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Serum lipidomics for exploring biomarkers of bortezomib therapy in patients with multiple myeloma

Keiko Maekawa[1,2] | Masaki Ri[3,4] | Miki Nakajima[5,6] | Akihiro Sekine[7] | Ryuzo Ueda[8] | Masahiro Tohkin[9] | Naoki Miyata[10] | Yoshiro Saito[2] | Shinsuke Iida[3]

[1]Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, Kyoto, Japan

[2]Division of Medical Safety Science, National Institute of Health Sciences, Kawasaki, Japan

[3]Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

[4]Division of Blood Transfusion, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

[5]Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan

[6]WPI Nano Life Science Institute, Kanazawa University, Kanazawa, Japan

[7]Center for Preventive Medical Science, Chiba University, Chiba, Japan

[8]Department of Tumor Immunology, Aichi Medical University School of Medicine, Nagakute, Japan

[9]Department of Regulatory Science, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan

[10]Institute of Drug Discovery Science, Nagoya City University, Nagoya, Japan

Correspondence
Yoshiro Saito, Division of Medical Safety Science, National Institute of Health Sciences, Kawasaki, Japan.
Email: yoshiro@nihs.go.jp

Shinsuke Iida, Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.
Email: iida@med.nagoya-cu.ac.jp

Abstract

Although the proteasome inhibitor bortezomib (BTZ) shows excellent efficacy in multiple myeloma (MM), a fraction of patients has a suboptimal or no response to this agent. In addition, BTZ-induced peripheral neuropathy (BiPN), a frequent side-effect of this therapy, limits its use in some patients. This study aimed to explore serum lipid biomarker candidates to predict the response to BTZ and the severity of BiPN. Fifty-nine serum samples were collected from patients with MM prior to receiving BTZ plus low-dose dexamethasone therapy. Serum levels of phospholipids, sphingolipids, neutral lipids, and polyunsaturated fatty acids and their oxidation products were measured by a comprehensive lipidomic study. Overall, 385 lipid metabolites were identified in patients' sera; lower levels of several glycerophospholipids, sphingolipids, and cholesteryl esters were associated with a poor treatment response. Metabolites related to platelet-activating factor biosynthesis and cholesterol metabolism appeared particularly relevant. Furthermore, several lysophosphatidylcholines, phosphatidylcholines, ceramides, neutral lipids, and oxidative fatty acids were significantly increased or decreased in patients with BiPN grades ranging from G0 to G3. Among these compounds, mediators reportedly inducing myelin breakdown and stimulating inflammatory responses were prominent. Although further study is necessary to validate these biomarker candidates, our results contribute to the

development of predictive biomarkers for response to BTZ treatment, or ensuing severe BiPN, in patients with MM.

1 | INTRODUCTION

The proteasome inhibitor bortezomib (BTZ) is widely used in the treatment of multiple myeloma (MM). This agent strongly inhibits proteasome activity, which results in homeostasis disruption between protein synthesis and destruction. Bortezomib treatment often yields excellent responses, both partial response (PR) and complete response (CR), not only in newly diagnosed MM, but also in patients who have relapsed or are refractory to other treatment. However, not all patients treated with this agent experience such a favorable outcome. Suboptimal or no responses to BTZ are seen in a fraction of patients, and the efficacy of the agent is unpredictable. In addition, BTZ-induced peripheral neuropathy (BiPN), an accompanying neuropathic pain and reflex suppression, is a frequent side effect of the therapy. Bortezomib-induced peripheral neuropathy is thought to occur at a certain treatment threshold (within 5 cycles, but rarely beyond) in 40%-60% of patients, of whom 15%-40% will develop severe peripheral neuropathy (grade 2 or higher).[1-5] As no effective prophylactic treatment is available, the onset of BiPN limits the use of BTZ in some patients. Therefore, identifying patients who have a potential drug resistance or high risk of developing BiPN is an important issue.

To date, a few potential biomarkers associated with the efficacy and toxicity of BTZ treatment have been proposed. A genome-wide association study identified that genetic polymorphisms of *PKNOX1* (transcription factor PBX/knotted 1 homeobox 1) and those in the immediate vicinity of *CBS* (cystathionine-β-synthetase) were associated with severe BTZ-induced toxicity.[6] Comparative proteomic profiling of sera from patients with refractory MM revealed 54 proteins that differentiated each analyzed experimental group, dividing them based on their response to therapy.[7] Zaal et al[8] aimed to identify the altered metabolic pathways that drove resistance in BTZ-resistant MM cells. As a result, they indicated that interfering with the serine metabolism could be a novel strategy to improve BTZ therapy, and identified 3-phosphoglycerate dehydrogenase, the rate-limiting enzyme of serine synthesis, as a potential biomarker for BTZ resistance.

Metabolomics is a powerful approach for exploring diagnostic biomarkers and druggable targets, because endogenous metabolites are responsible for phenotypic manifestations as a final expression of genomic information. Lipidomics, a lipid-targeting subarea of metabolomics, is an especially promising approach to biomarker discovery, because lipids are important players in a variety of complex physiological processes, executing important functions by acting as cellular membrane components, signaling mediators, energy reserve molecules, and endocrine regulators. We have already established global lipidomic analytical systems using liquid chromatography (LC)-time-of-flight mass spectrometry (TOFMS), or triple quadrupole mass spectrometry (MS/MS) to measure lipid (eg, phospholipids) metabolites in biological samples.[9] Applying this system to the study of disease metabolomics using clinical samples, the lipidomic signatures of clear cell renal cell carcinoma were obtained to reveal the metabolic changes in cancerous tissue and renal cell carcinoma pathophysiology.[10] In this study, we undertook a lipid metabolomics analysis of pretreatment sera from patients with MM who received BTZ plus low-dose dexamethasone (Bd) therapy, to identify predictive response markers for Bd therapy and BiPN severity.

2 | MATERIALS AND METHODS

2.1 | Clinical serum specimens

A total of 59 patients with MM were recruited between October 2007 and December 2014 at the Nagoya City University Hospital (Nagoya, Japan). Their venous blood was collected before Bd therapy in the morning after 14 hours fasting. Samples were centrifuged according to the manufacturer's instructions, and the serum was separated within 2 hours after blood sample collection. Patients were diagnosed according to the International Myeloma Working Group uniform response criteria[11] and divided into 6 response subcategories: CR, very good partial response (VGPR), PR, minor response (MR), stable disease (SD), and progressive disease (PD). The BiPN grades (grades 0-3) of the study subjects were diagnosed according to CTCAE version 4.0. The patient characteristics are summarized in Table 1, and their details are shown in Table S1. The Institutional Review Boards of the Nagoya City University and the National Institute of Health Sciences, Doshisha Women's College of Liberal Arts (Kyoto, Japan), approved this study. Informed consent was obtained from all patients.

2.2 | Metabolomic analysis of clinical serum samples

Detailed information regarding the extraction and measurement of lipid metabolites was described in our previous paper.[12] Briefly, lipid metabolites were extracted from 100 μL serum using the method described by Bligh and Dyer (BD),[13] with a few modifications in the presence of internal standards (IS). The lower organic layers were measured by ultraperformance liquid chromatography (UPLC)-TOFMS (Micromass LCT Premier XE;

TABLE 1 Clinical characteristics of multiple myeloma patients treated with bortezomib and low-dose dexamethasone

Characteristic	No. of patients (%)
Total number of patients	59
Age (y)	
Median	63
Range	36-85
Sex	
Male	30 (51)
Female	29 (49)
Bortezomib administration	
Intravenous injection	26 (44)
Subcutaneous injection	33 (56)
Best response to treatment[a]	
CR	1 (2)
VGPR	14 (24)
PR	21 (36)
MR	5 (8)
SD	7 12)
PD	6 (10)
NE[b]	5 (8)
Peripheral neuropathy	
Grade 0	34 (58)
Grade 1	11 (19)
Grade 2	9 (15)
Grade 3	5 (8)
Prior therapy	
Yes	36 (61)
No	23 (39)
M component	
IgG	27 (46)
IgA	14 (24)
IgD	2 (3)
BJP	16 (27)
ISS stage	
I	11 (19)
II	16 (27)
III	28 (47)
NE	4 (7)

[a]According to the International Uniform Response Criteria (Durie et al[11]).

[b]Five patients not evaluable for response were excluded.

BJP, Bence Jones protein; CR, complete response; ISS, International Staging System; MR, minor response; NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease; VGPR, very good partial response.

Waters) to analyze for glycerophospholipids (GPLs), sphingolipids (SLs), and neutral lipids (NLs). To distinguish plasmalogen and alkylacyl PL species with the same exact mass, such as 34:1

ethanolamine plasmalogen and 34:2 alkylacyl phosphatidylethanolamine (PE), a small aliquot of each BD sample was hydrolyzed using 1 N HCl as previously described and analyzed by UPLC-TOFMS. The upper aqueous layers were subjected to solid extraction to obtain polyunsaturated fatty acids (PUFAs) and their oxidative fatty acids (oxFAs), and then measured by UPLC-MS/MS using a 5500QTRAP quadrupole-linear ion trap hybrid mass spectrometer (AB Sciex) interfaced with an ACQUITY UPLC System (Waters). Structural analysis of PLs and SLs was carried out by LC-Fourier Transform Mass Spectrometry (LTQ Orbitrap XL; Thermo Fisher Scientific) as previously described.[12] The UPLC-TOFMS data were processed using the 2DICAL software (Mitsui Knowledge Industry), and the extracted ion peak heights were normalized using IS. The data for PUFAs and oxFAs from the UPLC-MS/MS were processed using MultiQuant software version 2.1 (AB Sciex), and the integrated peak area of each metabolite was normalized to IS.

2.3 | Statistical and multiple classification analyses

The lipid metabolite levels were relatively quantified as the ion count ratio of each metabolite to the internal standard. Statistical analyses were carried out using the Jonckheere-Terpstra trend test to examine ordered differences in metabolite levels for the 3 response subcategories (PD + SD, PR + MR, and CR + VGPR) or the 4 BiPN grades (G0, G1, G2, and G3). The R statistical environment (http://r-project.org/) software was used for the statistical analyses. Differences with P values <.005 were considered significant in accordance with the recent proposal on P value threshold for statistical significance by Benjamin et al.[14] The multiple testing correction was not applied, as metabolite levels are not exclusive but rather related to each other, and we focused on revealing the contributions of overall metabolic changes (such as pathways or metabolite groups) to the responses to Bd therapy in the serum of MM patients. Differences in the distribution of patient background characteristics between the analytical groups were assessed by the χ^2 test or Fisher's exact test and the Mann-Whitney U test for categorical and continuous variables, respectively. Differences with P values <.005 were considered statistically significant.[14]

3 | RESULTS

### 3.1 | Distribution of patient background characteristics

We first evaluated whether the patients' background characteristics were differently associated with the response to Bd therapy and BiPN severity. Concerning efficacy, there were no significant differences ($P < .005$) in background characteristics between responders (CR, VGPR, PR, and MR) and nonresponders (SD and PD), although both body mass index (BMI) ($P = .0296$) and serum albumin ($P = .0095$) tended to be higher in responders than in nonresponders (Table S2). For BiPN severity, the presence or absence of prior

TABLE 2 Lipid metabolites with significant *P* values in multiple myeloma patients classified as nonresponders, minor/partial responders, and good responders to treatment with bortezomib

Class	Subclass	Metabolites	Fatty acid composition of GPL and SL	*P* value	Median fold change (MR + PR vs PD + SD)	Median fold change (VGPR + CR vs PD + SD)
GPL	PC	PC(38:3) + H	PC(18:0/20:3)	.00433	1.41	2.31
GPL	PC	PC(38:5) + H	PC(16:0/22:5), PC(18:1/20:4)	.00482	1.05	1.33
GPL	Ether PE	PE(36:4e) + H	PE(18:2e/18:2)	.00003	1.22	1.75
GPL	Ether PE	PE(36:4p) + H	PE(16:0p/20:4)	.00457	1.68	2.06
GPL	Ether PE	PE(38:4p) + H	PE(18:0p/20:4)	.00312	1.70	2.12
GPL	Ether PE	PE(38:5p) + H	PE(18:1p/20:4)	.00176	1.22	1.79
GPL	Ether PE	PE(38:7e) + H	PE(18:1p/20:5) or PE(18:2e/20:5)	.00368	1.19	2.30
SL	SM	SM(39:1) + H	SM(d17:1/22:0), SM(d16:1/23:0)	.00457	1.21	1.77
NL	ChE	ChE(18:3) + NH_4	—	.00037	1.31	2.06
NL	ChE	ChE(20:3) + NH_4	—	.00166	1.40	1.71

Statistical analysis was carried out using the Jonckheere-Terpstra trend test for the 3 response subcategories: progressive disease (PD) + stable disease (SD), minor response (MR) + partial response (PR), and very good partial response (VGPR) + complete response (CR). *P* values <.005 were considered statistically significant.

ChE, cholesterolester; GPL, glycerophospholipid; NL, neutral lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SL, sphingolipid; SM, sphingomyelin.
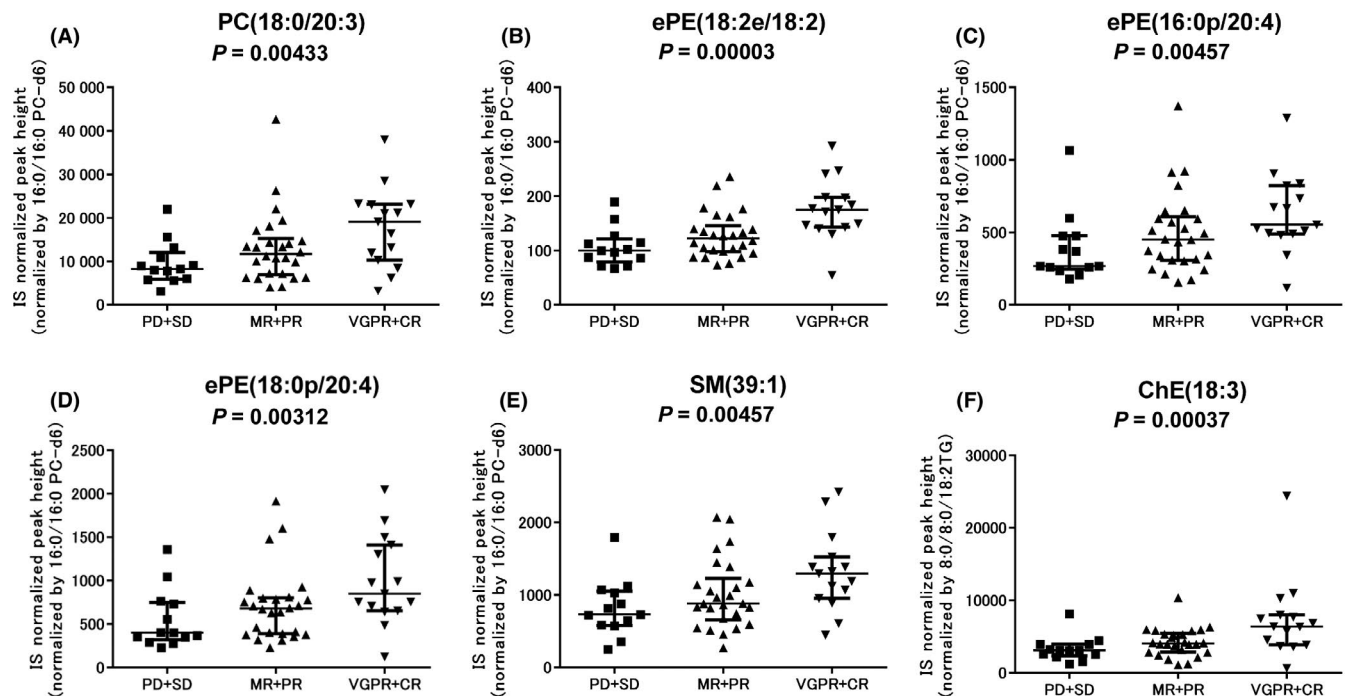
FIGURE 1 Comparison of lipid metabolite levels in multiple myeloma patients according to the response to bortezomib and low-dose dexamethasone therapy. Six representative metabolites associated with the response to bortezomib and low-dose dexamethasone therapy are shown. A, Phosphatidylcholine (PC)(18:0/20:3). B, Ether-type phosphatidylethanolamine (ePE)(18:2e/18:2). C, ePE(16:0p/20:4). D, ePE(18:0p/20:4). E, Sphingomyelin (SM)(39:1). F, Cholesterol ester (ChE)(18:3). Graphs show the medians and interquartile ranges. Statistical significance was assessed using the Jonckheere-Terpstra trend test. CR, complete response; IS, internal standard; MR, minor response; PD, progressive disease; PR, partial response; SD, stable disease; VGPR, very good partial response

TABLE 3 Lipid metabolites with significant *P* values between groups of multiple myeloma patients with 4 ordered grades (G0-G3) of peripheral neuropathy due to treatment with bortezomib and low-dose dexamethasone (BiPN)

Class	Subclass	Metabolites	Fatty acid composition of GPL	*P* value	Median fold change (G1/G0)	Median fold change (G2/G0)	Median fold change (G3/G0)
GPL	Ether LPC	LPC(16:0e) + H	—	.000534	1.34	1.57	1.29
GPL	PC	PC(36:6) + H	PC(14:0/22:6)	.003393	2.23	1.67	3.46
SL	Cer	Cer(41:2) + H	N.D.	.003228	1.56	1.43	1.24
NL	DG	DG(32:2) + NH4	—	.003228	1.48	2.19	1.28
NL	TG	TG(54:8) + NH4	—	.002378	1.74	1.20	2.35
oxFA	ALA	9-HOTrE	—	.000135	1.44	1.74	2.02
oxFA	AA	5-HETE	—	.002378	4.45	3.95	1.41
oxFA	AA	14,15-EpETrE	—	.001255	2.51	2.13	2.00
oxFA	AA	8,9-diHETrE	—	.003069	0.24	0.75	0.17
oxFA	AA	5,15-diHETE	—	.001188	2.21	2.00	1.44
oxFA	EPA	Eicosapentaenoic acid	—	.001646	1.65	1.66	1.77
oxFA	EPA	5-HEPE	—	.000045	2.50	1.92	2.89
oxFA	EPA	14,15-diHETE	—	.001063	1.33	1.35	1.65
oxFA	DHA	4-HDoHE	—	.001560	1.48	1.34	2.32

Statistical analysis was carried out using the Jonckheere-Terpstra trend test for 4 grades of BiPN (G0-G3). *P* values <.005 were considered statistically significant.

14,15-diHETE, 14,15-dihydroxyeicosatetraenoic acid; 14,15-EpETrE, 14,15-epoxyeicosatrienoic acid; 4-HDoHE, 4-hydroxyldocosahexaenoic acid; 5,15-diHETE, 5,15-dihydroxyeicosatetraenoic acid; 5-HEPE, 5-hydroxyeicosapentaenoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; 8,9-diHETrE, 8,9-dihydroxyeicosatrienoic acid; 9-HOTrE, 9-hydroxyoctadecatrienoic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; Cer, ceramide; DG, diacylglycerol; DHA, docosahexsaenoic acid; EPA, eicosapentaenoic acid; GPL, glycerophospholipid; LPC, lysophosphatidylcholine; N.D., MS2 or MS3 fragment ion was not detected in the structural analysis using LTQ Orbitrap; NL, neutral lipid; oxFA, oxidative fatty acid; PC, phosphatidylcholine; SL, sphingolipid; TG, triacylglycerol.

therapy showed a different distribution for patients with grade 2 or higher BiPN and patients with no or grade 1 BiPN (Table S3).

3.2 | Profiling of lipid metabolites measured by UPLC-TOFMS and UPLC-MS/MS

In total, 385 detected lipids were classified into 4 classes (120 GPLs, 49 SLs, 177 NLs, and 39 oxFAs), which were further divided into 20 subclasses (Table S4). The relative quantification levels of the GPLs, SLs, and NLs for each patient are shown in Table S5, and those of the FAs in Table S6.

3.3 | Lipid metabolites associated with response to Bd therapy

First, to identify the lipid metabolites associated with the response to Bd therapy, their serum levels were statistically compared among the 3 response groups (CR + VGPR, PR + MR, and SD + PD) (Table S7). Among the 346 lipid metabolites detected by LC-TOFMS, levels of 2 phosphatidylcholines (PCs), 5 ether-type PEs, 1 sphingomyelin (SM), and 2 cholesterolesters (ChEs) were increased progressively from the nonresponders (PD and SD), across the minor/partial responders (MR and PR), to the good responders (VGPR and CR) (Table 2 and Figure 1); their serum levels were 1.33 to 2.31-fold higher in the good responders than in the nonresponders. When focusing on the fatty acid compositions of GPLs showing significantly different levels, 2 PCs and 4 ether PEs contained PUFAs such as FA(20:3), FA(20:4), and FA(20:5). Regarding the 39 oxFAs (Table S8), none of the metabolites showed significantly different levels between nonresponders, minor/partial responders, and good responders.

3.4 | Lipid metabolites associated with severity of BiPN

Next, the association of lipid metabolite levels with the peripheral neuropathy grades was examined (Tables S7 and S8). When the levels of each metabolite were compared between the groups of patients with the 4 ordered BiPN grades (G0-G3), 14 metabolites showed significantly different levels (Table 3). They comprised 1 ether-type lysophosphatidylcholine (LPC), 1 PC, 1 ceramide, 1 diacylglycerol, 1 triacylglycerol, and 9 oxFAs. Their levels were increased progressively in patients with BiPN grades from G0 to G3, except for 8,9-diHETrE levels, which were decreased over the BiPN grades. The median fold change for the G3 group in comparison with the G0 group ranged from 1.24 to 3.46 for the 13 significantly increased metabolites and was 0.17 for 8,9-diHETrE. The representative metabolites with large fold changes in their levels are shown in Figure 2.
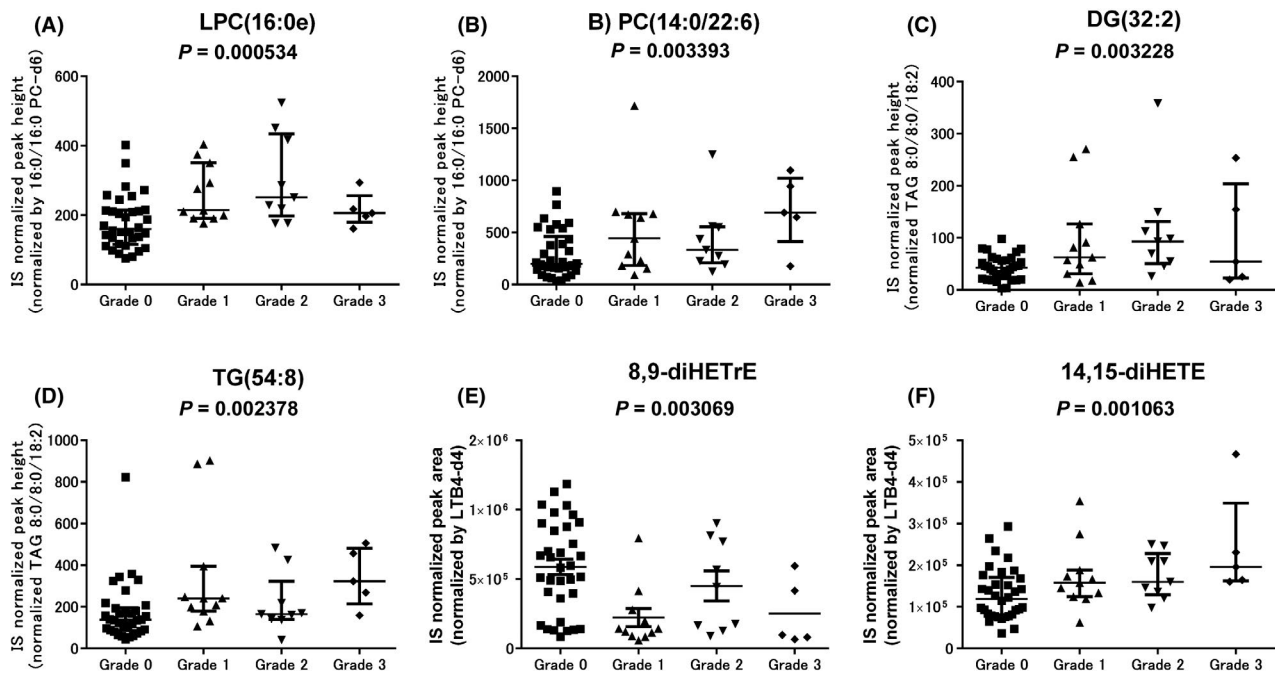
FIGURE 2 Comparison of lipid metabolite levels in multiple myeloma patients according to grades of bortezomib-induced peripheral neuropathy BiPN during treatment with bortezomib and low-dose dexamethasone. Six representative metabolites associated with BiPN severity are shown. A, Lysophosphatidylcholine (LPC)(16:0e). B, Phosphatidylcholine (PC)(14:0/22:6). C, Diacylglycerol (DG)(32:2). D, Triacylglycerol (TG)(54:8); E, Dihydroxyeicosatrienoic acid (8,9-diHETrE). F, Dihydroxyeicosatetraenoic acid (14,15-diHETE). Graphs show the medians and interquartile ranges. Statistical significance was assessed using the Jonckheere-Terpstra trend test. IS, internal standard

4 | DISCUSSION

Our study is the first comprehensive lipidomics analysis of pre-treatment sera in order to explore which biomarkers could predict an optimal response to BTZ-based chemotherapy and BiPN. The levels of 10 lipid metabolites, 2 PCs, 5 ether PEs, 1 SM, and 2 ChEs in the pretreatment sera were increased progressively from the nonresponders, across the minor/partial responders, to the good responders ($P < .005$). To identify potential confounding factors influencing lipid metabolite levels in the blood for our biomarker exploration study, we examined the association between patients' backgrounds and their response to Bd therapy. There were no differences in the total cholesterol and triglyceride levels and hemoglobin concentrations between responders and nonresponders, whereas serum albumin levels showed a trend towards decrease ($P = .0095$, not significant) in nonresponders. Serum albumin is the carrier of fatty acids in the blood and reflects the patients' nutritional status, suggesting that in nonresponders both the nutritional status was inferior, and fatty acids might not be supplied in sufficient amount for the biosynthesis of GPLs and NLs. Then, to assess the influence of the different nutritional status between responders and nonresponders on the lipid biomarker exploration for Bd response, we examined the association between serum albumin and the levels of 10 lipid biomarker candidates. There was no significant correlation ($P > .005$, Spearman rank correlation test) between the specific lipid levels and the serum albumin, with the exception of PE(38:7e) ($P = .0045$) and SM(39:1) ($P = .0014$),

indicating that at least 8 biomarker candidates are associated with the Bd response and not with the patients' nutritional status. In addition to serum albumin levels, the BMI tended to be lower in nonresponders than in responders ($P = .0296$). As the BMI has been extensively correlated with metabolite levels such as lipoprotein cholesterols and triacylglycerols,[15] we cannot rule out the possibility that our findings were confounded by the BMI. However, the levels of these 10 lipids and BMI were not significantly correlated ($P > .005$, Spearman rank correlation test). Therefore, the lower levels of both albumin and BMI in nonresponders are unlikely to be a confounding factor for the decreased levels of these lipid species, although a careful validation taking into account patients' backgrounds is needed for a few metabolites.

Several reports have indicated that MM pathophysiology is supported by a strong interaction between clonal plasma cells and the surrounding bone marrow microenvironment. Marrow fat might participate in the lipid metabolism by clearing and storing circulating triglycerides, thereby providing a localized energy reservoir for osteogenesis.[16] Ether PE species appear to be potential biomarker candidates for predicting BD responsiveness, whereas ether PC species and lyso platelet-activating factors (PAFs), including LPC(16:1e) and LPC(18:1e), are not, because they were present at similar levels between nonresponders and responders. The synthesis of ether PEs and PCs from 1-alkyl-2-acylglycerol shares a common enzyme, choline/ethanolamine phosphotransferase. Ether PCs are converted into lyso PAF by phospholipase A2 cleavage of the acetyl group at their sn-2 position, and its subsequent acetylation generates PAF.

Platelet-activating factor is an inflammatory phospholipid present in human bone marrow and reported to stimulate the Ig secretion of plasma cell lines initially derived from myeloma patients.[17] Serum ether PE levels seem to be an indicator of PAF production in the clonal plasma cells and stromal cells, which might affect Bd responsiveness.

Of the 14 ChE species detected in the patient sera, low levels of ChE(18:3) and ChE(20:3), which esterifies PUFA, might be possible biomarker candidates for predicting Bd nonresponders. Hypocholesterolemia has previously been described in MM patients, likely representing an increase in low-density lipoprotein clearance and lipid and cholesterol utilization by myeloma cells.[18] Łuczak et al[7] reported by comparative proteomic analysis that a higher abundance of apolipoprotein C1 in the pretreatment sera of patients with refractory/relapsed MM positively correlated with a better response to BTZ-containing therapy. Their results are in agreement with ours, because both apolipoprotein C1 and ChEs are major constituents of the lipoprotein particles carried in the blood, including high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein.

Higher levels of an ether-type LPC (lyso PAF), LPC(16:0e), were strongly associated with a more severe BiPN grade (P = .0005). In addition, other LPCs, such as LPC(17:1) (P = .0058), LPC(18:1e) (P = .0055), and LPC(20:5) (P = .0087), showed borderline significant association. These results implicate phospholipase A2, which hydrolyzes phospholipids to produce lysophospholipids and a free fatty acid, in BiPN.[19] Phospholipase A2 provides precursors for eicosanoid generation, when the cleaved fatty acid is arachidonic acid, or for PAF formation from ether-type PC, playing key roles in the inflammatory responses. Furthermore, LPC as a bioactive lipid, has been shown to enhance intracellular Ca^{2+} levels in many cell types, resulting in LPC-induced demyelination and axonal degeneration.[20,21] In addition, LPCs are known to cause neuropathic pain through the increase of neuronal nitric oxide synthase in the dorsal root ganglion.[22]

It was notable that differences in the levels of cytochrome P450 (P450) and/or the soluble epoxide hydrolase-mediated metabolites (14,15-epoxyeicosatrienoic acid [14,15-EpETrE], 8,9-dihydroxyeicosatrienoic acid [8,9-diHETrE], and 14,15-dihydroxyeicosatetraenoic acid [14,15-diHETE]) were associated with the BiPN grade. Interestingly, levels of 14,15-EpETrE and 14,15-diHETE were higher, whereas 8,9-diHETrE levels were lower in patients with severe BiPN. As endogenous signaling molecules, EpETrEs produce diverse effects in multiple biological systems including vasodilation, ion channel modulation, anti-inflammatory effects, and angiogenesis, which are attenuated following conversion to diHETrEs by soluble epoxide hydrolase.[23] 8,9-EpETrE, derived from arachidonic acid, is a natural agonist of the calcium channel transient receptor potential vanilloid-4 (TRPV4), and 8,9-diHETrE is a degradation product of 8,9-EpETrE.[23] Transient receptor potential vanilloid-4 has an important role through its expression in sensory neurons for the mechanotransduction of peripheral neuropathy-associated pain.[24] Furthermore, TRPV4 inhibition prevents paclitaxel-induced neurotoxicity in preclinical

models.[25] In contrast, epoxides from arachidonic acid and eicosapentaenoic acid, such as 14,15-EpETrE and 14,15-epoxy-eicosatetraenoic acid (14,15-EpETE), respectively, mediate cell signaling with anti-inflammatory, antihypertensive, and analgesic properties.[26] Although we could not detect 14,15-EpETE and 8,9-EpETrE in the patients' sera, because of their instability, high and low levels of their degradation products, 14,15-diHETE and 8,9-diHETrE, respectively, together with 14,15-EpETrE, could be plausible biomarkers of BiPN.

In conclusion, a comprehensive lipidomic study using pretreatment sera from MM patients undergoing Bd therapy revealed several biomarker candidates for predicting responses to BTZ and BiPN severity. The lipid metabolites that differentiated nonresponders from responders to Bd therapy were mainly involved in PAF biosynthesis and cholesterol metabolism. On the other hand, lysophosphatidylcholines and several oxFAs were examined as lipids associated with BiPN severity. Further studies are needed to validate these biomarker candidates together with their roles in MM pathophysiology and as Bd targets of action.

DISCLOSURE

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ORCID

Keiko Maekawa https://orcid.org/0000-0003-4857-4137
Masaki Ri https://orcid.org/0000-0002-9617-486X
Shinsuke Iida https://orcid.org/0000-0002-4951-960X

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SUPPORTING INFORMATION

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