EXPERT REVIEWS

Bortezomib for the treatment of multiple myeloma

Expert Rev. Hematol. 7(2), 173–185 (2014)

Sebastian Grosicki*[1], Agnieszka Barchnicka[1], Artur Jurczyszyn[2] and Anida Grosicka[3]

[1]*Department of Hematology, Community Hospital in Chorzow, Chorzow, Poland*
[2]*Department of Hematology, University Hospital, Cracow, Poland*
[3]*Department of Internal Medicine and Rheumatology, Silesian Medical University, Katowice, Poland*
**Author for correspondence:*
Tel.: +48 323 499 723
Fax: +48 323 461 471
sgrosicki@wp.pl

Bortezomib is the first proteasome inhibitor drug tested in human patients. Bortezomib demonstrates a particular clinical utility in the treatment of multiple myeloma (MM), where it is the only one of the new drugs administered as mono-therapy that prolongs survival. The significant problem for the consistent pursuit of bortezomib was neurotoxicity, which has been significantly reduced by registering subcutaneous administration or being administered once per week. Bortezomib is currently approved for the treatment of patients with progressive MM in mono-therapy and in combination with prednisone and melphalan in cases of untreated patients who are not candidates for autologous hematopoietic stem cell transplantation (AHSCT) and in combination with dexamethasone or dexamethasone and thalidomide in untreated MM patients, who are candidates for treatment AHSCT. Clinical research is focused on the combination of bortezomib with other new drugs with the hope of further optimizing the treatment of patients with multiple myeloma.

KEYWORDS: bortezomib • efficacy • guidelines • multiple myeloma • proteosome inhibitor • recommendations • safety • treatment

Multiple myeloma (MM) is a hematological cancer of patients with a median age of 72 years [1], and incidences of it are estimated at 6:100,000/year [2] characterized by clonal proliferation of atypical plasma cells, usually producing monoclonal protein [3].

Despite intensive clinical research into the pathogenesis conducted for the purpose of finding new target points for medicines, and more widespread introduction of them into clinical practice in recent years, MM remains an incurable disease [4–6].

The induction and consolidation chemotherapy regimens including autologous hematopoietic stem cell transplantation (AHSCT) in MM patients are well defined [7,8].

Currently, research is being focused on prolonged treatment including optimal maintenance treatment [9–14]. The introduction of immunomodulatory drugs [12–14] but above all, the first proteosome inhibitor bortezomib [9,10], has improved survival. In fact, only bortezomib monotherapy has improved the survival of patients with relapsed MM [15].

This article discusses the pharmacology of bortezomib and its clinical effect and toxicity when administered intravenously and subcutaneously as a single drug or in combination with different agents in patients with MM. It also raises the issue of the procedure needed in order to deal with the most important signs of intolerance to treatment with bortezomib.

Bortezomib

Chemistry & mechanism of action

Bortezomib is a modified dipeptide boronic acid analog that reversibly and very specifically inhibits β-subunit of the 26S proteasome (FIGURE 1) [9,16]. Bortezomib was the first proteasome inhibitor tested on humans.

The 26S proteosome is a multicatalytic enzyme that has been discovered in the nucleus and the cytoplasm of eukaryotic cells and is involved in the degradation of the pivotal proteins, which ultimately leads to cell death [17]. Inhibition of the 26S proteasome leads to the accumulation and disregulation of key proteins including p21 inhibitors [17] and p27 cyclin-dependent kinases [18], caspases [19], tumor suppressor p53 [20], B-cell leukemia/lymphoma 2 [21] oncogenes c-Myc, c-Fos and c-Jun [22], transcription factors E2A, E2F and STAT [23], Bax [24] and inhibitory κB protein (FIGURE 2) [25].

It was found that bortezomib was cytotoxic for various tumor cell types, including myeloma cells, when administered alone as well as in combination with other cytotoxic agents [19,26,27].

 10.1586/17474086.2014.899144 ISSN 1747-4086

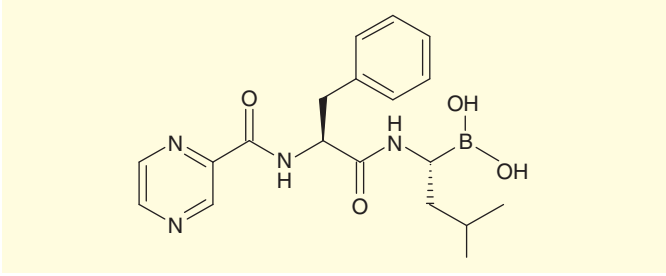

Figure 1. Bortezomib chemical structure.

Bortezomib restores the sensitivity of cell lines resistant to melphalan and dexamethasone [26,27]. *In vitro* studies bortezomib has a synergistic effect with other chemotherapeutic agents like melphalan [26], doxorubicin [27], arsenic trioxide [28] and further increases the activity of dexamethasone [19].

The maximum inhibition of the 20S proteasome occurs within an hour after the administration of bortezomib, then slowly decreases and returns to baseline after 72 h, according to the study of patients with hematological disorders treated with bortezomib at 0.4–1.38 mg/m^2 twice a week for 4 weeks [29].

Bortezomib also shows an anabolic effect on bone by inhibiting the activity of human osteoclasts by restraining their division, activity and resorbing action, and also in stimulating the function of osteoblasts [30–32]. Clinical studies have shown that bortezomib has a positive effect on bone formation, as evidenced by an increase in bone-specific alkaline phosphatase and osteocalcin [32–34], pro-collagen-type I N-terminal propeptide [34] and the parathyroid hormone levels [33] in the serum.

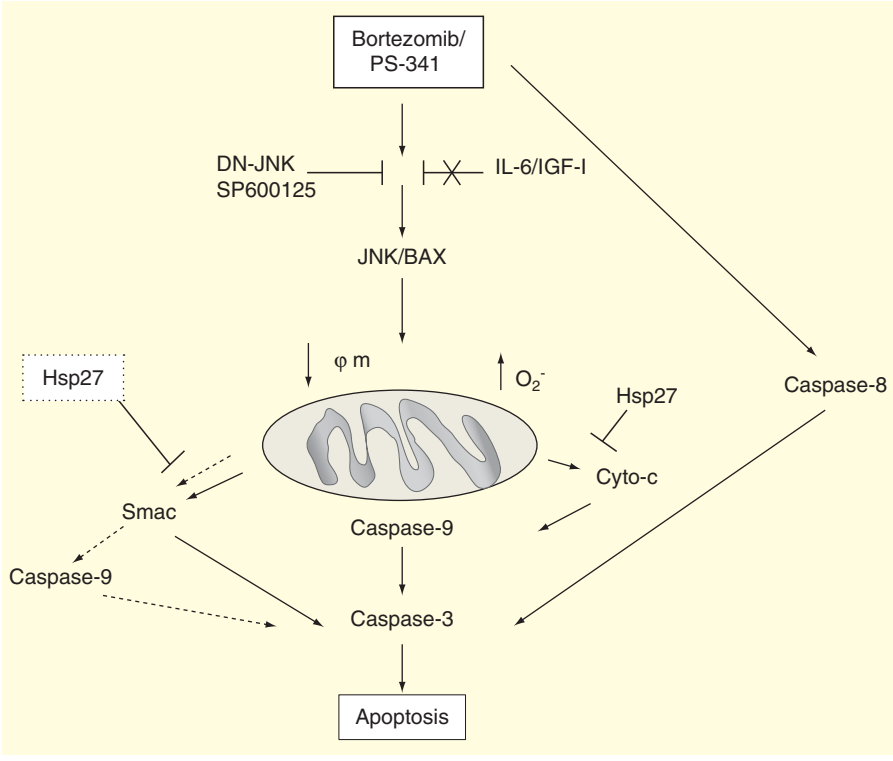

Figure 2. Bortezomib activates of c-Jun NH2-terminal kinase, which translocates to mitochondria and facilitates the release of cytochrome C and the second mitochondrial activator Kaspar from mitochondria to cytosol, followed by activation of caspase-9. Bortezomib also induces the activation of caspase-8. Activation of both caspase-8 and caspase-9 induces effector its sub-caspase-3 and the cutting of poly (ADP-ribose) polymerase. Blocking JNK, by either dominant-negative mutant or JNK inhibitor SP600125, abrogates both PK + bortezomib-induced release of cytochrome c/Smac and activation of caspase-9. Bortezomib-induced apoptosis is not inhibited by IL-6 and IGF-1. Ectopic expression of Hsp27 induced by bortezomib suppresses the release of cytochrome c Smac.
Cyto-C: Cytochrome C; DN-JNK: Dominant-negative mutant Jun NH2-terminal kinase; Smac: Second mitochondrial activator Kaspar.

Pharmacodynamics & pharmacokinetics

Average peak plasma concentrations of bortezomib in serum (C_{max}) after a single intravenous administration of 1.0 or 1.3 mg/m^2 were 57 or 112 ng/ml in patients with MM [16,35]. Similar C_{max} levels to those after a single dose were found after repeated administration of the drug. The time to C_{max} was observed 0.1–0.2 h after multiple doses of bortezomib at 1.3 mg/m^2.

C_{max} was 10-times lower after a subcutaneous injection of bortezomib than after intravenous administration with a longer exposure time to C_{max} (T_{max}) 0.5 h (Table 1). An average systemic exposure (AUClast) was similar after intravenous and subcutaneous administration [36]. The mean percentage inhibitions of the 20S proteasome (E_{max}) and the area under the effect-time curve (mean AUE72) were also similar in both methods of administration, and the time of the maximal inhibition of the 20S proteasome activity was longer in the used concentrations, following subcutaneous administration than intravenous administration (2.5 vs 1 mg/ml).

Bortezomib is primarily metabolized by cytochrome (CYP) P450 CYP3A4, CYP2C19 and CYP1A2 with a lower level of metabolism by CYP2D6 and CYP2C9. The basic metabolic pathway is 'oxidative deboratation' which is a form of two diastereomeric deboronated inactive metabolites then sequentially hydroxylated to other inactive metabolites.

Specific populations

Administration of a single dose of bortezomib at 1.0 or 1.3 mg/m^2 and the mean dose-normalized AUC and C_{max} were approximately 25% lower in 26 patients with MM aged <65 years than in 13 patients aged >65 years [16].

Similar values of dose-normalized AUC and C_{max} were observed in patients with normal kidney function and renal

impairment, where bortezomib was administered intravenously twice a week at 0.7–1.3 mg/m^2 [16,35]. The pharmacokinetics of bortezomib was not studied in patients with liver failure. The pharmacokinetic profile obtained in patients in Japan after a single dose of bortezomib and a number of doses were similar to that observed in Caucasian patients [16].

Table 1. Pharmacokinetic and pharmacodynamic parameters by rout of administration.

	Bortezomib subcutaneous (n = 17)	Bortezomib intavenous (n = 14)
Pharmacokinetics		
C_{max} (ng/ml)	20.4 (8–87)	223 (101)
T_{max} (min)	30 (5–60)	2 (2–5)
AUC_{last} (ng/h/ml)	155 (56.8)	151 (42.9)
Pharmacodynamics		
E_{max} (%)	63.7 (10.6)	69.3 (13.2)
T_{Emax} (min)	120 (30–1440)	5 (2–30)
AUE_{72} (%/h)	1714 (617)	1383 (767)

Data are mean (SD) or median (range).
AUC_{last}: Area under the concentration-time curve from time 0 to the last time point at which bortezomib was quantifiable; AUE_{72}: Area under the percentage inhibition-time curve from time 0–72 h; C_{max}: Maximum plasma concentration; E_{max}: Observed maximum percentage inhibition of 20S proteasome activity; T_{Emax}: Time to E_{max}; T_{max}: Time to C_{max}.
Data taken from [36].

Interaction

The data on the interaction of bortezomib with other drugs are ambiguous. When bortezomib was administered during treatment with another inhibitor of CYP3A4, such as ketoconazole, bortezomib AUC increased by 35%. This effect was not observed, however, during the administration of another potential CYP2C19 inhibitor – omeprazole [16]. The administration of bortezomib with melphalan and prednisone increases bortezomib AUC by 17%, but it does not translate into clinical effects [16].

Green tea extract epigallocatechin gallate, which had been expected to have a synergistic effect, was found by Encouse B. Golden *et al.* to reduce the effectiveness of bortezomib [37].

Clinical studies on bortezomib

The results of the first clinical trials as well as the first registration and introduction of bortezomib (Velcade) into clinical practice were a major breakthrough in the treatment of patients with MM, significantly improving response to treatment, but also overall survival (OS) [5,6,38,39].

Phase I trials

In 2002, Orlowski *et al.* published the results of the first Phase I study, where bortezomib was given to 27 patients with refractory hematological diseases at doses of 0.4, 1.04, 1.2 and 1.38 mg/m^2 intravenous (iv.) twice weekly for 3 weeks followed by a 1 week interval.

Obtaining complete remission (CR) to bortezomib treatment in one patient with MM, assessed by negative immunofixation, determined the decision to further clinical studies with bortezomib [38].

Phase II trials

In the first Phase II SUMMIT trial, the patients received bortezomib at a dose of 1.3 mg/m^2 daily as iv. bolus two-times a week for two consecutive weeks, followed by a 1 week interval. Treatment was continued for up to eight cycles and patients with a suboptimal response received additionally dexamethasone from the third cycle. The study enrolled 202 patients with relapsed or refractory MM. The rate of response to bortezomib was 35% [40].

Based on those very impressive results, bortezomib (Velcade) was approved by the US FDA on 13 May 2003 on an accelerated basis for treatment of MM patients with relapse after at least two previous lines of treatment [40].

Phase III studies in relapsed/refractory myeloma

The Phase III APEX trial compared bortezomib at a dose of 1.3 mg/m^2 on days 1, 4, 8 and 11 iv. for eight 3-week cycles followed by treatment on days 1, 8, 15 and 22 for three 5-week cycles with high doses of dexamethasone (40 mg orally) on days 1–4, 9–12 and 17–20 for four 5-week cycles and on days 1–4 for five 4-week cycles in 669 patients with relapsed MM. The study was halted after a partial analysis, which demonstrated that treatment with bortezomib is associated with a higher level of response 38 versus 18%, longer time to progression 6.22 versus 3.49 months and improvement of OS after 2 years, 29.8 versus 23.7 months (p = 0.02) [41].

In analyzing the tolerability, it was observed that bortezomib often causes thrombocytopenia, neutropenia, neuropathy and diarrhea [41]. It was also confirmed that bortezomib affects bone metabolism, which is manifested by an elevation in alkaline phosphatase. The drug inhibits the osteoclast function and directly induces bone formation [31].

In the next Phase III trial, 646 patients with MM were randomized to receive bortezomib in combination with liposomal doxorubicin on day 4 or in monotherapy. There was a statistically significant difference in the effect between the arms of treatment. Time of progression (9.3 vs 6.5 months, p = 0.000004) and OS of 15 months (76 vs 65%, p = 0.03) were better in the liposomal doxorubicin arm [42]. There was a higher incidence of grade 3/4 adverse events in the combination group (80 vs 64%). These complications were related to myelosuppression, constitutional and gastrointestinal symptoms and hand-foot syndrome [42].

In other studies, in refractory/relapse MM, bortezomib was combined with each of the classes of drugs with known activity

Table 2. Selected clinical trials of bortezomib in polychemotherapy regimens in relapse/refractory multiple myeloma patients.

Study (year)	Phase	Regimen	N	CR/nCR (%)	ORR (%)	Ref.
Pineda-Roman *et al*. (2008)	I/II	VT ± Dex	85	22	63	[81]
Palumbo *et al*. (2008)		PAD	64	25	67	[82]
Ciolli *et al*. (2008)	II	VTDD	42	52	74	[83]

CR: Complete remission; N: Number of evaluable patients; nCR: Near-complete remission; ORR: Overall response rate; PAD: Bortezomib–adriamycin–dexamethason; VT: Bortezomib–thalidomide; VTDD: Bortezomib–thalidomide–dexamethason–liposomal doxorubicin.

in MM such as steroids (dexamethasone, prednisone), immunomodulatory agents (thalidomide, lenalidomide) and conventional chemotherapeutic agents (melphalan, cyclophosphamide) (Table 2). The trials were related to 30–63 patients with refractory or recurrent MM. The response rates ≥ PR were 66–92%. The results of the bortezomib combination with cyclophosphamide and dexamethasone were particularly promising, where the ≥ PR was achieved in 82% (CR 16%). Addition of thalidomide to this regimen improved ≥ PR 92 and 52% CR. There are also studies ongoing on the combination of bortezomib with a variety of new drugs such as perifosine, tanespimycin, vorinostat, arsenic oxide, samarium SM 153 leksidrondam, siltiximab (CNTO 328) and panobinostat in patients with relapsed or refractory MM.

Phase III studies in newly diagnosed patients

Clinical research is focused on the effective and well-tolerated combination of bortezomib with other drugs with proved action in MM as induction therapy.

The Phase III clinical trial VISTA enrolled 682 previously untreated patients who were not candidates for AHSCT. The patients received MP (melphalan + prednisone) or VMP (MP + bortezomib).

The overall response rate was 71% for VMP and the MP 35% with high CR prevalence (respectively 30 vs 4%, $p < 0.001$) for both comparisons. All efficacy outcomes were also favorable for VMP relative to MP: median time to response (1.4 vs 4.2 months), duration of response (20 vs 13 months) and treatment-free interval (17 vs 9 months). Importantly, an improvement in the response was observed in all subgroups of patients, including those who were >75 years of age, in whom creatinine clearance was <60 and with high-risk cytogenetics t(4,14), t(14,16) or chromosome 17 deletion [43]. After 5 years of follow-up, improvement in OS in patients treated with VMP versus MP was reported (56.5 vs 43.1 months respectively; $p = 0.0004$) [44]. The improvement in survival was observed in all of these subgroups except for patients with confirmed high-risk cytogenetics [44]. Toxicities ≥ grade 3 were higher in the VMP arm, including neuropathy (14 vs 0%), nausea, vomiting/diarrhea, fatigue/asthenia and herpes zoster, which was observed in 14% of patients treated with VMP versus 4% in the MP arm. However, it was noted that in patients who received antiviral prophylaxis, only 3% developed shingles. An analysis of the incidence of zoster reactivation in APEX study also showed a significantly higher incidence of herpes zoster in the arm with bortezomib to dexamethasone (13 vs 5% respectively, $p = 0.0002$) [45]. Based on the above data, antiviral prophylaxis is recommended for all patients receiving chemotherapy regimens based on bortezomib. It may be acyclovir 400 mg/day, valacyclovir 250 or 500 mg daily and famciclovir 500 mg/day [46].

Data presented by Mateos *et al*. during the American Society of Hematology (ASH) Conference 2013 indicate that a higher cumulative bortezomib dose, reflecting prolonged treatment duration and/or dose intensity, is associated with improved OS [47]. Maintaining patients on bortezomib therapy, using dose/schedule modifications and adverse event management as required, is thus important in order to maximize the cumulative dose and to provide better OS [47].

The results of VISTA trial clearly indicate the improving effect of the VMP treatment without increased long-term toxicity after first-line treatment based on bortezomib.

There are also several Phase III trials evaluating the use of bortezomib as induction therapy with subsequent AHSCT.

Other two Phase III clinical trials comparing bortezomib in combination with doxorubicin and dexamethasone (bortezomib–adriamycin–dexamethason [PAD]) to traditional vincristine, doxorubicin, dexamethasone (VAD) regimen found improved levels of CR/near-complete remission (nCR) (30 vs 15%; $p < 0.015$) [10]. During the ASH Conference in New Orleans in 2013, Sonneveld *et al*. presented an update of the study VAD versus PAD after a median follow-up of 490/835 live patients 74 months and proved better OS after induction chemotherapy PAD versus VAD was superior after the whole treatment including AHSCT and maintenance with bortezomib in PAD arm and thalidomide in VAD arm ($p = 0.03$) [48].

This improved treatment efficacy after AHSCT in patients who were administered bortezomib-based induction therapy is significant. It was also shown in published, large studies, which compared the results of single and tandem transplantation that even if patients did not achieve CR/nCR after the first autologous transplant, they could benefit from a second transplant [49].

The current standard induction chemotherapy in MM includes three or four drugs with different mechanisms of action (Table 3). The efforts of investigators are still focused on finding combinations of drugs forming optimal induction regimens. In prospective, multicenter Phase II study, DVD regimen (liposomal pegylated doxorubicin, bortezomib, dexamethasone) proved to be well tolerated and effective in patients with untreated MM compared with the standard approach [50]. Thirty-five previously untreated MM patients were enrolled.

Table 3. Selected Phase III trials with bortezomib in previously untreated multiple myeloma.

Study	Regimen	N	CR/nCR	ORR (%)	Ref.
HOVON-655/GMMG-H4	PAD (vs VAD)	300	23% (vs 9%)	83	[10]
GIMEMA	VTD (vs TD)	460	55% (vs 32%)	94	[8]
VISTA	VMP (vs MP)	668	30% CR (vs 4%)	71	[43]
GIMEMA	VMPT (vs VMP)	354	39% CR (vs 21%)	87	[73]

CR: Complete remission; MP: Melphalan, prednisone; N: Number of evaluable patients; nCR: Near-complete remission; ORR: Overall response rate; PAD: Bortezomib–adriamycin–dexamethason; TD: Thalidomide, dexamethason; VAD: Vincristine, adriamycin, dexamethason; VGPR: Very good partial remission; VMP: Bortezomib–melphalan–prednisone; VMPT: Bortezomib–melphalan–prednisone–thalidomide; VTD: Bortezomib–thalidomide–dexamethason.

Dexamethasone iv. 40 mg, bortezomib 1 mg/m^2 and PLD 5 mg/m^2 were administered on days 1, 4, 8 and 11 of a 4 week cycle. Patients were treated to their maximum response plus two additional cycles. The treatment regimen was discontinued after a maximum of eight cycles. Our modified schedule and dosing regimen achieved a high overall response rate of 86%, while showing a marked decrease in the incidence and severity of peripheral neuropathy, palmar-plantar erythrodysesthesia and myelosuppression compared with the standard dosing on a 3 week cycle using these drugs [50]. The first results of using one of the most intensive induction RVDD regimen (lenalidomide, bortezomib, dexamethasone, doxorubicin, pegylated liposomal) in untreated MM patients were described by Jakubowiak *et al.* [51]. Patients received RVDD at four dose levels including the maximum tolerated dose (MTD). Patients with ≥ very good partial remission (VGPR) after cycle 4 proceeded to AHSCT or continued treatment. The primary objectives were MTD evaluation and response to RVDD after four and eight cycles. Seventy-two patients received a median of 4.5 cycles. The MTDs were lenalidomide 25 mg, bortezomib 1.3 mg/m^2, pegylated liposomal doxorubicin 30 mg/m^2 and dexamethasone 20/10 mg, as established with 3 week cycles. Results of the treatment are very impressive. Response rates after four and eight cycles were 96 and 95% partial response or better, 57 and 65% ≥ VGPR, and 29 and 35% complete or near-complete response, respectively. The estimated 18-month progression-free survival (PFS) and OS were 80.8 and 98.6%, respectively [51].

During the ASH Conference 2013, Kumar *et al.* presented very interesting data regarding the outcome of a two-centered, US-based, retrospective, observational study comparing the effectiveness and safety of bortezomib–cyclophosphamide–dexamethasone (VCD) and Bortezomib–lenalidomide–dexamethasone (VRD) as initial treatment for MM in routine oncology [52]. These data support comparable outcomes in terms of response, PFS and OS, well-tolerated safety profiles and lower drug costs per treatment course with VCD compared with VRD as initial therapy in MM. Similar outcomes were seen despite the higher proportion of patients in the VCD arm with renal failure, a feature typically associated with inferior outcomes [52].

This way of thinking, and subsequently of the approach, is assumed to lead to a maximal therapeutic effect with an acceptable toxicity in the greatest number of patients at the beginning of the treatment, which could prevent evaluation of disease resistance, inhibit the progression and complications and consequently extend the survival rate. Although, it is still hard to imagine the possibility of the eradication of myeloma clones by even the most radical treatment.

Maintenance therapy with bortezomib in MM

It is very important to schedule an extended treatment in myeloma multiple patients to maintain the effect of initial therapy. In recent years, a number of studies have been conducted on maintenance therapy with bortezomib. A randomized Phase III study PETHEMA group of patients with untreated MM ≥65 years found that the reduction of bortezomib dosing in subsequent treatments after one cycle of VTP (bortezomib, thalidomide, prednisone) or VMP (thalidomide instead of melphalan) to once per week resulted in a marked reduction of the neurotoxicity (Table 4) [11]. The continuation of initial treatment was the maintenance therapy with VP or VT, which increased CR level after induction from 24 to 42% [11]. The authors recommend a combination of bortezomib with thalidomide in the procedure for older patients with MM. A marked trend toward more favorable PFS and OS was noticed, but without reaching statistical significance [11].

In a randomized Phase III study, HOVON-65/GMMG-HD4 patients with MM aged <65 years were randomly assigned to receive induction therapy PAD or VAD with subsequent AHSCT. Afterward the PAD arm patients received maintenance therapy with bortezomib at a dose of 1.3 mg/m^2 every 2 weeks and the second arm received thalidomide 50 mg/day for 2 years (Table 4) [10]. It was found that the arm with bortezomib achieved a higher CR – 49 versus 34% in the second arm, but also a benefit in PFS and most importantly in OS [10]. A benefit is also related to the lower toxicity of the bortezomib arm, where due to adverse circumstances, maintenance therapy was discontinued in 9% of patients compared with 31% in the thalidomide arm.

Sonneveld *et al.* after a median observation of 41 months confirmed the favorable PFS and OS for the PAD arm followed by maintenance treatment with bortezomib administered once every 2 weeks [10]. What is particularly important, significant improvement in PFS, but also in the OS, was demonstrated for patients treated with bortezomib versus thalidomide in maintenance, respectively, 30 versus 13 months (p = 0.004)

Table 4. Results of prospective clinical trials on the use of bortezomib as a maintenance treatment of multiple myeloma.

Study (year)/trial	Auto-HSCT	Bortezomib in induction	Maintenance	N	OS (%)	PFS (%)	PN (%)	Ref.
Mateos *et al.* (2012)	No	Yes	Bortezomib + prednisone	89	50 (5 y)	32 m (5 y)	3	[11]
		Yes	Bortezomib + thalidomide	89	69 (5 y)	39 m (5 y)†	9	
Palumbo *et al.* (2010)	No	Yes	Bortezomib + thalidomide	139	59 (5 y)†	56 (3 y)†	ND	[53]
Palumbo *et al.* (2014)		No	Observation	139	46 (5 y)	41 (3 y)	ND	[9]
Sonneveld *et al.* (2012)	Yes	Yes	Bortezomib	410		28 m (3, 5 y)	ND	[10]
		No	Thalidomide	419	p = 0.049	35 m (3, 5 y)†	ND	

†Differences statistically significant.
Auto-HSCT: Autologous hematopietic stem cells transplantation; m: Months; med.: Median; N: Number of cases; ND: No data; OS: Overall survival; PFS: Progression-free survival; PN: Peripheral neuropathy; y: Years.

and 54 versus 21 months (p = 0.001) in patients with a particularly poor prognosis with hypercreatininemia >2.0 mg/dl and a similar advantage of this treatment in patients with deletion 17p13 [10].

Very important data regarding a randomized study bortezomib–melphalan–prednison–thalidomide followed by bortezomib–thalidomide maintenance (VMPT-VT) versus VMP were published this year by Palumbo *et al.* in not eligible transplantation of newly MM patients (Table 4) [9]. In the initial analysis with a median follow-up of 23 months, VMPT-VT improved the complete response rate from 24 to 38% and 3-year PFS from 41 to 56% compared with VMP. In this analysis, median follow-up was 54 months. The median PFS was significantly longer with VMPT-VT (35.3 months) than with VMP (24.8 months; hazard ratio [HR]: 0.58; p < 0.001). The time till the next therapy was 46.6 months in the VMPT-VT group and 27.8 months in the VMP group (HR: 0.52; p < 0.001). The 5-year OS was greater with VMPT-VT (61%) than with VMP (51%; HR: 0.70; p = 0.01). Survival from relapse was identical in both groups (HR: 0.92; p = 0.63) [9]. In the VMPT-VT group, the most frequent grades 3–4 adverse events included neutropenia (38%), thrombocytopenia (22%), peripheral neuropathy (11%) and cardiological events (11%). All of these, except for thrombocytopenia, were significantly more frequent in the VMPT-VT patients [9]. It was proved that bortezomib and thalidomide significantly improved OS in MM patients not eligible for transplantation.

The authors point out a significant reduction in the toxicity of bortezomib with weekly dosing, without a loss of efficacy [53].

In a study of 49 patients with advanced MM with the use of effective treatment with bortezomib and subsequently bortezomib at 1.3 mg/m^2 twice a month, with dexamethasone 20 mg/day on days 1–2, 15–16, very high tolerability was reported. The median time to progression was 16 months with a PFS of 61% after 1 year. The authors noted that the administration of bortezomib every 2 weeks was associated with no neurotoxicity ≥ grade 3 [33].

Bortezomib as a component of conditioning chemotherapy before AHSCT

In 2010, Lonial *et al.* published the results of a Phase I/II study, where bortezomib was added to chemotherapy conditioning before AHSCT [54]. The study enrolled patients with MM who had not attained VGPR before AHSCT. Bortezomib was administered as a single dose 24 h before or 24 h after high-dose melphalan at escalated dose of 1.0, 1.3 or 1.6 mg/m^2. The study included 39 patients. There was no increased toxicity of such treatment. The response was demonstrated in 87% of patients and 51% achieved at least a VGPR [54]. This observation encourages one to combine bortezomib with conditioning regimens before AHSCT as an effective and safe approach.

In 2010, Roussel *et al.* published the results of the study, where bortezomib at a dose 1 mg/m^2 four-times was added to the conditioning with melphalan 200 mg/m^2 prior to AHSCT [55]. The study included 54 patients, in which 70% received at least VGPR and 32% received CR after AHSCT. There was no increased toxicity or mortality associated with such an approach [55].

In 2013, authors from Japan published the results of a pilot study on the combination of bortezomib and high-dose melphalan as a conditioning regimen followed by an AHSCT [56]. The patients received two doses of 1.3 mg/m^2 of bortezomib on days –4 and –1 and 100 mg/m^2 of melphalan on days –3 and –2. Such treatment was used in 17 patients, and no negative impact on the course of AHSCT was observed [56].

Consolidation therapy with bortezomib

There are some observations with consolidation therapy using bortezomib-based therapy (Table 5).

In the first of them, patients achieving at least a very impressive partial response who had an available molecular marker based on the immunoglobulin heavy-chain rearrangement received four courses of treatment every month: four infusions

per month of bortezomib at 1.6 mg/m^2, thalidomide at 200 mg/day and dexamethasone at 20 mg/day on days 1–4, 8–11 and 15–18, respectively (Table 5) [57]. Patients were studied with tumor clone-specific primers by qualitative nested PCR and RQ-PCR. Of 39 patients enrolled, 31 received the four bortezomib–thalidomide–dexamethasone (VTD) courses. Immunofixation complete responses increased from 15% after auto-SCT to 49% after VTD. Molecular remissions were 3% after auto-SCT and 18% after VTD. The median time to maximum response was 3.5 months. So far, no patient in molecular remission has relapsed (median follow-up, 42 months). VTD consolidation induced an additional depletion of 4.14 natural logarithms of tumor burden by RQ-PCR. Patients with a tumor load less than the median value after VTD had outcomes better than those who had tumor loads above the median value after VTD (at median follow-up: PFS, 100 vs 57%; p = 0.001) [57].

In a randomized, Phase III study, superior CR/nCR rates and extended PFS were demonstrated with VTD versus TD as induction therapy before, and consolidation after, double AHSCT for newly diagnosed myeloma patients (Table 5) [8]. This per-protocol analysis (VTD, n = 160; TD, n = 161) specifically assessed the efficacy and safety of consolidation with VTD or TD. Before starting consolidation, CR/nCR rates were not significantly different in the VTD (63.1%) and TD arms (54.7%). After consolidation, CR (60.6 vs 46.6%) and CR/nCR (73.1 vs 60.9%) rates were significantly higher for VTD-treated patients versus TD-treated patients. VTD consolidation significantly increased CR and CR/nCR rates, but TD did not. With a median follow-up of 30.4 months from the start of consolidation, 3-year PFS was significantly longer for the VTD group (60 vs 48% for TD). Grade 2 or 3 peripheral neuropathy (8.1 vs 2.4%) was more frequent with VTD (grade 3, 0.6%) versus TD consolidation. The superior efficacy of VTD versus TD as induction was retained despite readministration as a consolidation therapy after a double autologous transplantation. VTD consolidation therapy significantly contributed to improved clinical outcomes observed for patients randomly assigned to the VTD arm of the study [8].

Another retrospective case series analysis included a total of 48 patients with newly diagnosed MM, who achieved CR or VGPR after bortezomib-based induction and were eligible for AHSCT. Twenty-four of these patients proceeded with AHSCT and the other 24 opted out of ASCT and received two additional cycles of bortezomib therapy as consolidation (Table 5) [58]. With a median follow-up of 28.5 months in the AHSCT group and 29 months in the consolidation group, no significant difference was seen in PFS and OS. This study may suggest that the continuation of effective chemotherapy as consolidation may promise a comparable late outcome as AHSCT [58].

Retreatment with bortezomib

In a Phase II study, Petrucci *et al.* showed that the retreatment with bortezomib ± dexamethasone may be effective in patients who relapsed 6 months after the end of treatment and who

Table 5. Selected studies with bortezomib-based consolidation therapy in previously untreated multiple myeloma.

Study (year)	N	Induction regimen	AHSCT	CR after AHSCT (%)	Consolidation regimen	CR after consolidation (%)	PFS	OS	Ref.
Ladetto *et al.* (2010)	39	VAD	Yes	15 (immunofixation) 3 (molecular)	4 × VTD (Bor 1,6 mg/m^2 1, 4, 8, 11; Thal. 200 mg/d, Dex. 20 mg/d)	49 (immunofixation) 18 (molecular)	Med 60 m	89% (after 3 y)	[57]
Cavo *et al.* (2012)	474	VTD	Yes	48.7*	VTD (Bor 1,3 mg/m^2 1, 4, 8, 11; Thal. 100 mg/d, Dex. 40 mg/d)	60.6**	60%*** (after 3 y)	90% (after 3 y)	[8]
		TD	Yes	40.4*	TD (Thal. 100 mg/d, Dex. 40 mg/d)	46.6**	48%*** (after 3 y)	88% (after 3 y)	
Gao *et al.* (2013)	48	Bor-based	Yes	ND	No		Med 39 m****	87.5%***** (after 3 y)	[58]
		Bor-based	No	ND	Bor-based		Med 32 m****	67.5%***** (after 3 y)	

*p = 0.1; **p = 0.01; ***p = 0.04; ****p = 0.82; *****p = 0.97.
AHSCT: Autologous hematopietic stem cells transplantation; Bor: Bortezomib; CR: Complete remission; d: Day; Dex: Dexamethasone; m: Months; med: Median; N: Number of cases; ND: No data; OS: Overall survival; PFS: Progression-free survival; Thal: Thalidomide; VAD: Vincristin–adriblastin–dexamethason; y: Years.

received in the past eight cycles of therapy with bortezomib [59]. About 40% of patients retreated with bortezomib had a response. There was no additional cumulative toxicity [59]. Currently, patients are enrolled to a randomized Phase III study of extended treatment with bortezomib.

Resistance to bortezomib treatment

In 2011, the results of Phase I/II Perifosine/bortezomib ± dexamethasone were reported [60]. Perifosine is a novel modulator of a signal transductor which in preclinical studies enhanced the antimyeloma effect of dexamethasone and bortezomib; 73% were refractory to bortezomib and 51% were refractory to bortezomib and dexamethasone. Complete response was demonstrated in 41% of patients including 65% in patients with relapsed MM and 31% with disease refractory to bortezomib. No significant toxicity and no treatment-related mortality were reported [60].

Another substance that gives hope in overcoming resistance to bortezomib is a panobinostat, pan-deacetylase inhibitor. Phase Ib studies demonstrated that the combination of bortezomib and panobinostat may be effective in patients with refractory or relapsed MM, also in case of prior resistance to bortezomib [61,62]. Currently, the next phases of research have begun to demonstrate the effect of treatment in randomized patients. Richardson *et al.* demonstrated in a Phase II study that the combination of bortezomib, dexamethasone, panobinostat is effective in heavily pretreated patients refractory to bortezomib. The level of response was 34.5% [61].

Bortezomib in MM patients with renal failure

Renal failure significantly worsens the prognosis in MM. Mild or moderate renal failure caused by accumulation and deposition of monoclonal light chains, which forms casts blocking the distal tubules of the kidney, is a relatively common complication of MM [63]. Light chains also interact directly toxic to the proximal renal tubules. In clinical trials, in patients with newly diagnosed myeloma, and in those with refractory disease, the treatment based on bortezomib resulted in the improvement of the renal function, with a rapid withdrawal of renal failure in many patients [64].

In the Phase III VISTA trial in patients with untreated MM, the withdrawal of renal failure was shown in 49 of 111 (44%) patients who received bortezomib with melphalan and prednisone compared with 40 of 116 (34%) patients receiving melphalan and prednisone ($p = 0.07$) [63].

A subgroup analysis dependent on the degree of renal function in the APEX study showed no difference in the results of the treatment of patients with creatinine clearance ≤50 ml/min compared with those who had >50 ml/min in the group treated with bortezomib. It was different in the group treated with dexamethasone, where OS was statistically shorter ($p = 0.003$) in patients with CC ≤50 ml/min. The profile of tolerance did not differ between patients with varying renal function treated with bortezomib. The authors noted that bortezomib is active against MM, regardless of the degree of renal insufficiency [65].

In 10 patients with a creatinine clearance (GFR) <30 ml/min who were enrolled in the study SUMMIT and CREST with the sponsor's approval, bortezomib was administered at a dose of 1.0 or 1.3 mg/m^2 per dose on days 1, 4, 8 11 in 21 day cycles. Seven of them completed eight planned treatments. PR was achieved in two patients with minimal PR in one patient. The results were similar to those obtained in the whole group of patients with MM [66].

Ludwig *et al.* demonstrated in a publication in 2010 that the combination of chemotherapy: bortezomib 1.0 mg/m^2 iv. on days 1, 4, 8 and 11, doxorubicin 9 mg/m^2 iv. on days 1, 4 and dexamethasone 40 mg/m^2 iv. on days 1, 4, 8 and 11 may improve renal function in patients with a light-chain disease and who had begun treatment with a reduced creatinine clearance <50 ml/min [67]. The median GFR increased from 20.5 to 48.4 ml/min. Improvement of renal function correlated with the treatment effect, and the greatest improvement of GFR to 59.6 ml/min was demonstrated in patients with a treatment effect level CR/nCR/VGPR [67].

Pönisch *et al.* in 2013 showed that the BPV program, which is a combination of bortezomib 1.3 mg/m^2 administered on days 1, 4, 8, 11, prednisone 100 mg on days 1, 2, 4, 8, 11 and bendamustine 60 mg/m^2 on days 1 and 2, is effective and well tolerated in patients with refractory/relapsed MM with renal damage induced by light chains [68]. The treatment resulted in a response in 67% of the patients and an improvement of renal function in >50% of treated patients. It was also demonstrated that chemotherapy with bortezomib before and after AHSCT reduces a worse prognosis in patients with renal insufficiency and a diagnosis of MM [69].

Bortezomib treatment in the elderly

In the elderly patients, a less intensive approach should be considered, in the presence of neuropathy, which can significantly affect the overall condition. In 2012, Sopeña *et al.* published the results of an analysis comparing bortezomib administered weekly in seven studies to the standard approach published in the VISTA study. It was demonstrated that dosing bortezomib once a week in elderly patients with relapsed/refractory MM resulted in lower toxicity without impairing its effectiveness. There was also the need to modify the treatment and thereby making such bortezomib treatment possibly more effective in older patients [70].

Pathophysiology & treatment of toxicity associated with bortezomib

Neuropathy

Another very significant bortezomib treatment complication is neuropathy. Clinically, it is important to note a baseline rate of neuropathy, especially in patients with relapsed/refractory MM. In the Phase II SUMMIT and CREST study with bortezomib, more than 80% of patients had symptoms of polyneuropathy, assessed by questionnaires and neurological examination [71]. It should be emphasized that serious neuropathy following treatment with bortezomib most frequently developed in patients

with previously peripheral neuronal damage. In the APEX study, of the 37% of patients with polyneuropathy, 9% had ≥ grade 3. The neuropathy was typically sensory although 2% of patients developed motor. It appears that the neuropathy was not dose dependent and typically developed during the five cycle and reaching a plateau by cycle 8, associated with a cumulative dose of 26 and 42 mg/m^2. It was noted that neuropathy was not related to age, previous treatment (including Vinca alkaloids and thalidomide) or diabetes.

It is worth noting that in patients who have symptoms of polyneuropathy, treatment should be matched to limit the possibility of worsening neuropathy. It is necessary to suitably combine chemotherapeutic agents and reduce the dose of bortezomib in a single dose, but also the cumulative dose over time, for example, by administering bortezomib once a week, which may reduce the risk of neuropathy, without loss of efficacy [72].

The prevalence of polyneuropathy in grade ≥3 in VMP regimen decreased from 14 to 2% when bortezomib was administered once, not twice a week, without loss of efficacy [73].

Currently, there is no proven effective prophylaxis for polyneuropathy. A variety of drugs are used to relieve the symptoms of bortezomib neuropathy including opioids, anticonvulsants, serotonin–norepinephrine reuptake inhibitors, nonsteroidal anti-inflammatory agents, vitamins, nutritional supplements as alpha-lipoic acid, glutamine and L-carnitine [74]. However, supplements should be administered with caution as there are some data suggesting that agents like vitamin C or green tea may reduce the effectiveness of treatment with bortezomib [75].

According to bortezomib neuropathy, results of the studies where bortezomib were administered subcutaneously became particularly relevant [36,76,77].

In 2008, the conclusions of a Phase I study were published, which showed in a group of 24 patients treated in 50% of an intravenously administered medication and 50% of the drug administered subcutaneously, similar systemic exposure in both groups of patients [76]. As a continuation, a multicenter, randomized Phase III study was conducted to confirm the hypothesis of comparable therapeutic effect with a similar toxicity for bortezomib administered intravenously to subcutaneously [36]. The study enrolled patients with relapsed or refractory, secretory MM. Two to three lines of treatment were allowed. The exclusion criteria were previous treatment with bortezomib as well as peripheral neuropathy ≥ grade 2 or neuropathic pain. Bortezomib was administered in the classical scheme with dexamethasone. Subcutaneous Velcade was prepared in a concentration of 2.5 mg/ml and then administered in the thigh or abdominal area. Injections in the same location were forbidden. The program allowed up to eight cycles of treatment, with the possibility of having two additional cycles for patients who were not confirmed or lost PR. Patients were randomized in a 2:1 ratio in favor of subcutaneous administration.

A tendency was noted to a higher incidence of adverse events ≥ grade 3 for the intravenous group – 70% compared with arm A – 57%, which resulted in the necessity of ending the treatment, respectively, in 27 and 22% of patients and to reduce the dose in 43 and 31% of patients. Serious adverse events were reported in a similar proportion of patients in both groups. In the subcutaneous group, it was 36% and in the intravenous group – 35% of patients.

The incidence of any symptoms of peripheral neuropathy, as well as polyneuropathy grades 2 and 3, was lower in the subcutaneous group compared with the intravenous, and it was 38%, 24.6 versus 53.41, 16%, respectively. The risk factors of such complications were balanced for both groups (Figure 2).

In 2012, a supplement of the study of subcutaneous versus intravenous bortezomib in MM patients was published, demonstrating no difference in PFS and OS in both groups of patients [77].

On the basis of these studies, the FDA in 23 January 2012 and the Committee for Medical Products for Human Use of the EMA in 26 June 2012 positively recommended administration of subcutaneous bortezomib, which was noted in the drug characteristics [78]. Also based on these studies, subcutaneous administration of bortezomib was registered, and due to the lower toxicity, it is recommended for all myeloma patients.

Other toxicities

Thrombocytopenia is a well-recognized problem associated with bortezomib treatment. In almost all patients, the platelets count drops during 1 and 14 days of treatment and then recovers to a baseline level by day 21 of the cycle. The mean reduction in relapsed/refractory myeloma patients of the platelets is 60%, and it appears to be independent of the baseline platelet count and indicators of disease activity. Murine studies showed no cytotoxic effect on megakaryocytes, therefore suggesting a distinct mechanism, perhaps that of the immune mechanism [79].

There have also been reported cases of acute pancreatitis induced by bortezomib treatment [80].

Current status

Bortezomib (Velcade) – registered indications

Velcade as monotherapy is indicated for the treatment of adult patients with progressive MM who have received at least one prior therapy and who have already undergone or are unsuitable for hematopoietic stem cell transplantation.

Velcade in combination with melphalan and prednisone is indicated for the treatment of adult patients with previously untreated MM who are not eligible for high-dose chemotherapy with hematopoietic stem cell transplantation.

Velcade in combination with dexamethasone, or with dexamethasone and thalidomide, is indicated for the induction treatment of adult patients with previously untreated MM who are eligible for high-dose chemotherapy with hematopoietic stem cell transplantation.

Expert commentary & five-year view

The introduction of bortezomib for the treatment of MM was undoubtedly a step forward in managing to extend the life of

the patients [5,6,38,39]. In recent years, bortezomib has become an essential element of both the induction as well as consolidating polychemotherapy of MM patients, when they are candidates for autologous stem cell transplantation as well as in the elderly and patients with comorbidities [9–11,38,40–43,49–51]. Research has been conducted on the optimization of the extended maintenance treatment with bortezomib. The chance for a more consistent bortezomib treatment increased after results of the studies related to subcutaneous bortezomib administration, which demonstrated that it is significantly better tolerated, particularly with regard to neurotoxicity, and retains the same pharmacokinetic and pharmacodynamic parameters and consequently, the same effect [36,77].

Of great importance is the efficacy of bortezomib in patients with a particularly poor cytogenetics prognosis and renal failure, regardless of its degree [44,66–69]. The problem that still remains is resistance to bortezomib treatment in some myeloma patients, thus research is focused on associations with overcoming agents [60–62]. Further research has been conducted on the optimization of treatment regimens based on bortezomib using new therapeutic agents.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Bortezomib is the first proteasome inhibitor drug tested in human patients, causing tumor cell death by degradation of key proteins. It acts in a very selective and reversible way.
- It was approved by the US FDA on an accelerated basis for treatment of relapsed or resistant multiple myeloma patients due to the very impressive results from Phase II trials, and currently also for the treatment of untreated patients.
- Bortezomib is widely used in induction and consolidation regimens. The research has been conducted on the optimal use in autologous hematopoietic stem cell transplantation and maintenance therapy.
- Demonstration of less neurological toxicity, particularly through the introduction of the subcutaneous administration of bortezomib, increases the chance of managing the intended treatment plan, and thus the effectiveness of therapy.
- Currently, further research is being conducted on the combination of bortezomib with new agents in multiple myeloma.

References

1. Ludwig H, Fritz E, Friedl HP. Epidemiologic and age-dependent data on multiple myeloma in Austria. J Natl Cancer Inst 1982;68:729-33
2. Harousseau JL, Dreyling M. ESMO Guidelines Working Group. Multiple myeloma: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 2008;19:1155-7
3. Anderson KC, Alsina M, Bensinger W, et al. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: multiple myeloma. J Natl Compr Canc Netw 2009;7:908-42
4. Giralt S, Stadtmauer EA, Harousseau JL, et al. IMWG International myeloma working group (IMWG) consensus statement and guidelines regarding the current status of stem cell collection and high-dose therapy for multiple myeloma and the role of plerixafor (AMD 3100). Leukemia 2009;23:1904-12
5. Dimopoulos MA, Richardson PG, Schlag R, et al. VMP (Bortezomib, Melphalan, and Prednisone) is active and well tolerated in newly diagnosed patients with multiple myeloma with moderately impaired renal function, and results in reversal of renal impairment: cohort analysis of the phase III VISTA study. J Clin Oncol 2009;27:6086-93
6. Jagannath S, Richardson PG, Barlogie B, et al. SUMMIT/CREST Investigators. Bortezomib in combination with dexamethasone for the treatment of patients with relapsed and/or refractory multiple myeloma with less than optimal response to bortezomib alone. Haematologica 2006;91:929-34
7. Harousseau JL, Attal M, Avet-Loiseau H, et al. 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 2010;28:4621-9
8. Cavo M, Pantani L, Petrucci MT, et al. GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) Italian Myeloma Network. Bortezomib-thalidomide-dexamethasone is superior to thalidomide-dexamethasone as consolidation therapy after autologous hematopoietic stem cell transplantation in patients with newly diagnosed multiple myeloma. Blood 2012;120:9-19
9. Palumbo A, Bringhen S, Larocca A, et al. Bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide compared with bortezomib-melphalan-prednisone for initial treatment of multiple myeloma: updated follow-up and improved survival. J Clin Oncol 2014;32(7):634-40
10. Sonneveld P, Schmidt-Wolf IG, van der Holt B, et al. Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/GMMG-HD4 trial. J Clin Oncol 2012;30:2946-55
11. Mateos MV, Oriol A, Martínez-López J, et al. Maintenance therapy with bortezomib plus thalidomide or bortezomib plus prednisone in elderly multiple myeloma patients included in the GEM2005MAS65 trial. Blood 2012;120:2581-8
12. Spencer A, Prince HM, Roberts AW, et al. Consolidation therapy with low-dose

thalidomide and prednisolone prolongs the survival of multiple myeloma patients undergoing a single autologous stem-cell transplantation procedure. J Clin Oncol 2009;27:1788-93

13. Ludwig H, Adam Z, Tothova E, et al. Thalidomide maintenance treatment increases progression-free but not overall survival in elderly patients with multiple myeloma. Haematologica 2010;95:1548-54
14. McCarthy P, Owzarb K, Hofmeister C, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. N Engl J Med 2012;366:1770-81
15. Richardson PG, Sonneveld P, Schuster MW, et al. Assessment of Proteasome Inhibition for Extending Remissions (APEX) Investigators. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med 2005;352:2487-98
16. Millennium Pharmaceuticals Inc. Velacade (bortezomib) for injection: US prescribing information [on-line]. Available from: www.fda.gov/cder/foi/label/2008/021602s015lbl.pdf [Last accessed 23 October 2008]
17. Sterz J, von Metzler I, Hahne JC, et al. The potential of proteasome inhibitors in cancer therapy. Expert Opin Investig Drugs 2008;17:879-95
18. Cayrol C, Ducommun B. Interaction with cyclin-dependent kinases and PCNA modulates proteasome-dependent degradation of p21. Oncogene 1998;17:2437-44
19. Hideshima T, Richardson P, Chauhan D, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res 2001;61:3071-6
20. Maki CG, Huibregtse JM, Howley PM. In vivo ubiquitination and proteasome-mediated degradation of p53(1). Cancer Res 1996;56(11):2649-54
21. Marshansky V, Wang X, Bertrand R, et al. Proteasomes modulate balance among proapoptotic and antiapoptotic Bcl-2 family members and compromise functioning of the electron transport chain in leukemic cells. J Immunol 2001;166:3130-42
22. Stancovski I, Gonen H, Orian A, et al. Degradation of the proto-oncogene product c-Fos by the ubiquitin proteolytic system in vivo and in vitro: identification and characterization of the conjugating enzymes. Mol Cell Biol 1995;15:7106-16
23. Kho CJ, Huggins GS, Endege WO, et al. The polymyositis-scleroderma autoantigen interacts with the helix-loop-helix proteins E12 and E47. J Biol Chem 1997;272:13426-31
24. Chang YC, Lee YS, Tejima T, et al. Mdm2 and bax, downstream mediators of the p53 response, are degraded by the ubiquitin-proteasome pathway. Cell Growth Differ 1998;9:79-84
25. Traenckner EB, Wilk S, Baeuerle PA. A proteasome inhibitor prevents activation of NF-kappa B and stabilizes a newly phosphorylated form of I kappa B-alpha that is still bound to NF-kappa B. EMBO J 1994;13:5433-41
26. Mitsiades N, Mitsiades CS, Richardson PG, et al. Molecular sequelae of histone deacetylase inhibition in human malignant B cells. Blood 2003;101:4055-62
27. Ma MH, Yang HH, Parker K, et al. The proteasome inhibitor PS-341 markedly enhances sensitivity of multiple myeloma tumor cells to chemotherapeutic agents. Clin Cancer Res 2003;9:1136-44
28. Yan H, Wang YC, Li D, et al. Arsenic trioxide and proteasome inhibitor bortezomib synergistically induce apoptosis in leukemic cells: the role of protein kinase Cdelta. Leukemia 2007;21:1488-95
29. Orlowski RZ, Stinchcombe TE, Mitchell BS, et al. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J Clin Oncol 2002;20:4420-7
30. Pennisi A, Li X, Ling W, et al. Inhibitor of DASH proteases affects expression of adhesion molecules in osteoclasts and reduces myeloma growth and bone disease. Br J Haematol 2009;145:775-87
31. Giuliani N, Morandi F, Tagliaferri S, et al. The proteasome inhibitor bortezomib affects osteoblast differentiation in vitro and in vivo in multiple myeloma patients. Blood 2007;110:334-8
32. Delforge M, Terpos E, Richardson PG, et al. Fewer bone disease events, improvement in bone remodeling, and evidence of bone healing with bortezomib plus melphalan-prednisone vs. melphalan-prednisone in the phase III VISTA trial in multiple myeloma. Eur J Haematol 2011;86:372-84
33. Zangari M, Esseltine D, Lee CK, et al. Response to bortezomib is associated to osteoblastic activation in patients with multiple myeloma. Br J Haematol 2005;131:71-3
34. Lund T, Søe K, Abildgaard N, et al. First-line treatment with bortezomib rapidly stimulates both osteoblast activity and bone matrix deposition in patients with multiple myeloma, and stimulates osteoblast proliferation and differentiation in vitro. Eur J Haematol 2010;85:290-9
35. European Medicines Agency, Velcade® - summary of product characteristics. Available from: www.emea.europa.eu/humandocs/PDFs/EPAR/velcade/H-539-PI-en.pdf [Last accessed 8 April 2009]
36. Moreau P, Pylypenko H, Grosicki S, et al. Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: a randomized, phase 3, non-inferiority study. Lancet Oncol 2011;12:431-40
37. Golden EB, Lam PY, Kardosh A, et al. Green tea polyphenols block the anticancer effects of bortezomib and other boronic acid-based proteasome inhibitors. Blood 2009;113:5927-37
38. Orlowski RZ, Stinchcombe TE, Mitchell BS, et al. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J Clin Oncol 2002;20:4420-7
39. Jagannath S, Barlogie B, Berenson JR, et al. Updated survival analyses after prolonged follow-up of the phase 2, multi-center CREST study of bortezomib in relapsed or refractory multiple myeloma. Br J Haematol 2008;143:537-40
40. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med 2003;348:2609-17
41. Richardson PG, Sonneveld P, Schuster MW, et al. Assessment of Proteasome Inhibition for Extending Remissions (APEX) Investigators. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med 2005;352:2487-98
42. Orlowski RZ, Nagler A, Sonneveld P, et al. Randomized phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: combination therapy improves time to progression. J Clin Oncol 2007;25:3892-901
43. San Miguel JF, Schlag R, Khuageva NK, et al. VISTA Trial Investigators. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med 2008;359:906-17
44. San Miguel JF, Schlag R, Khuageva NK, et al. Continued overall survival benefit after 5 Years' follow-up with bortezomib-melphalan-prednisone (VMP) versus melphalan-prednisone (MP) in patients with previously untreated multiple

myeloma, and no increased risk of second primary malignancies: final results of the phase 3 VISTA Trial. Blood 2011;118:476

45. Chanan-Khan A, Sonneveld P, Schuster MW, et al. Analysis of herpes zoster events among bortezomib-treated patients in the phase III APEX study. J Clin Oncol 2008;26:4784-90
46. Vickrey E, Allen S, Mehta J, Singhal S. Acyclovir to prevent reactivation of varicella zoster virus (herpes zoster) in multiple myeloma patients receiving bortezomib therapy. Cancer 2009;115:229-32
47. Mateos MV, Richardson P, Shi H. Higher cumulative bortezomib dose results in better overall survival (OS) in patients with previously untreated multiple myeloma (MM) receiving bortezomib-melphalan-prednisone (VMP) in the phase 3 VISTA Study. Blood (ASH Conference Presentation) 2013;122:1968
48. Sonneveld P, Scheid C, van der Holt B, et al. Bortezomib induction and maintenance treatment improves survival in patients with newly diagnosed multiple myeloma:extended follow-up of the HOVON-65/GMMG-HD4 trial. Blood 2013;122:404
49. Attal M, Harousseau JL, Facon T, et al. InterGroupe Francophone du Myélome. Single versus double autologous stem-cell transplantation for multiple myeloma. N Engl J Med 2003;349:2495-502
50. Berenson JR, Yellin O, Chen CS, et al. A modified regimen of pegylated liposomal doxorubicin, bortezomib and dexamethasone (DVD) is effective and well tolerated for previously untreated multiple myeloma patients. Br J Haematol 2011;155:580-7
51. Jakubowiak AJ, Griffith KA, Reece DE, et al. Lenalidomide, bortezomib, pegylated liposomal doxorubicin, and dexamethasone in newly diagnosed multiple myeloma: a phase 1/2 Multiple Myeloma Research Consortium trial. Blood 2011;118:535-43
52. Kumar S, Engebretson A, Buadi F, et al. Comparable Outcomes With Bortezomib-Cyclophosphamide-Dexamethasone (VCD) and Bortezomib-Lenalidomide-Dexamethasone (VRD) For Initial Treatment Of Newly Diagnosed Multiple Myeloma (MM). Blood 2013;122:3178
53. Palumbo A, Bringhen S, Rossi D, et al. Bortezomib-melphalan-prednisone-thalidomide followed by maintenance with bortezomib-thalidomide compared with bortezomib-melphalan-prednisone for initial treatment of multiple myeloma: a randomized controlled trial. J Clin Oncol 2010;28:5101-9
54. Lonial S, Kaufman J, Tighiouart M, et al. A phase I/II trial combining high-dose melphalan and autologous transplant with bortezomib for multiple myeloma: a dose- and schedule-finding study. Clin Cancer Res 2010;16:5079-86
55. Roussel M, Moreau P, Huynh A, et al. Intergroupe Francophone du Myélome (IFM). Bortezomib and high-dose melphalan as conditioning regimen before autologous stem cell transplantation in patients with de novo multiple myeloma: a phase 2 study of the Intergroupe Francophone du Myelome (IFM). Blood 2010;115:32-7
56. Miyamoto T, Yoshimoto G, Kamimura T, et al. Combination of high-dose melphalan and bortezomib as conditioning regimen for autologous peripheral blood stem cell transplantation in multiple myeloma. Int J Hematol 2013;98:337-45
57. Ladetto M, Pagliano G, Ferrero S, et al. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. J Clin Oncol 2010;28:2077-84
58. Gao W, Geng C, Zhang L, et al. Comparable outcome of stem cell transplant versus bortezomib-based consolidation in myeloma patients after major response to induction. Hematology (Am Soc Hematol Educ Program) 2013;18:341-7
59. Petrucci MT, Giraldo P, Corradini P, et al. A prospective, international phase 2 study of bortezomib re-treatment in patients with relapsed multiple myeloma. Br J Haematol 2013;160:649-59
60. Richardson PG, Wolf J, Jakubowiak A, et al. Perifosine plus bortezomib and dexamethasone in patients with relapsed/refractory multiple myeloma previously treated with bortezomib: results of a multi-center phase I/II trial. J Clin Oncol 2011;29:4243-9
61. Richardson PG, Schlossman RL, Alsina M, et al. PANORAMA 2: panobinostat in combination with bortezomib and dexamethasone in patients with relapsed and bortezomib-refractory myeloma. Blood 2013;122:2331-7
62. San-Miguel JF, Richardson PG, Günther A, et al. Phase Ib Study of Panobinostat and Bortezomib in Relapsed or Relapsed and Refractory Multiple Myeloma. J Clin Oncol 2013;31:3696-703
63. Dimopoulos MA, Kastritis E, Rosinol L, et al. Pathogenesis and treatment of renal failure in multiple myeloma. Leukemia 2008;22:1485-93
64. Terpos E, Katodritou E, Tsiftsakis E, et al. Greek Myeloma Study Group. Cystatin-C is an independent prognostic factor for survival in multiple myeloma and is reduced by bortezomib administration. Haematologica 2009;94:372-9
65. San-Miguel JF, Richardson PG, Sonneveld P, et al. Efficacy and safety of bortezomib in patients with renal impairment: results from the APEX phase 3 study. Leukemia 2008;22:842-9
66. Jagannath S, Barlogie B, Berenson JR, et al. SUMMIT/CREST Investigators. Bortezomib in recurrent and/or refractory multiple myeloma. Initial clinical experience in patients with impaired renal function. Cancer 2005;103:1195-200
67. Ludwig H, Adam Z, Hajek R, et al. Light chain-induced acute renal failure can be reversed by bortezomib-doxorubicin-dexamethasone in multiple myeloma: results of a phase II study. J Clin Oncol 2010;28:4635-41
68. Pönisch W, Moll B, Bourgeois M, et al. Bendamustine and prednisone in combination with bortezomib (BPV) in the treatment of patients with relapsed or refractory multiple myeloma and light chain-induced renal failure. J Cancer Res Clin Oncol 2013;139:1937-46
69. Scheid C, Sonneveld P, Schmidt-Wolf IG, et al. Bortezomib before and after autologous stem cell transplantation overcomes the negative prognostic impact of renal impairment in newly diagnosed multiple myeloma: a Subgroup Analysis From the HOVON-65/GMMG-HD4 Trial. Haematologica 2014;99(1):148-54
70. Sopeña M, Clavero EM, Villa P, Martínez-López J. Efficacy and safety of reduced-intensity induction therapy with a bortezomib-based regimen in elderly patients with multiple myeloma. Ther Adv Hematol 2012;3:147-54
71. Richardson PG, Briemberg H, Jagannath S, et al. Frequency, characteristics, and reversibility of peripheral neuropathy during treatment of advanced multiple myeloma with bortezomib. J Clin Oncol 2006;24:3113-20
72. Bringhen S, Larocca A, Rossi D, et al. Efficacy and safety of once-weekly bortezomib in multiple myeloma patients. Blood 2010;116:4745-53
73. Palumbo A, Bringhen S, Rossi D, et al. A prospective, randomized, phase III study of bortezomib, melphalan, prednisone (VMP) and thalidomide (VMPT) versus bortezomib, melphalan, prednisone (VMP)

in elderly newly diagnosed myeloma patients. Blood 2008;112:652

74. Argyriou AA, Iconomou G, Kalofonos HP. Bortezomib-induced peripheral neuropathy in multiple myeloma: a comprehensive review of the literature. Blood 2008;112: 1593-9
75. Perrone G, Hideshima T, Ikeda H, et al. Ascorbic acid inhibits antitumor activity of bortezomib in vivo. Leukemia 2009;23: 1679-86
76. Moreau P, Coiteux V, Hulin C, et al. Prospective comparison of subcutaneous versus intravenous administration of bortezomib in patients with multiple myeloma. Haematologica 2008;93:1908-11
77. Arnulf B, Pylypenko H, Grosicki S, et al. Updated survival analysis of a randomized phase III study of subcutaneous versus intravenous bortezomib in patients with relapsed multiple myeloma. Haematologica 2012;97:1925-8
78. Millennium Pharmaceuticals Inc. Velcade® (bortezomib) for injection: prescribing information. Rev. 13. Millennium Pharmaceuticals Inc, Cambridge, MA, USA. 2012.
79. Lonial S, Waller EK, Richardson PG, et al. SUMMIT/CREST Investigators. Risk factors and kinetics of thrombocytopenia associated with bortezomib for relapsed, refractory multiple myeloma. Blood 2005;106:3777-84
80. Wang HH, Tsui J, Wang XY, et al. Bortezomib-induced acute pancreatitis in a patient with multiple myeloma. Leuk Lymphoma 2013. [Epub ahead of print]
81. Pineda-Roman M, Zangari M, van Rhee F, et al. VTD combination therapy with bortezomib-thalidomide-dexamethasone is highly effective in advanced and refractory multiple myeloma. Leukemia 2008;22: 1419-27
82. Palumbo A, Gay F, Bringhen S, et al. Bortezomib, doxorubicin and dexamethasone in advanced multiple myeloma. Ann Oncol 2008;19:1160-5
83. Ciolli S, Leoni F, Casini C, et al. The addition of liposomal doxorubicin to bortezomib, thalidomide and dexamethasone significantly improves clinical outcome of advanced multiple myeloma. Br J Haematol 2008;141:814-19