CANCER TREATMENT REVIEWS 2003; **29** (Suppl. 1): 33–39
doi:10.1016/S0305-7372(03)00080-X

CANCER TREATMENT REVIEWS

# Clinical update: proteasome inhibitors in hematologic malignancies

**Paul Richardson**

*Dana-Farber Cancer Institute, Boston, MA 02115, USA*

The proteasome inhibitor bortezomib (VELCADE; formerly PS-341, LDP-341, MLN341) is a novel dipeptide boronic acid. In cell culture and xenograft models, bortezomib showed potent activity, enhanced the sensitivity of cancer cells to traditional chemotherapeutics, and appeared to overcome drug resistance. *In vitro*, bortzomib downregulated the NF-κB pathway. NF-κB is a transcription factor that enhances the production of growth factors (e.g., IL-6), cell-adhesion molecules, and anti-apoptotic factors, all of which contribute to the growth of the tumor cell and/or protection from apoptosis. Phase II trials have been conducted in patients with relapsed and refractory multiple myeloma (SUMMIT trial, 202 patients) or relapsed myeloma (CREST trial, $n = 54$) using a 1.3 mg/m$^2$ dose given twice weekly for 2 weeks (days 1, 4, 8, 11; rest days 12–21). Both trials showed responses (including complete responses) with manageable toxicities, forming the basis for an ongoing phase III trial comparing response to bortezomib versus high-dose dexamethasone.


**Key words:** Proteasome inhibition; bortezomib; multiple myeloma; hematologic malignancies.

## INTRODUCTION

The proteasome is a multicatalytic enzyme complex that degrades numerous types of proteins (reviewed by Adams in this issue), many of which are regulatory proteins that control the cell cycle or play a role in survival pathways. The coordinated expression and degradation of these proteins are essential for normal cellular function. Consequently, the proteasome plays a central role in up- or down-regulation of growth signaling pathways by removing key signals via protein degradation. Inhibition of the proteasome is therefore a promising approach for the treatment of cancer, a disease characterized by defects in growth signaling pathways that promote the hyperproliferation of aberrant cells.

Correspondence to: Paul Richardson MD, Jerome Lipper Multiple Myeloma Center, Division of Hematologic Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA. Tel.: 1-617-632-2104; Fax: 1-617-632-6624; E-mail: paul_richardson@dfci.harvard.edu

The proteasome inhibitor, bortezomib (VELCADE; formerly PS-341, LDP-341, MLN341), is a novel dipeptide boronic acid small molecule that has shown antitumor activity in preclinical studies and is the first such agent to have progressed to clinical trials. A phase III trial in patients with multiple myeloma (MM) is ongoing and several trials in patients with other hematologic malignancies or solid tumors are in progress. Here, we briefly review the preclinical rationale for bortezomib and the clinical trial status of bortezomib in patients with hematologic malignancies, with a focus on MM.

## PRECLINICAL RATIONALE FOR PROTEASOME INHIBITORS IN HEMATOLOGIC MALIGNANCIES

In cell culture and in xenografted tumors, bortezomib had potent activity, enhanced the sensitivity of cancer cells to traditional tumoricidal chemotherapeutics (1–4) and appeared to overcome drug resistance (5). This activity is at least in part mediated by

the activity of bortezomib against the NF-κB pathway. The NF-κB pathway is constitutively active in some cancer cells and is associated with proliferation, cell survival, and protection from chemotherapy-induced apoptosis (Figure 1). Proteasome inhibition has also been shown to block chemotherapy- and radiotherapy-induced activation of NF-κB in laboratory studies, resulting in enhanced sensitivity to these tumoricidal agents and increased apoptosis in cancer cells (1–3,6).

Activation of NF-κB signaling appears to be particularly important for the survival of multiple myeloma cells (MMCs): MMCs isolated from patients had constitutive NF-κB activity, MMCs and BMSCs showed enhanced NF-κB activity, and chemoresistant MMCs had increased NF-κB activity compared with chemosensitive lines (5,7). Bortezomib appeared not only to have activity against MMCs, but also to downregulate protective interactions with bone marrow stromal cells (BMSCs) in the bone marrow microenvironment (4), and to inhibit blood vessel development (8). Thus, bortezomib acts against MMCs by antagonizing the activation of protective NF-κB functions in not only MMCs but BMSCs.

Myeloma growth and resistance pathways become activated when MMCs bind to normal BMSCs. Myeloma cells express VLA-4 and VLA-5, which are receptors that allow myeloma to bind to the VCAM-1 receptor on BMSCs. Damiano and colleagues demonstrated that binding VLA receptors with fibronectin conferred protection against apoptosis, and Chauhan and colleagues have shown that the binding of myeloma cells to BMSCs resulted in the NF-κB-dependent secretion of IL-6 from BMSCs (9,10). These studies suggested that the interactions between myeloma cells and normal cells in the bone marrow microenvironment are important for the activation of resistance or growth-promoting mechanisms.

Preclinical studies with bortezomib showed direct cytotoxic activity against MMCs *in vitro*. In addition, bortezomib inhibited growth of dexamethasone-, doxorubicin-, and melphalan-resistant myeloma cells lines (4), reduced the expression of the BMSC adhesion receptor VCAM-1 (11), decreased adherence of myeloma cells to BMSCs (presumably due to downregulation of adhesion molecules), suppressed the NF-κB-dependent expression of IL-6 (a myeloma growth factor) (4), and had *in vivo* activity against human myeloma xenografts (8). Synergy with doxorubicin and melphalan but not dexamethasonewas observed in sensitive MMCs since low concentrations of bortezomib significantly reduced the $LD_{50}$ of either of these chemotherapies (12). In addition, MMCs from a heavily treated patient prior regimens included conventional and high dose chemotherapy, interferon-α, thalidomide, liposomal doxorubicin, and bortezomib as a single agent and in combination with dexamethasonedid not respond

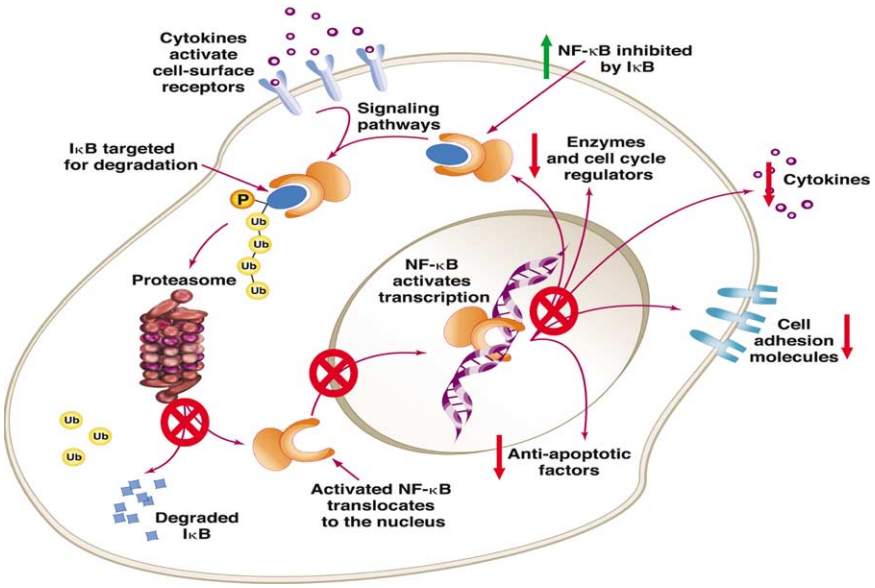


**Figure 1** Downregulation of the NF-κB pathway by bortezomib. NF-κB activation leads to phosphorylation and ubiquitination of IκB. Once ubiquitinated, IκB is bound by the proteasome and degraded, releasing NF-κB. Free NFκB readily enters the nucleus where it activates the transcription of promoters containing κB response elements. Some of the genes activated by NF-κB encode anti-apoptotic factors, cell adhesion molecules, cytokines, and cell cycle regulators. Bortezomib prevents IκB degradation, maintaining NF-κB in an inactive complex with IκB and preventing the downstream effects of NF-κB activation. Consequently, cancer cells that require NF-κB for survival or to prevent apoptosis following cytotoxic treatments experience a downregulation in NF-κB-activated gene expression.

well to bortezomib or doxorubicin monotherapy *in vitro*. However, combined treatment led to a 4–5-fold increase in cell killing. Further studies from this lab show that bortezomib can interfere with DNA repair pathways by promoting the cleavage of DNA PKcs (DNA protein kinase catalytic subunit) and/or ATM (13). In addition, bortezomib could increase MDM2 expression, thereby reducing the degradation of p53. Thus, while NF-κB may be a key target for bortezomib, inhibition of the proteasome affects numerous cellular pathways, thereby sensitizing the cell to apoptosis through several mechanisms.

Proteasome inhibitors have also demonstrated activity against other B-cell malignancies in laboratory studies, including mantle cell lymphoma (MCL) and Hodgkin's disease. An *in vitro* study of MCL cells treated with bortezomib resulted in stabilization of IκB and reduced binding of activated NF-κB to its promoter. In addition, bortezomib treatment *in vitro* led to cell growth inhibition and rapid induction of apoptosis of MCL cells. Bortezomib also exhibited activity in a xenograft model with MCL-xenografted, severe combined immunodeficiency mice evincing little or no gross MCL tumor involvement post-treatment compared to controls (14).

Other preclinical studies in diffuse large B-cell lymphoma (DLBCL) provide indirect evidence that proteasome inhibition may be a potential treatment approach in this tumor type as well (15). Research has indicated that DLBCL is composed of two subgroups – germinal center B-like (GCB) and activated B cell-like (ABC) – each of which present distinct pathogenetic mechanisms and clinical outcomes. Patients with ABC DLBLC have a poorer prognosis and ABC-type DLBCL is frequently refractory to chemotherapy. *In vitro*, ABC DLBCL cell lines had higher NF-κB activation, constitutive IκB kinase (IKK) activity, and IκBα degradation compared to GCB lines. Furthermore, ABC DLBCL cell lines treated with a ''super-repressor'' IκBα (unphosphorylatable by IKK and therefore non-degradable) showed cell death and G1-phase growth arrest. These results suggest that proteasome inhibition, via its downregulation of the NF-κB pathway, is a potential mechanism of action in DLBCL treatment (16).

While NF-κB is clearly important in bortezomib's mechanism of action, several studies suggest that other mechanisms act in parallel to NF-B to induce apoptosis in MMCs and other cancer cells. In early experiments, cell culture experiments uncovered evidence that proteasome inhibition altered the expression of proteins that control cell cycle and apoptosis (4,17,18). More recently, Hideshima *et al.* (12) used a specific inhibitor of the NF-κB pathway to investigate non-NF-κB-mediated mechanisms of cell killing by proteasome inhibitors. In contrast to bortezomib, PS-1145 specifically prevents NF-κB activation by preventing the phosphorylation of IκB. Specific pathways activated by NF-κ B were blocked by PS-1145: ICAM-1 and VCAM-1 expression were prevented in MMCs and IL-6 expression in BMSCs was eliminated. However, DNA synthesis in MMCs was incompletely blocked and PS-1145 was only capable of inhibiting cell growth in 20–50% of treated MMCs (compared to nearly 100% of bortezomib-treated cells). Since a specific NF-κB-inhibitor is not as potent an inducer of apoptosis as bortezomib, other mechanisms tied to proteasome inhibition must be involved in bortezomib-induced apoptosis.

## PHASE I: TRIAL OF BORTEZOMIB IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

Orlowski *et al.* conducted a phase I trial to determine the maximum-tolerated dose (MTD), dose-limiting toxicity (DLT), and phamacodynamics (PD) of bortezomib in patients with refractory hematologic malignancies (19) (Table 1). Patients ($N = 27$) were enrolled at 0.40 mg/m$^2$ ($n = 3$), 1.04 mg/m$^2$ ($n = 12$), 1.20 mg/m$^2$ ($n = 7$), or 1.38 mg/m$^2$ ($n = 5$). Bortezomib was administered twice weekly for 4 weeks followed by a 2-week rest. Participants received a total of 293 doses of bortezomib, including 24 complete cycles.

The MTD was determined to be 1.04 mg/m$^2$. DLTs possibly related to bortezomib included thrombocytopenia, malaise and fatigue, and electrolyte disturbances such as hyponatremia and hypokalemia. Ten patients developed grade 3 thrombocytopenia during cycle 1. Although the study protocol did not define thrombocytopenia as dose-limiting, it did influence dosing behavior. In the majority of cases, grade 3 thrombocytopenia occurred in patients who entered the trial with thrombocytopenia. Only 1 of 9 patients who entered the trial with normal platelet counts had dose withheld because of grade 3 thrombocytopenia. No episodes of febrile neutropenia were reported. Nor were any major bleeding events reported during this trial. Five patients developed a treatment-emergent peripheral neuropathy. Of the four grade 2 cases, three were thought to be related to bortezomib but were not dose-limiting; the sole grade 3 event was due to progressive disease.

Adverse events were reported in patients at all dose levels with cytopenias as the most common events (Table 2); these included thrombocytopenia (74% of patients), anemia and leukopenia (48%), and neutropenia (37%). Gastrointestinal events were also seen in many patients including nausea (52%) and constipation (30%). Fatigue was reported in 59% of patients.

**Table I** Phase I patient demographics

| Characteristics | Number | Percent |
|---|---|---|
| Number of patients | 27 | |
| MSKCC* | 7 | |
| UNC** | 20 | |
| Sex | | |
| Male | 17 | 63 |
| Female | 10 | 37 |
| Age (years) | | |
| Mean | 56 | |
| Range | 22–77 | |
| Race | | |
| Native American | 1 | 4 |
| African American | 7 | 26 |
| White | 19 | 70 |
| Diagnoses | | |
| Hodgkin's disease | 4 | |
| Non-Hodgkin's lymphoma | 10 | |
| SLL/CLL | 2 | |
| DLCL | 2 | |
| Mantle cell | 3 | |
| Plasma cell dycrasias | 12 | |
| Waldenstrom's | 1 | |
| Multiple myeloma | 11 | |
| MDS with excess blasts and POEMS | 1 | |
| ECOG performance scale | | |
| 0 | 6 | 22 |
| 1 | 16 | 59 |
| 2 | 5 | 19 |
| Prior therapy | | |
| Chemotherapy | 27 | |
| Median no. regimens | 3 | |
| Range | 1–12 | |
| Radiation therapy | 13 | |
| Marrow or stem-cell transplantation | 10 | |

Reprinted with permission from: 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**: 4420–4427.

* Memorial Sloan-Kettering Cancer Center.

** University of North Carolina.

Twelve patients with heavily pre-treated plasma cell dyscrasias were enrolled in the study, nine of whom completed at least one full cycle and were assessable for response. One patient who received a total of 4 cycles showed by the end of cycle 3 an immunofixation-negative complete response. There was also evidence of antitumor activity among the eight remaining patients, with reduction of paraprotein levels and/or marrow plasmacytosis. In addition, one patient with mantle cell lymphoma and another with refractory follicular lymphoma achieved a partial response with treatment at the 1.38 mg/m$^2$ level.

Orlowski *et al*. concluded that bortezomib can be administered at 1.04 mg/m$^2$, using a dose-intensive, twice-weekly administration for 4 weeks, followed a by a 2-week rest with concomitant monitoring for electrolyte abnormalities and late toxicities (19). However, induction of toxicity requiring the interruption of therapy in patients during their first cycle of therapy most commonly occurred during the third week of treatment. Twice-weekly therapy for 2-weeks with a third week off, delivering the same total dosage of over 6 weeks, was predicted to be better tolerated. Pharmacodynamic studies suggested that bortezomib induced 20S proteasome inhibition in a time- and dose-dependent manner. This study provided the first evidence of potential activity in myeloma and established the rationale for more comprehensive assessment in phase II (19).

## PHASE II: TRIALS OF BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA

The safety and efficacy of bortezomib have been assessed in two phase II trials of patients with relapsed and refractory MM. Patients in Study 025 (SUMMIT) were relapsed *and* refractory to their most recent therapy; those in Study 024 (CREST) were relapsed *or* refractory after front-line therapy. Patients in Study 025 thus had more advanced disease.

This study enrolled patients in two cohorts: the first cohort filled rapidly ($N = 78$), and, at the request of the investigators, enrollment continued to eventually enroll a total of 202 patients. The first phase I trials (19,20) were designed to test several dosing schedules for bortezomib of varying dose intensities (Table 3). These studies were still in progress at the initiation of Study 025 and preliminary indications from the study testing the 3-week cycle suggested this regimen offered a reasonable balance between toxicity and adequate dosing. At the time, dose-limiting diarrhea and peripheral neuropathy had been observed at 1.56 mg/m$^2$ on the 3-week cycle. Thus, 1.3 mg/m$^2$ bortezomib the previous dose level was administered by IV push on days 1, 4, 8, and 11 of a 21 day treatment cycle in Study 025. A total of eight treatment cycles were allowed. Patients with a suboptimal response were allowed to add dexamethasone to bortezomib after 2 (patients with progressive disease) or 4 cycles (patients with stable disease).

This trial employed strict response criteria [adapted from SWOG criteria and Blade *et al*. (21)] assessed by an independent review committee, and the population was heavily pretreated (the median number of prior treatments was 6) (22). For a com-

**Table 2** Bortezomib-related adverse events (⩾grade 3) during cycle 1

| Adverse event | Bortezomib dose level (mg/m$^2$) | | | | |
|---|---|---|---|---|---|
| | 0.40 ($n = 3$) | 1.04 ($n = 12$) | 1.20 ($n = 7$) | 1.38 ($n = 5$) | Total ($N = 27$) |
| ⩾ 1 adverse event | 0 | 7 | 6 | 4 | 17 |
| Blood and lymphatic system | 0 | 5 | 6 | 4 | 15 |
| Thrombocytopenia | 0 | 4 | 4 | 2 | 10 |
| Anemia | 0 | 1 | 3 | 1 | 5 |
| Neutropenia | 0 | 2 | 1 | 1 | 4 |
| Leukopenia | 0 | 2 | 1 | 0 | 3 |
| Metabolism and nutrition | 0 | 4 | 1 | 2 | 7 |
| Hyponatremia | 0 | 2 | 0 | 2 | 4 |
| Hypokalemia | 0 | 1 | 1 | 0 | 2 |
| Other | 0 | 0 | 1 | 0 | 1 |
| Malaise | 0 | 0 | 1 | 0 | 1 |

Reprinted with permission from: 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**: 4420–4427.

**Table 3** Dosing schedules in phase I bortezomib trials

| Treatment schedule | Cycle length (days) | Dose-limiting toxicities (mg/m$^2$) | Maximum tolerated dose (mg/m$^2$) |
|---|---|---|---|
| 1×/wk × 4 | 35 | ND[a] | ND[a] (25) |
| 2×/wk × 2 | 21 | 1.56[b] | 1.3 (20) |
| 2×/wk × 4 | 42 | 1.38[b] | 1.04 (19) |

Reprinted with permission from Adams J. Potential for proteasome inhibition in the treatment of cancer. *Drug Discovery Today*, 2003; in press.

[a] Not determined. MTD not reached at time of report.

[b] Enrollment suspended due to DLT; maximum dose reached.

plete response, no M protein was visible by immunofixation, fewer than 5% plasma cells were present in the bone marrow, and no new bone disease or plasmacytomas were present; responses were confirmed after 6 weeks. Preliminary data from the first cohort showed significant biologic activity with manageable toxicities, as demonstrated by reductions in M protein (23). A preliminary analysis of the full cohort ($n = 202$) confirming and extending these results was recently presented and is being prepared for publication (22). An integral component of Study 025 was the collection of bone marrow samples for pharmacogenomic analysis. Prior to treatment, bone marrow samples were collected from 126 consenting patients, and RNA from myeloma cells was purified from 64 of these. The gene expression profiles of responding patients are now being compared to the expression profiles of non-responders to identify expression patterns that are predictive of a response to bortezomib.

Study 024 allowed the assessment of a patient population with earlier stage disease (median prior therapies was 3) and applied the same treatment schedule and response criteria as in Study 025. A total of 54 patients were randomized to receive either 1.0 (28 patients) or 1.3 (26 patients) mg/m$^2$ bortezomib. Dexamethasone was also allowed for patients who had progressive (after cycle 2) or stable disease (after cycle 4). Preliminary results of this trial were recently reported and the analysis is ongoing (24).

## FUTURE DIRECTIONS

A phase III study comparing response to bortezomib versus high-dose dexamethasone is in progress. The multicenter Assessment of Proteasome Inhibition for Extending Remissions (APEX) trial will be conducted at more than 60 centers in the United States, Canada, and Europe. Over 600 patients with relapsed or refractory MM will be enrolled and randomize to either bortezomib or dexamethasone treatment arms. Response will be assessed by prolongation of time-to-disease progression and selected measures of clinical benefit. APEX will also study the potential of bortezomib as maintenance therapy. Survival, response rates, quality of life, and safety and tolerability in the two treatment arms will

DOCKET ALARM

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

**LAW FIRMS**
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

**FINANCIAL INSTITUTIONS**
Litigation and bankruptcy checks for companies and debtors.

**E-DISCOVERY AND LEGAL VENDORS**
Sync your system to PACER to automate legal marketing.