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Characterisation of haematological profiles and low risk of thromboembolic events with bortezomib in patients with relapsed multiple myeloma

Sagar Lonial,[1] Paul G. Richardson,[2] Jesús San Miguel,[3] Pieter Sonneveld,[4] Michael W. Schuster,[5] Joan Bladé,[6] Jamie Cavenagh,[7] S. Vincent Rajkumar,[8] Andrzej J. Jakubowiak,[9] Dixie-Lee Esseltine,[10] Kenneth C. Anderson[2] and Jean-Luc Harousseau[11]

[1]Emory University, Atlanta, GA, [2]Dana-Farber Cancer Institute, Boston, MA, USA, [3]Hospital Universitario Salamanca, CIC, IBMCC (USAL-CSIC), Spain, [4]University Hospital Rotterdam, Rotterdam, The Netherlands, [5]NY-Presbyterian Hospital, New York, NY, USA, [6]Haematology Department, IDIBAPS, Hospital Clinic, Barcelona, Spain, [7]St Bartholomew's Hospital, London, UK, [8]Mayo Clinic, Rochester, MN, [9]University of Michigan, Ann Arbor, MI, [10]Millennium Pharmaceuticals Inc., Cambridge, MA, USA, and [11]Hôpital Hotel-Dieu, Nantes, France

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Correspondence: Sagar Lonial, MD, The Winship Cancer Institute, Building C, 1365 Clifton Road, Atlanta, GA, 30322, USA.
E-mail: sloni01@emory.edu

Summary

Haematological toxicities and thromboembolic (TE) events are common complications of myeloma therapy. TE risk may be elevated with combination regimens, notably thalidomide/lenalidomide plus high-dose dexamethasone; concomitant erythropoietin appears to further increase the risk with lenalidomide–dexamethasone. We characterised thrombocytopenia and neutropenia in the phase 3 APEX (Assessment of Proteasome Inhibition for Extending Remissions) study of bortezomib *versus* high-dose dexamethasone in relapsed myeloma, and calculated the incidences of deep-vein thrombosis (DVT)/pulmonary embolism (PE) with: bortezomib or dexamethasone ± erythropoietin in APEX; bortezomib ± dexamethasone ± erythropoietin in two phase 2 studies of relapsed/refractory myeloma. Bortezomib-associated thrombocytopenia and neutropenia were transient, predictable and manageable; mean platelet and neutrophil counts followed a cyclical pattern, and improved over the treatment course. Grade 3/4 thrombocytopenia incidence was higher with bortezomib *versus* dexamethasone (26%/4% vs. 5%/1%), but significant bleeding events were comparable (4% vs. 5%). DVT/PE incidence was low (≤3·1%) in all analyses; addition of dexamethasone/erythropoietin did not affect TE risk. In APEX, TE risk appeared lower with bortezomib *versus* dexamethasone. Bortezomib caused transient and cyclical thrombocytopenia and was not associated with elevated TE risk, alone or with dexamethasone ± erythropoietin. Preliminary data suggest bortezomib may reduce the thrombogenic potential of combination regimens via inhibition of platelet function or other mechanism-specific effects on coagulation.

Keywords: bortezomib, haematological toxicity, multiple myeloma, thromboembolic event.

Haematological toxicities, including thrombocytopenia and neutropenia, are common complications of therapy for patients with multiple myeloma. Development of thrombocytopenia is often caused by the cytotoxic effects of agents on megakaryocytes (Zuckerman, 1998; Kaufman & Anderson, 2003), which can result in a prolonged reduction in platelet counts and associated bleeding complications. Similarly, neutropenia may arise due to cytotoxic disruption of haematopoiesis in the bone marrow (Zuckerman, 1998). Patients with multiple myeloma are also at increased risk of venous thromboembolic (TE) complications, notably deep-vein thrombosis (DVT) and pulmonary embolism (PE), due to underlying patient- and disease-related factors, as well as choice of therapy (Zangari *et al*, 2003, 2007a; Srkalovic *et al*, 2004; Hussein, 2006). The thrombogenic mechanisms associated with the disease itself are complex, and risk factors include a history of TE complications, acquired resistance to activated protein C and its regulation of coagulation, endothelial injury, a hypercoagulable state and advanced stage disease (Zangari *et al*, 2003, 2007a; Elice *et al*, 2006; Hussein, 2006; Auwerda *et al*, 2007). In addition, the risk of TE complications during therapy is elevated in the front-line setting and with the use of

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certain combination regimens (Rajkumar, 2005; Hussein, 2006; Zonder, 2006). Notably, although the immunomodulatory agents (IMiDs) thalidomide and lenalidomide do not appear to result in a significantly increased risk of TE complications as single agents, thalidomide combined with high-dose corticosteroids and lenalidomide combined with high-dose dexamethasone, especially with concomitant erythropoietin (EPO), are associated with substantially increased risks of TE complications (Rajkumar, 2005; Bennett *et al*, 2006; Hussein, 2006; Zonder, 2006; Zangari *et al*, 2007a). The addition of anthracyclines to thalidomide also increases the TE risk (Rajkumar, 2005; Bennett *et al*, 2006; Hussein, 2006; Zonder, 2006; Zangari *et al*, 2007a).

Bortezomib (VELCADE®; Millennium Pharmaceuticals, The Takeda Oncology Company, Cambridge, MA, USA, and Johnson & Johnson Pharmaceuticals Research & Development, L.L.C., Raritan, NJ, USA) is a first-in-class proteasome inhibitor that has activity in the settings of newly diagnosed and relapsed multiple myeloma. Bortezomib has a unique mechanism of action, affecting multiple signalling pathways through inhibition of the 26S proteasome. This mechanism of action may have an impact on haematopoietic function and the TE toxicity profile of bortezomib alone and in combination with other anti-myeloma agents.

In the Study of Uncontrolled Multiple Myeloma Managed with Proteasome Inhibition Therapy (SUMMIT) (Richardson *et al*, 2003) and Clinical Response and Efficacy Study of Bortezomib in the Treatment of Relapsing Multiple Myeloma (CREST) (Jagannath *et al*, 2004) phase 2 studies of bortezomib with or without added dexamethasone, thrombocytopenia and neutropenia were among the most common grade 3/4 toxicities, and the overall incidence of TE complications was low. Characterisation of bortezomib-associated thrombocytopenia in SUMMIT and CREST revealed the toxicity to be transient, cyclical and predictable, with patients' platelet counts decreasing during bortezomib treatment and then recovering rapidly during the 10-d rest period at the end of each cycle (Lonial *et al*, 2005). It was attributed to a reversible effect on megakaryocytes, probably a result of transient inhibition of platelet budding (Lonial *et al*, 2005). This contrasts with the kinetics and mechanism of thrombocytopenia with other cytotoxic therapies, which are associated with a longer recovery time and cytotoxic marrow injury. Toxicities reported in the phase 3 Assessment of Proteasome Inhibition for Extending Remissions (APEX) study of single-agent bortezomib *versus* high-dose dexamethasone were similar to those reported in phase 2 studies. Thrombocytopenia and neutropenia were again among the most common grade 3/4 toxicities in the bortezomib treatment arm, while TE events were uncommon (Richardson *et al*, 2005).

Here, thrombocytopenia and neutropenia reported with bortezomib and high-dose dexamethasone in APEX are characterised. In addition, in light of the elevated risk of TE complications reported with many commonly used anti-myeloma regimens, we assessed the incidences of DVT and PE with bortezomib or dexamethasone, with or without EPO, in APEX, and with bortezomib ± dexamethasone, with or without EPO, in SUMMIT and CREST.

Design and methods

Study design

Full details of APEX, SUMMIT and CREST have been published previously (Richardson *et al*, 2003, 2005; Jagannath *et al*, 2004). Briefly, in the international APEX study, 669 patients with relapsed multiple myeloma following one to three prior therapies were randomised (1:1) to receive bortezomib 1·3 mg/m^2 on days 1, 4, 8 and 11 for eight 3-week cycles, and then on days 1, 8, 15 and 22 for three 5-week cycles (n = 333), or dexamethasone 40 mg on days 1–4, 9–12 and 17–20 for four 5-week cycles, and then on days 1–4 for five 4-week cycles (n = 336) (Richardson *et al*, 2005).

In SUMMIT, patients with relapsed and/or refractory multiple myeloma received bortezomib 1·3 mg/m^2 on days 1, 4, 8 and 11 for up to eight 3-week cycles (n = 202) (Richardson *et al*, 2003). Dexamethasone 20 mg on the day of and day after bortezomib could be added for patients with suboptimal response to bortezomib alone (n = 78). In CREST, patients with relapsed multiple myeloma received bortezomib 1·0 or 1·3 mg/m^2 on the same schedule (n = 54); as in SUMMIT, dexamethasone could be added for suboptimal response to bortezomib alone (n = 28) (Jagannath *et al*, 2004). In all three studies, EPO use and prophylactic anticoagulation were not mandated by protocol. However, a proportion of patients received concomitant EPO; similarly, a proportion of patients received aspirin or low-molecular-weight heparin, but these were not necessarily administered prophylactically. All studies were conducted according to the Declaration of Helsinki, the International Conference on Harmonisation and the Guidelines for Good Clinical Practice.

Assessments

Safety was assessed throughout each study and until 20 (SUMMIT and CREST) or 30 (APEX) d following the last dose of study drug. Adverse events were graded according to the National Cancer Institute's Common Toxicity Criteria, version 2·0 (http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf). In the APEX study, platelet count and absolute neutrophil count (ANC) were recorded throughout, and incidences of thrombocytopenia and neutropenia were calculated in the safety populations of the bortezomib and dexamethasone arms. Associated rates of platelet transfusions, clinically significant bleeding events (including any grade ≥3 bleeding events, bleeding events of any intensity that were reported as serious, cerebral haemorrhage, regardless of intensity and seriousness), febrile neutropenia and use of granulocyte colony-stimulating factor (G-CSF) were also recorded.

The incidences of DVT and PE were calculated for patients receiving bortezomib or dexamethasone with/without EPO in APEX, and patients receiving bortezomib with/without added dexamethasone and/or concomitant EPO in the SUMMIT and CREST studies. The severity of these toxicities was also recorded.

Statistical analysis

A logistic regression model was used to describe the risk of DVT/PE adjusted for treatment arm and EPO use in the APEX study. Odds ratios and corresponding 95% confidence intervals and *P*-values were calculated; *P* values <0·05 were considered statistically significant.

Results

Baseline patient characteristics and haematological status for each of the study populations are shown in Table I. In APEX, patients in the bortezomib arm received a median of six 3-week cycles of treatment, and patients in the dexamethasone arm received a median of three 5-week cycles. In SUMMIT/CREST, patients received a median of six 3-week cycles of bortezomib ± dexamethasone.

Thrombocytopenia and neutropenia in APEX

The incidence of thrombocytopenia and neutropenia are shown in Table II. Although grade 3/4 thrombocytopenia was more common with bortezomib (26%/4%) than dexamethasone (5%/1%), the incidence of clinically significant bleeding events was similar (4% and 5% respectively). Two patients in the dexamethasone arm died as a result of a significant bleeding event, whereas no deaths associated with bleeding events were reported in the bortezomib arm. Grade 3/4 neutropenia was also more common with bortezomib (12%/2%) compared with dexamethasone (1%/0%); febrile neutropenia was reported in only one patient in the bortezomib arm. Bortezomib-associated thrombocytopenia and neutropenia were transient (Fig 1). The mean platelet count and ANC followed cyclical patterns, with recovery towards baseline during the rest period of each cycle. The nadir mean platelet count was approximately 36% of the baseline value. Mean platelet count and ANC for all patients increased over the eight cycles of therapy.

Analysis of TE events

APEX. The incidence of DVT/PE was low in the bortezomib and dexamethasone arms, with or without use of concomitant EPO (Table III). The use of EPO appeared to have no impact on the rate of TE events [overall odds ratio (OR) for the risk of DVT/PE according to EPO use = 1·670; 95% confidence interval (CI): 0·500–5·580; *P* = 0·4051]. Furthermore, there appeared to be a decrease in TE events with bortezomib compared with dexamethasone, controlling for EPO use (OR: 0·207; 95% CI: 0·044–0·971; *P* = 0·0459). In total, 54 (16%) patients in the bortezomib arm and 72 (22%) patients received aspirin or low-molecular-weight heparin during their treatment course.

Table I. Baseline patient characteristics.

	APEX: bortezomib arm *n* = 333*	APEX: dexamethasone arm *n* = 336*	SUMMIT and CREST: all patients, *n* = 256
Median age, years	62	61	60
Male, %	56	60	56
Myeloma type IgG/IgA, %	60/23	59/24	60/24
Median β_2-microglobulin, mg/l	3·7	3·6	3·6
Karnofsky performance score ≥70, %	94	96	91
Median prior therapies, *n*	2	2	5
Prior steroids, %	98	99	99
Prior thalidomide, %	48	50	72
Median platelet count (range), $\times 10^9$/l	192·5 (24–523)	188 (8–488)	168 (NA)
Platelets $<75 \times 10^9$ (grade ≥2), %	6	4	NA
Platelets $<50 \times 10^9$ (grade ≥3), %	2	3	NA
Median ANC (range), $\times 10^9$/l	2·73 (0·12–8·46)	2·75 (0–8·67)	NA
Median haemoglobin (range), g/l	108 (73–155)	109 (67–174)	NA

*Intent-to-treat population.

ANC, absolute neutrophil count; NA, not available; APEX, Assessment of Proteasome Inhibition for Extending Remissions; SUMMIT, Study of Uncontrolled Multiple Myeloma Managed with Proteasome Inhibition Therapy; CREST, Clinical Response and Efficacy Study of Bortezomib in the Treatment of Relapsing Multiple Myeloma.

Table II. Haematological toxicities and associated interventions during the APEX study.

	Bortezomib $n = 331$	Dexamethasone $n = 332$
Thrombocytopenia, *n* (%)		
All grades	115 (35)	36 (11)
Grade 3 (platelets ≥10 – <50 × 10^9/l)	85 (26)	18 (5)
Grade 4 (platelets <10 × 10^9/l)	12 (4)	4 (1)
Clinically significant bleeding events, *n* (%)	13 (4)	15 (5)
Patients requiring platelet transfusions, cumulative incidence, %	15	1
Neutropenia, *n* (%)		
All grades	62 (19)	5 (2)
Grade 3 (ANC ≥0·5 – <1·0 × 10^9/l)	40 (12)	4 (1)
Grade 4 (ANC <0·5 × 10^9/l)	8 (2)	0
Febrile neutropenia, *n* (%)	1 (<1)	0
Patients requiring G-CSF support, *n* (%)	20 (6)	2 (<1)

APEX, Assessment of Proteasome Inhibition for Extending Remissions; ANC, absolute neutrophil count; G-CSF, granulocyte colony stimulating factor.

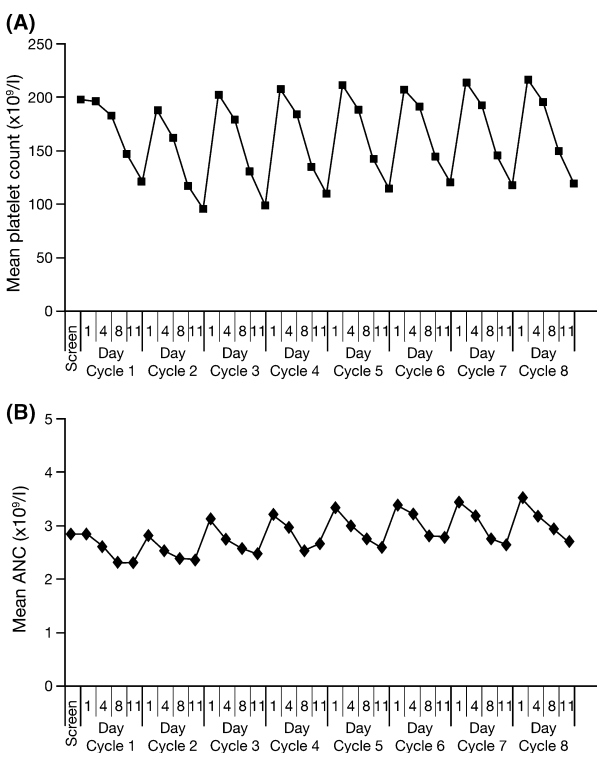

Fig 1. Mean platelet count (A) and mean absolute neutrophil count (ANC) (B) among 331 patients during eight 3-week cycles of treatment with bortezomib in the APEX study.

SUMMIT/CREST. The incidences of DVT/PE were also low among patients receiving bortezomib in SUMMIT and CREST (Table III). The addition of dexamethasone due to suboptimal response to bortezomib alone did not appear to affect the incidence of TE events. Similarly, the use of concomitant EPO did not affect DVT/PE incidence, either in patients receiving bortezomib alone or in those receiving bortezomib plus dexamethasone. In total, 49 (19%) patients received aspirin or low-molecular-weight heparin during their treatment course.

Discussion

This analysis, characterising the haematological profiles in patients treated with bortezomib in the international phase 3 APEX study, demonstrated that bortezomib-associated thrombocytopenia and neutropenia were transient and cyclical, with rapid recovery. These findings were similar to our previously published experience in two phase 2 studies (Lonial *et al*, 2005), confirming the cyclical and reversible pattern of bortezomib-associated thrombocytopenia. Mean platelet count and ANC improved over the course of bortezomib treatment for the entire patient population in APEX. Importantly, despite the higher incidence of thrombocytopenia with bortezomib *versus* dexamethasone, the incidence of significant bleeding events was comparable. Due to the transient nature of bortezomib-associated thrombocytopenia, this toxicity alone may not require treatment delays or dose reductions, except if associated with bleeding events, and may instead be managed with platelet transfusions as clinically indicated to maximise dosing and response to therapy. Similarly, bortezomib-associated neutropenia may be managed with growth factor support, with dosing holds only required in patients with febrile neutropenia, an uncommon event with bortezomib reported in only two of 589 (0·3%) patients treated in APEX, SUMMIT and CREST.

The results from our analyses of TE complications in APEX, SUMMIT and CREST demonstrate that bortezomib in combination with dexamethasone and/or concomitant EPO does not appear to be associated with an elevated risk of DVT or PE. It should be noted that these studies primarily involved single-agent bortezomib therapy, whereas studies of other therapies indicated that the risk of TE events is elevated with combination therapy (Rajkumar, 2005; Hussein, 2006). Nevertheless, our findings are supported by data from two phase 2 studies of bortezomib with or without dexamethasone (Jagannath *et al*, 2006) and bortezomib plus dexamethasone (Harousseau *et al*, 2006) in the front-line setting, in which no TE complications were reported in a total of 99 patients, a proportion of whom also received concomitant EPO. Additionally, data from two large randomized phase 3 trials demonstrated a low incidence of TE complications with bortezomib plus dexamethasone (Harousseau *et al*, 2007) and bortezomib–thalidomide–dexamethasone (Cavo *et al*, 2007) among newly diagnosed patients.

Our findings contrast with the experience with thalidomide and lenalidomide. High rates of DVT and PE have been reported when these drugs are used in combination with agents including doxorubicin, melphalan and high-dose

Table III. Incidence of DVT and PE with bortezomib or dexamethasone, with or without concomitant EPO, in APEX, and with bortezomib ± dexamethasone ± EPO in SUMMIT and CREST.

	Bortezomib			Dexamethasone		
	+EPO	No EPO	Total	+EPO	No EPO	Total
APEX						
Patients, *n*	137	194	331	106	226	332
DVT, *n* (%)	1 (0·7)	0	1* (0·3)	2 (1·9)	4 (1·8)	6† (1·8)
PE, *n* (%)	1 (0·7)	0	1‡ (0·3)	1 (0·9)	4 (1·8)	5§ (1·5)
DVT/PE, *n* (%)	2 (1·5)	0	2 (0·6)	3 (2·8)	6¶ (2·7)	9¶ (2·7)
	Bortezomib alone			Bortezomib + dexamethasone		
SUMMIT/CREST						
Patients, *n*	158	98	256	74	32	106
DVT**, *n* (%)	3 (1·9)	0	3 (1·2)	0	0	0
PE††, *n* (%)	1 (0·6)	1 (1·0)	2 (0·8)	0	1 (3·1)	1 (0·9)
DVT/PE, *n* (%)	3‡‡ (1·9)	1 (1·0)	4‡‡ (1·6)	0	1 (3·1)	1 (0·9)

*Grade 2.
†Five grade 3, one grade 4.
‡Grade 3.
§One grade 2, two grade 3, two grade 4.
¶Two patients had both DVT and PE.
**All grade 3.
††Two grade 1, 1 grade 3.
‡‡One patient had both DVT and PE.
EPO, erythropoietin; DVT, deep-vein thrombosis; PE, pulmonary embolism.
APEX, Assessment of Proteasome Inhibition for Extending Remissions; SUMMIT, Study of Uncontrolled Multiple Myeloma Managed with Proteasome Inhibition Therapy; CREST, Clinical Response and Efficacy Study of Bortezomib in the Treatment of Relapsing Multiple Myeloma.

dexamethasone, both in the front-line and relapsed settings (Osman *et al*, 2001; Zangari *et al*, 2001, 2002; Rajkumar *et al*, 2002, 2006; Urbauer *et al*, 2002; Weber *et al*, 2003, 2007; Barlogie *et al*, 2006; Hussein *et al*, 2006; Knight *et al*, 2006; Niesvizky *et al*, 2006, 2008; Palumbo *et al*, 2006; Dimopoulos *et al*, 2007). Additionally, *post hoc* analyses of data from two studies of lenalidomide–dexamethasone in the relapsed setting found a substantial increase in TE risk with the use of concomitant EPO (Knight *et al*, 2006; Niesvizky *et al*, 2006).

Recently, it has been demonstrated that the IMiDs CC-4047 and lenalidomide upregulate the potent platelet activator Cathepsin G *in vitro* and *in vivo* respectively (Pal *et al*, 2007). This might contribute to the development of TE complications in patients receiving lenalidomide, and suggests a rationale for why aspirin may be effective as TE prophylaxis (Pal *et al*, 2007). However, the mechanisms by which thalidomide and lenalidomide cause thrombosis in combination regimens remain unconfirmed.

Preliminary data from small phase 1 and 2 studies of combination regimens including bortezomib, thalidomide or lenalidomide, doxorubicin, melphalan and dexamethasone or prednisone, suggest that the risk of TE complications may be markedly lower when bortezomib is used in combination with regimens of known thrombogenic potential, in both the front-line and relapsed settings, even in the absence of anticoagulant prophylaxis (Zangari *et al*, 2005; Chanan-Khan *et al*, 2006, 2007; Richardson *et al*, 2006, 2007a,b; Terpos *et al*, 2006; Ciolli *et al*, 2007; Jagannath *et al*, 2007; Palumbo *et al*, 2007a; Wang *et al*, 2007). Furthermore, data from a preplanned interim analysis of a phase 3 study in newly diagnosed multiple myeloma patients showed that bortezomib plus thalidomide and dexamethasone was associated with a significantly lower rate of grade 3/4 DVT (3% vs. 6·5%, $P = 0·01$) than thalidomide and dexamethasone alone (Cavo *et al*, 2007) and, in another phase 3 study in the front-line setting, bortezomib plus dexamethasone was associated with a significantly lower rate of thrombosis than vincristine, doxorubicin and dexamethasone (3·8% vs. 8·4%, $P = 0·04$) (Harousseau *et al*, 2007). In addition, Palumbo *et al* (2007b) reported a lower incidence of TE complications in patients receiving bortezomib in preliminary results of a phase 3 study of anticoagulant prophylaxis for patients treated with thalidomide-based therapy. These data suggest that bortezomib may mitigate the thrombogenic potential of other agents.

Interestingly, the novel kinetics of bortezomib-associated thrombocytopenia and the hypothesised reduced incidence of TE complications may be partly due to the same aspect of the unique mechanism of action of bortezomib. Through inhibition of the 26S proteasome, bortezomib substantially inhibits the activity of nuclear factor-κB (NF-κB) in cells by preventing proteasomal degradation of I-κB, which binds to and inhibits NF-κB (Baldwin, 2001; Adams & Kauffman, 2004). NF-κB is a transcription factor that is involved in numerous cellular signalling pathways. Platelet budding from megakaryocytes

depends partly on NF-κB (Liu *et al*, 2002); the transient inhibition of NF-κB transcription by bortezomib may result in inhibition of platelet budding and the subsequent transient, cyclical thrombocytopenia reported here in APEX, and in SUMMIT and CREST (Lonial *et al*, 2005). Furthermore, NF-κB has been shown to be involved in downregulation of the coagulation inhibitor thrombomodulin and expression of thrombogenic factors, such as tissue factor and plasminogen activator inhibitor type I, through the induction of cytokines including tumour necrosis factor-α (Parry & Mackman, 1995; Hamaguchi *et al*, 2003; Sohn *et al*, 2005). Therefore, bortezomib-induced inhibition of NF-κB may affect these aspects of the complex coagulation system and contribute to a reduced risk of TE complications. This idea is supported by a preclinical study of bortezomib as treatment for embolic stroke in rats: bortezomib was shown to reduce the risk of secondary thrombosis and inflammatory responses through upregulation of endothelial nitric oxide synthesase expression and inhibition of NF-κB activation (Zhang *et al*, 2006). Furthermore, in a recent study of 10 relapsed/refractory multiple myeloma patients who had normal coagulation parameters at baseline, Zangari *et al* (2007b) showed that bortezomib significantly decreased adenosine diphosphate (ADP)-, epinephrine- and ristocetin-induced platelet aggregation in samples of platelet-rich plasma, and suggested that inhibition of platelet function by bortezomib may give rise to an anti-thrombotic effect. Avcu *et al* (2008) also found that bortezomib inhibited ADP-induced platelet aggregation, as well as collagen-induced adenosine triphosphate release of platelets, in human platelet-rich plasma.

In conclusion, the unique mechanism of action of bortezomib appears to be associated with the novel kinetics of bortezomib-associated thrombocytopenia, and may also be the reason for the low incidence of TE complications reported with bortezomib alone and in combination. Further studies are required to confirm the hypothesis that bortezomib provides a protective, anti-thrombotic effect in combination with regimens of known thrombogenic potential, as well as to elucidate the mechanisms by which bortezomib affects the coagulation pathway in patients with multiple myeloma.

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