M. Cavo
M.C. Pallotti
L. Pantani
A. Petrucci
A. Brioli
C. Terragna
N. Testoni
G. Marzocchi
S. Durante
M. Ceccolini
G. Perrone
P. Tacchetti
E. Zamagni
P. Tosi
M. Baccarani

Seràgnoli Institute of Hematology and Medical Oncology Bologna University School of Medicine
Bologna, Italy

# Proteasome inhibitors: bortezomib in multiple myeloma

Over the past decade, new insights into the biology of multiple myeloma (MM) have provided the framework for the development of novel therapies to overcome drug resistance. In particular, recognition of the pivotal role of bone marrow microenvironment in promoting myeloma cell growth, survival, drug resistance, and migration has allowed for identification of specific therapeutic strategies targeting myeloma–stromal cell interactions and cytokine secretion in the bone marrow milieu. The first-in-class proteasome inhibitor bortezomib is an excellent example of this novel class of agents that has quickly translated from the bench to the bedside.

## Preclinical studies with bortezomib in multiple myeloma

Preclinical studies with bortezomib conducted on human myeloma cell lines and on freshly isolated cells from patients with MM showed that pharmacologically achievable doses of this agent directly inhibited the proliferation of human myeloma cell lines which were both sensitive and refractory to cytotoxic agents, and induced caspase-dependent apoptosis of myeloma cell lines and primary patient MM cells.[1] Bortezomib also inhibited NF-κB activation in tumor necrosis factor (TNF)-α–treated MM cells by blocking the degradation of the inhibitor protein I-κBα and overcame the resistance to apoptosis in MM cells conferred by IL-61.[2] Furthermore, bortezomib inhibited binding of MM cells to bone marrow stromal cells and abrogated the NF-κB–dependent transcription and secretion of IL-6 in bone marrow stromal cells1. Inhibition of NF-κB activity by bortezomib markedly suppressed the *in vitro* growth of primary patient MM cells and of MM cell lines,[1] an effect furtherly enhanced by dexamethasone. In addition, bortezomib markedly improved the sensitivity to doxorubicin and melphalan in both drug-sensitive and resistant MM cell lines and of primary patient MM cells.[2,3]

## Registrative studies of single-agent bortezomib in advanced refractory and/or relapsed multiple myeloma

Results from preclinical studies and phase I clinical trials[4] showing the activity of bortezomib against MM prompted the initiation of phase II and III studies aimed at investigating the safety and activity of this agent in patients in whom prior treatments strategies, including stem-cell

transplantation, had failed.[5-7] The phase II SUMMIT[5] and CREST[6] trials provided the first demonstration that bortezomib was an effective salvage therapy for approximately one third to one half of patients with refractory or relapsed MM. Based on these favorable results, bortezomib received accelerated approval by the FDA and the EMEA for clinical use in patients who have received at least two prior therapies and who have experienced progressive disease on their last therapy. Following these studies, the phase III APEX trial of single-agent bortezomib vs high-dose dexamethasone for relapsed MM[7] was initiated. Results of this study showed the superiority of bortezomib over dexamethasone in terms of increased ≥ partial remission (PR) rate (38%, including 6% complete remission [CR]), extended time to progression (TTP) (6.22 months), and longer duration of overall survival (OS) (1-year rate, 80%). The clinical benefits of single-agent bortezomib for the treatment of relapsed MM were recently confirmed with an extended follow-up of 22 months.[8] In addition, a subgroup analysis provided demonstration that benefits from bortezomib were the greatest among patients who experienced first relapse.[9] Among these patients, the overall probability of ≥ PR was 45% with bortezomib compared with 26% with dexamethasone alone, median TTP was 7 months *vs*. 5.6 months, respectively, and 1-year probability of OS was 89% *vs.* 72%, respectively. Based on these results, in 2005 the FDA and the EMEA approved an expanded indication for bortezomib use in MM patients who have received at least one prior line of therapy.

### Adverse events and toxicities

Toxicities attributable to bortezomib as single-agent therapy for patients with advanced relapsed and/or refractory MM are generally manageable and reversible. Most common side effects include gastrointestinal symptoms, fatigue, thrombocytopenia, sensory neuropathy, vomiting, and anorexia.[5-7] Several of these toxicities, such as diarrhea, peripheral neuropathy, and vomiting, were observed more frequently with the dose of 1.3 mg/m$^2$ compared with 1.0 mg/m.[2,6] Gastrointestinal symptoms were generally mild or moderate and could be managed with routine support. Thrombocytopenia was the most common severe adverse event, with an average occurrence in approximately 30% of patients; it occurred more frequently in patients with low platelet counts at baseline and was typically transient, with recovery of platelet counts toward baseline during the rest period of each cycle.[10] Clinically, cumulative peripheral neuropathy was the most important toxicity of bortezomib therapy for patients with advanced and refractory MM. Combined safety data from two phase II clinical trials reported a 35% frequency of treatment-emergent neuropathy (grade 1-2: 22%; grade 3-4: 13.4%), including a 37% value among patients receiving bortezomib 1.3 mg/m$^2$ and 21% among those receiving bortezomib 1.0 mg/m$^2$ 11. The incidence of ≥ grade 3 peripheral neuropathy, occurred more frequently in patients with neurological symptoms at baseline.[5,7] Among patients requiring treatment discontinuation due to ≥ grade 3 neuropathy, resolution to baseline or improvement occurred in 71% of cases.[11] Thus, with early detection of peripheral neuropathy and the use of an algorithm for dose reductions or discontinuation, most patients can promptly improve or recovery from their neurological symptoms.

## Studies of bortezomib combined with other agents in advanced refractory/relapsed multiple myeloma

Different combinations of bortezomib with

dexamethasone and/or cytotoxic drugs and/or novel agents have been explored in an attempt to expand the therapeutic armamentarium for patients with advanced refractory and/or relapsed MM. The rationale for exploring the activity of these novel regimens relied upon preclinical data showing that a) bortezomib enhances the activity of both anthracyclines and alkylating agents, b) bortezomib is not cross-resistant with thalidomide, c) bortezomib and lenalidomide trigger dual apoptotic pathways, including caspase-8- and caspase-9-mediated cell death. In the phase II SUMMIT and CREST studies, patients with suboptimal response to 2-4 courses of single-agent bortezomib were permitted to subsequently receive added dexamethasone (20 mg) on the day of and after each bortezomib dose.[5,6] An improved response (≥ minimal response [MR]) was observed in 18%5 and 37%6 of patients, respectively, confirming the additive effect of these two agents previously found in preclinical studies. Additional phase I-II studies conducted in the same setting of patients showed encouraging results with 3- or 4-drug combinations, including bortezomib-cyclophosphamide-dexamethasone/prednisone,[12,13] bortezomib-thalidomide-dexamethasone,[14] bortezomib-lenalidomide-dexamethasone[15] and bortezomib-thalidomide-melphalan-prednisone.[16] The DOXIL-MMY-3001 trial was a large phase III study aimed at prospectively comparing the combination of bortezomib with pegylated liposomal doxorubicin versus bortezomib alone in patients with primary refractory MM or who had relapsed after a single line of prior therapy.[17] Although not effecting superior rates of response, combination therapy significantly improved TTP (9.3 months median), progression-free survival (PFS) (9.0 months median) and the 15-month OS rate (76%) in comparison with bortezomib alone. Importantly, superior activity of combination therapy was maintained across all subgroup analyses, including those at high risk for disease progression.

## Studies of bortezomib up-front combined with other agents as induction therapy in younger MM patients eligible for autologous stem-cell transplantation

Following the remarkable success and tolerable toxicity profile of bortezomib in advanced refractory and/or relapsed MM, numerous clinical trials have been designed to explore the role of this agent, either alone or in combination with other drugs, in patients with newly diagnosed disease. In younger patients who are eligible for autologous stem-cell transplantation (ASCT), great efforts have been devoted to the development of bortezomib-based regimens aimed at enhancing the rate of CR, which has become a well established early end point surrogate for prolonged OS. In addition to response, important end points also included safety and toxicity profile, with particular considerations for peripheral blood stem-cell (PBSC) harvesting. In some phase II studies, the rate of CR increased from 10-12.5% with bortezomib alone or alternating bortezomib and dexamethasone18 to 21% (including also near CR) with combined bortezomib-dexamethasone.[19] The CR + near CR rate was furtherly enhanced, up to the 29% range, by the addition of a third drug, such as doxorubicin20 or lenalidomide.[21] Two large phase III trials performed in France and Italy evaluated the efficacy of bortezomib as part of induction therapy in preparation for subsequent ASCT.[22,23] In the IFM 2005/01 study, the combination of bortezomib plus dexamethasone (VD) was significantly superior to the standard VAD regimen22. Following induction, the CR + near CR rates were 19% with VD vs 8% with VAD ($p$=0.0004) and ≥ very good partial response (VGPR) rates were 47% vs 19%, respectively

($p$<0.0001). Following the first ASCT, 35% of patients receiving VD were in CR + near CR vs 23% of patients in the VAD arm of the study ($p$=0.0056). The ≥VGPR rates were 63% vs 44% ($p$<0.0001), respectively, demonstrating the activity of the VD regimen. These results furtherly support the notion that in the era of novel agents VAD will no longer be preferred as induction therapy for patients who are eligible for ASCT. In another phase III study conducted by the Italian Myeloma Network GIMEMA,[23] the combination of bortezomib with thalidomide and dexamethasone (VTD) was compared with thalidomide plus dexamethasone (TD) which was previously demonstrated to be superior to VAD.[24] Both VTD and TD were given before and after double ASCT. Overall, the VTD regimen resulted in high CR + near CR and ≥VGPR rates, both pre- and post-ASCT.[23] Following induction therapy, 60% of patients randomized to receive VTD achieved at least a VGPR compared with 27% of patients receiving TD ($p$<0.001). The CR + nCR rate was 36% *vs.* 9%, respectively ($p$<0.001). Following the first ASCT, at least a VGPR was observed in 77% of patients in the VTD arm vs 54% of patients receiving TD ($p$=0.003), with a CR + nCR rate of 57% vs 28%, respectively ($p$<0.001). In both studies, the use of bortezomib in preparation for ASCT did not adversely affect the efficiency of PBSC collection, with median CD $34^+$ cell yields ($x10^6$/kg) of 7.7 in the French study22 and of 9.2 in the Italian study.[23]

## Studies of bortezomib up-front combined with melphalan-prednisone for elderly multiple myeloma patients

Age remains a significant risk factor with conventional therapy for MM therapy. However, recent developments in the treatment of elderly patients have demonstrated that outcomes can be improved by combining novel agents with the traditional melphalan-prednisone (MP) regimen. Three randomized studies compared MP with MP plus thalidomide (MPT),[25-27] but only in two of them there was a survival benefit from MPT.[25,26] The large VISTA trial of MP vs MP plus bortezomib (MPV) showed the significant superiority of MPV in terms of response and time-to-event end points, including response rate, CR rate, TTP, OS and time to next therapy.[28] The high CR rate of 30% is unprecedented in this patient population. Although median OS was not reached, MPV resulted in a significantly higher 3-year OS rate (72%) in comparison with MP (59%), despite 45% of patients treated on MP received bortezomib following progression. Importantly, in the trial one third of patients were older than 75 years and, remarkably, efficacy in these patients was comparable to that seen in patients aged less than <75 years. Based on results of the VISTA trial, the FDA has recently approved the use of bortezomib for patients with previously untreated MM. Taken together, the results of the above mentioned studies indicate that MP should no longer be considered the standard treatment for elderly MM patients and, importantly, that in the era of novel agents age is no longer an adverse prognostic factor in the treatment of MM.

## Studies with bortezomib in high-risk multiple myeloma

Another important benefit from novel agents in the treatment of MM is in the area of high-risk disease. Clinical evidence is accumulating to suggest that bortezomib is able to overcome the poor prognosis associated with chromosomal abnormalities. Indeed, a number of studies in the up-front setting have shown that response rate, TTP and OS with bortezomib

are not influenced by the presence of chromosome 13 deletion, translocation (t) t(4;14) and t(14;16), and chromosome 17p deletion.[18,22,23,28] In addition, other high-risk features, such as increased β2 microglobulin or low albumin levels, do not negatively affect bortezomib efficacy.[22,28] Importantly, these benefits in patients with high-risk disease were seen in both young and elderly patients.

Renal impairment is another scenario that can be associated with complications and poor outcome. Bortezomib has demonstrated a clear benefit for these patients, since it results in response rates, TTP and OS that are comparable to those of patients with normal renal function. Importantly, bortezomib has also been shown to reverse renal failure in about 40% of patients.[29]

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