ARTICLE

Human leukocyte antigen supertype matching after myeloablative hematopoietic cell transplantation with 7/8 matched unrelated donor allografts: a report from the Center for International Blood and Marrow Transplant Research

Aleksandr Lazaryan,[1] Tao Wang,[2] Stephen R. Spellman,[3] Hai-Lin Wang,[2] Joseph Pidala,[4] Taiga Nishihori,[4] Medhat Askar,[5] Richard Olsson,[6] Machteld Oudshoorn,[7] Hisham Abdel-Azim,[8] Agnes Yong,[9] Manish Gandhi,[10] Christopher Dandoy,[11] Bipin Savani,[12] Gregory Hale,[13] Kristin Page,[14] Menachem Bitan,[15] Ran Reshef,[16] William Drobyski,[17] Steven GE Marsh,[18] Kirk Schultz,[19] Carlheinz R. Müller,[20] Marcelo A. Fernandez-Viña,[21] Michael R. Verneris,[1] Mary M. Horowitz,[2] Mukta Arora,[1] Daniel J. Weisdorf,[1] and Stephanie J. Lee[22]

[1]University of Minnesota Medical Center, Fairview, Minneapolis, MN, USA; [2]Center for International Blood and Marrow Transplant Research, Milwaukee, WI, USA; [3]Center for International Blood and Marrow Transplant Research, Minneapolis, MN, USA; [4]H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA; [5]Baylor University Medical Center, Dallas, TX, USA; [6]Karolinska University Hospital, Centre for Allogeneic Stem Cell Transplantation, Stockholm, Sweden; [7]Leiden University Medical Centre, The Netherlands; [8]Children's Hospital of Los Angeles, CA, USA; [9]Royal Adelaide Hospital / SA Pathology, Australia; [10]Mayo Clinic, Rochester, MN, USA; [11]Cincinnati Children's Hospital Medical Center, OH, USA; [12]Vanderbilt University Medical Center, Nashville, TN, USA; [13]All Children's Hospital, St. Petersburg, FL, USA; [14]Duke University Medical Center, Pediatric Blood and Marrow Transplant, Durham, NC, USA; [15]Tel-Aviv Sourasky Medical Center, Israel; [16]Columbia University Medical Center, New York, NY, USA; [17]Froedtert Memorial Lutheran Hospital, Milwaukee, MN, USA; [18]Anthony Nolan Research Institute & University College London Cancer Institute, Royal Free Campus, UK; [19]British Columbia's Children's Hospital, Vancouver, British Columbia, Canada; [20]Zentrales Knochenmarkspender-Register Deutschland, Ulm, Germany; [21]Stanford University Medical Center, CA, USA; and [22]Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Haematologica 2016
Volume 101(10):1267-1274

Correspondence:

alazarya@umn.edu

Received: February 12, 2016.

Accepted: May 25, 2016.

Pre-published: May 31, 2016.

doi:10.3324/haematol.2016.143271

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/101/10/1267

ABSTRACT

The diversity of the human leukocyte antigen (HLA) class I and II alleles can be simplified by consolidating them into fewer supertypes based on functional or predicted structural similarities in epitope-binding grooves of HLA molecules. We studied the impact of matched and mismatched HLA-A (265 *versus* 429), -B (230 *versus* 92), -C (365 *versus* 349), and -DRB1 (153 *versus* 51) supertypes on clinical outcomes of 1934 patients with acute leukemias or myelodysplasia/myeloproliferative disorders. All patients were reported to the Center for International Blood and Marrow Transplant Research following single-allele mismatched unrelated donor myeloablative conditioning hematopoietic cell transplantation. Single mismatched alleles were categorized into six HLA-A (A01, A01A03, A01A24, A02, A03, A24), six HLA-B (B07, B08, B27, B44, B58, B62), two HLA-C (C1, C2), and five HLA-DRB1 (DR1, DR3, DR4, DR5, DR9) supertypes. Supertype B mismatch was associated with increased risk of grade II-IV acute graft-*versus*-host disease (hazard ratio =1.78, *P*=0.0025) compared to supertype B match. Supertype B07-B44 mismatch was associated with a higher incidence of both grade II-IV (hazard ratio=3.11, *P*=0.002) and III-IV (hazard ratio=3.15, *P*=0.01) acute graft-*versus*-host disease. No significant associations were detected between supertype-matched *versus* -mismatched groups at other HLA loci. These data suggest that avoiding HLA-B supertype mismatches can mitigate the risk of grade II-IV acute graft-*versus*-host disease in 7/8-mismatched unrelated donor hematopoietic cell transplantation when multiple HLA-B supertype-matched donors are available. Future studies are needed to define the mechanisms by which supertype mismatching affects outcomes after alternative donor hematopoietic cell transplantation.

Introduction

Cellular immune responses are mediated in part by cytotoxic T lymphocytes that recognize peptide molecules bound to human leukocyte antigen (HLA) on the surface of antigen-presenting cells. HLA molecules are extremely polymorphic[1] and most of their variation is centered within the peptide-binding grooves that accommodate the primary anchor positions of the peptides displayed for immune recognition.[2] Discovery of HLA supertypes almost a decade ago offered a simplification of diverse HLA nomenclature by consolidating individual HLA class I and II alleles into fewer supertype clusters based on functional or predicted structural similarities in epitope-binding specificities of HLA molecules.[3] HLA alleles belonging to each particular supertype have either experimentally proven or predicted ability to present antigenic peptides with similar anchoring amino acids at the second (B-pocket) and C-terminal (F-pocket) positions of the peptide molecules.[4] Although HLA class I and II supertypes have been increasingly studied in association with immune susceptibility to infection[5-7] and cancer,[8,9] the significance of individual allele mismatching within and outside of HLA class I or II supertypes remains unknown in the context of allogeneic hematopoietic cell transplantation (alloHCT).

Recent encouraging outcomes in fully HLA-matched unrelated donor (MUD) HCT[10,11] have contributed in part to the steady rise of MUD allografts that now outnumber related donor transplants reported annually to the Center for International Blood and Marrow Transplant Research (CIBMTR). Allografts mismatched at a single HLA-A, -B, -C, or -DRB1 locus [i.e. 7/8 mismatched unrelated donor (MMUD) HCT] were previously reported to be associated with lower overall and disease-free survival, higher treatment-related mortality, and more acute graft-*versus*-host disease (GVHD) compared to outcomes of 8/8 MUD allografts.[12-14] Despite these risks, 7/8 MMUD grafts remain a viable option for HCT, particularly in minorities who lack suitable donors or in patients with aggressive hematologic malignancies for whom the risks of disease progression due to delays in identifying optimal donors[15] is offset in part by the benefits of earlier transplantation with a 7/8 MMUD alloHCT.

Although multiple strategies have been sought to identify "permissible mismatches" associated with improved outcomes of a single-allele MMUD HCT,[13,16-19] the clinical significance of clustering mismatched alleles within HLA class I or II supertypes has not been established. We therefore conducted a large registry analysis of the CIBMTR database of single-allele mismatched myeloablative allografts to determine whether HLA class I or II supertype mismatching is associated with worse outcomes after 7/8 MMUD alloHCT.

Methods

Study design and patient selection

The study base population consisted of 2218 recipients of myeloablative conditioning followed by 7/8 HLA MUD bone marrow or peripheral blood stem cell allografts for acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and myelodysplastic syndrome between 1999 and 2011. The patients' data were reported to the National Marrow Donor Program (NMDP)/CIBMTR, and subjects were excluded if: (i) they did not consent to participate (n=55); (ii) they had fewer than 100 days of post-transplant follow up (n=11); (iii) their disease status prior to alloHCT was missing (n=32); or (iv) they had undergone *ex vivo* T-cell depletion (n=186). Recipients of prior HCT were excluded. Allografts performed for lymphoid malignancies and non-malignant disorders were also excluded in order to enhance the overall homogeneity of the study population. All eligible adult and pediatric study participants (n=1934) from 175 transplant centers and 16 countries provided informed consent to participate in NMDP/CIBMTR research. This was a retrospective observational study approved by NMDP/CIBMTR's Institutional Review Board. Standard methods of NMDP/CIBMTR data analysis were used to mitigate any bias related to exclusion of non-consenting study candidates.[12]

HLA class I and II typing and supertype assignment

High-resolution allele-level typing at HLA-A, -B, -C, and -DRB1 loci was performed through the NMDP's high-resolution HLA typing project according to well established and validated DNA-based techniques as previously reported.[20] Single allele mismatch at HLA-A, -B, -C, or -DRB1 was defined as a "7/8 match". The assignment algorithm for HLA-A and -B supertypes *(Online Supplementary Table S1)* was based on an updated supertype classification with revised main HLA anchor specificities.[21] This method extends the previously described nine HLA-A and -B supertype designations[3] (A1, A2, A3, A24, B27, B44, B58, and B62) to 12 supertype groups (A01, A01A03, A01A24, A02, A03, A24, B07, B08, B27, B44, B58, B62), mostly due to the fact that certain HLA-A alleles were found to have peptide-binding repertoires with overlapping supertype specificities thereby resulting in newly defined A01A03 and A01A24 supertype categories. This revised classification of HLA-A and -B supertypes captured 99% of the allelic diversity of allograft recipients and their donors. The remaining 1% of unclassified HLA-A and -B alleles were grouped into supertypes using bioinformatics methods.[22] Two HLA-C supertypes (C1 and C2) were derived from hierarchical cluster analysis[22] with distinct amino-acid fingerprints in protein structure for HLA-C1 (Ser^{77}) and -C2 (Asn^{77}), which also coincide with killer Ig-like receptor binding specificities for HLA-C.[23] The grouping of HLA-DRB1 alleles into supertypes was accomplished according to previously described *in-silico* methods on the basis of common structural and functional features of HLA class II molecules.[24] The significance of alternative supertype designations and individual supertype effects was further assessed in the *post-hoc* exploratory analysis.

Study endpoints

The primary comparison between the 7/8 supertype-matched and 7/8 supertype-mismatched allografts was conducted across major clinical endpoints including overall survival, disease-free survival, relapse, treatment-related mortality, acute GVHD, chronic GVHD, and time-to-neutrophil recovery (absolute neutrophil count ≥ $0.5x10^9$/L). Overall survival corresponded to the time from transplantation to death from any cause and surviving patients were censored at the time of their last follow-up. Disease-free survival was defined as the time between transplantation and relapse or death from any cause; patients who remained alive and in remission were censored at the time of their last follow-up. Clinical relapse of the primary disease and treatment-related mortality were defined by established CIBMTR criteria with the latter defined as death while in continuous remission. Relapse was therefore considered a competing risk endpoint for treatment-related mortality, and treatment-related mortality was considered a competing risk for relapse. The onset of grades II-IV or III-IV

acute GVHD was determined based on the Consensus criteria[25] while the onset of chronic GVHD was determined based on the Seattle criteria.[26] Neutrophil engraftment was defined as time-to-neutrophil recovery. Death was considered a competing risk endpoint for engraftment and GVHD.

Statistical analysis

Descriptive frequency estimates and comparisons for HLA alleles and supertypes as well as non-HLA study variables were obtained through the standard methods of categorical and continuous data analysis. Univariate probabilities for overall and disease-free survival were calculated using the Kaplan-Meier estimator,[27] whereas probabilities of treatment-related mortality, relapse, acute GVHD, chronic GVHD, and neutrophil engraftment were calculated as cumulative incidence rates while accounting for competing risks.[28] Survival curves were compared by the log-rank test. Multivariate models for overall survival, disease-free survival, relapse, treatment-related mortality, acute GVHD, chronic GVHD and neutrophil engraftment were built using Cox proportional hazards models. All clinical variables were tested for the affirmation of the proportional hazards assumption. Variables that were found to violate this assumption were adjusted for by stratification. Final outcome-specific models were developed using a stepwise model building procedure with the threshold of α=0.05 for both entry and retention of co-variates in the model. Main variables, including HLA supertypes, were forced into the models with the interactions between the main variables and the adjusted covariates being tested at the significance level of α=0.01. Given the multiple testing, *P* values <0.01 were considered statistically significant.

Results

HLA class I and II alleles and supertypes

For 1934 recipients of 7/8 MMUD alloHCT, single-allele mismatches occurred within the HLA-A (36%), -B (17%), -C (37%), and -DRB1 (11%) loci. Individual HLA-A, -B, -C, and -DRB1 allele-level mismatches were matched by corresponding HLA supertypes in 38%, 71%, 51%, and 75%, respectively (Table 1). Overall, supertype-level matching at any one of the four HLA loci was observed in 52% of study subjects.

Non-HLA characteristics

Baseline patient and clinical characteristics are summarized in Table 2. In brief, the patients' median age was 35 years (range, 1-70), and less than 20% of the study population was of non-Caucasian background. Acute myeloid leukemia and acute lymphoblastic leukemia accounted for 76% of all hematologic malignancies with over half of patients classified as having intermediate or advanced risk disease. Peripheral blood stem cell allografts were used in 56% of all transplant procedures. Conditioning regimens for alloHCT included total body irradiation in 58% of cases, whereas anti-thymocyte globulin or alemtuzumab was incorporated into conditioning regimens in 36% of cases. The majority of GVHD prophylactic regimens included tacrolimus (62%) or cyclosporine (36%). The median follow-up of surviving patients was 54 months (range, 3-149) after alloHCT. In the crude comparisons of supertype-matched (any locus) *versus* –mismatched 7/8 allografts, significant differences were observed in underlying hematologic malignancies, conditioning regimens and timing of alloHCT (all P<0.01). Specifically, the supertype-matched group contained a greater proportion of total body irradiation-based conditioning regimens (62% *versus* 54%, P<0.001) and a smaller proportion of *in vivo* T-cell-depleted grafts (32% *versus* 40%, P<0.001). Supertype-mismatched grafts were also more common in recent years (P<0.001).

HLA supertype-matched and -mismatched outcomes

Univariate analyses of post-transplant outcomes based on HLA supertype matching are summarized in Table 3. Recipients of HLA supertype B-mismatched allografts (n=62) had a significantly higher cumulative incidence of grade II-IV acute GVHD than did recipients of HLA-B supertype-matched (n=174) allografts (67% *versus* 47%, respectively) (Figure 1, log-rank P=0.007). This association was primarily driven by an excess in grade II acute GVHD as no difference was found in the incidence of severe grade III-IV acute GVHD with supertype-B mismatching. The independent effect of HLA-B supertype matching on grade II-IV acute GVHD was confirmed by the multivariable analysis [hazard ratio (HR)=1.78; 95% confidence interval (CI), 1.23-2.59; P=0.0025] adjusting for age, gender, disease type, ABO-mismatch, graft source, and *in vivo* T-cell depletion (Figure 3). No other class I supertype mismatch (including supertype mismatch at any locus) was found to be significantly associated with any of the study endpoints (engraftment, chronic GVHD, relapse or death) at the pre-specified statistical threshold.

HLA-B supertype mismatches involving B07-B44, B27-B44, and B07-B62 were found to be the most prevalent and these individual mismatches were subsequently examined in the *post-hoc* analysis for their association with acute GVHD. In contrast to all other HLA-B mismatched supertypes, B07-B44 mismatched allografts were associated with a higher incidence of both grade II-IV (HR=3.11; 95% CI, 1.54-6.28, P=0.002) and III-IV acute GVHD (HR=3.15; 95% CI, 1.30-7.65, P=0.01).

Table 1. HLA class I/II allele- and supertype-level distribution.

Single allele-mismatch	N	HLA supertype	
		Matched (%)	Mismatched (%)
HLA-A	694	265 (38.2)	429 (61.8)
HLA-B	322	230 (71.4)	92 (28.6)
HLA-C	714	365 (51.1)	349 (48.9)
HLA-DRB1	204	153 (75)	51 (25)
Any allele	1934*	1000 (51.7)	921 (48.3)

**Missing supertype assignment for 13 patients.*

Table 2. Non-HLA characteristics of the study population.

Characteristic	All	Supertype-matched	Supertype-mismatched	*P* value
Number of patients	1934*	999	922	
Number of centers	175	161	148	
Age, median (range), years	35 (1-70)	35 (1-69)	37 (1-70)	0.16
Age at alloHCT, years				0.51
<18 years old	410 (21%)	212 (21%)	194 (21%)	
19-35 years old	541 (28%)	292 (29%)	246 (27%)	
36-55 years old	758 (39%)	386 (39%)	367 (40%)	
>55 years old	225 (12%)	109 (11%)	115 (12%)	
Gender				0.95
Male	1090 (56%)	563 (56%)	521 (57%)	
Female	844 (44%)	436 (44%)	401 (43%)	
KPS prior to alloHCT				0.11
< 90	510 (26%)	259 (26%)	248 (27%)	
90-100	1303 (67%)	666 (67%)	627 (68%)	
Missing	121 (6%)	74 (7%)	47 (5%)	
Race of recipient				0.12
Caucasian	1574 (81%)	824 (82%)	740 (80%)	
African-American	175 (9%)	75 (8%)	100 (11%)	
Asian / Pacific Islander	62 (3%)	35 (4%)	27 (3%)	
Hispanic	71 (4%)	41 (4%)	28 (3%)	
Native American	52(3%)	7 (<1%)	7 (<1%)	
CMV Donor/Recipient				0.43
D-/R-	533 (28%)	294 (29%)	235 (25%)	
D-/R+	602 (31%)	300 (30%)	298 (32%)	
D+/R-	272 (14%)	137 (14%)	133 (14%)	
D+/R+	499 (26%)	254 (25%)	242 (26%)	
Disease				0.006
Acute myeloid leukemia	870 (45%)	427 (43%)	436 (47%)	
Acute lymphoblastic leukemia	609 (31%)	345 (35%)	262 (28%)	
Chronic myeloid leukemia	274 (14%)	147 (15%)	124 (13%)	
Myelodysplastic syndrome	181 (9%)	80 (8%)	100 (11%)	
Disease risk[¥]				0.03
Early	813 (42%)	447 (45%)	362 (39%)	
Intermediate	612 (32%)	310 (31%)	298 (32%)	
Advanced	509 (26%)	242 (24%)	262 (28%)	
Donor parity				0.03
Male or non-parous female	1389 (72%)	692 (69%)	688 (75%)	
Parous female	443 (23%)	246 (25%)	193 (21%)	
Missing	102 (5%)	61 (6%)	41 (4%)	
Donor/Recipient sex match				0.43
Male / male	657 (34%)	329 (33%)	325 (35%)	
Male / female	467 (24%)	233 (23%)	228 (25%)	
Female / male	433 (22%)	234 (23%)	196 (21%)	
Female / female	377 (19%)	203 (20%)	173 (19%)	
Graft source				0.12
Bone marrow	845 (44%)	452 (45%)	385 (42%)	
Peripheral blood stem cells	1089 (56%)	547 (55%)	537 (58%)	
Conditioning with TBI	1127 (58%)	623 (62%)	498 (54%)	<0.001
In vivo T-cell depletion[§]	693 (36%)	316 (32%)	372 (40%)	<0.001
GVHD prophylaxis				0.64
Tacrolimus-based	1193 (62%)	625 (63%)	560 (61%)	
Cyclosporin A-based	689 (36%)	346 (35%)	338 (37%)	
Other[‡]	52 (3%)	28 (3%)	24 (3%)	
Year of alloHCT				<0.001
1999-2002	405 (21%)	234 (23%)	167 (18%)	
2003-2006	633 (33%)	351 (35%)	278 (30%)	
2007-2011	896 (46%)	414 (41%)	477 (52%)	
Follow up, median (range), months	54 (3-149)	60 (3-149)	48 (3-145)	

*alloHCT: allogeneic hematopoietic cell transplantation; CMV: cytomegalovirus; D: donor; R: recipient; TBI: total body irradiation; GVHD: graft-*versus*-host disease; KPS: Karnofsky performance score; *Including 13 cases with missing supertypes. [¥]According to ASBMT 2006 definitions. [§]Antithymocyte globulin or alemtuzumab. [‡]Mycophenolate mofetil + other (n=5); methotrexate + other (n=10); antithymocyte globulin ± corticosteroid (n=6); sirolimus (n=1); unknown (n=30).*

Table 3. Univariate probabilities of clinical outcomes between HLA supertype-matched (M) and –mismatched (MM) 7/8 unrelated donor allografts.

Outcomes	Timing post-HCT	HLA-A ST M *vs.* MM	HLA-B ST M *vs.* MM	HLA-C ST M *vs.* MM	HLA-DRB1 ST M *vs.* MM	Any HLA ST M *vs.* MM
Acute GVHD II-IV	Day 100	54% *vs.* 54% *P*=0.95	47% *vs.*67% *P*=0.006	51% *vs.* 48% *P*=0.56	50% *vs.* 56% *P*=0.6	51% *vs.* 53% *P*=0.53
Acute GVHD III-IV	Day 100	27% *vs.* 32% *P*=0.26	31% *vs.* 32% *P*=0.84	25% *vs.* 26% *P*=0.73	22% *vs.* 22% *P*=0.98	26% *vs.* 29% *P*=0.27
Any chronic GVHD	2 years	47% *vs.* 44% *P*=0.39	51% *vs.* 42% *P*=0.14	45% *vs.* 37% *P*=0.03	43% *vs.* 46% *P*=0.78	47% *vs.* 41% *P*=0.012
ANC recovery	Day 28	95% *vs.* 94% *P*=0.68	94% *vs.* 95% *P*=0.78	94% *vs.* 93% *P*=0.58	91% *vs.* 94% *P*=0.5	94% *vs.* 94% *P*=0.91
Treatment-related mortality	3 years	45% *vs.* 40% *P*=0.19	45% *vs.* 37% *P*=0.25	37% *vs.* 40% *P*=0.42	31% *vs.* 50% *P*=0.03	40% *vs.* 40% *P*=0.89
Relapse	3 years	27% *vs.* 28% *P*=0.87	20% *vs.* 26% *P*=0.28	30% *vs.* 33% *P*=0.31	27% *vs.* 19% *P*=0.26	26% *vs.* 29% *P*=0.16
Disease-free survival	3 years	27% *vs.* 32% *P*=0.23	35% *vs.* 37% *P*=0.81	33% *vs.* 27% *P*=0.06	42% *vs.* 31% *P*=0.2	34% *vs.* 30% *P*=0.14
Overall survival	3 years	31% *vs.* 38% *P*=0.09	40% *vs.* 41% *P*=0.88	39% *vs.* 30% *P*=0.02	46% *vs.* 30% *P*=0.05	38% *vs.* 35% *P*=0.12

ST: supertype; ANC recovery: absolute neutrophil count over 0.5x10⁹/L.

Since the impact of single-allele mismatching was most apparent in patients with early and intermediate risk disease, as demonstrated in the prior large NMDP analysis, we analyzed the effect of HLA-B supertype mismatching within the subset of patients with early and intermediate risks. Similar to our major finding, compared to HLA-B-matched supertype grafts, HLA-B-mismatched supertype grafts were associated with an increased risk of grade II-IV acute GVHD (HR=1.84; 95% CI, 1.20-2.84, *P*<0.01).

Although HLA-DRB1 supertype-mismatched transplants (n=51) were associated with faster neutrophil engraftment (median 12 *versus* 16 days, Figure 2), this early difference in engraftment kinetics was not evident by day 28 after the transplant (94% *versus* 90%, *P*=0.4). Notably, the relatively slower neutrophil engraftment among HLA-DRB1 supertype-matched allograft recipients had no adverse influence on treatment-related mortality or other major post-transplant outcomes. On the contrary, mismatching at HLA-DRB1 supertypes was associated with a trend towards higher treatment-related mortality (HR=1.64; 95% CI, 0.99-2.74, *P*=0.057) and inferior overall survival (HR=1.58; 95% CI 1.04-2.38, *P*=0.037) compared to that associated with HLA-DRB1 supertype-matched allografts.

Discussion

In this large registry analysis of the CIBMTR database of single allele mismatched myeloablative allografts, we found a significant increase in the hazard of grade II-IV acute GVHD among HLA-B supertype-mismatched compared to HLA-B supertype-matched recipients of 7/8 MMUD allografts. Allele-level 7/8 HLA-B mismatch was proven in the past to be associated with a higher incidence of acute GVHD compared to 8/8 HLA-match [estimated 28% (95% CI, 26%-30%) incidence of grade III-IV acute GVHD].[12] In our cohort, the cumulative incidence rates of grades II-IV and III-IV acute GVHD among HLA-B allele-MMUD allograft recipients were 53% (95% CI, 48%-59%) and 31% (95% CI, 25%-37%), respectively. This study has further extended the significance of HLA-B mismatch in regards to acute GVHD at the supertype level for 7/8 allele-mismatched allografts. This observation conforms to our primary hypothesis of adverse post-transplant outcomes with mismatched HLA supertypes and it further supports the notion of increased alloreactivity with HLA-B supertype-mismatched 7/8 MMUD transplants as opposed to supertype-level mismatches at HLA-A, -C, or -DRB1 loci.

There are several possible explanations for our findings. First, HLA-B alleles in humans have the highest degree of described polymorphism relative to other class I or II alleles,[29] likely as a result of the evolutionary pressures from various infectious pathogens. The contribution of HLA supertypes to immune-mediated responses against a number of viral infections was well established by prior studies.[5-7,30-32] It is therefore possible that early post-transplant inflammatory responses mediated by mismatched HLA-B supertypes could perpetuate alloreactive immune responses such as acute GVHD. Although addressing this hypothesis was beyond the scope of this study, this could be tested in future studies. Second, it is possible that the supertype categorization algorithm used in this study could have obscured some of the underlying true associations between class I and II supertypes with major clinical outcomes after alloHCT. Considerable diversity and a variable degree of overlap exist between major HLA class I and II supertype classifications. In this study we used the revised supertype assignment algorithm proposed by Sette and Sidney for HLA class I A- and B-supertypes.[21] As opposed to other alternatives,[33-37] our chosen algorithm provided successful supertype classification for the entire study population with most of the HLA-A and -B alleles classified based on experimentally established motifs in epitope-binding pockets of HLA molecules. Our supertype assignment strategy therefore ensured the most stringent selection of corresponding alleles, and by doing so it strengthened the internal validity of the study. In addition, our *post-hoc* exploratory analysis of alternative HLA-A, -B,

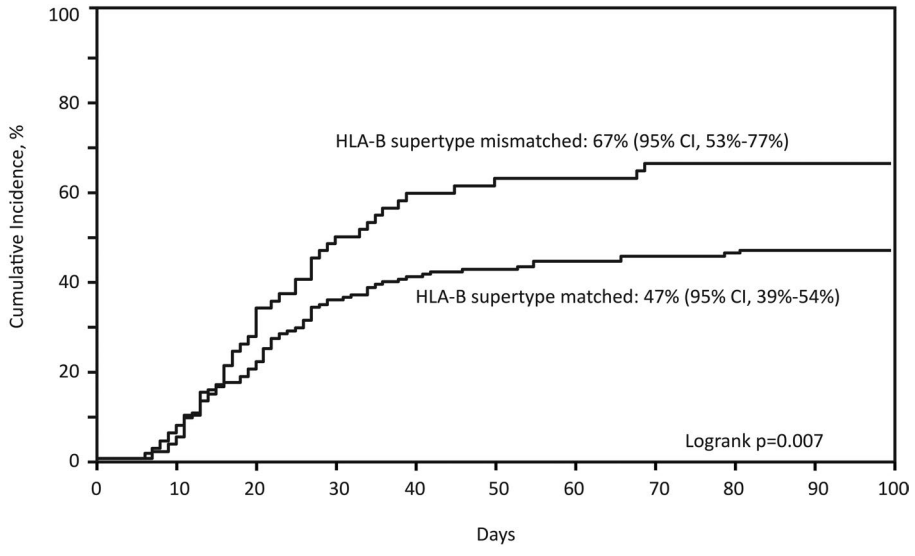

Figure 1. Cumulative incidence of grade II-IV acute GVHD according to HLA-B supertypes.

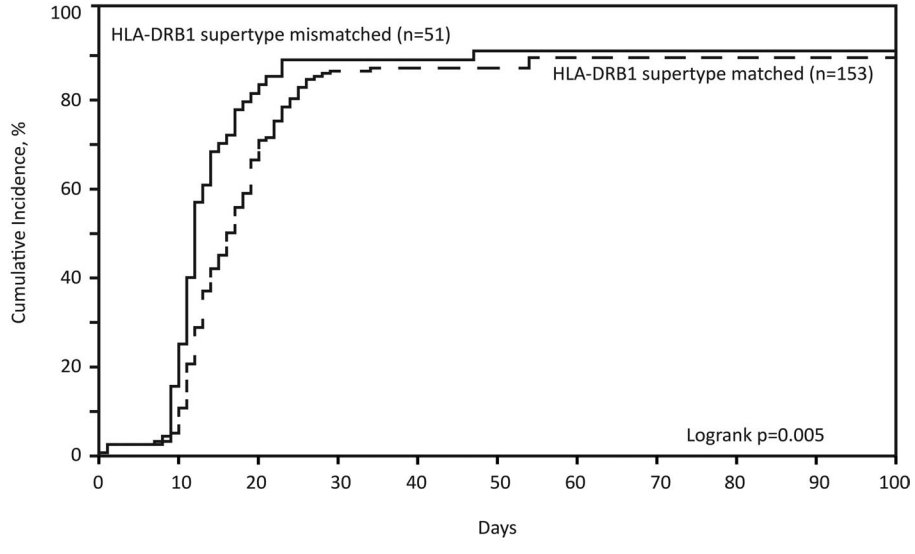

Figure 2. Neutrophil recovery according to HLA-DRB1 supertypes.

Note: Median time-to-absolute neutrophil recovery (≥500/μL) was 12 days for DRB1-mismatched versus 16 days for –matched allografts

and -DRB1 supertype classifications (*data not shown*) revealed either limited capacity of other algorithms to cluster allelic diversity of our study population into supertype categories (e.g. the algorithms by Reche,[37] Harjanto,[34] Hertz/Yanover,[35] and Greenbaum[33]), or significant overlap between our supertype assignment algorithm and other classifications such as those proposed by Lund[36] or Doytchinova.[22] Furthermore, accounting for the mismatch vector direction (i.e. graft-*versus*-host or host-*versus*-graft) did not further influence or enrich the findings from this study.

Future practical implications of HLA-B supertype-matched donor selection of 7/8 HLA-B MMUD allografts can be expected to lower the incidence of grade II-IV acute GVHD for a modest fraction (5%) of all 7/8 MMUD HCT according to the donor selection practices reflected in this study. Avoidance of B07-B44 supertype mismatches should be interpreted with caution given the small number of allografts (n=9) in that subset analysis of the individual HLA-B supertype mismatches. Nevertheless, all but one B07-B44 supertype mismatched allografts were complicated by grade II-IV acute GVHD with over half classified as severe acute GVHD.

Major limitations of this study are inherent to its retrospective design and in statistical challenges of analyzing multiple endpoints across various HLA class I supertypes. Consequently, we found faster neutrophil engraftment among HLA-DRB1 supertype-mismatched allograft recipients than among HLA-DRB1 supertype-matched allograft recipients to be more controversial and difficult to explain. Although recipients of HLA-DRB1 supertype-mismatched allografts achieved neutrophil recovery on average 4 days earlier, they demonstrated a trend towards inferior overall survival. Notably, the median estimated time of neutrophil engraftment for DRB1-matched supertypes (16 days), which accounted for 75% of all 7/8 DRB1 allele-mismatched allografts, was overall comparable to data reported for 8/8 MUD HCT.[38] In contrast, DRB1 supertype

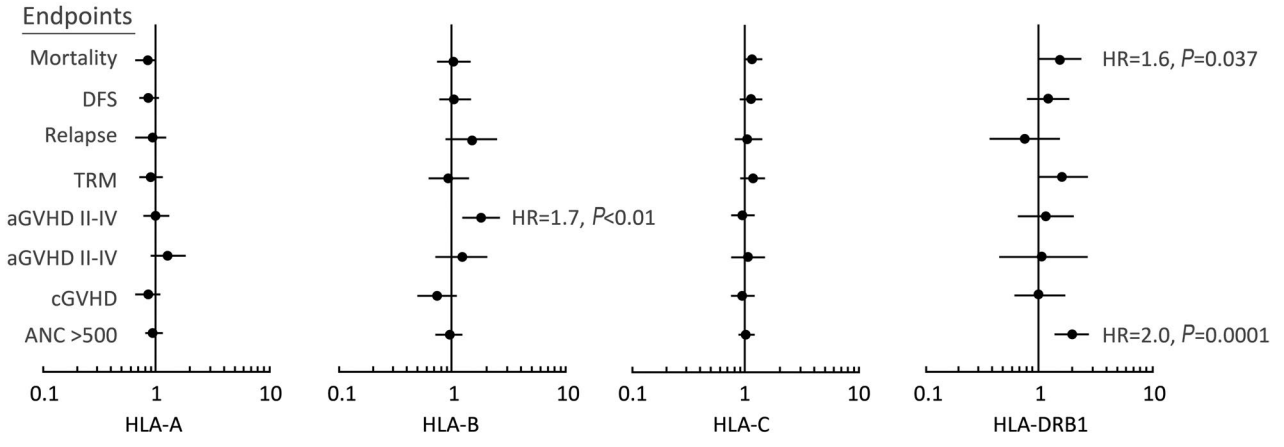

Figure 3. Multivariate analysis of the impact of supertype mismatching at HLA-A, -B, -C, and -DRB1. DFS: disease-free survival; TRM: treatment-related mortality; aGVHD: acute graft-*versus*-host disease; cGVHD: chronic graft-*versus*-host disease; ANC>500: absolute neutrophil count > $0.5x10^9$/L.

mismatches accounted for only 2.6% of all 7/8 MMUD HCT in this study thereby raising the possibility of a random effect in lieu of a less plausible cause-and-effect relationship between DRB1 supertype mismatch and neutrophil engraftment kinetics. Further studies are needed to provide definitive guidance on incorporating DRB1 supertype matching in donor selection algorithms as increased treatment-related mortality and inferior overall survival, albeit not statistically significant in this dataset, are concerning.

This large observational study has provided the first evidence of "permissible" supertype-based donor selection of optimal 7/8 MMUD for myeloablative alloHCT. Pending validation in an independent dataset, our findings suggest that avoiding HLA-B supertype mismatch can serve as a novel strategy to mitigate the risk of grade II-IV acute GVHD in 7/8 MMUD HCT when multiple potential HLA-B supertype-matched donors are available. This study offers new insights and testable hypotheses for future studies on the role of HLA supertypes among recipients of reduced intensity MMUD HCT and recipients of other mismatched alternative donor allografts such as umbilical cord blood or haploidentical HCT.

Acknowledgments

The authors gratefully acknowledge Katharina Fleischhauer, Neng Yu, Biju George, and Jason Dehn for their participation in the study protocol review, and Michael Franklin, MS, for his assistance in editing the manuscript. The CIBMTR is supported by Public Health Service Grant/Cooperative Agreement 5U24-CA076518 from the National Cancer Institute (NCI), the National Heart, Lung and Blood Institute (NHLBI) and the National Institute of Allergy and Infectious Diseases (NIAID); a Grant/Cooperative Agreement 5U10HL069294 from NHLBI and NCI; a contract HHSH250201200016C with Health Resources and Services Administration (HRSA/DHHS); two grants N00014-14-1-0028 and N00014-15-1-0848 from the Office of Naval Research; and grants from Alexion; Amgen, Inc.; Anonymous donation to the Medical College of Wisconsin; Be the Match Foundation; Bristol Myers Squibb Oncology; Celgene Corporation; Chimerix, Inc.; Fred Hutchinson Cancer Research Center; Gamida Cell Ltd.; Genentech, Inc.; Genzyme Corporation; Gilead Sciences, Inc.; Health Research, Inc. Roswell Park Cancer Institute; HistoGenetics, Inc.; Incyte Corporation; Jazz Pharmaceuticals, Inc.; Jeff Gordon Children's Foundation; The Leukemia & Lymphoma Society; The Medical College of Wisconsin; Merck & Co, Inc.; Mesoblast; Millennium: The Takeda Oncology Co.; Miltenyi Biotec, Inc.; National Marrow Donor Program; Neovii Biotech NA, Inc.; Novartis Pharmaceuticals Corporation; Onyx Pharmaceuticals; Optum Healthcare Solutions, Inc.; Otsuka America Pharmaceutical, Inc.; Otsuka Pharmaceutical Co, Ltd. – Japan; Oxford Immunotec; Perkin Elmer, Inc.; Pharmacyclics; Sanofi US; Seattle Genetics; Sigma-Tau Pharmaceuticals; Spectrum Pharmaceuticals, Inc.; St. Baldrick's Foundation; Sunesis Pharmaceuticals, Inc.; Swedish Orphan Biovitrum, Inc.; Telomere Diagnostics, Inc.; TerumoBCT; Therakos, Inc.; University of Minnesota; and Wellpoint, Inc. 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.

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