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HLA genotyping in Japanese patients with multiple myeloma receiving bortezomib: An exploratory biomarker study of JCOG1105 (JCOG1105A1)

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Abstract

Bortezomib (Btz) shows robust efficacy in patients with multiple myeloma (MM); however, some patients experience suboptimal responses and show specific toxicities. Therefore, we attempted to identify specific HLA alleles associated with Btz-related toxicities and response to treatment. Eighty-two transplant-ineligible patients with newly diagnosed MM enrolled in a phase II study (JCOG1105) comparing two less intensive melphalan, prednisolone, plus Btz (MPB) regimens were subjected to HLA typing. The frequency of each allele was compared between the groups, categorized based on toxicity grades and responses to MPB therapy. Among 82 patients, the numbers of patients with severe peripheral neuropathy (PN; grade 2 or higher), skin disorders (SD; grade 2 or higher), and pneumonitis were 16 (19.5%), 15 (18.3%), and 6 (7.3%), respectively. Complete response was achieved in 10 (12.2%) patients. Although no significant HLA allele was identified by multiple comparisons, several candidates were identified. HLA-B*40:06 was more prevalent in patients with severe

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PN than in those with less severe PN (odds ratio [OR] = 6.76). HLA-B*40:06 and HLA-DRB1*12:01 were more prevalent in patients with SD than in those with less severe SD (OR = 7.47 and OR = 5.55, respectively). HLA-DRB1*08:02 clustered in the group of patients with pneumonitis (OR = 11.34). Complete response was achieved in patients carrying HLA-DQB1*03:02, HLA-DQB1*05:01, and HLA-DRB1*01:01 class II alleles. HLA genotyping could help predict Btz-induced toxicity and treatment efficacy in patients with MM, although this needs further validation.

KEYWORDS

bortezomib, HLA, Japanese, multiple myeloma, peripheral neuropathy

1 | INTRODUCTION

Recent progress in therapeutic strategies, characterized by the clinical use of three agents, proteasome inhibitors, immunomodulatory drugs, and mAbs, have substantially improved the outcome of patients with multiple myeloma (MM).[1,2] Among these agents, bortezomib (Btz) is the first proteasome inhibitor recognized as a key drug for the treatment of MM, including newly diagnosed, relapsed, and refractory cases. Although Btz shows excellent efficacy in patients with MM, some patients experience a suboptimal or no response to this agent. In addition, Btz-induced toxicities, such as Btz-induced peripheral neuropathy (BiPN), skin disorders (SD), and Btz-related pneumonitis, limit the use of Btz in some patients.[3,4] No predictive biomarkers for the efficacy or toxicity of Btz-containing treatments have been developed to date.

The melphalan, prednisolone, plus Btz (MPB) regimen was established as a standard treatment for patients who are transplant-ineligible and newly diagnosed with MM in a randomized phase III study comparing MPB with melphalan plus prednisolone (VISTA study).[5,6] In the ALCYONE study, the daratumumab and MPB regimen (D-MPB) significantly improved progression-free survival and minimal residual disease-negativity compared with the control, that is, MPB therapy alone.[7,8] In the study, D-MPB was proven to have a similar toxicity profile to MPB alone, except for the infusion reaction [9-11]; therefore, the management of the toxicities of MPB, especially those of Btz, is indispensable to maintain the dose intensity or prevent the discontinuation of D-MPB therapy for transplant-ineligible, newly diagnosed MM patients.

Previously, we reported the results of a randomized phase II study to determine the optimal dose and schedule of MPB therapy (JCOG1105) by comparing two less intensive MPB regimens for the treatment of transplant-ineligible MM cases.[12] In the study, we concluded that twice weekly dosing of Btz in the first cycle, along with a higher dose of melphalan and a higher cumulative dose of both Btz and melphalan, influenced the efficacy of the modified MPB regimen as an induction treatment. In terms of toxicities of MPB therapy, although BiPN incidence tended to be higher in the group treated twice weekly with Btz, no significant factors related to the severity of Btz-induced toxicities were identified.

Several studies have suggested that specific HLA alleles are strongly associated with idiosyncratic adverse reactions induced by specific drugs.[13,14] Therefore, we planned an ancillary study of JCOG1105 (JCOG1105A1) and attempted to identify specific HLA alleles associated with Btz-related toxicities and treatment responses to Btz-containing therapy among Japanese patients with MM.

2 | MATERIALS AND METHODS

2.1 | Study information

JCOG1105 (jRCTs031180097) was an open-label, multicenter, randomized phase II trial undertaken by the Lymphoma Study Group (LSG) of the Japan Clinical Oncology Group (JCOG). Patients who participated in JCOG1105 were randomly assigned at a 1:1 ratio to Arm A (less intensive, known as PETHEMA/GEM05 MPB, twice weekly Btz administration during cycle 1 of a 6-week cycle, followed by once weekly Btz during cycles 2-9 of a 5-week cycle) or Arm B (further less intensive MPB, once weekly Btz administration during cycles 1-9 of a 4-week cycle).[12] An ancillary study of JCOG1105 (JCOG1105A1) was planned to identify any biomarkers associated with the efficacy and toxicities of MPB therapy by analyzing peripheral blood samples collected from patients enrolled in JCOG1105. The study protocol of JCOG1105A1 was approved by the JCOG Protocol Review Committee and the respective institutional review board.

In JCOG1105, 91 patients were enrolled and randomized to Arm A (45 patients) and Arm B (46 patients) from 41 institutions of JCOG-LSG. Written informed consent to participate in the JCOG BioBank Japan Biorepository (JCOG BioBank), a Japanese biorepository project, was obtained from the patients prior to sample collection. Peripheral blood was collected from the patients.

Adverse events (AEs) were recorded and graded according to the Common Terminology Criteria for Adverse Events version 4.0.

Treatment responses were assessed according to the International Myeloma Working Group criteria.[15]

2.2 | DNA extraction and sequencing and HLA-typing from PBMCs

Peripheral blood samples were centrifuged according to the manufacturer's instructions and used to separate mononuclear cells. Separated plasma was frozen and stored at −80°C in the JCOG BioBank according to the common banking protocol in JCOG. Genomic DNA was also extracted from the PBMC sample of each patient and stored at 4°C in the JCOG BioBank. A part of stored genomic DNA (1 μg) was used for this study as the subjects of HLA genotyping using the next-generation sequencing method, targeting class I (HLA-A, -B, and -C) and class II (HLA-DPB1, -DRB1, and -DQB1) alleles. Each HLA allele was amplified with locus-specific primers using a long-range PCR method. The primers were designed to anneal to conserved regions (Doc. S1). Each 10-μL PCR mixture contained 10 ng genomic DNA, 1 unit of PrimeSTAR GXL DNA polymerase (Takara Bio), 1× PrimeSTAR GXL buffer (Mg^{2+}, 1 mmol/L), 0.2 mmol/L of each dNTP, and 0.2 μmol/L of each primer.[16] The cycling conditions were as follows: initial denaturation at 94°C for 2 minutes, followed by 30 cycles at 98°C for 10 seconds, 60°C for 15 seconds, and 68°C for 10 minutes. DNA libraries of these PCR products were prepared using the transposase-mediated library preparation method with the Nextera DNA Sample Preparation Kit or Nextera XT DNA Library Prep Kit (Illumina). The library was subjected to multiplex sequencing using a MiSeq sequencer (Illumina). To identify the HLA genotype, sequencing reads were aligned using the CLC Bio Genomics Workbench (version 8.5.1; CLC Bio).

2.3 | Association analysis and statistical methods

The frequency of each HLA allele was compared between the groups categorized based on toxicity grade and response to MPB therapy, that is, grade 0-1 vs grade 2 or higher BiPN and SD, grade 0 vs grade 1 or higher pneumonitis, and non-complete response (CR) vs. CR, using Fisher's exact test. The odds ratio (OR) and 95% confidence interval (CI) were calculated. Results with *P* values less than .05 were considered statistically significant by Fisher's exact test. In the multiple comparison test, *P* values were adjusted by Bonferroni's correction, and results with *P* values less than .00053 (.05/95) were regarded as statistically significant.

After the identification of candidate HLA allele markers involved in the toxicity or response to MPB therapy, candidate HLA alleles and several extraneous factors were applied to the univariable and multivariable analyses. In the univariable analysis, *P* values less than .05 were considered statistically significant by Fisher's exact test. In a multivariable analysis, a stepwise multivariable logistic regression using the model selection by the Akaike information criterion was adopted.

All statistical analyses were undertaken with SAS version 9.4 (SAS Institute).

3 | RESULTS

3.1 | Background information of patients

Of the 91 patients enrolled in JCOG1105, 85 patients (93%) participated in the JCOG BioBank. Among them, three patients were excluded due to ineligibility. Therefore, in JCOG1105A1, 82 samples were subjected to HLA allele typing and evaluated for their association with Btz-induced toxicities and responses to MPB therapy. The patient characteristics are shown in Table 1. The proportions of patients with severe BiPN (grade 2 and higher), severe SD (grade 2 and higher), pneumonitis, and CR were 19.5%, 18.3%, 7.3%, and 12.2%, respectively. The proportion of Btz-related toxicities observed in this study was not significantly different from that reported previously.[6,17,18]

3.2 | Identification of HLA alleles

HLA types, HLA allele frequency, and HLA alleles of all 82 cases are summarized in Tables S1 and S2. All class I (HLA-A, -B, and -C) and three class II (HLA-DPB1, -DQB1, and -DRB1) types were identified in all 82 cases. Due to insufficient amplification of the PCR products, HLA-DPB1 and HLA-DRB1 were not detected in three cases and one case, respectively.

In total, 52 types of class I alleles (HLA-A, 14 types; HLA-B, 25 types; and HLA-C, 13 types) and 43 types of class II alleles (HLA-DPB1, 11 types; HLA-DQB1, 12 types; and HLA-DRB1, 20 types) were identified. No biased distribution was observed for most HLA alleles from the 82 cases, except for six alleles, compared to the publicly available data on HLA allele frequency in the Japanese population from the HLA Laboratory (http://hla.or.jp/med/frequency_search/en/allele/; data not shown). Pearson's χ^2 test was used to determine the deviation from the normal population.

3.3 | HLA alleles associated with severity of toxicities or responses to MPB therapy

Although no significant HLA alleles were detected by multiple comparisons, several candidates associated with the response or toxicity of MPB therapy were identified from the HLA alleles (Tables 2-4).

As shown in Table 2, carriers of HLA-B*40:06 were more prevalent in the group of patients who developed severe BiPN than in the group of patients who did not (OR = 6.76, *P* = .025). Conversely, HLA-DPB1*05:01 was less prevalent in the group of patients who developed severe BiPN (OR = 0.17, *P* = .011). HLA-B*40:06 and -DR*12:01 were more prevalent in the group that showed severe SD than in the group that did not (OR = 7.47, *P* = .019 and OR = 5.55,

TABLE 1 Baseline characteristics of 82 Japanese patients with multiple myeloma treated with bortezomib enrolled in JCOG1105A1

Number of patients	n = 82 (100%)
Sex, n (%)	
Male	48 (58.5)
Female	34 (41.5)
Age, years	
Median	72
Range	65-79
M protein, n (%)	
IgG	53 (64.6)
IgA	21 (25.6)
IgM	0 (0.0)
IgD	1 (1.2)
BJP	7 (8.6)
ISS stage, n (%)	
I	27 (32.9)
II	40 (48.8)
III	15 (18.3)
Translocation (quantitative PCR and FISH)	
CCND1 positive	27
FGFR3 positive	8
cMAF positive	2
Not expressed	23
Not evaluated	23
G-banded karyotype, n (%)	
Normal	62 (75.6)
Abnormal	20 (24.4)
Comorbidity, n (%)	
Peripheral neuropathy	5 (6.1)
Pathologic fractures	30 (36.6)
Bone pain	33 (40.2)
Diabetes	10 (12.2)
Hypertension	32 (39.0)
Treatment schedule, n (%)	
Arm A	41 (50.0)
Arm B	41 (50.0)
Bortezomib administration, n (%)	
Intravenous injection	0 (0)
Subcutaneous injection	82 (100)
BiPN, n (%)	
Grade 0-1	66 (80.5)
Grade 2 and over	16 (19.5)
Skin disorders, n (%)	
Grade 0-1	67 (81.7)
Grade 2 and over	15 (18.3)
Pneumonitis, n (%)	
No	76 (92.7)
Yes	6 (7.3)
Best response to treatment, n (%)	
CR	10 (12.2)
Non-CR	66 (80.5)
NE	6 (7.3)

Abbreviations: BiPN, bortezomib-induced peripheral neuropathy; CR, complete response; ISS, International Staging System; NE, not evaluable.

P = .034, respectively). HLA-DRB1*08:02 was abundant in the group that developed pneumonitis (OR = 11.34, *P* = .041).

HLA-B*40:06 was commonly observed in two groups, that is, severe BiPN and severe SD, whereas other HLA alleles were not commonly observed in these groups. Among seven patients carrying HLA-B*40:06, four patients had both severe BiPN and SD simultaneously, and the other three patients had neither severe BiPN nor SD as AEs of MPB therapy (Table 3).

With regard to the response to MPB therapy (Table 4), the frequency of the three class II alleles (DQB1*03:02, DQB1*05:01, and DRB1*01:01) was higher in the CR group than in the other groups with AEs (OR = 5.43, *P* = .033; OR = 4.69, *P* = .046; and OR = 4.69, *P* = .046, respectively). Two HLA class II alleles, DQB1*05:01 and DRB1*01:01, were considered to be involved in linkage disequilibrium (LD), which is the nonrandom association of alleles at different loci. In the current study, of all 82 cases examined, 13 cases expressed both DQB1*05:01 and DRB1*01:01. Therefore, 13 overlapping cases were identified. DQB1*05:01 and DRB1*01:01, considered to be in LD, would be a part of a haplotype. These LD cases did not result in misleading information or overestimation in the identification of HLA allele candidates in our association analysis.

All seven HLA alleles identified as being associated with any toxicity or response to MPB therapy showed no biased distribution among the 82 cases when compared to the publicly available data of HLA allele frequency in the Japanese population.

3.4 | Multivariable analysis including HLA alleles and background factors in the severity of BiPN

Because the carriers of HLA-B*40:06 showed a high odds ratio (6.76) for the risk of developing severe BiPN, univariable and multivariable analyses of HLA-B*40:06, including several background factors, were carried out. As shown in Table 5, three factors, HLA-B*40:06, female sex, and Arm A (treatment course), based on a *P* value of less than .05 were significantly associated with the risk of developing severe BiPN. In the multivariable analysis, four factors, HLA-B*40:06, female sex, Arm A (treatment course), and bone

TABLE 2 Specific HLA alleles associated with toxicities during treatment with the melphalan, prednisolone, plus bortezomib regimen in Japanese patients with multiple myeloma

A, BiPN: Case (grade 2-4), n = 16; Control (grade 0-1), n = 66										
	Allele (+)		Allele (−)				95% CI		Bonferroni-corrected 95% CI	
HLA	Case, n (%)	Control, n (%)	Case, n (%)	Control, n (%)	Odds ratio	*P* value*	Lower	Upper	Lower	Upper
B4006	4 (57.1)	3 (42.9)	12 (16.0)	63 (84.0)	6.760	.025	1.313	38.489	0.356	182.509
DP0501	3 (7.9)	38 (92.1)	13 (31.7)	28 (68.3)	0.174	.011	0.039	0.659	0.007	1.514
B, Skin disorders: Case (grade 2-4), n = 15; Control (grade 0-1), n = 67										
	Allele (+)		Allele (−)				95% CI		Bonferroni-corrected 95% CI	
HLA	Case, n (%)	Control, n (%)	Case, n (%)	Control, n (%)	Odds ratio	*P* value*	Lower	Upper	Lower	Upper
B4006	4 (57.1)	3 (42.9)	11 (14.7)	64 (85.3)	7.466	.019	1.439	42.869	0.390	203.792
DR1201	4 (50.0)	4 (50.0)	11 (14.9)	63 (85.1)	5.553	.034	1.164	26.740	0.270	116.918
C, Pneumonitis: Case (grade 1-4) n = 6, Control (grade 0) n = 76										
	Allele (+)		Allele (-)				95% CI		Bonferroni-corrected 95% CI	
HLA	Case, n (%)	Control, n (%)	Case, n (%)	Control, n (%)	Odds ratio	*P* value*	Lower	Upper	Lower	Upper
DR0802	2 (40.0)	3 (60.0)	4 (5.2)	73 (94.8)	11.341	.041	1.130	96.675	0.098	634.254

Note:: In the multiple significance tests carried out using Bonferroni's method, $P < .00053$ (≒.05/95) was considered statistically significant.

Abbreviation: BiPN, bortezomib-induced peripheral neuropathy.

*$P < .05$, significant level by Fisher's exact test.

pain, were chosen as explanatory variables using the stepwise method and were then subjected to logistic regression analysis (Table 5). As a result, all four factors were significantly associated with the risk of BiPN development, and the odds ratios of the four factors were as follows: HLA-B*40:06, 8.14; female sex, 5.56; Arm A (treatment course), 7.47; and bone pain, 0.19. Among them, the carriers of HLA-B*40:06 showed the highest odds ratio for the risk of BiPN development (Figure 1).

4 | DISCUSSION

The identification of patients with a high potential risk of Btz-induced severe toxicities is clinically important to improve the management of Btz-containing therapy for transplant-ineligible patients, such as elderly individuals or unfit/frail patients. Importantly, a better understanding of the etiology of Btz-induced toxicities could contribute to the development of a combination of this agent with other agents, such as cytotoxic agents, immunomodulatory drugs, and mAbs, for the clinical management of patients with MM. Regarding BiPN, there are several potential explanations regarding how factors, including cytokines and genes, might be associated with PN onset and worsening. In our study, although several specific HLA alleles were identified to be associated with the severity of Btz-induced toxicities, how these HLA alleles are linked to the severity of any toxicity remains unclear. A possible mechanism for the association of a specific HLA with BiPN development is hypothesized to be the alteration of inward systematic inflammatory processes, as reported previously.[19-21] According to these reports, Btz treatment could induce changes in the expression of genes and production of inflammatory cytokines in immunological cells. Therefore, it can be speculated that lymphocytes of any type with a specific HLA are susceptible to Btz-induced alteration of inflammatory processes, leading to the frequent onset or worsening of BiPN through a systematic inflammatory reaction.[20,22] However, the precise mechanisms underlying these effects should be characterized in future studies.

HLA-mediated immunity, repertoires, and levels of peptides presented by HLA class I molecules are regulated by multiple factors, including proteasomal and nonproteasomal degradation. Previous studies have shown that inhibition of the proteasome has a mixed effect on the levels and production rates of HLA peptides; some HLA peptides are increased, and others are decreased.[23] Hence, altered peptide antigen presentation on HLAs (HLA-binding peptidome) might be involved in the development of severe SD through the generation of specific T cells during exposure to proteasome inhibitors. However, to date, there are no related studies focusing on the correlation of the altered HLA peptidome with the generation of T cell-mediated adverse drug reactions (ADRs) by bortezomib treatment. In addition, no common histological features in the SD induced by Btz have been identified[24,25]and there are no reports of T cell-mediated drug reactions

TABLE 3 Association of HLA-B4006 with three toxicities observed in 82 cases of multiple myeloma treated with bortezomib

	(Case)	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22	#23	#24	#25	#26	#27	#28
BiPN	Grade 2-4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Skin disorders	Grade 2-4	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−
Pneumonitis	Grade 1-4	−	−	−	−	−	−	−	−	+	−−	−	−	−	−	−	−	+	−	−	−	−	−	-	+	+	+	+	−
HLA-B4006	n = 7		o		o	o			o																				

		#29	#30	#31	#32	#33	#34	#35	#36	#37	#38	#39	#40	#41	#42	#43	#44	#45	#46	#47	#48	#49	#50	#51	#52	#53	#54	#55	#56
BiPN	Grade 2-4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Skin disorders	Grade 2-4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pneumonitis	Grade 1-4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HLA-B4006	n = 7																												

		#57	#58	#59	#60	#61	#62	#63	#64	#65	#66	#67	#68	#69	#70	#71	#72	#73	#74	#75	#76	#77	#78	#79	#80	#81	#82
BiPN	Grade 2-4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Skin disorders	Grade 2-4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pneumonitis	Grade 1-4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HLA-B4006	n = 7		o			o													o							

TABLE 4 Specific HLA alleles associated with treatment response during the melphalan, prednisolone, plus bortezomib regimen for multiple myeloma

Case (CR) n = 10, Control (non-CR) n = 66										
	Alle (+)		Alle (−)				95% CI		Bonferroni-corrected 95% CI	
HLA	Case, n (%)	Control, n (%)	Case, n (%)	Control, n (%)	Odds ratio	*P* value*	Lower	Upper	Lower	Upper
DQ0302	4 (36.3)	7 (63.7)	6 (9.2)	59 (90.8)	5.431	.033	1.173	24.699	0.273	86.962
DQ0501	4 (33.3)	8 (66.7)	6 (9.3)	58 (90.7)	4.692	.046	1.034	21.066	0.242	70.962
DR0101	4 (33.3)	8 (66.7)	6 (9.3)	58 (90.7)	4.692	.046	1.034	21.066	0.242	70.962

Note: In the multiple significance tests carried out using Bonferroni's method, $P < .00053(\fallingdotseq .05/95)$ was considered statistically significant.

*$P < .05$, significant level by Fisher's exact test.

TABLE 5 Association of background factors with the severity of bortezomib-induced peripheral neuropathy in patients with multiple myeloma

Univariable analysis			
	Grade 2-4 (n = 16)	Grade 0-1 (n = 66)	*P* value
HLA-B4006, n (%)	4 (25.0)	3 (4.5)	.025*
Sex			
Male/female	5/11	43/23	.022*
Arm A, n (%)	13 (81.3)	28 (42.4)	.011*
Age, y			
Average (range)	72.5 (66-78)	71.9 (65-79)	.594
Comorbidity, n (%)			
Peripheral neuropathy	1 (6.3)	4 (6.1)	1.000
Pathologic fractures	4 (25.0)	26 (39.4)	.389
Bone pain	3 (18.8)	30 (45.5)	.086
Diabetes	0 (0.0)	10 (15.2)	.197
Hypertension	4 (25.0)	28 (42.4)	.259
G-banded karyotype, n (%)			
Abnormal	5 (31.3)	15 (22.7)	.522

Multivariable analysis				
	Grade 2-4 (n = 16), n (%)	Grade 0-1 (n = 66), n (%)	Odds ratio	*P* value
HLA-B4006	4 (25.0)	3 (4.5)	8.14	.040*
Female	11 (68.8)	23 (34.8)	5.56	.015*
Arm A	13 (81.3)	28 (42.4)	7.47	.011*
Bone pain	3 (18.8)	30 (45.5)	0.19	.034*

*$P < .05$: significant level by Fisher's exact test.

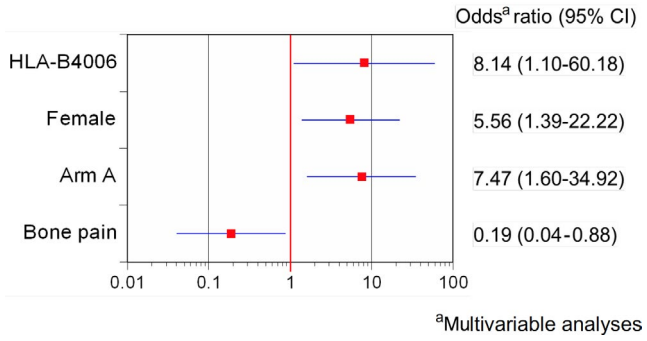

FIGURE 1 Logistic regression analysis of bortezomib-induced peripheral neuropathy in patients with multiple myeloma from four factors: HLA-B*40:06, female sex, Arm A, and bone pain. CI, confidence interval

resulting in skin lesions. Hence, the relevance of an altered HLA peptidome with immune-mediated ADRs in the Btz-containing therapy remains unclear. Further studies are required to elucidate these issues.

Among several immune-mediated ADRs, T cell-mediated ADR is associated with phenotypically distinct clinical diagnoses and can vary from a mild delayed rash to a life-threatening cutaneous, systemic, or organ disease.[26] It is also strongly linked to the presence of particular HLA risk alleles. In the case of abacavir hypersensitivity and HLA-B*57:01, abacavir binds noncovalently within the HLA-B*57:01 peptide-binding groove, which leads to a change in the binding properties of HLA-B*57:01, thereby altering the repertoire of peptides capable of binding to a specific allele. For Btz-containing therapy, there have been no reports of Btz binding to specific HLA molecules, and their peptide-binding pockets have not been identified thus far. Further analysis evaluating the possibility of T cell-mediated drug hypersensitivity to Btz, including binding to the peptide pocket in specific HLA molecules, is encouraged.

According to previous studies,[27-30] proteasome inhibition regulates the expression level of several HLA phenotypes in MM cells, and this has been attributed to natural killer (NK) cell-mediated killing of MM cells evading the killing by proteasome inhibition alone. Shi et al[30] reported that after Btz treatment, MM cells from patients showed the downregulation of HLA class I expression, and the patients were susceptible to both allogeneic and autologous NK cell-mediated killing in a Btz dose-dependent manner. Recently, Carlsten et al[27] proposed the Btz-induced loss of HLA-E expression on MM cells in an endoplasmic reticulum stress mechanism, facilitating NK cell-mediated tumor lysis. In our study, several HLA types were frequently observed in the group that achieved CR; however, the critical role of these HLA phenotypes in the efficacy of MPB therapy was unclear. We assumed that specific HLA phenotypes might be highly sensitive to Btz-induced downregulation of

HLA expression and subjected to enhanced NK cell lysis. However, we only checked the germline expression of HLA molecules, and the status of HLA molecules on MM cells was not obtained in the current study; therefore, the role of somatic HLA expression and mutation in tumor immunity is unclear. In subsequent studies, the precise mechanisms responsible for the association between specific HLA types and the efficacy of Btz-containing treatment should be elucidated considering the expression levels and mutations in MM cells.

Our study has a major limitation: no significant HLA alleles were detected when applied in the multiple comparisons by Bonferroni's correction. This could be due to the small size of the cohort in our study. Therefore, a further examination supported by a larger patient cohort is needed to evaluate the reliability and utility of several HLA alleles identified in this study to predict the toxicities and efficacy of Btz-containing treatment for the elderly or unfit/frail patients with MM.

In conclusion, specific HLA alleles might be associated with the severity of Btz-induced toxicities and treatment responses in patients with MM. HLA genotyping could serve as a potential biomarker for predicting Btz-induced toxicity and treatment response in Japanese patients with MM before the initiation of Btz-containing therapy. Although our exploratory results need to be confirmed, our findings indicate that HLA phenotypes might contribute to the identification of patients at a higher risk of Btz-specific toxicity and development of personalized treatment regimens for each patient. As a further study, we are planning to examine the utility of HLA alleles in the patients enrolled in both JCOG Biobank and JCOG1911 (jRCTs031200320, https://jrct.niph.go.jp/latest-detail/jRCTs031200320), our ongoing phase III study of daratumumab (D) versus bortezomib plus D as a maintenance therapy after D-MPB for elderly or nonelderly patients refusing transplantation with untreated MM (B-DASH study).

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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