95–4·09), p=0·070

NA, not applicable

*Models including HLA-matched transplants adjusted for year of transplantation, type of transplant, disease status, cytomegalovirus serologic status, patient age, donor age, donor sex, patient sex, total body irradiation, T-cell depletion, HLA-DPB1 mismatching, patient race, donor race, mean HLA-A and mean HLA-C expression.

† Models exclusive to HLA-B-mismatched transplants: Models for MM patients adjusted for patient age and distance. Models for TT patients adjusted for year of transplantation, type of transplant, disease status, cytomegalovirus serologic status, patient age, donor age, source of cells, donor sex, patient sex, total body irradiation, T-cell depletion, HLA-DPB1 mismatching, donor race, patient race, mean HLA-A and HLA-C expression, HLA-E and distance. Models for MT patients adjusted for year of transplantation, type of transplant, total body irradiation, cytomegalovirus serologic status, disease status, patient age, source of cells, patient sex, T-cell depletion, donor race, patient race, HLA-DPB1 mismatching and mean HLA-A and HLA-C expression.