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A Phase I/II, Open-Label, Prospective, Multicenter Study to Evaluate the Efficacy and Safety of Lower Doses of Bortezomib Plus Busulfan and Melphalan as a Conditioning Regimen in Patients with Multiple Myeloma Undergoing Autologous Peripheral Blood Stem Cell Transplantation: The KMM103 Study

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A B S T R A C T

A phase I/II trial was conducted to explore the safety and activity of the addition of bortezomib on days -6, -3, and +1 relative to the day of autologous stem cell transplantation (ASCT) to a conditioning regimen with busulfan and melphalan (BuMel; 3.2 mg/kg/day busulfan on days -5 to -3 and 140 mg/m^2/day melphalan on day -2) in patients with multiple myeloma (MM) following bortezomib-based induction chemotherapy. In phase I, doses of bortezomib (.7, 1.0, and 1.3 mg/m^2) with BuMel were administered to groups of 3 patients each. No dose-limiting toxicities were observed. The maximum tolerated dose of bortezomib was 1.3 mg/m^2/day. A subsequent cohort with 41 patients was analyzed in a phase II trial to identify safety and efficacy. The phase II trial showed a 75% response rate, including very good partial response (VGPR) or better, and a 55% rate of complete response (CR) at 3 months; For post-transplantation best response, an 83% rate of VGPR or better (68% CR) was observed. With a median follow-up of 31.4 months, the median progression-free survival (PFS) was 26.8 months. The probability of 2 year-PFS was 56.5%, and median overall survival (OS) could not calculated. Specifically, high-risk cytogenetics were associated with adverse survival outcomes compared with standard-risk cytogenetics (median PFS, 12.2 months versus 35.7 months, $P = .039$; median OS, 26.7 months versus 73.3 months; $P = .086$). With a median of 11 days to neutrophil engraftment and 10 days for platelet engraftment, no graft failure or delayed engrafting were observed. The most common grade 3 or severe nonhematologic adverse events included neutropenic fever (73.2%) and stomatitis (14.6%). Except for 3 patients with transplantation-related mortality due to sepsis, other adverse events were manageable. These findings demonstrate that bortezomib is safe and has a potential role in conditioning regimens in combination with BuMel for patients with transplantation-eligible MM.

INTRODUCTION

Single-agent, high-dose melphalan is a standard conditioning option in patients with multiple myeloma (MM) who are eligible for autologous stem cell transplantation (ASCT) [1,2]. Despite the advantages of ASCT using melphalan at a high dose of 200 mg/m^2 over the nontransplantation approach [3], the results of ASCT in MM are still unsatisfactory because this approach is not curative, and inevitable relapse remains the

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primary cause of death. Few alternatives to ASCT have been explored to improve the antimyeloma activity of classic conditioning with high-dose melphalan [4-7].

Busulfan and melphalan are myeloablative chemotherapeutic agents, and previous studies have proposed a combination of busulfan and melphalan (BuMel) as an attractive conditioning regimen for ASCT compared with high-dose melphalan [4,8]. Moreover, encouraging results from a phase III trial showed that the conditioning regimen of BuMel was safe and associated with improved PFS (65 months versus 34 months) compared with high-dose melphalan [9]. However, most patients still experienced relapse post-ASCT despite enhanced activity of BuMel conditioning. Thus, further improvements of the conditioning regimens are needed to improve the response and delay disease relapse.

Bortezomib (Velcade), a first-in-class proteasome inhibitor, has powerful antimyeloma activity, including disturbance of the cell cycle and induction of apoptosis of MM cells, alteration of the bone marrow microenvironment, and inhibition of nuclear factor κB signaling [10,11]. Bortezomib has shown efficacy in both front-line and relapsed/refractory settings and is considered a backbone therapy for MM. Based on interesting results of a phase I/II study showing that the combination of bortezomib and melphalan is synergistic, especially when bortezomib is administered after high-dose melphalan [12], a follow-up phase III study was conducted by a American group to assess whether the addition of bortezomib to high-dose melphalan conditioning followed by ASCT is effective.

In a preliminary report of this study, addition of bortezomib to high-dose melphalan was not associated with significant improvements in strength of the hematologic response or survival outcomes [13]; however, given the aforementioned more powerful antimyeloma activity of BuMel compared with high-dose melphalan, we hypothesized that the addition of bortezomib to BuMel (V-BuMel) would improve the response and survival outcomes without significant toxicity.

Thus, we conducted a phase I study to identify the maximum tolerated dose (MTD) of bortezomib and a subsequent phase II study to evaluate the efficacy and toxicity of the regimen, followed by ASCT involving the use of V-BuMel. Recently, the Revised International Staging System (R-ISS) [14] and the International Myeloma Working Group [15] proposed high-risk cytogenetic abnormality defined as presence of t(4;14) and/or t(14;16) and/or del(17p). R-ISS was even well validated as a robust tool either in the ASCT setting or under novel treatments, including thalidomide, lenalidomide, and bortezomib [16-18]. In this respect, we were also interested in the possible role of the V-BuMel regimen in patients harboring high-risk cytogenetic abnormalities.

METHODS

Study Design and Endpoints

The study was designed as a phase I/II, open-label, multicenter trial. Patients were recruited from 8 participating centers of the Korea Multiple Myeloma Working Party. The primary endpoint of the phase I study was to determine the MTD of V-BuMel based on a standard 3 + 3 dose escalation (.7, 1.0, and 1.3 mg/m^2/day of bortezomib) study design until the onset of dose-limiting toxicity (DLT) at day +28. All grade 3 or higher toxicities based on the National Cancer Institute Common Terminology Criteria for Adverse Events (AEs) version 4.0 [19] were considered DLT except the following: nausea, weakness, hair loss, loss of sexual affinity, amenorrhea, neutropenic fever, infection, anorexia, depression, anxiety, grade 3 stomatitis, and grade 3 or 4 hematologic toxicity. If a DLT was encountered at a particular dose, the cohort at this dose was expanded to 6 patients. If 2 DLTs occurred at the same dose, the previous dose level was defined as the MTD. If no DLT was observed at any dose, the MTD of bortezomib was determined to be 1.3 mg/m^2/day.

After the MTD was defined, a subset of patients in the phase I study who received the MTD of bortezomib and new patients were enrolled in a subsequent phase II study. The primary endpoint of the phase II study was to identify response rates at 3 months from ASCT (i.e., very good partial response [VGPR] or better). Secondary endpoints of the phase II study were the best overall response rate until disease progression, progression-free survival (PFS), overall survival (OS), and toxicity profiles.

The Institutional Review Board at each participating hospital approved the study protocol. All of the procedures were conducted in accordance with the principles of the Declaration of Helsinki and local regulations. All patients provided written informed consent before enrollment. The study was registered at ClinicalTrials.gov (NCT 01255527).

Patient Eligibility

Patients were eligible to participate if they had been newly diagnosed with symptomatic MM, had previously been treated with induction chemotherapy using bortezomib, were age $\geq$20 and $\leq$65 years, had a life expectancy of $\geq$6 months, and had at least 2×10^6 CD34$^+$ hematopoietic stem cells/kg mobilized in preparation for ASCT. Patients were required to have measurable disease before initiation of induction chemotherapy, with a serum M-protein level $>$1 g/dL or a 24-hour urine M-protein level $>$200 mg/day. Additional inclusion criteria were Eastern Cooperative Oncology Group performance status 0 to 2, left ventricular ejection fraction $\geq$50%, adequate hepatic function with serum aspartate aminotransferase and alanine aminotransferase $<$3 times the upper limit of normal, serum total bilirubin $<$2 times the upper limit of normal, and adequate hematologic function with absolute neutrophil count $\geq 1.0 \times 10^9$/L, platelet count $\geq 75 \times 10^9$/L, and hemoglobin $\geq$8.0 g/dL. Patients were excluded who had systemic amyloidosis, plasma cell leukemia, known HIV infection, active hepatitis B or C infection, significant peripheral neuropathy, nonhematologic invasive malignancy within the past 3 years, history of kidney transplantation, myocardial infarction within 6 months of registration, New York Heart Association class 3 or 4 congestive heart failure, or uncontrolled angina, or if they were pregnant or nursing.

Conditioning Regimen and Post-Transplantation Treatment

As shown in Supplementary Figure 1, increasing doses of bortezomib (i.v. on days -6, -3, and +1 relative to transplantation) were added to the combination of busulfan (i.v., 3.2 mg/kg/day from days -5 to -3) and melphalan (i.v., 140 mg/m^2/day on day -2). Except for the conditioning regimen, other supportive care, including prophylactic antibiotics, prophylaxis of veno-occlusive disease, granulocyte colony-stimulation factor, and bisphosphonates, were administered according to institutional policy. Administration of maintenance treatment (MT) after ASCT was also based on institutional policy.

Definitions and Statistical Analysis

Stage was classified by the International Staging System (ISS) for multiple myeloma [20], and the response to treatment was evaluated using the International Myeloma Working Group's response criteria [21]. Response rates before ASCT and 3 months after ASCT were evaluated. The best response after ASCT until disease progression was also assessed. High-risk cytogenetic abnormalities, defined as the presence of any 1 or more of the following, were detected by interphase fluorescein in situ hybridization (FISH) method: del(17p), t(4;14), or t(14;16) [14]. Regarding the absence of a standardized panel for FISH probes in Korea, FISH was performed using corresponding probes, not using a comprehensive panel, according to the manufacturer's protocol in each institution. The time to neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $>.5 \times 10^9$/L. The time to platelet engraftment was defined as the first of 7 consecutive days with a platelet count $>20 \times 10^9$/L without platelet transfusion. Veno-occlusive disease was diagnosed using both Seattle and Baltimore criteria [22,23]. Categorical variables were compared using Fisher's exact test. The Kaplan-Meier method was used to analyze time-to-event endpoints, including PFS and OS. All statistical analyses were conducted using R 3.1.1 statistical software (http://cran.r-project.org/).

RESULTS

Patient Characteristics

Nine patients were enrolled between June 2011 and December 2011 and treated according to the phase I design in 3 consecutive cohorts. Patient characteristics are summarized in Table 1. None of the 9 patients developed DLT (Supplementary Table 1); therefore, 1.3 mg/m^2 of bortezomib was determined as the MTD, and that dose of bortezomib was administered on days -6, -3, and +1 in combination with BuMel in the subsequent phase II study. There was a total of 43 patients recruited to participate in the phase II study between June 2011 and September 2016. One patient withdrew consent before initiation of conditioning, and 1 patient became ineligible because a collected dose of CD34$^+$

Table 1
Patient Characteristics

Characteristic	Phase I (N= 9)	Phase II (N= 41)	Total (N = 47)*
Age yr, median (range)	59 (34-65)	56 (34-64)	57 (34-65)
Male sex, n (%)	5 (55.5)	29 (70.7)	32 (68.1)
Type of myeloma, n (%)			
Ig A	3 (33.3)	8 (19.5)	11 (23.4)
Ig G	2 (22.2)	23 (56.1)	25 (53.2)
Ig D	0 (.0)	1 (2.4)	1 (2.1)
Light chain disease	4 (44.4)	8 (19.5)	9 (19.1)
Unknown	0 (.0)	1 (2.4)	1 (2.1)
ECOG performance status at transplantation, n (%)†			
0	0 (.0)	15 (36.6)	15 (31.9)
1	8 (88.9)	25 (61.0)	31 (66.0)
2	1 (11.1)	1 (2.4)	1 (2.1)
Cytogenetic status, n (%)			
Standard risk	6 (66.7)	25 (61.0)	28 (59.6)
High risk	1 (11.1)	7 (17.1)	8 (17.0)
t(4;14)	1 (11.1)	4 (9.8)	5 (10.6)
t(14;16)	1 (11.1)	1 (2.4)	2 (4.3)
del(17p)	0 (.0)	2 (4.9)	2 (4.3)
Unknown	2 (22.2)	9 (22.0)	11 (23.4)
Presence of extramedullary disease, n (%)			
Present	4 (44.4)	8 (19.5)	9 (19.1)
Unknown	0 (.0)	6 (14.6)	6 (12.8)
Lactate dehydrogenase level, n (%)			
> Upper limit of normal	3 (33.3)	10 (24.4)	12 (25.5)
Normal	6 (66.7)	27 (65.9)	31 (66.0)
Unknown	0 (.0)	4 (9.8)	4 (8.5)
ISS stage at diagnosis, n (%)			
I	3 (33.3)	11 (26.8)	12 (25.5)
II	2 (22.2)	14 (34.1)	16 (34.0)
III	4 (44.4)	14 (34.1)	17 (36.2)
Unknown	0 (.0)	2 (4.9)	2 (4.3)
Response status at transplantation, n (%)			
CR	2 (22.2)	15 (36.6)	15 (31.9)
VGPR, n (%)	4 (55.6)	12 (29.3)	16 (34.0)
PR, n (%)	1 (11.1)	13 (31.7)	14 (29.8)
Stable disease, n (%)	2 (22.2)	1 (2.4)	2 (4.3)
Time to ASCT from diagnosis, mo, median (range)	8.4 (3.6-8.4)	6.7 (3.5-13.0)	6.7 (3.5-13.0)
Creatinine clearance before ASCT, mL/min, median (range)	111.1 (53.9-326.2)	104.7 (22.1-326.2)	109.0 (22.1-326.2)
Creatinine clearance >60 mL/min, n (%)	7 (77.8)	38 (92.7)	42 (89.4)
Creatinine clearance ≥30 to <60 mL/min, n (%)	2 (22.2)	2 (4.9)	4 (8.5)
Creatinine clearance <30 mL/min, n (%)	0 (.0)	1 (2.4)	1 (2.1)
Induction treatment, n (%)			
Bortezomib-thalidomide-dexamethasone	0 (.0)	29 (70.7)	29 (61.7)
Bortezomib-dexamethasone	6 (66.7)	5 (12.2)	10 (21.3)
Bortezomib-doxorubicin-dexamethasone	2 (22.2)	3 (7.3)	4 (8.5)
Bortezomib-cyclophosphamide-dexamethasone	0 (.0)	2 (4.9)	2 (4.3)
Bortezomib-melphalan-prednisolone	1 (11.1)	2 (4.9)	2 (4.3)
Infused $CD34^+$ cell dose, $\times 10^6$/kg, median, (range)	6.1 (3.9-16.7)	6.0 (2.5-35.6)	6.0 (2.5-35.6)
Post-transplantation maintenance therapy, n (%)	5 (55.6)	10 (24.4)	13 (27.7)

ECOG indicates Eastern Cooperative Oncology Group; ISS, International Staging System.
* Three patients in the phase I study were also enrolled in the phase II study.
† ECOG performance as published by the ECOG.

before ASCT was $<2 \times 10^6$ cells/kg. Therefore, a final cohort of 41 patients was analyzed in the phase II study.

In the phase II cohort, the median patient age was 56 years (range, 34 to 64 years). Cytogenetic risk stratification was available for 32 patients. Seven patients (17.1%) had high risk-cytogenetic status. Risk group was assessed by the ISS at diagnosis, and 11 patients (26.8%) were in stage I, 14 (34.1%) were in stage II, and 14 (34.1%) were in stage III. The status of 2 patients could not be confirmed using ISS criteria. Regarding the response status at transplantation, 15 patients (36.6%) had a complete response (CR), 12 (29.3%) had a VGPR, 13 (31.7%) had a PR, and 1 (2.4%) had stable disease. The regimens used

for induction chemotherapy included bortezomib-thalidomide-dexamethasone (n = 29; 70.7%), bortezomib-dexamethasone (n = 5; 12.2%), bortezomib-doxorubicin-dexamethasone (n = 3; 7.3%), bortezomib-cyclophosphamide-dexamethasone (n = 2; 4.9%), and bortezomib-melphalan-prednisolone (n = 2; 4.9%). A total of 10 patients (24.4%) received MT using thalidomide and/or prednisolone, but no patients administered bortezomib as MT. Other characteristics are summarized in Table 1.

Post-Transplantation Responses and Survival Outcomes

Serial pretransplantation/post-transplantation responses in evaluable patients are shown in Figure 1. The overall response of VGPR or better at 3 months after ASCT (primary endpoint of this study) was achieved in 30 patients (75%); 55% (n = 22) had CR, and 20% (n = 8) had VGPR. Two patients who had PR and VGPR pretransplantation experienced early disease progression after 1.1 months and 2.4 months, respectively. The post-transplantation overall best response rate of PR or better was 95%, which included 68% (n = 28) with CR, 15% (n = 6) with VGPR, and 12% (n = 5) with PR. The post-transplantation CR rate (68%) was significantly increased by 31% ($P = .008$) compared with the pretransplantation CR rate (37%).

The median follow-up duration of all patients was 31.4 months (95% confidence interval [CI], .9 to 81.4 months). Three patients in the phase II cohort died of sepsis related to pneumonia, at .9 months, 4.0 months, and 7.4 months after ASCT. With a median PFS of 26.8 months (95% CI, 38.9 to 70.8 months), the probability of 2-year PFS was 56.5% (95% CI, 38.9% to 70.8%). Although the median OS could not be calculated, the probability of 2-year OS was 70.0% (95% CI, 53.2% to 81.8%) (Figure 2).

Subgroup Analysis

Subgroup analyses of PFS are summarized in Supplementary Table 2. Age, disease type, ISS score, lactate dehydrogenase, presence of extramedullary disease, response status at ASCT, or time to transplantation from diagnosis had no significant effect on PFS. However, the median PFS of patients with high-risk cytogenetics was significantly lower than that of patients with standard-risk cytogenetics (12.2 months [95% CI, 7.7 months to not available] versus 35.7 months [95% CI, 17.6 to 48.2 months]; $P = .039$) (Figure 3A). Median OS also showed a different trend according to cytogenetic status (26.7 months [95% CI, 16.7 months to not available] in high-risk patients versus 73.3 months [95% CI, 49.5 months to not available] in standard-risk patients; $P = .086$) (Figure 3B). In a subgroup analysis of 23 patients who had available cytogenetic data and did not receive MT, median OS (23.9 months [95% CI, 16.7 months to not available] in high-risk patients versus 73.3 months [95% CI, 49.5 months to not available] in standard-risk patients; $P = .005$) and PFS (10.3 months [95% CI, 7.7 months to not available] in high-risk patients versus 35.1 months [95% CI, 17.6 months to not available] in standard-risk patients; $P = .036$) were significantly shorter in patients with high-risk cytogenetics compared with those with standard-risk cytogenetics (Figure 3C and D).

Engraftment and Toxicity Profiles

Neither graft failure nor delayed engraftment was observed in the total cohort. Neutrophil engraftment and platelet engraftment were achieved at a median of 11 days (range, 9 to 23 days) and 10 days (range, 1 to 35 days), respectively (Figure 4). Nonhematologic AEs are listed in Table 2. The most common grade 3 or severe AE was neutropenic fever (n = 30; 73.2%), followed by stomatitis (n = 6; 14.6%), nausea or vomiting (n = 4; 9.8%), diarrhea (n = 2; 4.9%), and pulmonary dysfunction (n = 1; 2.4%). Except for 1 patient's grade 4 pulmonary dysfunction (pulmonary edema with pneumonia) and hypokalemia, which were related to septic pneumonia and the cause of early death at .9 month, other AEs were successfully managed by supportive care. No second primary malignancies or veno-occlusive disease had occurred in the total cohort at the time of this report.

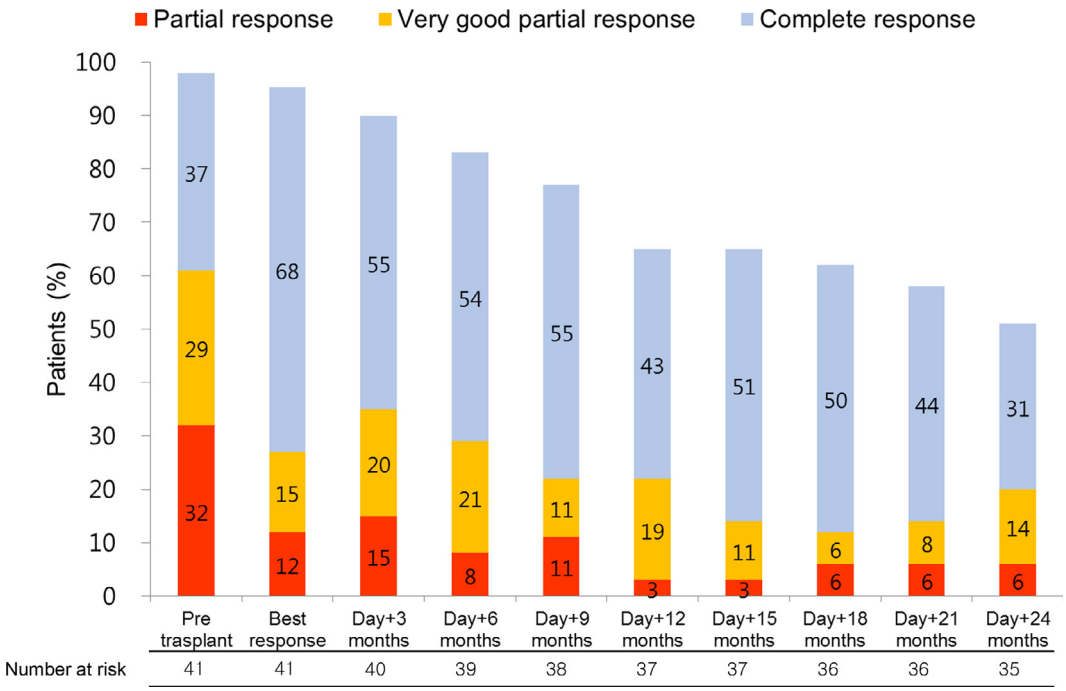

Figure 1. Serial pretransplantation and post-transplantation responses.

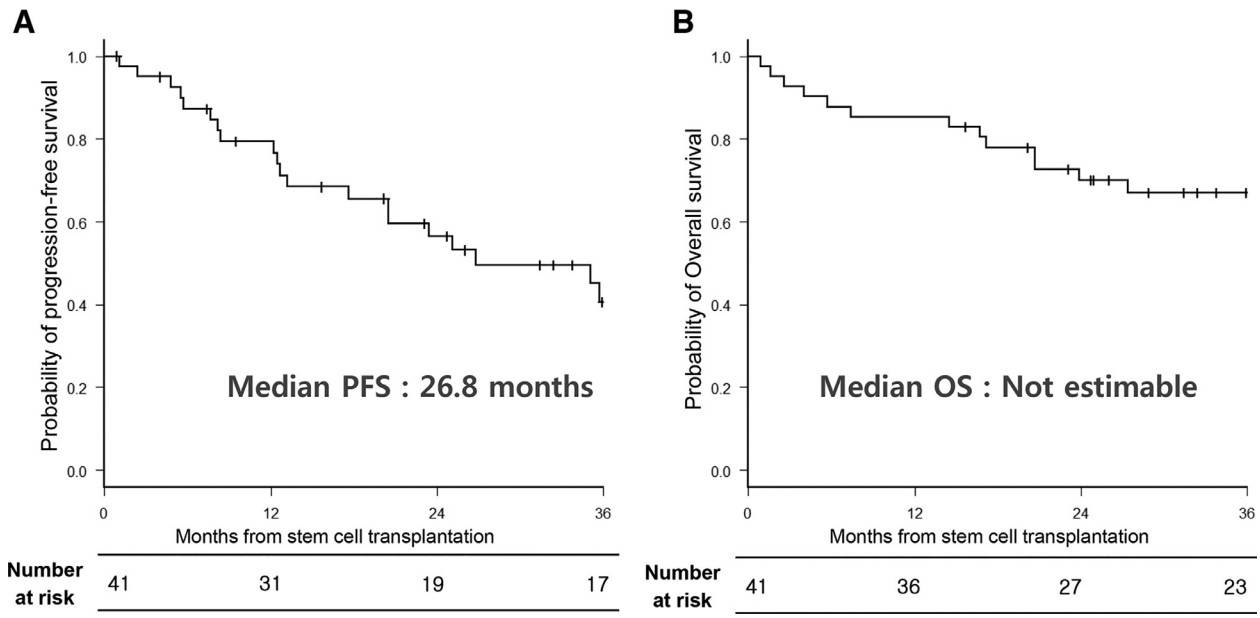

Figure 2. Survival outcomes. (A) PFS. (B) OS.

DISCUSSION

Growing clinical evidence indicates that BuMel is more effective than high-dose melphalan for ASCT without increasing unexpected transplantation-related mortality [9]. Nevertheless, no conditioning regimen for ASCT has been accepted as optimal in transplantation-eligible patients with MM. We explored whether bortezomib could be safely added to the BuMel regimen in this phase I study. Bortezomib with a dose of 1.3 mg/m^2 on days -6, -3, and +1 was determined to be the MTD, and the efficacy and toxicity profiles were consequently evaluated in a phase II study. The phase II study showed that V-BuMel led to CR in 55% of patients and VGPR or CR in 75% of patients at 3 months after ASCT. In terms of post-transplantation best response, we found a 68% rate of CR and an 83% rate of VGPR or better, respectively.

As shown in Table 3, compared with the post-transplantation strength of response after conditioning with melphalan plus busulfan and/or bortezomib in recently reported prospective trials [4,8,12,22,24,25], a triplet regimen with V-BuMel in the present study appeared to deliver relatively favorable response rates. Although a high rate of CR was achieved, the superiority of PFS (median of 26.8 months) was not evident compared with the previous trials. Considering the fact that this was a phase I/II study with relatively short follow-up, the influence of V-BuMel on PFS

Table 2
Nonhematologic Adverse Events of the Phase II Study (Total Patients, N = 41)

Adverse Event	Any Grade, n (%)	Grade 3, n (%)	Grade 4, n (%)
Neutropenic fever	36 (87.8)	30 (73.2)	0 (.0)
Stomatitis	29 (70.7)	6 (14.6)	0 (.0)
Nausea or vomiting	30 (73.2)	4 (9.8)	0 (.0)
Diarrhea	23 (56.1)	2 (4.9)	0 (.0)
Pulmonary dysfunction	3 (7.3)	1 (2.4)	1 (2.4)
Hypokalemia	2 (4.9)	1 (2.4)	1 (2.4)
Hepatic dysfunction	13 (31.7)	1 (2.4)	0 (.0)
Indigestion	9 (22.0)	1 (2.4)	0 (.0)
Cardiac dysfunction	5 (12.2)	1 (2.4)	0 (.0)
Hemorrhage	5 (12.2)	1 (2.4)	0 (.0)
Skin rash	14 (85.4)	0 (.0)	0 (.0)
Psychiatric disturbance	2 (4.9)	0 (.0)	0 (.0)
Benign prostatic hyperplasia	2 (4.9)	0 (.0)	0 (.0)
Neuropathy	2 (4.9)	0 (.0)	0 (.0)
General ache	2 (4.9)	0 (.0)	0 (.0)
General weakness	1 (2.4)	0 (.0)	0 (.0)
Constipation	1 (2.4)	0 (.0)	0 (.0)
Gastric ulcer, n (%)	1 (2.4)	0 (.0)	0 (.0)
Edema	1 (2.4)	0 (.0)	0 (.0)
Renal dysfunction	0 (.0)	0 (.0)	0 (.0)
Veno-occlusive disease	0 (.0)	0 (.0)	0 (.0)
Central nerve system dysfunction	0 (.0)	0 (.0)	0 (.0)

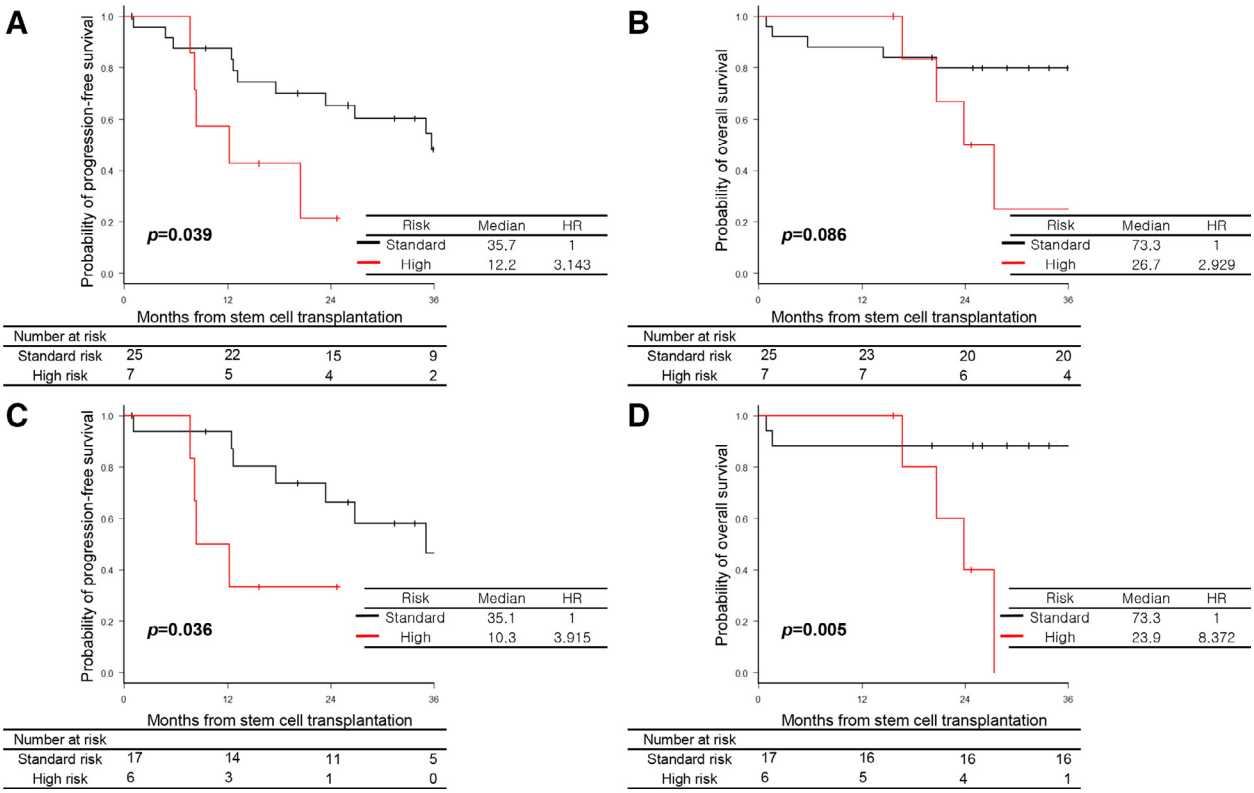

Figure 3. Survival outcomes according to cytogenetic risk. PFS (A) and OS (B) according to cytogenetic risk in all available patients (N = 32). PFS (C) and OS (D) according to cytogenetic risk in a subcohort without maintenance therapy (N = 23). HR indicates hazard ratio.

can be clarified in future phase III clinical trials with larger sample sizes and longer follow-up durations. In line with our results, a previous phase I/II study by Tulio et al [25] also showed the efficacy of adding bortezomib to BuMel as conditioning for ASCT in patients with MM. The authors used bortezomib at a dose of 1.6 mg/m^2/day on day -1 combined with BuMel and compared the efficacy of V-BuMel with that of high-dose melphalan in a matched control cohort extracted from the Center for International Blood and Marrow Transplant Research database. Impressive response rates following the V-BuMel regimen were also observed, with a VGPR rate of at least 70% and a CR rate of 42%. Interestingly, the 1-year probability of PFS was significantly higher in the V-BuMel arm compared with the high-dose melphalan arm (90% versus 77%; $P = .02$).

Considering the evident association between stronger post-transplantation response and better survival outcomes [26], the results of the present phase II study support a future randomized trial to determine whether the V-BuMel regimen could enhance antimyeloma activity compared with a high-dose melphalan or BuMel regimen.

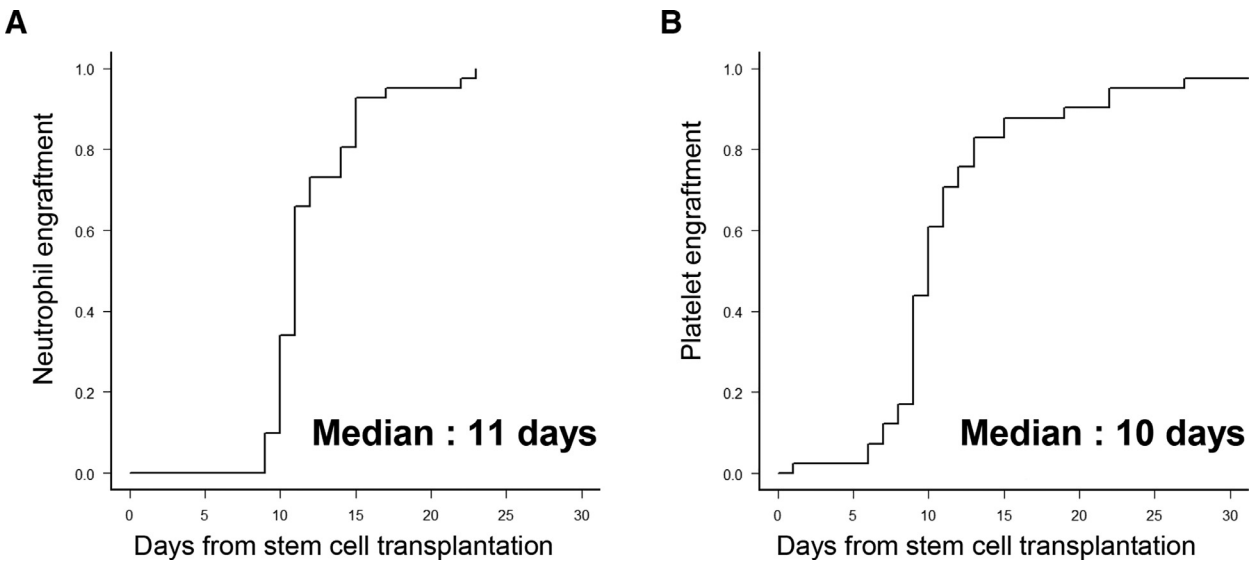

Figure 4. Cumulative incidence of neutrophil (A) and platelet (B) engraftment.

Table 3
Efficacy of Conditioning Regimen Consisting of Melphalan with or without Busulfan and/or Bortezomib in Recent Clinical Trials

Study Design (Sample Size)	Conditioning Regimen	Response at 3 mo	Best Response	Median Follow-Up, mo	Median PFS, mo	Reference
Phase I/II (N = 41)	Busulfan i.v. 3.2 mg/kg/d for 3 d; melphalan 140 mg/m^2/d for 1 d; bortezomib 1.3 mg/m^2/d for 3 d	≥CR 55%; ≥VGPR 75%	≥CR 68%; ≥VGPR 83%	31.4	26.8	Present study
Prospective nonrandomized (N = 225)	Busulfan orally 3 mg/kg/d for 4 d; melphalan 140 mg/m^2/d for 1 d	-	≥CR 38%; ≥VGPR 51%	72	41	[4]
Case-control analysis (N = 51)	Busulfan i.v. 3.2 mg/kg/d for 3 d; melphalan 140 mg/m^2/d for 1 d	-	≥CR 23.5%; ≥VGPR 51%	50	33	[8]
Phase II (N = 99)	Busulfan i.v. 3.2 mg/kg/d for 3 d; melphalan 140 mg/m^2/d for 1 d	-	≥CR 43.5%; ≥VGPR 66.7%	26.1	27.2	[24]
Phase I/II (N = 39)	Bortezomib 1, 1.3, or 1.6 mg/m^2 for 1 d; melphalan 100 mg/m^2/d for 2 d	≥CR 21%; ≥VGPR 72%	-	17.3	15.3	[12]
Phase III (N = 154)	Bortezomib 1 mg/m^2 on days -6, -3, +1, and +4; melphalan 200 mg/m^2/d for 1 d	CR 23% (at day +60)	≥CR 44%; ≥VGPR 86%	14	76% at 18 mo	[13]
Phase I/II (N = 43)	Busulfan i.v. 130 mg/m^2/d for 2 d, followed by adjusted dose targeting total area under the curve of 20,000 M for 2 d; melphalan 140 mg/m^2/d for 1 d; bortezomib 1.6 mg/m^2/d for 1 d	-	≥CR 42%; ≥VGPR 70%	25	90% at 12 mo	[25]

Despite the addition of bortezomib to BuMel conditioning, the median times to neutrophil engraftment and platelet engraftment in the present study (11 days for neutrophil and 10 days for platelet engraftment) were comparable to those in earlier trials that used BuMel conditioning (ie, 10 to 12 days for neutrophil and 9 to 16 days for platelet engraftment) [4,8,24]. As expected, neutropenic fever and stomatitis were the most common grade 3 or greater nonhematologic toxicities. With 3 deaths from sepsis related to pneumonia in a phase II cohort of 41 patients, the treatment-related mortality seems to be high; nevertheless, in studies with small samples, a few subjects can have a substantial effect on the overall results. Except for these 3 cases with fatal sepsis associated with pneumonia (Supplementary Table 3), other AEs were generally manageable. Despite the well-known significant AEs of peripheral neuropathy related to bortezomib [27] and veno-occlusive disease associated with busulfan [4], the toxicity profiles of the present study showed neither grade 3 or severe peripheral neuropathy nor veno-occlusive disease. The use of bortezomib in conjunction with BuMel appeared to be safe and to provide enhanced antimyeloma activity.

Interestingly, despite the small number of participants in subgroups, a subset with high-risk cytogenetic abnormalities had worse PFS and OS compared with those with standard risk, regardless of MT. Regarding the scanty incidence of t (14;16), described in just 2% to 4% of patients with MM [28], it is not surprising that there have been no data from an MM cohort harboring t(14;16) and undergoing ASCT. Previous trials and guidelines were based on the idea that bortezomib with standard induction or MT can improve ASCT outcomes, but there are insufficient prognostic implications of t(4;14) and del (17p) [28-31]. On the other hand, our study was designed to enroll patients who received bortezomib as part of a conditioning regimen for ASCT as well as induction chemotherapy before ASCT. Apart from the relatively homogenous protocol surrounding ASCT, we allowed MT according to each institution's policy. For this reason, only some subjects received MT using thalidomide and/or prednisolone without bortezomib; in Korea, the use of bortezomib for MT has been limited owing to insufficient insurance coverage. In line with previous studies [32], our results indicate that bortezomib-only-based induction and conditioning regimens are insufficient to abolish the adverse impact of high-risk cytogenetic abnormalities. Therefore, given the potential advantage of bortezomib maintenance for MM harboring t(4;14) and/or del(17p) according to previous reports [33-35], a future phase III study is warranted to clarify outcomes of ASCT following V-BuMel conditioning with a homogeneous protocol of MT using bortezomib.

This study has several limitations. One major pitfall is the heterogeneous induction regimens and MT. Other important shortcomings are the relatively short follow-up duration and small sample size, which preclude us from drawing definite conclusions reflecting PFS or OS. Furthermore, previous studies have demonstrated that the presence of minimal residual disease at the traditional day +100 assessment post-ASCT independently predicts for both PFS and OS [36,37]. Although the effect of MRD on outcomes is reliable in patients achieving a CR as well as in those with both high-risk and standard-risk cytogenetics, we did not provide for the role of MRD as a surrogate endpoint for survival in this clinical trial.

In summary, the present study shows that bortezomib can be safely added to BuMel in a conditioning regimen for ASCT. Based on the encouraging improved response, including VGPR or CR, our findings support a future randomized trial to assess whether the V-BuMel regimen has enhanced efficacy compared

with a classical conditioning regimen, such as high-dose melphalan or BuMel. However, considering the unsatisfactory outcome for those with a high-risk cytogenetic abnormality despite ASCT with V-BuMel, future studies should include a rigorous post-transplantation protocol for MT using bortezomib in addition to the design of the present study.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.bbmt.2019.03.016.

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