HLA-C expression levels define permissible mismatches in
hematopoietic cell transplantation

Short title: HLA-C Expression Scientific Category: Transplantation

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Key Points

- The expression level of patients' HLA-C allotypes affects GVHD and mortality after HCT from HLA-C mismatched unrelated donors.
- Transplant outcome can be improved by avoiding high-risk HLA-C mismatched donors when no matched stem cell source is available.
Abstract

Life-threatening GVHD limits the use of HLA-C-mismatched unrelated donors in transplantation. Clinicians lack criteria for donor selection when HLA-C mismatched donors are a patient’s only option for cure. We examined the role for HLA-C expression levels to identify permissible HLA-C mismatches. The median fluorescence intensity (MFI), a proxy of HLA-C expression, was assigned to each HLA-C allotype in 1975 patients and their HLA-C-mismatched unrelated transplant donors. The association of outcome with the level of expression of patients’ and donors’ HLA-C allotypes was evaluated in multivariable models. Increasing expression level of the patient’s mismatched HLA-C allotype was associated with increased risks of grades III-IV acute GVHD [odds ratio (OR), 1.34; 95% confidence interval (CI), 1.10-1.62; \( P = .003 \)], non-relapse mortality [hazard ratio (HR), 1.22; 95% CI, 1.06-1.39; \( P = .005 \)] and mortality (HR, 1.15; 95% CI, 1.03-1.27; \( P = .009 \)). Increasing expression level among HLA-C mismatches with residue 116 or residues 77/80 mismatching was associated with increased non-relapse mortality (HR, 1.31 and 1.38, respectively; 95% CI, 1.09-1.58 and 1.14-1.67, respectively; \( P = .004 \) and .0009, respectively). The immunogenicity of HLA-C mismatches in unrelated donor transplantation is influenced by the expression level of the patient’s mismatched HLA-C allotype. HLA-C expression levels, provide new information on mismatches that should be avoided, and extend understanding of HLA-C-mediated immune responses in human disease.
Introduction

The transplantation barrier is defined by the human leucocyte antigen (HLA) genes that are responsible for tissue histocompatibility.\textsuperscript{1-7} Mismatching for HLA-C allotypes between patients and unrelated donors generally leads to very high risks of acute graft-versus-host disease (GVHD) and mortality after hematopoietic cell transplantation, although risks to individual patients may vary.\textsuperscript{3-7} The success of transplantation for a given patient may depend on the unique features of the HLA-C mismatch itself. Three different models of HLA-C mismatching shed light on the variability of individual risks. Mismatching can occur between allotypes that elicit an antibody (serologic) response ("antigen" mismatches) or between allotypes that differ for limited nucleotide sequence variation ("allele" mismatches). The similarity of sequence features between allele mismatches may contribute to their lower immunogenicity.\textsuperscript{3-7}

A second model of HLA-C alloreactivity entails mismatching for amino acid residues that determine the repertoire of peptides presented to T-cells. Patient-donor differences at several residues of the class I molecule might significantly affect the immunogenicity of HLA-C mismatches and of these residues, residue 116 in the F pocket of the peptide binding groove has a high frequency of patient-donor mismatching and consistently shows an effect on transplant outcome.\textsuperscript{8-10} HLA-C mismatched patients who are residue 116-mismatched have higher risks of acute GVHD and mortality than HLA-C matched patients,\textsuperscript{10-12} observations that support a critical role for T-cell recognition of class I-peptide complexes.\textsuperscript{13-14} Most recently, a third model has been proposed in which transplant outcome may depend on the regulation of donor NK cell responses against patient cells.\textsuperscript{15} Amino acid substitutions at HLA-C residues 77 and 80 define two mutually exclusive groups of ligands, each recognized by different killer immunoglobulin-like receptors (KIRs). HLA-C-mismatched patients who are residues 77/80-mismatched may have different transplant outcomes than HLA-C mismatched patients who are residues 77/80-matched.\textsuperscript{15-20}
Each of the three mismatch models suggests that some HLA-C mismatches are less risky than others, and therefore represent mismatches that could be considered when matched donors are not available. The high overall risks associated with transplantation of HLA-C-mismatched unrelated donors have led some clinicians to abandon the use of such donors altogether. Clinical practice is heterogeneous because the features that define permissive HLA-C mismatches remain ill-defined.

Recently, the range of expression across HLA-C allotypes has been elucidated. Each serologically-defined HLA-C allotype has a characteristic median fluorescence intensity (MFI) of cell surface expression which is reproducible in both healthy and HIV infected cells in vitro. The MFI coefficient is superior to any other marker of expression level, including the previously described single nucleotide polymorphism that resides 35 kb upstream of HLA-C, because the MFI provides direct allotype-specific measurement of HLA-C surface expression. Expected levels of HLA-C cell surface expression based on the sum of the two allelic MFI coefficients was shown to predict observed HLA-C expression levels among individuals in two cohorts, indicating that MFI coefficients can be assigned to each HLA-C allotype in lieu of direct ascertainment of expression. Thus, the clinical importance of HLA-C expression can be determined in large-scale retrospective outcome studies where appropriate materials for measuring HLA-C expression directly are not available. Using this approach, higher MFI levels were shown to correlate with better control of HIV viral load and slower progression to HIV-AIDS across ethnic groups, but with increased susceptibility to Crohn’s disease, solidifying the role for HLA-C expression levels in modulating the strength of immune responses. Accordingly, we applied the MFI as a quantitative proxy of HLA-C expression level (simply termed “expression level” throughout the manuscript) to assess the clinical significance of the level of HLA-C expression in an exceptionally large international population of patients and unrelated transplant donors whose only HLA mismatch was a single HLA-C allotype.
Materials and Methods

Study population, HLA and MFI

To test the hypothesis that the permissivity of HLA-C mismatches depends on the level of expression of the patient’s and donor’s mismatched HLA-C allotypes, we retrospectively analyzed 1975 patients who received a hematopoietic cell transplant from an HLA-A,-B,-DRB1,-DQB1-matched but single HLA-C-mismatched unrelated donor as previously defined (Table 1).

Restriction of the study population to pairs with only one HLA-C mismatch removes any contribution of disparity at other HLA loci and addresses whether among the spectrum of HLA-C mismatches, there are combinations of mismatches that are better tolerated than others.

HLA-A,-C,-B,-DRB1,-DQB1 allotypes were typed at high-resolution for 1959 pairs and medium-resolution for 16 pairs. Fresh blood and skin, liver and gastrointestinal biopsy specimens are not available for large-scale historic transplant recipients and unrelated donors for direct HLA-C expression analysis in peripheral blood and GVHD-affected organ sites.

However, the legitimacy of the predictive algorithm for MFI values for each serologic-equivalent HLA-C*01-18 allotype has been described and is concordant between healthy cells and cells infected with HIV in vitro. Not only are expression levels reproducible, but predicted expression levels based on HLA-C allotype correlate strongly and significantly with observed expression values, the level of expression of a given HLA-C allotype is consistent across ethnic groups, and measurement of HLA-C transcript levels across allotypes replicates the same hierarchy of HLA-C cell surface expression levels. Since the MFI is a proxy for HLA-C expression levels, and fresh cells are not available on historic transplant pairs, HLA-C expression levels were imputed using the MFI for each patient and donor HLA-C allotype according to previously published quantitative measurements of MFIs.

Each pair was matched for one HLA-C (“shared” allotype) and mismatched for the second HLA-C (“non-shared” allotype). Each non-shared HLA-C mismatch was defined as an
allele or antigen mismatch.3 Protein sequences of non-shared allotypes may have identical
amino acids at certain hypervariable residues, but different (non-identical) amino acids at other
residues.28 Any non-identity at residue 116, 77 and 80 was determined by alignment to
reference HLA-C sequences.28

Consent from patients and donors was obtained in accordance with the Declaration of
Helsinki. The work was approved by the National Institutes of Health Office of Human Subjects
Research Protections and the Institutional Review Board, Fred Hutchinson Cancer Research
Center. HLA and clinical data were contributed by participants of the International
Histocompatibility Working Group in Hematopoietic Cell Transplantation.26

**Biostatistical analysis**

We hypothesized that the level of expression of the patient’s mismatched allotype, the donor’s
mismatched allotype, and/or the shared allotype, influences the risks of grades III-IV acute
GVHD, relapse, non-relapse mortality (death without a preceding recurrence of the underlying
disease) and overall mortality (henceforth mortality). Since previous studies demonstrate a
correlation between the sum of the MFIs of the two HLA-C allotypes and HIV outcomes,22 we
examined the sum of the MFIs of patients and of donors’ HLA-C allotypes. Expression level was
modeled as a continuous linear variable in Cox (for relapse, non-relapse mortality and mortality)
and logistic regression models (for acute GVHD). Under this assumption, hazard and odds
ratios from these regression models are presented in terms of the increase in risk of failure
associated with an increase in expression level of 100 fluorescence intensity units. All models
were adjusted for age, source of stem cells, disease severity, T-cell depletion, and year of
transplant (Table 1). Statistical interactions between expression level and mismatching defined
according to 3 different models (allele versus antigen, residue 116, residues 77/80) were
assessed by including appropriate terms into regression models, and interactions between
expression levels were assessed in the same way. Expression levels were dichotomized as “low” (C*07, C*03) or “high” (C*01, C*14) in order to demonstrate the interaction between expression level and mismatch model when appropriate. Mean expression levels were compared between groups for each mismatch model with the two-sample t-test. No adjustments for multiple comparisons were made and \( P \)-values between .01 and .05 should be considered suggestive, as opposed to conclusive, evidence of an association.

**Results**

The clinical characteristics of the patients and donors in the study population were consistent with the worldwide experience in unrelated donor hematopoietic cell transplantation (Table 1). Increasing expression level of the patient’s non-shared HLA-C was significantly associated with increased risks of acute GVHD, non-relapse mortality and mortality but not disease relapse (\( P = .003, .005, .009 \) and .76, respectively) (Figure 1, Table 2). There was no suggestion that these effects differed across the various years of transplant included in the study for any of these endpoints (interaction tests between expression level and year of transplant, \( P = .56, .69, .42, \) and .55, respectively).

Increasing expression level of the donor’s non-shared HLA-C was associated with increased non-relapse mortality (\( P = .04 \)) and mortality (\( P = .01 \)), though with borderline significance. The sum of the MFIs of patients’ and donors’ non-shared allotypes was significantly associated with non-relapse mortality (\( P = .002 \)) and mortality (\( P = .001 \)); there was no statistically significant association between donors’ expression level and acute GVHD or relapse (Table 2). The strong effect of patients’ HLA-C expression level on GVHD risk suggests enhanced graft-versus-host recognition of highly expressed patient allotypes by the donor graft.
Three mismatch models have been previously proposed to explain why some HLA-C mismatches are better tolerated than others.\textsuperscript{3-7,9-12,15-20} Since the expression level of the patient’s non-shared allotype correlated significantly with transplant outcome, we evaluated whether expression levels play a role in each of these mismatch models.

**Model 1: allele and antigen mismatches**

HLA-C allele mismatches may be better tolerated than antigen mismatches.\textsuperscript{3-7} We found striking differences in the mean HLA-C MFIs of the patients who had an allele mismatch relative to their donors (123.2 on average) as compared to those who had an antigen mismatch relative to their donors (176.7 on average; \( P < .0001 \); Table 3) and the same was true for donors’ mismatched HLA-C allotypes (data not shown). The lower mean MFI of allele mismatches resulted from the overwhelming representation of common low-expression C*07 and C*03 mismatches (C*07:01/07:02; C*03:03/03:04), whereas antigen mismatches were more uniformly distributed across all expression levels. Thus, allele mismatches differ from antigen mismatches in the degree of sequence similarity\textsuperscript{3,5} and in expression levels, and these features may contribute to the historically lower risks of allele compared to antigen mismatches. These results are consistent with recent studies demonstrating that the HLA-C*03:03/03:04 mismatch is a well-tolerated high-frequency mismatch\textsuperscript{24,25} and provide a potential mechanism (i.e. low expression) for why this particular mismatch is permissive.

The difference in GVHD between antigen and allele mismatches in our study was not as large as previously reported\textsuperscript{6,7} (OR, 1.27; 95% CI, 0.96-1.68; \( P = .09 \)). The virtual absence of higher expression allele mismatches precluded comparison of the effects of expression levels on outcome in allele \textit{versus} antigen-mismatched patients. However, since the full range of HLA-C allotypes were represented among patients mismatched for an HLA-C antigen (Table 3), the
importance of expression on outcome can be defined in antigen-mismatched transplants, thereby removing the allele/antigen effect. Among patients mismatched for one HLA-C antigen, increasing expression level of the patient’s mismatched HLA-C were associated with increased risk of acute GVHD (OR, 1.36; 95% CI, 1.09-1.69; \( P = .006 \)).

Moreover, among patients mismatched for the lowest expressing allotypes (C*07, C*03), the risk of GVHD was similar between antigen and allele mismatches (OR, 1.07; 95% CI, 0.75-1.53; \( P = .70 \)). These results suggest that antigen mismatches are tolerated if the patient’s mismatched antigen is expressed at low levels.

The risk of non-relapse mortality was increased for antigen versus allele mismatches but not statistically significantly so (HR, 1.16; 95% CI, 0.95-1.41; \( P = .13 \)); the risk of mortality was also marginally increased (HR, 1.17; 95% CI, 1.01-1.35; \( P = .04 \)). Among antigen mismatches, non-relapse mortality was significantly increased (HR, 1.23; 95% CI, 1.06-1.44; \( P = .007 \)), as was mortality, albeit with borderline significance (HR, 1.13; 95% CI, 1.01-1.27; \( P = .03 \)) as the expression level of the patient’s mismatched HLA-C increased. Among the lowest expression mismatches (C*07, C*03), the risk of non-relapse mortality was similar among antigen and allele mismatches (HR, 1.06; 95% CI, 0.83-1.35; \( P = .66 \)) as was the risk of mortality (HR, 1.11; 95% CI, 0.93-1.33; \( P = .26 \)).

These data suggest that risks may be defined more by the expression level of the patient’s mismatched allotype than by the allele or antigen designation. Low-expression antigen mismatches appear to be as readily tolerated as allele mismatches.

**Model 2: residue 116 mismatches**

Residue 116 plays a key role in determining the peptide repertoire of class I allotypes\(^8,13-14\) and patient-donor mismatching at this position may influence transplant outcome.\(^9-12\) The mean MFIs of patients’ (and donors’) non-shared allotypes were statistically significantly lower among
Residue-matched compared to residue-mismatched pairs (Table 3); the mean MFIs for the shared allotype were similar. These results might explain the observation of lower risks after residue-matched compared to residue-mismatched transplantation.9-12

Residue-mismatched patients had an increased risk of acute GVHD compared to residue-matched patients, but the difference was not statistically significant (OR, 1.14; 95% CI, 0.91-1.41; P = .26). There was no evidence to suggest that the association between expression level and GVHD was different among residue-mismatched and residue-matched patients (interaction P = .55), therefore separate analyses of expression levels and GVHD risk were not conducted for these groups.

The risk of overall mortality was slightly higher in residue-mismatched patients (HR, 1.12; 95% CI, 1.00-1.26; P = .05), but an interaction test between expression levels and mismatching was not statistically significant (P = .21).

The risk of non-relapse mortality was higher among residue-mismatched patients than among residue-matched patients with borderline significance (HR, 1.21; 95% CI, 1.04-1.40; P = .02), but the impact of residue mismatching on non-relapse mortality depended on the expression level (interaction P = .05). Specifically, non-relapse mortality increased as expression levels increased among residue-mismatched patients (HR, 1.31; 95% CI, 1.09-1.58; P = .004), but not among residue-matched patients (HR, 0.98; 95% CI, 0.78-1.23; P = .88).

One specific demonstration of this interaction is seen when patients whose non-shared allotype falls at the lower end of the MFI scale (C*07 or C*03, or “low expression”) are compared to those whose non-shared allotype falls at the higher end of the MFI scale (C*01 or C*14, or “high expression”). The negative impact of high expression (relative to low expression) on non-relapse mortality was seen among residue-mismatched patients (HR, 1.64) but not residue-matched patients (HR, 0.85). Similarly, the negative impact of residue mismatching (relative to residue matching) on non-relapse mortality was observed among patients with high-expression (HR, 1.97) but not among patients with low-expression mismatches (HR, 1.02).
These results were virtually identical for antigen-mismatched patients (data not shown), and they suggest that the increased risk of non-relapse mortality associated with high-expression mismatches is confined to patients who are mismatched at residue 116 and vice versa.

**Model 3: Residues 77/80 mismatches**

The mean MFIs of non-shared allotypes were significantly lower among residues 77/80-matched compared to residues 77/80-mismatched patients (Table 3), and might explain lower risks with residues 77/80 matching in some studies.\(^\text{15-20}\)

There was no association between residues 77/80 mismatching and acute GVHD (OR, 1.02; 95% CI, 0.82-1.27; \(P = .83\)). The magnitude of the association between the expression level of the patient's mismatched HLA-C and GVHD was larger among residue 77/80-mismatched patients than among residues 77/80-matched patients, but this difference was not statistically significant (interaction \(P = .11\)) and therefore separate analyses of residues 77/80 matching are not presented.

The risk of overall mortality was suggestively higher in residues 77/80-mismatched patients (HR, 1.13; 95% CI, 1.01-1.26; \(P = .04\)), but an interaction test between expression and mismatching was not statistically significant (\(P = .20\)).

There was no statistically significant association between residues 77/80-mismatching and the risk of non-relapse mortality (HR, 1.09; 95% CI, 0.94-1.27; \(P = .24\)); however, the association between residues 77/80-mismatching and non-relapse mortality appeared to depend on the expression level of the patient’s non-shared HLA-C (interaction \(P = .02\)). In particular, non-relapse mortality increased as expression levels increased among residues 77/80-mismatched patients (HR, 1.38; 95% CI, 1.14-1.67; \(P = .0009\)), but not among residues 77/80-matched patients (HR, 1.01; 95% CI, 0.82-1.24; \(P = .91\)).
Similar to residue 116, evaluation of patient mismatches with extreme expression levels provides a specific demonstration of the interaction between expression level and residues 77/80 match status (Figure 2). The negative effect of high expression (relative to low MFI) on non-relapse mortality was evident among residues 77/80-mismatched patients (HR, 1.74) but not residues 77/80-matched patients (HR, 0.97); similarly, the negative impact of residues 77/80 mismatching (relative to residues 77/80 matching) was observed among patients with high-expression (HR, 1.66) but not low-expression mismatches (HR, 0.92). When restricted to patients with antigen mismatches, the results were qualitatively the same (data not shown). These data suggest that MFIs provide information on the permissiveness of residues 77/80 mismatches. Whereas low- or high-expression residues 77/80 matches and low-expression residues 77/80 mismatches are well tolerated (permissible), high-expression residues 77/80 mismatches are non-permissible.

The impact of expression levels on grades III-IV acute GVHD was similar when other variables were taken into account such as source of stem cells, use of T-cell depletion, year of transplant, and severity of disease (i.e. no statistically significant interactions were observed between expression level and any of these factors). Collectively, all three models of transplant alloreactivity demonstrate that the expression level of the patient's non-shared HLA-C allotype is associated with transplant outcome.

Discussion

An unmet need in transplantation is a functional measure of HLA-C mismatching that can be shown to be associated with clinical outcome. The availability of such a tool would provide options for the use of selected HLA-C mismatched unrelated donors without increasing life-threatening acute GVHD and mortality. The recent discovery that HLA-C expression levels have direct consequences on HIV-AIDS progression and susceptibility to Crohn’s disease, provides
a framework for studying HLA-C in transplantation. To test our hypotheses, we leveraged an expansive international collaboration among immunogenetic laboratories, transplant centers and transplant/donor registries to identify patients who have only one HLA-C mismatch with their donor. This unique genetic relationship between the transplant patient and donor permitted the effect of HLA-C expression levels to be examined for single HLA-C molecules.

The collection of archived DNA samples from patients and donors is a unique and precious international scientific resource. As is true for virtually all large retrospective disease cohorts, no viable cells are available for *in vitro* HLA cell surface measurements of expression. Notably, such measurements require fresh cells, as freezing causes a significant artifactual decrease in HLA expression. Given the shortfalls of direct *in vitro* measurement of HLA-C expression in historic transplant study subjects, the use of inferred expression data is needed. As previously demonstrated, HLA-C expression levels, as defined by MFI, correlate with the HLA-C allotype. Use of an HLA-C-specific antibody that binds all HLA-C allotypes equally has shown that in a panel of fresh cells from both African Americans and European Americans, HLA-C expression levels reproducibly correlate strongly and significantly with the HLA-C allotype, and the expression level of a given allotype is consistent across diverse populations. Furthermore, HLA-C transcript levels across allotypes replicate the same hierarchy of HLA-C expression levels. Given these results and the lack of the ability to directly measure HLA-C expression levels, we used MFI as a proxy for expression and examined the association of MFI with outcome.

The MFI is superior to any other genetic proxy for HLA-C expression when studying disease association and provides allotype-specific information for each transplant patient and donor. We found that the expression level of the patient’s mismatched HLA-C is a key determinant of transplant outcome, where mismatches involving alleles that have, on average, higher expression levels are more poorly tolerated than mismatches involving alleles
that have, on average, lower expression levels. Moreover, the expression levels of patients’ mismatches allotypes distinguish permissible (lower MFI) from non-permissible (higher MFI) antigen, residue 116 and residues 77/80 mismatches. Hence, HLA-C expression may introduce a new principle to the paradigm of alloreactivity in transplantation.

The graft-versus-leukemia effect describes a lower risk of relapse among patients with clinical GVHD when compared to patients without GVHD.\textsuperscript{32} In the current analysis, we observed higher risks of acute GVHD, non-relapse mortality and mortality associated with increasing expression levels of the patient’s non-shared HLA-C, without a lowered risk of disease relapse. These results suggest that GVHD-independent relapse may involve the level of surface expression of HLA, and may help to explain why some patients relapse despite developing clinical GVHD. The role of HLA expression in the graft-versus-leukemia effect merits further investigation in the future, when sufficiently large numbers of patients that encode the full range of HLA-C allotypes can be examined.

We surmise that mismatched allotypes expressed at lower levels are more likely to escape detection by the donor, as indicated by the decreased risk of poor outcomes among patients who are mismatched for low-expression allotypes. Our results suggest that avoidance of mismatching for higher expression allotypes in patients may help to lower overall risks after transplantation. When matched donors are not available, mismatching for the lower-expression allotype in the patient may lower risks of GVHD and mortality. Avoidance of HLA-C mismatched donors altogether for patients with two highly expressed allotypes is advisable.

The residue mismatch model posits that donor T-cells recognize differences in the HLA class I/peptide complex of patients.\textsuperscript{8,13,14} When HLA-C mismatches involve differences at residues 77 – 80, they may provoke recognition of donor KIR for patient’s HLA-C ligands that are not self.\textsuperscript{15} A given HLA-C allotype in the patient could generate \textit{in vivo} T-cell as well as NK responses from the donor, but the balance of T and NK reactivity may depend on many factors including those that influence the maturation of NK cells in the donor.\textsuperscript{33-35} In HLA-C-mismatched
unrelated donor transplantation, both T- and NK-mediated allorecognition may contribute to transplant-associated risks. Patient-donor variation at several key residues of class I molecules might be associated with transplant outcome, and of these, residue 116 has received particular attention because of its importance in the peptide-anchoring F pocket of the class I molecule and its high frequency of mismatching among transplant pairs. Residues 77 and 80 reside with residue 116 in the F pocket and influence the size, shape and charge of the peptide-binding groove and the carboxy-terminal peptide anchor. Hence, the same residues that define the cognate ligands of KIRs may also influence the nature of the HLA-C/peptide complex. As any residues 77/80 mismatch is also an allele or antigen mismatch, the specific contribution to non-relapse mortality by T or NK pathways are almost certainly intertwined. The importance of patient-donor mismatching at other class I residues is also of interest. Many HLA-C mismatch combinations involve concurrent mismatching at multiple residues, but few examples exist in the clinical population where allotypes differ at only one of these positions, which is essential for appropriate comparison of residue-specific risks. In addition to residue-specific effects, the role of expression in influencing the immunogenicity of a given HLA allotype in the context of its repertoire of minor histocompatibility antigens is an important question. Such studies may be feasible in the future if and when a sufficiently larger transplant experience is available.

This study provides new insight into the strength of the immune response of HLA-C in transplantation and a platform for exploring the mechanistic basis of T-cell and NK recognition of HLA-C allotypes. Application of the findings in the current study can be envisioned for future patients who do not have HLA-matched donors as an option. The effects of differential allotype expression levels at other HLA loci may further delineate and broaden the pool of acceptable donors for patients, and given the results presented herein, characterizing such effects is warranted.
Acknowledgments

Members of the International Histocompatibility Working Group in Hematopoietic Cell Transplantation who contributed data to this study are listed in Supplemental table 1.

Funding

E.W.P., T.A.G., M.M., S.R.S., M.D.H., and M.M.H. are supported by AI069197 from the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), USA; E.W.P., T.A.G., and M.M. by CA18029 from the National Cancer Institute (NCI); M.M.H. by CA76518 from NCI and by HHSH234200637015C from the Health Resources and Services Administration; S.R.S., M.D.H., and M.M.H. by U24-CA76518 from the NCI, the National Heart, Lung and Blood Institute (NHLBI) and NIAID and by 5U01HL069294 from the NHLBI and NCI and by N00014-12-1-0142 and N00014-13-1-0039 from the Office of Naval Research, USA; A.P.G. by The Associazione Italiana Ricerca sul Cancro, Milano, Italy; P.J. by Project ED2.1.00/03.0076 from the European Regional Development Fund, T.A. ČR TA01010342, and GACR NT/12454 – 5, Czech Republic; O.R. by The Swedish Cancer Society, The Swedish Research Council, The Children’s Cancer Foundation, The Cancer Society in Stockholm and Karolinska Institutet, Stockholm, Sweden; J.M.T. by The Swiss National Science Foundation, grant N°310030-146306/1, Geneva, Switzerland; M.C. by The Frederick National Laboratory for Cancer Research Contract No. HHSN261200800001E and by The Intramural Research Program of the NIH, Frederick National Laboratory, Center for Cancer Research, USA. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. The views expressed in this article do not reflect the official policy or position of the National Institute of Health, the
Department of the Navy, the Department of Defense, or any other agency of the U.S. Government.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Authorship and Conflict-of-interest Statement**
Contribution: E.W.P. conceived and designed the experiments; E.W.P. and M.C. performed the experiments; T.A.G. analyzed the data; E.W.P., A.P.B., A.C., E.D.T., G.E., T.E., G.F.F., T.G., M.D.H., M.M.H., K.H., P.J., A.M., O.R., M.L.S., S.R.S., J.M.T., A.V., C.S.W. contributed reagents/materials/analysis tools; M.M. managed data; E.W.P. wrote the first draft of the manuscript; E.W.P., T.A.G., and M.C. contributed to the writing of the manuscript; M.C., C.O’H. and R.A. contributed MFI values and intellectual content.
Conflict-of-interest disclosure: The authors declare no competing financial interests.

**References**


Table 1. Demographics of the study population

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<th>Characteristic</th>
<th>Transplants (N = 1975)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient age, median years (range)</strong></td>
<td>36.8 (0.19-72.4)</td>
</tr>
<tr>
<td><strong>Donor age, median years (range)</strong></td>
<td>36.1 (18-61.1)</td>
</tr>
<tr>
<td><strong>Year of transplantation</strong></td>
<td></td>
</tr>
<tr>
<td>1983-1992</td>
<td>74 (4%)</td>
</tr>
<tr>
<td>1993-1999</td>
<td>548 (28%)</td>
</tr>
<tr>
<td>2000-2005</td>
<td>880 (45%)</td>
</tr>
<tr>
<td>2006-2011</td>
<td>436 (22%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>37 (2%)</td>
</tr>
<tr>
<td><strong>Patient-donor sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male-male</td>
<td>762 (39%)</td>
</tr>
<tr>
<td>Male-female</td>
<td>397 (20%)</td>
</tr>
<tr>
<td>Female-male</td>
<td>433 (22%)</td>
</tr>
<tr>
<td>Female-female</td>
<td>366 (19%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>17 (&lt;1%)</td>
</tr>
<tr>
<td><strong>Disease/early, intermediate, late or advanced, other or unknown</strong>†</td>
<td></td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>974 (49%)/325, 347, 251, 51</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td>374 (19%)/237, 95, 21, 21</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>241 (12%)/45, 0, 99, 97</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>192 (10%)/ 6, 3, 45, 138</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>27 (1%)/ 1, 0, 18, 8</td>
</tr>
<tr>
<td>Category</td>
<td>Value</td>
</tr>
<tr>
<td>------------------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Other malignancies</td>
<td>12 (&lt;1%), NA</td>
</tr>
<tr>
<td>Non-malignancies</td>
<td>155 (8%), NA</td>
</tr>
<tr>
<td><strong>Transplant type</strong></td>
<td></td>
</tr>
<tr>
<td>Myeloablative/ No TBI</td>
<td>409 (21%)</td>
</tr>
<tr>
<td>Myeloablative/ TBI</td>
<td>967 (49%)</td>
</tr>
<tr>
<td>Reduced-intensity/non-myeloablative</td>
<td>482 (24%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>117 (6%)</td>
</tr>
<tr>
<td><strong>Source of cells</strong></td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1157 (59%)</td>
</tr>
<tr>
<td>Peripheral blood stem cells</td>
<td>779 (39%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>39 (2%)</td>
</tr>
<tr>
<td><strong>GVHD prophylaxis</strong></td>
<td></td>
</tr>
<tr>
<td>Any single agent by itself</td>
<td>42 (2%)</td>
</tr>
<tr>
<td>Two or more agents mixed together</td>
<td>1004 (51%)</td>
</tr>
<tr>
<td>T-cell depletion</td>
<td>798 (40%)</td>
</tr>
<tr>
<td>Other combinations</td>
<td>29 (2%)</td>
</tr>
<tr>
<td>Missing</td>
<td>102 (5%)</td>
</tr>
<tr>
<td><strong>Allele and antigen mismatches</strong></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td>389 (20%)</td>
</tr>
<tr>
<td>Antigen</td>
<td>1582 (80%)</td>
</tr>
<tr>
<td>Unknown‡</td>
<td>4 (&lt;15)</td>
</tr>
<tr>
<td><strong>Residue 116 status of the non-shared patient-donor allotypes</strong></td>
<td></td>
</tr>
<tr>
<td>Matched</td>
<td>847 (43%)</td>
</tr>
<tr>
<td>Mismatched</td>
<td>1108 (56%)</td>
</tr>
</tbody>
</table>
Unknown 20 (<1%)

**Residues 77/80 status of the non-shared**
**patient-donor allotypes**

- Matched 955 (48%)
- Mismatched 996 (50%)
- Unknown 24 (1%)

*Additional characteristics are provided in Supplemental table 2.

†Disease status prior to transplant is categorized as early (first complete remission [CR] of acute myeloid leukemia [AML] or acute lymphoblastic leukemia [ALL]; first chronic phase [CP] of CML; refractory anemia [RA]; refractory anemia with ring sideroblasts of myelodysplastic syndrome [MDS]; non-Hodgkin lymphoma in first CR; chronic lymphoid leukemia in CR); intermediate (second or higher CR of AML or ALL; second or higher CP or accelerated phase of CML; Hodgkin lymphoma in third CR); late or advanced (primary induction failure or first or higher relapse of AML or ALL; blast phase of CML; MDS RA with excess blasts or excess blasts in transformation; non-Hodgkin lymphoma in relapse; Hodgkin lymphoma in third relapse; myeloma; unnamed MDS or unknown).

Other malignancies included breast cancer, renal/kidney carcinoma, hepatoblastoma. Non-malignancies included severe aplastic anemia, Shwachman-Diamond anemia, Diamond-Blackfan anemia, adrenoleukodystrophy, Wiskott Aldrich syndrome, hyper IgM syndrome, hemoglobinopathy, chronic granulomatous disease, familial erythro hemophagocytic lymphocytosis, paroxysmal nocturnal hemoglobinuria, metachromatic leukodystrophy, immunodysregulation polyendocrinopathy enteropathy X-linked like syndrome, Fanconi anemia, bone marrow aplasia, idiopathic bone marrow failure, sickle cell disease, immune deficiency disorder, thalassemia, inherited abnormalities of erythrocyte differentiation or function, other
immune system disorders, inherited abnormality of platelets, inherited disorder of metabolism, histiocytic disorders and other non-malignancies. NA, not applicable.

‡ Four individuals each encoded novel HLA-C sequences that have not yet been characterized using serological reagents.
Table 2. The level of HLA-C expression influences acute GVHD, non-relapse mortality and overall mortality, but not relapse

<table>
<thead>
<tr>
<th>Non-shared HLA-C allotype</th>
<th>Acute GVHD†</th>
<th>Non-relapse mortality†</th>
<th>Overall mortality†</th>
<th>Relapse†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient’s mismatch</td>
<td>OR (95% CI)</td>
<td>P</td>
<td>HR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>1.34 (1.10-1.62)</td>
<td>.003</td>
<td>1.22 (1.06-1.39)</td>
<td>.005</td>
</tr>
<tr>
<td>Donor’s mismatch</td>
<td>1.07 (0.88-1.30)</td>
<td>.49</td>
<td>1.15 (1.01-1.31)</td>
<td>.04</td>
</tr>
<tr>
<td>Sum of mismatched allotypes</td>
<td>1.16 (1.03-1.32)</td>
<td>.02</td>
<td>1.15 (1.06-1.25)</td>
<td>.002</td>
</tr>
</tbody>
</table>

The level of expression of the patient’s mismatch, the donor’s mismatch and the sum of these mismatched allotypes, were each modeled as a continuous linear variable. ORs and HRs are presented as an increase in risk of failure associated with each increase in expression of 100 fluorescence intensity units.

† For the shared (matched) allotype, the OR for acute GVHD was 0.90 (95% CI, 0.71-1.13; P = .36), the HR for non-relapse mortality was 0.85 (95% CI, 0.72-1.00; P = .05), the HR for overall mortality was 0.94 (95% CI, 0.84-1.07; P = .34), and the HR for relapse was 1.10 (95% CI, 0.90-1.34; P = .34).
†Numbers denote the number of patients with failure for each of the four endpoints of all patients with clinical data for the given endpoint.
Table 3. Distribution of HLA-C allotypes according to three models of HLA-C mismatching

<table>
<thead>
<tr>
<th>Patient's non-shared allotype*</th>
<th>Median fluorescence intensity</th>
<th>HLA-C mismatch model for the non-shared patient allotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allele versus Antigen†</td>
</tr>
<tr>
<td></td>
<td>N = 1971</td>
<td>N = 1955</td>
</tr>
<tr>
<td></td>
<td>Allele</td>
<td>Antigen</td>
</tr>
<tr>
<td></td>
<td>N = 389</td>
<td>N = 1582</td>
</tr>
<tr>
<td>C*07</td>
<td>111</td>
<td>104 (27)†</td>
</tr>
<tr>
<td>C*03</td>
<td>114</td>
<td>238 (61)†</td>
</tr>
<tr>
<td>C*17</td>
<td>115</td>
<td>0</td>
</tr>
<tr>
<td>C*05</td>
<td>154</td>
<td>5 (1)</td>
</tr>
<tr>
<td>C*02</td>
<td>164</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>C*08</td>
<td>176</td>
<td>5 (1)</td>
</tr>
<tr>
<td>C*16</td>
<td>180</td>
<td>10 (3)</td>
</tr>
<tr>
<td>C*12</td>
<td>193</td>
<td>4 (1)</td>
</tr>
<tr>
<td>HLA-C Allotype</td>
<td>Mean MFI of the Patient's Mismatched HLA-C Allotype</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>C*04 200</td>
<td>5 (1)</td>
<td>161 (10)</td>
</tr>
<tr>
<td>C*15 223</td>
<td>11 (3)</td>
<td>111 (7)</td>
</tr>
<tr>
<td>C*06 225</td>
<td>5 (&lt;1)</td>
<td>63 (4)</td>
</tr>
<tr>
<td>C*18 239</td>
<td>0</td>
<td>2 (&lt;1)</td>
</tr>
<tr>
<td>C*01 254</td>
<td>0</td>
<td>152 (10)</td>
</tr>
<tr>
<td>C*14 294</td>
<td>1 (&lt;1)</td>
<td>85 (5)</td>
</tr>
</tbody>
</table>

Mean MFI of the patient's mismatched HLA-C allotype: 123.2, 176.7, 148.6, 179.8, 154.2, 177.4

Patient allele mismatches as a group had significantly lower mean MFIs than patient antigen mismatches. The mean MFIs of the patients’ non-shared HLA-C allotypes were significantly different between allele and antigen mismatches, between residue 116-matches and residue 116-mismatches, and between residues 77/80-matches and residues 77/80-mismatches. Similar results were observed for donors’ non-shared allotypes (123.2 and 176.5, \( P < .0001 \) for allele and antigen mismatches; 147.0 and 179.9, \( P < .0001 \) for residue 116-matches and residue 116-mismatches; 150.5 and 180.2, \( P < .0001 \) for residues 77/80-matches and residues 77/80-mismatches). The mean MFIs of the shared matched allotypes, however, did not differ from one another (150.0 and 155.0, \( P = .06 \); 155.2 and 153.3, \( P = .44 \); 152.5 and 155.6, \( P = .15 \), respectively).

*Patients’ mismatched (non-shared) allotypes are listed in order from lowest (C*07) to highest (C*14) MFI.*

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*From www.bloodjournal.org by guest on April 6, 2017. For personal use only.*
†HLA allele and antigens were defined according to WHO HLA Nomenclature.29 Four individuals each encoded novel HLA-C sequences that have not yet been characterized using serological reagents; these individuals were not included in the allele/antigen mismatch analysis.

‡Patients’ and donors’ non-shared HLA-C allotypes can be either matched or mismatched at residue 116. A total of 20 transplants were not included in the residue 116 analysis because they lacked sequence information at this position.

§Patient’s and donors’ non-shared HLA-C allotypes can be either matched or mismatched at residues 77-80 that define the KIR C1 and C2 ligand groups. A total of 24 transplants were not included in the residues 77/80 analysis because they lacked sequencing information for residue 77 and/or 80 or did not have 77S-80N (C1) or 77N-80K (C2).

‖The most common patient-donor mismatch was C*07:01 versus C*07:02 or C*07:02 versus C*07:01; N = 79/104 (76%).

¶The most common patient-donor mismatch was C*03:03 versus C*03:04 or C*03:04 versus C*03:03; N = 216/238 (91%).
Figure Legends

Figure 1. The level of expression of the patient’s mismatched HLA-C allotype associates with transplant outcome. ORs of grades III-IV acute GVHD (A) and HRs of non-relapse mortality (B) for each mismatched HLA-C allotype in the patient is shown relative to C*07. The expression of the patient’s mismatched allotype was defined by its median fluorescence intensity as determined previously. The size of each circle is proportional to the number of patients with the indicated allotype. The least-squares line shown is weighted by the number of observations at each expression level.

Figure 2. Demonstration of interaction between expression level of patients’ non-shared HLA-C and residue 116 (A) and residues 77/80 match status (B) for non-relapse mortality. Patients’ low-expression mismatched allotypes were defined as HLA-C*07 and C*03; high-expression mismatched allotypes were defined as HLA-C*01 and C*14. Each 95% CI is denoted by black bars. Numbers in parentheses indicate the patients who died of non-relapse causes of the total number of patients in each of the four groups as defined by residue 116 match status, residues 77/80 match status and level of expression of the patients’ mismatched HLA-C.
Figure 2

(A) Residue 116 match status-expression level

- Match-low (N = 173/441)
- Mismatch-low (N = 97/267)
- Match-high (N = 22/56)
- Mismatch-high (N = 74/145)

P = 0.003

(B) Residues 77/80 match status-expression level

- Match-low (N = 182/459)
- Mismatch-low (N = 87/247)
- Match-high (N = 30/78)
- Mismatch-high (N = 66/122)

P = 0.001

P = 0.47

P = 0.89

P = 0.88

P = 0.55
HLA-C expression levels define permissible mismatches in hematopoietic cell transplantation


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