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Genetics of HLA Peptide Presentation and Impact on Outcomes in HLA-Matched Allogeneic Hematopoietic Cell Transplantation

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Abstract

Minor histocompatibility antigens (mHAs), recipient-derived peptide epitopes presented on the cell surface, are known to mediate graft-versus-host disease (GVHD); however, there are no current methods to associate mHA features with GVHD risk. This deficiency is due in part to the lack of technological means to accurately predict, let alone confirm, the tremendous number of potential mHAs in each individual transplant. Previous studies have shown that different HLA molecules present varying fractions of candidate peptide epitopes; however, the genetic "distance" between HLA-matched donors and recipients is relatively constrained. From these 2 observations, it is possible that the HLA type for a donor-recipient pair (DRP) would provide a surrogate measurement of the number of predicted mHAs, which could be related to GVHD risk. Because different HLA molecules present variable numbers of peptide antigens, a predicted cumulative peptide-binding efficiency can be calculated for individual DRP based on the pair's HLA type. The purpose of this study was to test whether cumulative peptide-binding efficiency is associated with the risk of acute GVHD (aGVHD) or relapse. In this retrospective Center for International Blood and Marrow Transplant Research study, a total of 3242 HLA-matched DRPs were analyzed for predicted cumulative peptide-binding efficiency using their HLA types and were divided into tertiles based on their scores. Univariable and multivariable analyses was performed to test for associations between cumulative peptide-binding efficiency for DRPs, divided into the HLA-matched related donor (MRD) and HLA-matched unrelated donor (MUD) cohorts, and the primary outcomes of aGVHD and relapse. Secondary outcomes investigated included overall survival, disease-free survival, and transplantation-related mortality. Using a computationally generated peptidome as a test dataset, the tested series of HLA class I displayed peptide-binding frequencies ranging from 0.1% to 3.8% of the full peptidome, and HLA class II molecules had peptide-binding frequencies of 12% to 77% across the HLA-DRB1 allotypes. By increasing binding efficiency tertile, the cumulative incidence of aGVHD at 6 months for MUD patients was 41%, 41%, and 45% for HLA class I (P = .336) and 44%, 41%, and 42% for HLA class II (P = .452). The cumulative incidences of relapse at 3 years for MUD transplant recipients were 36%, 38%, and 38% for HLA class I (P = .533) and 37%, 37%, and 38% for HLA class II (P = .896). The findings were similar for MRD transplant recipients. Multivariable analysis did not identify any impact of peptide-binding efficiency on aGVHD or relapse in MUD or MRD transplant recipients. Whereas GVHD is mediated by minor antigen mismatches in the context of HLA-matched allo-HCT, peptide-binding efficiency, which was used as a surrogate measurement for predicted number of binding antigens, did not provide additional clinical information for GVHD risk assessment. The negative result may be due to the limitations of this surrogate marker, or it is possible that GVHD is driven by a subset of immunogenic mHAs. Further research should be directed at direct mHA epitope and immunogenicity prediction.

Keywords

HLA; Minor histocompatibility; antigen; Peptide epitope binding; Graft-versus-host disease

INTRODUCTION

Graft-versus-host disease (GVHD) is a common complication of allogeneic hematopoietic cell transplantation (alloHCT), even in the setting of HLA-identical transplantation. Acute GVHD (aGVHD) is initiated by the activation of host-derived antigen-presenting cells, which stimulates donor-derived (graft) T cells to differentiate, proliferate, and subsequently coordinate an immune response against host tissues [1].

Previous Center for International Blood and Marrow Transplant Research (CIBMTR) analyses have reported an aGVHD incidence of 33% to 39% following HLA-matched related donor (MRD) transplantation and a higher incidence (51% to 59%) following HLA-matched unrelated donor (MUD) transplantation [2,3]. The incidence of aGVHD increases with increasing degrees of HLA-mismatch in addition to other risk factors, including older age of either recipient or donor, female donor for male recipient, myeloablative conditioning, and use of peripheral blood grafts [2,4–9]. However, even when these features are considered, in the setting of fully HLA-matched alloHCT, the ability to predict which patients will develop clinically significant aGVHD remains limited.

Minor histocompatibility antigens (mHAs) are small peptides derived from recipient-encoded proteins that are presented by class I and class II HLA and recognized by donor-derived T cells [10–16]. Alloreactivity occurs because the donor-derived T cell population was never exposed to these antigens during T cell development in the donor thymus. Clinical observations demonstrate that mHAs mediate an increased risk of GVHD and decreased risk of leukemia relapse (ie, graft-versus-leukemia [GVL] effect) [17–19]. In any given HLA-matched transplant, there are potentially thousands of mHAs capable of mediating either GVL or GVHD [20,21]; however, it is uncertain whether the total number of mHA between a donor and recipient is associated with clinical outcome, or if GVL and/or GVHD are mediated primarily through a few immunodominant mHAs in a more patient-specific manner [21,22]. Detailed characterization of all possible mHAs across multiple transplant donor-recipient pairs (DRPs) is not feasible at present.

The netMHCpan algorithm can predict the binding of potential peptide antigens to a multitude of common HLA types, and thus can be used to generate possible mHAs and other HLA-restricted peptide antigens from various protein sources [23–25]. In addition to predicting individual HLA-restricted peptide antigens, netMHCpan also can quantify the number or frequency of predicted peptide binding to a particular HLA if given a set of peptides, with the expectation that different HLA types will present a varying frequency of peptide antigens. Because high-resolution data allowing the prediction of mHAs are not available to thoroughly test the hypothesis that the number of mHAs is associated with risk of aGVHD, we hypothesized that HLA peptide-binding frequency estimated by HLA genotype may be a useful surrogate for actual mHA number. If this is so, then patients with cumulative higher peptide binding would be at increased risk of GVHD and decreased risk of relapse.

A small single-institution study failed to show an association [26]; therefore, we examined this hypothesis in a large cohort of patients reported to the CIBMTR with the primary

objective of determining whether the cumulative peptide-binding fraction of an HLA genotype is associated with the incidence of aGVHD and relapse with either related or unrelated donors.

METHODS

Clinical Data Source

The CIBMTR is a working group of more than 300 transplant centers worldwide that contribute detailed data on HCT recipients to the statistical center at the Medical College of Wisconsin. Participating centers are required to report all consecutive transplantations and to follow patients longitudinally. Computerized checks for discrepancies, physician reviews of submitted data, and onsite audits of participating centers ensure data quality.

The CIBMTR performs observational studies in compliance with all applicable federal regulations pertaining to the protection of human research participants. The CIBMTR collects data at two levels: Transplant Essential Data (TED) and Comprehensive Report Form (CRF) data. TED data include disease type, age, sex, pretransplantation disease stage and chemotherapy responsiveness, date of diagnosis, graft type (bone marrow- and/or peripheral blood-derived stem cells), conditioning regimen, post-transplantation disease progression and survival, development of new malignancy, and cause of death. All CIBMTR centers contribute TED data. A subset of registered patients selected by weighted randomization have CRF data, which include more detailed disease and pretransplantation and post-transplantation clinical information, including infection data. TED- and CRF-level data are collected pretransplantation, 100 days and 6 months post-transplantation, and annually thereafter or until death [27]. All included patients provided written informed consent. The Institutional Review Boards of the National Marrow Donor Program and the Medical College of Wisconsin approved this study.

Peptide-Binding Fraction Determination

A dataset of all 8-, 9-, 10-, 11-, 16-, 20-, and 24-mer amino acid sequences containing a nonsynonymous coding single nucleotide polymorphism (cSNP) at each possible peptide position for both alleles (ie, 196 donor and recipient peptides per cSNP) was computationally generated for the set of 9575 cSNPs captured in the genetic variation of 101 DRPs genotyped on the Illumina NS-12 cSNP array (Illumina, San Diego, CA) as part of an Institutional Review Board-approved protocol (LAB99–062) at the University of Texas at Houston School of Medicine microarray core using the ENSEMBL Variant Effect Predictor and the human reference transcriptome (Gencode GRCh37.p13) [20,21,28]. This analysis generated a potential peptidome of 1,655,540 peptides (Figure 1) [21].

The fraction of this peptidome dataset that could bind (defined as predicted $K_d < 500$ nM) the HLA class I and II alleles (A, B, C, and DRB1) was analyzed using netMHCpan v2.8 and netMHCIIpan v3.0 software. This analysis was performed only on 30 HLA class I and 20 HLA class II alleles that were each present in at least 3 DRPs, hereinafter referred to as "informative" HLA alleles [21,29,30]. There was a >10-fold difference between the fraction of peptides in the dataset that were predicted to be bound and potentially presented across

this series of HLA class I, with peptide-binding frequencies ranging from 0.1% to 3.8% (Figure 2A,B). HLA class II molecules were generally predicted to bind to and present a higher fraction of the peptidome dataset, with peptide-binding frequencies of 12% to 77% across the HLA-DRB1 allotypes (Figure 2A). The fraction of the computational peptidome predicted to bind to a given HLA allele closely correlated to the ratio of predicted mHAs to the number of peptides in the test set containing a cSNP allele (and thus potential mHAs) for a given HLA (Figure 2C,D). Because these 2 calculated values were so closely associated, the median value for predicted mHAs per cSNP was used as a more intuitive scalar for the remainder of the analysis [21].

Patients

The study population comprised adult patients undergoing a fully (10/10) HLA MRD (n = 467) or MUD (n = 2775) alloHCT between 2005 and 2016 for acute myelogenous leukemia, acute lymphoblastic leukemia, or myelodysplastic syndrome. For MRDs, high-resolution typing was available only starting in 2008. Patients received either peripheral blood stem cells or bone marrow stem cells only with either myeloablative or reduced-intensity conditioning [31,32]. GVHD prophylaxis was restricted to calcineurin inhibitor-based GVHD prophylaxis. Patients who received in vivo or ex vivo T cell depletion or post-transplantation cyclophosphamide were excluded.

Included patients required an adequate representation of "informative" HLA alleles. DRPs with fewer than 4 HLA class I or no HLA class II informative HLA alleles were excluded. An overall peptide-binding efficiency score was calculated for each haplotype by averaging the predicted mHAs per cSNP values for the known informative alleles (Figure 2C,D).

Endpoints

The co-primary endpoints of this analysis were the cumulative incidence of grade II-IV aGVHD and relapse. AGVHD was defined by accepted clinical criteria [33]. Relapse was defined as the development of hematologic relapse according to the CIBMTR: 5% blasts in bone marrow or peripheral blood or extramedullary disease following a previous assessment of complete remission. Secondary endpoints included overall survival (OS), disease-free survival (DFS), and transplantation-related mortality (TRM). For OS, surviving patients were censored at the time of last follow-up. DFS was defined as time to disease relapse or death from any cause. TRM was defined as death without evidence of disease relapse.

Statistical Analysis

Patient clinical data, including demographics, disease-related, and transplantation-related factors, were described using median and range for continuous variables and frequency for categorical variables (Table 1). All analyses were performed with the unrelated DRPs analyzed separately from the related DRPs because of the known increased genetic distance, and thus the increased number of potential mHAs, in HLA-MUD HCT compared with HLA-MRD HCT [21].

For all outcomes, HLA class I and class II peptide-binding efficiency scores were examined separately. OS and DFS were determined using the Kaplan-Meier estimator. Outcomes of

GVHD and relapse were measured using cumulative incidence estimates to account for competing risks.

Multivariable models for the outcomes of aGVHD, relapse, TRM, DFS, and OS were built using the Cox proportional hazards model with the main effect variable of cumulative peptide-binding efficiency score. All clinical variables were tested for affirmation of the proportional hazards assumption. Factors violating the proportional hazards assumption were adjusted through stratification. Then a stepwise model building procedure was used to develop models for each outcome, with a threshold of 0.05 for both entry and retention in the model. To adjust for multiple testing, a threshold of 0.01 was used to determine the significance of the main testing variable. Interactions between the main variable and the adjusted covariates were tested at the significance level of 0.01, and no significant interactions were detected. For completeness, peptide-binding efficiency score was examined both as a continuous variable and in tertiles. The findings were similar, and the data presented here are from the tertile analysis of low, moderate, and high binding efficiency, according to the original statistical plan.

RESULTS

DRPs Can Be Categorized According to Cumulative Predicted Peptide-Binding Frequency Based on Estimated Median mHAs per cSNP

The CIBMTR dataset included 2775 unrelated DRPs and 467 related DRPs (total of 3242 DRPs) with sufficient informative HLA alleles. HLA class I and class II allotypes were considered separately. The mHA per cSNP values for all 6 HLA class I alleles for each DRP were summed to yield cumulative predictive peptide-binding efficiencies following the same procedure used for summing the 2 HLA-DRB1 alleles. DRPs who were homozygous for an HLA allele had their predictive peptide-binding efficiencies count twice toward the cumulative efficiency total. For DRPs with uninformative alleles, the average predicted peptide-binding efficiency per HLA was calculated using only the informative alleles. This average was subsequently multiplied by 6 for HLA class I analysis and by 2 for HLA class II analysis.

The distributions of patients in low, moderate, and high binding tertiles by HLA class I and class II for MRDs and MUDs are shown in Table 2. The tertiles by estimated binding efficiency were determined irrespective of donor type. For class I HLAs, the median estimated binding efficiency scores, determined as the number of peptide epitopes per SNP, were 0.3019 (range, 0.0729 to 0.3706) for low-affinity binding, 0.4351 (range, 0.3707 to 0.4965) for moderate-affinity binding, and 0.5843 (range, 0.4968 to 0.8768) for high-affinity binding. For class II, the binding affinities were much higher, and the tertiles of low, moderate, and high were 19.20 (range, 0 to 21.61), 22.41 (range, 21.70 to 24.87), and 28.47 (range, 24.89 to 41.19).

Lack of Association Between Peptide-Binding Efficiency and aGVHD

The study cohorts had rates of aGVHD that were consistent with previous reports. The cumulative incidence of grade II- IV aGVHD at 6 months was 42% (95% confidence

interval [CI], 41% to 44%) for the MUD cohort and 36% (95% CI, 32% to 41%) for the MRD cohort. Figure 3A,B shows the cumulative incidence of aGVHD for the MUD cohort, examined by binding affinity strata for HLA class I and class II (HLA class I: low, 41% [95% CI, 38% to 44%], versus moderate, 41% [95% CI, 38% to 45%], versus high, 45% [95% CI, 41% to 45%], P = .336; HLA class II: low, 44% [95% CI, 41% to 47%], versus moderate, 41% [95% CI, 38% to 44%], versus high, 42% [95% CI, 39% to 46%], P = .452). The findings were similar for the MRD cohort (Supplementary Figure S1). As expected, based on the univariate findings, the multivariable analysis did not identify any impact of peptide-binding efficiency on aGVHD for MUD or MRD patients (Table 3). Factors associated with the development of aGVHD in the MUD cohort included conditioning intensity, disease, donor age, graft type, type of GVHD prophylaxis, and Disease Risk Index (DRI). For the MRD cohort, only disease and donor-recipient sex match were associated with aGVHD. Analyzing peptide binding as a continuous variable yielded similar results.

Lack of Association Between Peptide-Binding Efficiency and Relapse

The study cohorts had rates of relapse that were consistent with previous reports. The cumulative incidence of relapse at 3 years was 37% (95% CI, 35% to 39%) for the MUD cohort and 39% (95% CI, 34% to 43%) for the MRD cohort. Figure 3C,D shows the cumulative incidence of relapse for MUD patients, examined by binding affinity strata for HLA class I and class II (class I: low, 36% [95% CI, 33% to 39%], versus moderate, 38% [95% CI, 34% to 41%], versus high, 38% [95% CI, 35% to 42%], P = .533; class II: low, 37% [95% CI, 34% to 40%], versus moderate, 37% [95% CI, 34% to 41%], versus high, 38% [95% CI, 34% to 41%], P = .896). The findings were similar for MRD patients (Supplementary Figure 1). As expected, based on the univariate analysis findings, the multivariable analysis did not identify any impact of peptide-binding efficiency on relapse in either the MUD or MRD cohort (Tables 3 and 4). Analyzing peptide binding as a continuous variable yielded similar results. Factors associated with relapse in the MUD cohort included conditioning intensity and year of transplantation. The sole factor associated with relapse in the MRD cohort was the time from diagnosis to transplantation (Table 4).

Survival and TRM—As with aGVHD and relapse outcomes, there was no association of peptide epitope- binding affinity with OS, DFS, or nonrelapse mortality. Supplementary Table S1 shows the multivariable analysis results for OS in MUD and MRD HCTs. For the MUD cohort, survival was associated with disease, recipient age, GVHD prophylaxis regimen, DRI, Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI), Karnofsky Performance Status, and year of transplantation. For the MRD cohort, only DRI, HCT-CI, and year of transplantation were associated with survival. For DFS, recipient age, GVHD prophylaxis regimen, and year of transplantation in the MUD cohort and only time from diagnosis to transplantation in the MRD cohort were associated with survival (data not shown). Supplementary Table S2 presents the data for nonrelapse mortality.

DISCUSSION

Several risk factors for the development of GVHD are well described. HLA mismatch, donor age, recipient age, sex mismatch (ie, female donor-male recipient), and conditioning

regimen intensity have all shown significant association with GVHD incidence [2,4–7,9]; however, even when these risks are minimized, GVHD remains a common and significant complication of HCT. Identification of additional risk factors for the development of GVHD in HLA-identical transplantations may potentially allow clinicians to further ameliorate GVHD-related morbidity and mortality by making different clinical decisions about conditioning regimen intensity, donor graft source, and GVHD prophylaxis. Conversely, this knowledge also may allow the clinician to mitigate relapse by such maneuvers as decreasing GVHD prophylaxis more rapidly in patients at low risk of GVHD.

This large CIBMTR analysis sought to determine whether a relatively simple measurement of potential antigen binding capacity, or "peptide-binding efficiency," derived from already existing HLA-typing data could provide further clinical information with respect to the risk of aGVHD and relapse in MUD and MRD patients, with the hypothesis that patients with higher peptide-binding efficiency scores would present more mHAs and be at higher risk of aGVHD and lower risk of relapse. This study was designed based on the computational observation that HLA molecules when exposed to a large number of potential peptides will bind to, and theoretically present, vastly different fractions of the overall peptide pool (Figure 2), which would allow some approximation of the number of potential mHAs that could be presented in a DRP based solely on HLA type which is known at high resolution for all allogeneic HCTs. Despite the large sample size, our data demonstrate no association of peptide-binding efficiency with aGVHD or relapse. Furthermore, results were similar for survival and TRM. There are several possible explanations for this lack of association.

mHAs are peptide epitopes encoded by the recipient genome but not present in the genome of the donor. There are many mechanisms for minor antigen reactivity in the setting of HCT. A classic example of mHA occurs in the setting of a male recipient receiving a graft from a female donor. In that situation, the T cell repertoire from the female donor has not been tolerized against epitopes encoded by genes expressed on the Y antigen [9,12,34]. Similar minor antigen reactivity has been described in the context of whole gene deletions and alternative splicing occurring in the stem cell donor [35,36]; however, the most common genetic cause of minor antigen reactivity is a result of simple differences in nonsynonymous coding single nucleotide polymorphisms (cSNPs) between the donor and recipient, that is, the recipient expresses an allele that is not encoded in the donor's genome [13]. For any HLA-matched transplant, there are thousands of cSNP disparities that could encode mHA and lead to alloreactivity [21].

Although mHAs have been recognized as important mediators of GVHD for many decades now [10–14,34,37,38], their individual and cumulative impacts on clinical outcomes have been difficult to measure [22,35,39–41]. Syngeneic HCT, which would be expected to have very few if any minor antigen mismatches, has historically been associated with an extremely low incidence of GVHD without any GVHD prophylaxis [18], and early investigations in the use of HLA-matched unrelated donor grafts did reveal higher rates of GVHD compared with rates observed in MRD alloHCT [2,3]. Because the HLA-matched unrelated donors are genetically more "distant" (ie, have greater genetic disparity) than HLA-matched related donors, a general idea of increasing numbers of minor antigens being

associated with increased risk of GVHD and reduced risk of relapse is plausible; however, additional factors likely overwhelm this effect.

To extensively test the hypothesis that the actual number of mHAs in an HCT is associated with the risk of cGVHD (or reduced risk of relapse), high-resolution genotyping and antigen prediction would need to be performed on a large number of HCT patients. This has not been performed to date; however, data that could allow for these studies are available [42]. The closest study to this type of analysis, although not directly interrogating predicted peptide mHAs, is a single-center analysis of 3057 DRPs performed at the Fred Hutchinson Cancer Center. That study showed an association between genetic distance between HLA-matched related DRPs and aGVHD but no such association for HLA-matched unrelated DRPs [22]. Of note, in that analysis it was found that mismatches in the HLA-DP locus in MUD HCT accounted for the increased GVHD risk between MRD and MUD patients, and the greater genetic distance observed in MUD versus MRD transplants was not a significant clinical feature. Our study focused on 10/10 MUD transplants without stratification for HLA-DP, which could have led to the blunting of any signal from peptide-binding efficiency [43,44]. In addition, mismatches at other HLA loci, including the HLA-DRB3/4/5 locus, which occur in ~15% of HCTs, could have influenced the results from this analysis [45,46]. Finally, a significant technical limitation of this study is the fact that the predicted peptide-binding efficiency scores could not be well defined for all HLA types. As a result, the predicted cumulative peptide-binding efficiencies for each DRP often contained incomplete data that required normalization of the efficiency score based on the number of informative HLA alleles present in each DRP. The study included only DRPs with at least 4/6 HLA class I and $1/2$ HLA class II informative HLA alleles; however, the potential clinical impact from the other "uninformative" alleles cannot be determined accurately.

Although the present study sought to develop an easy-to-measure score that relates HLA-type to potential antigen presentation, the actual biology of antigen presentation and immune response is far more complicated. The peptide-binding efficiency score provided an assessment of one necessary feature for antigen recognition, binding of the peptide epitope to its HLA molecule; however, many additional biochemical reactions and intracellular shuttling processes are necessary for the successful presentation of antigens and their subsequent ability to stimulate immune responses [47,48]. For HLA class I antigen presentation, endogenous proteins must be ubiquitinated, digested in the proteasome, and transported through the Golgi/endoplasmic reticulum complex before presentation, and any of these processes can influence whether a peptide is actually presented on the cell surface [49]. Because of these processes, and others, only a subset of the proteome can be presented by HLA molecules [50], and this feature is not accounted for in our model. This differential antigen presentation is further complicated by the fact that gene expression and protein processing can be dramatically affected by the cells environment at a particular time, so that it is likely that some minor antigens will be presented at higher levels and potentially be more immunogenic at certain time points post-transplantation (eg, during an inflammatory response) than at others [51–53]. Furthermore, different mHAs will exhibit different magnitudes of immune response based on such features as the chemical differences between the 2 alleles that influence either peptide binding to the HLA or the affinity of binding between the peptide/mHA complex and a donor-derived T cell receptor [54–56].

This study provides further evidence that GVHD risk across a population is not strongly associated with the relatively small differences in total numbers of mHA mismatches among DRPs, particularly in the setting of HLA-matched unrelated donor transplantation. Instead, it is more likely that GVHD risk is more strongly associated with such features as mismatching at non-classical HLAs, a much more common occurrence in unrelated donor HCT. Alternatively, it could be driven by a subset of highly immunogenic mHAs within the total pool of candidate mHAs, which would dilute the clinical significance of the actual number of mHAs. The clinical significance of non-classical HLAs can be measured using large datasets, such as the CIBMTR database; however, methods to predict and confirm multiple immunodominant mHAs in a given DRP have not yet been developed. Detailed genotyping studies of large numbers of DRPs have been performed, and computational mHA prediction studies are underway. These studies could lead to further our understanding of GVHD risk in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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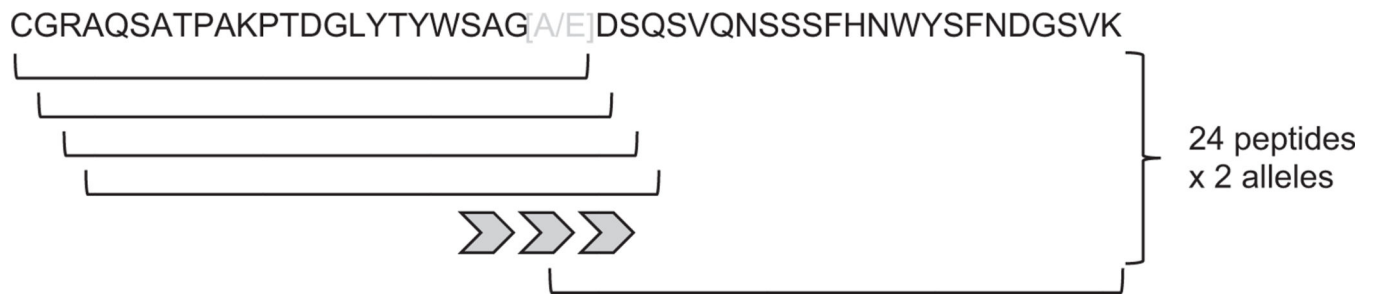
Figure 1.
Computational generation of a peptidome dataset. For each cSNP (in this case rs9876490, allelic amino acids are A/E), the peptide sequence was extended 24 amino acids N terminal and C terminal from the cSNP. A "sliding window" method was used to identify each 24-mer peptide that contained the cSNP., which yielded 24 unique peptides for each allele. This process was repeated to identify all 8-, 9-, 10-, 11-, 16-, 20-, and 24- mer peptides, resulting in a total of 196 peptides for each cSNP.
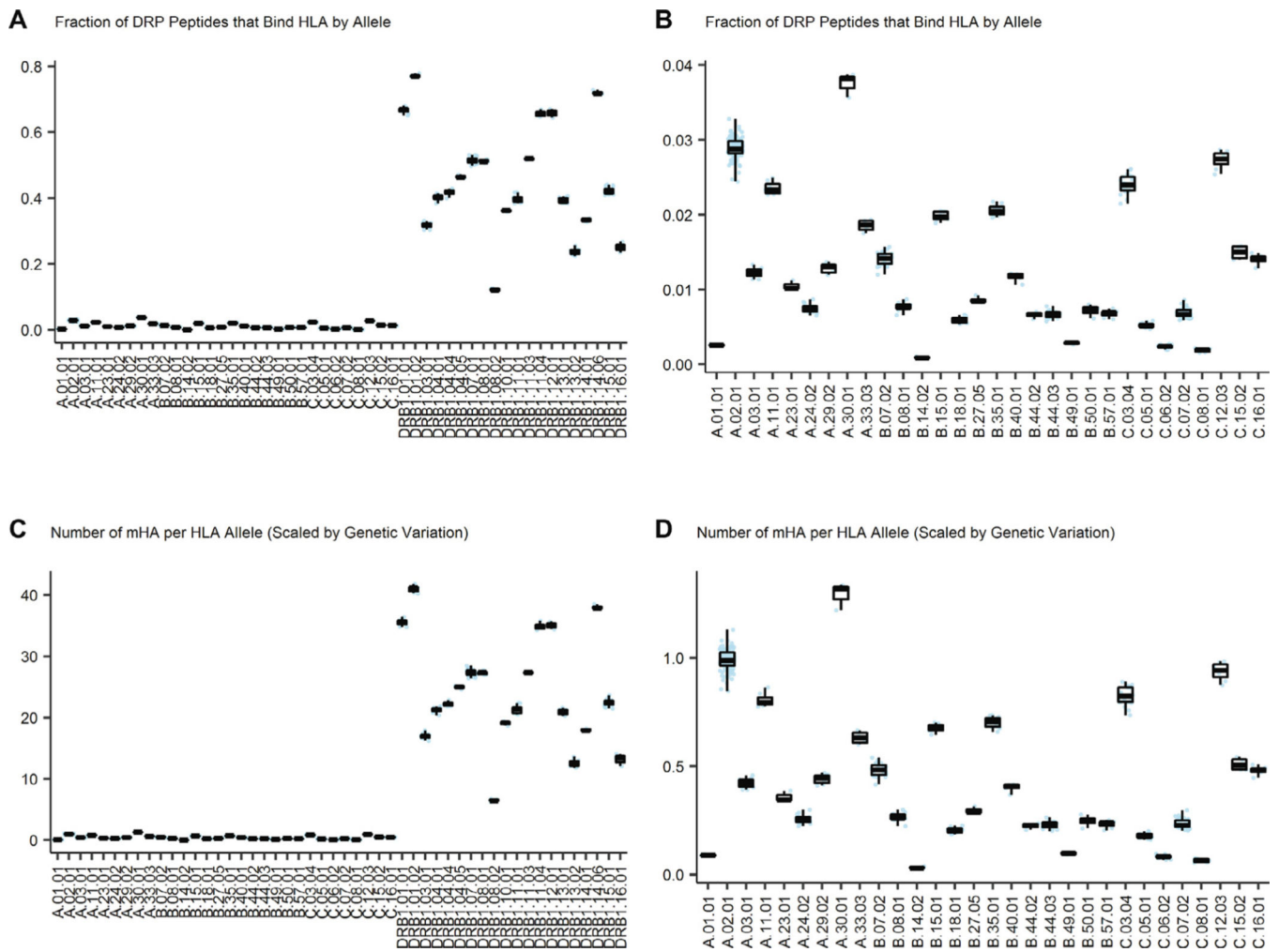
Figure 2.
Variability of peptide/HLA-binding efficiency according to HLA type. (A) The variation in peptide-binding efficiency demonstrated by HLA class I and class II. HLA class II can present a higher fraction of antigenic peptides compared with HLA class I. (B) The peptide-binding fraction by allele for the HLA class I alone. (C) The median number of mHAs estimated by HLA allele for HLA class I and class II alleles scaled by cSNP to control for genetic variation. (D) The median number of mHAs estimated by HLA class I alleles scaled by cSNP.
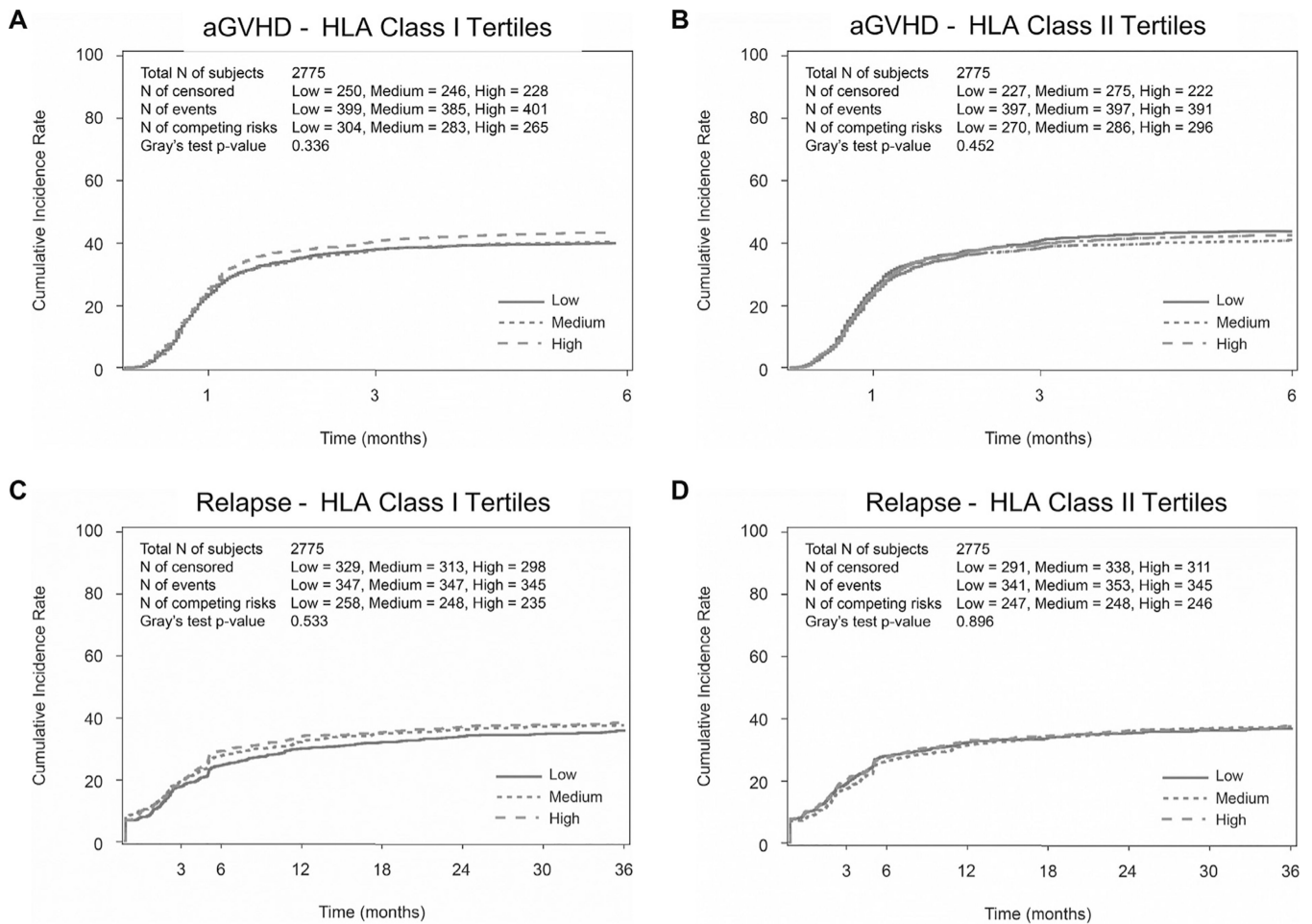
Figure 3.
Cumulative incidence curves for aGVHD and relapse for MUD alloHCT. (A) The cumulative incidence of aGVHD according to HLA class I peptide-binding efficiency. (B) The same incidence according to HLA class II peptide-binding efficiency. (C) The cumulative relapse rate according to HLA class I peptide-binding efficiency. (D) The cumulative relapse rate according to HLA class II peptide-binding efficiency.

Table 1

Patient Characteristics

Donor Type	10/10 Related (n = 467)	10/10 Unrelated (n = 2775)
Number of Centers	63	116
Patient Age (median and range)	55 (18–74)	55 (18–78)
Recipient Sex, Male	269 (58%)	1566 (56%)
Graft type, PBSC	451 (97%)	2304 (83%)
Years of transplant		
2005 – 2007	0[*]	790 (28%)
2008 – 2010	126 (27%)	611 (22%)
2011 – 2013	131 (28%)	506 (18%)
2014 – 2016	210 (45%)	868 (32%)
HCT-CI		
0	107 (23%)	420 (15%)
1	72 (15%)	272 (10%)
2	73 (16%)	326 (12%)
3+	215 (46%)	960 (35%)
Missing/unavailable	0[*,£]	797 (29%)[£]
Disease type		
AML	259 (55%)	1565 (56%)
ALL	63 (13%)	424 (15%)
MDS	145 (31%)	786 (28%)
Disease Risk Index		
low	19 (4%)	135 (5%)
intermediate	256 (55%)	1389 (50%)
high	170 (36%)	997 (36%)
very high	6 (1%)	113 (4%)
missing/unavailable	16 (4%)	141 (5%)
Karnofsky Performance Score		
0–80	195 (42%)	1133 (41%)
90–100	265 (57%)	1570 (57%)
Missing	7 (2%)	72 (3%)
Time from Dx to HCT (months)		
< 6 months	269 (58%)	1325 (48%)
6 – 12 months	87 (19%)	709 (26%)
12 – 24 months	61 (13%)	396 (14%)
> 24 months	50 (10%)	343 (12%)
Missing	0 (0%)	2 (0%)
Conditioning Intensity		

Donor Type	10/10 Related (n = 467)	10/10 Unrelated (n = 2775)
MAC	322 (69%)	1749 (63%)
NMA/RIC	145 (31%)	1017 (37%)
TBD (under review)	0	9 (<1%)
Donor/Recipient Sex match		
F to M	117 (25%)	344 (12%)
Donor Age at HCT	Not available	
Median (range)		29 (18–62)
18–29		1542 (56%)
30–39		716 (26%)
40–49		377 (14%)
50–59		106 (4%)
60–69		2 (<1%)
Missing		32 (1%)
CMV status: (- / -)	113 (24%)	853 (31%)
GVHD prophylaxis (PTCY excluded)		
CNI + MMF +- others (no MTX)	91 (19%)	624 (22%)
CNI + MTX +- others (no MMF)	313 (67%)	1864 (67%)
CNI + others (excluding above)	54 (12%)	223 (8%)
CNI alone	9 (2%)	64 (2%)
Median survivor f/u, months	48 (11–118)	63 (3–151)
% Informative alleles Class I		
4	210 (45%)	1154 (42%)
5	194 (42%)	1137 (41%)
6	63 (13%)	484 (17%)
% Informative alleles Class II (DRB1)		
1	69 (15%)	303 (11%)
2	398 (85%)	2472 (89%)

All patients had AML/ALL/MDS and underwent a first allogeneic 10/10 HLA-matched bone marrow transplantation with a minimum of 4/6 informative HLA class I alleles and 1/2 HLA class II alleles.

PBSCs indicates peripheral blood setm cells; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; MAC, myeloablative conditioning; NMA, nonmyeloablative; RIC, reduced-intensity conditioning; TBD, to be determined; CMV, cytomegalovirus; PTCY, post-transplantation cyclophosphamide; CNI, calcineurin inhibitor (tacrolimus or cyclosporine A); MMF, mycophenolate mofetil; MTX, methotrexate.

[*] Related donor repository not available prior to 2007, so no samples available for high resolution typing.

[£] Data for HCT-CI were not collected prior to 2007. CNI: Calcineurin inhibitor (TAC or CSA). PTCY: Post-transplant cyclophosphamide

Table 2

Distribution of Patients by Binding Tertile by Donor Type and HLA Class I and Class II Analyses

	MRD, n (%)		MUD, n (%)	
Binding Affinity	**HLA Class I**	**HLA Class II**	**HLA Class I**	**HLA Class II**
Low	126 (27)	176 (38)	945 (34)	893 (32)
Moderate	158 (34)	142 (30)	912 (33)	943 (34)
High	183 (39)	149 (32)	889 (32)	910 (33)

Table 3

Multivariate Analysis Results for Grade II-IV aGVHD

Variable	MUD (n = 2775)				MRD (n = 467)			
	HLA Class I (99% CI)	P Value	HLA Class II (99% CI)	P Value	HLA Class I (99% CI)	P Value	HLA Class II (99% CI)	P Value
Binding tertile		.2218		.3168		.381		.8675
Low	1.00		1.00		1.00		1.00	
Moderate	1.01 (0.89–1.14)	.917	0.92 (0.82–1.03)	.132	1.04 (0.67–1.61)	.8518	1.06 (0.75–1.49)	.7529
High	1.09 (0.98–1.22)	.1179	0.96 (0.83–1.11)	.6081	0.82 (0.57–1.19)	.3062	0.95 (0.70–1.28)	.7317
Conditioning intensity		<.0001		<.0001		NS		NS
MAC	1.00		1.00					
RIC/NMA	0.62 (0.50–0.76)		0.62 (0.50–0.76)					
Disease		<.0001		<.0001		.0226		.0244
AML	1.00		1.00		1.00		1.00	
ALL	1.43 (1.21–1.69)	<.0001	1.43 (1.21–1.69)	<.0001	0.98 (0.61–1.58)	.9299	0.98 (0.60–1.60)	.9379
MDS	1.40 (1.16–1.69)	.0005	1.40 (1.16–1.69)	.0004	1.47 (1.11–1.94)	.0065	1.47 (1.11–1.94)	.0067
Donor age, yr		.0002		.0001		NA		NA
18–29	1.00		1.00					
30–39	1.05 (0.93–1.19)	.4008	1.06 (0.94–1.9)	.3704				
40–49	1.03 (0.88–1.20)	.7201	1.03 (0.88–1.20)	.7331				
50	1.64 (1.32–2.04)	<.0001	1.65 (1.33–2.04)	<.0001				
Missing	0.64 (0.32–1.31)	.2234	0.63 (0.31–1.32)	.2221				
Graft type		.001		.0012		NS		NS
Marrow	1.00		1.00					
PBSC	1.33 (1.12–1.59)	.001	1.33 (1.12–1.58)	.0012				
GVHD prophylaxis		.0186		.0186		NS		NS
CNI + MMF ± other	1.00		1.00					
CNI + MTX ± other	0.80 (0.66–0.97)	.0241	0.80 (0.66–0.97)	.0243				
CNI + other	0.74 (0.54–1.02)	.0658	0.74 (0.54–1.02)	.0683				
CNI alone	0.42 (0.23–0.76)	.0043	0.42 (0.23–0.76)	.0044				
DRI		.0295		.029		NS		NS

Variable	MUD (n = 2775)				MRD (n = 467)			
	HLA Class I (99% CI)	P Value	HLA Class II (99% CI)	P Value	HLA Class I (99% CI)	P Value	HLA Class II (99% CI)	P Value
Low	1.00		1.00					
Intermediate	1.10 (0.85–1.43)	.4623	1.01 (0.87–1.17)	.8947				
High	0.92 (0.69–1.21)	.5296	0.85 (0.73–0.99)	.0326				
Very High	0.72 (0.45–1.17)	.5296	0.98 (0.75–1.28)	.8833				
Missing	1.09 (0.78–1.54)	.6098	2.58 (1.38–4.83)	.0029				
Donor recipient sex match		NS		NS		.0094		.0094
Male-male					1.00		1.00	
Male-female					1.13 (0.77–1.67)	.5256	1.14 (0.77–1.69)	.5031
Female-male					1.13 (0.77–1.67)	.1115	0.78 (0.55–1.12)	.1833
Female-female					1.13 (0.77–1.67)	.0082	0.51 (0.31–0.83)	.0070

NS indicates not significant; NA, not applicable.

Multivariate Analysis Results for Relapse

Table 4

Variable	MUD (n = 2775)				MRD n = 467			
	HLA Class I (99% CI)	P Value	HLA Class II (99% CI)	P Value	HLA Class I (99% CI)	P Value	HLA Class II (99% CI)	P Value
Binding tertile		.4061		.5684		.7497		.6516
Low	1.00		1.00		1.00		1.00	
Moderate	1.07 (0.91–1.27)	.4045	0.94 (0.83–1.06)	.3312	0.99 (0.64–1.51)	.9533	1.07 (0.79–1.44)	.669
High	1.11 (0.95–1.29)	.1796	1.00 (0.88–1.14)	.9834	0.89 (0.62–1.26)	.5074	0.90 (0.63–1.29)	.5767
Conditioning intensity		.0001		.0001		NS		NS
MAC	1.00		1.00					
RIC/NMA	1.40 (1.19–1.65)	.0001	1.39 (1.19–1.64)	.0001				
Year of transplantation		.0038		.0043		NS		NS
2014–2016	1.00		1.00					
2011–2013	1.15 (0.96–1.37)	.1271	1.14 (0.96–1.36)	.1321				
2008–2010	1.30 (1.12–1.51)	.0007	1.29 (1.11–1.50)	.0008				
2005–2007	1.25 (1.07–1.46)	.0043	1.24 (1.07–1.45)	.0051				
Time from diagnosis to HCT		NS		NS		.0022		.0018
mo					1.00		1.00	
6–12 mo					1.44 (1.02–2.05)	.0387	1.45 (1.03–2.03)	.0347
12–24 mo					0.78 (0.52–1.18)	.2389	0.81 (0.54–1.22)	.3187
mo					0.56 (0.33–0.97)	.0368	0.56 (0.33–0.96)	.0362