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Proteasome inhibition in multiple myeloma

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ABSTRACT

The ubiquitin-proteasome pathway is the major cellular degradative system for various proteins critical for proliferation, survival and homing of myeloma cells. Bortezomib is the first specific and reversible proteasome inhibitor for clinical application in humans. Phase I studies have defined the maximum tolerated dose and suggested activity against multiple myeloma. From single agent phase II studies, a rate of at least partial responses ranging from 27% for relapsed and refractory to 38% for second-line patients was derived. In comparison with pulsed dexamethasone, bortezomib enabled a higher response rate, a longer time to myeloma progression and a longer survival for patients after one to three prior lines of therapy. Preclinical and clinical phase I studies as well as initial phase II studies combining bortezomib with conventional chemotherapy or thalidomide support the assumption that bortezomib sensitizes myeloma cells to these drugs resulting in additive or synergistic activity.

1. Introduction

The ubiquitin-proteasome pathway was discovered in the 1970s during the search for an adenosine triphosphate (ATP)-dependent, non-lysosomal system for regulated protein breakdown. The proteasome emerged as a highly conserved supramolecular protease complex with an unusual threonine-based proteolytic mechanism degrading primarily proteins which have been previously tagged by polyubiquitination.[1] The ubiquitin-proteasome pathway is involved in the degradation of most cell proteins, short-lived mainly regulatory proteins as well as long-lived proteins. Moreover, specialized forms of the 26S proteasomes, often referred to as immunoproteasomes, incorporating three alternative interferon-γ-inducible β-subunits (LMP2, LMP7, MECL1) are involved in the generation of antigenic peptides from intracellular non-native proteins for major histocompatability complex (MHC) class I molecule-bound presentation to cytotoxic T-lymphocytes.[2,3]

Three types of enzymes activate ubiquitin molecules (E1), transfer (E2) and covalently link them to proteins which are to be degraded (E3). There are at least 20–30 E2s and some hundred ubiquitin ligases (E3s) providing the substrate specificity for the regulated degradation process (Table 1).[4]

The 26S proteasome itself is a very large (~2.5 MDa) cylindrical shaped protease complex composed of 44 polypeptides which are present in all eukaryotic cells.[5] It is responsible for more than 80% of intracellular protein degradation.[6] The proteasome's centre is capped by one or two 19S (890 kDa) regulatory complexes which unfold globular proteins and inject them into a 20S (720 kDa) core.[7] Prior to this, isopeptidases from the lid of the regulatory complex disassemble the polyubiquitin chain which makes the ubiquitin molecules available for reuse. The base of the regulatory complex binds polypeptide substrates, unfolds globular proteins, triggers opening of the gate to the core, and catalyses protein translocation into the core. The core, a hollow cylindrical particle, is composed of two outer alpha- and two inner β-rings, each composed of 7 homologous subunits. The alpha rings form a narrow channel, whose traverse requires unfolding of tightly packed globular proteins.[8] The β-subunits contain the proteolytic sites, each two "chymotrypsin-like", "trypsin-like"

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Table 1 – Functional components of the ubiquitin-proteasome pathway

Function	Component	Activity
Polyubiquitination	Enzyme E1 Ub-activating enzyme Enzyme E2 Ub-carrier protein Enzyme E3 Ub-ligase	• Activates ubiquitin • Transfers ubiquitin • Covalently links ubiquitin to substrate
Regulation of proteolysis	19S lid 19S base	• Isopeptidases disassemble ubiquitin chains • ATPases open channel to proteolytic chamber • Unfold globular proteins • Catalyse protein translocation into the 20S core
Degradation	20 S core, α-rings 20 S core, β-rings	• Restrict translocation to unfolded proteins • Proteolytic activity

Ub, Ubiquitin; ATP, adenosine triphosphate.

and "caspase-like" where polypeptides are processively digested into small peptides with a median size of 6–7 residues (range 2–24).[7,9]

Various short-lived regulatory proteins involved in proliferation and apoptosis are known substrates of the ubiquitin-proteasome's proteolytic activity, including many transcription factors, oncogene products, tumour suppressors, cell-cycle regulatory proteins (e.g. various cyclins and cyclin-dependent kinase-inhibitors) and rate-limiting enzymes (Table 2).[4,10] Proteasomes degrade abnormal secretory and membrane proteins, e.g. proteins not properly folded or failing to bind cofactors or form oligomeric structures.[11] Inhibition of the proteasome's activity stabilizes transcription factors of heat-shock proteins which are usually short-lived, thus enhancing protective cellular responses against exogenous stressors.[12] However, 80–90% of long–lived proteins are also degraded by the proteasome pathway.[3]

2. Natural product and synthetic proteasome inhibitors

Inhibitors of the proteasome have initially been used to study its biological role *in vitro* and *in vivo*. Several natural source inhibitors were identified, e.g. lactacystin, epoxomicin and

Table 2 – Physiologic substrates of the proteasome and their function

Function	Substrate	Physiologic function	Ref.
Cell cycle regulatory proteins	Cyclins (A, B, C, D)	Control the cell cycle	[94]
	CDK inhibitors (p21, p27)	Inhibit CDK activity	[94]
	Phosphatases (cdc25A, cdc25B, cdc25C)	Control CDKs and transitions through the cell cycle	[95]
Transcriptional regulators	I-κB/NF-κB	Control transcription of gene products involved in inflammation	[96]
		Control transcription of gene products involved in cell adhesion, metastasis and angiogenesis (ICAM-1, VCAM-1, E-selectin)	[97]
		Control transcription of Bcl-2 family proteins	[98]
	β-catenins	Control cell cycle checkpoints	[99]
	HIF1	Initiates molecular events required for the adaptation of tumour cells to hypoxia	[100]
	ATF2	Transcription factor for protein kinases	[1]
	STAT proteins	Regulate many pathways important in oncogenesis	[101]
Oncogenic products and tumour suppressors	Oncogenes (c-fos, c-myc, c-jun, c-Mos) p53 and MDM2 E2A proteins	Regulate oncogene activity	[102–104]
Apoptosis	Inhibitors of apoptosis (IAPs; XIAP, cIAP)	Regulation of transcription	[105]
Antigen presentation	Microbial antigens	Generation of antigenic peptides binding to MHC class I molecules	[2,3]

Adapted from Richardson.[106]
CDK, cyclin dependent kinase; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; Bcl-2, B cell lymphoma-2; HIF1, hypoxia-inducible factor 1; ATF2, activating transcription factor 2; IRF2 – iron regulatory protein 2.

the family of peptide epoxyketone natural products, macrocyclic compounds, gliotoxin and polyphenols.[13] Initially, synthetic inhibitors of the ubiquitin proteasome pathway were developed with the goal of reducing the excessive proteolysis in atrophying muscle or chachexia and inhibition of MHC class I antigen presentation, all of which depend on proteasome function.[14] Synthetic proteasome inhibitors can be divided in reversible (e.g. peptide aldehydes, amides, boronic esters and acids) as well as irreversible inhibitors (e.g. epoxy ketones and vinyl sulfones). However, covalent binding of inhibitors to the active β-subunits of the proteasome usually results in induction of apoptosis.[15]

Bortezomib, a dipeptide boronic acid derivative is the first representative of a class of drugs, which highly, specifically and reversibly inhibit proteasome activity. Inhibition by bortezomib is due to rapid but reversible binding to a single threonine in the active site of the 20S proteolytic core.[16] *In vitro* studies have shown that bortezomib has significant activity against numerous tumour cell lines, and animal studies have demonstrated marked effectiveness against human prostate cancer, adult T-cell leukaemia[17] and several B-cell lymphomas.[18] When screened *in vitro* for activity against a National Cancer Institute's panel of 60 human tumour cell lines, bortezomib exhibited a novel pattern of cytotoxicity compared with historical compounds.[16] Malignant cells independent from their proliferative capacity emerged to be more sensitive to inhibition of proteasome activity than their benign counterparts.[6]

3. Mechanisms of action of bortezomib

Bortezomib inhibits the chymotryptic-like peptidase activity of the proteasome.[19] *In vivo* measurements of proteasome inhibition after bortezomib application demonstrated proteasome inhibition of bortezomib to be dose dependent and reversible across species.[20]

Inhibition of the proteasome in multiple myeloma (MM) cells affects various growth and survival signalling mechanisms and interferes with myeloma cell adhesion mediated drug resistance.[21] The major mechanism, by which bortezomib acts as a growth inhibitor might be by blocking of inhibitor-kappa B (IκB) thereby abrogating nuclear factor-kappa B (NFκB) signalling. Myeloma cells have enhanced NFκB activity compared with normal haematopoietic cells.[22] NFκB signalling results in the production of cytokines like interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNFα), survival factors (IAPa, Bcl-XI), and adhesion molecules like intracellular adhesion molecule (ICAM), vascular adhesion molecule (VCAM) and E-selectin.[23] Moreover, preclinical studies indicate that proteasome inhibition potentiates the activity of several conventional antitumour agents. The chemosensitivity of resistant myeloma cells to doxorubicin and melphalan was increased when combined with bortezomib.[22,24] In solid tumour xenograft models, bortezomib enhanced the efficacy of gemcitabine in pancreatic cancer,[25] CPT11 in colon and pancreatic cancer models[26,27] and docetaxel in ovarian cancer.[28]

However, since specific inhibition of IκB (by PS1145) only partially exerts the growth inhibiting effect of bortezomib, NFκB seems not to be the only mechanism by which proteasome inhibitors act. In fact, bortezomib triggers apoptotic signalling through a variety of additional mechanisms including activation of heat shock proteins,[29–31] C-Jun-NH2-terminal kinase (JNK),[32] and caspase-8 as well as caspase-3,[33] alteration of mitochondrial membrane potential and generation of reactive oxygen species,[34] and induction of intrinsic and extrinsic death pathways. Inhibition of JNK activity abrogates PS-341–induced MM cell death.[35] Bortezomib induces p53 and MDM2 protein expression as well as the phosphorylation (Ser15) of p53 protein. Moreover, bortezomib down-regulates the expression of several proteins involved in DNA repair, e.g. cleaves the DNA-dependent protein kinase catalytic subunits ATM and MDM2.[24]

Mechanisms mediating bortezomib resistance are not well defined up to now. It is likely that Hsp27 and Bcl2 protein family members confer drug resistance to bortezomib in MM.[30,36] Moreover, inhibitors of apoptosis proteins may play a role in bortezomib resistance.[29]

4. Pharmacokinetics and pharmacodynamics of bortezomib

Following intravenous (i.v.) bolus administration, plasma concentrations of bortezomib decline in a biphasic manner with a rapid distribution phase followed by a longer terminal elimination phase. Greater than 90% of bortezomib is rapidly (within 15 minutes) cleared from the plasma and distributed to all tissues, including the bone marrow.[37] However, the drug does not cross the blood–brain or blood–testis barriers and does not reach various regions of the eye and optic nerve.[16] Bortezomib yields a reversible and time-limited inhibition of the proteasome's chymotrypsin-like activity by 60–80%. Proteasome function recovers with a half-life of approximately 24 h, returning towards baseline by 48 to 72 h. In animal models, sustained inhibition produced marked toxicity and a twice-weekly dosing schedule was selected for clinical studies to allow recovery between doses and to minimize toxicity.[37,38] Bortezomib is inactivated through oxidative deboronation by both cytochrome P450 mediated (mainly CYP3A4 and 2C19) and nonenzymatic mechanisms.[39] After that, the drug undergoes secondary metabolism and is ultimately excreted in the bile and urine. Bortezomib is a poor inhibitor and not an inducer of cytochrome P450 isoenzymes. It is therefore unlikely that bortezomib changes the pharmacology of concomitant medications. However, the opposite (change of bortezomib pharmacology by concomitant medication) has not been assessed.[40] There are no data on drug pharmacology and toxicology in patients with serious hepatic insufficiency. Clinical experience in a limited number of myeloma patients with impaired renal function (creatinine clearance 14–29 ml/min; $n = 10$) or on dialysis ($n = 15$) suggests that bortezomib can be administered to these patients with similar efficacy and a comparable adverse event (AE) profile.[41,42]

There are no reports on the treatment of pregnant or lactating women as well as children with bortezomib.

5. Phase I clinical trials

5.1. *Bortezomib as a single agent*

Three schedules for intravenous dosing of bortezomib have been evaluated in patients with malignant disease up to date.

One schedule administered the drug once weekly for four weeks, another twice weekly for two weeks and the third twice weekly for four weeks, each followed by a recovery period of one to two weeks. Finally, a schedule with twice weekly injections for two weeks followed by one week rest was selected for further clinical development in MM.

Based on promising preclinical data the maximum tolerated dose (MTD) for bortezomib as a single agent was evaluated in patients with advanced malignancies. In a phase I study on solid tumours the MTD was explored in a three week schedule with bortezomib injections at doses ranging from 0.13 to 1.56 mg/m^2/dose on days 1, 4, 8 and 11 followed by a 10 days rest. Recommended dose with this schedule in advanced solid malignancies was 1.56 mg/m^2/dose limited by diarrhoea (n = 2/12) and sensory neuropathy (n = 2/12) each of which reaching grade 3.[43]

In a parallel study on advanced haematologic malignancies, 27 patients received the drug twice weekly for 4 weeks followed by a two week rest period. The MTD with this dosing schedule emerged to be 1.04 mg/m^2. Dose limiting toxicities in these entities and above this threshold comprised electrolyte disturbances (hyponatraemia, hypokalaemia), fatigue and malaise. Since many of these AEs occurred during the third or fourth week of treatment and resolved during the rest period, a two week treatment schedule with applications twice weekly followed by a ten day recovery period at an intermediate dose of 1.3 mg/m^2 was selected for phase II evaluation.[38]

In the latter study including patients with advanced haematologic malignancies all nine evaluable patients with plasma cell dyscrasias exhibited evidence of treatment response either as a decline in serum monoclonal immunoglobulin or a reduction in plasma cell infiltration of the bone marrow. This secondary result identified MM as the most promising disease entity for phase II evaluation. In addition, each one patient in this study with follicular lymphoma and mantle cell lymphoma achieved a durable partial response (PR).

5.2. *Phase I evaluations of bortezomib in combination with established anti-myeloma agents*

Phase I studies combining bortezomib with established anti-myeloma drugs were initiated based on xenograft models of solid tumours pointing to an enhancement of the effects of standard chemotherapies when combined with bortezomib. This holds true for combinations with gemcitabine (in pancreatic cancer),[25] CPT-11 (in colon and pancreatic cancer)[26,27] and docetaxel (in ovarian cancer).[28] In MM, bortezomib has been shown to restore melphalan as well as doxorubicin sensitivity to resistant cell lines and to synergise with melphalan in killing myeloma cells.[22,24]

In 42 patients with advanced haematologic malignancies, including 24 patients with MM, the MTD for bortezomib given on days 1, 4, 8 and 11 escalated from 0.90 to 1.50 mg/m^2 and combined with a fixed dose of pegylated liposomal doxorubicin (PegLD) of 30 mg/m^2 was determined in a 21-day cycle. Grade 4 haematologic toxicity or grade 3 non-haematologic toxicity (apart from alopecia) was considered dose-limiting. The most frequent AEs of at least grade 3 were thrombocytopenia (43%), lymphopenia (40%), neutropenia (17), fatigue (14%), pneumonia (14%), peripheral neuropathy (12%), febrile neutropenia (10%) and diarrhoea (10%). Frequency and intensity of side-effects was as expected from single agent studies without evidence of potentiation of toxicities. Formal MTD for bortezomib based on the first treatment cycle was 1.50 mg/m^2. However, due to frequent dose reductions and treatment delays in subsequent cycles recommended dose for continuous treatment from this study was 1.3 mg/m^2 in combination with 30 mg/m^2 PegLD. Concerning efficacy, patients were evaluable if they had received at least two treatment cycles. Five out of 22 evaluable MM patients achieved complete response (CR) and an additional 11/22 partial response (PR) (European Group for Blood and Marow Transplant (EBMT) criteria).[44]

Berenson and co-workers explored oral melphalan at 0.025, 0.05, 0.1, 0.15 and 0.25 mg/kg on days 1–4 every 4 weeks for up to 8 cycles combined with bortezomib 0.7 and 1.0 mg/m^2 in patients with relapsed or refractory myeloma. Bortezomib 1.0 mg/m^2 in combination with melphalan 0.1 mg/kg was assigned as the MTD with grade 4 neutropenia emerging as the dose-limiting toxicity. Responses $\geqslant$ minor response (MR) occurred in 68% of patients including five of six assessable patients treated at the MTD level.[45]

Zangari added thalidomide at incremental doses (50, 100, 150, 200 mg daily) per cohorts of at least ten patients with the start of the second treatment cycle to bortezomib initially at a dose of 1.0 and 1.3 mg/m^2 days 1, 4, 8 and 11 every 21 days. The MTD was reached at bortezomib 1.3 mg/m^2 and thalidomide 150 mg. 55% of the patients achieved a PR and 15% a minor response (MR). Patients with abnormal cytogenetics or prior thalidomide experienced shorter event-free survival (EFS) and overall survival (OS).[46]

In addition, interim results of further phase I studies have recently been presented which evaluated bortezomib in combination with glucocorticoids and one or more of the aforementioned drugs as well as thalidomide, lenalidomide, and KOS-953, a selective inhibitor of heat shock protein (HSP)-90 (Table 4). HSP-90 which is induced by and confers resistance to bortezomib[47] can be inhibited by KOS-953, a novel formulation of 17-AAG (17-allylamino-17-demethoxygeldanamycin). In a phase I dose escalation study, bortezomib (1.0 or 1.3 mg/m^2) and the immunomodulatory thalidomide derivative CC-5013/lenalidomide (5–20 mg/day per OS (PO)) could be combined at active doses and showed an encouraging activity (64% $\geqslant$PR).[48] Further phase I/II trials define the MTD of standard regimen bortezomib in combination with low-dose intravenous melphalan + dexamethasone,[49] oral cyclophosphamide + prednisone[50] as well as melphalan, prednisone and thalidomide (V-MPT).[51] A combination of bortezomib with doxorubicin, thalidomide and dexamethasone might revert resistance to one or more of the single drugs since responses during phase I were observed among patients previously resistant to bortezomib or thalidomide.[52] *In vitro* studies and experimental data on human MM in severe combined immunodeficient (SCID) mice suggest that bortezomib in combination with arsenic trioxide and ascorbic acid my have synergistic antimyeloma effects. This finding is somewhat surprising because other preclinical data demonstrated an inactivation of bortezomib by vitamin C in human cancer cells.[53] Interim results of a clinical phase I/II study indicate that this combination is well tolerated and has some efficacy in heavily pretreated patients.[54] As a single agent KOS-953 has

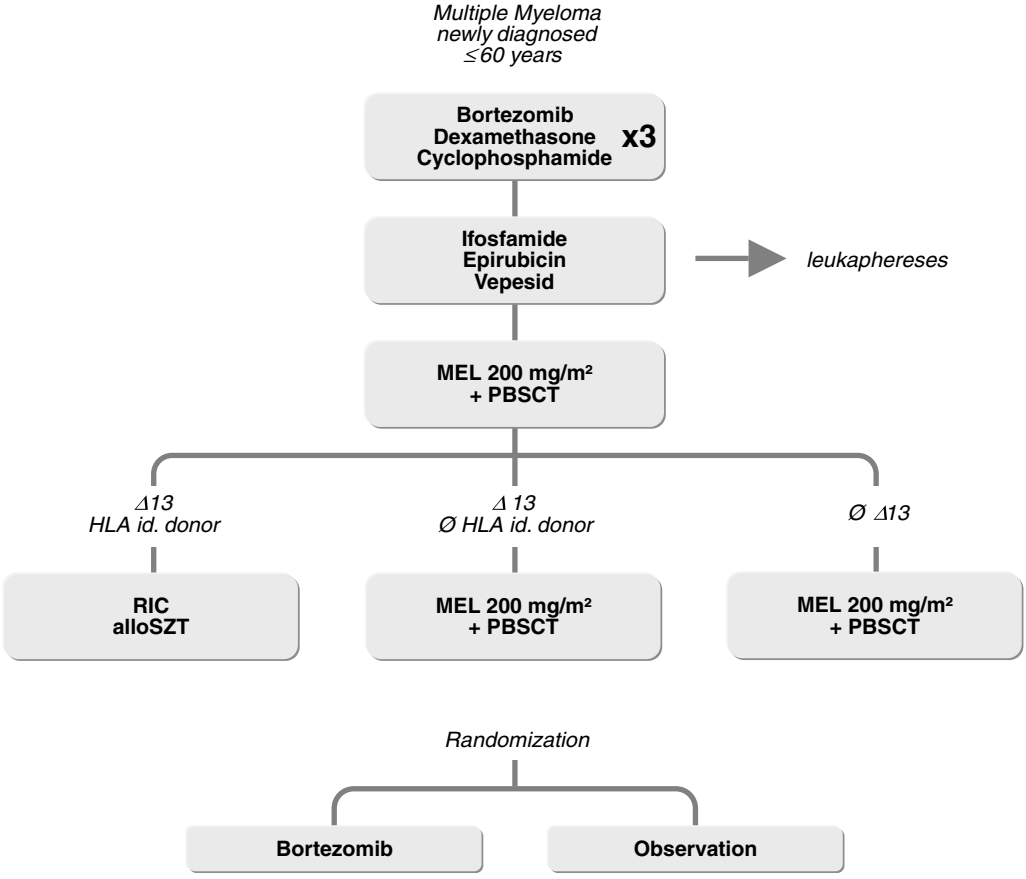

Fig. 1 – Treatment schema of *the Deutsche Studiengruppe Multiples Myeloma* (DSMM) XI protocol. MEL, melphalan; PBSCT, peripheral blood stem cell transplantation; Δ 13, partial or complete deletion of chromosome 13 on molecularcytogenetic analysis; HLA, human leukocyte antigen; id., identical; RIC, reduced-intensity conditioning.

significant activity in heavily pretreated MM patients with manageable toxicity but without treatment-emergent neuropathy.[55] The optimal dose of both drugs when combined is currently defined in a phase I clinical trial which stepwise escalates both agents (bortezomib 0.7, 1.0 and 1.3 mg/m^2; KOS-953 100, 150 and 220 mg/m^2). Dose escalation is ongoing at bortezomib 1.3 mg/m^2/KOS-953 150 mg/m^2 and interim results of clinical activity even in bortezomib refractory patients are encouraging.[56] In Germany, two phase I trials have currently been launched. Based on favourable single centre experience in relapsed MM[57] the MTD of bendamustin in combination with bortezomib and prednisolone will be defined. The investigators apply a cohort-wise dose escalation of bendamustin (60–100 mg/m^2) in combination with standard regimen bortezomib (1.3 mg/m^2 days 1, 4, 8 and 11 every 21 days) and prednisolone 100 mg PO on the day of each bendamustin or bortezomib injection in patients with MM after 1–3 relapse. Moreover, the next first-line trial of the 'Deutsche Studiengruppe Multiples Myelom' (DSMM) defines the optimal dose of cyclophosphamide in combination with bortezomib and dexamethasone for pre-transplant induction in younger patients. Eligible patients with newly diagnosed MM receive up to three 3-week cycles of bortezomib 1.3 mg/m^2 days 1, 4, 8 and 11 combined with dexamethasone 40 mg on the day of bortezomib injection and the day thereafter, and stepwise escalated intravenous cyclophosphamide on day 1. Cyclophosphamide dose levels are 600, 900, 1200 and 1500 mg/m^2, respectively. Following peripheral blood stem cell mobilization by ifosfamide/epirubicin/vespesid (IEV), patients receive melphalan 200 mg/m^2-based tandem transplants with subsequent randomisation between a bortezomib consolidation and observation without maintenance/consolidation. High-risk patients identified by a 13q-deletion are offered a reduced–intensity conditioning allogeneic stem cell transplantation instead of a second autologous transplantation (SCT) (Fig. 1). The German-speaking Myeloma Multicenter Group (GMMG) compares an anthracycline/dexamethasone-based induction followed by two autologous transplants and thalidomide maintenance with the same sequence supplemented for bortezomib during induction and bortezomib maintenance instead of thalidomide (Fig. 2). In this trial patients with defined risk factors and a sibling donor are scheduled for a reduced-intensity conditioning allogeneic transplant as well.

6. Phase II clinical trials in multiple myeloma

6.1. *Bortezomib ± dexamethasone in relapsed multiple myeloma*

Two phase II studies evaluating bortezomib as a single agent in patients with relapsed MM have been performed (Table 3).

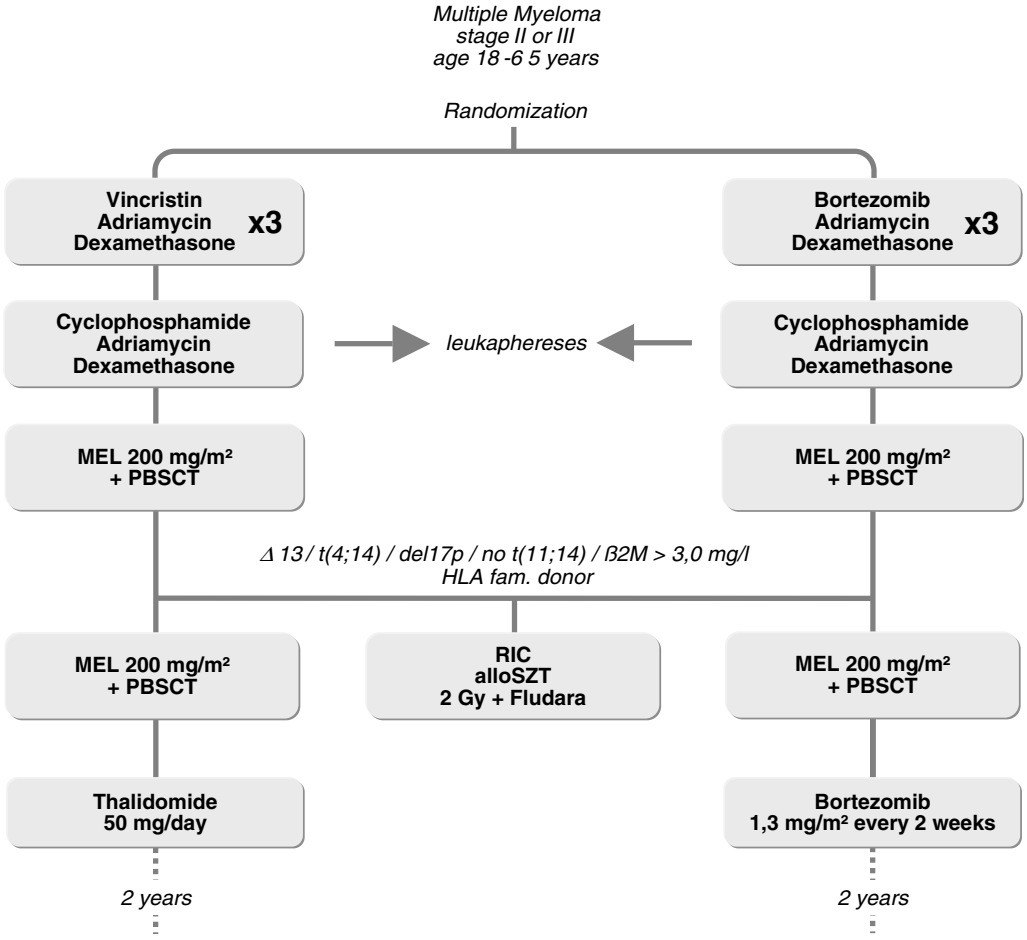

Fig. 2 – Treatment schema of the GMMG-HD-4/HOVON-64 study protocol. β2M, beta-2 microglobulin; fam., family.

Study 024 ("CREST") included patients who were relapsed or refractory after front-line therapy whereas patients in study 025 ("SUMMIT") had relapsed and refractory MM. Patients included in the latter trial had not only a disease relapse but the most recent therapy had failed to induce a sustained response (⩾60 days).

In the SUMMIT trial, 202 heavily pretreated MM patients received bortezomib 1.3 mg/m^2 given on day 1, 4, 8 and 11 of a 21-day cycle for up to 8 cycles. Patients with progressive disease (PD) after two cycles or stable disease (SD) after four cycles were eligible for an addition of 20 mg oral dexamethasone on the day of each bortezomib dose and the day thereafter.[58] CREST was a prospective randomised comparison of two bortezomib doses (1.0 or 1.3 mg/m^2) using the same schedule and optional dexamethasone addition as in SUMMIT.[59]

In the SUMMIT trial the overall response rate (ORR) among 193 evaluable patients who received bortezomib alone was 35%, including 4% CR, 24% PR and 7% MR. Dexamethasone added to clinical anti-myeloma activity of bortezomib by inducing 18% responses in patients with either SD or PD on bortezomib alone. Responses were generally rapid, with a median time to response of 38 days. Median time to progression among all 202 patients was 7 months and 13 months among those with a PR or CR. Median OS with bortezomib alone was 16 months.

Factors predictive of a lower response rate were age ⩾65 years and plasma cell infiltration in the bone marrow >50%. An increased C-reactive protein and abnormal cytogenetics (but not chromosome 13 deletion) were associated with a shorter time to progression. Survival was mainly influenced by parameters which are thought to reflect tumour burden like bone marrow plasma cell infiltration, level of albumin, platelet count as well as Karnofsky performance score. Remarkably, factors historically considered to be adverse prognostic factors in myeloma patients like chromosome 13 deletion and elevated β2-microglobulin were not predictive of poor outcome with bortezomib in the SUMMIT trial.[60]

In the CREST trial, comparing two doses of bortezomib (1.3 mg/m^2 and 1.0 mg/m^2) ORR among 53 evaluable patients was higher and time to progression (TTP) longer in the 1.3 mg/m^2 group than in the 1.0 mg/m^2 group (ORR 50% *versus* 33%; TTP 11.0 months *versus* 7.0 months), suggesting a potential dose-response relationship. Twenty-eight patients received additional dexamethasone which resulted in additional responses.

We evaluated a primary combination of bortezomib and dexamethasone in 30 consecutive patients who experienced their second untreated or refractory relapse in an attempt to improve disease response. Patients were offered additional support (e.g. blood component support) instead of treatment

exclusion for poor peripheral blood counts. Bortezomib dosage was 1.3 mg/m^2 administered i.v. on days 1, 4, 8 and 11 on a 21-day cycle for up to 8 cycles; dexamethasone (DEX) (20 mg orally once daily) was administered on the day of bortezomib injection and the day thereafter. ORR was 80% (7% CR; 67% PR; 7% MR); 9 of 13 patients with a chromosome 13 deletion achieved at least a PR. Median time to response was 3 weeks and responses were independent of prognostic parameters. However, remissions were often not durable (median TTP 4 months; median OS 14 months).[61,62] In this context, weekly bortezomib with or without glucocorticoids may as well be an appropriate schedule with similar efficacy.[63]

Clinical experience in a limited number of patients ($n = 10$) with impaired renal function (creatinine clearance 14–29 ml/min) suggests that bortezomib can be administered to these patients with similar efficacy and a comparable AE profile.[64] However, there was a trend toward a higher frequency of serious AE with decreasing renal function. There was no association between inhibition and recovery of proteasome activity and renal function in this trial.

Preliminary analyses of cell lines and clinical experience in single patient point to a role of bortezomib in plasma cell leukaemias.[65–68]

6.2. *Phase II evaluations of bortezomib in combination with chemotherapy and/or thalidomide in relapsed multiple myeloma*

Three phase II clinical trials on bortezomib in combination with chemotherapy or thalidomide have been reported up to now (Table 4).

In a DSMM trial, 50 patients with advanced MM were scheduled to receive bortezomib 1.3 mg/m^2 i.v. on days 1, 4, 8 and 11 q 3 weeks for 8 cycles in combination with DEX 20 mg PO on the day of bortezomib injection and the day thereafter and cyclophosphamide (CY) 50 mg PO daily; followed by three cycles of bortezomib 1.3 mg/m^2 i.v. on days 1, 8, 15 and 22 q 5 weeks in combination with the same DEX and CY schedule. The ORR achieved was 90% including 6 CRs (12%).[69]

Interim results of two further phase II trials have recently been presented. Both trials aim at targeting myeloma cells and tumour microenvironment with a combination of bortezomib with thalidomide to overcome drug resistance. Terpos and colleagues showed that bortezomib in combination with melphalan, dexamethasone and intermittent thalidomide induced responses in 56% of patients.[70] Moreover, this combination reduced sRANKL and MIP-1α levels after 4 cycles. Pegylated liposomal doxorubicin combined with bortezomib and thalidomide appears to be a highly active salvage regimen with response in 13/13 evaluable patients despite prior failure of steroids, thalidomide or adriamycin.[71]

6.3. *Phase I/II clinical trials in* de-novo *multiple myeloma*

Currently, a broad clinical development program is proceeding which aims at defining the impact of bortezomib on the treatment of newly diagnosed MM (Table 5). Taken together, recently reported interim results indicate a single agent activity ($\geqslant$PR) of bortezomib in induction treatment of around 38–50%,[72,73] which can be improved to 80–90% by the addition of dexamethasone in patients with suboptimal response on bortezomib alone.[73–76] In clinical trials combining bortezomib ± glucocorticoids with thalidomide,[77] doxorubicin,[78,79] melphalan,[80] DT-PACE (dexamethasone/thalidomide – cisplatinum/adriamycin/cyclophosphamide/etoposide)[81] or incorporating the latter into a total therapy program[82] response rates can be further increased. It remains to be shown whether the impressive response rates from preliminary results of these trials translate into prolonged post-transplant progression-free or overall survival. Moreover, since a substantial proportion of patients with newly diagnosed MM exhibits signs of neuropathy at baseline,[72] combination of bortezomib with chemotherapy for pretransplant induction might enable a dose reduction for bortezomib and thereby reduce bortezomib-associated neuropathy.[79] An induction treatment incorporating bortezomib does not prejudice subsequent stem cell mobilization or engraftment after high-dose treatment and SCT.[78,82,83] After allogeneic transplant, bortezomib can yield a further paraprotein decline but is associated with considerable toxicity.[84]

7. Phase III clinical trial – assessment of proteasome inhibition for extending remissions (APEX)

Bortezomib was compared in an international, randomised, open-label phase III trial to pulsed DEX. Patients were eligible if they were relapsing after 1–3 prior lines of treatment, and had adequate bone marrow (platelets $\geqslant 50 \times 10^3$/mm^3) renal (creatinine clearance $\geqslant$ 20 ml/min) and liver function. Subjects assigned to bortezomib received the drug for 8 three-week cycles (1.3 mg/m^2 days 1, 4, 8, 11) followed by 3 five-week cycles (1.3 mg/m^2 days 1, 8, 15, 22), whereas those randomised to the standard arm initially received DEX 40 mg orally days 1–4, 9–12 and 17–20 for 4 five-week cycles followed by 5 four-week-cycles with DEX on days 1–4. At a predefined interim analysis both a significant prolongation of TTP as well as OS for patients treated with bortezomib were observed. As a consequence, all patients treated with DEX were offered bortezomib in a companion study. At final analysis patients initially treated with bortezomib had a response rate more than double that of DEX (38% *vs.* 18%; $P < 0.001$), a longer TTP (6.22 *vs.* 3.49 months; $P < 0.001$) and a higher rate of OS at one year (80% *vs.* 66%; $P = 0.003$). There was neither a difference in the time to a first skeletal event nor in the incidence of $\geqslant$°3 infections.[85] A recent update confirmed a further increase in the response rate to 43% with bortezomib and, despite >62% of DEX patients crossing over to bortezomib, a 6-month improvement in median survival from 23.7 months with initial DEX treatment to 29.8 months with initial bortezomib. Median TTP remained unchanged.[86]

Currently, the international randomised VISTA trial compares a combination of melphalan/prednisone/bortezomib to standard melphalan/prednisone as first-line treatment in patients with newly diagnosed MM who are not eligible for high-dose treatment. The VISTA trial is intended to be the basis for first-line approval of bortezomib.

Table 3 – Efficacy of bortezomib alone or in combination with dexamethasone for relapsed or refractory multiple myeloma

Trial First Author/ Year of publication	SUMMIT Richardson, 2003[58]	CREST Jagannath, 2004[59]		APEX Richardson, 2005[85,86]	Bortezomib/Dexamethasone Kropff, 2005[61,62]
Patients	Relapsed or refractory	Relapse during/ following front-line therapy		1–3 previous therapies	$\geqslant 2^{nd}$ relapse; irrespective of pretreatment PBC
No. of patients evaluable	202	53		315	30
		27	26		
Median age (years)	60	63		62	63
Median duration from diagnosis (years)	4,0	2.0		3.5	3,1
Bortezomib schedule	1,3 mg/m^2 days 1, 4, 8, 11 q 3 weeks × 8 cycles	1,0 mg/m^2 *vs.* 1,3 mg/ m^2 days 1, 4, 8, 11 q 3 weeks × 8 cycles		1,3 mg/m^2 days 1, 4, 8, 11 q 3 weeks × 8 cycles followed by 1,3 mg/m^2 days 1, 8, 15, 22 q 5 weeks × 3 cycles	1,3 mg/m^2 days 1, 4, 8, 11 q 3 weeks × 8 cycles followed by 1,3 mg/m^2 days 1, 8, 15, 22 q 5 weeks × 3 cycles
Dexamethasone schedule	Permitted for PD $\geqslant$ 2 cycles or SD $\geqslant$ 4 cycles	Permitted for PD $\geqslant$ 2 cycles or SD $\geqslant$ 4 cycles		–	20 mg on the day of bortezomib injection and the day thereafter
Endpoints					
– Primary	– ORR (CR+PR + MR) to bortezomib alone	– ORR (CR + PR + MR) to bortezomib alone		– TTP (compared with DEX)	– ORR
– Secondary	– TTP, OS, safety, ORR to bortezomib in combination with DEX, quality of life	–TTP, OS, safety, ORR to bortezomib in combination with DEX		– OS – survival at 1 year – response rate (CR + PR) – duration of response – time to $\geqslant$°3 infection – incidence of $\geqslant$°3 infection – time to next skeletal event	– TTP – OS – safety
Response rate (ORR; %)	**35**	**33**	**50**	**43**	**60**
– CR	4	4	4	9	3
– PR	24	26	35	34	47
– MR	7	4	12	8[a]	10
– NC	24	26	19	43[a]	13
– PD	41	41	31	7[a]	27
Median TTP (months)	7.0	7.0	11.0	6.22	4
Median OS (months)	16	26,7	N.R.	29.8	14

PBC, peripheral blood count, PD, progressive disease; SD, stable disease; ORR, overall response rate; CR, complete response; PR, partial response; MR, minor response; TTP, time to progression; DEX, dexamethasone; OS, overall survival; N.R., not recorded.

a Results from Ref. [85] not updated in Ref. [86].

Table 4 – Summary of phase I/II data on bortezomib in combination with chemotherapy or thalidomide for relapsed multiple myeloma

Reference	Patients	Schedule	Result		Outcome	Remarks
Orlowski (2005)[44]	$n = 42$ (24 MM, 22/24 evaluable for response)	Bortezomib 0.9–1.5 mg/m^2 IVP d 1, 4, 8, 11 Doxil 30 mg/m^2 IV d 4 q 21 d	CR PR	5/22 11/22		Bortezomib 1.3 mg/m^2 recommended for further study Responses in 8/13 MM patients after prior anthracyclines
Berenson (2006)[45]	$n = 35$	Bortezomib 0.7/1.0 mg/m^2 IVP d 1, 4, 8, 11 Melphalan 0.025–0.25 mg/kg PO d 1–4 q 28 d X $\leqslant$ 8 cycles followed by Bortezomib 1.3 mg/m^2 IVP every two weeks	CR PR MR	6% 41% 21%	Median PFS 8 mo.	$\geqslant$°3 tox. mostly myelosuppression DLT: °4 neutropenia 11/35 treatment-emergent neuropathy, 1/35 °3 s
Zangari ASH (2005)[46]	$n = 85$	Bortezomib 1.0/1.3 mg/m^2 IVP d 1, 4, 8, 11 Thalidomide 50–200 mg/d PO d1–4, 17–20 added at cycle 2 q 21 d X $\leqslant$ 8 cycles DEX permitted for suboptimal response after $\geqslant$ 3 cycles	MTD bortezomib 1.3 mg/m^2/thalidomide 150 mg PR	55%	Median EFS 9 mo. Median OS 22 mo.	Superior 12 mo. EFS and OS with bortezomib 1,3 mg/m^2 No apparent thalidomide dose effect on EFS or OS EFS and OS significantly shorter with prior thalidomide or cytogenetic abnormalities
Richardson ASH (2005)[48]	$n = 24$	Bortezomib 1.0/1.3 mg/m^2 IVP d 1, 4, 8, 11 Lenalidomide 5/10/15/20 mg/d PO d 1–14 q 21 d until PD DEX 20 mg PO d 1, 2, 4, 5, 8, 9, 11, 12 permitted for PD	ORR	67%		Accrual ongoing at bortezomib 1.0/1.3 mg/m^2/lenalidomide 20 mg No significant neuropathy or fatigue DLTs: Hyponatremia, treatment delay due to HZV infection
Popat ASH (2005)[49]	$n = 18$	Bortezomib 1.3 mg/m^2 IVP d 1, 4, 8, 11 Melphalan 2.5–10 mg/m^2 IV d 2 q 28 d X $\leqslant$ 8 cycles DEX 20 mg PO d 1, 2, 4, 5, 8, 9, 11, 12 permitted for PD after 2 cycles or SD after 4	MTD not yet defined ORR 50% (75% with DEX)		Median TTP and OS after a median follow-up of 3 mo. not reached. Accrual ongoing	Most common °3/4 AE: Myelosuppression
Reece ASH (2005)[50]	$n = 20$	Bortezomib 0.7–1.3 mg/m^2 IVP d 1, 4, 8, 11 or 1, 8, 15 Prednisone 100 mg PO every other morning	CR PR MR	– 9/20 4/20		Accrual ongoing, MTD not reached DLT: Anemia, leukopenia, neutropenia, thrombocytopenia, infection, nausea/vomiting, hypophosphatemia, hyperglycemia
		Cyclophosphamide 150 => 300 mg PO d 1, 8, 15, 22 q 28d X $\leqslant$ 8 cycles	SD PD	2/20 5/20		
Palumbo ASH (2005)[51]	$n = 20$ 9/20 2nd line 11/20 3rd line	Bortezomib 1.0/1.3/1.6 mg/m^2 IVP d 1, 4, 15, 22 Melphalan 6 mg/m^2 PO d 1–5 Prednisone 60 mg/m^2 PO d 1–5 Thalidomide 100 mg QD PO d1–5 q 35 d X $\leqslant$ 6 cycles	MTD bortezomib not defined PR	67%	n.a. accrual ongoing	Most common °3 AE: Myelosuppression, infection, fatigue, vasculitis

(*continued on next page*)

Table 4 – *continued*

Reference	Patients	Schedule	Result		Outcome	Remarks
Hollmig ASH (2004)[52]	*n* = 20 heavily pretreated	Bortezomib 1.0/1.3 mg/m^2 IVP d 1, 4, 8, 11 Adriamycin 2.5 => 10 mg/m^2/day CI Thalidomide 50–100 mg QD PO DEX 20 or 40 mg QD PO d 1–4, 9–12 Dose escalation schema not detailed	ORR	63%	At 11 mo. median follow-up, 13 patients alive	Responses seen in patients previously resistant to bortezomib or thalidomide
Berenson ASH (2005)[54]	*n* = 18	Bortezomib 0.7 => 1.3 mg/m^2 IVP d 1, 4, 8, 11 Arsenic trioxide 0.125/0.25 mg/kg i.v. d 1, 4 8, 11 Ascorbic acid 1000 mg i.v. d 1, 4, 8, 11 q 21 d X $\leqslant$ 8 cycles followed by maintenance: Same treatment once every other week	PR MR	2/15 5/15		
Chanan- Khan ASH (2005)[56]	*n* = 15 Median no. of prior regimens 4	Bortezomib 0.7 => 1.3 mg/m^2 i.v. d 1, 4, 8, 11 KOS-953 100 => 220 mg/m^2 i.v. d 1, 4, 8, 11 q 21 d	Accrual ongoing at bortezomib 1.3 mg/m^2/KOS-953 150 mg/m^2			Responses in 3/4 bortezomib- naïve and 6/12 bortezomib- refractory pts. No additive toxicities or PK interaction.
Kropff ASH (2005)[69]	*n* = 50 $\geqslant$ 2nd line	Bortezomib 1.3 mg/m^2 i.v. d 1, 4, 8, 11	CR	12%	At 15 mo. Median follow-up, median OS not reached	
		DEX 20 mg PO d 1, 2, 4, 5, 8, 9, 11, 12 Cyclophosphamide 50 mg PO continuously q 21 d X $\leqslant$ 8 cycles followed by Bortezomib 1.3 mg/m^2 i.v. d 1, 8, 15, 22 DEX 20 mg PO d 1, 2, 8, 9, 15, 16, 22, 23 Cyclophosphamide 50 mg PO continuously q 21 d X $\leqslant$ 3 cycles	PR MR SD PD	70% 8% 6% 4%		
Terpos ASH(2005)[70]	*n* = 31 pretreated, 20/31 resistant relapse	Bortezomib 1.0 mg/m^2 i.v. d 1, 4, 8, 11 Melphalan 0.15 mg/kg PO d 1–4 Thalidomide 100 mg QD PO d 1–4, 17–20 DEX 12 mg/m^2 PO d 1–4, 17–20 q 21 d X $\leqslant$ 8 cycles	CR PR MR SD	8% 48% 8% 20%	Median PFS 9.6 months	°3/4 neutropenia 8% °3/4 thrombocytopenia 12% Fatigue 56% °1/2 neuropathy (Ø °3) 48% Infection 36%
Chanan-Khan ASH (2004)[71]	*n* = 18 Median prior therapies: 2	Bortezomib 1.3 mg/m^2 IVP d 1, 4, 15 18 Doxil 20 mg/m^2 i.v. d 1, 15 Thalidomid 200 mg/day PO continuously q 28 d X 4–6 cycles	13/18 completed $\geqslant$ 1 cycle PR MR	 5/13 8/13	1 patient died of sepsis during cycle 1	Low-dose coumadin (1-2 mg PO/day) prevented VTE

IVP, intravenous push injection; PO, per os; CI, continuous infusion; VTE, venous thromboembolic events; nCR, near complete response; mo, months: d, day; n.a., not applicable; PFS, progression free survival.

Table 5 – Summary of phase I/II data on bortezomib alone or in combination for newly diagnosed multiple myeloma

Reference	Patients	Schedule	Result		Comment
Richardson ASH (2005)[72]	$n = 66$	Bortezomib 1.3 mg/m^2 d 1, 4, 8, 11 q 21 d X $\leqslant$ 8 cycles	CR PR MR SD PD	10% 28% 25% 32% 5%	Neurophysiologic testing at baseline and throughout follow-up period Defined pharmacologic interventions 55% °1/2 treatment emergent PNP at one site
Jagannath ASH (2005)[73,74]	$n = 50$	Bortezomib 1.3 mg/m^2 d 1, 4, 8, 11 q 21 d X $\leqslant$ 6 cycles DEX 40 mg added on the day of and day after each bortezomib dose for < PR after 2 cycles or < CR after 4 cycles	*Bortezomib alone:* CR + PR *Bortezomib/DEX:* CR + PR Median PFS 15 mo. Estimated OS at 1 year 93%	 50% 90%	Stem cell harvest successful and engraftment prompt AE predictable and manageable
Harrouseau ASH (2004)[75]	$n = 47$ (18 evaluable)	Bortezomib 1.3 mg/m^2 d 1, 4, 8, 11 DEX 40 mg PO d 1–4, 9–12 q 21 d X 2 cycles, followed by Bortezomib 1.3 mg/m^2 d 1, 4, 8, 11 DEX 40 mg PO d 1–4 q 21 d X 2 cycles	CR ORR	17% 83%	Stem cell collection adequate in all patients Randomised phase III trial VAD vs. bortezomib/dexamethasone planned
Dispenzieri ASH (2005)[76]	$n = 43$ (high risk) (19 evaluable)	Bortezomib 1.3 mg/m^2 d 1, 4, 8, 11 q 21 d X $\leqslant$ 8 cycles Maintenance: Bortezomib 1,3 mg/m^2 every other week indefinitely	CR PR MR	– 14/19 1/19	Non-haematologic AEs $\geqslant$ °3: Hyponatremia, diarrhoea, fatal heart block and asystole in 1 patient after 2 doses
Wang[77]	$n = 38$ (35/38 evaluable)	Bortezomib 1.3/1.5/1.7 mg/m^2 Thalidomide 100/150/200 mg/d DEX 20 mg/m^2 d 1–4, 9–12, 17–20 q 28 d, median 2 cycles followed by SCT	CR PR	18% 74%	LMW heparin or coumadin °3/4 AE: Myelosuppression, infection, neuropathy, DVT/PE
Oakervee, Popat (2005)[78,79]	$n = 21$	Bortezomib 1.3 mg/m^2 d 1, 4, 8, 11 DEX 40 mg/m^2 d 1–4, 8–11, 15–18 (day 1–4 during cycles 2–4) Doxorubicin 0/4.5/9.0 mg/m^2 d 1–4 q 21 d X 4 cycles	CR PR	24% 71%	20/21 pts. had PBSC mobilised 18/20 received MEL200/PBSCT
Mateos ASH (2005)[80]	$n = 60$ 12 for phase I 48 for phase II	Bortezomib 1.0/1.3 mg/m^2 d 1, 4, 8, 11, 22, 25, 29, 32 Melphalan 9 mg/m^2 PO d 1–4 Prednisone 60 mg/m^2 PO d 1–4 d 42 d X 4 cycles followed by Bortezomib 1.0/1.3 mg/m^2 d 1, 8, 15, 22 Melphalan 9 mg/m^2 PO d 1–4 Prednisone 60 mg/m^2 PO d 1–4 q 35 d X 5 cycles	No MTD defined CR PR 18 mo. EFS 85% 18 mo. PFS 93%	 30% 56%	*AE* $\geqslant$ °3: Myelosuppression, peripheral neuropathy, infection, and diarrhoea

(*continued on next page*)

Table 5 – *continued*

Reference	Patients	Schedule	Result		Comment
Badros ASH (2005)[81]	n = 12	Bortezomib 0.7/1.0/1.3 mg/m^2 d 1, 4, 8 Dexamethasone 40 mg/d × 4 d Thalidomide 100–200 mg/d × 4 d Cisplatinum 10 mg/m^2 IVCI × 4 d Adriamycin 10 mg/m^2 IVCI × 4 d Cyclophosphamide 400 mg/m^2 IVCI × 4 d Etoposide 40 mg/m^2 IVCI × 4 d q 35 d	CR PR	1/12 9/12	Rapid response favourable in comparison with DT-PACE historical data Adequate stem cell mobilisation and timely engraftment post-transplant
Barlogie ASH(2005)[82]	n = 162 (156 completed induction)	2 cycles VDT-PACE (PBSC collection after the 1st cycle) Melphalan 200 mg/m^2-based tandem transplants with peritransplant T + D 2 consolidation cycles with VDT-PACE 1 year maintenance with VTD 2 years maintenance with T + D	nCR at 12 mo. 81%		TT3: Tandem transplants faster than in TT2 More patients completed tandem transplants 12 mo. treatment-related mortality 4% (vs. 6% with TT2 + thalidomide) Too early to assess CR-rate and 2-year

IVP, intravenous push injection; PBSC, peripheral blood stem cells; IVCI, intravenous continuous infusion; LMW, low molecular weight; SCT, stem cell transplantation.

8. Adverse events in completed clinical trials on bortezomib ± dexamethasone in relapsed multiple myeloma

Grade 3 adverse events (AEs) were reported in 61% of the APEX patients. The most common grade 3 AE in completed phase II/III trials were thrombocytopenia (13–29%), fatigue (5–12%), peripheral neuropathy (4–15%), weakness (4–11%), and neutropenia (11–30%) (Table 6). 14% of patients in the SUMMIT and APEX trials developed grade 4 AEs, mainly thrombocytopenia, neutropenia, and diarrhoea.[85] Adverse events were the primary cause of premature study discontinuation in 22% of the SUMMIT patients (in 18% considered to be drug related) and 37% of the APEX patients with peripheral neuropathy being the most frequent AE requiring treatment discontinuation.

The mean platelet count decreased by approximately 60% during each treatment cycle and recovered to baseline during the rest period. Less than 10% of patients with baseline platelet counts $\geqslant 70 \times 10^9$/L developed grade 4 thrombocytopenia in phase II studies.[58,59] Among responders, the platelet count increased significantly during subsequent treatment cycles. Preclinical data are compatible with a temporary, reversible impairment of megakaryocytic function rather than megakaryocyte cytotoxicity or thrombopoietin deficiency.[87]

The overall incidence of peripheral neuropathy in the phase II studies was 35%; 13% and <1% of patients developed grade 3 and 4 peripheral neuropathy, respectively. Five percent of patients discontinued bortezomib therapy due to peripheral neuropathy.[58,59,88] Of note, most of the patients who experienced peripheral neuropathy following bortezomib had previously received neurotoxic therapy and 81% had symptoms of peripheral neuropathy at baseline. The incidence of grade 3 peripheral neuropathy was 16% among patients with peripheral neuropathy at baseline compared with 3% in patients without peripheral neuropathy at baseline.[88] Peripheral neuropathy associated with bortezomib treatment required dose modification and/or treatment interruption, and was to a large degree reversible. In phase II studies, peripheral neuropathy improved or resolved in 71% of patients who developed significant symptoms, with a median time to resolution of 47 days from the end of treatment.[88]

Significant renal, hepatic and cardiac toxicity is rare with bortezomib. Overall, the incidence of cardiac events during treatment with bortezomib was 15% with no particular cardiac disorder accounting for an incidence of more than 10%.[85] Rarely, tumour lysis syndrome[68,89,90] and bilateral hearing loss have been described in association with bortezomib treatment.[91] From a distinct ethnical entity of Japanese patients a high incidence of serious lung injury of unknown etiology has been reported.[92]

In the CREST trial the incidences of diarrhea, nausea and peripheral neuropathy emerged to be dose-dependent with lower frequencies in the 1.0 mg/m^2 group while fatigue, constipation and thrombocytopenia occurred at a similar rate in both dose groups. Arthralgia and peripheral oedema were more common in the lower-dose group.

Table 6 – Grade 3 and grade 4 adverse events in completed trials on bortezomib alone or in combination with dexamethasone

Trial First Author	SUMMIT Richardson, 2003		CREST Jagannath, 2004				APEX Richardson, 2005		Bortezomib/Dexamethasone Kropff, 2005	
Patients	Relapsed or refractory		Relapse during/following front-line therapy				1–3 previous therapies		$\geqslant 2^{nd}$ relapse; irrespective of pre-treatment peripheral blood counts	
No. of patients evaluable	202		27		53 26		315		30	
	Grade 3 (%)	Grade 4 (%)	Grade 3 (%)	Grade 4 (%)	Grade 3 (%)	Grade 4 (%)	Grade 3 (%)	Grade 4 (%)	Grade 3 (%)	Grade 4 (%)
Diarrhoea	7	1					7	0	0	0
Nausea	6	0					2	0	0	0
Fatigue	12	0					5	<1	10	0
Constipation	2	0					2	0	0	0
Peripheral neuropathy	12	0	4	4	15	0	7	1	7	0
Vomiting	8	<1					3	0	0	0
Pyrexia	4	0					2	0		
Thrombocytopenia	28	3	29	0	19	4	26	4	13	33
Anemia	8	0					9	1	17	10
Headache	3	0					1	0		
Anorexia	2	0					3	0		
Paresthesia							2	0		
Dyspnea							5	<1		
Neutropenia	11	3	11	0	23	0	12	2	30*	3
Rash	<1	0					1	0		
Insomnia							<1	0		
Abdominal pain							2	0		
Bone pain							4	0		
Pain in limb	7	0	11	0	8	0	2	0		
Muscle cramp							1	0		
Dizziness	1	0								
Weakness	11	5	4	0	12	0				
Pneumonia (NOS)			0	0	15	0				
Hyponetraemia			11	0	8	0				
Infection									2	0
Herpes zoster									1	0

9. Summary and future prospects

The ubiquitin-proteasome pathway is the major intracellular pathway for the degradation of proteins, many of which are essential for proliferation of malignant cells. Preclinical studies have demonstrated remarkable antitumour activity of proteasome inhibitors *in vitro* as well as in animal models. Bortezomib synergizes with various established antitumour agents thus overcoming many forms of drug resistance. Clinical experience from the SUMMIT phase II study demonstrated significant activity in relapsed and refractory MM with considerable but manageable toxicities. The CREST trial indicates that treatment-related side effects can be reduced by a dose reduction while retaining efficacy. Addition of DEX to bortezomib for patients not adequately responding to bortezomib therapy alone increased response rates on both dose levels and in primary treatment as well as in relapsed myeloma but does not appear to prolong progression-free survival (PFS) or OS. Renal function does not seem to have a major impact on response rate to bortezomib or its toxicity.

Early phase I and II data demonstrate remarkable activity of bortezomib in combination with anthracyclines, melphalan, cyclophosphamide, and thalidomide. Enhanced efficacy from combinations may open the way to dose limitations of bortezomib by this way preventing premature treatment discontinuations.[79] In this context and taking into account that treatment emergent neuropathy is the clinically most relevant adverse event with limited options for supportive care, the impact of vinca alkaloids in myeloma treatment should once more be critically questioned.

Currently available proteasome inhibitors are comparably unselective because they interact with active sites of the 20S particle. Thus, they affect a wide spectrum of proteins with diverse functions instead of targeting specific cellular proteins or associated functions. However, active sites in the 19S regulatory cap of the proteasome or substrate-specific E3 enzymes of the ubiquitin conjugation cascade might theoretically be more preferable targets for a selective and specific inhibition of the proteasome's activity. As long as specific inhibitors of proteasome functions are not available for *in vitro* testing, preclinical development for the near future focuses on the development of second generation, orally available proteasome inhibitors with improved toxicity profiles.[93] Moreover, data from pharmacogenomic studies will help to understand mechanisms or primary and acquired bortezomib resistance and guide the way to improved drug combinations which hold the potential to overcome resistance to single agents.

Conflict of interest statement

M. Kropff has received a research grant from ORTHO BIOTECH and research funding for participation in the APEX and VISTA clinical trials. He also received speaker's honoraria from ORTHO BIOTECH and Millennium.

W.E. Berdel is a member of an advisory board for ORTHO BIOTECH.

J. Kienast received speaker's honoraria from ORTHO BIOTECH.

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