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LMP7 polymorphism may modify the presentation and clinical impact of minor histocompatibility antigens in matched related hematopoietic stem cell transplantation

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ABSTRACT

Differential expression of minor histocompatibility antigens between the recipient and donor determines their disparity and can be modified by immunoproteasomes that regulate their processing and presentation. We examined the impact of HA-1 and HA-8 disparity, and immunoproteasome *LMP7* polymorphism in 130 pairs. In multivariate analysis, HA-1 disparity showed a statistically significant association with an increased incidence of acute graft-versus-host disease (aGVHD) II–IV ($p = 0.043$, HR: 3.71, 95%CI = 1.04–13.26), while *LMP7-Q/Q* showed a trend toward increased incidence of aGVHD compared to *LMP7-Q/K* and *K/K* genotypes ($p = 0.087$, HR: 2.36, 95%CI = 0.88–6.31). All HA-1 and HA-8 disparate patients who developed aGVHD had the *LMP7-Q/Q* genotype. No significant association could be detected between HA-1, HA-8, or *LMP7* and chronic GVHD, relapse-free survival (RFS), overall survival (OS), or transplant-related mortality (TRM). In conclusion, we suggested an association between the HA-1 disparity and the risk of developing aGVHD with a possible modifying effect of LMP7.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (AHSCT) is a curative treatment for hematological malignancies. Differential expression of the endogenously generated polymorphic minor histocompatibility antigens (mHags) presented in the context of shared HLA molecules results in disparities between the recipient and donor after HLA-matched AHSCT [1]. Disparities in the mHags can induce specific alloimmune responses in the recipient involving CD8+ cytotoxic T cells, CD4+ T helper (Th) cells and T regulatory (Treg) cells [2]. Disparity may mediate graft-versus-host disease (GVHD) and graft-versus-leukemia (GVL) effects [3]. Discrepancies have been reported in the impact of disparities in the mHags on the transplantation outcome pointing to the possible contribution of factors related to their binding affinity and presentation in the context of the HLA molecules as well as factors related to the alloreactive response [4–6]. The alteration in the processing of mHags rather than the diversity of their interaction with the major histocompatibility complex (MHC) molecule or the T- cell receptor (TCR) can result in disparity [1]. The presentation of these peptides in the context of MHC class I molecules involves their processing through the proteasome. The constitutively expressed proteasome plays a central role in the degradation and presentation of cytosolic proteins to CD8+ cytotoxic T cells [7,8]. The catalytic immunoproteasome subunit (low molecular mass polypeptide) LMP7 (b5i) is expressed in monocytes and inducible in lymphocytes and non-hematopoietic cells by interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNFα) under inflammatory conditions [8–11]. LMP7 has distinct cleavage preferences over the constitutive proteasome, generating a different pool of peptides better suited to bind to MHC molecules. Therefore, LMP7 induces a more efficient immune response than the constitutive proteasome and participates in shaping the repertoire of peptides presented in the context of MHC class I to cytotoxic T cells [10,12–15]. LMP7 also affects Th cell differentiation and the production of proinflammatory cytokines and has been implicated in T cell-mediated autoimmune diseases [16,17].

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The differential tissue distribution of the mHag allows dissection between disparity induced GVHD and GVL effects [18]. mHags exclusively expressed on hematopoietic cells can induce the GVL effect and are considered ideal targets for immunotherapy in patients post-transplantation [19,20]. T cells that recognize broadly expressed mHags can also induce harmful GVHD [21]. The autosomal HLA-A2 restricted hematopoietic HA-1 mHags (HMHA1) and the ubiquitously expressed HA-8 mHags have been identified based on their binding affinity to class I MHC [1,22,23]. A common SNP in the HA-1 encoding gene (rs1801284) creates a histidine (HA-1^{H}) to arginine (HA-1^{R}) substitution. The immunogenic HA-1^{H} encoded peptide has a higher stability than its non-immunogenic counterpart HA-1^{R} allele [22]. Another SNP in the HLA-A2 restricted HA-8 mHags, (rs2173904), generates peptides with either arginine (HA-8^{R}) or proline (HA-8^{P}). The immunogeneic HA-8^{R} peptide is more efficiently transported by the transporter associated with antigen processing (TAP) protein and subsequently presented on the cell surface compared to the variant HA-8^{P} peptide [1].

Selective inhibition of LMP7 improved acute GVHD in murine mainly by reducing the presentation of mHags [24]. LMP7 is encoded by the proteasome subunit beta-type 8 (*PSMB8*) gene located in the short arm of chromosome 6 within the MHC class II region [10]. Genetic variants of *LMP7* may modify the presentation of mHAgs in the context of class I MHC. The *LMP7* polymorphism (rs2071543) leads to glutamine (Q) to lysine (K) substitution at codon 145 and is associated with the regulation of gene transcription [25]. Our study aimed to examine the impact of HA-1 and HA-8 mHag disparities and *LMP7* polymorphism on transplantation outcome. We also investigated the possible modifying effect of LMP7 on the impact of mHags in matched related AHSCT.

2. Material and methods

2.1. Patients

The study included 130 recipients and their respective HLA- matched sibling hematopoietic stem cell donors. Transplants were performed between 2015 and 2018 at Nasser Institute, Ministry of Heath, Egypt. The study was approved by the institutional review board. Informed consent was obtained. They were 81 (62.3%) males and 49 (37.7%) females. Their median age was 30.5 with range (4–56) years. They included 19 children and 111 adults. Patient characteristics are summarized in table 1. Diagnoses included acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and myelodysplastic syndrome (MDS). Patients received conditioning regimens and GVHD prophylaxis as defined in table 1. Acute GVHD was graded according to ‘‘the 1994 Consensus Conference on Acute GVHD Grading’’ [26]. Classification of cGVHD was performed using the ‘‘National Institutes of Health Consensus Development Project Criteria’’’ [27]. Disparity in the GVHD direction was identified when the recipient had the immunogenic allele and the donor was homozygous for the non-immunogenic allele (HA-1^{H}:immunogenic, HA-1^{R} : non-immunogenic and HA-8^{R} : immunogenic, HA-8^{P}: non-immunogenic). Analysis was performed in the presence of the appropriate HLA restriction molecule (HLA-A*02-restricted). Analysis in the presence or absence of HLA-A*02 was also performed (HLA-A*02-unrestricted).

2.2. Methods

Genotyping was performed in the Bone Marrow Transplantation Laboratory at the National Cancer Institute, Cairo University for the mHags HA-1 and HA-8 for recipients and donors by sequence-specific primers-polymerase chain reaction (SSP-PCR) method using allele-specific primers as previously described [28,29]. *LMP7* polymorphism was investigated in recipients and donors using the amplification refractory mutation system (ARMS) PCR according to Lim et al. [30] to identify the *LMP7-Q* and *K* alleles. HLA-A*02 typing was performed using reverse sequence-specific oligonucleotide (rSSO) (Innogenetics, Belgium and One Lambda, USA).

Table 1
Patients’ characteristics.

Characteristics	Number (%)
Age*	30.5 (4–56) years
Gender	
Male	81 (62.3)
Female	49 (37.7)
Disease at transplantation	
Acute myeloid leukemia	83 (63.8)
Acute lymphoblastic leukemia	29 (22.4)
Chronic myeloid leukemia	13 (10.0)
Myelodysplatic syndrome	5 (3.8)
Stage of the disease	
Low, intermediate	98 (73.4)
High	32 (24.6)
Gender mismatch between recipient and donor	
Female to male	33 (25.3)
Other combinations	97 (74.6)
Conditioning regimen	
Busulfan/Cyclophosphamide	74 (56.9)
Fludarabin-based	
Fludarabin/Alkylating agent	9 (6.9)
Fludarabin/Busulfan	31 (23.8)
Fludarabin/Cyclophosphamide	3 (2.3)
TBI/Cyclophosphamide	13 (10)
Graft-versus- host disease prophylaxis	
Cyclosporine, Methotrexate	102 (78.5)
Cyclosporine, Mycophenolate mofetil	11 (8.5)
Cyclosporine, post-Cyclophosphamide	17 (13.1)
Acute graft-versus-host disease	
Grade 0-I	105 (80.8)
Grade II-IV	25 (19.2)
Chronic graft-versus-host disease	
No or mild	115 (88.5)
Extensive	15 (11.5)

*Median (range).

2.3. Statistical analysis

Statistical analysis was done using IBM SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA). Ages were expressed as median and range. Qualitative data were expressed as frequency and percentage. Pearson’s chi-square test or Fisher’s exact test was used to examine the relation between qualitative variables. Survival analysis was done using the Kaplan-Meier method and comparison between two survival curves was done using log-rank test. Multivariate analysis was done using Cox-proportional Hazard regression model for the factors affecting survival on univariate analysis. Hazard ratio (HR) with it 95% confidence interval (CI) was used for risk estimation. All tests were two-tailed. A p-value <0.05 was considered significant.

3. Results

Patient characteristics are presented in Table 1.

3.1. HA-1, HA-8 disparity, and LMP7 polymorphism

The distribution of HA-1, and HA-8, and *LMP7* genotypes in recipients and donors was in Hardy-Weinberg equilibrium. HLA-A*02-unrestricted HA-1 disparity in the GVHD direction was detected in 13/130 recipient/donor pairs (10%), and HA-8disparity in 20/130 (15.4%). HLA-A*02-restricted HA-1 and HA-8 disparities were reported in 7/130 (5.3%) and 6/130 (4.6%) patients, respectively. The frequency of *LMP7*

Table 2
Association between HA-1and HA-8 disparities and *LMP7* polymorphism with transplantation outcome in 130 recipients after matched related transplantation.

Parameters	aGVHD *P value*, HR, 95%CI	cGVHD *P value*, HR, 95%CI	RFS *P value*, HR, 95%CI	OS *P value*, HR, 95%CI	TRM *P value*, HR, 95%CI
HA-1 disparity-HLA-A*02- restricted vs No disparity	**0.090**	0.357	0.107	0.302	0.374
	2.72 0.81–9.11	2.01 0.45–8.93	NC	NC	NC
HA-1 disparity$^{\neq}$ vs No disparity	0.774	0.996	0.258	0.283	0.229
	1.91 0.35–3.98	1.04 0.22–4.45	0.449 0.10–1.86	0.45 0.11–1.90	NC
HA-8 disparity-HLA- A*02- restricted vs No disparity	0.309	0.529	0.143	0.347	0.416
	2.07 0.49–8.82	NC	NC	NC	NC
HA-8 disparity$^{\neq}$ vs No disparity	0.328	0.305	0.433	0.240	0.247
	0.49 0.11–2.09	0.34 0.45–2.63	0.662 0.23–1.87	0.49 0.15–1.60	0.42 0.10–1.80
LMP7- Q/Q vs *Q/K, K/K*	**0.043**	0.338	0.512	0.685	0.954
	2.63 0.99–7.03	5.53 0.18–1.79	0.795 0.39–1.83	0.86 0.43–1.74	0.97 0.44–2.13
HA-1 disparity$^{\neq}$/*LMP7- Q/Q* vs Others	0.172	0.431	0.318	0.352	0.355
	2.26 0.67–7.56	1.81 0.41–8.06	0.378 0.05–2.75	0.38 0.05–2.84	NC
HA-1 disparity$^{\neq}$/*LMP7- Q/K,K/K* vs Others	0.470	0.393	0.607	0.615	0.453
	NC	NC	0.97 0.82–4.35	0.60 0.08–4.38	NC
HA-8 disparity$^{\neq}$/*LMP7- Q/Q* vs Others	0.648	0.996	0.579	0.856	0.561
	1.40 0.33–5.94	0.99 0.13–7.57	1.39 0.421–4.54	0.87 0.21–3.65	0.53 0.7–4.07
HA-8 disparity$^{\neq}$/*LMP7- Q/K,K/K* vs Others	0.303	0.393	0.157	0.207	0.337
	NC	NC	0.263 0.36–1.92	0.27 0.03–2.03	0.05–2.77

Bold p values indicate statistical significance or near significance.
$^{\neq}$ HLA-A*02-unrestricted (included recipients with and without HLA-A*02). Association with HA-1 and HA-8 disparity (HLA-A*02-restricted and HLA-A*02-unrestricted) was tested in the GVHD direction. aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; RFS, relapse-free survival; OS, overall survival; TRM, transplant-related mortality; HR, hazard ratio; CI, confidence interval; NC: Not calculated. In case all patients were censored, the hazard ratio (HR) could not be calculated and was marked as NC, and the Kaplan-Meier method was used to calculate the p-values.

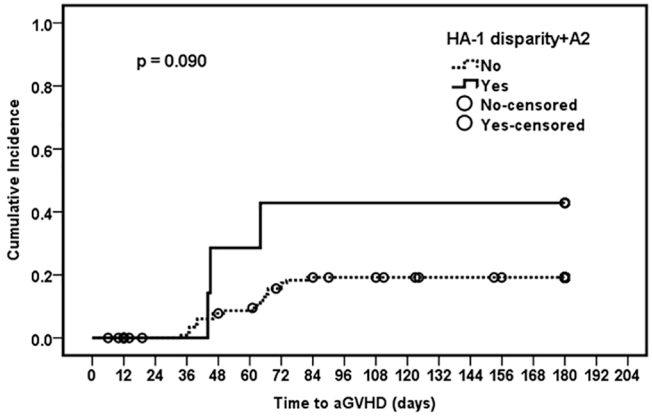

Fig. 1. Association between HA-1 disparity and cumulative incidence of aGVHD at 6 months (HA-1 disparity versus no disparity).

Table 3
Multivariate Cox-proportional hazard regression analysis of risk factors associated with aGVHD including HA-1 disparity.

Factor	p-value	HR	95% CI
Age (≥40 vs <40)	**0.030**	2.67	1.10–6.50
Gender mismatch (female donor to male recipient) vs other combinations	0.963	0.97	0.40–2.37
Conditioning regimen (Bu/Cy, Fludarabine-based vs TBI/CY)	0.233	3.39	0.45–25.40
GVHD prophylaxis (CSA, MTX vs CSA, post-CY, MMF)	**0.016**	12.40	1.61–95.44
HA-1 disparity (Disparate vs non-disparate)	**0.043**	3.71	1.04–13.26

Bu, Busulfan; TBI, total body irradiation; CSA, Cyclosporine; MTX, methotrexate; post-CY, post-cyclophosphamide; MMF, Mycophenolate mofetil; HR, hazard ratio; CI, confidence interval.
Bold p values indicate statistical significance or near significance.

genotypes in recipients and their HLA-matched identical siblings was 83/130 (63.9%) for *LMP7-Q/Q*, 41/130 (31.5%) for *LMP7-Q/K* and 6/130 (4.6%) for *LMP7-K/K*.

3.2. Association with acute and chronic graft-versus-host diseases

Acute GVHD II-IV occurred in 25/130 (19.2%) of the transplanted patients. Association between HA-1, HA-8 disparities, and *LMP7* polymorphism with aGVHD is shown in Table 2. Recipients with HA-1 disparity showed a trend toward an increased cumulative incidence of aGVHD II–IV at 6 months compared to those without disparity (p = 0.090, HR: 2.72, 95%CI = 0.81–9.11) as shown in Fig. 1. In multivariate analysis adjusted for other risk factors, HA-1 disparity was an independent risk factor for developing aGVHD II–IV (p = 0.043, HR: 3.71, 95%CI = 1.04–13.26). The use of cyclosporine (CSA) and methotrexate (MTX) as GVHD prophylaxis resulted in a significantly higher incidence of aGVHD in comparison to post-transplant cyclophosphamide (post-CY) and mycophenolate mofetil (MMF) based regimens (p = 0.016, HR: 12.4, 95%CI:1.61–95.44) as shown in the Table 3. The risk of aGVHD was also increased with age (Table 3).

Recipients with HA-8 disparity did not show a significant association with the incidence of aGVHD II–IV compared to those without disparity (p = 0.309, HR: 2.07, 95%CI = 0.49–8.82). Patients with *LPM7-Q/Q* genotype showed a significant association with increased cumulative incidence of aGVHD II–IV compared to those with *LMP7-Q/K* or *K/K* genotypes (p = 0.043, HR: 2.63, 95%CI = 0.99–7.03) as shown in Table 2 and Fig. 2. In multivariate analysis, *LMP7-Q/Q* showed only a trend toward an increased incidence of aGVHD (p = 0.087, HR: 2.36, 95%CI = 0.88–6.31) (Table 4). All HA-1 and HA-8 disparate recipients

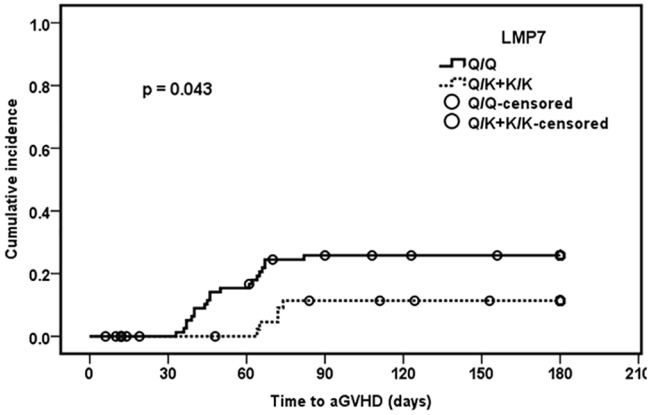

Fig. 2. Association between *LMP7* polymorphism and cumulative incidence of aGVHD at 6 months (*LMP7-Q/Q* versus *LMP7-Q/K* and *K/K* genotype).

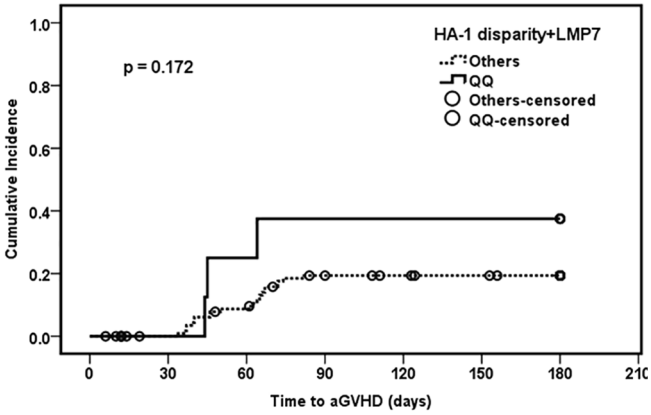

Fig. 4. Association of the combined effect of HA-1 disparity (HLA-A*02-unrestricted) and *LMP7* polymorphism with cumulative incidence of aGVHD at 6 months (HA-1 disparity combined with *LMP7- Q/Q* genotype versus others).

Table 4
Multivariate Cox-proportional hazard regression analysis of risk factors associated with aGVHD including *LMP7* polymorphism.

Factor	p-value	HR	95% CI
Age (≥40 vs <40)	**0.044**	2.41	1.02–5.69
Gender mismatch (female donor to male recipient) vs other combinations	0.814	1.11	0.46–2.68
Conditioning regimen (Bu/Cy, Fludarabine-based vs TBI/CY)	0.254	3.21	0.43–23.89
GVHD prophylaxis (CSA, MTX vs CSA, post-CY, MMF)	**0.019**	11.70	1.48–92.07
LMP7 *(Q/Q vs Q/K and K/K)*	**0.087**	2.36	0.88–6.31

Bu, Busulfan; TBI, total body irradiation; CSA, Cyclosporine; MTX, methotrexate; post-CY, post-cyclophosphamide; MMF, Mycophenolate mofetil; HR, hazard ratio; CI, confidence interval.

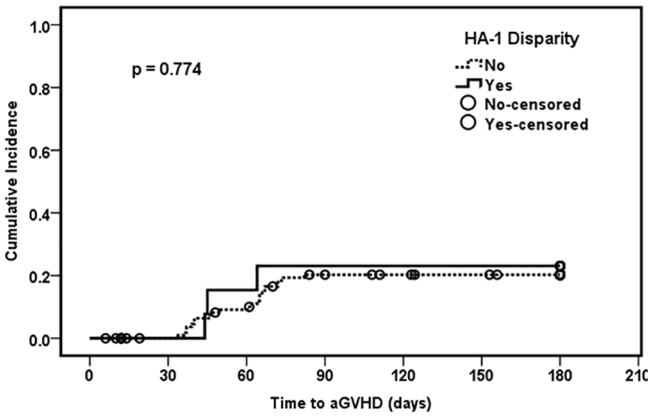

Fig. 3. Association between HA-1 disparity (HLA-A*02-unrestricted) and cumulative incidence of aGVHD at 6 months (HA-1 disparity-HLA-A*02-unrestricted versus no disparity).

who developed aGVHD had the *LMP7-Q/Q* genotype. The 2 patients with combined HA-1 and HA-8 disparities who did not develop aGVHD had the *LMP7-K* allele. HA-1 disparity (HLA-A*02-unrestricted) did not show a significant association with aGVHD (p = 0.774, HR:1.91, 95%CI = 0.35–3.98), while HA-1 disparity combined with *LMP7-Q/Q* genotype showed a near significant association with aGVHD (p = 0.172, HR:2.26, 95%CI = 0.67–7.56) compared to HA-1 disparity combined with *LMP7-Q/K* or *K/K* genotypes (p = 0.470) (Table 2, Figs. 3 and 4).

Extensive chronic GVHD (cGVHD) occurred in 15/130 (11.5%) patients. The cumulative incidence of extensive cGVHD at 2 years was not significantly different in recipients with HA-1 or HA-8 disparity compared to those without disparity (p = 0.347, p = 0.338, respectively) (Table 2). Patients with the *LMP7- Q/Q* genotype did not show a significant difference in the incidence of cGVHD compared to those with the *LMP7-Q/K* and *K/K* genotypes (p = 0.331). A combined analysis of *LMP7* polymorphism and HA-1 or HA-8 disparity also did not show an association with cGVHD (Table 2).

3.3. Association with relapse-free survival, overall survival and transplant- related mortality

The median follow-up period was 19.4 (0.2–49.6) months. In case all patients were censored, the HR could not be calculated and was marked as not calculated (NC), and then the Kaplan-Meier method was used to calculate p-values as shown in table 2. The rate of relapse-free survival (RFS) at 2 years was 71.7%. HA-1 disparity did not show a significant association with RFS (84.6% in the disparate patients versus 70.2% in the non-disparate group, p = 0.258) or HA-8 disparity (79.7% in the disparate patients versus 70.3% in the non-disparate group, p = 0.433) as shown in Table 2. *LMP7* polymorphism did not show a significant association with RFS (68.5% in patients with *LMP7-Q/Q* versus 75.2% in patients with *LMP7-Q/K* or *K/K* genotypes (p = 0.512) (Table 2). Recipients with combined HLA-A*02-unrestricted HA-1or HA-8 disparity combined with *LMP-* Q/Q or *LMP7-K/Q* and *K/K* genotypes did not show a significant association with RFS compared with others as shown in Table 2. The rate of overall survival (OS) at 2 years was 73.1% and was not influenced by HA-1 disparity (100% in the disparate patients versus 73% in the non-disparate group; p = 0.283), HA-8 disparity (100% in the disparate patients versus 73.8% in the non-disparate group; p = 0.240) or *LMP7* polymorphism (70.3% in patients with *LMP7-Q/Q* versus 76.4% in those with *LMP7-Q/K* and *K/K* genotypes (p = 0.685) (Table 2). Recipients with combined HLA-A*02-unrestricted HA-1or HA-8 disparity combined with *LMP7-Q/Q* or *LMP7-Q/K* and *K/K* genotypes did not show a significant association with OS compared with others as shown in Table 2. The cumulative incidence of transplant-related mortality (TRM) was 20.1%. We did not detect a statistically significant association between TRM and HA-1 disparity (none in the disparate patients versus 21.3% in the non-disparate group, p = 0.229), HA-8 (none in the disparate patients versus 21.1% in the non-disparate group, p = 0.247) or with *LMP7* polymorphism (20.9% in patients with *LMP7-Q/Q* genotype versus 18.6% in patients with *LMP7- Q/K* or *K/K* genotype, p = 0.954) (Table 2).

4. Discussion

Discrepancies have been reported in the association of disparities in the mHags between recipients and donors with transplantation outcome. The differential expression of the polymorphic mHags in the context of HLA molecules determines their disparity and can be modified by the immunoproteasomes that regulate their processing and presentation. We investigated the impact of *LMP7* polymorphism on transplantation outcome, and examined its potential modifying effect on the hematopoeitic-restricted HA-1 and the broadly expressed HA-8 mHags in matched related HSCT.

In our cohort, HA-1 disparity was an independent risk factor for the development of aGVHD II–IV ($p = 0.043$, HR: 3.71, 95%CI = 1.04–13.26). Previous reports showed discrepancies in the association of HA-1 with aGVHD. A similar association with increased risk of aGVHD has been observed in Tunisian, Spanish, French, Swiss and American [31–35]. Despite the restricted expression of HA-1 in hematopoietic cells, the association with aGVHD has been attributed to its expression on the residual recipient antigen presenting cells in the aGVHD target tissues after AHSCT [22,32]. In HLA-A*02-unrestricted analysis, we could not find a significant association between the HA-1 disparity and aGVHD, in contrast to the data reported by Mutis et al. [35] in vitro. Alternatively, Spierings et al. [36] did not find a significant association between the HA-1 disparity and aGVHD in their multicenter study that included different ethnic groups. This was consistent with previous studies by Lin et al. [37] among Americans, Katageri et al. [38] in Japanese and Mutis et al. [39] in patients with CML from 20 European centers. The HA-8 disparity did not show a significant association with aGVHD in our cohort. The HA-8 disparity has been studied by fewer groups, Turpeinen et al. [40] did not reveal a significant association with aGVHD in Finnish, whereas Spierings et al. [36], reported an increased risk with HA-8 disparity in line with Akatsuka et al. [41] in Caucasians and Pérez-García et al. [42] in severe aGVHD in Spanish.

In our study, *LMP7-Q/Q* genotype showed a trend toward an increased risk of developing aGVHD II- IV compared to the *LMP7-Q/K* and *K/K* genotypes in multivariate analysis adjusted for other risk factors ($p = 0.087$, HR:2.36, 95%CI = 0.88–6.31). Interestingly, in patients with HA-1 or HA-8 disparity, aGVHD developed exclusively in those with *LMP7-Q/Q* genotype. Moreover, in the combined analysis of mHags disparity and *LMP7* polymorphism, HA-1-HLA-A*02-unrestricted disparity, showed a trend toward an increased risk of aGVHD when combined with *LMP7-Q/Q* genotype compared to *LMP7-K/Q* and *K/K* genotypes ($p = 0.172$ and $p = 0.470$, respectively). We also observed that despite the reported increased risk of development of GVHD with an increasing number of disparate mHags [21–43], patients with combined HA-1 and HA-8 disparities who did not develop aGVHD had the *LMP7-K* allele. The suggested association between *LMP7* polymorphism and aGVHD is probably related to its role in the processing and presentation of peptides. Nicholls et al. [22] had elaborated on the possible role of the inflammation-induced immunoproteasome associated with the conditioning regimen in mediating the HA-1-specific- immune response post ASCT. Lipopolysaccharide (LPS) that leaks from the gastrointestinal tract into the systemic circulation following tissue damage plays an important role in the initiation of aGVHD [44]. A study with cells derived from mouse model confirmed that overexpression of the LMP7 in antigen-presenting cells as a result of LPS exposure and LMP7 expression in peripheral target cells contributes to CD8+ *T*-cell autoreactivity. This result indicates a different role for peptides derived from the immunoproteasome compared to those generated by the constitutive proteasome [45].

The association between LMP7 and aGVHD may also be linked to its role in promoting T cell differentiation into Th1 and Th17 and production of proinflammatory cytokines namely TNF, IL-6, IL-17 and IL-23 [16,46]. This goes in line with the reported association between the *LMP7* polymorphism and *T*-cell mediated autoimmune and inflammatory diseases, including juvenile rheumatoid arthritis and colitis [47,48]. Alternatively, Kang et al. [49], observed that the level of expression of MHC class I on the cell surface was associated with a defect in the *LMP7* gene rather than *LMP2*, transporter associated with antigen processing (*TAP1*, *TAP2*), or *HLA* genes. *LMP7-K* allele may reduce the formation of immunoproteasome and hence peptide processing and presentation [25]. The potential modifying effect of LMP7 on the impact of HA-1 disparity rather than HA-8 is probably related to the mechanism of immunogenicity, which differs between the two peptides.

Previous studies have suggested the use of proteasome and immunoproteasome inhibitors as a potential therapeutic modality for GVHD. The proteasome inhibitor bortezomib has been found to inhibit dendritic cells in addition to its immunomodulatory effects on alloreactive T cells and has been used as a GVHD prophylaxis [50,51]. However, delayed use of bortezomib posttransplantation has been associated with the aggravation of GVHD [52]. Other regimens combining posttransplant cyclophosphamide with the proteasome inhibitors bortezomib and ixazomib have been suggested for GVHD prophylaxis and have shown promising results in human and animal models [53,54].

The selective LMP7 inhibitor ONX 0914 (epoxyketone inhibitor) improved GVHD in mHag disparate MCH matched transplantation in murine. Data suggested its role in regulating the allogeneic response mainly by reducing the presentation of mHag to cytotoxic CD8+ T cells and the production of cytokines [24]. Selective inhibition of LMP7 alters the differentiation of Th1 and Th17 cells and the secretion of proinflammatory cytokines, while promoting Treg cells and has shown a beneficial effect in autoimmune and inflammatory diseases [11,16,17,55]. ONX 0914 has also been shown to suppress LPS-induced secretion of proinflammatory cytokines using human and mouse-derived cells [55].In a mixed lymphocyte reaction (MLR) model using human cells, ONX-0914 was superior to the proteasome inhibitor CEP-18770 in suppressing cellular immunity. Since the MLR mainly represents a direct allorecognition model, this result may indicate a quantitative effect of the immunoproteasome on antigen processing and subsequent presentation with MHC molecules on the cell surface [56]. Selective LMP7 inhibition is also expected to be associated with less toxicity than proteasome inhibition because of its selective expression in immune cells and under inflammatory conditions [55,56].

In our cohort, the HA-1 disparity and the *LMP7-Q/Q* genotype did not show a significant association with cGVHD despite their association with aGVHD. The lack of association between HA-1 disparity and cGVHD is consistent with previous studies [32,37–39,41,42]. We also did not report a significant association between HA-8 disparity and cGVHD in contrast to Turpeinen et al. [40] who found a significant association with cGVHD in the absence of aGVHD. The combined analysis of the HA-1 or H-8 disparity with the *LMP7* polymorphism also did not show an association with cGVHD. The lack of association between the *LMP7* polymorphism and cGVHD can be attributed to the difference in immunobiology of cGVHD, which primarily involves Th2 cytokines rather than Th1.

No association could be detected between the HA-1 disparity and RFS or OS in accordance with previous reports by Gallardo et al. and Lin et al. [32,37]. On the other hand, Mutis at al., [39] found a reduced risk of relapse in HA-1 disparate patients exclusively in those with aGVHD. This association has been translated into prolonged RFS and OS. In our cohort, the HA-8 disparity was also not associated with RFS or OS. A similar observation has been reported by Akatsuka et al. [41]. Contrary to our finding, the HA-8 disparity was associated with a reduced risk of relapse exclusively in those with aGVHD as reported by Spierings et al. [36], and resulted in prolonged RFS and OS. In another study, the HA-8 disparity showed an association with reduced RFS and worse OS [42]. In our study, the *LMP7* polymorphism was not associated with RFS or OS. The combined analysis of the HA-1 or H-8 disparity with the *LMP7* polymorphism also did not show an association with RFS or OS. The reported association between the *LMP7* polymorphism and aGVHD rather than the GVL effect may be related to its role in promoting the differentiation of T cells into Th1 and Th17 and the production of

proinflammatory cytokines [57]. Finally, we also did not report any association between the HA-1 and HA-8 disparities and TRM in agreement with other studies [37,41,42], nor between the *LMP7* polymorphism and TRM.

The low frequency of HA-1 and HA-8 disparities along with the small number of events in disparate patients and HLA presentation restrictions has limited the power of the study to detect statistically significant associations. Therefore, the possible significant association between HA-1 and HA-8 disparities and other transplantation outcomes in the presence or absence of the *LMP7* effect cannot be excluded and would merit further studies in larger patient cohorts.

In conclusion, we reported an association between HA-1 disparity and an increased risk of developing aGVHD. To the best of our knowledge, we have suggested for the first time an association between the *LMP7* polymorphism and the risk of developing aGVHD. We have also highlighted the possible modifying effect of the *LMP7* polymorphism on the presentation and the impact of mHags on the transplantation outcome that must be proven in a large cohort. Our suggestion paves the way for the study of variation in other genes involved in the processing and presentation of different mHags. This "research" may help existing comprehensive computational analysis models predict alloreactivity to mHags.

CRediT authorship contribution statement

Ghada I. Mossallam: Conceptualization, Methodology, Investigation, Writing - review & editing. **Raafat Abdel Fattah:** Data curation. **Mahmoud Bokhary:** Data curation. **Manar Moneer:** Formal analysis. **Hossam K. Mahmoud:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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