

Pharmacokinetic and pharmacodynamic study of two doses of bortezomib in patients with relapsed multiple myeloma

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Abstract

Purpose Characterize bortezomib pharmacokinetics/pharmacodynamics in relapsed myeloma patients after single and repeat intravenous administration at two doses.

Methods Forty-two patients were randomized to receive bortezomib 1.0 or 1.3 mg/m², days 1, 4, 8, 11, for up to eight 21-day treatment cycles ($n = 21$, each dose group). Serial blood samples for pharmacokinetic/pharmacodynamic analysis were taken on days 1 and 11, cycles 1 and 3. Observational efficacy and safety data were collected.

Results Twelve patients in each dose group were evaluable for pharmacokinetics/pharmacodynamics. Plasma clearance decreased with repeat dosing (102–112 L/h for

first dose; 15–32 L/h following repeat dosing), with associated increases in systemic exposure and terminal half-life. Systemic exposures of bortezomib were similar between dose groups considering the relatively narrow dose range and the observed pharmacokinetic variability, although there was no readily apparent deviation from dose-proportionality. Blood 20S proteasome inhibition profiles were similar between groups with mean maximum inhibition ranging from 70 to 84% and decreasing toward baseline over the dosing interval. Response rate (all 42 patients) was 50%, including 7% complete responses. The safety profile was consistent with the predictable and manageable profile previously established; data suggested milder toxicity in the 1.0 mg/m² group.

Conclusions Bortezomib pharmacokinetics change with repeat dose administration, characterized by a reduction in

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plasma clearance and associated increase in systemic exposure. Bortezomib is pharmacodynamically active and tolerable at 1.0 and 1.3 mg/m² doses, with recovery toward baseline blood proteasome activity over the dosing interval following repeat dose administration, supporting the current clinical dosing regimen.

Keywords Pharmacodynamics · Pharmacokinetics · Bortezomib · Multiple myeloma · Proteasome inhibition

Introduction

The first-in-class proteasome inhibitor bortezomib (VELCADE®; Millennium Pharmaceuticals, Inc., Cambridge, MA, and Johnson & Johnson Pharmaceutical Research & Development L.L.C., Raritan, NJ) is approved in the US for the treatment of patients with multiple myeloma and for the treatment of patients with mantle cell lymphoma following at least one prior therapy [1], in the EU [2] and Canada for the treatment of previously untreated patients with multiple myeloma in combination with melphalan and prednisone, and in these and other countries worldwide for the treatment of patients with multiple myeloma following at least one prior therapy. Bortezomib inhibits the ubiquitin–proteasome pathway, which is responsible for the degradation of most intracellular proteins [3], by blocking the activity of the 26S proteasome [1, 4]. Consequently, bortezomib disrupts multiple downstream signaling pathways in cells and the bone marrow microenvironment, inducing apoptosis and inhibiting cell-cycle progression, angiogenesis, cell adhesion, and proliferation, and resulting in anti-tumor activity [4, 5]. Bortezomib alone and in combination has shown substantial activity in patients with newly diagnosed [6–9] and relapsed/refractory [10–15] MM. In the VISTA phase 3 study in previously untreated patients with MM ineligible for stem cell transplantation, the addition of bortezomib to melphalan–prednisone resulted in significantly superior response rates, time to progression, and overall survival [9, 16]. In the APEX phase 3 study in patients with relapsed MM following 1–3 prior therapies, bortezomib demonstrated superiority versus high-dose dexamethasone in terms of response rate, time to progression, and overall survival [13]; at an updated analysis, the response rate to bortezomib was 43%, including 15% complete or near-complete responses (CR/nCR), and bortezomib showed a 6-month survival benefit versus dexamethasone (median 29.8 vs. 23.7 months) [15].

The recommended standard dose and schedule for single-agent bortezomib is 1.3 mg/m² intravenously (IV) on days 1, 4, 8, and 11 of a 21-day cycle, for up to 8 cycles [1]. The twice-weekly schedule was selected based on the findings of animal studies of inhibition of proteasome activity,

which showed activity returning toward baseline within 48–72 h following inhibition by bortezomib dosing [17]. The standard 1.3 mg/m² dose and schedule was shown to be active and well tolerated in the APEX phase 3 [13] and SUMMIT [12] and CREST [10] phase 2 studies in patients with relapsed/refractory MM. A lower dose of 1.0 mg/m² was also investigated, using the same schedule, in the CREST study [10].

The pharmacokinetic profile of bortezomib is characterized by a two-compartment model, with a rapid initial distribution phase followed by a longer elimination phase and a large volume of distribution [18]. In vitro studies have shown the primary metabolic pathway of bortezomib to be hepatic oxidative deboronation by multiple cytochrome P450 enzymes, including CYP3A4, CYP2C19, CYP1A2, CYP2D6, and CYP2C9 [1, 19–21].

The primary objectives of this study were to characterize the pharmacokinetics and pharmacodynamics of bortezomib after single and repeat IV administration at the two-dose levels explored in clinical trials, 1.0 and 1.3 mg/m², in patients with relapsed MM. Observational efficacy and safety data were also collected.

Materials and methods

Patient selection

Patients aged ≥18 years with relapsed MM following ≥1 prior line of chemotherapy were eligible. Additional eligibility criteria included Karnofsky performance score (KPS) ≥70%, life expectancy ≥3 months, the resolution of toxicities related to prior therapy, and, at screening, aspartate transaminase (AST) and alanine transaminase (ALT) ≤2 × upper limit of normal (ULN), total bilirubin ≤1.5 × ULN, hemoglobin ≥10 g/dl, platelets ≥50 × 10⁹/L, absolute neutrophil count (ANC) ≥1,000/μl, calculated creatinine clearance ≥50 ml/min, and normal serum calcium (8.6–10.3 mg/dl).

Patients were excluded if they had significant cardiac disease, an active systemic infection requiring treatment, serious medical/psychiatric illness, grade ≥2 neuropathy (National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE] version 3.0), active hepatitis, HIV disease, a secondary malignancy, plasma cell leukemia, or polyneuropathy, organomegaly, M-protein, skin changes (POEMS) syndrome. Patients were also excluded if they were transfusion-dependent or had received extensive radiation therapy (per investigator's discretion), systemic chemotherapy, other antineoplastic therapy, or another investigational agent within 4 weeks, or thalidomide within 2 weeks, of enrollment. Concurrent treatment with CYP3A4 inhibitors/inducers and corticoste-

roids, except prednisone or equivalent ≤ 10 mg/day, was prohibited.

Study design

This prospective, multicenter, randomized study was performed in five centers in the USA and two centers in Canada between February 2004 and August 2006. The study was conducted in accordance with the Declaration of Helsinki and with the International Conference on Harmonisation Good Clinical Practice. The protocol was approved by an independent ethics committee/institutional review board at each participating institution. All patients provided written informed consent.

Patients were randomized (1:1) to receive bortezomib at a dose of 1.0 or 1.3 mg/m² on days 1, 4, 8, and 11, for up to eight 21-day cycles (or more at Canadian sites, where bortezomib was not available commercially at the time of this study). Patients discontinued treatment if they had unacceptable toxicity, progressive disease, lack of efficacy, or a significant intercurrent illness, or by patient/investigator decision. Dose reductions (1.3–1.0, and 1.0–0.7 mg/m²; further reduction from 0.7 to 0.4 mg/m² permitted if patient experiencing benefit) were specified for grade ≥ 3 neutropenia with fever, grade 4 neutropenia for >7 days, thrombocytopenia with platelets $<10 \times 10^9$ /L, or any grade ≥ 3 non-hematologic toxicity considered related to bortezomib, except peripheral neuropathy, which was managed according to established dose modification guidelines [1]. Toxicities were graded according to NCI CTCAE v3.0. Dose escalation was not allowed during cycles 1–3. After cycle 3, dose escalation to 1.3 mg/m² was permitted for patients in the 1.0 mg/m² dose group. Dose escalation was not permitted for patients in the 1.3 mg/m² dose group. Patients in both groups could receive added dexamethasone, from treatment cycle 4 onward, at the discretion of the investigator.

Pharmacokinetic and pharmacodynamic assessments

Blood samples for the pharmacokinetic and pharmacodynamic analyses were taken at the following time points: prior to, and at 5, 15, and 30 min, and 1, 2, 4, 6, 8, 12, 24, and 48 h following dosing on days 1 and 11, cycles 1 and 3; prior to dosing on days 4 and 8 and on days 15, 17, and 19, cycles 1 and 3; and prior to dosing on day 1, cycles 2 and 4. Bortezomib plasma concentrations were analyzed by Advion (Ithaca, NY, USA) by a validated liquid chromatography coupled with tandem mass spectrometry method. The following pharmacokinetic parameters for days 1 and 11, cycles 1 and 3, were estimated by non-compartmental analysis of plasma concentration–time data: maximum concentration ($C_{\max}$), area under the curve (AUC) from time 0

to 48 h (AUC_{48h}), AUC from time 0 to the last measurable concentration (AUC_{last}), terminal phase half-life ($t_{1/2}$), total body clearance (CL), and beta-phase volume of distribution (V_{β}). Dose-proportionality assessment was based on $C_{\max}$ and AUC_{48h} values. Percent inhibition of the activity of the 20S proteasome, a subunit of the 26S proteasome, relative to baseline was determined using a whole-blood 20S proteasome-specific activity inhibition assay [22]. The following pharmacodynamic parameters for days 1 and 11, cycles 1 and 3, were calculated: maximum percent inhibition of 20S proteasome activity ($E_{\max}$), time to $E_{\max}$ ($T_{\max}$), and area under the percent inhibition–time curve from time 0 to 48 h (AUE_{48h}).

Efficacy and safety assessments

M-protein levels were quantified in serum and 24-h urine samples collected at screening and during the rest period of cycles 2, 3, 4, 6, and 8. Response was determined by investigators in accordance with the European Group for Blood and Marrow Transplantation (EBMT) criteria [23]. Immunofixation was only performed for patients considered to have achieved CR.

A complete medical history was taken and a complete physical examination performed at screening. Vital signs, patient weight and body surface area, KPS, and clinical and laboratory parameters were analyzed at screening and at specified time points during cycles 1–4. Safety was monitored throughout the study until 30 days after the last dose of bortezomib. Treatment-emergent adverse events (AEs) were recorded up to day 1, cycle 4, and graded according to NCI CTCAE v3.0. Neurotoxicity AEs and serious AEs (SAEs) were recorded for the entire study duration. Patient-reported symptoms of peripheral neuropathy were assessed using the neurotoxicity subscale of the Functional Assessment of Cancer Therapy scale/Gynecologic Oncology Group (FACT/GOG-Ntx) [24], which comprises 11 questions evaluating the presence of peripheral neuropathy symptoms on a scale of 0 (not at all) to 4 (very much), with a total score of 0–44. Patients completed this questionnaire at screening and on day 1 of each treatment cycle.

Statistical methods

Approximately 40 patients were to be enrolled to obtain 24 patients evaluable for pharmacokinetic and pharmacodynamic analyses, 12 in each dose group; this sample size per dose group was considered sufficient for characterizing the pharmacokinetic and pharmacodynamic profiles of bortezomib and to estimate the inter-individual variation in pharmacokinetics. Patients who completed bortezomib dosing through day 11, cycle 3, without a dose modification, and who had sufficient samples for non-compartmental

Table 1 Baseline demographics and disease characteristics of patients in the bortezomib 1.0 and 1.3 mg/m² dose groups, and overall

	1.0 mg/m ² (<i>n</i> = 21)	1.3 mg/m ² (<i>n</i> = 21)	Total (<i>n</i> = 42)
Median age (years)	62	61	61.5
Male [<i>n</i> (%)]	12 (57)	11 (52)	23 (55)
White [<i>n</i> (%)]	15 (71)	14 (67)	29 (69)
Median body mass index (kg/m ²)	25.6	27.8	27.2
KPS ≥ 90% [<i>n</i> (%)]	13 (68)	11 (52)	24 (60)
Type of myeloma IgG/IgA [<i>n</i> (%)]	12 (57)/5 (24)	14 (67)/5 (24)	26 (62)/10 (24)
Median time from diagnosis (years)	3	3	3
Median calculated creatinine clearance, median (ml/min)	64.3	75.9	70.4
Median hemoglobin (g/L)	107	104	106
Median platelets (×10 ⁹ /L)	147	222 ^c	179.5
Symptoms of peripheral neuropathy [<i>n</i> (%)] ^a	14 (67)	16 (76)	30 (71)
Median no. of prior lines of therapy	3	2	2
Received prior therapy containing [<i>n</i> (%)] ^b			
Steroids	20 (95)	21 (100)	41 (98)
Alkylating agent	19 (90)	20 (95)	39 (93)
Anthracycline	15 (71)	13 (62)	28 (67)
Platinum/vincristine/taxanes	14 (67)	13 (62)	27 (64)
Stem cell transplant/high-dose therapy	15 (71)	11 (52)	26 (62)
Thalidomide	13 (62)	11 (52)	24 (57)
Experimental or other therapy	6 (29)	7 (33)	13 (31)
Lenalidomide	2 (10)	0	2 (5)

BMI body mass index, *KPS* Karnofsky performance status, *Ig* immunoglobulin

^a Any score > 0 on questions Ntx1–4, 8, or 9 of the FACT/GOG-Ntx questionnaire

^b Prior therapies could have included regimens containing more than one of the agents listed

^c *P* < 0.05 for comparison between dose groups; there were no other statistically significant differences

estimation of pharmacokinetic parameters were considered evaluable for these analyses. Pharmacokinetic parameters were calculated using SAS software (Windows Version 8.2) and calculations were verified using WinNonlinTM software (Version 4.0.1.a). Pharmacokinetic and pharmacodynamic parameters were summarized using mean and standard deviation as descriptive statistics for each dose group on days 1 and 11, cycles 1 and 3. Mean plasma concentration–time and percent inhibition–time profiles were plotted for each dose group on days 1 and 11, cycles 1 and 3. The efficacy and safety populations comprised all patients who received at least one dose of bortezomib. The study was not designed to compare efficacy and safety between the two dose groups.

Results

Patient characteristics and disposition

A total of 42 patients were enrolled, 21 to each dose group. Demographics and disease characteristics were similar between the two dose groups (Table 1). Fifteen patients

(71%) in each dose group completed treatment cycles 1–3, the pharmacokinetic–pharmacodynamic portion of the study, of whom 12 (57%) in each dose group were evaluable for analysis; three patients had dose holds (two in the 1.0 mg/m² dose group and one in the 1.3 mg/m² dose group), one patient in the 1.0 mg/m² dose group had a dose increase, one patient in the 1.3 mg/m² dose group had a dose reduction, and one patient in the 1.3 mg/m² dose group had incomplete data, and were therefore not evaluable for pharmacokinetic and pharmacodynamic analyses.

Pharmacokinetics

Mean plasma concentration–time profiles on days 1 and 11 of cycles 1 and 3 in each dose group are shown in Fig. 1, panels a and b. Plasma concentrations typically declined multi-exponentially with time, with a rapid initial decline in concentrations of over 10-fold within the first hour post-dose (Fig. 1 inset plots). Pharmacokinetic parameters following administration of bortezomib 1.0 and 1.3 mg/m² at the four time points are shown in Table 2. An increase in systemic exposure (AUC_{48h}) was seen following multiple-dose (day 11, cycle 1 onward) versus single-dose (day 1,

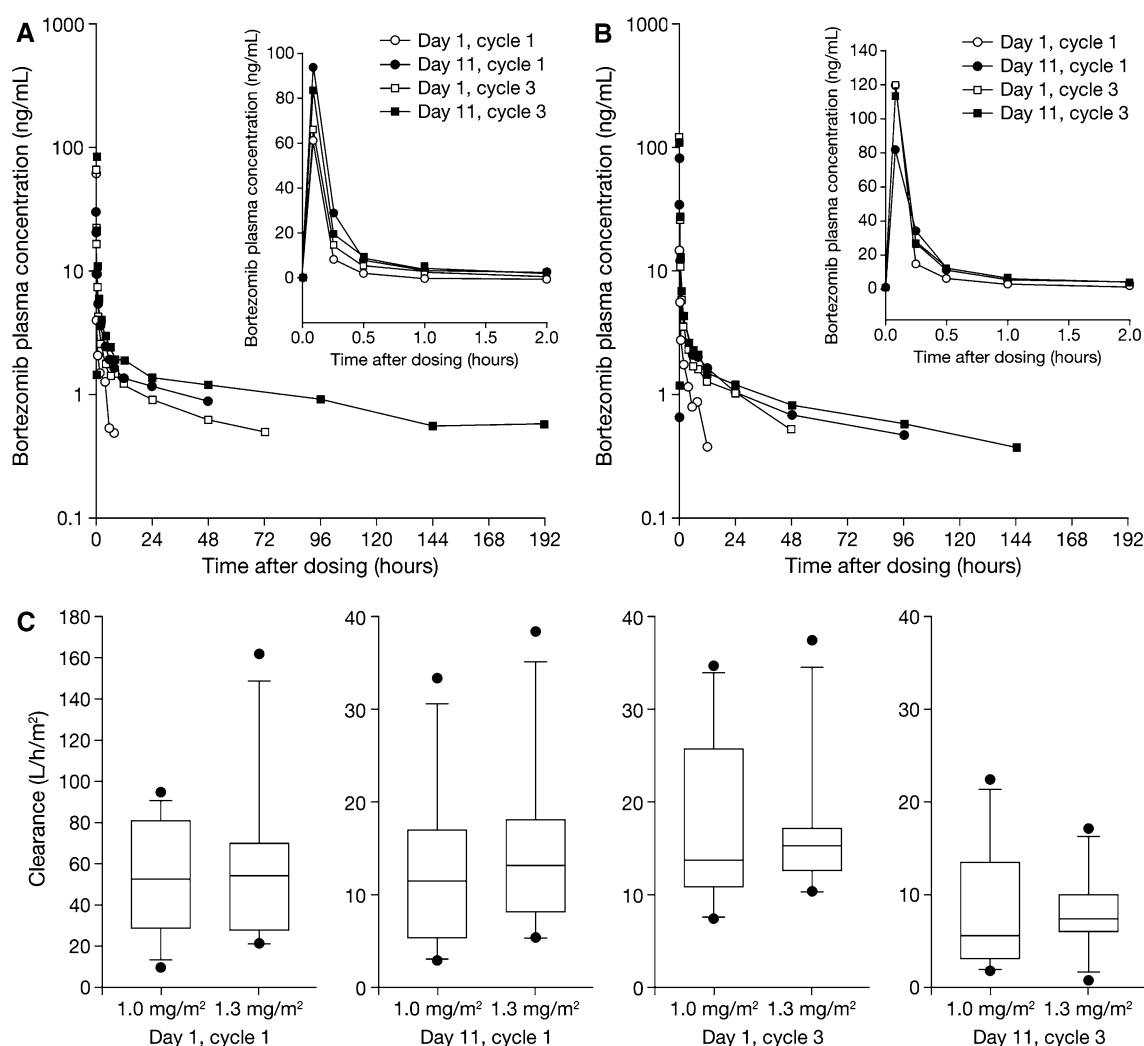


Fig. 1 Mean concentration–time profiles of bortezomib in plasma following administration of **a** 1.0 mg/m² and **b** 1.3 mg/m² ($n = 11$ or 12 per profile). **c** Box plots of body surface area-normalized bortezomib clearance over the duration of pharmacokinetic assessment

cycle 1) administration, and was associated with a substantial decrease in bortezomib clearance (102–112 L/h for the first dose; 15–32 L/h following repeat dosing) and an increase in terminal half-life. The clearance following bortezomib administration at the three multiple-dosing time points (day 11, cycle 1; days 1 and 11, cycle 3) was generally comparable indicating that although clearance decreased following repeat dose administration, there were no additional changes in bortezomib clearance beyond day 11, cycle 1. Mean volume of distribution was consistently high ($\geq 1,659$ L) across all time points in both dose groups, indicating extensive peripheral tissue distribution of bortezomib.

Pharmacokinetic variability was modest to large, with coefficients of variation in clearance ranging from 47 to 92% over the course of pharmacokinetic evaluation in this study. Plasma concentrations of bortezomib and systemic exposure (mean $C_{\max}$ and AUC) were therefore similar

following administration of the 1.0 and 1.3 mg/m² doses, when considered in context of the observed pharmacokinetic variability. However, there was no readily apparent deviation from dose-proportionality based on the lack of any readily discernible differences between the observed distributions of estimated plasma clearance values at the two dose levels studied (Fig. 1c).

Pharmacodynamics

Pharmacodynamic parameters following administration of bortezomib 1.0 and 1.3 mg/m² on days 1 and 11 of cycles 1 and 3 are shown in Table 3. Mean percent 20S proteasome inhibition–time profiles at these four time points in each dose group are shown in Fig. 2. $E_{\max}$ was most frequently observed 5 min after bortezomib administration (i.e., the first sampling time) in both dose groups, consistent with rapid inhibition of the 20S proteasome in blood following

Table 2 Pharmacokinetic parameters (mean $\pm$ standard deviation) of bortezomib in plasma following administration of 1.0 or 1.3 mg/m²

Parameter	1.0 mg/m ² dose group			
	Day 1, Cycle 1 (<i>n</i> = 11) ^a	Day 11, Cycle 1 (<i>n</i> = 12)	Day 1, Cycle 3 (<i>n</i> = 12)	Day 11, Cycle 3 (<i>n</i> = 12)
<i>C</i> _{max} (ng/ml)	56.7 $\pm$ 36.3	106.2 $\pm$ 46.7	66.5 $\pm$ 42.6	83.9 $\pm$ 69.3
<i>AUC</i> _{48h} (ng h/ml)	26.5 $\pm$ 12.4	82.8 $\pm$ 35.9 ^c	66.4 $\pm$ 24.1	101.6 $\pm$ 58.2 ^c
<i>AUC</i> _{last} (ng h/ml)	22.6 $\pm$ 12.6	137 $\pm$ 106	74.8 $\pm$ 35.8	227 $\pm$ 181
<i>CL</i> (L/h)	102.1 $\pm$ 48.1	23.2 $\pm$ 17.8	32.2 $\pm$ 19.0	15.1 $\pm$ 13.9
<i>CL</i> (L/h/m ²)	57.1 $\pm$ 25.3	12.9 $\pm$ 9.0	17.9 $\pm$ 9.4	8.3 $\pm$ 6.9
<i>V</i> _{ss} (L)	1,547 $\pm$ 2143 ^b	1,421 $\pm$ 684 ^d	1,621 $\pm$ 695 ^e	3,105 $\pm$ 2790 ^c
<i>V</i> _{ss} (L/m ²)	796 $\pm$ 1061 ^b	800 $\pm$ 388 ^d	923 $\pm$ 468 ^e	1,783 $\pm$ 1560 ^c
<i>V</i> _β (L)	1,976 $\pm$ 2498 ^b	1,659 $\pm$ 752 ^d	1,852 $\pm$ 951 ^e	3,294 $\pm$ 2993 ^c
<i>V</i> _β (L/m ²)	1,019 $\pm$ 1236 ^b	929 $\pm$ 427 ^d	1,057 $\pm$ 613 ^e	1,884 $\pm$ 1674 ^c
<i>t</i> _{1/2} (h)	30.7 $\pm$ 44.8 ^b	78.9 $\pm$ 50.9 ^d	39.9 $\pm$ 14.4 ^e	193 $\pm$ 169 ^c

Parameter	1.3 mg/m ² dose group			
	Day 1, Cycle 1 (<i>n</i> = 12)	Day 11, Cycle 1 (<i>n</i> = 12)	Day 1, Cycle 3 (<i>n</i> = 12)	Day 11, Cycle 3 (<i>n</i> = 11) ^a
<i>C</i> _{max} (ng/ml)	112 $\pm$ 122	88.6 $\pm$ 47.6	120.3 $\pm$ 70.7	114.9 $\pm$ 98.3
<i>AUC</i> _{48h} (ng h/ml)	34.6 $\pm$ 19.8	82.4 $\pm$ 28.6 ^c	79.4 $\pm$ 24.5 ^c	85.2 $\pm$ 18.7 ^g
<i>AUC</i> _{last} (ng h/ml)	31.5 $\pm$ 18.6	122.2 $\pm$ 67.4	86.0 $\pm$ 27.6	160.7 $\pm$ 67.1
<i>CL</i> (L/h)	111.6 $\pm$ 73.6	28.0 $\pm$ 19.8	32.1 $\pm$ 15.4	18.2 $\pm$ 9.2
<i>CL</i> (L/h/m ²)	58.2 $\pm$ 41.8 ^c	14.9 $\pm$ 9.4 ^c	17.1 $\pm$ 7.6 ^c	8.9 $\pm$ 3.6 ^d
<i>V</i> _{ss} (L)	1,540 $\pm$ 2730 ^e	1,972 $\pm$ 1,347	1,613 $\pm$ 1,125	2,213 $\pm$ 1,442
<i>V</i> _{ss} (L/m ²)	285 $\pm$ 266 ^f	1,070 $\pm$ 712 ^c	925 $\pm$ 684 ^c	1,231 $\pm$ 843 ^d
<i>V</i> _β (L)	2,015 $\pm$ 2974 ^e	2,415 $\pm$ 1,711	2,059 $\pm$ 1,231	2,505 $\pm$ 1,641
<i>V</i> _β (L/m ²)	498 $\pm$ 425 ^f	1,309 $\pm$ 873 ^c	1,182 $\pm$ 751 ^c	1,389 $\pm$ 950 ^d
<i>t</i> _{1/2} (h)	11.5 $\pm$ 12.7 ^e	75.6 $\pm$ 49.9	49.1 $\pm$ 34.6	108.6 $\pm$ 64.8

*C*_{max} maximum observed plasma concentration, *AUC*_{48h} area under plasma–concentration time curve from time zero to scheduled 48-h measurement, *AUC*_{last} area under plasma–concentration time curve from time zero to the last non-zero concentration, *CL* clearance, *V*_{ss} steady-state volume of distribution, *V*_β beta-phase volume of distribution, *t*_{1/2} terminal half-life

^a Results are presented from the subset of evaluable patients. Data were excluded for one patient on day 1, cycle 1 and one patient on day 11, cycle 3 due to outlying plasma concentrations

^b *n* = 5

^c *n* = 11

^d *n* = 10

^e *n* = 7

^f *n* = 6

^g *n* = 9

intravenous bolus injection of bortezomib. The mean *E*_{max} following single- or multiple-dose administration was 70–84% in the 1.0 mg/m² dose group and 73–83% in the 1.3 mg/m² dose group. As noted for systemic exposure of bortezomib, mean *E*_{max} and *AUE*_{48h} values were higher on day 11, cycle 1, versus day 1, cycle 1, with smaller differences observed between values at the three multiple-dosing time points (day 11, cycle 1, and days 1 and 11, cycle 3). More patients (four vs. one in each dose group) exhibited >90% inhibition after multiple- versus single-dose administration. No substantial differences in 20S proteasome inhibition–time profiles were observed between the two dose groups. As drug concentrations declined, reversibility of

20S proteasome inhibition was demonstrated; mean inhibition of 22–48% was observed 48 h post-administration of either dose, which decreased further to 11–35% after the 10-day washout period between cycles (i.e. in pre-dose samples collected on day 1, cycles 2 and 4).

Response to bortezomib

Among all 42 patients enrolled in the study, the overall response rate (CR + partial response [PR]) was 50%, including 7% CR. Activity was similar in the two dose groups. In the 1.0 mg/m² dose group, overall response rate was 48%, including two (10%) CR and eight (38%) PR; a

Table 3 Pharmacodynamic parameters (mean $\pm$ standard deviation) of bortezomib in whole blood following administration of 1.0 or 1.3 mg/m²

Parameter	1.0 mg/m ² dose group			
	Day 1, Cycle 1 (<i>n</i> = 11) ^a	Day 11, Cycle 1 (<i>n</i> = 12)	Day 1, Cycle 3 (<i>n</i> = 11) ^a	Day 11, Cycle 3 (<i>n</i> = 12)
<i>E</i> _{max} (%)	69.7 $\pm$ 11.0	83.4 $\pm$ 7.02 ^b	78.3 $\pm$ 7.09	83.5 $\pm$ 7.05 ^b
<i>AUE</i> _{48h} (% h)	1,649 $\pm$ 473	2,567 $\pm$ 489 ^c	2,537 $\pm$ 647	2,796 $\pm$ 717 ^c
Parameter	1.3 (mg/m ²) dose group			
	Day 1, Cycle 1 (<i>n</i> = 12)	Day 11, Cycle 1 (<i>n</i> = 12)	Day 1, Cycle 3 (<i>n</i> = 11) ^a	Day 11, Cycle 3 (<i>n</i> = 11) ^a
<i>E</i> _{max} (%)	73.0 $\pm$ 10.8	78.6 $\pm$ 6.50	78.2 $\pm$ 9.27	82.9 $\pm$ 9.46
<i>AUE</i> _{48h} (% h)	1,426 $\pm$ 717	2,278 $\pm$ 748 ^b	1,824 $\pm$ 1047 ^d	2,386 $\pm$ 577 ^e

*E*_{max} maximum 20S proteasome inhibition, *AUE*_{48h} area under 20S proteasome inhibition–time curve from time zero to scheduled 48-h measurement

^a Results are presented from the subset of evaluable patients. Data were excluded from the calculations for one patient in the 1.0 mg/m² dose group on day 1, cycle 1, and day 1, cycle 3, and for one patient in the 1.3 mg/m² dose group on day 1, cycle 3, and day 11, cycle 3, due to outlying values

^b *n* = 11

^c *n* = 9

^d *n* = 10

^e *n* = 8

further two (10%) patients had a minimal response and four (19%) had stable disease. In the 1.3 mg/m² dose group, overall response rate was 52%, including one (5%) CR and ten (48%) PR; a further four (19%) patients had a minimal response and three (14%) had stable disease.

Safety

Overall, 17 (40%) patients completed the protocol-specified eight cycles of treatment, 11 (52%) in the 1.0 mg/m² dose group and 6 (29%) in the 1.3 mg/m² dose group. The most common reasons for discontinuing treatment prior to completing eight cycles were occurrence of an AE (four and six

patients in the 1.0 and 1.3 mg/m² dose groups, respectively) and lack of efficacy (four and five patients, respectively). Patient disposition is summarized in the Supplemental Figure. Patients received a median of 5.5 treatment cycles (range, 1–24) with medians of 8.0 (range, 1–24) and 5.0 (range, 1–24) in the 1.0 and 1.3 mg/m² dose groups, respectively. Ten (48%) patients in the 1.0 mg/m² dose group had dose increases to 1.3 mg/m², including four (19%) prior to the start of cycle 4, and six (29%) from cycle 9 onwards. Dose reductions occurred in two (10%) and six (29%) patients in the 1.0 and 1.3 mg/m² dose groups, respectively. Dexamethasone was added for 1 (5%) patient in the 1.0 mg/m² dose group and no patients in the 1.3 mg/m² dose group; a

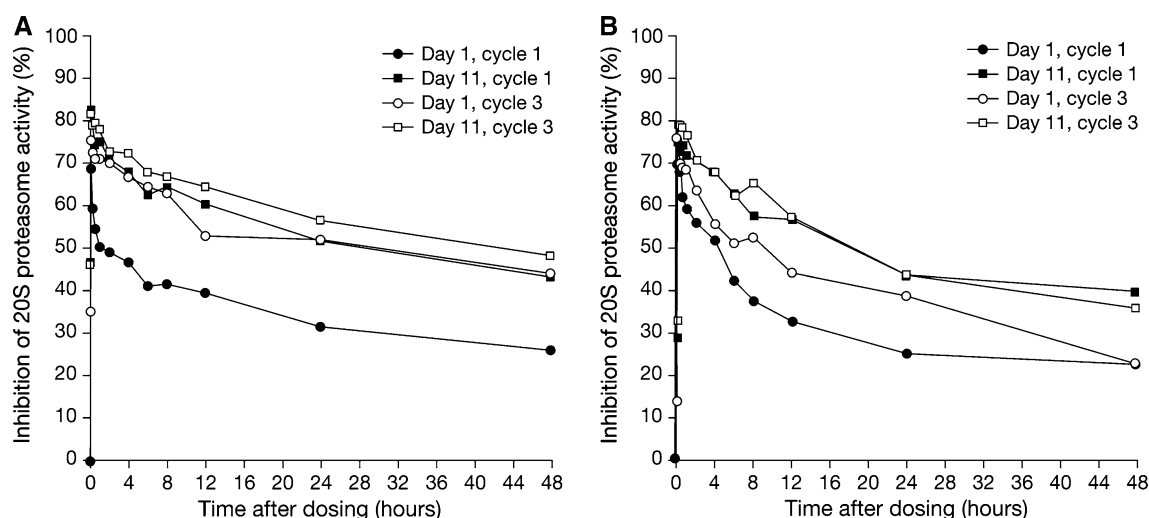


Fig. 2 Mean percent 20S proteasome inhibition in whole blood following administration of bortezomib at **a** 1.0 mg/m² and **b** 1.3 mg/m² (*n* = 11 or 12 per profile)

Table 4 Summary of adverse events (AEs) reported during the first three treatment cycles in patients receiving bortezomib 1.0 mg/m² or 1.3 mg/m², including the most common AEs ($\geq 20\%$) and grade 3/4 AEs ($\geq 5\%$), plus serious AEs (SAEs) and AEs resulting in discontinuation reported in more than one patient throughout the duration of study treatment

	1.0 mg/m ² (n = 21)	1.3 mg/m ² (n = 21)	Total (n = 42)
At least one AE, n (%)	29 (95)	21 (100)	41 (98)
Constipation	8 (38)	10 (48)	18 (43)
Fatigue	9 (43)	8 (38)	17 (40)
Nausea	5 (24)	10 (48)	15 (36)
Musculoskeletal pain	6 (29)	9 (43)	15 (36)
Anemia	4 (19)	7 (33)	11 (26)
Thrombocytopenia	2 (10)	9 (43)	11 (26)
Peripheral neuropathy	5 (24)	5 (24)	10 (24)
Diarrhea	3 (14)	6 (29)	9 (21)
At least one drug-related AE, n (%)	17 (91)	20 (95)	37 (88)
At least one grade 3/4 AE, n (%)	7 (33)	10 (48)	17 (40)
Thrombocytopenia	2 (10)	7 (33)	9 (21)
Anemia	2 (10)	2 (10)	4 (10)
Acute renal failure	1 (5)	1 (5)	2 (5)
Angina pectoris	1 (5)	1 (5)	2 (5)
Thrombocytopenia aggravated	2 (10)	0	2 (5)
At least one SAE, n (%)	8 (38)	7 (33)	15 (36)
Pneumonia	1 (5)	3 (14)	4 (10)
Acute renal failure	1 (5)	2 (10)	3 (7)
Pathological fracture	1 (5)	1 (5)	2 (5)
AE resulting in discontinuation, n (%)^a	5 (24)	7 (33)	12 (29)
Peripheral neuropathy	2 (10)	4 (19)	6 (14)
Acute renal failure	0	2 (10)	2 (5)

^a Includes 1 additional patient in each dose group discontinuing due to an AE compared with disposition data due to recording of these AEs beyond cycle 4, day 1, when protocol-specified recording of AEs (other than neurotoxicity and SAEs) was to stop

further 2 (10%) patients in each group received dexamethasone after the last dose of bortezomib, having received 1, 1, 2, and 2 cycles of bortezomib, respectively.

The safety profiles in the two bortezomib dose groups and overall are summarized in Table 4, including rates of AEs and grade 3/4 AEs reported through day 1, cycle 4, and rates of SAEs and AEs resulting in discontinuation reported during all study treatment. The rates of AEs, drug-related AEs, grade 3/4 AEs, and AEs resulting in discontinuation appeared lower in the 1.0 mg/m² versus the 1.3 mg/m² dose group; the AEs with the greatest differences in incidence between dose groups were thrombocytopenia (10 vs. 43%), nausea (24 vs. 48%), and diarrhea (14 vs. 29%). Similarly, the incidence of grade 3/4 thrombocytopenia appeared lower in the 1.0 mg/m² versus the 1.3 mg/m² dose group (10 vs. 33%). By contrast, the rates of SAEs were comparable between dose groups. One death, of a patient in the 1.0 mg/m² dose group, occurred during the study (≤ 30 days after last bortezomib dose) due to disease progression with accompanying complications and was considered unrelated to study treatment.

The incidence of peripheral neuropathy during the entire study was 31%, including seven (33%) and six (29%) patients in the 1.0 and 1.3 mg/m² dose groups. Three (7%) patients experienced grade 3 peripheral

neuropathy: two in the 1.0 mg/m² group and one in the 1.3 mg/m² group. Peripheral neuropathy resulted in treatment discontinuation in six (14%) patients, including two (10%) and four (19%) in the 1.0 and 1.3 mg/m² dose groups. Median values of maximum AUC_(0–t) (215.0 vs. 138.1 ng h/ml) and maximum C_{max} (143.0 ng/ml and 97.3 ng/ml) appeared to be higher for patients reporting any neuropathy than for those with no neuropathy. Maximum percent 20S proteasome inhibition also appeared higher (88.4 vs. 83.8%).

Median FACT/GOG-Ntx summary scores on day 1, cycle 4, were 7.0 and 11.0 among patients in the 1.0 (n = 13) and 1.3 (n = 15) mg/m² dose groups, respectively, representing a median change from baseline of 1.0 and 2.0, respectively. Higher scores reflect greater symptoms of peripheral neuropathy.

Discussion

This study represents the first comprehensive multi-cycle characterization of the single-and repeat-dose pharmacokinetics and pharmacodynamics of intravenously administered bortezomib in patients with relapsed MM, during

administration of a twice-weekly regimen at doses of 1.0 and 1.3 mg/m². The results of this study contribute the core body of clinical pharmacokinetic and pharmacodynamic data on bortezomib [1].

Consistent with the single-dose pharmacokinetic profile reported in a previous study in patients with androgen-independent prostate cancer treated with a weekly dosing regimen of intravenous bortezomib [18] and following intravenous administration in a study of subcutaneous versus intravenous administration of bortezomib in patients with relapsed or refractory MM [25], plasma bortezomib concentrations declined in a multi-exponential manner, with a rapid initial phase followed by a prolonged terminal phase and extensive peripheral tissue distribution (Fig. 1). Although this general feature of the pharmacokinetic profile was noted at all time points of pharmacokinetic characterization (days 1 and 11, cycles 1 and 3), a noteworthy finding of this study is that the pharmacokinetics of bortezomib displayed time-dependent changes, characterized by an approximately fourfold decrease in systemic plasma clearance and resultant increases in systemic plasma exposure and terminal half-life following repeat dose administration in relation to the first dose of the first treatment cycle (Table 2). Although the pharmacokinetics on day 1, cycle 1 were clearly distinguishable from those of later doses studied (day 11, cycle 1, days 1 and 11, cycle 3), there were no readily apparent differences between the observed concentration–time profiles and pharmacokinetic parameters among the latter three time points of pharmacokinetic evaluation, indicating that the pharmacokinetic changes that occur following repeat dosing were achieved at least as early as the administration of the last dose of cycle 1 (Fig. 1 and Table 2). These pharmacokinetic changes in systemic plasma clearance and exposure are consistent with observations following intravenous administration in the study of subcutaneous versus intravenous bortezomib in patients with relapsed or refractory MM [25].

Total variability in bortezomib exposure was modest to large. As a consequence, mean values of exposure parameters at the 1.0 and 1.3 mg/m² dose levels were not distinguishable, which is consistent with the relatively narrow dose range studied (1.3-fold) in relation to the observed pharmacokinetic variability (47–92% coefficient of variation in clearance) and sample size in the evaluable population ($n = 12$ per dose group). However, it should be noted that there was no readily apparent deviation from dose-proportionality based on comparable distributions of clearance values (i.e., inverse of dose-normalized exposure) between the two dose levels (Fig. 1c).

Pharmacodynamic analysis using a whole-blood assay of 20S proteasome activity inhibition revealed rapid attainment of maximum inhibitory effect (70–84% inhibition) following IV bolus administration of bortezomib, and a

reversal of pharmacodynamics over the dosing interval (Fig. 2), consistent with previous observations [18, 25, 26]. These observations confirm the reversibility of bortezomib-associated 20S proteasome inhibition, both between doses during twice-weekly dosing and, more importantly, during the 10-day rest period at the end of a standard treatment cycle. A modest increase in pharmacodynamic effect ($E_{\max}$ and AUE) was observed following repeat dosing of bortezomib compared with the first dose of cycle 1 (Table 3), a finding that is consistent with the previously discussed time-dependent pharmacokinetic changes that result in a greater-than-expected drug accumulation with repeat dose administration. Consistent with the pharmacokinetic observations, the pharmacodynamic parameters of bortezomib were not distinguishable between the 1.0 and 1.3 mg/m² dose levels in this study (Table 3).

Bortezomib was active and tolerable in both dose groups in the present study; the relatively small numbers of patients preclude any definitive conclusions regarding the comparative activity and safety at the two different dose levels investigated. Other factors confounding comparisons between dose groups include the permitted bortezomib dose increases seen in patients in the 1.0 mg/m² group, the option for all patients to receive added dexamethasone following cycle 3, and the differences in overall exposure to treatment between dose groups.

The overall response and CR rates seen in the present study reflect the activity reported in phase 2 and phase 3 studies in patients with relapsed and/or refractory MM [10–13, 15]. Response rates appeared similar in the 1.0 and 1.3 mg/m² dose groups. However, the findings of the non-comparative phase 2 CREST study, in which both doses were investigated, suggest that the standard 1.3 mg/m² dose may result in somewhat greater efficacy, in terms of both response rate [10] and, as reported recently, overall survival [11]. This is supported by the results of a phase 1/2 study of bortezomib, doxorubicin, and dexamethasone (PAD) in newly diagnosed patients with MM, in which two different PAD regimens, including bortezomib at 1.0 or 1.3 mg/m², were investigated; overall response and CR rates appeared higher, and there seemed to be a trend toward prolonged progression-free survival, time to subsequent therapy, and overall survival among patients who received PAD with the higher bortezomib dose [27].

The safety profile of bortezomib observed in this study was consistent with the predictable and manageable profile established in phase 2 and phase 3 studies [10, 12, 13, 28]. No new or unexpected toxicities were observed. Although the study was not designed to compare toxicities in the two dose groups, there appeared to be a trend toward a milder toxicity profile in the 1.0 mg/m² dose group. This suggestion is supported by similar trends between patients receiving the two doses of bortezomib in the phase 2 CREST

study [10] and the phase 1/2 study of PAD [27]. Maximum plasma concentration, total systemic exposure, and maximum percent blood 20S proteasome inhibition did appear higher in patients who experienced peripheral neuropathy of any grade, which is consistent with the cumulative, dose-related nature of this toxicity reported in analyses of SUMMIT and CREST [29] and APEX [30]. The efficacy and safety data from the present study in combination with those from the phase 2 CREST study [10] and the phase 1/2 study of PAD [27] suggest that use of the standard dose of bortezomib 1.3 mg/m², with dose reduction to 1.0 mg/m² if required to manage AEs while retaining substantial activity, represents an appropriate dosing strategy.

In conclusion, bortezomib pharmacokinetics changes with repeat dose administration, characterized by a reduction in plasma clearance and an associated increase in systemic exposure. Bortezomib is pharmacodynamically active and tolerable at both the 1.0 and 1.3 mg/m² doses, with recovery toward baseline blood proteasome activity over the dosing interval following repeat dose administration, which in theory allows cells to recover proteasome activity for normal cellular housekeeping functions between doses, supporting the current clinical dosing regimen.

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