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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

A randomized phase II, open-label and multicenter study of combination regimens of bortezomib at two doses by subcutaneous injection for newly diagnosed multiple myeloma patients

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Abstract

Purpose Combinations of bortezomib (Velcade), cyclophosphamide and dexamethasone have shown significant efficacy and safety for patients of newly diagnosed multiple myeloma (NDMM). In this study, we compared the efficacy and safety of modified VCD regimens with novel changes in bortezomib dose and schedule for NDMM.

Methods Eighty-five NDMM patients from multiple centers were randomly assigned to a high-dose (1.6 mg/m^2) (group A) or a low-dose (1.3 mg/m^2) (group B) bortezomib, administrated on days 1, 6, 11, and 16 subcutaneously in a 4-week cycle for nine cycles, combined with 40 mg dexamethasone on bortezomib days and cyclophosphamide 300 mg/m^2 on days 1–3 intravenously.

Results After four cycles, complete response (CR) or better in group A (43.6%) was higher than that in group B (12.8%) ($P=0.002$). During induction, for patients with R-ISS stage III, the CR or better rate in group A was superior to that in group B ($P=0.01$). Of patients <65, the CR or better rate of group A was superior to that of group B ($P=0.004$). Rapid onset of CR occurred in group A ($P<0.01$). Meanwhile, rate of 3–4 diarrhea was higher in group A ($P=0.03$), which caused higher rate of dose reduction for patients ≥ 65 ($P=0.041$). No significant difference between the two groups in PFS and OS.

Conclusions The studied high-dose VCD as induction regimen had an improved CR rate, especially in patients <65 or with R-ISS stage III, and is feasible for young and high-risk patients.

Trial registration ClinicalTrials.gov: NCT02086942.

Keywords Multiple myeloma · Bortezomib · Subcutaneous injection · Different doses · Efficacy

Introduction

Multiple myeloma (MM) is the second most common hematologic malignancy. The treatment goals for both young and elderly patients should be to prolong survival by achieving the best possible treatment response, while ensuring quality of life. Novel agents such as thalidomide, bortezomib and lenalidomide have greatly advanced MM treatment during the past decade (Cavo et al. 2011; Richardson et al. 2007). These agents have been incorporated into induction treatments to increase rates of response (≥ the rate of very good partial response [VGPR]) before autologous stem cell transplantation (ASCT) and thereby improved post transplantation outcomes (Cavo et al. 2010; Harousseau et al. 2010). Bortezomib contained regimens as induction treatments for newly diagnosed MM (NDMM) patients were associated with higher response rate, especial deeper response, and rapider onset than other available regimens in China (He et al. 2014). Recent studies indicated that three-drug combination regimens of proteasome inhibitors and immunomodulatory drugs yielded deep responses in NDMM patients (Mai et al. 2015). In China, bortezomib-contained regimens have been the preferred induction regimens. Triple combinations of

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bortezomib–cyclophosphamide–dexamethasone (VCD), bortezomib–doxorubicin–dexamethasone (PAD) and bortezomib–thalidomide–dexamethasone (VTD) are three of the most frequently used induction regimens prior to ASCT that are recommended by the National Comprehensive Cancer Network (NCCN) guidelines version 1.2019. For treatment of transplant-ineligible patients, only VCD regimen is recommended by NCCN.

Recently, a phase III clinical trial showed that VCD and PAD were similarly effective as judged by the response rates and toxicity profile (Mai et al. 2015). More frequent leukocytopenia/neutropenia (≥ Grade 3) occurred in the VCD group (35.2% vs. 11.3%, $P < 0.001$) while neuropathy rates (≥ Grade 2) were higher in the PAD group (14.9 vs. 8.4%, $P = 0.03$). But overall serious adverse events and those related to thromboembolic events were higher in the PAD group than in the VCD group (32.7 vs. 24.0%, $P = 0.04$ and 2.8 vs. 0.4%, $P = 0.04$). Furthermore, the collection of stem cells was not hindered by VCD. Therefore, VCD is preferred to PAD as induction therapy (Mai et al. 2015). In addition, the tolerance of VCD as consolidation regimen was superior to that of VTD or PAD, and VCD is less expensive than either VTD or PAD. A study in China (He et al. 2014) compared the efficacy of four bortezomib-based therapies as induction regimens including bortezomib (1.3 mg/m^2, given intravenously on days 1, 4, 8, and 11) plus dexamethasone (PD) or PD and either adriamycin (PAD), cyclophosphamide (PCD) or thalidomide (PTD) combination for every 28 days, and found that overall response rate (ORR) of PCD, PAD, PTD, and PD were 97.4%, 93.2%, 85.3%, and 77.8%, respectively, while the rates of VGPR or better were 63.7%, 62.7%, 44.2%, and 37.8%, respectively. The incidence of peripheral neuropathy (PN), especially grade 2–3 in PTD group was significantly higher than the other three groups. These results indicated that bortezomib-based regimens were effective and well tolerated in the studied Chinese population, especially with respect to PCD. Bortezomib of dose 1.3 mg/m^2 giving as an intravenous bolus injection (taking 3–5 s to administer) twice weekly for 2 weeks (on days 1, 4, 8 and 11) in a 21-day cycle was a preferred MM treatment (Richardson et al. 2003). The survival, response, and time-to-progression data indicated that the above intravenous bortezomib regimen for a maximum of eight cycles surpassed that of the dose 1.0 mg/m^2 (Jagannath et al. 2004). This would suggest that higher dose of bortezomib may be beneficial for increasing patient response rate and survival, though the main adverse event of bortezomib is dose-depending PN, which limits the optimal use of bortezomib (Richardson et al. 2006). Studies also have shown that weekly use of bortezomib and subcutaneous administration significantly improved drug tolerability with no adverse effect on efficacy (Mateos et al. 2010; Moreau et al. 2011). Based on these findings, we speculated that higher dose of bortezomib and subcutaneous administration could benefit response rate while maintaining the level of treatment-related toxicity.

The recommended standard quantity of bortezomib in relevant guidelines is 1.3 mg/m^2, twice weekly. Considering the point of response, drug density and adverse events, we set the elevated dose of bortezomib to 1.6 mg/m^2 in this study. We also modified the dosing interval to once every 5 days via subcutaneous administration. The selection of 1.6 mg/m^2 once every 5 days was based on the fact that the cumulative dose of 1.6 mg/m^2 once every 5 days of four cycles (a total of 112 days) is equal to five cycles of the recommended regimen of bortezomib 1.3 mg/m^2, twice weekly (a total of 105 days). To test the effect of bortezomib use rate (dose), we hypothesized that bortezomib 1.6 mg/m^2, once every 5 days subcutaneously would be superior to that of 1.3 mg/m^2 as induction or consolidation therapy. To validate this hypothesis and further verify treatment efficacy and tolerability, we designed this multicenter, open-label randomized phase 2 trial with patients of NDMM.

Materials and methods

Study design

This trial was done at 10 centers in China to determine the safety and efficacy of two doses of bortezomib (1.6 mg/m^2, group A and 1.3 mg/m^2, group B) by subcutaneous injection combined with cyclophosphamide and dexamethasone for NDMM patients. Declaration of Helsinki and the principles of Good Clinical Practice were followed. The study protocol was approved by the institutional ethics committee of Jinling Hospital. All participating patients provided written consents. Patients were enrolled between April 2013 and May 2017. Clinical data cutoff was August 1, 2018.

Patients

Patients aged 18–75 years with NDMM having measurable disease were enrolled. Key inclusion criteria included the Karnofsky performance status ≥ 50%, adequate hematological and hepatic function. Exclusion criteria included active infectious disease; active peptic ulcer; medical history of venous thrombosis; pregnant females; PN of grade 2 or worse; myocardial infarction; unstable angina within 4 months or other clinically significant heart diseases.

Procedures

Eligible patients were randomly assigned at 1:1 ratio to receive two doses of bortezomib, administered on days 1, 6, 11, and 16 by subcutaneous injection in a 4-week cycle for maximum nine cycles. Dexamethasone 40 mg on the

days of bortezomib and cyclophosphamide 300 mg/m^2 on days 1–3 were administered by intravenous infusion. After four cycles of induction, transplant-eligible patients received either stem cell transplant or chemotherapy on their own choice. Treatment was interrupted when grade 3 or worse drug-related non-hematological adverse events or grade 4 drug-related hematological adverse events occurred, and resumed at 20% dose reduction upon resolution of the toxicity to grade 2 or better. Subsequent maintenance therapy included cyclophosphamide 0.2 g/day for 1–7 days alone or combined with prednisone 60 mg/day up to 2 years if no relapse or progression occurred.

Assessment

Assessments started at the beginning of each cycle during induction, consolidation, and every three cycles of maintenance treatment. Fluorescence in situ hybridization (FISH) was used for *t* (4:14), *t* (11:14), *t* (14;16), del13, +1q21, and del17p. The primary endpoint was CR-evaluated according to the International Myeloma Working Group (IMWG) criteria. The secondary endpoints included PFS and OS, ORR, VGPR or better, and safety evaluations. Adverse events (AEs) were graded by NCI-CTCAE Version 3.0.

Pharmacodynamic assessments

Inhibition of 20S proteasome as bortezomib mechanism of action (Blaney et al. 2004; Moreau et al. 2011) was evaluated using a previously described assay (Lightcap et al. 2000). Blood samples (2 mL) of selected patients were obtained before and at 1, 2, 4, 24, and 48 h after the first dose, and before and 1, 2, and 24 h after the second, third and fourth dose during the first cycle.

Statistical analysis

Simon Minimax two-stage design was used. The maximum CR rate considered of low interest was 30% (P0) and the minimum was 50% (P1). The target enrollment ($\alpha = 0.05$; $\beta = 0.20$) was estimated as 39 patients per group. In the first stage, 19 patients per group were considered and at least seven CRs in group A were needed to complete the accrual. At least 17 CRs after therapy completion were necessary to demonstrate efficacy of group A.

Patients received at least one dose of bortezomib were included in intention-to-treat (ITT) and safety analyses. All patients who completed four or nine cycles were included in per-protocol analysis. PFS and OS were analyzed using Kaplan–Meier method. Response rate differences were analyzed with the χ^2 or Fisher's exact test. All analyses were done with the Statistical 4.0 for Windows.

Results

Study population

Overall, 85 NDMM patients were enrolled between April 2013 and May 2017. Forty-two patients were randomly assigned to receive bortezomib 1.6 mg/m^2 (Group A) and 43 were to receive bortezomib 1.3 mg/m^2 (Group B). The demographics and cytogenetic characteristics were generally well balanced between the two groups (Table 1). The patients received a median of 4.5 treatment cycles (range: 1–9) in group A and 4 (range: 1–9) treatment cycles in group B. In group A, 37 (88.1%) patients completed two cycles, 29 (69%) patients received at least four cycles (induction) and 19 (45.2%) patients completed all nine cycles (induction plus consolidation). In group B, 38 (88.4%) patients completed two cycles, 30 (69.8%) patients received at least four cycles and 15 (34.9%) patients completed all nine cycles. Only three patients in group A and one in group B have undergone stem cell transplantation (ASCT) after four cycles (induction). The patients who completed all nine cycles in the two groups have received maintenance therapy. The total treatment cycles of the two groups were similar with 228.25 cycles for group A and 225 cycles for group B. The median cumulative dose of bortezomib and dose intensity (mg/m^2 per cycle, cycle ≥ 5) also were similar between the groups ($P = 0.12$, Table 1). The mean bortezomib dose intensity (mg/m^2 per cycle) received by patients of cycles 1–4 in group A was higher than that of group B (mean dose intensity 5.2 vs. 4.5, $P = 0.03$, Table 1). At data cutoff (August 1, 2018), the median follow-up of all patients was 31.87 months (range: 1.83–61.3). The most common causes of therapy interruption were progressive disease, adverse events, insurance issue, and refusal (Fig. 1).

Efficacy

Treatment response was evaluated for 39 patients in both group A and group B. Seven patients were not evaluable because of missing assessment following cycle 1. At the first stage of the Simon Minimax design, 11 (57.9%) of the 19 patients in group A and 6 (31.6%) of the 19 patients in group B achieved CR. The result of interim analysis after the completion of the first stage suggested proceeding with the second stage to complete the planned accrual. At the end of the second stage, 17 patients assigned to 1.6 mg/m^2 (group A) and 10 patients assigned to 1.3 mg/m^2 (group B) achieved CR or better after therapy completion (nine cycles), with the rate of 43.6% and 25.6% ($P = 0.076$), respectively, statistically similar (Table 2).

Table 1 Patient demographics and baseline disease characteristics

Variable	Group A $N=42$	Group B $N=43$	P
Gender (male, %)	25 (59.5)	26 (60.5)	0.553
Median age, years (range)	59 (43–74)	60 (40–80)	> 0.05
≥ 65 years, *n* (%)	11 (26.2)	11 (25.6)	0.572
M isotype, *n* (%)			
IgA	10 (23.8)	9 (20.9)	0.477
IgG	21 (50)	24 (55.8)	0.375
IgD	2 (4.8)	1 (2.3)	0.491
Light chains	9 (21.4)	9 (20.9)	0.582
Albumin (g/L) ≤ 35, *n* (%)	24 (57.1)	30 (69.8)	0.163
Creatinine ≥ 176 μmol/L, *n* (%)	11 (26.2)	8 (18.6)	0.282
Elevated LDH, *n* (%)	10 (23.8)	13 (30.2)	0.337
DS, *n* (%)			0.14
I + II	2 (4.8)	6 (14)	
III	40 (95.2)	37 (86)	
ISS, *n* (%)			0.215
I + II	15 (35.7)	20 (46.5)	
III	27 (64.3)	23 (53.5)	
R-ISS, *n* (%)			0.442
I + II	28 (66.7)	27 (62.8)	
III	14 (35)	16 (39)	
Cytogenetic abnormality, *n* (%)			
Del(13q)	14 (33.3)	15 (34.9)	0.531
Del(17p)	3 (7.1)	4 (9.3)	0.513
Amp(1q21)	18 (42.9)	16 (37.2)	0.378
t (4:14)	9 (21.4)	11 (25.6)	0.423
t (11:14)	4 (9.5)	6 (14)	0.384
t (14:16)	1 (2.4)	2 (4.7)	0.509
Baseline diabetes mellitus, *n* (%)	6 (14.3)	5 (11.6)	0.483
Baseline cardiopathy, *n* (%)	4 (9.5)	3 (7)	0.487
Median cycles, *n* (range)	4 (1–9)	4 (1–9)	1
Bortezomib cumulative dose (mean, mg/m^2)	34.1 (6.3–57.6)	28.2 (5.2–46.8)	0.12
Bortezomib dose intensity (mg/m^2 per cycle, mean, cycles 1–4)	5.2 (1.6–6.4)	4.5 (1.3–5.2)	0.03*
Bortezomib dose intensity (mg/m^2 per cycle, mean, cycle ≥ 5)	5.3 (1.28–6.4)	4.7 (0.78–5.2)	0.12

LDH serum lactate dehydrogenase, *ISS* international staging system, *DS* Durie–Salmon, *R-ISS* revised international staging system

*$P \leq 0.05$

The CR rate after two induction cycles of bortezomib 1.6 mg/m^2 (group A) was higher than that of bortezomib 1.3 mg/m^2 (group B). Seven (17.9%) of the 39 patients in group A had a CR compared with 0 (0%) of the 39 patients in group B ($P = 0.006$) (Table 2). The difference in CR or better between group A and B after the four induction therapies was 43.6% vs. 12.8% ($P = 0.002$). Moreover, 30 patients in group A and 20 patients in group B achieved VGPR or better after induction therapy, with statistical difference between them (76.9% vs. 51.3%, $P = 0.016$). The ORR after the induction therapy was similar, 79.5% for group A and 76.9% for group B. The response rates of different subgroups are shown in Table 2, including subgroups based on age (≥ 65 years vs. < 65 years) and R-ISS (I + II vs. III). Of patients with R-ISS stage III, the rate of CR or better in group A was superior to that in group B ($P = 0.01$) after the four induction therapies. Of patients with R-ISS stage I and II, after the four induction therapies the VGPR or better in group A was superior to that in group B ($P = 0.016$). Meanwhile, of patients younger than 65 years, after the four induction therapies the rate of CR or better and the rate of VGPR or better in group A was superior to that in group B ($P = 0.004$, $P = 0.03$). After completion of induction and consolidation therapy

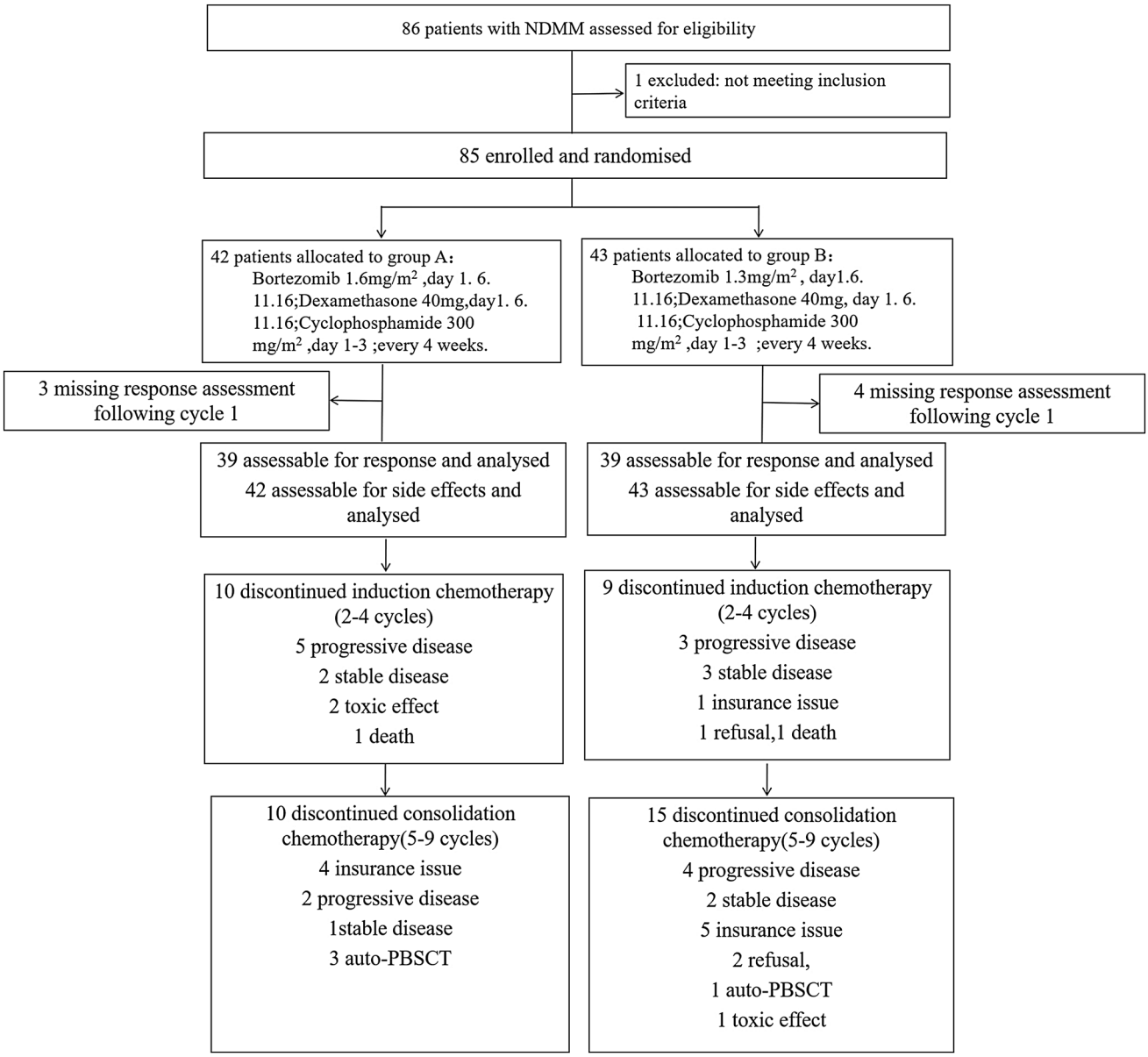

Fig. 1 Consort diagram of the study

(9 cycles), the rate of CR or better of patients younger than 65 years in group A was superior to that in group B ($P = 0.046$). In group A, 72.4% of patients were transplant-eligible (< 65 years old, VGPR or better), while 44.8% of patients in group B were transplant-eligible ($P = 0.03$). However, the outcomes after completion of induction and consolidation therapy (9 cycles) showed that response rates including VGPR, PR and ORR of patients in group A were similar as patients in group B ($P > 0.05$) (Table 2). Interestingly, after induction course no further tumor response was observed in group A,suggesting maximum efficacy achieved during induction (Table 2). On the other hand, 7 of 30 patients in group B with PR or VGPR after the induction chemotherapy had a further tumor volume reduction after chemotherapy completion, including four sCR, one CR and two VGPR.

For the responding patients, the median time to PR (response-evaluable analysis) was 1.18 months ($n = 33$, range 1–3) in group A and 1.35 months ($n = 31$, range 1–4) in group B ($P = 0.29$). The median time to VGPR was 2.1 months ($n = 32$, range 1–4) and 2.61 months ($n = 23$, range 1–8) for group A and B, respectively ($P = 0.16$) (data not shown). In addition, the median time to best response was 2.9 months ($n = 33$, range 1–4) in group A and 3.3 months ($n = 30$, range 1–8) in group B ($P = 0.31$). The median time to CR in group A was 3.11 months ($n = 18$,

Table 2 The therapy outcome after different cycles

Outcome	Group A (1.6 mg/m^2) ($N=39$)			Group B (1.3 mg/m^2) ($N=39$)		
	2 cycles	4 cycles	9 cycles	2 cycles	4 cycles	9 cycles
sCR	0	5 (12.8%)	8 (20.5%)	0	1 (2.6%)	5 (12.8%)
CR	7 (17.9%)*	12 (30.8%)*	9 (23.1%)	0	4 (10.3%)	5 (12.8%)
VGPR	16 (41%)	13 (33.3%)	11 (28.2%)	15 (38.5%)	15 (38.5%)	12 (30.8%)
PR	8 (20.5%)	1 (2.6%)*	1 (2.6%)	13 (33.3%)	10 (25.6%)	6 (15.4%)
≥CR	7 (17.9%)*	17 (43.6%)*	17 (43.6%)	0	5 (12.8%)	10 (25.6%)
≥VGPR	23 (59%)	30 (76.9%)*	28 (71.8%)	15 (38.5%)	20 (51.3%)	22 (56.4%)
ORR	31 (79.5%)	31 (79.5%)	29 (74.4%)	28 (71.8%)	30 (76.9%)	28 (71.8%)
≥CR/R-ISS						
I+II		10/25 (40%)	10/25 (40%)		4/23 (17.4%)	5/23 (21.7%)
III		7/14 (50%)*	7/14 (50%)		1/16 (6.3%)	5/16 (31.3%)
≥VGPR/R-ISS						
I+II		22/25 (88%)*	20/25 (80%)		13/23 (56.5%)	14/23 (60.9%)
III		8/14 (57.1%)	8/14 (57.1%)		7/16 (43.8%)	8/16 (50%)
≥CR/age						
≥65 years		4/10 (40%)	4/10 (40%)		2/10 (20%)	4/10 (40%)
<65 years		13/29 (44.8%)*	13/29 (44.8%)*		3/29 (10.3%)	6/29 (20.7%)
≥VGPR/age						
≥65 years		8/10 (80%)	8/10 (80%)		7/10 (70%)	8/10 (80%)
<65 years		21/29 (72.4%)*	20/29 (69%)		13/29 (44.8%)	14/29 (48.3%)
Median cycles to CR		3.11 (2–4) ($n=18$)*			4.77 (3–6) ($n=13$)	
Median cycles to the best response		2.9 (1–4) ($n=33$)			3.3 (1–8) ($n=30$)	

*$P \leq 0.05$

range 2–4) and 4.77 months ($n=13$, range 3–6) in group B ($P<0.01$). According to the PP analysis, both the rate of CR or better and VGPR or better after induction therapy (4 cycles) were significantly higher in group A (58.6% vs. 16.6%, $P=0.001$; 93.1% vs. 66.7%, $P=0.012$). No significant difference was observed in ORR between the two groups (96.6% vs. 90%, $P=0.319$). After the completion of the therapies (9 cycles), no significant differences were observed in CR, VGPR and ORR between the two groups ($P>0.05$) (Table 3).

Pharmacodynamic

Preliminary test showed that the maximum 20S proteasome inhibition occurred at 2 h after bortezomib administration. In our study, this maximum inhibition activity was increased as the dose increases ($P=0.02$) for those tested patients

Table 3 Responses to therapy

	Induction therapy		Induction and consolidation therapy	
	Group A (1.6 mg/m^2)	Group B (1.3 mg/m^2)	Group A (1.6 mg/m^2)	Group B (1.3 mg/m^2)
ITT	$n=39$	$n=39$	$n=39$	$n=39$
≥CR	17 (43.6%)	5 (12.8%)*	17 (43.6%)	10 (25.6%)
≥VGPR	30 (76.9%)	20 (51.3%)*	28 (71.8%)	22 (56.4%)
≥PR	31 (79.5%)	30 (76.9%)	29 (74.4%)	28 (71.8%)
Per protocol	$n=29$	$n=30$	$n=19$	$n=15$
≥CR	17 (58.6%)	5 (16.6%)*	13 (68.4%)	6 (40%)
≥VGPR	27 (93.1%)	20 (66.7%)*	18 (94.7%)	12 (80%)
≥PR	28 (96.6%)	27 (90%)	19 (100%)	15 (100%)

*$P \leq 0.05$

Table 4 Percentage 20S proteasome inhibition following bortezomib dosing

Dose and patient no.	Percentage proteasome inhibition after bortezomib								
	Day 1					Day 6 (2 h postdose)	Day 11 (2 h postdose)	Day 16 (2 h postdose)	Patient average
	1 h postdose	2 h postdose	4 h postdose	24 h postdose	48 h postdose				
1.3 mg/m^2									
1	51	71.5	44	7.6	3.8	ND	ND	ND	71.5
2	43	58.3	53.6	15	0	ND	ND	ND	58.3
3	ND	62.5	40.6	14.8	0	73.2	64	82.6	70.6
4	ND	46.4	27.1	10.4	0	72.7	82	64.8	66.5
5	ND	48.4	47.8	29.1	25.1	50.6	49.6	61.4	52.5
6	ND	44.8	34.9	4.8	0	66.3	76.3	73.6	65.3
7	ND	40.3	31.7	34.7	12.8	63.1	55.3	59.7	54.6
Average	47	53.2	40	16.6	6	65.2	65.4	68.4	62.8
SD	4	11.2	9.3	11.2	9.7	8.2	12.2	8.6	7.6
1.6 mg/m^2									
1	63	77	52.8	21.6	24.9	ND	ND	ND	77
2	50	75	47	19	6.4	ND	ND	ND	75
3	ND	59.7	57.6	39.7	18.4	68.8	70.5	67.3	66.6
4	ND	77.8	31.3	47.2	34.5	74.6	66.4	69.1	72
Average	56.5	72.4	47.2	31.9	21.1	71.7	68.5	68.2	72.7
SD	6.5	8.5	11.4	13.8	11.8	2.9	2.1	0.9	4.5
P	ND	0.02*	0.35	0.06	0.05*	ND	ND	ND	0.02*

h hour, *ND* not done, *SD* standard deviation

*$P \leq 0.05$

(Table 4). Significant difference in the mean percentage of proteasome inhibition at 48 h after the first dosing on day 1 also was observed between the two groups ($P = 0.05$). In addition, there was no significant difference in the mean percentage of proteasome inhibition at 0 h (before dosing) on days 1, 6, and 16 between the two treatment groups ($P > 0.05$, data not shown), which demonstrated a complete recovery to baseline activity between dosing. However, this observation should be interpreted cautiously because of the limited number of patients and dose levels in this study.

Safety

Eighty-five patients who received at least one dose of bortezomib were evaluable for safety. The safety profiles were summarized in Table 5. The rates of all grade hematological toxicity and grade 3 or higher non-hematological toxicities were similar between the two treatment groups. Rates of diarrhea events of any grade, and of severity grade 3 and higher were higher in group A than in group B (59.5% vs. 32.6%, $P = 0.011$, and 23.8% vs. 7.0%, $P = 0.03$, respectively). However, there were no significant differences in rates of peripheral sensory neuropathy or all grades of burbulence events between the two groups. No patient in the study died of toxic effects. Two older-than-65 patients discontinued the treatment due to peripheral sensory neuropathy of grade 3 or higher in group A during induction stage. One patient aged 65 discontinued the treatment due to infection of grade 3 or higher in group B during consolidation stage. Bortezomib dose modifications occurred in a higher proportion of patients in groups A when compared with group B (28.6% vs. 7.0%, $P = 0.009$). Dose reductions of 25% or more were implemented for 12 patients in the higher dose group (A) and for three patients in the lower dose group (B). In group A, bortezomib dose reduction occurred for eight patients (23.5%) during the induction therapy, including seven patients due to grade 3 diarrhea (6 of them were older than 65) and one patient (younger than 65) due to grade 3 burbulence, while four patients had bortezomib dose reduction during the consolidation therapy, including three due to grade 3 diarrhea (all younger than 65) and one due to grade 3 burbulence (younger than 65). In group B, two patients (4.9%, one older than 65) had bortezomib dose reduction due to grade 3 diarrhea during induction therapy and one patient (younger than 65) had dose reduction due to grade 3 diarrhea during consolidation therapy. During induction, the rate of dose reduction of 25% or more due to 3–4 diarrhea or burbulence were was significantly higher for higher dose group (A) than that of lower dose group (B) ($P = 0.041$).

Table 5 Most common adverse events by treatment group

Adverse event type	Group A ($n=42$)		Group B ($n=43$)	
	All grades	Grade ≥ 3	All grades	Grade ≥ 3
Hematologic				
Leukopenia	14 (33.3%)	3 (7.1%)	15 (34.9%)	4 (9.3%)
Thrombocytopenia	17 (40.4%)	6 (14.3%)	15 (34.9%)	7 (16.3%)
Neutropenia	14 (33.3%)	3 (7.1%)	15 (34.9%)	4 (9.3%)
Nonhematologic				
Fatigue	13 (31%)	0	17 (39.5%)	1 (2.3%)
Peripheral sensory neuropathy	12 (28.6%)	2 (4.8%)	10 (23.3%)	1 (2.3%)
Nausea	9 (21.4%)	1 (2.4%)	6 (14%)	0
Diarrhea	25 (59.5%)*	10 (23.8%)*	14 (32.6%)	3 (7%)
Constipation	5 (11.9%)	2 (4.8%)	7 (16.3%)	0
Vomiting	6 (14.3%)	2 (4.8%)	4 (9.3%)	0
Edema limbs	2 (4.8%)	0	3 (7%)	1 (2.3%)
Creatinine increased	1 (2.4%)	1 (2.4%)	0	0
Infection	16 (38.1%)	16 (38.1%)	20 (46.5%)	19 (44.2%)
Burbulence	11 (26.2%)	2 (4.8%)	5 (11.6%)	0
Asthenia	6 (14.3%)	1 (2.4%)	5 (11.6%)	0
Anorexia	15 (35.7%)	2 (4.8%)	18 (41.9%)	1 (2.3%)
Treatment discontinuations because of AEs		2 (4.8%)		1 (2.3%)
Bortezomib dose reductions because of AEs		12 (28.6%)*		3 (7%)

*$P \leq 0.05$

Survival analysis

First, we analyzed PFS and OS between group A and group B with ITT. Between median follow-up of 29 months (IQR 2.5–61.3) in group A and 33.9 months (1.8–60.2) in group B, we noted no significant differences in PFS (median 30.6 months vs. 28.8 months, $P=0.481$) and OS (2-year survival 75.8%, vs. 78.3%; $P=0.995$) (Fig. 2a, b). We further analyzed the effect of prognosis stratification on PFS and OS (Fig. 2c, d). Unexpectedly, we found that the patients in group A with R-ISS III stage had inferior PFS to those in group A with R-ISS I or II stage (median 20.0 vs. 35.7 months, $P=0.038$). However, the patients in group B with R-ISS III stage had similar PFS to those in group B with R-ISS I or II stage (median 29.7 vs. 27.5 months, $P=0.8$). In addition, patients in group A with R-ISS III stage or R-ISS I or II stage had similar PFS to those in group B (R-ISS III stage, median 20 vs. 29.7 months, $P=0.363$; R-ISS I or II stage, median 35.7 vs. 27.5 months, $P=0.128$). Numerically, the median PFS of patients with R-ISS I or II stage was higher for group A (35.7 months) than that for group B (27.5 months). The opposite is true for the patients with R-ISS III stage. OS was not significantly different among the four subgroups ($P>0.05$).

Second, PFS and OS of patients who completed the total nine cycles without bortezomib dose reductions were analyzed. There were 27 such patients including 12 in group A and 15 in group B. As shown in Fig. 3, no significant differences in PFS (median 39.2 vs. 30.8 months, $P=0.149$) and OS (median 48 vs. 52.5 months, $P=0.838$) were observed between these two subsets of patients.

Discussion

The results from this open-label and multicenter randomized phase II study of NDMM patients showed that the modified VCD regimen with bortezomib 1.6 mg/m^2 on days 1, 6, 11, and 16 by subcutaneous injection (group A) as induction regimen was associated with higher CR or VGPR rates, especially for patients < 65 years old or with R-ISS stage III, than that of bortezomib 1.3 mg/m^2 (group B). Patients younger than 65 years also were benefited by the higher dose of bortezomib during consolidation therapy. This efficacy advantage of high-dose bortezomib might be explained by the findings of the pharmacodynamic analyses. The time to Emax (maximum proteasome inhibition) was 2 h after bortezomib dosing in both groups, which is similar with the results reported by Moreau et al. in patients with relapsed MM (Lightcap et al. 2000). This maximum inhibition increased with the dose increased, and could explain the faster onset response and a better response rate in group A than that of group B. However, at the completion of all nine cycles (induction + consolidation), we observed similarity between the two groups across all efficacy endpoints, including rates of VGPR, PR, and ORR. In addition, PFS and OS

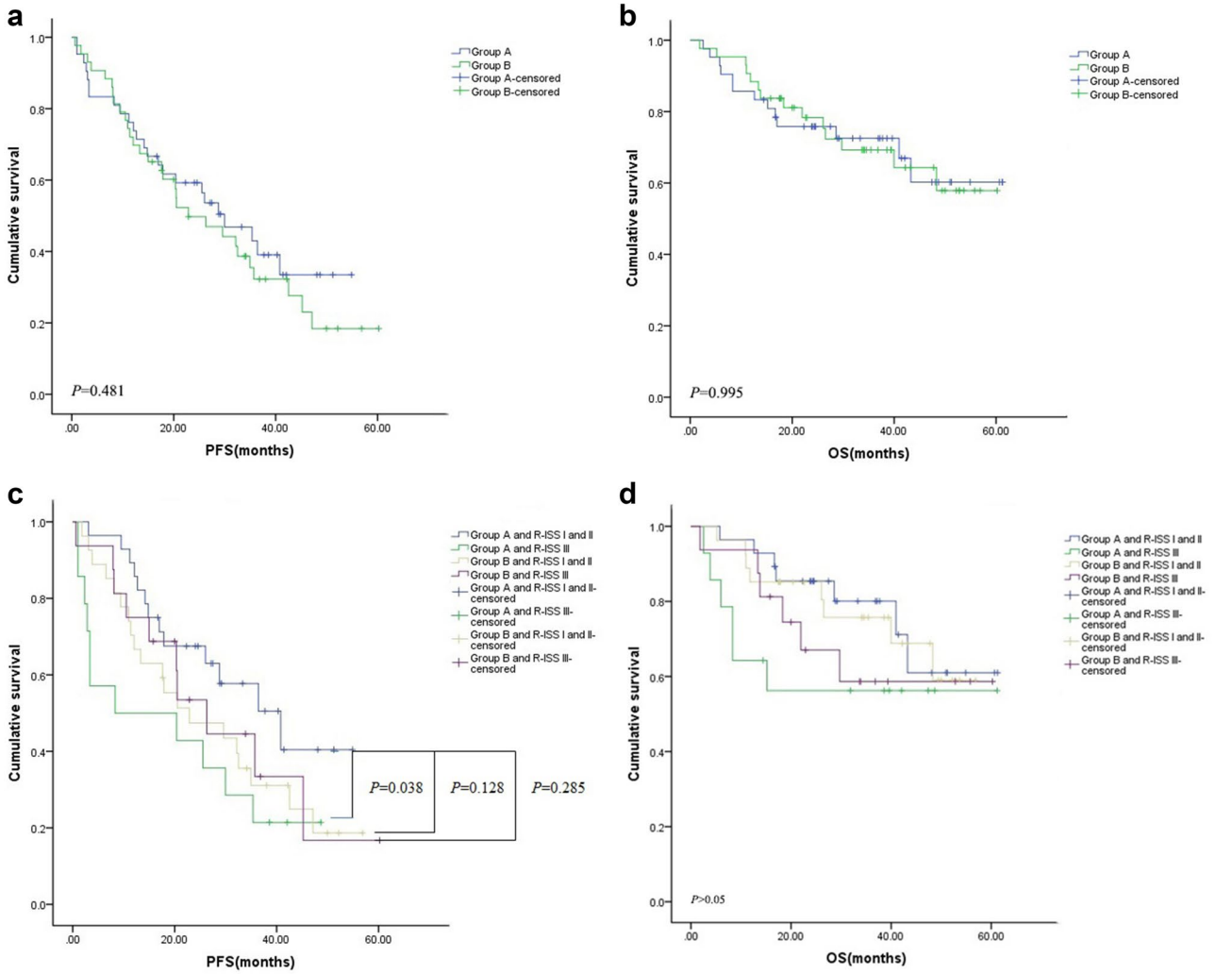

Fig. 2 PFS and OS of group A and group B (ITT). There were no significant differences in PFS ($P=0.481$) and OS ($P=0.995$) between the two groups (**a**, **b**). The effect of prognosis stratification on PFS and OS was also analyzed (**c**, **d**). Group A with R-ISS I or II stage ($n=28$): blue curve; group A with R-ISS III stage ($n=14$): green curve; group B with R-ISS I or II stage ($n=27$): brown curve; group B with R-ISS III stage ($n=16$): purple curve. Group A patients with R-ISS III stage had inferior PFS to those with R-ISS I or II stage ($P=0.038$). However, the patients with R-ISS III stage in group B had similar PFS to those with R-ISS I or II stage ($P=0.8$). The patients in group A with R-ISS III stage had similar PFS to those in group B with R-ISS III stage ($P=0.363$). The patients in group A with R-ISS I or II stage had similar PFS to those in group B with R-ISS I or II stage (median 35.7 vs. 27.5 months, $P=0.128$). There was no significant difference in OS among the four subgroups ($P>0.05$)

were not significantly different between the two treatment groups after a median follow-up of 29 months in group A and 33.9 months in group B. Furthermore, the higher bortezomib dose also seemed more beneficial for R-ISS I or II stage patients than for R-ISS III patients indicated by higher PFS median.

CR achievement after both induction therapy and autologous stem cell transplantation with minimal side effects is one of the most powerful predictors for long-term outcome and represents a major endpoint of the treatment strategy in younger MM patients (Tricot et al. 1995). The current study of VCDs as induction therapies in transplant-eligible NDMM patients was heterogeneous in terms of treatment dosing and schedules. It was worth mentioning that 72.4% of the patients were transplant-eligible (VGPR or better) after four cycles of induction therapy in our trial groups, which is higher than other studies (Reeder et al. 2009, 2010; Bensinger et al. 2010; Kumar et al. 2012; Moreau et al. 2016; Tanake et al. 2019) listed in Table 6 (Supplementary material). In addition, the rate of CR or better was similar in our trial (44.8%) to that reported in Reeder's trial with higher cumulative doses of cyclophosphamide or dexamethasone (Reeder et al. 2010). In the meantime, the rate of grade 3 or worse PN in our study was not higher than that in the studies

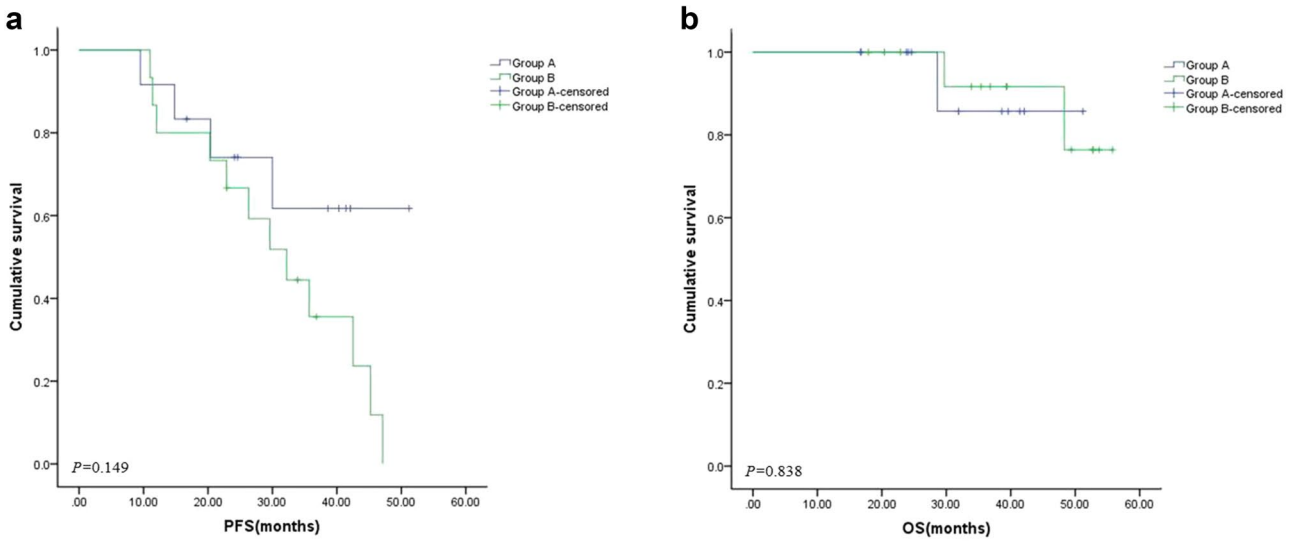

Fig. 3 PFS and OS of group A and group B with patients who completed the total nine cycles of treatment without bortezomib dose reductions. No significant differences in PFS ($P=0.149$) (**a**) and OS ($P=0.838$) (**b**)

listed in Table 6. Whereas the rate of diarrhea of grade 3 or worse in our study was higher but manageable than that of the studies listed in Table 6. The diarrhea was controlled by Loperamide during the study period. The higher rates of diarrhea in group A of the current study could be explained by the higher mean percentage of proteasome inhibition at 48 h after dosing in group A and/or other factors. Between the two groups of our study, bortezomib dose reduction occurred with a higher proportion in groups A than in group B during induction and after therapy completion, especially for patients older than 65 during induction. Considering the overall adverse events, the modified VCD regimen of bortezomib 1.6 mg/m^2 on days 1, 6, 11, and 16, by subcutaneous injection was good for patients younger than 65 as induction, but was not well tolerated as induction for patients older than 65 or as consolidation regimens for all patients. Moreau et al. (2016) reported that VTD was superior to VCD prior to intensive therapy in multiple myeloma. The VTD treatment in the study consisted of four 3-week cycles of bortezomib 1.3 mg/m^2 administered subcutaneously (SC) on days 1, 4, 8, and 11, dexamethasone 40 mg on days 1–4, 9–12, plus thalidomide 100 mg/day administered orally. The rate of VGPR or better after four cycles of induction therapy of transplant-eligible NDMM patients in VTD regimen was 66.3%, which was lower than 72.4% in our trial groups. The rate of CR or better after four cycles of induction therapy of transplant-eligible NDMM patients in VTD regimen was 13%, which was lower than 44.8% in our trial groups. The rate of 3–4 grade PN was 7.7% in the VTD arm, which was higher than 4.8% in our trial groups. On the other hand, the modified VCD regimen with bortezomib 1.3 mg/m^2 on days 1, 6, 11, and 16, by subcutaneous injection was well tolerated as consolidation regimens especially in elderly patients. For patients older than 65 years in the 1.3 mg/m^2 dose group (B), the rate of VGPR or better and CR or better after induction and consolidation therapies (9 cycles) was 80% and 40%, respectively. This result of our study is better than those reported recently by Tuchman et al. (2017), with the rate of VGPR or better and CR or better was 41% and 17.9%, respectively. Jimenez-Zepeda et al. (2017) also suggested that CyBorD regimen was a feasible and tolerated regimen for the treatment of transplant-ineligible patients. The CyBorD of the study consisted of a 28-day cycle where bortezomib was given SC or IV at 1.3–1.5 mg/m^2 weekly for 3–4 weeks, cyclophosphamide 300 mg/m^2 PO once weekly for 3–4 weeks, and dexamethasone 20–40 mg PO once weekly. Thirteen of 42 patients were treated with subcutaneous bortezomib. The rate of VGPR or better and CR or better after induction and consolidation therapies (median 6 cycles) was 76.1% and 19%, respectively, which was inferior to ours. Based on these results, we suggest that bortezomib high-dose strategy (1.6 mg/m^2) can apply to young and high-risk patients as induction regimen before transplantation. The modified VCD regimen with 1.3 mg/m^2 bortezomib on days 1, 6, 11, and 16 by subcutaneous injection seems a feasible therapy for patients unfit for transplant.

After a median follow-up of 29 months in group A and 33.9 months in group B, PFS and OS were not significantly different in ITT analysis. As expected, this study showed that the rate of CR in high-risk patients (R-ISS III stage) after induction therapy was improved by the high-dose bortezomib (group A). Unexpectedly, we found that the R-ISS III stage patients in group A had inferior PFS to those of R-ISS I or II stage, while the patients in group

B with R-ISS III stage had similar PFS to those in group B with R-ISS I or II stage. Furthermore, PFS of R-ISS III stage between group A and group B were statistically similar (median 20.0 vs. 29.7 months, $P = 0.363$). The patients with R-ISS III stage in group A had the shortest PFS in all subgroups, which might due to having more risk factors such as high plasma cell labelling index (PCLI) (Larsen et al. 2011), high-risk signature in gene expression profile (GEP) (Mitra et al. 2017) or soft-tissue extramedullary infiltration (Pour et al. 2014). Unfortunately, the risk factors were not assessed in this study. Future studies should consider using GEP and 18F-fluoro-deoxyglucose positron emission tomography-computed tomography (18F-FDG-PET/CT) to further investigate the prognostic stratification of patients with MM (Chng et al. 2016; Zamagni et al. 2011). In addition, these high-risk patients in group A had not undergone stem cell transplantation (ASCT) (Lee et al. 2015). This may be another important reason for the shorter PFS of the patients with R-ISS III stage in group A. Recent studies indicated that maintenance therapy with bortezomib represented a safe, well tolerated, and efficacious option for patients with high-risk cytogenetics, renal insufficiency or another high risk factor (Chakraborty et al. 2018; Sivaraj et al. 2017). In our study, all patients who completed induction and consolidation chemotherapy were given cyclophosphamide alone or in combination with prednisone as maintenance therapy. We did not select different maintenance regimens based on risk stratification, such as bortezomib for patients with high risk factors, which might be one more reason for the short PFS of patients with R-ISS III stage in group A. In the current study, a total of 27 patients completed the total nine cycles of treatment without bortezomib dose reductions including 12 patients in group A and 15 patients in group B. Therefore, it was worth mentioning that the benefit of high bortezomib dose was observed in this study, though there was no statistically significant difference in PFS and OS, which may be attribute to the limit sample size.

In conclusion, the modified VCD regimen (bortezomib 1.6 mg/m^2 on days 1, 6, 11, and 16, by subcutaneous injection) as induction regimen was associated with rapid CR and higher CR rate, especially for patients < 65 years or with R-ISS stage III. Moreover, the regimen had a quick onset, but it is not well tolerated as consolidation regimens. This high-dose strategy seems feasible for young and high-risk patients for induction regimen before stem cell transplant. This conclusion needs further verification by phase III clinical trials. On the other hand, the modified VCD regimen (bortezomib 1.3 mg/m^2 on days 1, 6, 11, and 16, by subcutaneous injection) might be considered as an induction and consolidation regimen for patients who are unfit or will not undergo transplant, which also should be further assessed in future randomized trials.

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Author contributions FL designed and conducted the experiments and wrote the manuscript. FL, F-SY, X-JZ, W-YG, X-HW, BC, D-PH, J-HD, T-QW, YZ, QZ, Y-MT, PS, X-GZ, Z-MA, XG, X-LW, LZ and X-BX carried out the collection of samples and clinical data. FL conducted the analysis of the clinical data. Y-PZ contributed to the experimental design, the review and revision of the manuscript, and the final approval of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that no conflicts of interest exist.

Ethical statements All patients provided written informed consent for the use of their samples and data, and the study was approved by the Ethics Committee of Jinling Hospital, Nanjing, China.

References

Bensinger WI, Jagannath S, Vescio R et al (2010) Phase 2 study of two sequential three-drug combinations containing bortezomib, cyclophosphamide and dexamethasone, followed by bortezomib, thalidomide and dexamethasone as frontline therapy for multiple myeloma. Br J Haematol 148:562–568

Blaney SM, Bernstein M, Neville K et al (2004) Phase I study of the proteasome inhibitor bortezomib in pediatric patients with refractory solid tumors: a Children's Oncology Group study (ADVL0015). J Clin Oncol 22:4804–4809

Cavo M, Tacchetti P, Patriarca F et al (2010) Bortezomib with thalidomide plus dexamethasone compared with thalidomide plus dexamethasone as induction therapy before, and consolidation therapy after, double autologous stem-cell transplantation in newly diagnosed multiple myeloma: a randomised phase 3 study. Lancet 376:2075–2085

Cavo M, Rajkumar SV, Palumbo A et al (2011) International Myeloma Working Group consensus approach to the treatment of multiple myeloma patients who are candidates for autologous stem cell transplantation. Blood 117:6063–6073

Chakraborty R, Muchtar E, Kumar SK et al (2018) Outcomes of maintenance therapy with lenalidomide or bortezomib in multiple myeloma in the setting of early autologous stem cell transplantation. Leukemia 32:712–718

Chng WJ, Chung TH, Kumar S et al (2016) Gene signature combinations improve prognostic stratification of multiple myeloma patients. Leukemia 30:1071–1078

Harousseau JL, Attal M, Avet-Loiseau H et al (2010) Bortezomib plus dexamethasone is superior to vincristine plus doxorubicin plus dexamethasone as induction treatment prior to autologous stem-cell transplantation in newly diagnosed multiple myeloma: results of the IFM 2005-01 phase III trial. J Clin Oncol 28:4621–4629

He J, Yang L, Han X et al (2014) The choice of regimens based on bortezomib for patients with newly diagnosed multiple myeloma. PLoS One 9:e99174

Jagannath S, Barlogie B, Berenson J et al (2004) A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol 127:165–172

Jimenez-Zepeda VH, Duggan P, Nerl P et al (2017) Bortezomib-containing regimens (BCR) for the treatment of non-transplant eligible multiple myeloma. Ann Hematol 96:431–439

Kumar S, Flinn I, Richardson PG et al (2012) Randomized, multicenter, phase 2 study (EVOLUTION) of combinations of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide in previously untreated multiple myeloma. Blood 119:4375–4382

Larsen JT, Chee CE, Lust JA et al (2011) Reduction in plasma cell proliferation after initial therapy in newly diagnosed multiple myeloma measures treatment response and predicts improved survival. Blood 118:2702–2707

Lee SE, Kim JH, Jeon YW et al (2015) Impact of extramedullary plasmacytomas on outcomes according to treatment approach in newly diagnosed symptomatic multiple myeloma. Ann Hematol 94:445–452

Lightcap ES, McCormack TA, Pien CS et al (2000) Proteasome inhibition measurements: clinical application. Clin Chem 46:673–683

Mai EK, Bertsch U, Durig J et al (2015) Phase III trial of bortezomib, cyclophosphamide and dexamethasone (VCD) versus bortezomib, doxorubicin and dexamethasone (PAd) in newly diagnosed myeloma. Leukemia 29:1721–1729

Mateos MV, Oriol A, Martinez-Lopez J et al (2010) Bortezomib, melphalan, and prednisone versus bortezomib, thalidomide, and prednisone as induction therapy followed by maintenance treatment with bortezomib and thalidomide versus bortezomib and prednisone in elderly patients with untreated multiple myeloma: a randomised trial. Lancet Oncol 11:934–941

Mitra AK, Harding T, Mukherjee UK et al (2017) A gene expression signature distinguishes innate response and resistance to proteasome inhibitors in multiple myeloma. Blood Cancer J 7:e581

Moreau P, Pylypenko H, Grosicki S et al (2011) Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: a randomised, phase 3, non-inferiority study. Lancet Oncol 12:431–440

Moreau P, Hulin C, Macro M et al (2016) VTD is superior to VCD prior to intensive therapy in multiple myeloma: results of the prospective IFM2013-04 trial. Blood 127:2569–2574

Pour L, Sevcikova S, Greslikova H et al (2014) Soft-tissue extramedullary multiple myeloma prognosis is significantly worse in comparison to bone-related extramedullary relapse. Haematologica 99:360–364

Reeder CB, Reece DE, Kukreti V et al (2009) Cyclophosphamide, bortezomib and dexamethasone induction for newly diagnosed multiple myeloma: high response rates in a phase II clinical trial. Leukemia 23:1337–1341

Reeder CB, Reece DE, Kukreti V et al (2010) Once- versus twice-weekly bortezomib induction therapy with CyBorD in newly diagnosed multiple myeloma. Blood 115:3416–3417

Richardson PG, Barlogie B, Berenson J et al (2003) A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med 348:2609–2617

Richardson PG, Briemberg H, Jagannath S et al (2006) Frequency, characteristics, and reversibility of peripheral neuropathy during treatment of advanced multiple myeloma with bortezomib. J Clin Oncol 24:3113–3120

Richardson PG, Mitsiades C, Schlossman R et al (2007) New drugs for myeloma. Oncologist 12:664–689

Sivaraj D, Green MM, Li Z et al (2017) Outcomes of maintenance therapy with bortezomib after autologous stem cell transplantation for patients with multiple myeloma. Biol Blood Marrow Transplant 23:262–268

Tanake K, Toyota S, Akiyama M et al (2019) Efficacy and safety of a weekly cyclophosphamide–bortezomib–dexamethasone regimen as induction therapy prior to autologous stem cell transplantation in japanese patients with newly diagnosed multiple myeloma: a phase 2 multicenter trial. Acta Haematol 141:111–118

Tricot G, Jagannath S, Vesole D et al (1995) Peripheral blood stem cell transplants for multiple myeloma: identification of favorable variables for rapid engraftment in 225 patients. Blood 85:588–596

Tuchman SA, Moore JO, DeCastro CD et al (2017) Phase II study of dose-attenuated bortezomib, cyclophosphamide and dexamethasone ("VCD-Lite") in very old or otherwise toxicity-vulnerable adults with newly diagnosed multiple myeloma. J Geriatr Oncol 8:165–169

Zamagni E, Patriarca F, Nanni C et al (2011) Prognostic relevance of 18-F FDG PET/CT in newly diagnosed multiple myeloma patients treated with up-front autologous transplantation. Blood 118:5989–5995

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