Specific class I HLA supertypes but not HLA zygosity or expression are associated with outcomes following HLA-matched allogeneic hematopoietic cell transplant

Christine Camacho-Bydume, MD[1], Tao Wang, PhD[2], Jennifer A. Sees, MPH[3], Marcelo Fernandez-Viña, PhD[4], Muhammad Bilal Abid, MD, MRCP[5], Medhat Askar, MD, PhD[6], Amer Beitinjaneh, MD, MSc, MPH[7], Valerie Brown, MD, PhD[8], Paul Castillo, MD[9], Saurabh Chhabra, MD, MS[10], Shahinaz M. Gadalla, MD, PhD[11], Jing-Mei Hsu, MD, PhD[12], Malek Kamoun, MD, PhD[13], Aleksandr Lazaryan, MD, MPH, PhD[14], Taiga Nishihori, MD[14], Kristin Page, MD, MHS[15], Johannes Schetelig, MD, MSc[16], Katharina Fleischhauer, MD[17], Steven G. E. Marsh, BSc, PhD, ARCS, FRCPath[18,19], Sophie Paczesny, MD, PhD[20], Stephen R. Spellman, MBS[3], Stephanie J. Lee, MD, MPH[21,22], and Katharine C. Hsu, MD, PhD[23-25]

[1]Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY

[2]Division of Biostatistics, Medical College of Wisconsin, Milwaukee, WI

[3]Center for International Blood and Marrow Transplant Research, National Marrow Donor Program/Be The Match, Minneapolis, MN

[4]Department of Pathology at Stanford University School of Medicine, Stanford, CA

[5]Divisions of Hematology/Oncology and Infectious Diseases, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI

[6]Department of Pathology and Laboratory Medicine, Baylor University Medical Center, Dallas, TX

[7]Department of Medicine, Division of Transplantation and Cellular Therapy, University of Miami, Miami, FL

[8]Division of Pediatric Hematology/Oncology, Department of Pediatrics, Penn State Hershey Children's Hospital and College of Medicine, Hershey, PA

[9]Division of Pediatric Hematology/Oncology, Department of Pediatrics, University of Florida Health Shands Children's Hospital, Gainesville, FL

[10]Division of Hematology/Oncology, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI

[11]Division of Cancer Epidemiology and Genetics, NIH-NCI Clinical Genetics Branch, Rockville, MD

[12]Division of Hematology/Oncology, Department of Medicine, Sandra and Edward Meyer Cancer Center, Weill Cornell Medicine/New York Presbyterian Hospital, New York, NY

[13]Deparment of Pathology and Laboratory Medicine, Perelman School of Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA

[14]Department of Blood and Marrow Transplant and Cellular Immunotherapy, Moffitt Cancer Center, Tampa, FL

[15]Division of Pediatric Blood and Marrow Transplantation, Duke University Medical Center, Durham, NC

[16]Department of Internal Medicine I, University Hospital Carl Gustav Carus Dresden, Dresden, Germany

[17]Institute for Experimental Cellular Therapy, University Hospital Essen, Essen, Germany

[18]Anthony Nolan Research Institute, Royal Free Hospital, London, UK

[19]UCL Cancer Institute, London, UK

[20]Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC

[21]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA

[22]Department of Medicine, University of Washington, Seattle, WA.

[23]Department of Medicine, Adult Bone Marrow Transplantation Service, Memorial Sloan Kettering Cancer Center, New York, NY

[24]Department of Medicine, Weill Cornell Medical College, New York, NY

[25]Human Oncology & Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY

Corresponding Author:

Dr. Katharine Hsu

Memorial Sloan Kettering Cancer Center

1275 York Avenue

New York, NY 10065

Phone: 646-888-2667

Email: hsuk@mskcc.org

Short Title: HLA supertypes impact allogeneic HCT outcomes

Abstract: 248

Manuscript: 3,685

Tables: 4

Figures: 2

Supplementary tables: 2

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HIGHLIGHTS

- HLA zygosity, supertypes, and expression are evaluated in post-transplant outcomes
- HLA-B62 supertype is associated with reduced transplant-related mortality
- HLA-B27 supertype is correlated with worse disease free-survival in AML
- No association exists between HLA zygosity or expression and transplant outcomes

ABSTRACT

Maximizing the probability of antigen presentation to T cells through diversity in human leukocyte antigens (HLA) can enhance immune responsiveness and translate into improved clinical outcomes, as evidenced by the association of heterozygosity and supertypes at HLA class I loci with improved survival in patients with advanced solid tumors treated with immune checkpoint inhibitors. We investigated the impact of HLA heterozygosity, supertypes, and surface expression on outcomes in adult and pediatric patients with AML, MDS, ALL, and NHL who underwent 8/8 HLA-matched, T cell replete, unrelated, allogeneic hematopoietic cell transplant (HCT) from 2000 to 2015 using patient data reported to the Center for International Blood and Marrow Transplant Research. HLA class I heterozygosity and HLA expression were not associated with overall survival, relapse, transplant-related mortality (TRM), disease-free survival (DFS), and acute graft-versus-host disease following HCT. The HLA-B62 supertype was associated with decreased TRM in the entire patient cohort (HR=0.79, 95% CI 0.69 – 0.90, P=0.00053). The HLA-B27 supertype was associated with worse DFS in patients with AML (HR=1.21, 95% CI, 1.10-1.32, P=0.00005). These findings suggest that the survival benefit of HLA heterozygosity seen in solid tumor patients receiving immune checkpoint inhibition does not extend to patients undergoing allogeneic HCT. Certain HLA supertypes, however, are associated with TRM and DFS, suggesting that similarities in peptide presentation between supertype members play a role in these outcomes. Beyond implications for prognosis following HCT, these findings support the further investigation of these HLA supertypes and the specific immune peptides important for transplant outcomes.

KEYWORDS: Hematopoietic cell transplant (HCT); allogeneic HCT; human leukocyte antigen (HLA); HLA heterozygosity; HLA supertypes

INTRODUCTION

Eliciting an adaptive immune response against infection or malignancy requires precise interaction of T cell receptors on cytotoxic T lymphocytes with peptides bound to human leukocyte antigen (HLA) expressed on the surface of antigen-presenting cells[1, 2]. Effective immune surveillance benefits from extensive polymorphism within the HLA genetic region encoding amino acid residues centered in the peptide-binding groove, where diversity expands the overall repertoire of HLA-bound peptide ligands and their respective antigen-specific T cells[2]. A more diverse repertoire of HLA molecules capable of binding tumor peptides for presentation to T cells may therefore potentially increase the likelihood of engendering an immune response and ultimately improving clinical outcomes[2, 3].

Heterozygosity at the HLA class I loci of HLA-A, -B, and -C is associated with improved survival in patients with advanced solid tumors treated with immune checkpoint inhibitors when compared to patients with homozygosity in at least one HLA class I locus[4]. Similarly, in cases of human immunodeficiency virus (HIV), HLA locus heterozygosity in African American and Caucasian patients is associated with slower progression to acquired immunodeficiency syndrome (AIDS) and improved overall survival (OS)[5]. The protective effect of HLA locus heterozygosity on survival has been attributed to the greater likelihood of tumor- or pathogen-derived peptide presentation within a more diverse HLA landscape to autologous T cells, leading to an effective immune response[4,5].

While considerable genetic diversity has generated thousands of HLA class I alleles, multitudes of HLA molecules can be functionally classified into HLA supertypes, distinct groupings of HLA-A and -B alleles that share chemical specificities in the B and F binding pockets of the peptide-binding groove, leading to overlapping peptide-binding specificities[3]. HLA

supertypes have been associated with disease outcomes[6-10] and recently have been shown to impact survival based on differences in their predicted capacities for tumor and viral antigen presentation[4]. Among melanoma patients treated with immune checkpoint inhibitors, those expressing the HLA-B44 supertype experienced improved survival, presumably due to shared binding properties to a large number of melanoma antigens[4]. In contrast, those patients exhibiting the HLA-B62 supertype experienced reduced survival, an association primarily influenced by the member allele, HLA-B*15:01. Notably, the HLA molecule encoded by HLA-B*15:01 is distinguished by an amino acid bridge in the peptide-binding groove that impairs the strength of interaction of a bound peptide within HLA to T cells, which is presumed to interfere with antigen recognition and immune responsiveness[4].

Surface expression of HLA has also been implicated in dictating immune responsiveness[11-14]. Development of autoimmune diseases has been linked to increased surface expression of HLA class I alleles, while certain malignancies are capable of evading immune detection via downregulation of HLA[11, 12]. Elevated HLA-C expression is associated with improved viral control of HIV in African and European Americans due to increased strength and likelihood of cytotoxic T cell responses to the virus[12]. In the setting of mismatched allogeneic hematopoietic cell transplant (HCT), lower expression of the mismatched HLA-C allele is associated with improved survival and decreased risk of acute graft-versus-host disease (aGVHD) and non-relapse mortality, presumably due to lower levels of immune detection[13]. Therefore, HLA surface expression directly titrates opportunity for antigen presentation and calibrates the strength of the immune response, thereby influencing clinical outcomes.

Although HLA heterozygosity, supertypes, and expression levels have been evaluated separately for their role in immune responsiveness, their effects have not been investigated in the

setting of HLA-matched allogeneic HCT. Because donor T cells are a significant contributor to graft-versus-leukemia (GVL) effect in allogeneic HCT, we hypothesize that HLA class I heterozygosity, supertype, and increased HLA expression will influence disease control and survival through their effect on improved antigen presentation to antigen-specific T cells.

MATERIALS AND METHODS

Study Design

We retrospectively analyzed 7,474 pediatric and adult patients with a diagnosis of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), or non-Hodgkin lymphoma (NHL), who underwent an 8/8 HLA-matched (HLA-A, -B, -C, and -DR) first allogeneic HCT from an unrelated donor between 2000 to 2015. Patients were included if they received T cell replete bone marrow or peripheral blood stem cell allografts following myeloablative conditioning. The exception were patients with NHL, who more commonly receive reduced-intensity conditioning (RIC) or non-myeloablative (NMA) regimens. All patients had high-resolution allele-level HLA typing performed prior to HCT.

Patients were excluded if they received a previous HCT, underwent *ex vivo* T cell depletion (CD34 selection), received post-transplant cyclophosphamide, received RIC or NMA conditioning for AML, MDS, or ALL, had less than 100 days of follow up post-transplant, or did not consent to participate in research. All patient data were collected from the National Marrow Donor Program (NMDP)/Center for International Blood and Marrow Transplant Research (CIBMTR). Patients represented a total of 314 transplant centers, and all provided informed consent to participate in NMDP/CIBMTR research. This retrospective study was approved by the

NMDP/CIBMTR's Institutional Review Board, and standard methods of NMDP/CIBMTR data collection and analysis were employed.

HLA Class I Typing and Designation of HLA Zygosity, Supertypes, and Expression Levels

HLA high resolution allele typing for HLA-A, -B, -C and -DR was performed, confirmed, and provided by the NMDP[15]. Patients were categorized as HLA heterozygous if they differed at the allele-level for all HLA class I loci of HLA-A, -B, and -C. HLA homozygous patients were those who shared the same alleles in at least one HLA class I locus of HLA-A, -B, or -C.

Assignment of patient HLA supertypes was based on the updated classification by Sidney and colleagues of 945 HLA-A and -B alleles[3] (Supplemental Table 1A and 1B). Among patient HLA genotypes, 80% of alleles could be categorized based on shared chemical specificities of the B and F binding pockets into one of the twelve established supertypes[3]. The remaining HLA-A and -B alleles that did not belong to a specific supertype were designated as unclassified and excluded from this analysis.

We explored the effects of expression of HLA-Bw4 and HLA-C alleles based on available expression data[12-14, 16-21]. HLA alleles bearing the Bw4 epitope as well as HLA-C alleles were stratified into high versus low expression subtypes based on previously published surface expression data using median fluorescence intensity (MFI) as a comparative measure[9,10,13]. High-expressing HLA-Bw4 and -C alleles were identified as those with MFIs greater than the overall mean, while low expressing alleles were those with MFIs less than the mean. Patient genotypes were categorized into high, mixed (high/low), or low Bw4 or C expression subgroups based on the presence of 1 or 2 copies of HLA-Bw4 or HLA-C alleles with known expression data and analyzed for their association with clinical outcomes post-HCT. Patients homozygous for Bw6 alleles or for

Bw4 alleles with unknown expression data were excluded. Patients homozygous for HLA-C alleles with unknown expression data were also excluded from this portion of analysis.

Statistical Analysis

The primary study outcomes of OS and relapse were evaluated among the entire patient cohort as well as among individual disease subgroups. OS was defined as the time from HCT to death from any cause. Relapse represented the time from HCT to disease recurrence. Patients who received a second HCT or were lost to follow-up were censored at the time of the second transplant or last follow-up, respectively. Secondary outcomes included transplant-related mortality (TRM), disease-free survival (DFS), and aGVHD. TRM was described as time to death without evidence of disease recurrence and patients were censored at time of relapse or last follow-up; relapse was a competing risk. DFS was defined as the time to treatment failure due to death or relapse with patients censored at the time of last follow-up. aGVHD was the cumulative incidence of Grade II-IV and III-IV GVHD per consensus criteria at day 100 post-HCT. Multivariate analyses for OS, relapse, DFS, TRM, and aGVHD were performed using the Cox proportional hazards model. All variables were tested for the affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption were adjusted through stratification. A stepwise variable selection procedure was then used to develop multivariable models for the primary and secondary outcomes. For the multivariable analysis of HLA heterozygosity, a P-value of < 0.01 was considered significant when analyzed for the entire patient cohort. When evaluating disease subgroups individually, a P-value of <0.00125 was considered significant to account for multiple comparisons. For outcomes association studies of HLA supertype and HLA-Bw4 and -C allele expression, each HLA supertype, HLA-Bw4, and C-allele expression variable was tested

separately after adjusting for the selected patient and clinical variable. To adjust for multiple testing, a P-value of < 0.00059 for the entire cohort and P-value of <0.000147 for disease subgroups were considered significant. All P-values were raw and 2-sided. Statistical analyses were done using SAS version 9.4.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. A total of 7,474 patients were included in the study. The median patient age was 45.5 years (range 0-76). Males comprised 56% of the study population, and Caucasians represented 88% of the study population. A diagnosis of AML accounted for nearly half of all malignancies for patients undergoing HCT while 16% of patients were diagnosed with NHL. Peripheral blood stem cells were used for 69% of all HCT allografts. The majority of patients (77%) were classified as heterozygous to the allele-level at all HLA class I loci of HLA-A, -B, and -C. HLA homozygosity was present in 22% of the total study population with 46% of those patients homozygous at the HLA-A locus only (data not shown). Only 12% of patients possessed a full homozygous haplotype at HLA-A, -B, and -C loci (data not shown).

HLA Heterozygosity

In an 8/8-HLA matched allogeneic HCT setting, there were no significant differences observed in clinical outcomes for patients who were homozygous for at least one HLA class I locus when compared to those who were heterozygous at all HLA class I loci of HLA-A, -B, and -C. In multivariate analysis, OS was not associated with HLA homozygosity (HR=0.96; 95% CI, 0.88 – 1.04, *P*=0.33) for the entire patient cohort (Table 2A) or disease subgroups (Table 2B and data not

shown). There were no differences observed for relapse, TRM, DFS, and aGVHD in patients with HLA homozygosity when compared to patients with HLA heterozygosity (Table 2A) both among the entire cohort and within individual disease subgroups (Table 2B and data not shown).

HLA Supertypes

In our study population, 1% of patients were categorized as unclassified for both of their HLA-A alleles, while 32% of patients remained unclassified for their HLA-B alleles[3]. Those patients with HLA-A or -B alleles that were unclassified (i.e. did not belong to a defined HLA supertype) were excluded from each corresponding analysis. There was a total of 16 patients who exhibited both unclassified HLA-A and -B alleles and thus were not included in the outcome analyses based on supertypes. No associations were observed for OS, relapse, and aGVHD based on HLA-A or -B supertypes within the entire patient cohort (Table 2A) or within disease subgroups (Table 2B and data not shown).

The presence of the HLA-B62 supertype was significantly associated with decreased TRM among the entire cohort when compared to patients without the HLA-B62 supertype (HR=0.79; 95% CI, 0.69 – 0.90, *P*=0.00053) (Table 2A and Figure 1). Lower risk of TRM among patients with AML and ALL subgroups was also observed among patients with the HLA-B62 supertype (*P*=0.015 and *P*=0.0077, Table 2B and data not shown, respectively), although the analysis did not achieve statistical significance per p-values established for this study.

The HLA-B62 supertype was previously found to be associated with reduced OS in patients with melanoma treated with immune checkpoint inhibitors, an outcome attributed to poor antigen presentation due to an encoded amino acid bridge in the peptide-binding groove of its primary member allele, HLA-B*15:01[4]. We hypothesized that this structural feature may also impact

presentation of antigens related to TRM leading to weaker interactions with T cells, decreased antigen recognition, and diminished immune activation. We investigated whether the protective effect on TRM varied depending upon the presence of the HLA-B*15:01 allele among patients with HLA-B62 supertype. We found that there was a similarly decreased risk of TRM in patients with HLA-B62 supertype with the HLA-B*15:01 allele (HR=0.81; 95% CI, 0.69 – 0.96, *P*=0.013) and in patients with HLA-B62 supertype without the HLA-B*15:01 allele (HR=0.72; 95% CI, 0.50 – 1.04, *P*=0.081) when compared to patients without HLA-B62 supertype (Table 3). These results further illustrate that the protective effect on TRM is impacted by the presence of the HLA-B62 supertype and is not an effect restricted to its most frequent allele member. We also evaluated specific causes of death in our patient population based on the presence or absence of the HLA-B62 supertype (Table 4). Interestingly, disease progression as a cause of death was significantly more prevalent in patients with the HLA-B62 supertype (51%) when compared to those without HLA-B62 supertype (44%) (*P*=0.001).

Within the AML cohort, DFS was significantly decreased in patients with HLA-B27 supertype (HR=1.21; 95% CI, 1.10 - 1.32, *P*=0.00005, Table 2B and Figure 2) when compared to patients without HLA-B27 supertype. A similar trend of DFS and HLA-B27 supertype was detected in the entire cohort (HR=1.12; 95% CI, 1.04 - 1.20, *P*=0.0034) (Table 2A).

Expression Levels of HLA-Bw4 and -C Alleles

In our study, 1% of patients failed to be stratified by HLA expression due to the absence of expression data for their HLA-B and -C alleles[12, 16]. Among all patients, 58% had at least one HLA-B allele with the Bw4 epitope: 39% were considered to have low Bw4 expression (Bw4 low/low or Bw4 low/Bw6), 5% exhibited mixed Bw4 expression (Bw4 low/high), and 13% had

high Bw4 expression (Bw4 high/high or Bw4 high/Bw6). Patients homozygous for Bw6 accounted for 42% of the entire cohort and were therefore excluded from this portion of the analysis. Within the entire cohort, patients with high HLA-Bw4 expression had a trend of higher risk of relapse (HR=1.20; 95% CI, 1.05 - 1.39, P=0.0096) when compared to those with low HLA-Bw4 expression. We found no association between HLA-Bw4 expression and OS, DFS, TRM, and aGVHD for the entire patient cohort (Table 2A) or within disease subgroups (Table 2B and data not shown). Upon evaluation for HLA-C expression, most patients were classified as having low HLA-C expression (54%) while 8% of patients showed exclusively high HLA-C expression. The remainder of patients (38%) were identified as mixed HLA-C expression. We found no association of HLA-C expression and outcomes of OS, relapse, DFS, TRM, and aGVHD both for the entire cohort (Table 2A) and within disease subgroups (Table 2B and data not shown).

DISCUSSION

Our study was designed to investigate several mechanisms by which differences in HLA, including heterozygosity, supertypes, and surface expression, impact antigen presentation to T cells. Each of these mechanisms could potentially lead to differences in adaptive immune response and influence clinical outcomes following allogeneic HCT. In this retrospective analysis of 8/8-HLA matched unrelated HCT for myeloid and lymphoid malignancies, we did not identify an effect of HLA heterozygosity on OS, relapse, TRM, DFS, or aGVHD post-HCT when analyzed in the entire patient cohort or within disease subgroups. Differences in HLA surface expression also were not significantly associated with post-transplant outcomes. We did observe that the HLA supertypes HLA-B62 and HLA-B27 were significantly associated with specific outcomes post-transplant. In the entire patient cohort, the HLA-B62 supertype was associated with decreased TRM, while the

HLA-B27 supertype correlated with worse DFS in the AML subgroup, potentially revealing how shared binding capacities of HLA molecules may influence antigen presentation and immune response in allogeneic HCT. It is notable that both effects of HLA-B62 and -B27 supertype of TRM and DFS, respectively, occurred early in the post-transplant course within the first six months and persisted for the remainder of the follow-up period (Figures 1-2).

The classification of HLA supertypes allows large numbers of HLA alleles with shared peptide-binding specificities to be grouped together for more streamlined analysis of HLA effects[22]. Unlike the highly polymorphic individual HLA alleles that vary among different ethnicities, HLA supertypes have more consistent expression among populations providing a more stable and representative group for analysis[22, 23]. The HLA-B62 supertype, which is comprised largely of HLA-B15 alleles, has previously been associated with outcomes in HIV-infected individuals and in melanoma patients treated with immune checkpoint inhibitors via separate immune mechanisms. In HIV-infected patients, the HLA-B62 supertype is associated with lower HIV viral load and delayed progression to AIDS,[8, 9, 23, 24] a finding attributed to its binding of conserved viral epitopes that are less likely to mutate and escape immune recognition[8]. Given the equivalent infection-related deaths among those patients with and without HLA-B62 supertype, it is possible that a recently identified structural feature of a prominent member of the HLA-B62 supertype is responsible for the observed HCT outcomes[4].

As the prevalent member allele of HLA-B62 supertype, HLA-B*15:01 is an appropriate representative of its shared peptide-binding capacities[25, 26]. HLA-B*15:01 was found in approximately 80% of patients with HLA-B62 supertype in our study population. Among patients with melanoma treated with immune checkpoint inhibitors, the association of HLA-B62 supertype with decreased OS was primarily influenced by HLA-B*15:01[4]. HLA-B*15:01 encodes an amino

acid bridge that impedes antigen binding, leading to decreased strength of tumor antigen presentation and reduced anti-tumor response[4].

In our study, the association of decreased TRM with HLA-B62 supertype was further investigated by evaluating the effect of the member allele HLA-B*15:01. We found that both HLA-B62 supertype subgroups with and without the HLA-B*15:01 allele offered comparable protection from TRM. It is notable that this association was significant in the subgroup with HLA-B*15:01 present, which suggests that this allele strongly impacts the supertype's overall effect. We also evaluated TRM by analyzing the causes of death of patients based on the presence of HLA-B62 supertype. There was a trend towards lower GVHD-related deaths in patients with HLA-B62 supertype and a significantly higher rate of deaths secondary to disease progression. Like for melanoma, it is possible that HLA-B62 members exhibit hindered presentation of GVHD-related antigens as well as tumor antigens, leading to decreased TRM and increased disease-related death.

Although the effect on clinical outcomes following immune checkpoint inhibitors and allogeneic HCT may seem paradoxical, both the negative effect on OS and protective effect on TRM, respectively, could be attributed to the same underlying mechanism of structural impedance encoded by the primary member allele of the HLA-B62 supertype, hampering antigen binding, decreasing strength of cellular interactions during antigen presentation, and reducing immune surveillance[4].

The association of HLA-B27 supertype with worse DFS in patients with AML was a unique finding in our study that was not previously demonstrated in analysis of patients with advanced solid tumors treated with immune checkpoint inhibitors[4]. The HLA-B27 supertype is comprised predominantly of HLA-B27 alleles, an intriguing and dichotomous HLA molecule

recognized for its well-established association with multiple autoimmune diseases and its protective effect in viral infections, such as HIV and hepatitis C[3, 27, 28]. Due to its high level of cell surface expression and narrow antigenic repertoire, HLA-B27 likely provides protection against HIV by acting as a "specialized" HLA capable of recognizing unique antigens pathogenic to HIV and typically difficult for the virus to mutate in order to evade immune detection, thereby conferring more stable protection against the virus[29]. In our study, the association of HLA-B27 supertype with worse DFS in AML may be explained by the same "specialized" role of its HLA-B27 member alleles. It is possible that the HLA-B27 allotypes may not bind tumor antigens associated with AML, resulting in decreased tumor immune surveillance, higher rates of disease progression, and worse DFS.

Our study did not demonstrate a similar protective effect of HLA heterozygosity on disease control following HCT as was found for solid tumors treated with checkpoint blockade. It is possible that the differences in post-HCT microenvironment, including a limited T cell repertoire, dissimilar tumor antigens, alloreactivity of T cells, and presence of minor histocompatibility complex antigens, overrides any anti-tumor effect seen in treatment of solid tumor patients with immune checkpoint inhibitors[30, 31]. The protective effect of HLA heterozygosity also may be more relevant in the setting of immunotherapy where autologous T cell reactivity and activation thresholds have been altered by the treatment.

A predicted increase in tumor antigen presentation offered by high surface expression of HLA was not identified to be associated with outcomes following allogeneic HCT. Surprisingly, we found a trend of higher risk of relapse in patients with high HLA-Bw4 expression when compared to patients with low expression in the entire cohort, although not to a statistically significant level. Given that HLA-Bw4 is a ligand for the inhibitory killer immunoglobin-like

receptor KIR3DL1 on natural killer (NK) cells, it is possible that NK cell reactivity plays a greater role than T cell reactivity in tumor surveillance in this setting. Strong inhibitory interactions between KIR3DL1 and specific HLA-Bw4 allotypes have been shown to be associated with higher rates of relapse in patients with AML[17].

Limitations of our study may be due to the inherent challenges related to retrospective analysis. The inclusion of patients who underwent HCT during a 15-year time span from 2000 to 2015 may confound results due to differences in conditioning regimens and medical management. Although our overall study population was large, subgroup analysis may have lacked the power to detect significant differences.

Our study sought to identify how differences in HLA diversity, antigen binding, and expression may influence antigen presentation, calibrating the strength of the immune response and impacting clinical outcomes following allogeneic HCT. While we did not observe an impact of zygosity for HLA class I alleles on HCT outcomes, we did find associations of HLA supertypes. The association of the HLA supertype HLA-B62 with TRM and disease-related death is intriguing. Because this supertype has been found to influence outcomes in solid tumor patients treated with immune checkpoint inhibitors, this supertype and, in particular, its member allele HLA-B*15:01, may have far-reaching implications for immune surveillance due to a structural peculiarity. The finding that the HLA supertype HLA-B27 is associated with lower DFS in AML patients may similarly have prognostic utility. Although further validation studies are needed, consideration of the differential immunogenic potentials of HLA supertypes on immune surveillance may impact donor selection to minimize morbidity and mortality associated with allogeneic HCT.

ACKNOWLEDGMENTS

This work was supported by funding from NIH U01 AI069197 and Leukemia & Lymphoma Society to KH and the Cancer Center Core grant (NIH P30 CA008748) to MSKCC.

The CIBMTR is supported primarily by Public Health Service U24CA076518 from the National Cancer Institute (NCI), the National Heart, Lung and Blood Institute (NHLBI) and the National Institute of Allergy and Infectious Diseases (NIAID); U24HL138660 from NHLBI and NCI; OT3HL147741, R21HL140314 and U01HL128568 from the NHLBI; HHSH250201700006C, SC1MC31881-01-00 and HHSH250201700007C from the Health Resources and Services Administration (HRSA); and N00014-18-1-2850, N00014-18-1-2888, and N00014-20-1-2705 from the Office of Naval Research; Additional federal support is provided by P01CA111412, R01CA152108, R01CA215134, R01CA218285, R01CA231141, R01HL126589, R01AI128775, R01HL129472, R01HL130388, R01HL131731, U01AI069197, U01AI126612, and BARDA. Support is also provided by Be the Match Foundation, Boston Children's Hospital, Dana Farber, Japan Hematopoietic Cell Transplantation Data Center, St. Baldrick's Foundation, the National Marrow Donor Program, the Medical College of Wisconsin and from the following commercial entities: AbbVie; Actinium Pharmaceuticals, Inc.; Adaptive Biotechnologies; Adienne SA; Allovir, Inc.; Amgen, Inc.; Anthem, Inc.; Astellas Pharma US; AstraZeneca; Atara Biotherapeutics, Inc.; bluebird bio, Inc.; Bristol Myers Squibb Co.; Celgene Corp.; Chimerix, Inc.; CSL Behring; CytoSen Therapeutics, Inc.; Daiichi Sankyo Co., Ltd.; Gamida-Cell, Ltd.; Genzyme; GlaxoSmithKline (GSK); HistoGenetics, Inc.; Incyte Corporation; Janssen Biotech, Inc.; Janssen Pharmaceuticals, Inc.; Janssen/Johnson & Johnson; Jazz Pharmaceuticals, Inc.; Kiadis Pharma; Kite Pharma; Kyowa Kirin; Legend Biotech; Magenta Therapeutics; Mallinckrodt LLC; Medac GmbH; Merck & Company, Inc.; Merck Sharp & Dohme Corp.;

Mesoblast; Millennium, the Takeda Oncology Co.; Miltenyi Biotec, Inc.; Novartis Oncology; Novartis Pharmaceuticals Corporation; Omeros Corporation; Oncoimmune, Inc.; Orca Biosystems, Inc.; Pfizer, Inc.; Phamacyclics, LLC; Regeneron Pharmaceuticals, Inc.; REGiMMUNE Corp.; Sanofi Genzyme; Seattle Genetics; Sobi, Inc.; Takeda Oncology; Takeda Pharma; Terumo BCT; Viracor Eurofins and Xenikos BV. 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, Health Resources and Services Administration (HRSA) or any other agency of the U.S. Government.

AUTHORSHIP CONTRIBUTIONS

CCB and KH devised the study design, interpreted the data, and wrote the manuscript. Members of the CIBMTR Immunobiology working committee assisted in the study design. TW and JAS provided statistical support. All authors interpreted and discussed the results and reviewed the manuscript.

CONFLICT OF INTEREST DISCLOSURE

SP has a patent on "Methods of detection of graft-versus-host disease" licensed to Viracor-IBT Laboratories. TN has research with Novartis and Karypopharm. All other authors have no other relevant conflicts of interest to declare.

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Table 1. Characteristics of Study Population

Characteristic	Heterozygous at all HLA class I loci N (%)	Homozygous at ≥ 1 HLA class I locus N (%)	P-value[a]
Number of Recipients	5775	1699	
Number of Centers	175	139	
Recipient Age, median (range), years	45 (0-75)	46 (1-76)	0.14
Race of Recipient			0.18
Caucasian, non-Hispanic	5074 (90)	1487 (90)	
Recipient Gender			0.53
Male	3268 (57)	947 (56)	
Female	2507 (43)	752 (44)	
Disease			0.30
AML	2896 (50)	839 (49)	
ALL	1386 (24)	384 (23)	
MDS	570 (10)	178 (10)	
NHL	923 (16)	298 (18)	
Stem Cell Source			0.33
Marrow	1781 (31)	503 (30)	
PBSC	3994 (69)	1196 (70)	
Year of Transplant			<0.001
2000 – 2005	1212 (21)	287 (17)	
2006 – 2010	1997 (35)	603 (35)	
2011 – 2015	2566 (44)	809 (48)	
CMV Serostatus Donor/Recipient			0.92
+/+	1323 (24)	387 (24)	
+/-	546 (10)	152 (9)	
-/+	2077 (37)	620 (37)	
-/-	1679 (30)	498 (30)	
Unknown	150 (N/A)	42 (N/A)	
Donor/Recipient Sex Match			0.18
Male/Male	2437 (42)	728 (43)	
Male/Female	1597 (28)	502(30)	
Female/Male	831 (14)	219 (13)	
Female/Female	910 (16)	250 (15)	
Conditioning Regimen			0.15
Myeloablative	5209 (90)	1512 (89)	
RIC/NMA	566 (10)	187 (11)	
GVHD Prophylaxis			0.04
Tacrolimus + MMF ± others	696 (12)	225 (13)	
Tacrolimus + MTX ± others (except MMF)	3444 (60)	1040 (61)	
Tacrolimus + others (except MTX, MMF)	351 (6)	83 (5)	
Tacrolimus alone	123 (2)	44 (3)	
CSA + MMF ± others (except Tacrolimus)	222 (4)	77 (5)	
CSA + MTX ± others (except Tacrolimus, MMF)	857 (15)	207 (12)	
CSA + others (except Tacrolimus, MTX, MMF)	43 (1)	11 (1)	
CSA alone	39 (1)	12 (1)	
ATG/Campath Usage			0.54
ATG + CAMPATH	2 (<1)	1 (<1)	
ATG alone	1682 (29)	525 (31)	

Characteristic	Heterozygous at all HLA class I loci N (%)	Homozygous at ≥ 1 HLA class I locus N (%)	P-value[a]
CAMPATH alone	201 (3)	59 (3)	
No ATG or CAMPATH	3882 (67)	1113 (66)	
Unknown	8 (N/A)	1 (N/A)	
Median Follow-up of Survivor, months (range)	63 (6-216)	61 (3-193)	0.11

[a] The Pearson chi-square test was used for comparing discrete variables; the Kruskal-Wallis test was used for comparing continuous variables.

HLA, human leukocyte antigen; N, number; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; PBSC, peripheral blood stem cells; CMV, cytomegalovirus; RIC, reduced intensity conditioning; NMA, non-myeloablative; GVHD, graft-versus-host disease; MMF, mycophenolate mofetil; MTX, methotrexate; CSA, cyclosporine; ATG, anti-thymocyte globulin

Table 2. Multivariate results for (A) the entire cohort and (B) the AML subgroup. No differences were seen for the ALL, MDS and NHL subgroups (data not shown).

2A. Entire Cohort

	N	HCT Outcome	HR	95% CI	P-value
HLA Zygosity					
Heterozygous	5775	-	1.00	-	-
Homozygous	1699	OS	0.96	0.88 – 1.04	0.33
		Relapse	0.96	0.88 – 1.04	0.30
		DFS	0.94	0.88 – 1.01	0.11
		TRM	1.06	0.95 – 1.18	0.30
		aGVHD II – IV	1.05	0.96 – 1.16	0.30
		aGVHD III – IV	1.16	1.01 – 1.34	0.035
HLA Supertype*					
A01	3249	OS	1.07	1.00 – 1.14	0.057
		Relapse	0.91	0.85 – 0.98	0.013
		DFS	1.03	0.97 – 1.09	0.39
		TRM	1.15	1.05 – 1.26	0.0022
		aGVHD II – IV	0.97	0.88 – 1.06	0.46
		aGVHD III – IV	0.89	0.79 – 0.99	0.037
A02	3954	OS	0.97	0.91 – 1.03	0.27
		Relapse	1.07	0.99 – 1.16	0.093
		DFS	0.99	0.93 – 1.04	0.65
		TRM	0.90	0.82 – 1.00	0.040
		aGVHD II – IV	1.08	0.99 – 1.17	0.083
		aGVHD III – IV	1.07	0.94 – 1.21	0.31
A03	3537	OS	1.01	0.95 – 1.09	0.69
		Relapse	1.04	0.96 – 1.14	0.35
		DFS	1.02	0.95 – 1.09	0.61
		TRM	0.97	0.87 – 1.07	0.54
		aGVHD II – IV	0.97	0.88 – 1.07	0.59
		aGVHD III – IV	0.95	0.82 – 1.09	0.45
A24	1453	OS	0.97	0.91 – 1.04	0.40
		Relapse	1.10	1.00 – 1.20	0.044
		DFS	1.00	0.94 – 1.07	0.91
		TRM	0.88	0.78 – 0.98	0.018
		aGVHD II – IV	0.95	0.86 – 1.06	0.38
		aGVHD III – IV	0.95	0.80 – 1.12	0.51
A01A03	248	OS	0.89	0.73 – 1.09	0.27
		Relapse	0.85	0.63 – 1.13	0.27
		DFS	0.97	0.78 – 1.21	0.77
		TRM	1.14	0.80 – 1.64	0.46

		aGVHD II – IV	1.17	0.94 – 1.46	0.17
		aGVHD III – IV	1.05	0.75 – 1.49	0.76
A01A24	558	OS	0.87	0.77 – 0.99	0.041
		Relapse	0.96	0.81 – 1.13	0.60
		DFS	0.89	0.77 – 1.04	0.16
		TRM	0.87	0.70 – 1.08	0.21
		aGVHD II – IV	1.03	0.88 – 1.20	0.75
		aGVHD III – IV	1.20	0.93 – 1.55	0.16
B07	3862	OS	1.03	0.96 – 1.10	0.44
		Relapse	1.09	1.01 – 1.17	0.029
		DFS	1.02	0.96 – 1.10	0.49
		TRM	1.01	0.92 – 1.10	0.84
		aGVHD II – IV	0.99	0.92 – 1.08	0.89
		aGVHD III – IV	0.86	0.75 – 0.98	0.025
B27	1510	OS	1.08	1.00 – 1.17	0.045
		Relapse	1.07	0.98 – 1.17	0.14
		DFS	1.12	1.04 – 1.20	0.0034
		TRM	1.04	0.93 – 1.16	0.53
		aGVHD II – IV	1.01	0.90 – 1.13	0.87
		aGVHD III – IV	0.96	0.81 – 1.13	0.61
B44	3659	OS	1.04	0.97 – 1.12	0.23
		Relapse	0.97	0.89 – 1.06	0.56
		DFS	1.01	0.94 – 1.09	0.76
		TRM	1.00	0.90 – 1.10	0.97
		aGVHD II – IV	0.96	0.88 – 1.04	0.32
		aGVHD III – IV	1.06	0.91 – 1.24	0.44
B58	770	OS	1.02	0.90 – 1.15	0.79
		Relapse	1.10	0.96 – 1.25	0.17
		DFS	1.02	0.91 – 1.15	0.73
		TRM	0.88	0.74 - 1.06	0.18
		aGVHD II – IV	0.93	0.81 – 1.06	0.29
		aGVHD III – IV	1.06	0.87 – 1.29	0.59
B62	979	OS	0.87	0.78 – 0.97	0.0093
		Relapse	1.03	0.92 – 1.15	0.60
		DFS	0.90	0.82 – 0.99	0.032
		TRM	**0.79**	**0.69 – 0.90**	**0.00053**
		aGVHD II – IV	0.95	0.83 – 1.08	0.43
		aGVHD III – IV	0.93	0.76 – 1.15	0.51
HLA Expression Level					
HLA-B					

Bw4 Low	2901	-	1.00	-	-
Bw4 High	1008	OS	1.05	0.94 – 1.18	0.39
		Relapse	1.20	1.05 – 1.39	0.0096
		DFS	1.09	0.97 – 1.23	0.15
		TRM	0.90	0.76 – 1.07	0.24
		aGVHD II – IV	0.84	0.72 – 0.98	0.025
		aGVHD III – IV	0.85	0.67 – 1.07	0.17
Bw4 High/low	345	OS	1.11	0.93 – 1.33	0.26
		Relapse	1.13	0.92 – 1.40	0.25
		DFS	1.03	0.87 – 1.23	0.72
		TRM	0.89	0.70 – 1.12	0.32
		aGVHD II – IV	0.91	0.73 – 1.13	0.40
		aGVHD III – IV	0.91	0.64 – 1.30	0.61
HLA-C					
C High	573	-	1.00	-	-
C Low	4054	OS	0.97	0.84 – 1.11	0.63
		Relapse	0.92	0.80 - 1.06	0.24
		DFS	0.97	0.85 – 1.10	0.61
		TRM	1.06	0.86 – 1.30	0.61
		aGVHD II – IV	0.97	0.81 – 1.15	0.71
		aGVHD III – IV	1.16	0.87 – 1.54	0.31
C High/Low	2845	OS	0.92	0.79 – 1.08	0.31
		Relapse	0.92	0.80 - 1.06	0.25
		DFS	0.98	0.84 – 1.14	0.81
		TRM	1.08	0.86 – 1.35	0.51
		aGVHD II – IV	1.04	0.87 – 1.25	0.67
		aGVHD III – IV	1.21	0.89 – 1.65	0.22

*No patients expressed alleles belonging to the HLA-B08 supertype. B08 supertype is not included in this table of multivariate results.

Abbreviations: N, number; HCT, hematopoietic cell transplant; HR, hazard ratio; CI, confidence interval; HLA, human leukocyte antigen; OS, overall survival; DFS, disease-free survival; TRM, transplant-related mortality; aGVHD, acute graft-versus-host disease; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma;

2B. AML Subgroup

	N	HCT Outcome	HR	95% CI	P-value
HLA Zygosity					
Heterozygous	2896	-	1.00	-	-
Homozygous	839	OS	0.96	0.85 - 1.08	0.49
		Relapse	0.92	0.81 – 1.03	0.16
		DFS	0.95	0.85 – 1.05	0.29
		TRM	1.09	0.94 – 1.26	0.27
		aGVHD II – IV	1.08	0.93 – 1.24	0.31
		aGVHD III – IV	1.25	1.02 – 1.54	0.029
HLA Supertype*					
A01	1618	OS	0.99	0.90 – 1.08	0.79
		Relapse	0.89	0.80 – 0.99	0.030
		DFS	0.98	0.89 – 1.07	0.59
		TRM	1.12	0.98 – 1.27	0.088
		aGVHD II – IV	0.95	0.83 – 1.09	0.45
		aGVHD III – IV	0.77	0.64 – 0.94	0.011
A02	1961	OS	1.02	0.93 – 1.11	0.70
		Relapse	1.07	0.95 – 1.21	0.24
		DFS	1.00	0.92 – 1.08	0.93
		TRM	0.95	0.84 – 1.07	0.40
		aGVHD II – IV	1.06	0.95 – 1.19	0.32
		aGVHD III – IV	0.99	0.80 – 1.23	0.94
A03	1802	OS	1.04	0.95 – 1.13	0.40
		Relapse	1.07	0.95 – 1.20	0.26
		DFS	1.06	0.98 – 1.15	0.17
		TRM	0.99	0.86 – 1.14	0.91
		aGVHD II – IV	1.01	0.86 – 1.18	0.92
		aGVHD III – IV	1.10	0.87 – 1.39	0.41
A24	705	OS	0.98	0.87 – 1.09	0.66
		Relapse	1.12	0.95 – 1.32	0.18
		DFS	1.02	0.91 – 1.15	0.73
		TRM	0.83	0.70 – 1.00	0.044
		aGVHD II – IV	0.95	0.82 – 1.11	0.54
		aGVHD III – IV	0.93	0.71 – 1.23	0.63
A01A03	131	OS	1.04	0.82 – 1.33	0.74
		Relapse	0.83	0.56 – 1.24	0.37
		DFS	1.11	0.86 – 1.44	0.43
		TRM	1.55	1.05 – 2.28	0.027
		aGVHD II – IV	1.11	0.74 – 1.68	0.60
		aGVHD III – IV	1.30	0.79 – 2.13	0.31

A01A24	264	OS	0.87	0.69 – 1.09	0.22
		Relapse	0.92	0.71 – 1.18	0.50
		DFS	0.85	0.68 – 1.07	0.17
		TRM	0.81	0.60 – 1.09	0.17
		aGVHD II – IV	1.02	0.79 – 1.31	0.90
		aGVHD III – IV	1.05	0.67 – 1.64	0.85
B07	1917	OS	0.98	0.89 – 1.08	0.72
		Relapse	1.00	0.89 – 1.12	0.99
		DFS	0.97	0.88 – 1.06	0.52
		TRM	0.95	0.83 – 1.08	0.39
		aGVHD II – IV	1.04	0.92 – 1.17	0.54
		aGVHD III – IV	0.92	0.75 – 1.13	0.44
B27	759	OS	1.15	1.04 – 1.28	0.0074
		Relapse	1.19	1.04 – 1.36	0.012
		DFS	**1.21**	**1.10 – 1.32**	**0.00005**
		TRM	1.06	0.88 – 1.28	0.52
		aGVHD II – IV	1.02	0.87 – 1.21	0.80
		aGVHD III – IV	0.97	0.75 – 1.26	0.83
B44	1822	OS	1.09	0.97 – 1.21	0.13
		Relapse	1.02	0.89 – 1.16	0.82
		DFS	1.03	0.92 – 1.14	0.61
		TRM	1.05	0.90 – 1.21	0.55
		aGVHD II – IV	0.96	0.85 – 1.09	0.55
		aGVHD III – IV	1.04	0.81 – 1.33	0.77
B58	408	OS	1.04	0.87 – 1.24	0.68
		Relapse	1.19	1.01 – 1.41	0.039
		DFS	1.08	0.93 – 1.27	0.32
		TRM	0.97	0.76 – 1.24	0.80
		aGVHD II – IV	0.81	0.68 – 0.98	0.029
		aGVHD III – IV	0.86	0.62 – 1.21	0.40
B62	496	OS	0.85	0.74 – 0.97	0.018
		Relapse	0.93	0.80 – 1.07	0.30
		DFS	0.91	0.79 – 1.04	0.15
		TRM	0.76	0.60 – 0.95	0.015
		aGVHD II – IV	0.92	0.77 – 1.10	0.36
		aGVHD III – IV	0.92	0.67 – 1.27	0.61
HLA Expression Level					
HLA-B					
Bw4 Low	1446	-	1.00	-	-
Bw4 High	516	OS	1.01	0.88 – 1.16	0.87
		Relapse	1.13	0.94 – 1.35	0.18
		DFS	1.06	0.92 – 1.22	0.40

		TRM	0.91	0.74 – 1.12	0.35
		aGVHD II – IV	0.84	0.70 – 1.01	0.064
		aGVHD III – IV	0.88	0.64 – 1.23	0.47
Bw4 High/low	179	OS	1.16	0.92 – 1 .46	0.21
		Relapse	1.38	1.08 – 1.76	0.011
		DFS	1.16	0.93 – 1.46	0.18
		TRM	0.90	0.64 – 1.25	0.52
		aGVHD II – IV	1.16	0.86 – 1.58	0.33
		aGVHD III – IV	1.09	0.66 – 1.82	0.73
HLA-C					
C High	283	-	1.00	-	-
C Low	2047	OS	0.94	0.80 – 1.11	0.49
		Relapse	0.97	0.77 – 1.22	0.80
		DFS	0.93	0.78 – 1.11	0.43
		TRM	0.93	0.71 – 1.20	0.56
		aGVHD II – IV	0.92	0.73 – 1.15	0.47
		aGVHD III – IV	0.84	0.59 – 1.19	0.32
C High/Low	1404	OS	0.91	0.75 – 1.09	0.30
		Relapse	0.97	0.75 – 1.25	0.81
		DFS	0.94	0.77 – 1.16	0.59
		TRM	0.89	0.67 – 1.19	0.44
		aGVHD II – IV	1.01	0.80 – 1.27	0.92
		aGVHD III – IV	0.89	0.59 – 1.36	0.60

*No patients expressed alleles belonging to the HLA-B08 supertype. B08 supertype is not included in this table of multivariate results.

Abbreviations: N, number; HCT, hematopoietic cell transplant; HR, hazard ratio; CI, confidence interval; HLA, human leukocyte antigen; OS, overall survival; DFS, disease-free survival; TRM, transplant-related mortality; aGVHD, acute graft-versus-host disease; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma;

Table 3. Transplant-Related Mortality Based on Member Alleles of HLA-B62 Supertype in the Entire Cohort

Outcome	HLA Supertype Subgroup	N	HR	95% CI	P-value
TRM					0.0025
	All other HLA supertypes excluding B62	6328	1.00		.
	B62 supertype without HLA-B*15:01 allele	212	0.72	0.50 - 1.04	0.081
	B62 supertype with HLA-B*15:01 allele	742	0.81	0.69 - 0.96	0.013
	B62 supertype: with vs without HLA-B*15:01 allele		1.13	0.73 - 1.74	0.59

Abbreviations: N, number; HR, hazard ratio; CI, confidence interval;

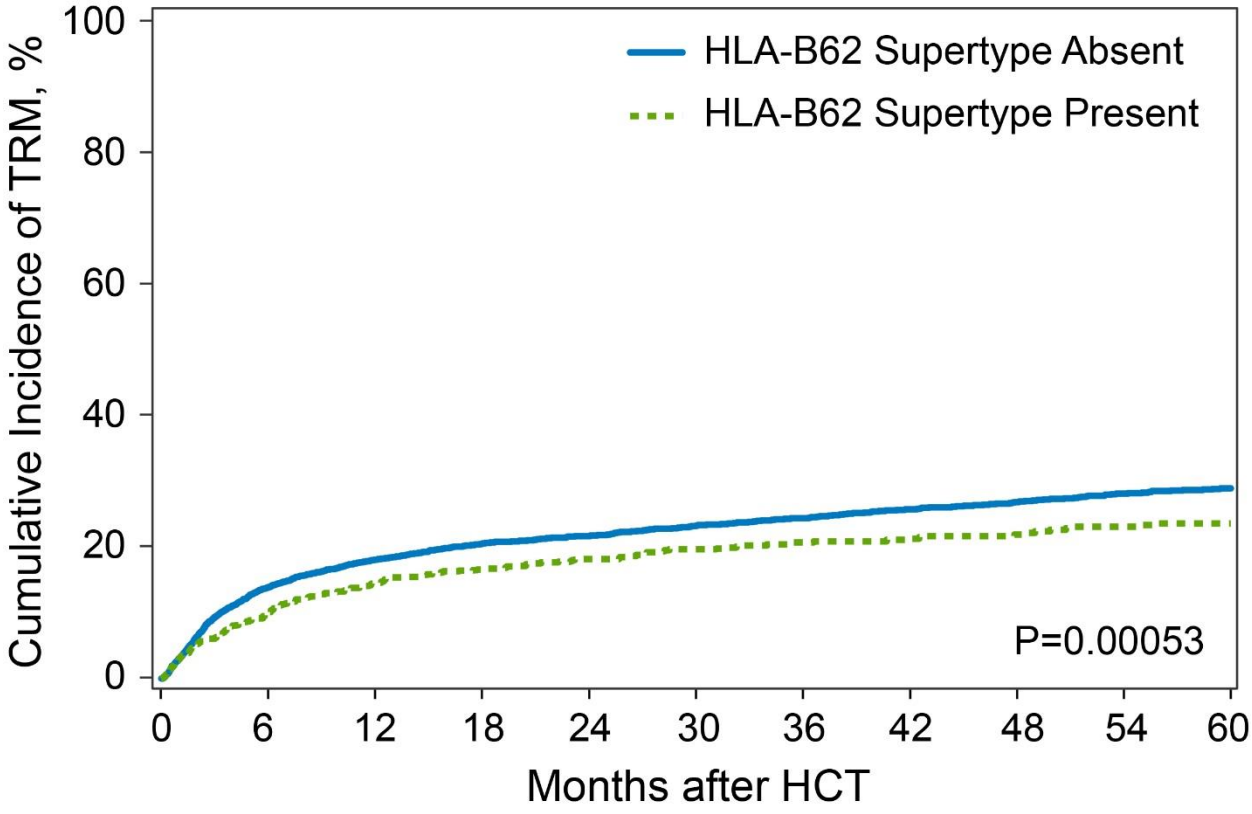
Table 4. Causes of Death Based on HLA-B62 Supertype in the Entire Cohort

	B62 Supertype Absent N (%)	B62 Supertype Present N (%)	P-value[a]
Number of Patients	3672	515	
Causes of Death			0.02
Primary Disease	1601 (44)	263 (51)	0.001
Graft Failure	30 (1)	0	0.04
GVHD	557 (15)	64 (12)	0.10
Infection	528 (14)	80 (16)	0.49
IPn/ARDS	145 (4)	18 (3)	0.62
Organ Failure/Toxicity	458 (12)	55 (11)	0.25
Secondary Malignancy	79 (2)	5 (1)	0.07
Other Causes	116 (3)	12 (2)	0.31
Unknown	158 (4)	18 (3)	0.39

[a] The Pearson chi-square test was used for comparing discrete variables; the Kruskal-Wallis test was used for comparing continuous variables.

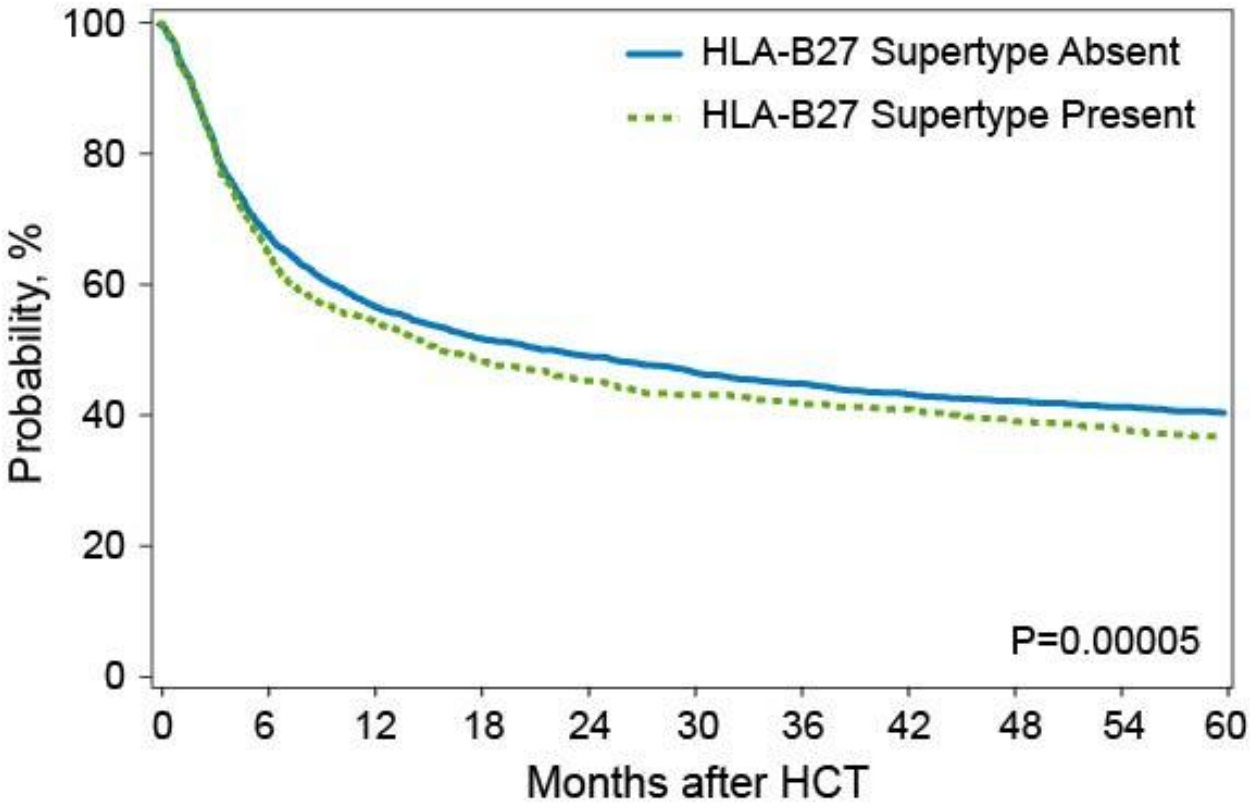
Abbreviations: N, number; GVHD, graft-versus-host disease; IPN, Interstitial Pneumonitis; ARDS, acute respiratory distress syndrome

Figure 1. Transplant-Related Mortality in Entire Cohort Based on HLA-B62 Supertype



The cumulative incidence for transplant-related mortality is shown above for recipients with at least one copy of HLA-B62 supertype versus recipients without HLA-B62 supertype. Recipients with HLA-B62 supertype had significantly decreased TRM (HR 0.79, 95% CI 0.69-0.90, *P*=0.00053).

Figure 2. Disease-Free Survival in AML Subgroup Based on HLA-B27 Supertype



The disease free-survival for the AML subgroup is shown for recipients with at least one copy of HLA-B27 supertype versus recipients without HLA-B27 supertype. Recipients with HLA-B27 supertype had worse DFS (HR 1.21, 95% CI 1.10-1.32, P=0.00005).