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ORIGINAL PAPER

Transplantation and Cellular Therapy

Indirect presentation of mismatched human leukocyte antigen-B associates with outcomes of cord blood transplantation

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Summary

Cord blood transplantation (CBT) is a valuable donor source for patients without human leukocyte antigen (HLA)-matched donors. While CBT has a lower risk of graft-versus-host disease and requires less stringent histocompatibility, it is associated with a higher transplantation-related mortality (TRM) compared to other donor sources. We hypothesized that assessing the immunogenicity of mismatched HLA could reveal non-permissive mismatches contributing to increased TRM. We retrospectively analysed 1498 single-unit CBT cases from 2000 to 2018 across eight Japanese institutions, evaluating the immunogenicity of mismatched HLA using the PIRCHE algorithm to examine binding affinities of HLA-derived epitopes to donor or recipient HLA. Results indicated that Class I epitopes from mismatched recipient HLA-B were significantly associated with poor outcomes due to higher TRM and lower neutrophil engraftment, particularly when presented on matched HLA class I. Notably, epitopes from HLA-B exon 1 showed stronger prognostic significance, with HLA-B alleles carrying M-type leader peptides exhibiting higher affinity for these epitopes. Patients with a matched M-type HLA-B and Class I epitopes derived from mismatched HLA-B exon 1 had worse outcomes. These findings suggest that immunogenicity-informed donor selection could improve CBT outcomes.

KEYWORDS
cord blood transplantation, donor selection, HLA, transplantation-related mortality

INTRODUCTION

The most significant issue in cord blood transplantation (CBT) is a higher incidence of treatment-related mortality (TRM) primarily due to a greater frequency of lethal infections compared to other donor sources.[1,2] This increased frequency of infections is largely caused by delayed immune reconstitution and a high incidence of engraftment failure.[3–5] The mechanisms underlying poor engraftment include immunologic rejection due to the donor-specific human leukocyte antigen (HLA) antibodies[6–8] and haemophagocytic syndrome (HPS) induced by severe pre-engraftment immune reaction (PIR).[9–12] While the intricate mechanisms underlying PIR, including the identification of causative immunogens, remain unveiled, the rapid expansion of donor CD8+ T cells in the graft is recognized as a contributing.[13,14] To overcome the low engraftment rates, grafts with a higher number of total nucleated cells (TNC) and CD34+ cells relative to patients' body weights should be considered.[15,16] However, this approach encounters a significant limitation especially in adult cases, as securing an adequate cell count only from a single unit is sometimes difficult. Moreover, even when a sufficient cell dosage is obtained, the incidence of TRM remains comparatively higher than transplantation from haploidentical-related donors.[17,18] To improve outcomes, the causes of immunological rejections after CBT should be determined, thereby expanding the application of cord blood as a donor source.

In CBT, a less stringent level of histocompatibility is required because the risk of acute and chronic graft-versus-host disease (GVHD) is lower than the other donor sources.[19,20] The prognostic impact of the total number of allelic mismatches is controversial.[21,22] However, it is generally recommended to select a donor with up to two mismatches, where HLA-A and HLA-B are evaluated at the serological split level and HLA-DRB1 at the high-resolution level.[23,24] Moreover, all HLA mismatches are generally counted equally as one mismatch, regardless of their composition. However, each pair of mismatches could possess a different immunogenicity[25] and some previous studies have highlighted the clinical significance of functionally classifying HLAs rather than their allele sequence.[26–30] From a functional perspective, T-cell immunity targeting mismatched HLA is one of the most crucial aspects of alloimmunity. T-cell responses towards mismatched HLA can be divided into two types: direct recognition and indirect recognition of mismatched HLA.[31,32] Direct recognition, where donor T cells directly recognize mismatched HLA expressed as intact proteins on recipient antigen-presenting cells (APCs), is considered one of the main mechanisms of PIR.[13,14,33] However, none of the in silico predictive models for direct recognition so far have predicted the clinical outcomes in haematopoietic stem cell transplantation (HSCT),[34,35] except for DPB1 mismatch defined by the T cell epitope model.[29] Indirect recognition, where donor T cells recognize the epitopes derived from mismatched HLAs presented by cell-surface HLA of APCs, is possibly an alternative mechanism for PIR pathogenesis, but not proven. PIRCHE[36] is a useful algorithm to estimate the impact of indirect recognition of mismatched HLAs. In the field of solid organ transplantation, the number of epitopes derived from donor-mismatched HLA presented on recipient HLA Class II has repetitively been identified as a significant predictive factor of graft rejection due to its effect on the production of the HLA antibodies[37–41] and likely on promoting T-cell-mediated rejection.[42,43] In the field of HSCT, the total number of epitopes derived from recipient-mismatched HLA presented on donor HLA has been reported as prognostic; these epitopes are associated with a higher incidence of severe acute GVHD in the bone marrow[44,45] and peripheral blood stem cell transplantation,[45–48] and lower relapse rates in paediatric patients who received CBT.[49] However, there is no report analysing the impact of T-cell epitopes derived from each locus separately because such analyses require larger numbers of patients in the study. The current study aimed to evaluate the immunogenicity of mismatched HLA in CBT and to identify factors related to outcomes of CBT.

METHODS

Data collection

We collected data from 1716 patients who received single-unit CBT between 2000 and 2018 in the Fukuoka Blood and Marrow Transplantation Group and Toranomon Hospital. Exclusion criteria were incomplete HLA-A, -B, -C and HLA-DRB1 allele data ($n=208$); ≥2 previous HSCT ($n=5$); aged over 75 or under 16 ($n=6$). Consequently, a total of 1498 patients were included in the current study (Figure S1). Data on pretransplant complications were collected to calculate the haematopoietic cell transplantation-specific comorbidity index (HCT-CI).[50] To adjust the prognostic impact of disease type and status, we also collected information on disease status at transplantation and calculated the disease risk index (DRI).[51] The current study was approved by the institutional ethics committee of Kyushu University Graduate School of Medical Sciences and Toranomon Hospital.

Definitions

Progression-free survival (PFS) was defined as the number of days from transplantation to disease progression or death

from any cause. TRM was defined as any death related to transplantation toxicity without disease progression. The day of sustained engraftment was defined as the first of three consecutive days with an absolute neutrophil count greater than 0.5×10^9/L. Acute GVHD was defined and graded using the standard criteria, and only patients who experienced engraftment were evaluated. Chronic GVHD was evaluated according to standard criteria in patients who survived for over 100 days after transplantation. We classified conditioning regimens as either full-intensity conditioning (FIC) or reduced intensity conditioning (RIC) based on previous proposals.[52,53] In the current study, conditioning regimens that included ≥8 Gy of total body irradiation in multiple fractions, intravenous busulfan at >6.4 mg/kg or melphalan at >140 mg/m^2 were classified as FIC, while all other regimens were classified as RIC. Alleles at the HLA-A, HLA-B, HLA-C and HLA-DRB1 loci were identified by high-resolution DNA typing.

Immunogenicity of mismatched HLA

Using the PIRCHE algorithm (PIRCHE AG, Berlin), the variety of mismatched HLA-derived epitopes that should be presented on HLA was predicted. The immunogenicity was quantified separately for the mismatched loci (HLA-A, HLA-B, HLA-C and HLA-DRB1) and the presenting HLA (class I or II, matched or mismatched HLA). In the analysis of graft-versus-host direction, we evaluated the binding affinities of peptides derived from recipient mismatched HLA to donor HLA using NetMHCpan[54] and NetMHCIIpan.[55] Conversely, for the host-versus-graft analysis, we assessed the binding of peptides derived from donor mismatched HLA to recipient HLA. Epitopes were classified as immunogenic if their IC_{50} values were <500 nM for class I and <1000 nM for class II (Figure 1).

Statistical analysis

The primary comparison between cases with and without Class I epitopes or HLA class II epitopes from each HLA locus was conducted across major clinical end-points, including PFS, relapse, TRM, acute and chronic GVHD and time-to-neutrophil recovery (absolute neutrophil count of ≥0.5×10^9/L). The Benjamini–Hochberg method was used for multiple comparison corrections. The probability of PFS was estimated using the Kaplan–Meier method. The cumulative incidence (CI) rates of TRM, relapse, acute grade II–IV and III–IV GVHD and neutrophil engraftment were estimated via CI function analysis, wherein the competing risks included relapse; TRM; mortality or disease progression without acute grade II–IV GVHD; mortality or disease progression without acute grade III–IV GVHD; and mortality or disease progression within 50 days after transplantation without neutrophil engraftment respectively. The univariate and multivariate models were established using the Cox proportional hazards models.

Cytometry by time of flight (CyTOF) analysis

All available cryopreserved bone marrow samples from patients who received CBT at Kyushu University Hospital were analysed ($n = 12$). The cells were stained with metal-labelled antibodies targeting 33 antigens according to the manufacturer's instructions. Data analysis was conducted by Cytobank (Beckman Coulter, CA). Details are described in the Supplementary Methods.

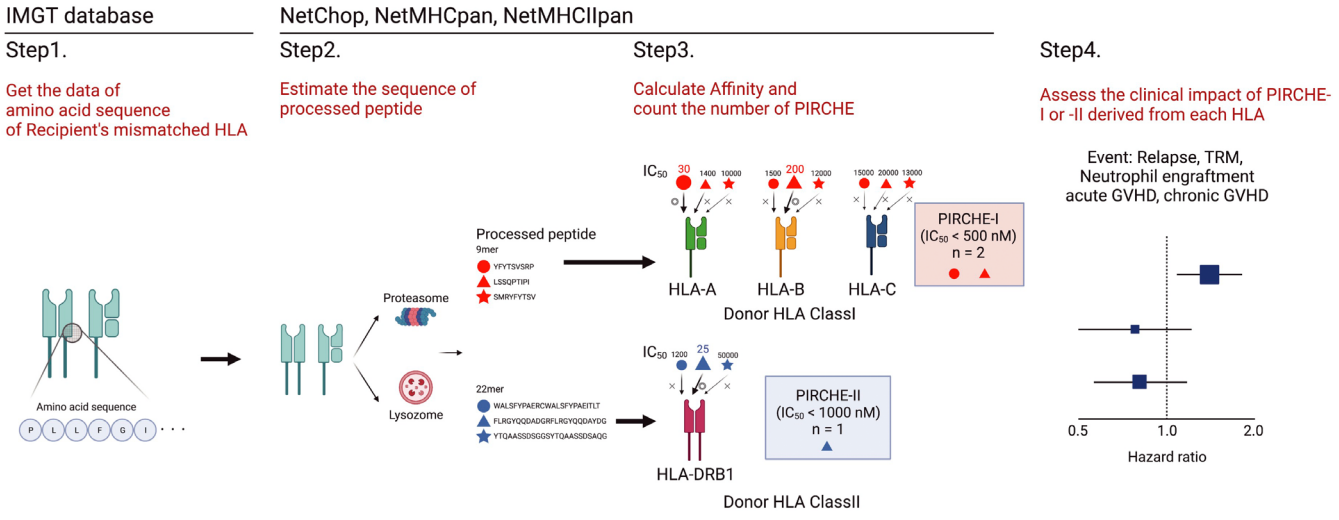

FIGURE 1 Workflow of the current study.

TABLE 1 Patient backgrounds.

Variable	Group	Value
Median observation time (years)		3.8 (0.12–12.9)
Age, years (range)		57 (16–75)
Sex (%)	Female	581 (38.8)
	Male	918 (61.2)
Disease (%)	ALL/LBL	170 (11.3)
	AML/MDS	984 (65.6)
	ATL	91 (6.1)
	Lymphoma	199 (13.3)
	MPN/MDS	40 (2.7)
	Myeloma	15 (1.0)
Disease status (%)	Untreated	258 (17.2)
	Relapse/refractory	797 (53.2)
	SD/PD	12 (0.8)
	CR	340 (22.7)
	PR	44 (2.9)
	CML AP/BP	24 (1.6)
	CML CP	16 (1.1)
	Unknown	7 (0.5)
DRI, disease risk (%)	High	509 (35.0)
	Intermediate	843 (58.0)
	Low	101 (7.0)
DRI, stage risk (%)	High	798 (57.0)
	Low	603 (43.0)
HCT CI (%)	0	553 (36.9)
	1	191 (12.7)
	2	247 (16.5)
	3	274 (18.3)
	4	101 (6.7)
	≥5	133 (8.9)
Past HSCT (%)	No	1156 (77.1)
	Yes	343 (22.9)
GVHD prophylaxis (%)	CNI + MMF	801 (53.5)
	CNI + MTX	396 (26.5)
	CNI only	300 (20.0)
Conditioning, RIC (%)		619 (41.3)
	Bu + CY	2 (0.1)
	Flu + Bu	5 (0.3)
	Flu + Mel	137 (9.1)
	Flu + Bu + CY	21 (1.4)
	Flu + Bu + Mel	80 (5.3)
	Flu + TBI	5 (0.3)
	CY + TBI	28 (1.9)
	Mel + TBI	4 (0.3)
	Flu + Bu + TBI	43 (2.9)
	Flu + Mel + TBI	288 (19.2)
	Flu + CY + TBI	2 (0.1)
	Others	4 (0.3)
Conditioning, FIC (%)		878 (58.7)
	Bu + CY	5 (0.3)
	Flu + Bu	8 (0.5)
	Flu + Bu + CY	19 (1.3)
	Flu + Bu + Mel	418 (27.9)
	Flu + TBI	5 (0.3)
	CY + TBI	166 (11.1)
	Mel + TBI	6 (0.4)
	Flu + Bu + TBI	176 (11.7)
	Flu + Mel + TBI	68 (4.5)
	Flu + CY + TBI	7 (0.5)
Total nucleated cells	$\times 10^7$ cells/kg	2.64 (0.02–24.5)
CD34+ cells	$\times 10^5$ cells/kg	0.91 (0.05–12.4)

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; AP/BP, accelerated phase/blastic phase; ATL, adult T-cell leukemia/lymphoma; Bu, busulfan; CML, chronic myeloid leukaemia; CNI, calcineurin inhibitors; CP, chronic phase; CR, complete remission; CY, cyclophosphamide; DRI, disease risk index; FIC, full intensity conditioning; Flu, fludarabine; GVHD, graft-versus-host disease; HCT-CI, haematopoietic cell transplantation-specific comorbidity index; HSCT, haematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; Mel, melphalan; MMF, mycophenolate mofetil; MPN, myeloproliferative neoplasm; MTX, methotrexate; PD, progressive disease; PR, partial remission; RIC, reduced intensity conditioning; SD, stable disease; TBI, total body irradiation.

RESULTS

Class I epitopes derived from recipient HLA-B are associated with TRM

Table 1 and Table S1 show the characteristics and HLA matching status of the patients respectively. The numbers of the immunogenic epitopes derived from each HLA locus had zero-inflated distributions (Figure S1). Therefore, univariate Cox hazard analysis between patients with or without immunogenic epitopes derived from each HLA locus was performed. Results showed that Class I epitopes derived from recipient-mismatched HLA-B (recipient HLA-B) were strongly associated with a lower PFS (hazard ratio [HR] = 1.298 [95% confidence interval, CI: 1.146–1.47], $p = 0.00079$), higher TRM rate (HR = 1.351 [95% CI: 1.138–1.604], $p = 0.0118$) and lower neutrophil engraftment rate (HR = 0.865 [95% CI: 0.776–0.963], $p = 0.03$) (Figure 2A). Bacterial infection was the leading cause of mortality in patients with recipient HLA-B-derived Class I epitopes. By contrast, the presence of recipient HLA-B-derived Class I epitopes was not associated with increased severe acute or chronic GVHD (Figure S2). In HLA-B allele-mismatched cases, patients with recipient HLA-B-derived Class I epitopes were more likely to present with higher TRM and

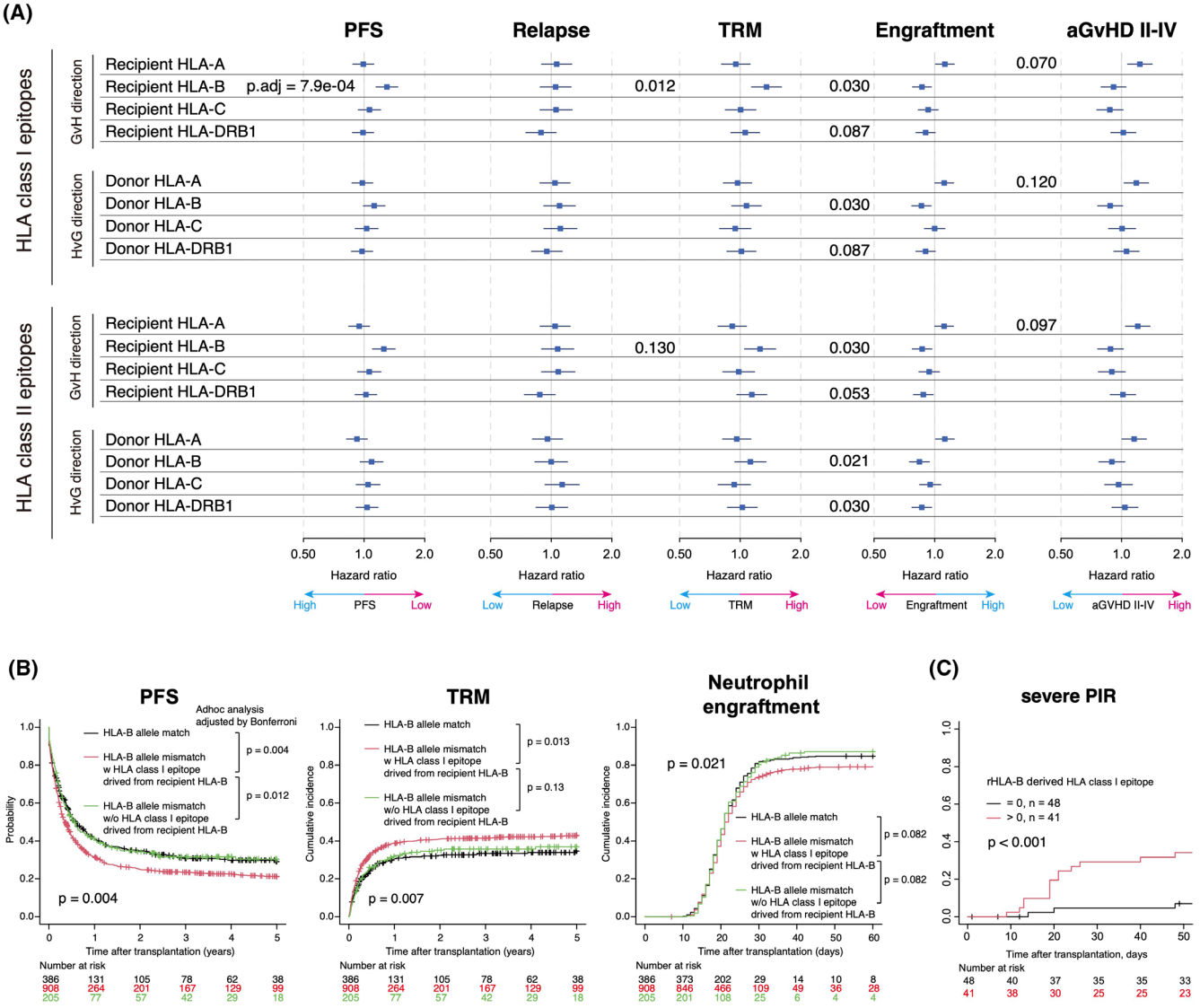

FIGURE 2 Univariate analysis of the PIRCHE derived from each HLA locus. (A) The forest plot shows the hazard ratio of each clinical event in patients with immunogenic epitopes derived from each HLA locus. The upper plots show the data of Class I epitopes and the lower plots indicate the results of HLA class II epitopes. (B) The survival and cumulative incidence curves for each event stratified by the HLA-B allele mismatch status and Class I epitopes derived from recipient HLA-B. (C) Cumulative incidence curve of severe PIR stratified by recipient HLA-B-derived Class I epitopes. The *p*-values for ad hoc analysis to compare the outcomes of each group are adjusted using the Benjamini–Hochberg method. aGVHD, acute graft-versus-host disease; PFS, progression-free survival; PIR, pre-engraftment immune reaction; TRM, transplantation-related mortality.

engraftment failure rates, and a significantly lower PFS rate (Figure 2B). Detailed analysis of 89 patients treated at Kyushu University Hospital revealed that the patients with recipient HLA-B-derived Class I epitopes had a significantly higher rates of organ dysfunction and HPS due to severe PIR (HR = 5.704 [95% CI: 1.675–19.42], $p = 0.0054$), leading to the need for treatment with a higher dose of steroid (methylprednisolone at a dose of 1 mg/kg/day) (Figure 2C). Additionally, multivariate analysis was conducted alongside key prognostic factors, including HCT-CI, DRI and CD34+ cell count. The presence of recipient HLA-B-derived Class I epitopes was significantly associated with higher TRM, lower engraftment rate and PFS, independent of these factors (Figure 3).

Importance of antigen presentation by HLA that is matched between donor and recipient

Next, we analysed the prognostic impact of HLA molecules presenting recipient HLA-B-derived epitopes (Figure 4A). In the univariate and stratified analyses, recipient HLA-B-derived Class I epitopes were associated with TRM and engraftment failure only when they were presented on donor HLA shared with the recipient (Figure 4B; Figure S3). In the multivariate analysis, only the recipient HLA-B-derived Class I epitopes presented on matched HLA were found to be associated with higher TRM, engraftment failure and a lower PFS (Figure 4C). Furthermore, in the subgroup analysis of 89 patients with detailed clinical information

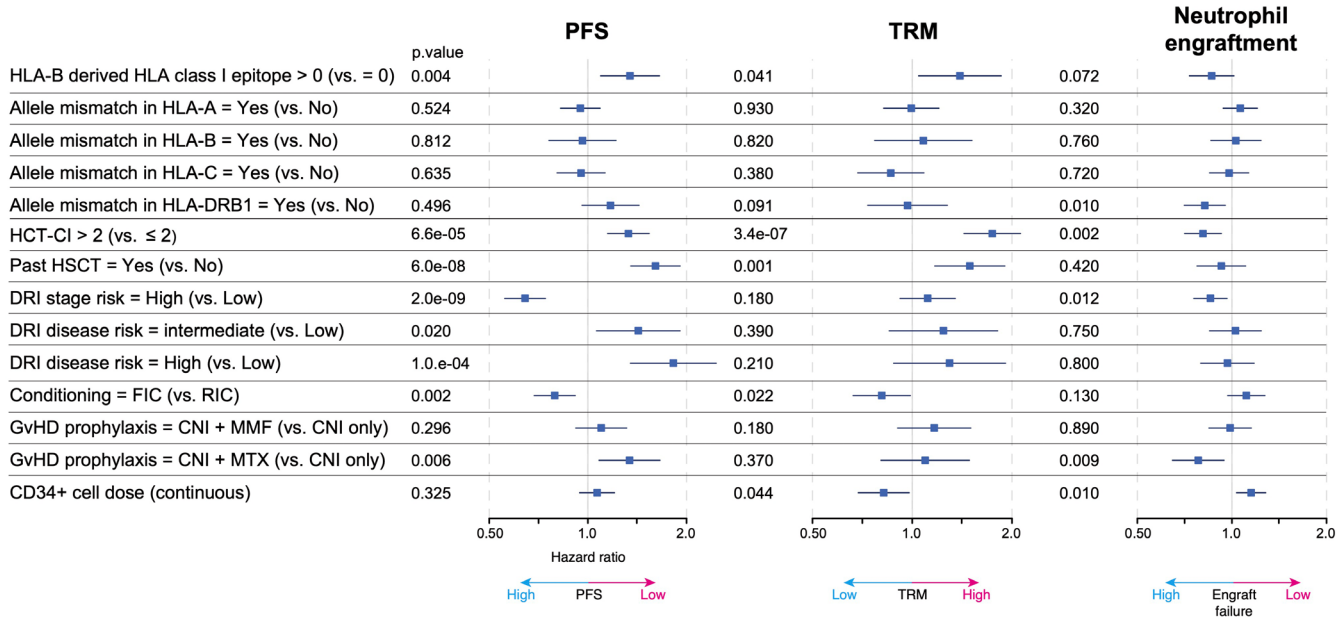

FIGURE 3 Multivariate analysis of Class I epitopes derived from HLA-B with other prognostic factors. PFS, progression-free survival; TRM, transplantation-related mortality.

treated at Kyushu University Hospital, the cumulative incidence of severe PIR was significantly increased only when recipient HLA-B-derived Class I epitopes were presented by matched HLA (Figure 4D).

Association between positions 7–15 of exon 1 and higher TRM and engraftment failure rates

We differentially analysed the prognostic impact of immunogenic epitopes derived from different exons of HLA-B. Among 478 patients with HLA-B exon 1-derived Class I epitopes, 311 (65.2%), 244 (51%), 72 (15.1%) and 174 (36.4%) patients also had Class I epitopes derived from exons 2, 3, 4 and 5, respectively, at the same time. Additionally, 66 (13.8%) patients had epitopes derived exclusively from exon 1 (Figure S4A). In the univariate analysis, Class I epitopes derived from all exons except exon 4 were associated with a low PFS. Meanwhile, those derived from exons 1 and 2 were associated with a low neutrophil engraftment rate. In the multivariate analysis, only the Class I epitopes derived from exon 1 were significantly associated with a high TRM rate and a low neutrophil engraftment rate (Figure 5A). Class I epitopes from HLA-B exon 1 were strongly associated with TRM, regardless of the presenting HLA locus presenting it as antigens. However, for PFS and neutrophil engraftment, it was significant only when they were presented on matched HLA-B (Figure S4B,C). Almost all patients with a mismatch in the leader peptide of HLA-B (TM-leader mismatch) had recipient HLA-B-derived Class I epitopes presented on matched alleles and high TRM (Figure S4D). Nearly two-thirds of patients without a TM-leader mismatch (659/967) had recipient HLA-B-derived class I epitopes on matched alleles, which were associated with higher TRM and lower neutrophil engraftment rates (Figure S4D,E). Additionally, shared M-type HLA-B is significantly more likely to present antigens derived from mismatched HLA-B than shared T-type HLA-B (M type, 45.5% vs. T type, 19.4%; Figure S4D). Especially, this difference was pronounced when we only focused on the frequency of the patients with Class I epitopes derived from HLA-B exon 1 (M type, 34.8% vs. T type, 0.32%; Figure 5B). In the current study, the patients with HLA-B mismatch and M-type-shared HLA-B had significantly lower PFS rates (Figure S4F). Moreover, they were more likely to have a lower neutrophil engraftment rate than patients with HLA-B mismatch and shared T-leader, only when they presented Class I epitopes derived from HLA-B exon 1 on M-type-shared HLA-B (Figure 5C; Figure S4F).

Figure 5D and Table S2 show the variation in the peptide sequence of HLA-B exon 1. HLA-B exon 1 is classified into T and M types based on the peptide in position 2 (rs1050458 dimorphism) and serves as a prognostic factor in HSCT.[26,56] In this cohort, 14 HLA-B alleles belonged to the M type and 44 to the T type. Among M-type HLA-B, exon 1 sequences were identical, while T-type HLA-B could be divided into five groups based on the polymorphisms between positions 7 and 15 (Figure 5D; Table S2). Of the 536 patients with Class I epitopes from HLA-B exon 1, 332 had epitopes from position 2, 400 from positions 7–15, and 196 from both (Figure 5E). In multivariate analysis, only antigens derived from positions 7–15 were strongly associated with high TRM and engraftment failure rates (Figure 5F). Furthermore, an analysis of T-type HLA-B subtypes, classified as T1 to T5 based on sequences from positions 7 to 15, showed that matching at these positions did not impact prognosis (Figure S4G). Among

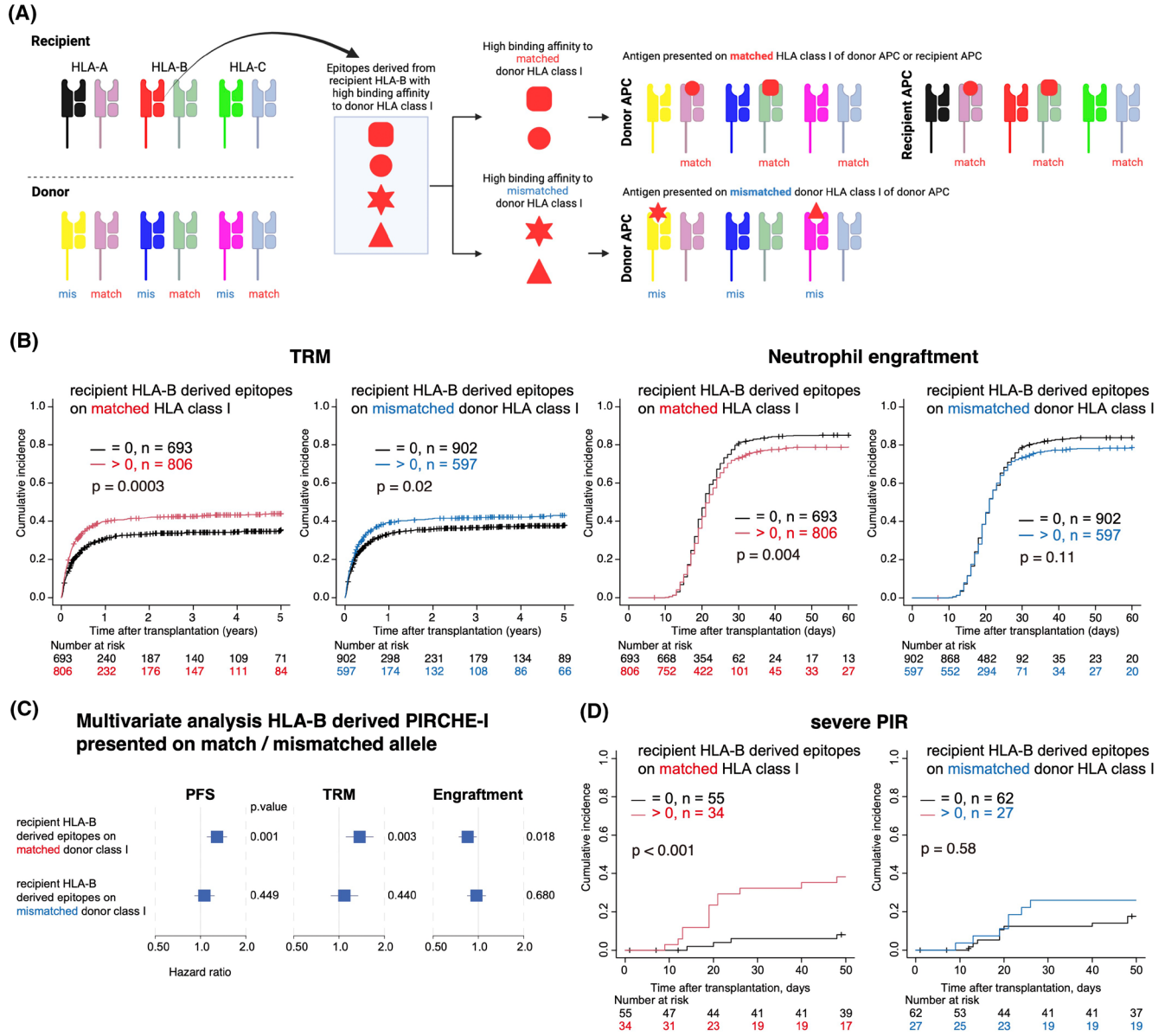

FIGURE 4 Differences in significance of epitopes derived from HLA-B by the presenting HLA (matched donor—HLA class I versus mismatched donor—HLA class I). (A) A conceptual figure for the analysis of the prognostic significance of recipient HLA-B-derived epitopes presented on mismatched donor HLA or matched HLA. (B) Cumulative incidence curve of transplantation-related mortality (TRM) and neutrophil engraftment stratified by the presence of Class I epitopes derived from recipient HLA-B presented on matched or mismatched HLA. (C) Multivariate analysis of Class I epitopes derived from recipient HLA-B presented on matched and mismatched HLA. (D) Cumulative incidence curve of severe PIR stratified by Class I epitopes derived from recipient HLA-B presented on matched or mismatched HLA for patients who can be evaluated. PFS, progression-free survival; PIR, pre-engraftment immune reaction.

patients with Class I epitopes derived from recipient HLA-B, those with epitopes derived from positions 7 to 15 of HLA-B exon 1 had a higher TRM rate (Figure 5G).

Impact of recipient HLA-B epitopes on CD8+ memory T-cell proportion

We performed immune phenotyping of bone marrow samples collected on days 24–42 post-transplantation from 12 patients by CyTOF. All samples were stained with 38 antibodies (Table S3), and immune cell phenotyping was performed using the gating strategy, as shown in Figure S5. Results showed that the proportion of CD8+ memory T cells increased with a higher number of Class I epitopes derived from recipient HLA-B values (Figure 6). Hence, Class I epitopes derived from recipient HLA-B may promote alloimmune reactions mediated by CD8+ T cells.

DISCUSSION

The higher incidence of TRM and engraftment failure remains significant problems in CBT.[3] Here, we report the

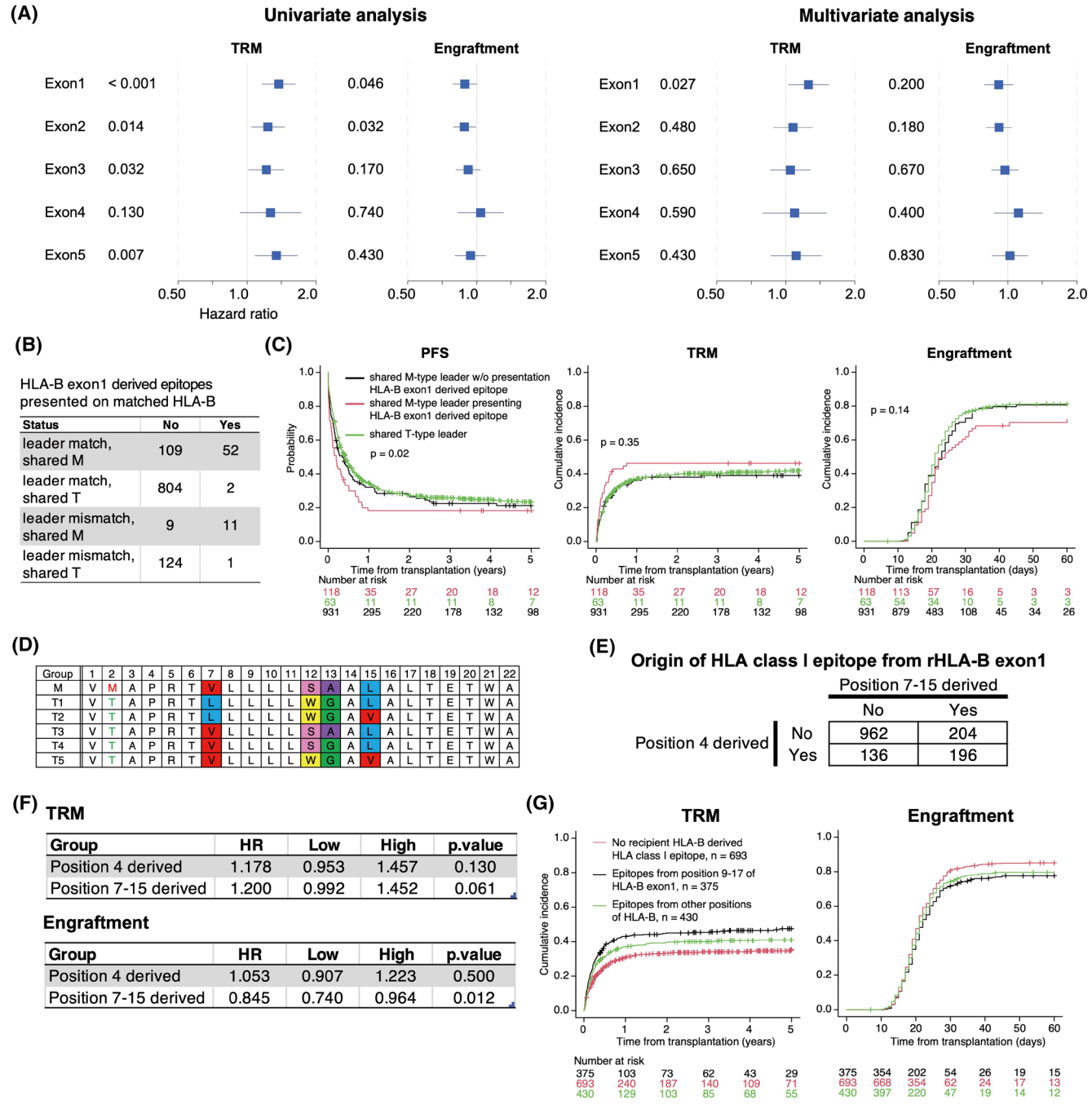

FIGURE 5 Differential prognostic significance of HLA-B-derived epitopes according to their exons of origin. (A) The forest plot of the univariate and multivariate analyses of TRM and neutrophil engraftment by the exon of origin for Class I epitopes derived from recipient HLA-B. (B) The association between HLA-B exon 1-derived Class I epitopes presented on matched HLA-B and leader-type matching status in mismatched HLA-B or the type of leader peptide (T or M) for matched HLA-B. (C) Survival curve and cumulative incidence curve of TRM and neutrophil engraftment stratified by the presence of Class I epitopes derived from recipient HLA-B presented on matched HLA-B and the type of leader peptide (T or M) for matched HLA-B. (D) Classification of the peptide sequence of exon 1 of the observed HLA-B. (E) Distribution of Class I epitopes derived from recipient HLA-B exon 1 according to the site of origin. (F) Multivariate analysis of TRM and neutrophil engraftment by Class I epitopes derived from recipient HLA-B exon 1 from different sites of origin. (G) Cumulative incidence of TRM and neutrophil engraftment for patients with Class I epitopes derived from positions 7 to 15 in exon 1 of HLA-B (black) and patients with (green) and without (red) Class I epitopes derived from the other portions of HLA-B. HR, hazard ratio; PFS, progression-free survival; TRM, transplantation-related mortality.

first in-depth evaluation of the immunogenicity of mismatched HLA in relation to engraftment using the data from a large cohort of CBT. Furthermore, epitopes derived from recipient-mismatched HLA-B were associated with decreased neutrophil engraftment and increased TRM, leading to poor prognosis. Importantly, the antigenicity of HLA-B mismatch, not HLA-B allele mismatch per se, is associated with clinical outcomes. Our findings underscore the

(A)

Correlation coefficeient between recipient HLA-B derived HLA class I epitope and frequency of immune cell subsets at day 30

Population	Correlation coefficeient
CD20+ CD38+ B cell	-0.28
B cell	-0.25
CD20- CD38+ B cell	-0.18
CD20- CD38- B cell	-0.15
CD20+ CD38- B cell	-0.05
CD4-CD8- T cell	0.11
Th1	0.13
CD4+ Tnaive	0.14
Treg	0.22
CD8+ Tnaive	0.23
CD56dim CD57+ NK cell	0.29
CD4+ Tem	0.32
CD4+ T cell	0.35
NK cell	0.39
Th2	0.40
CD56dim CD57- NK cell	0.41
CD56bright NK cell	0.46
CD8+ Teffe	0.50
T cell	0.50
CD4+ Tcm	0.50
Th1/17	0.53
Th17	0.54
CD4+ Teffe	0.58
CD8+ T cell	0.62
CD8 Tem	0.63
CD8 Tcm	0.70

(B)

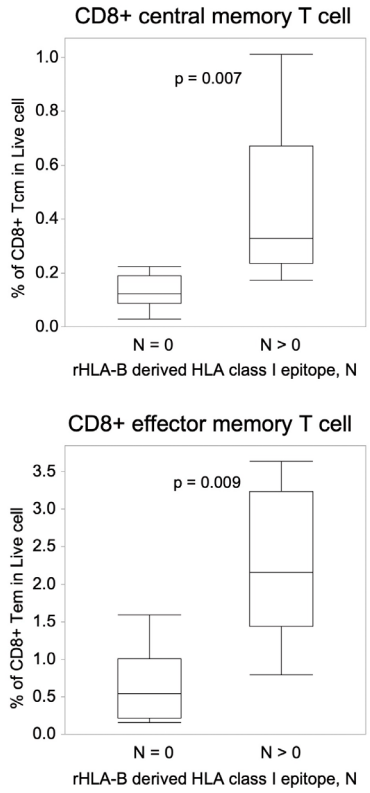

FIGURE 6 Association between HLA-B-derived epitopes and the composition of the immune cells after transplantation. (A) Correlation coefficient between Class I epitopes derived from recipient HLA-B and the percentage of immune cell fraction of bone marrow approximately 30 days after transplantation. (B) Box plot of the percentage of CD8+ central and effector memory T cells in patients with and without Class I epitopes derived from recipient HLA-B.

importance of evaluating HLA immunogenicity in donor selection in CBT. In the current donor selection strategy for CBT, TNC and CD34+ cell doses were considered the most critical factors in graft selection, and strict histocompatibility is not required.[23,24] However, the immunogenicity of mismatched HLA-B was significantly associated with prognosis independent of key prognostic factors including CD34+ cell dose.

Interestingly, the prognostic significance of HLA-derived epitopes varies depending on the presenting HLA. In this analysis, Class I epitopes from recipient HLA-B were associated with outcomes only when presented on alleles shared between donor and recipient. One of the main differences between matched and mismatched HLA is that only matched HLA is expressed on both donor and recipient APCs (Figure 4A). Consequently, recipient HLA-B-derived epitope presented on matched allele could have been presented by recipient APCs as well as those of the donors. Previous reports have shown that recipient APCs utilize matched HLA to present alloantigen and induce alloimmunity immediately after transplantation due to various reasons. First, immediately after transplantation, recipient APCs play an important role in alloimmune reaction because donor APCs will be reconstituted for approximately 2 months.[57,58] Second, immediately after transplantation, donor T cells are mainly derived from peripheral T cells included in the graft, which expanded in the inflammatory milieu in the patient's body, and they can efficiently recognize the donor HLA-restricted antigens only because they received positive selection in the fetal thymus.[13,14,59] Therefore, Class I epitopes from recipient HLA-B are presented on the recipient APCs using matched HLA to induce severe alloimmune reaction leading to engraftment failure. Our results can provide an indirect proof of a previously considered hypothesis and important insights about the mechanism of alloimmunity after allogeneic transplantation.[60] In addition, CyTOF analysis showed that T-cell expansion was enhanced in the patients with Class I epitopes derived from recipient HLA-B. It has already been reported that severe PIR is accompanied by a rapid proliferation of donor CD8+ T cells,[14] suggesting that Class I epitopes derived from recipient HLA-B may be responsible for inducing PIR.

Our data revealed the important prognostic role for epitopes originating from positions 7 to 15 of HLA-B exon 1. Previous reports have focused primarily on the prognostic impact of dimorphism (M or T) at position 2 of exon 1.[26,56] Patients with M-type leaders had an increased risk of severe acute GVHD, likely due to differential expression of leader-associated HLA-E affecting the suppressive

NKG2A-mediated response.[26] In CBT, cord blood with leader mismatch along with the shared M-type leader HLA-B lowered non-relapse mortality.[56] In this cohort, patients with HLA-B mismatch and shared M-leader were more likely to have lower neutrophil engraftment rates and higher TRM rates and had significantly lower PFS compared to those with shared T-leader. These differences may stem from patient characteristics, including lack of ATG, older age or ethnic and genetic backgrounds. Our analysis also identified that almost all leader mismatched cases had Class I epitopes from recipient HLA-B and that only M-type HLA-B can present epitopes from HLA-B exon 1 as antigen. While these findings need validation in larger cohorts due to multiple comparison limitations, they suggest a novel link between HLA-B leader peptide type and prognosis.

In conclusion, if an HLA-B mismatched donor is considered a candidate, evaluation for immunogenicity of recipient HLA-B should be performed. A major limitation of this study is that the cohort includes patients with substantial variability in clinical characteristics and conditioning regimens. Therefore, further validation is required to validate the efficacy of this approach. The findings of this study could be useful in improving the outcomes of CBT, which can cause major complications, specifically engraftment failure that is partly attributed to HLA-B-derived epitopes.

AUTHOR CONTRIBUTIONS

TS, K. Miyawaki, NU, K. Mori, MN and KK designed experiments. TS and NU collected the clinical data. TS, MN, ES, K. Miyawaki and KK analysed the data. TS, MN, ES and KK wrote the manuscript. YO, TE, YM, GY, Y. Kikushige, Y. Kunisaki, SM, KN, HI, TK, RO, TM, ST and KA reviewed the data.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The patient and transplantation data used in this study are not publicly available due to privacy and confidentiality concerns.

ETHICS APPROVAL STATEMENT

The current study was approved by the institutional ethics committee of Kyushu University Graduate School of Medical Sciences and Toranomon Hospital.

PATIENT CONSENT STATEMENT

Informed consent was obtained from all patients involved in this study, including consent for the analysis of transplantation-related information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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