SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Velcade. This scientific discussion has been updated until 1 May 2004. For information on changes after this date please refer to module 8.

1. Introduction

Multiple myeloma

Multiple myeloma is a B-cell malignancy of the plasma cell and represents the second most common hematological malignancy, with non-Hodgkin's lymphoma being the most common. The incidence and prevalence of multiple myeloma are similar in the US and Europe. In 2000, there were approximately 19,000 cases diagnosed in the European Economic Area (Globocan, 2000), with a 5-year prevalence of approximately 47,000 cases.

The course of multiple myeloma is characterized by an asymptomatic or subclinical phase before diagnosis (possibly for several years), a chronic phase lasting several years, and an aggressive terminal phase. Multiple myeloma leads to progressive morbidity and eventual mortality by lowering resistance to infection and causing significant skeletal destruction (with bone pain, pathological fractures, and hypercalcemia), anemia, renal failure, and, less commonly, neurological complications and hyperviscosity. From the time of diagnosis, the survival without treatment is between 6 to 12 months and extends to 3 years with chemotherapy. Approximately 25% of patients survive 5 years or longer, with fewer than 5% surviving longer than 10 years.

Approved anticancer agents for the treatment of myeloma in the US include melphalan (1992), carmustine (BCNU, 1977) and cyclophosphamide (1959) and in addition, in Europe, epirubicin is also approved for treatment of this disease.

At the time of diagnosis, multiple myeloma is a heterogeneous disease, with a course that varies on the basis of both disease- and host-related factors (e.g., age, renal function, stage, alpha2- microglobulin, chromosomal abnormalities). Most patients with myeloma receive multiple treatments over the course of their disease, and the precise sequence of therapy and regimens used can be quite variable.

Spontaneous remissions do not occur in multiple myeloma and no placebo effect on response has been noted. Standard therapy for myeloma currently consists of 4 classes of agents: corticosteroids, alkylating agents, anthracyclines, and more recently investigational thalidomide. A fifth treatment class available to patients under the age of 65 years is high-dose chemotherapy and bone marrow transplantation.

Patients have a variable response to conventional treatments after their initial therapy, but the probability of response and the duration of response decrease with each successive treatment. Eventually, all patients will become refractory to therapy. This transition to the refractory state may occur after 1 treatment or after many. Regardless if it occurs in the first year of treatment or after many years, the occurrence of progression on therapy (refractory) conveys biological homogeneity characterized by a short terminal phase. These patients are typically offered either palliative care or experimental agents. Their prognosis is poor and they represent a group of patients with few treatment options and short survival (6 to 9 months).

Bortezomib

Bortezomib is a novel cytotoxic chemical entity that potently and specifically inhibits the proteolytic activity of the proteasome and thus the degradation of poly-ubiquitinated proteins destined for catalysis by the proteasome. Bortezomib is a modified dipeptidyl boronic acid derived from leucine and phenylalanine. The chemical name for bortezomib, the monomeric boronic acid, is [(1R)-3-methyl-1-[[(2S)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl)amino] propyl]amino]butyl]boronic acid. Bortezomib is the first anti-neoplastic proteasome inhibitor.

The following mechanisms of action are thought to be relevant to the effect of bortezomib in multiple

myeloma:

- Induction of apoptosis of multiple myeloma cells.
- Inhibition of activation of nuclear factor-κB (NF-κB) in multiple myeloma cells and in tumour microenvironment.
- Reduction of adherence of myeloma cells to bone marrow stromal cells.
- Blocks production and intracellular signaling of interleukin-6 (IL-6).
- Blocks production of angiogenic factors.
- Overcomes defects in apoptotic regulators, such as B-cell lymphoma-2 (Bcl-2) overexpression and alterations in tumour suppressor protein p53.
- Unaffected by drug efflux pumps.
- Activity is cell-cycle independent.

Bortezomib (PS-341) has shown cytotoxic activity against a variety of multiple myeloma cell lines and in freshly isolated cells from patients. Bortezomib is a selective and reversible inhibitor of the 26S proteasome holoenzyme. It affects the degradation of poly-ubiquitinated proteins destined for catalysis by the proteasome. Over 80% of all cellular proteins are processed by the 26S proteasome complex. There are multiple functions of the proteasome in the control of protein degradation, particularly of regulatory proteins controlling cell-cycle and apoptosis, such as cyclins, p53, p27, inhibitor protein kappa B (I□B) and nuclear factor kappa B (NF□B). The proteasome 26S complex is a large, multiprotein particle present in both the cytoplasm and the nucleus of all eukaryotic cells. It is composed of two functional components: a 20S core catalytic complex and the 19S regulatory subunit (for reviews see Adams, 2002; Adams, 2003; Ben-Neriah, 2002). Proteins aimed to be degraded are marked with ubiquitin chains, which bind to a receptor on the 19S complex. Ubiquitin is a small protein of 76 amino acids and it serves as a tag that marks proteins for degradation by the proteasome. The tagging of a substrate is exquisitely regulated by a multi-enzyme system. Once recognized by the regulatory complex, the ubiquitin chain is removed and the protein denaturated for degradation. The protease activity resides in a channel at the center of the 20S complex. Inside the catalytic chamber proteins are surrounded by six protease-active sites. Within the 20S core are three proteolytic activities: a) trypsin-like, b) post-glutamyl hydrolyzing activity, and c) chymotrypsin-like. Bortezomib inhibits specifically the chymotrypsin-like activity, but this inhibition is sufficient to block all proteasomal catalytic activity. The proteasome protease functions similarly to serine proteases, but is unique, since it relies on a threonine residue in the active site. Proteins processed by proteasome are reduced to small polypeptides 3 to 22 residues in length.

Quality aspects

Introduction

Velcade is a sterile, lyophilized formulation in a single-use 10ml glass vial with bromobutyl stopper, containing 3.5mg of bortezomib with mannitol as bulking agent, in an atmosphere of nitrogen.

When reconstituted with 3.5ml 0.9 % Sodium Chloride for Injection, each ml contains 1mg bortezomib and 10mg mannitol.

Drug Substance

- Manufacture

Bortezomib is a trimeric anhydride of a dipeptide of a boronic amino acid prepared by synthesis. During lyophilisation performed for preparing the drug product, the active ingredient forms a diester with mannitol used as excipient. From this ester, the active boronic acid is obtained by reconstitution of the drug product in saline solution for injection. In water, an equilibrium between the boronic ester and acid is present. The terms bortezomib and drug substance are used loosely in this report to indicate any of the above species, depending on the relevant context.

Four processes for the manufacture of bortezomib have been used during clinical development. These processes have been named as Processes A, B, C, and D. The drug substance synthesis uses standard peptide chemistry, involving a sequence of coupling, deprotection, coupling, and deprotection steps. Process D, the commercial manufacturing process, includes 4 steps and a final recrystallisation.

It is a chiral molecule arising from the starting materials (e.g. a chirally-defined boronate and the L-amino acids leucine and phenylalanine), and the structure has been well characterised.
In particular, ^{1}H and ^{13}C NMR spectroscopy studies have been performed, including NOESY (Nuclear Overhauser Effect Spectroscopy) experiments.

- Specification

The specification includes validated tests and limits for appearance (visual), ID (IR, HPLC), Assay (% anhydrous basis), impurities (HPLC), chiral purity (HPLC), resdual solvents (GC), moisture (Karl Fischer), specific rotation, heavy metals, bacterial endotoxins and bioburden (PhEur where relevant).

Batch analysis results indicate that the final process D is under control and results in consistent quality, and final specifications are based solely on data from process D.

Impurities
A number of impurities, including chiral impurities, arising from the synthesis of bortezomib can be present in the active ingredient and limits have been included in the specification. Stereoisomers include an enantiomer and two diastereomers. They are derived from the coupling of the stereoisomers of the starting materials.

Furthermore, several degradation products have been reported as possible contaminants in the specification. Oxidation is the primary degradative pathway observed for bortezomib drug substance, and a total of fourteen distinct impurities have been identified.

Qualification of impurities is considered to be performed correctly using batches prepared for toxicological studies. These batches contained the same levels of impurities as those contained in subsequent lots and in commercial batches.
A complete batch analysis has been included for batches prepared with process C and D and these data indicate clear improvement in the quality of the batches obtained from process D in comparison to the one from process C.During development the initial specifications for assay and structurally related impurities have been revised and lower limits introduced in line with the most recent batch analyses. The applicant also commits to re-evaluate specifications once additional data from commercial lots are available.

- Stability

Stress testing and accelerated stability studies have shown the drug substance to be sensitive to all the usual factors tested; it appears to be intrinsically unstable. Accordingly, storage under refrigeration and protection from light is needed. Data are presented to support the retest period of nine months required with a labeled storage condition of -20°C ± 5°C, protected from light. These results are concluded valid for stability evaluation, and allow concluding the proposed retest period acceptable.

Drug Product

- Pharmaceutical Development

A lyophilized dosage form was developed to overcome the stability problems encountered with a liquid formulation used in the early clinical studies and to allow rapid reconstitution in 0.9% saline to be achieved. Mannitol, 1% (w/v), was selected due to its reconstitution properties and enhanced stability when freeze-dried with bortezomib.
Mannitol is the only excipient cited in the composition. Water for Injection and *tertiary*-Butyl Alcohol are co-solvents used in the compounding process, which are removed during lyophilization. There are no overages in the commercial formulation of bortezomib drug product.
The lyophilized product is the 1:1 mannitol boronic ester and after reconstitution consists of an equilibrium between the mannitol ester and boronic acid. NMR and mass spectrometry have effected the structural characterization of the lyophilized product.

A complete characterization of the boronic ester:boronic acid equilibrium in the reconstituted product has been performed and it has been concluded that the equilibrium is established in less than ten minutes after dissolution, and that the equilibrium remains unchanged over a 12-hour period.

The drug product manufacturing process has been developed passing from process I (preparation of a liquid dosage form) to a classical lyophilization process (II to V). Process V is the presently proposed commercial manufacturing process.

The sterilization method using filtration through a microbically retentive filter and aseptic processing is justified by the lyophilized dosage form which cannot be terminally sterilized by moist heat and the physico-chemical properties of the drug product that melts at the temperatures required for dry heat sterilization and is photosensitive. Furthermore, Gamma irradiation has been shown to significantly degrade the drug product, as expected.

The suitability of the container closure system is demonstrated by the stability studies. The secondary packaging, which includes a paperboard carton provides protection from light exposure, as demonstrated in photostability studies. The integrity of the container closure system has been demonstrated.

- Manufacture of the Product

The manufacture of the formulation is a standard procedure entailing compounding of the solution of mannitol and drug substance in Water for Injections. The final solution is sampled and tested for appearance, UV assay, density, and bioburden. The compounded solution is then filtered aseptically. Once filtration is initiated, the filtrate is sampled and tested for appearance, UV assay, and density. Appropriate filter testing is performed.

Critical manufacturing parameters have been identified.and are controlled in line with the relevant Guideline.

The overall manufacturing process is considered a non-standard process (aseptic filtration connected to lyophilisation), and has been validated in line with relevant EU guidelines.

- Product Specification

The drug product is tested for appearance (visual), ID (IR, HPLC), assay (% label claim), impurities (HPLC), moisture (Karl Fischer), Residual *t*-Butanol (GC), content uniformity (HPLC), sterility, Bacterial Endotoxins,. Additional for the reconstituted vial: reconstitution time (visual), colour/clarity of solution (visual, EP), particulate matter, pH . Methods are adequately validated, and PhEur methods applied where relevant.

- **Stability of the Product**

Data are available for more than 12 months data at long term storage condition for three primary lots as well as 9 months for further two primary lots. In addition, 6-month data at accelerated conditions (40°C/75% RH) are available for all batches. Studies were carried out under ICH conditions and the results are considered adequate to support the proposed shelf life and storage conditions as defined in the SPC.

The compatibility of Velcade with the recommended reconstitution diluent, 0.9%w/v sodium chloride solution for injection, has been demonstrated in reconstitution stability studies, and compatibility with common dosage administration devices (e.g. syringes) has also been demonstrated

- **Discussion on chemical, pharmaceutical and biological aspects**

Bortezomib is a trimeric anhydride of a dipeptide of a boronic amino acid prepared by synthesis using standard peptide chemistry. The different molecular forms of the active substance have been well characterised, and the manufacture and control have been shown to deliver a substance of satisfactory and reproducible quality. Because of an inherent instability and photosensitivity, it must be stored under refrigeration.

A thorough discussion has taken place on identification and quantification of impurities as well as their origin and reflection in the specifications.

The applicant has committed to further investigate analysis of impurities when more experience has been gained with commercial lots.

Since the overall manufacturing process of the finished product is considered a non-standard process (aseptic filtration connected to lyophilisation), therefore, full process validation data have been requested from three consecutive batches at production scale.

The manufacturing process for the product has been validated and batch analytical results indicate a product of reliable uniformity at the point of manufacture. Stability studies have been planned taking into account the known instability of the active substance, but the formulated product has been shown to be more stable, both in the dry state and after reconstitution.

At the time of the CPMP Opinion, a number of minor quality issues were not resolved, and the applicant gave a commitment to resolve these afterwards by means of Follow Up Measures.

3. Toxico-pharmacological aspects

Pharmacodynamics

- Primary pharmacodynamics

Available information on the pharmacodynamics of bortezomib was derived from studies conducted by the applicant, and supportive data from the literature.

The ubiquitin-proteasome pathway (UPP) plays an essential role in the maintenance of homeostasis in cells and organisms. The 26S proteasome plays an essential role in the UPP by acting as the final proteolytic effector of cellular proteins targeted by polyubiquitination for destruction. The 26S proteasome contains a 20S catalytic core, composed of four stacked seven-membered rings (De Martino et al.1999). Bortezomib is a selective and reversible inhibitor of the proteasome 20S activity (Adams et al 1998). The boronic acid moiety of bortezomib is thought to form a stable tetrahedral complex with the active site N-terminal threonine residue of the 20S β 5 subunit. The applicant has investigated the reversibility of this binding. The K_{off} (rate constant for dissociation of inhibitor from enzyme-inhibitor complex) was approximately 6 x 10^4/sec with a t½ of 20 minutes. Bortezomib does not apparently interact with usual surface and steroid hormone receptors.

The proteasome exists in mammalian cells in a number of isoforms. Studies presented by the applicant showed that isoform composition did not influence cell sensitivity to proteasome inhibition.
Numerous published reports analysed the bortezomib *in vitro* system effects, and demonstrated activity in the concentration range of 0.1 and 30nM, with cancer cells being more sensitive to the pro apoptotic effects of proteasome inhibition than normal cells (Adam et al. 1999, Hideshima et al.2001, Ma et al. in press, Soengas et al.2002, Singhal et al. 1999, Barlogie et al 2001). *In vitro* pharmacodynamics studies conducted by the applicant showed that bortezomib did not appear to be a substrate of any of the three efflux pumps involved in multi-drug resistance (MDR). Resistant cells generated from two MM cell lines (RPMI and MM1) showed a 300-1000 fold resistance to bortezomib, and closely related boronate analogs. No over expression of PgP (P-glycoprotein), MRP3 and MRP5 (MDR, associated proteins) was found, suggesting that an alternative mechanism confers resistance to bortezomib. This *in vitro* phenotype was not sustained *in vivo* in a xenograft model. The cells did not lose their *in vitro* resistance by being passed through the animals. In another study, analysis of expression of selected UPP in human solid tumours showed that the representative subset of UPP genes includes 4 proteasome subunits, 5 conjugating enzymes, and 4 U ligases. Genes encoding every component examined were elevated in one or more tumour types.

In all examined *in vivo* models using human tumour cell line xenografts, antitumour activity [tumour volume (TV) and delay in tumour growth (DTG), and apoptosis] and 20S proteasome inhibition have been observed at the higher dose used (1mg/kg), corresponding to 3mg/m^2, which is near the MTD. In a study in LLC mice using bortezomib i.v..,4 days after implantation 0.3, 0.6 and 1.0mg/kg (0.9, 1.8 and 3mg/m^2) 2 x week for two weeks, the proteasome inhibition was higher in whole blood (WB) compared with liver tissue. The low levels of bortezomib in tumour tissue was nevertheless sufficient to inhibit proteasome and reduced TV. A greater effect on TV was shown after combined administration with other chemotherapeutics, which do not seem to influence the intrinsic activity of bortezomib on the 20S proteasome.

Bortezomib was investigated to exclude the potential for a negative response in MM patients who subsequently undergo autologous stem cell transplantation. Bortezomib treatment of mice did not affect the ability of hematopoietic stem cell to form colonies *in vitro*. Cells from bortezomib treated animals were functional in a bone marrow transplant to completely repopulate peripheral blood cells in lethality irradiation recipient mice.

A series of impurities (A, E, F, G, H, I, K, J) structurally similar to bortezomib, occurred at level greater than 0.1% (in any batches of the bortezomib drug substance) were synthesised and analysed in 20S proteasome assay, and the second order rate constant inhibition ($K_{ob}s$/[I]) was used as an approximation of binding affinity, in comparison with bortezomib. Both the non-boronate compounds (impurity K and A) had undetectable activity. Of the boronate compounds, all but two had $K_{ob}s$/[I]over 200 fold lower than bortezomib, making them essentially inactive (impurities I,G,H,E). Two boronate tripeptides impurities F and J, however had a $K_{ob}s$/[I] values 3-4 fold greater than bortezomib, so they were analysed in a more detailed way in order to identify the inhibition K_os and K_{off} and the dissociation constant Ki. For impurities F and J, Ki values were 0.23 and 0.18nM respectively; thus 3-4 fold more potent as inhibitors than PS-341 showing in this study a Ki of 0.69nM. These impurities are present in lots of the drug substance at levels of 1%, thus their contribution to the overall activity of the drug would not seem significantly relevant. In any case, a representative PS-341 preparation (98% pure) was directly compared in the 20S assay with a highly purified bortezomib (>99% pure) sample, and plotting the K_{obs} versus [I] showed identical lines, indicating the same affinity for the 20S proteasome. Moreover, the potency too seems the same, since the Ki values for the two preparations were 0.60 and 0.74nM.

- Safety pharmacology

Only cardiovascular effects were evaluated in 3 non-GLP studies using telemetered (2 studies) and unconscious (1 study) Cynomolgus monkeys (1-2/groups) treated with doses ranging between 2.4-3.6mg/m^2. In all studies, the animals poorly tolerated the high doses used. At acutely lethal single doses bortezomib induced hypotension and initial tachycardia followed by progressive hypotension, bradycardia, hypothermia and eventual death.especially in the first hours post dose. Mechanistic studies were performed to investigate the hypotensive effects of bortezomib, indicating that bortezomib promotes robust expression of PGE_2 and PGI_2 associated with induction of Cox-2, but not Cox-1, mRNA and protein.

- Pharmacodynamic drug interactions

In vitro on MM cells, bortezomib increased the sensitivity of chemo resistant MM cells to melphalan, doxorubicin, and mitoxantrone. *In vivo* in LLC model, oral and i.p. bortezomib administration in combination with 5-FU, adriamycin, cisplatin, and Taxol indicated that greater reduction in TV was observed when drugs were co-administered, and combination treatments had no effects on proteasome activity and did not alter the inhibition of 20S by bortezomib.

Pharmacokinetics

The non-clinical PK, distribution, metabolism, and excretion properties of bortezomib were determined in rats and cynomolgus monkeys. Toxicokinetic (TK) studies were conducted in rats, rabbits and cynomolgus monkeys.

- Absorption- Bioavailability

Bortezomib is intended only for intravenous administration in MM patients. Therefore, the applicant has not conducted pharmacokinetic studies elucidating absorption of bortezomib.

- Distribution

After single i.v. administration to rats and cynomolgus monkeys, plasma bortezomib concentrations declined in a bi-phasic manner with rapid distribution phase which was followed by a longer terminal elimination phase. The elimination half-time in monkeys was 8 to 10 hours. The AUC increased in a dose-dependent manner over the dose range of 0.05mg/kg to 0.20mg/kg in rats and from 0.05 to 0.10mg/kg in monkeys. After multiple doses, the elimination half-life increased in monkeys by 3 to 4-fold that paralleled by decrease in total body clearance resulting in several fold increase in AUC.
The Vd is large (30l/kg at week 38 in monkeys and 600l at week 2 in humans). There is a trend to a decreased Cl and accumulation with repeated doses. Bortezomib is bound 70-85% to plasma proteins. In all species, bortezomib concentrations are higher in plasma than in RBC (by 43%, 15% and 12% in rats, monkeys and humans, respectively). Bortezomib is rapidly distributed into most tissues. The tissue/plasma concentration ratio in most tissues was >1, indicating extensive movement of [^{14}C]-bortezomib-derived radioactivity from the vascular compartment into the tissues, mostly in the liver, adrenals and kidney. Important exceptions are brain, testis and some parts of eye and optic nerve. At 144 hours post dose, radioactivity is almost completely eliminated in liver and kidney, but reaches its highest concentrations in the lymph nodes, spleen and thymus. In the brain of monkeys, at 144 hours the level was 0.08 g eq., and the CNS/blood concentration ratio was <1 (0.77).

- Metabolism

The metabolism of bortezomib was investigated in laboratory animals and in man. The metabolite profiles between rat, monkey and man are very similar. Over 30 metabolites were identified, deboronated metabolites M1 and M2 accounting over 90% of the drug metabolism. The deboronated metabolites were shown not be pharmacologically active. M1 and M2 were subsequently hydroxylated to form M5, M6 and M8, or dealkylated to form M3 followed by deamination to M4. Bile collected from bortezomib-treated monkeys did not elicit inhibitory activity against the 20S proteasome. The major part of the metabolism is accounted by CYP3A4 and CYP2C19. Bortezomib itself was a poor inhibitor of human recombinantly expressed CYP enzymes 1A2, 2C9, 2C19, 2D6 and 3A4.

- Excretion

A preliminary partial comparative biotransformation pathway has been created using hepatic microsomes. Biliary excretion is the major route of elimination in the rat, with about 39% and 21% of the administered radioactivity excreted in the faeces and urine respectively. The elimination is slow in rats and monkeys: 20-30% of the administered radioactivity was found in the carcass after 72 and 144 hours. The excretion pattern seems to be different in rodents and in monkeys, being mainly biliary in rats and both biliary and urinary in monkeys. It is not clear whether human elimination resembles the rat or the monkey.

Toxicology

- Single dose toxicity

Studies were conducted in mice, rats, dogs and monkeys with bortezomib administered i.v.., intraperitoneally (i.p.) and orally. In the majority of the studies, bortezomib was dissolved in dimethyl sulfoxide (DMSO). Following i.v.. administration (bolus or infusion), the acute MTD is 0.6mg/m^2 for rats, and 1.2mg/m^2 for monkeys, corresponding to 0.46 and 0.92 times the proposed human dose (1.3mg/m^2). The dog was the species better tolerating the bortezomib with an MTD of 3.6mg/m^2. No substantial different effects were observed after various routes of administration. No information on exposure is available, since in rats plasma and blood levels were only measurable at the higher dose used. The single treatment does not induce microscopic findings in rats and monkeys. In this species however, microscopic lesions were observed in animals in moribund conditions, probably due to drug toxicity, and included pulmonary venous embolism and congestion, lymphoid necrosis of the spleen and lymphnodes, focal myocardial degeneration, focal hepatic necrosis and skeletal muscle

haemorrhage.

- Repeat dose toxicity

A series of repeated dose studies were performed with different routes of administration in mice, rats, rabbits, dogs and monkeys in order to establish doses and timing of dosage for subsequent main toxicity GLP studies. The main GLP studies were performed in rats for 2 and 26 weeks and in monkeys for 4 and 38 weeks, using only i.v. route.

After repeated administration, the GI tract (associated with emesis and diarrhoea in non rodents) with mucosal hyperplasia, and lymphoid tissues (with decreased lymphocytes counts in non rodent) are target organs of toxicity both in rats and in monkeys. In addition, in rodents decreased platelet counts (sometimes with bone marrow hyperplasia), increased liver weight associated with hypertrophy of hepatocytes and vacuolisation were also observed. In monkeys, severe anaemia and thrombocytopenia associated with bone marrow suppression, kidney tubular degeneration/hypertrophy and/or glomerulopathy, peripheral neuropathy characterized by axonal degeneration of sensory neurons were also observed. In rat studies, the NOAEL has not been determined either after 5 or 9 cycles, although the lowest dose used, 0.05mg/kg (0.3mg/m^2) induced minimal alteration in red blood cells, blood glucose and hepatocellular/vacuolisation hypertrophy, which do not compromise animal conditions. The MTD based on mortality was 0.6mg/m^2, that is 0,46 times the proposed human dose [1.3mg/m^2 associated with a Cmax of 10.7 and 10.9ng/ml (5 and 9 cycles respectively) and AUC $_{0\text{-}24}$ of 145 and 134ng.h/ml (5 and 9 cycles respectively)], and with a mean peak 20S Proteasome inhibition of 80% at weeks 14 and 26. Microscopic changes were found in the spleen (at all doses), in kidneys (MD, HD) in bone marrow, nasal cavity, reproductive tract, thymus and caecum (mainly at HD). In monkeys the NOAEL was not determined after bortezomib i.v.. administration for 13 cycles. The MTD based on mortality was 0.6mg/m^2, that is 0,46 times the proposed human dose (1.3mg/m^2 associated with a Cmax of 48.2ng/ml and AUC $_{0\text{-}24}$ of 83.1ng.h/ml, and with a mean peak 20S Pr inhibition of 76%at week 38. Microscopic changes were found in the GI tract, bone marrow, kidney, PNS and lymphoid organs. In rats and monkeys, 8 weeks were not sufficient for recovery of all alterations.

Genotoxicity *and* carcinogenicity

The genotoxicity of bortezomib was evaluated using the bacterial reverse mutation assay, the *in vitro* chromosomal aberration in CHO and *in vivo* erythrocyte micronucleous assay in mice. Bortezomib was not mutagenic in assay systems using variety strains of Salmonella typhimurium and Escherichia coli. Bortezomib shows *in vitro* clastogenic properties inducing structural *in vitro* chromosomal aberrations in CHO cell assay at concentrations higher than those reached at the MTD. These findings were considered consistent with the intrinsic bortezomib mechanism of action, involving proteasome inhibition. In the *in vivo* test, up to 1mg/kg (3mg/m^2) bortezomib did not induce a significant increase in micronucleated polychromatic erythrocytes in either male or female mice. No carcinogenic studies have been done to assess possible consequences of clastogenicity *in vivo*, in agreement with Note for guidance on the pre-clinical evaluation of anticancer medicinal products (CPMP/SWP/997/96).

- Reproductive and developmental studies

No interspecies comparison of reproductive and developmental toxicity due to different dosing regimen (once daily in animals, twice weekly in humans), and to the unsuitable exposure and PD activity data have been available. NOAEL in rat and rabbit teratology studies are 0.3mg/m^2/day and 0.28mg/m^2/day, respectively, being 0.23 times the proposed twice weekly human dose (1.3mg/m^2). Bortezomib caused developmental toxicity in neither species at the highest maternally tolerated dosages. No evaluation of effects on fertility or peri- and postnatal development is available. The 6-month rat study showed testicular seminiferous tubule degeneration in males at the highest dosage (0.20/0.15mg/kg), and ovarian luteal cell necrosis in females at all dosages (≥0.05mg/kg). Therefore, bortezomib is likely to have a potential effect on fertility. No studies were performed to investigate the absorption across placenta and in lactating animals.

- Local tolerance

A study was performed to investigate the potential venous and tissue irritancy of bortezomib following

a single injection i.v.., perivascular (p.v.) subcutaneous (s.c.), intraperitoneally (i.p.) and intramuscular (i.m.) route of 0.1mg/kg (1.1mg/m^2) in New Zealand male rabbits, at the same dosing concentration (1mg/ml) used in clinical testing. An additional group of rabbits received the bortezomib excipient D-mannitol reconstituted with 0.9% Sodium Chloride for injection USP. D-mannitol i.m., and bortezomib i.v.. and i.m. administrations induced erythema and oedema (reaction of grade 0-3). Microscopic changes with p.v. administration were characterised by moderate perivascular inflammation, oedema and necrosis of isolate keratinocytes in the stratum spinosum. In addition, after i.m. administration, locally extensive moderate degeneration and necrosis of myofibers were observed. All microscopic alterations were reversible in 72 hours.

- Other toxicity studies

Two studies have been performed to investigate potential immunotoxicity. One study was conducted in BALB/c mice treated twice-weekly i.p. at 0.3 and 0.6mg/kg (0.9 and 1.8mg/m^2) for 18 days. There were no systemic toxicity and immunotoxicological effects, with a NOAEL of 0.6mg/kg (1.8mg/m^2), approximately 38% higher than the intended clinical dosage (1.3mg/m^2). Another study was conducted using an immunization model of experimental allergic encephalomyelitis with SJL/7J 0.5 female mice receiving 2mg/kg (1.5, 6.0mg/m2) for 28 days. No toxic or alterations of exomide parameters were observed at 0.5mg/kg (1,5mg/m^2), which is 15% higher than the proposed human dose.

A toxicological drug interaction study was performed in order to determine possible additive or synergistic effects between bortezomib and other chemotherapeutic agents: 5, fluorouracil (5-FU), Cytoxan or irinotecan. With any combinations of bortezomib with 5-FU, Cytoxan and irinotecan, addictive but not synergistic effects on mortality and body weight loss was observed. In another study with i.v. administration of bortezomib and gemcitabine alone or in combination and to determine possible additive or synergistic effects of co-administration, the toxicity of co-administered bortezomib and Gemcitabine was additive but not synergistic. The MTD for bortezomib in this study was 1-0mg/kg (3.0mg/m^2), approximately 33% of the proposed human dose. Fourteen non-GLP compliant studies conducted in mice and rats early in the development of bortezomib in which mortality and body weight assessment were recorded, indicated additive toxicity with cyclophosphamide, Cytoxan, doxorubicin, irinotecan and Taxol.

A report was submitted discussing proteasome inhibition and the risk for prion disease following publication of two papers [Ma et al., Science 298, 1781-5 and 1785-8, 2002] investigating the role of the ubiquitine-proteasome system in the degradation of prion-related protein (PP). A United States National Cancer Institute expert group had concluded that these papers "raised questions that may not be completely answered, if ever, until there have been many years of post-treatment evaluation of large patient cohorts possibly achievable only with Phase 4 post-marketing accrual." The Cancer Evaluation Treatment Program (CTEP) of the NCI noted that it was prudent to consider investigating the implications of the two papers. They also noted that the available preclinical and clinical data do not suggest that patients treated with proteasome inhibitors are at increased risk of developing human prion disease and that there is no suggestion of a generalized public health hazard associated with the clinical use of bortezomib. Overall, the applicant concluded that prion disease remains a theoretical but thus far unproven risk for patients taking bortezomib, and that concern for this risk is low in people with multiple myeloma and other life-threatening diseases.

An environmental risk assessment has been submitted, from which it results that bortezomib has a octanol-water partition coefficient (Ko) = 100.87, that would suggest some partitioning to soils, but using the "worst case scenario" the $_{PEC surface\ water}$ would be 8.9 x 10^{-5}µg/l, orders of magnitude below 0.01µg/l. Based on the limited quantities expected to be used in Europe, no environmental impact is foreseen for bortezomib.

Discussion on the non-clinical aspects

Pharmacodynamics
Bortezomib showed a selective and reversible interaction with the 20S proteasome. Ki is 0.62nM, about 1400-14000 fold lower than in the Kis measured against a panel of 11 known proteases. The results of *in vitro* and *in vivo* studies show specific anticancer effects and seem to indicate that bortezomib induces anticancer activity with multiple mechanisms of action. *In vitro* inhibition of growth, proliferation, activation of constitutive factors, influence on efflux pump, resistant cells, cytotoxicity, overexpression of UPP genes, and PrP synthesis were reported in the concentration range of 0.1-30nM. An increased dependence on UPP had been considered as a part of the mechanism for enhanced sensitivity of cancer cells to proteasome inhibition.

In xenograft models *in vivo,* reductions in TV, TGD, and effects on tumour survival and proliferation were noted. All effects were achieved at 1mg/kg (3mg/m^2) that is almost the MTD for the model, but is about 5 times higher than the MTD (0.6mg/m^2) in rats and monkeys.

Bortezomib was not expected to have any significant direct secondary pharmacodynamic actions and the results from the multiple receptor screening system are consistent with this view. The primary action is 20S proteasome inhibition but there could be multiple secondary pharmacodynamic actions in cells.

In multi-cycle general toxicity studies conducted in the rat and monkey, the principal target organs included the gastrointestinal tract resulting in vomiting and/or diarrhoea, hematopoietic and lymphatic tissues resulting in peripheral blood cytopenias and lymphoid tissue atrophy and hematopoietic bone marrow hypocellularity, peripheral neuropathy (observed in monkeys, mice and dogs) involving sensory nerve axons, and mild changes in the kidneys. All these target organs have shown partial to full recovery following discontinuation of treatment.

Based on animal studies, the penetration of bortezomib through the blood-brain barrier appears to be limited, if any and the relevance to humans is unknown. However, the neurological dysfunctions reported in dogs and the neurotoxicity reported in humans made the issue about the potential penetration of bortezomib in the nervous system of some concern. The applicant has provided additional reassuring information on the penetration of bortezomib into the CNS but some concerns remain on this topic, which the applicant committed to carefully monitor in man. Studies are ongoing to clarify the mechanism responsible for the peripheral neuropathy observed in monkeys, mice and dogs, believed to be due to a dose-related disruption of the normal homeostasis of some of the cytosolic proteins involved in axonal transport.

Pharmacokinetics
Bortezomib bioavailability appears to be low, regardless of administration route and species. However, the exposures achieved in the chronic toxicity studies were comparable to those obtained in cancer patients.

The conventional ADME studies have been done for distribution, metabolism and elimination, but not for absorption, since specific PK data after single and repeated doses are lacking. TK exposure data are available for rats and monkeys. They appear adequate to determine systemic exposure of bortezomib in toxicity studies, but not to assess its complete PK behaviour, the plasma concentration time curves and the linearity of kinetics.

The plasma profile in rats, monkeys and humans receiving multiple doses (0.9, 1,2 and 1mg/m^2 respectively) are quite similar, with a rapid distribution half-life (about 30 minutes) and a slower elimination phase.

Bortezomib is highly bound (≥80%) to plasma proteins, as determined from both *in vitro* and *in vivo* studies. After labelled bortezomib administration, radioactivity was not detectable in the brain and spinal cord of rats but it has been observed the brain of monkeys at levels equivalent to those seen in fat tissue and higher than in bone marrow. Some concerns remain on the real penetration into brain in animal species used and if there is a quantitative different penetration in rat and monkey brain.

The CYP 450 dependent hepatic metabolism was only investigated *in vitro* inhibition and induction

studies, and no formal drug-drug interactions studies have been conducted. The P450 metabolism is high. All tested CYP, but CYP 2C19, contribute to form M1 and M2 metabolites up to a 76%, while the remaining 24% of bortezomib was hydroxylated with retention of the boron moiety. Bortezomib seems a poor inhibitor and inductor of P450 system enzymes. Phase II pattern does not seem to have a major role in the metabolism of bortezomib. All metabolites seen in pre-clinical animal models have also been seen in humans, in whom M2 is the more abundant metabolite, while M4 is the main metabolite in rats and monkeys.

Tissues and carcass contained about 36 % of radioactivity at 72 hours after administration of bortezomib indicating that large proportion of radioactivity is retained in tissues.

The overall picture of bortezomib PK behaviour, the main differences in animal models, and their relevance in humans are not completely elucidated. This is probably due to the lack of PK data, the high variability of human data and the poor information on hepatic biotransformation (only addressed in rodents and human microsomes).

Toxicology
In all species examined, no substantial different effects were observed after various routes of administration (index of a good absorption and solubility of the product). It was not possible to have information on exposure, since in rats the plasma and blood levels were measurable only at the higher dose used. In monkeys, the TK profiles seems to have very little relationship to dose.

There is a sufficient overlap between findings in pre-clinical models, especially in monkeys, and the most common adverse effects reported in humans (Study M34100-025).

Due to different sensitivity and validation of analytical methods, TK and PK data are not sufficient to allow a final safety margin (SF) calculation based on exposure. In any case, the SF based on AUC are 2.5 and 1.5 fold for rats and monkeys, respectively, while SF based on Cmax are considerably less than 1 (0.08 and 0.038 in rats and monkeys, respectively). PD activity data are limited and calculation of SF is complicated by the fact that the relationship between plasma concentrations and proteasome inhibition is characterized by a Emax model, in which the plateau effect is achieved at inhibition greater than 80%, that is the percentage inhibition reported at the rat and monkey MTD.

The dosage (mg/m^2) remains the only possible determinant of SF, resulting in a SF of less than 1. The MTD values are 0.6mg/m2 for rats and monkeys, compared with proposed human dose of $1.3mg/m^2$ with a resulting SF of 0.46. In addition, bortezomib appears active in xenograft models at doses ($3mg/m^2$) 5 times higher than MTD in rats and monkeys. Although bortezomib should be considered rather toxic, it is to be noted that SF less than 1 is not uncommon for anticancer drugs.

Peripheral neurotoxicity, primarily in sensory neurons, was observed both in rats and monkeys as well as in man. It was classified mainly as axonal degeneration. Studies are ongoing aiming to clarify the mechanism responsible for the peripheral neuropathy observed, believed to be due to a dose-related disruption of the normal homeostasis of some of the cytosolic proteins involved in axonal transport. The applicant has committed to submit the results of these studies as a follow-up measure.

The Applicant has adequately investigated genotoxicity of bortezomib in various in vitro and in vivo models. Bortezomib was positive for clastogenic activity (structural chromosomal aberrations) in the *in vitro* chromosomal aberration assay using Chinese hamster ovary cells at concentrations as low as 3.125µg/ml, which was the lowest concentration evaluated. Bortezomib was not genotoxic when tested in the *in vitro* mutagenicity assay (Ames assay) and *in vivo* micronucleus assay in mice. Currently, the in vivo consequences of clastogenicity are not fully known, and no carcinogenic studies have been performed to assess the possible consequences. Positive genotoxicity results are common for cytotoxic agents, and could be acceptable in the overall evaluation of risk/benefit ratio for a product proposed for MM cancer treatment. The Applicant has not performed any formal carcinogenicity studies, which may be considered acceptable for the intended clinical use of bortezomib.

Developmental toxicity studies in the rat and rabbit have shown embryo-fetal lethality at maternally toxic dosages, but no direct embryo-foetal toxicity below maternally toxic dosages. Fertility studies were not performed but evaluation of reproductive tissues has been performed in the general toxicity studies. In the 6-month rat study, degenerative effects in both the testes and the ovary have been

observed. It is, therefore, likely that bortezomib could have a potential effect on either male or female fertility. Peri- and postnatal development studies were not conducted.

Bortezomib is moderately irritant (at approximately 85% of the proposed human dose) when administered i.v., p.v. and i.m. but not s.c and i.p.

Bortezomib seems devoid of potential adverse interactions on the immune system at doses up to 1.5 and 1.8mg/m^2.

Bortezomib showed additive but not synergistic effects with other anticancer drugs. Therefore, caution is needed if bortezomib is co-administered with these chemotherapeutic agents.
No environmental impact is expected with the use of bortezomib.

4. Clinical aspects

The clinical development program for bortezomib included Phase I dose-escalation studies of both bortezomib monotherapy (either on solid tumours and in haematological malignancies patients), and bortezomib in combination with other chemotherapeutics in solid tumours (e.g., gemcitabine, irinotecan, and docetaxel). The data to support the anti-tumour activity of bortezomib in patients with relapsed and refractory multiple myeloma are derived from two multi-centre, open-label, non-comparative studies (M34100-024, M34100-025). The first study is a Phase II randomised single-agent study of two doses of bortezomib (1,0 or 1,3mg/m^2 IV), the second is a single-agent study testing one dose only (1,3mg/m^2 IV); the administration was identical in both studies: bortezomib was administered to patients twice per week for two weeks (days 1,4,8,11) followed by a 10-day rest period, for a maximum of up to 8 cycles of treatment. Pharmacodynamic (PD) and pharmacokinetic (PK) data are available from these Phase I and II studies using both conventional (intense sampling) and population (sparse sampling) approaches. Sparse pharmacodynamic and limited pharmacokinetic data are also available from two Phase II studies. A Phase III study of bortezomib compared with high dose dexamethasone for patients with relapsed myeloma (1 to 3 prior therapies) (Study M34101-039) and a non-comparative study (Study M34101-040) were ongoing at the time of submission of the marketing authorisation application.

Pharmacokinetics

Two bortezomib formulations have been developed for intravenous bolus administration. Initially, a liquid formulation was used in 17 patients enrolled in the two earliest Phase I trials (Study 98-104 and Study 98-194). This formulation was subsequently replaced in April 1999 with the final lyophilized drug product formulated with mannitol in order to increase stability and solubility when reconstituted in normal saline. This formulation has been used in all subsequent Phase I and II studies, it is currently being used in the Phase III study and it is the intended to-be-marketed formulation. All the pharmacokinetic data have been obtained with the current lyophilized formulation, thus bioequivalence studies have not been conducted.

Single-dose kinetics have been obtained in solid tumour patients during Phase I studies and in multiple myeloma patients in Phase II studies. The initial understanding of multiple-dose pharmacokinetics of bortezomib is derived from a study in patients with solid tumours receiving the drug in combination with gemcitabine. An increase in the AUC (approximately 2-fold) was observed following the third dose of bortezomib as compared to the first dose. In parallel, clearance decreased by approximately 50% and t½ increased roughly 2-fold. No PK data available over the entire 11-day cycle.

The pharmacokinetic studies have been performed in patients participating phase I or II, receiving bortezomib by i.v. route, by using a validated LC/MS/MS analytical method, with a limit of quantitation of 0.5ng/mL. None of the pharmacokinetic studies presented included sampling at time points between 6 and 24h, thus making impossible an accurate and precise estimate of the terminal half-life and clearance of bortezomib.

Data on pharmacokinetics in special populations, and interaction studies have not been presented. Population pharmacokinetic analysis is being performed in the ongoing Phase III study (M34101-039) in multiple myeloma patients.

Pharmacodynamics

Pharmacodynamic assessment was performed in Phase I studies DM98-194, LCCC9834/00-31, and 98-104 A, and in Phase II studies M34100-025, and M34100-02. Although the pharmacodynamic data are indicative of a biological effect of the drug that is consistently observed in blood 1h after treatment with doses of bortezomib higher than 1.0mg/m^2, it seems that, at least with the assay developed so far to evaluate 20S proteasome activity, the quantitative characterization of the kinetic of recovery and the correlations between pharmacokinetic and pharmacodynamic of bortezomib were not precisely defined. A very large variability of the effect, both in terms of levels of inhibition and in terms of its kinetic of recovery was observed. The control 20S proteasome values before treatment appeared very variable too. This variability was attributed to the variability of the assay. It was claimed that at low levels of inhibition of 20S proteasome activity the variability is very high, whereas when there is a high level of inhibition (i.e. at doses greater than 1.0mg/m^2) the assay were claimed to be robust.

Discussion on clinical pharmacology

Pharmacokinetics
The characterisation of the pharmacokinetics of bortezomib in patients with cancer and in special patient groups is insufficient. Given the toxicity of bortezomib, healthy volunteer studies are not feasible. The analytical assay for the determination of bortezomib, developed and validated by applicant is certainly adequate in terms of precision and accuracy, but the lower limit of quantitation (i.e. 0.5mg/mL) is not low enough for a reliable determination of bortezomib at long-time intervals after i.v. doses of 1.0-1.3mg/m^2. None of the pharmacokinetic studies presented showed an adequate sampling time protocol (i.e. no time points between 6 and 24h), making impossible an accurate and precise estimate of the terminal half-life and clearance of bortezomib.

No data are available to describe the repeated dose pharmacokinetics of bortezomib when used as recommended in the SmPC and as used in the clinical studies (1.3mg/m^2 twice weekly for two weeks, followed by 10-day rest period). The very small treatment groups and the narrow dose-range studied preclude a conclusive assessment of dose-dependence of the pharmacokinetics of bortezomib. Plasma bortezomib vs. time data are only available in 8 patients who participated in the pivotal Phase II trial -025 and received the dose level intended for clinical use. The data show remarkable inter-individual variability.

There are no data on dose-dependency following repeated administration of bortezomib alone. The only available PK data on repeated administration of bortezomib come from study -027 where bortezomib was co-administered with gemcitabine. The possibility of a PK interaction with gemcitabine has not been totally ruled out. More clarification is expected from the population PK analysis of an ongoing study in multiple myeloma.

The observed changes in bortezomib clearance after repeated doses are a matter of concern, as they might require dose and treatment schedule adjustments during the therapy. The mechanisms underlying the observed clearance changes were not investigated and elucidated.

There are no data on the actual routes of elimination and the relative contribution of renal and biliary excretion routes in man. The data are derived from animal studies. Biliary elimination predominated in rats, whereas both urinary and biliary routes were prominent in the cynomolgus monkey.

The effects of gender, age or race on the PK of bortezomib have not been investigated. Bortezomib has not been formally studied in children and in patients with impaired renal or hepatic function. The scarce data on the effect of mild to moderate renal impairment suggest that exposure is higher in these patients compared with those with normal renal function. However, the data are inconclusive. No data are available on the effects of liver impairment, although it is very likely that liver impairment would result in a clinically significant impact. The lack of data in special populations has been adequately reflected in the SPC.

The applicant has committed to conduct further studies to investigate the PK in patients with renal or hepatic impairment compared with patients with normal organ function. As an adjunct to the population PK study nested in ongoing Phase III trial, the applicant has committed to conduct a

clinical PK study in 24 patients with multiple myeloma receiving bortezomib at the doses of 1.0 and 1.3mg/m^2, twice a week for two weeks followed by a rest period. The aim of this study, which is expected to be performed in 2004 and completed by the end of 2005, is to investigate (a) bortezomib PK after single and multiple dose treatment; (b) the mechanisms behind the observed changes in the PK parameters after repeated dosing; (c) any clinically plausible intrinsic factors that may explain and reduce variability; (d) the relationship between body weight/body surface area/lean body weight) and PK parameters since dosing regimen is based on BSA; (e) the concentration-proteasome inhibition relationship using PK/PD-modelling.

In vitro bortezomib is a relatively weak inhibitor of CYP1A2, 2C19, 2D6 and 3A4: this has been confirmed by the fact that newly provided IC50 and Ki for CYP isoenzymes in human liver microsomes are much higher than bortezomib maximal plasma levels. However, clinically relevant interactions cannot be totally excluded. In fact, more than 10-fold differences in C_{max} are observed in the available limited data on single-dose PK. Furthermore, pre-clinical data clearly show that bortezomib has a high Vd and that tissue concentrations are higher than plasma concentrations. Formal interaction studies cannot be carried out in healthy volunteers. The SmPC now includes appropriate general warnings.

The major metabolic pathway, presumably mediated by CYP3A4, leads to the formation of a metabolite with no observed proteasome inhibition properties. This is clearly a risk factor in terms of potential pharmacokinetic interactions. Inhibitors of CYP3A4, such as macrolides, fluconazole, itraconazole, ketoconazole and fluoxetine, which may be frequently used by cancer patients, might increase the risk of toxicity of bortezomib on one hand. On the other hand, CYP3A4 inducers, such as dexamethasone (frequently used in myeloma patients), rifampicin, phenytoin and carbamazepine might lead to loss of efficacy. The lack of reduced activity in patients receiving dexamethasone in combination cannot be taken as a demonstration of the lack of interaction. The drug-drug interaction will be further investigated in PK and PK/PD studies of bortezomib and inhibitors (e.g., ketoconazole) of CYP3A4 and CYP2C19.

Pharmacodynamics

The primary pharmacodynamic effect of bortezomib is proteasome inhibition. The plasma concentration-proteasome inhibition relationship suggests a rapid transition from high level of inhibition (hours 1-6) to low level of inhibition by 24 hours. However, the relationship is based on a small number of patients with available PK/PD data. The dose-proportionality of proteasome inhibition both at peak and at trough together with dose-dependence of exposure to bortezomib within a relatively narrow dose-range suggests that the level of proteasome inhibition could be affected by pharmacokinetic interactions, renal impairment and liver impairment. The clinical relevance of such interferences is not known. The evaluation of proteasome inhibition data in the planned PK study in patients with multiple myeloma, in the PK studies in special patient groups and in the ketoconazole interaction study will hopefully provide some more information.

The results of the Phase II study M34100-025 in myeloma patients receiving bortezomib alone (1.3mg/m^2 on Days 1, 4, 8, and 11 followed by 10-day rest period) or with dexamethasone suggest only a slight accumulation of the peak and trough 20S proteasome-inhibiting activity between the first and the fourth dose of a cycle. The mean peak inhibition was 61% (range 14-97%) at 1 hour after the injection. However, as samples were only collected at pre-dose, 1 hour post-dose, and 24 hours post-dose (in a subset of patients), it cannot be concluded that the peak inhibition values represent true peak inhibition. The quantitative characterization of the kinetic of recovery and the correlations between pharmacokinetics and pharmacodynamics of bortezomib were not precisely defined. In a subset of only 9 patients, the percent inhibition of 20S proteasome activity was decreasing at 24 hours, but nevertheless exceeded 50% in 2 of these patients and it suggests high variability in recovery (from approximately 10% to 60% inhibition remained at 24 hours). It is remarkable that an approximately 20% inhibition, with a high degree of variability, was observed before the first dose of Cycle 7, i.e. after the 10-day rest period. This clearly suggests that recovery of proteasome activity can be very slow. Possible reasons for the high degree of variability, including the low precision of the assay to determine low levels of 20S proteasome activity inhibition, should be discussed. While it is feasible to identify a pattern of gene expression that is related to the sensitivity or resistance to bortezomib, at this stage the results obtained are too preliminary for any realistic clinical application. The pharmacodynamic studies suggest that bortezomib causes a transient partial inhibition of 20S

proteasome activity. The interval of bortezomib doses at which a sufficiently high level of inhibition was observed appears to be between 1.0mg/m^2 and 2.0mg/m^2. Within this interval of doses, the experimental data do not suggest a clear increase in the inhibitory activity. No statistical analysis support the contention of a correlation between bortezomib doses and the inhibition of 20S proteasome activity.

Clinical efficacy

- **Dose response**

The dose of bortezomib selected for Phase II studies in multiple myeloma was 1.3mg/m^2, and the dose regimen was a 3-week treatment cycle consisting of bortezomib administration twice weekly for 2 weeks (Days 1, 4, 8, and 11) followed by a 10-day rest period, for a maximum of up to 8 cycles of treatment. This dose regimen was suggested by pre-clinical and clinical data.

Three Phase I, dose-escalation studies of bortezomib monotherapy have been conducted in patients with advanced malignancies either in solid and non-solid cancers, for a total of 123 patients. In the Phase I single agent studies, bortezomib was evaluated at doses ranging from 0.13 to 2.0mg/m^2 administered as a bolus IV injection either once or twice per week. Additional information on the disposition of bortezomib after repeated dose administration was collected from a Phase 1 study of bortezomib with gemcitabine in patients with solid tumours (M34100-027). Although a novel PD assay (inhibition of 20S proteasome activity) has been developed as a guide to dose-escalation in Phase I studies, the evaluation of the dose-limiting toxicity was based on clinical observation (DLT and MTD).

The study DM98-194, (initiated October 1998) was a Phase I, dose escalation study designed to determine the dose-limiting toxicity (DLT), the maximum tolerated dose (MTD), the pharmacokinetics and pharmacodynamics of bortezomib administered as IV bolus once weekly for 4 consecutive weeks (on Days 1, 8, 15, and 22), followed by a 2-week rest period in patients with advanced solid tumours (primarily prostate cancer patients). The study was conducted at MDACC in Houston, Texas in 53 patients, and provided the first assessment of the pharmacokinetics of bortezomib in 24 patients.

Study 98-104A (initiated February 1999) was a Phase I, single-centre, dose escalation study designed to determine the DLT, MTD, pharmacokinetics and pharmacodynamics of bortezomib administered as IV bolus in 3-week treatment cycles consisting of bortezomib administration twice weekly for two consecutive weeks (on Days 1, 4, 8, and 11) followed by a 10-day rest period in patients with advanced solid tumours. The study was conducted at University of North Carolina at Chapel Hill and Memorial Sloan-Kettering Cancer Center (MSKCC) in 43 patients.

Study LCCC9834/00-31 (initiated November 1999) was a Phase I, two-centre, dose escalation study designed to determine the DLT, MTD, and pharmacodynamics of bortezomib administered as an IV bolus in treatment cycles consisting of bortezomib administration twice weekly for four consecutive weeks (on Days 1, 4, 8, 11, 15, 18, 22, and 25) followed by a 14- to 17-day rest period in patients with advanced haematological malignancies. This study was conducted at MSKCC in 27 patients, and it was the first study conducted in patients with haematologic malignancies, with a total of 13 cases with multiple myeloma or related plasma cell dyscrasia.

DLT was defined as any Grade 4 haematologic toxicity, Grade 4 hyperbilirubinemia, and any ≥ Grade 3 non-haematologic toxicity (with the exception of alopecia and Grade 3 hyperbilirubinemia). By convention, any of the above events were to have occurred during Cycle 1 and be considered by the investigator to be at least possibly related to the study drug in order to be considered a DLT. The intensity of toxicities was assessed according to the National Cancer Institute Common Toxicity Criteria (NCI CTC), version 2.0.

In Studies LCCC9834/00-31 and 98-104A, the MTD was defined as the highest dose studied for which the incidence of DLT was ≤33%. In Study DM98-194, in which the continual reassessment method was used for dose escalation, the MTD was defined as the dose level having a mean posterior DLT probability closest to 25%. In Study DM98-194, in which bortezomib was administered once weekly for four weeks to patients with solid tumours (mostly prostate cancer), DLTs were observed in a total of 6 patients treated at doses ≥1.6mg/m^2. DLTs reported among these 6 patients included Grade

3 diarrhoea (4 patients), Grade 3 hypotension or orthostatic hypotension (2 patients), and Grade 3 tachycardia NOS, vision abnormal NOS, and syncope (1 patient each). The MTD in this study was determined to be 1.6mg/m^2. In Study 98-104A, in which bortezomib was administered twice weekly for 2 weeks to heavily pretreated patients with advanced solid tumours, DLTs were observed in a total of 3 patients treated at a dose of 1.56mg/m^2. DLTs reported for these 3 patients included Grade 3 diarrhoea (3 patients) and Grade 3 peripheral sensory neuropathy (1 patient). Based on these findings, the MTD was established at 1.3mg/m^2.

The MTD was lower, 1.04mg/m^2, when bortezomib was administered twice per week for four weeks to patients with haematologic malignancies, including multiple myeloma (Study LCCC9834/00-31). Utilizing this treatment schedule, DLTs were observed at 1.04, 1.20 and 1.38mg/m^2 and included Grade 3 hyponatremia (4 patients), Grade 3 hypokalemia (2 patients), and Grade 3 malaise (1 patient). No haematologic DLTs (Grade 4) were reported in any Phase I study.

The 3 Phase I studies in monotherapy (DM98-194, 98-104, LCCC 9834/00-31) demonstrated an MTD of bortezomib of 1.6, 1.3 and 1.04mg/m^2, respectively.

Dose limiting toxicities of diarrhoea and peripheral sensory neuropathy were observed at a dose of 1.56mg/m^2 (MTD 1.3mg/m^2) using a regimen identical to that evaluated in Phase II of twice weekly doses for 2 weeks followed by a 10-day rest period. Two of the Phase I studies evaluated the twice-weekly dosing schedule, 1 with dosing over a 2-week period and 1 with dosing over 4 weeks. These studies suggested that the earlier rest period, i.e., after 2 weeks of twice weekly dosing, appeared to be better tolerated.

Evidence of anti-tumour activity in patients with multiple myeloma had been seen in 6 of 10 patients at doses at or above 1.04mg/m^2 in the Phase I study including patients with haematologic malignancies (LCCC 9834/00-31, N. 27). Thus, doses at or above 1.0mg/m^2 were anticipated to be active in patients with multiple myeloma.

Overall, 66 (54%) of the 123 patients enrolled in Phase I studies withdrew prematurely. The most common reason (37%) was progressive disease. The proportion of withdrawals due to disease progression was lower in the >1.3mg/m^2 group (10 of 43 patients; 23%) than in the <0.7mg/m^2 group (12 of 29 patients; 41%) and 0.7 to 1.3mg/m^2 group (24 of 51 patients; 47%). An apparent dose-relationship was noted with regard to the proportion of withdrawals due to adverse events/toxicity (19, 15%), with 1 (3%) of the 29 patients in the <0.7mg/m^2 group, 5 (10%) of the 51 patients in the 0.7 to 1.3mg/m^2 group, and 13 (30%) of the 43 patients in the >1.3mg/m^2 group.

The regimen selected to be further tested in Phase II and Phase III studies (1.0 or 1.3mg/m^2 administration twice weekly for 2 weeks (Days 1, 4, 8, and 11) followed by a 10-day rest period) was consistent with preliminary preclinical data and with results from formal Phase I studies.

The intermittent and high inhibition of 20S proteasome enzyme activity was believed to account for the antiproliferative and apoptotic effect of bortezomib. The return toward pre-treatment level and the complete recovery of proteasome activity between doses and cycle, respectively, were considered important to minimize toxicity in view of the physiological function of this ubiquitous enzyme.

The novel PD assays, that was developed and used as a guide to dose escalation and to the development of dose regimen strategies in MM patients, was not actually used for the evaluation of dose-limiting toxicity for the limitation of clinical pharmacology tests (limited time points collections during therapy and variability of the assay that was reported to be low when there is a high level of inhibition and high when the level of inhibition is low). Traditional clinical DLT and MTD parameters, together with standard evaluation of toxicity/side effects were actually used to define optimum anti-tumour doses and schedule to take forward into Phase II and Phase III trials.

- **Main studies**

The data supporting the anti-tumour activity of bortezomib in patients with relapsed and refractory multiple myeloma come from two multi-centre, open-label, non-comparative studies (M34100-024, M34100-025). Study M34100-024 initiated in May 2001 and was completed in July 2002, while study M34100-025 initiated in January 2001 and was completed in June 2002. The Applicant has conducted

an efficacy Phase III, multi-centre, international, randomised study of bortezomib compared with high dose dexamethasone for patients with relapsed myeloma (1 to 3 prior therapies) (Study M34101-039), which has been terminated early, reportedly because of bortezomib better efficacy; a companion non-comparative study (Study M34101-040) is also ongoing.

Methods

- **Study Participants**

In Study M34100-024, bortezomib was administered at doses of 1.0 and 1.3mg/m^2 given alone or in combination with dexamethasone in case of inadequate response to bortezomib monotherapy, to patients with multiple myeloma who had failed to respond to or had relapsed following either conventional or high-dose front-line therapy. Patients who, in the investigator's opinion, were benefiting from bortezomib treatment in the current study were eligible to continue treatment in an extension study (M34101-029) outside the auspices of this protocol.

In study M34100-025, bortezomib was administered at the dose of 1.3mg/m^2 given alone or in combination with dexamethasone in case of inadequate response to bortezomib monotherapy, to patients with multiple myeloma who had relapsed disease after initial front-line therapy and were refractory to their most recent therapy, whether or not containing systemic corticosteroids.

Patients were heavily pretreated and in resistant relapse (progressing on their most recent therapy) after having received conventional and novel treatments for their disease. More than 99% of patients had received prior treatment with steroids, 92% had received prior alkylating agent therapy, 81% had received prior anthracyclines and 83% had received prior therapy with thalidomide. Among all 202 patients, 98% had received at least 2 of these 4 types of chemotherapeutic agents, 92% had received 3 of these 4 agents, and 66% had received prior therapy with all 4 chemotherapeutics. In addition, 64% of patients had received prior high dose therapy including bone marrow transplant. Nine percent of patients did not meet the entry criteria: 91% of the 202 patients had progression of disease as assessed by the investigator while receiving their last therapy and an additional 4% exhibited transient minimal or partial response with relapse within 3 months or less, a time period generally considered consistent with refractory disease. The most common treatments administered immediately prior to study entry were thalidomide, administered in 45% of patients and combination, or single agent, chemotherapy given in 20%. Evidence of the progressive state of their disease included a median of one month from last therapy to study entry with only 4% of patients ending therapy ≥6 months prior to bortezomib. Table 1 reports the basic summary statistics regarding prior treatments received for myeloma:

Table 1. Previous Therapy for Multiple Myeloma (All Patients; Studies M34100-025 and M34100-024)

Therapy	Study M34100-025 (1.3 mg/m^2) by Cohort and Overall			Study M34100-024		
	Cohort 1 (n=78)	Cohort 2 (n=124)	Total (n=202)	1.0 mg/m^2 (n = 28)	1.3 mg/m^2 (n = 26)	Total (n = 54)
Any prior steroids (e.g., dexamethasone, VAD)	78 (100)	123 (99)	201 (>99)	27 (96)	26 (100)	53 (98)
Any prior alkylating agents (e.g., MP, VBMCP)	71 (91)	115 (93)	186 (92)	21 (75)	18 (69)	39 (72)
Any prior anthracyclines (e.g., VAD, mitoxantrone)	65 (83)	98 (79)	163 (81)	12 (43)	17 (65)	29 (54)
Any prior thalidomide therapy	58 (74)	110 (89)	168 (83)	9 (32)	7 (27)	16 (30)
Received ≥ 2of the above[a]	77 (99)	121 (98)	198 (98)	25 (89)	24 (92)	49 (91)
Received ≥ 3 of the above[a]	68 (87)	117 (94)	185 (92)	14 (50)	14 (54)	28 (52)
Received all 4 of the above[a]	49 (63)	85 (69)	134 (66)	2 (7)	4 (15)	6 (11)
Any prior stem cell transplant	44 (56)	85 (69)	129 (64)	15 (54)	11 (42)	26 (48)
Pts ≤65 yrs at entry w/ BMT	39/54 (72)	63/82 (77)	102/136 (75)	10/15 (67)	9/18 (50)	19/33 (58)
Pts >65 yrs at entry w/ BMT	5/24 (21)	22/42 (52)	27/66 (41)	5/13 (38)	2/8 (25)	7/21 (33)
Prior experimental/other therapy	34 (44)	55 (44)	89 (44)	3 (11)	3 (12)	6 (11)
No. prior regimens of treatment						
N	78	124	202	28	26	54
Mean (□SD)	6 (2.4)	6 (2.9)	6 (2.8)	3 (1.8)	3 (1.6)	3 (1.7)
Median	5	6	6	3	3	3
Minimum, maximum	2, 15	2, 15	2, 15	1, 7	1, 7	1, 7

Abbreviatoions: MP = melphalan and prednisone; VAD = vincristine, doxorubicin, and dexamethasone; VBMCP = vincristine, carmustine, melphalan, Cytoxan, prednisone. These are distinct regimens.

a Received 2, 3 or 4 of the following: dexamethasone, alkylating agents, anthracyclines, or thalidomide.

- **Treatments**

In study M34100-024, all patients were to receive bortezomib. Bortezomib doses were 1.0 or 1.3mg/m^2 (based on random assignment) administered as a rapid intravenous (IV) bolus twice per week for two weeks (on Days 1, 4, 8, and 11) followed by a 10-day rest period (Days 12 to 21). This 3-week period was to be considered a treatment cycle; Cycle 2 was to commence on Day 22 (Cycle 2, Day 1). A complete treatment cycle was comprised of 4 bortezomib doses. Patients were to receive a maximum of eight treatment cycles. Patients who experienced progressive disease (PD) after receiving bortezomib 1.3mg/m^2 alone in Cycles 1 and 2, or PD or no change (NC) after receiving bortezomib 1.3mg/m^2 alone in Cycles 3 and 4 (as compared with their status at the end of Cycle 2) or Cycles 5 and 6 (compared with their status at the end of Cycle 4) were to start treatment with bortezomib 1.3mg/m^2 plus dexamethasone. Dexamethasone 20mg was to be administered orally (PO) four times per week on each day of and on day after bortezomib administration for two consecutive weeks (on Days 1, 2, 4, 5, 8, 9, 11, and 12). Thus, for every dose of bortezomib 1.3mg/m^2, patients were to receive a total of 40mg dexamethasone.
In study M34100-025, patients were to receive bortezomib 1.3mg/m^2 administered following the same scheme used for M34100-024.

In both studies, patients were to receive a maximum of eight 3-week treatment cycles; therefore, the maximum duration of treatment in this study was 24 weeks (~6 months). The actual number of cycles administered for each patient was based on the response to therapy.

- **Objectives**

The primary objective of both studies was to determine the response rate (the combined CR + PR + MR) Secondary objectives were:

- To assess the safety and tolerability of bortezomib 1.3mg/m^2/dose alone and in combination with dexamethasone in patients with multiple myeloma;
- To obtain additional pharmacodynamic information for bortezomib 1.3mg/m^2/dose in patients with multiple myeloma as assessed by the proteasome inhibition assay;
- To obtain additional genomic information on multiple myeloma and its response to drugs. Special assays (e.g., IL-6) were to be done to obtain information on multiple myeloma disease markers and mechanisms of action of bortezomib in this disease. Pharmacogenomic findings are presented outside the auspices of this report;
- To pilot a composite quality of life (QOL) instrument [European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ)-C30, QLQ-MY24 Multiple Myeloma modules and Functional Assessment of Cancer Therapy (FACT)/Gynecology Oncology Group (GOG) and Functional Assessment of Chronic Illness Therapy (FACIT) surveys of neurotoxicity and fatigue, respectively)] in evaluating potential changes in QOL during treatment and correlating QOL to response data.

- **Outcomes/endpoints**

The protocol-defined primary endpoint for both Study M34100-025 and M34100-024 was the proportion of patients with an objective response to treatment, that is complete remission (CR), partial response (PR), or minimal response (MR). The responses were based on the stringent Blade criteria and were determined by an independent review committee (IRC) comprised of three experts in the diagnosis and treatment of myeloma. Secondary efficacy endpoints included time to progression, reduction in myeloma paraprotein levels, time to response, duration of response, overall survival, and measures of clinical benefit including quality of life.

- **Sample size**

Study M34100-024 was to enrol 32 patients into each of the 2 dose groups (1.0 and 1.3mg/m^2), for a total of 64 patients. This sample size estimate was based on the aim to determine if the true rate of response to bortezomib alone was at least 20%, at the one-sided alpha-level of 0.05 and having at least 80% power to conclude that the rate of response was 40% or more. This sample size was to accommodate stratification within each treatment group.

For study M34100-025, a total of up to 200 patients were to be enrolled. The original protocol was planned to include 75 patients and was amended to enrol a second cohort of 125 patients. The primary conclusions regarding treatment efficacy, as prospectively defined in the protocol, were to be based on the initial planned cohort of 75 patients, with corroborative evidence of effectiveness and characterization of the safety profile derived from the second cohort.

- **Randomisation**

Study M34100-024 was a prospective, randomised, open-label, multicentre study designed to evaluate the efficacy and safety of 2 dose levels of bortezomib. Randomisation was stratified at each study centre according to stage of disease and type of front-line chemotherapy administered (conventional or high dose). Study M34100-025 was a single arm trial.

- **Blinding (masking)**

In an effort to minimize bias due to the uncontrolled open-label design, an IRC consisting of three independent physicians with expertise in multiple myeloma was employed for the assessment of patients' response to treatment. The IRC's assessment was used in all statistical analyses of disease response. The IRC had written operating procedures.

- **Statistical methods**

For both studies, the statistical analysis was to focus on the need to estimate response rates within specified limits of accuracy in order to determine if bortezomib alone or in combination with dexamethasone was sufficiently efficacious to warrant further clinical study. The primary efficacy analysis was to be performed on disease response to the initial regimen of bortezomib alone, where a responder was defined as a CR, PR, or MR, based on the results of the IRC's independent majority disease response assessment. In addition, the CR rate was to be presented. Two-sided 90% confidence limits on the percentage of responders were to be established to determine if the lower bound exceeded 20% (study M34100-024) or 10% (study M34100-025) In the analysis, when patients failed to respond to bortezomib alone (regardless of their potential subsequent response to the combination therapy), this was considered as treatment failure.

The final efficacy analysis was performed using three definitions of complete remission:

- CRBlade: (CR by complete Blade criteria): 100% reduction in serum and urine M protein by both protein electrophoresis and immunofixation negative status on two determinations at least 6 weeks apart, a bone marrow examination with <5% plasma cells, stable bone disease and normal calcium levels.
- CRIF+: PR by Blade criteria: i.e., a 100% reduction in serum and urine M protein (with >95% reduction for confirmation) independent of immunofixation status; stable bone disease and normal calcium levels.
- CRSWOG: PR by Blade criteria: a ≥75% reduction in paraprotein levels confirmed at a 6 week interval along with stable bone disease and normal calcium levels.

Efficacy analyses were performed using the IRC evaluated population for primary efficacy and "all treated patients" population for secondary efficacy measures. In study M34100-024, all analyses were conducted by dose group.

The primary efficacy analysis was performed on disease response to the initial regimen of bortezomib alone, where a responder was defined as a patient who achieved CR, PR, or MR, based on the results of the IRC's independent two-thirds majority disease response assessment. The criteria used by the IRC were according to Blade *et al.*, 1998. Efficacy analyses were performed on the rate of responders for the intent-to-treat (ITT) population, where a responder was defined for this analysis as a patient who at any point in the study achieved a CR, PR, or MR from bortezomib alone.

At variance with what established in the protocol, for analysis of rates of response, a patient was included in the category CR if the patient achieved either a CRBlade or a CRIF+; since the CRIF+ classification is being recently used in published trials, this overall CR rate was felt most relevant by the applicant. Two-sided 90% confidence limits on the percentage of responders were established. In this analysis, all patients in the IRC Evaluated population who failed to respond to bortezomib alone, regardless of their potential subsequent response to the combination therapy, were considered treatment failures.

Additional analyses consisted of evaluations for each dose group of time to and duration of response (overall and CR) for the IRC evaluated population, and time to disease progression and survival for the All Treated Patients population. Time-to-event analyses used standard survival analysis techniques, such as Kaplan-Meier test methods. These analyses were performed for those patients who received bortezomib alone and for all patients (regardless of receipt of combination therapy).

Duration of response on bortezomib alone was defined as the time from first diagnosis of response to time of progression. Patients who went on to receive dexamethasone in combination with bortezomib were censored in this analysis at the last evaluation prior to starting combination treatment. Patients who went on to receive additional bortezomib in the extension study M34101-029 were also censored in the analysis at the last evaluation prior to starting additional therapy.

Duration of response on bortezomib alone or in combination was defined similarly, as the time from first diagnosis of response to time of progression. For those patients who progressed during the treatment phase of the study, the date of progression was based on review by the IRC and for those patients who progressed off treatment, the date was determined by the study centre investigator.

Patients who went on to receive additional bortezomib in the extension study M34100- 029 were also censored in the analysis at the last evaluation prior to starting additional therapy. The response rate to bortezomib alone (CR + PR rate) relative to all other responses (MC, NC, PD and NE) was assessed in univariate and multivariate analyses, as functions of a pre-specified number of prognostic factors. The categorical factors were analysed using the Fisher's Exact Test, and continuous variables were analysed with logistic regression. In addition, a multivariate logistic regression analysis was conducted using firstly all prognostic factors in the model, and then using a step-wise selection method where terms were retained at the 0.20 significance level.

Survival was defined from the date of first dose of bortezomib to the date of death, regardless of treatment received. Patients who had not died were censored for this analysis at the last documented date at which the patient was known to be alive.

Quality of Life assessment based on the EORTC QLQ-C30 and EORTC QLQ-MY24 Multiple Myeloma modules and FACT/GOG and FACIT surveys of neurotoxicity and fatigue, respectively, were analysed for each dose group to determine if response to therapy was accompanied by measurable improvement in quality of life. The analysis was performed on summary scores, as specified in the literature.

Results

- **Participant flow**

Study M34100-024

A total of 58 patients with multiple myeloma were screened for enrolment in this clinical study and 54 patients were enrolled and treated at 10 study centres in the USA. Four patients were screen failures. Twenty-eight patients were enrolled and treated in the 1.0mg/m^2 dose group and 26 in the 1.3mg/m^2 dose group.

Twenty-four (86%) of the 28 patients in the 1.0mg/m^2 dose group and 19 (73%) of 26 patients in the 1.3 mg/m^2 dose group completed 4 or more treatment cycles. Patients in the 1.0mg/m^2 dose group were more likely to receive at least one dose in Cycle 8 and were more likely to complete the study through Cycle 8. Dexamethasone was added to bortezomib in 16 (57%) of the 28 patients in the 1.0mg/m^2 dose group and in 12 of the 26 (46%) patients in the 1.3mg/m^2 dose group. Combination therapy was initiated in Cycles 2 to 4 for 6 patients in the 1.0mg/m^2 dose group and for 3 patients in the 1.3mg/m^2 dose group and in Cycles 5 to 8 for 10 patients in the 1.0mg/m^2 dose group and for 9 patients in the 1.3mg/m^2 dose group The investigator could elect to place patients with PD or NC in disease status on combination treatment at protocol-specified time points, that is after 4 cycles if NC or PD or (by amendment) for PD after 2 cycles.

Twenty-five patients were reported to have terminated the study prematurely, including 9 patients in the 1.0mg/m^2 dose group and 16 patients in the 1.3mg/m^2 dose group. Among the 9 patients who terminated early in the 1.0mg/m^2 dose group, the majority (6 patients) terminated prior to Cycle 5 and 3 terminated after that time. In the 1.3mg/m^2 dose group, 8 of the 16 patients terminated prior to Cycle 5, and 8 terminated after that time. The reasons reported for early termination, i.e., termination other than for CR prior to completion of 8 treatment cycles, were similar for the 2 dose groups, with the exception of AE, which was more frequently in the 1.3mg/m^2 dose group. A total of 9 (35%) of the 26 patients in the high-dose group compared to 3 (11%) of 28 in the low-dose group terminated the study due to AEs.

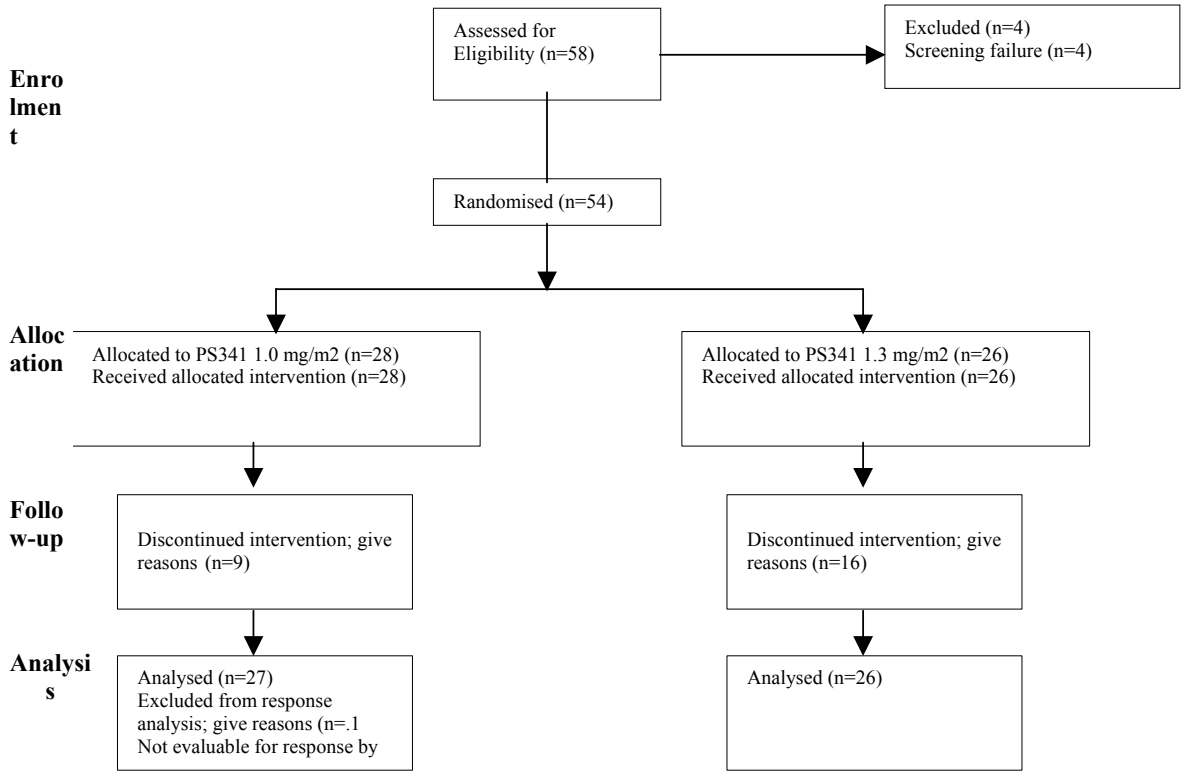
A total of 28 (52%) of the 54 patients had at least one bortezomib dose held for AEs during the study; the proportion of patients with doses held was higher in the 1.3mg/m^2 dose group (17 of 26 patients, 65%) compared with the 1.0mg/m^2 dose group (8 of 28 patients, 29%). Similarly, a higher proportion of patients in the 1.3mg/m^2 dose group had a dose reduction due to AEs (9 of 26 patients, 35%) compared with the lower dose group (11 of 28 patients, 39%).

Four of the 54 patients did not receive correct random assignment. . Data from all 4 of these patients were tabulated under dose received.
The population for analysis of primary efficacy results, i.e., response to treatment, included a total of

53 patients, 27 from the 1.0mg/m^2 dose group and 26 from the 1.3mg/m^2 dose group. Secondary efficacy analyses, including time to progression, survival and clinical benefit analyses, were conducted on the All Treated Patients population (N = 54).

Table 2. Summary of the population for analysis and participants flow for study M34100-024

Population	1.0 mg/m^2 n (%)	1.3 mg/m^2 n (%)	Total n (%)
All Treated Patients	28 (100%)	26 (100%)	54 (100%)
IRC Evaluated Population	27 (96%)	26 (100%)	53 (98%)
Excluded from the IRC population	1 (4%)	0	1 (2%)
Pharmacodynamic Population	15 (54%)	11 (42%)	26 (48%)
Pharmacokinetic Population[a]	10 (36%)	6 (23%)	15 (28%)



Study M34100-025

A total of 214 patients were recruited, of which 202 patients with relapsed/refractory multiple myeloma were enrolled in this study at 14 study centres in the USA, 78 patients in Cohort 1 and 124 patients in Cohort 2. The first patient received the first dose of bortezomib on 26 February 2001 and the last patient received the first dose on 10 December 2001. The initial cohort of 78 patients was based on all patients who received their first dose of bortezomib as of 23 July 2001.

The two Cohorts were similar with regard to patient disposition. A total of 196 (97%) of the 202 patients completed at least one treatment cycle, with 83% completing at least two cycles of treatment. Seventy-eight (39%) of the 202 patients were reported to have completed the study through Cycle 8 (i.e., received dosing in Cycle 8). Dexamethasone was added to bortezomib in 78 (39%) of the 202 patients; combination therapy was initiated in Cycles 2 to 4 for 27 patients and in Cycles 5 to 8 for 51 patients.

Early termination from the study, i.e., termination for reasons other than CR before completion of 8 treatment cycles, was reported for 122 (60%) of the 202 patients; the main reasons for early termination were lack of efficacy (54 patients; 27%) and adverse event (45 patients; 22%). Five additional patients (2%) terminated the study because they missed 3 of the 4 doses within a treatment cycle due to toxicity (required by protocol) and 4 (2%) terminated because of overcome illness. Other reasons for early termination included patient request (8 patients; 4%), lost to follow-up (4 patients; 2%) and non-compliance/administrative reasons (2 patients; <1%). A total of 13 patients were assigned patient numbers but did not receive any dose of bortezomib in this study. Of these 13 patients, 11 did not meet the protocol entry criteria, 1 patient withdrew consent to participate, and for 1 patient the reason was not specified.

Data from all the 202 patients enrolled and treated in this study were included in the safety analyses. Primary efficacy analyses were conducted on the ITT population. The study protocol (by amendment) allowed for patients with non-measurable disease to be enrolled. These patients could have been enrolled into the study with non-measurable serum and urine M protein, defined as serum IgG <10g/L, serum IgA <5g/L, and urine M protein <0.2g/24 hrs. As patients with non-measurable disease are difficult to evaluate for response, the statistical analysis section of the protocol provided that primary efficacy analyses would be conducted only on patients with measurable serum or urine M protein. The protocol also noted that the IRC would not review data from patients with non-measurable disease. However, in order to provide a more complete review of the results from all patients treated in this protocol, all data for all patients were reviewed by the IRC, including the data from 13 patients with non-measurable disease at baseline. Following IRC review, patients with non-measurable disease who the IRC determined could not be assessed for response were excluded from the analysis. Patients with measurable disease who were determined by the IRC not to be assessed for efficacy were included in the analyses as non-responders. A total of 9 patients with non-measurable disease were excluded from the efficacy analyses, including 2 (3%) of the 78 patients in Cohort 1 and 7 (6%) of the 124 patients in Cohort 2, as the IRC could not evaluate their response to treatment. Thus, the ITT population for analysis of primary efficacy results, i.e., response to treatment, includes a total of 193 patients, 76 from Cohort 1 and 117 from Cohort 2. Secondary efficacy analyses, including time to progression, survival and clinical benefit analyses were conducted on the All Treated Patients population (N = 202).

Table 3. Summary of the population for analysis and participants flow for study M34100-024

Population	Cohort 1 n (%)	Cohort 2 n (%)	Total n (%)
All Treated Patients	78 (100%)	124 (100%)	202 (100%)
Safety Population	78 (100%)	124 (100%)	202 (100%)
ITT Population	76 (97%)	117 (94%)	193 (96%)
Excluded from the ITT population	2 (3%)	7 (6%)	9 (4%)
Pharmacodynamic Population	53 (68%)	88 (71%)	141 (70%)
Pharmacokinetic Population	0	8 (6%)	8 (4%)

- **Baseline data**

In study M34100-024, the two dose groups were similar with regard to most of the demographic and baseline characteristics. The majority of the 54 patients treated in this study were female (31 patients, 57%) and white (45 patients, 83%); mean age was 64 years in the 1.0mg/m^2 dose group and 60 years in the 1.3mg/m^2 dose group, with a range of 30 to 84 years. The majority of patients (31 of 53, 57%) had a KPS score of 90 to 100 reported by the investigator at baseline, including 17 (61%) of the 28 patients in the 1.0mg/m^2 dose group and 14 (54%) of the 26 in the 1.3mg/m^2 dose group. Only 7 (13%) patients had a Karnofsky performance status score of 70. Serum M-protein was of the immunoglobulin G (IgG) subtype in 32 (59%) of the 54 patients including 15 (54%) of the 28 patients in the 1.0mg/m^2 dose group and 17 (65%) of the 26 in the 1.3mg/m^2 dose group. Fourteen (26%) of the 54 patients had serum M-protein of the immunoglobulin A (IgA) subtype; 7 (13%) patients had light chain disease and 1 (2%) patient had non-secretory myeloma at study entry.

Most patients (31 of 53 patients with data, 58%) had advanced stage disease (Durie Salmon stage IIIA

or IIIB) at diagnosis; this was similar across treatment groups. Median duration between diagnosis of multiple myeloma and first dose of bortezomib was 2.0 years in both the 1.0mg/m^2 and 1.3mg/m^2 dose groups, with mean duration since diagnosis of 3.9 and 2.3 years, respectively. Seven (25%) of the 28 patients in the 1.0mg/m^2 dose group were more than 5 years from diagnosis at the time of study entry compared with 2 (8%) of the 26 patients in the 1.3mg/m^2 dose group. The proportion of patients <1year from diagnosis was lower in the 1.0mg/m^2 dose group (6 of 28, 21%) than in the 1.3mg/m^2 dose group (8 of 26, 31%).

Among patients with IgG multiple myeloma and data available for assessment, mean serum IgG was 32.5g/L in the 1.0mg/m^2 dose group (n = 10) and 24.4g/L in the 1.3mg/m^2 dose group (n = 15) at baseline. Among patients with IgA multiple myeloma and data available, mean serum IgA was 18.2g/L in the 1.0mg/m^2 dose group (n = 7) and 24.2g/L in the 1.3mg/m^2 dose group (n = 5) at baseline.

Four of the 28 (14%) patients in the 1.0mg/m^2 dose group had significant renal impairment (creatinine 2mg/dL) compared with none of the 26 patients in the 1.3mg/m^2 dose group; serum creatinine was 1.5mg/dL in 21% and 8% of patients in the 1.0mg/m^2 and 1.3mg/m^2 dose groups, respectively. In addition, mean and median creatinine clearance were lower in patients in the 1.0mg/m^2 dose group compared with patients in the 1.3mg/m^2 dose group.

Median â$_2$-microglobulin at study entry was 8.4mg/L in the 1.0mg/m^2 dose group; in approximately 58% of patients, this prognostic factor was 4mg/L at baseline and in 50%, â$_2$-microglobulin was 6mg/L. In the 1.3mg/m^2 dose group, median baseline â$_2$-microglobulin was lower at 3.6mg/L, and a lower proportion of patients in this dose group had â$_2$-microglobulin 4mg/L (58%) and 6mg/L (39%), compared with the 1.0mg/m^2 dose group. Eighty-nine percent (89%) of patients had an abnormal skeletal survey at screening, including 82% (23 of 28 patients) in the 1.0mg/m^2 dose group and 96% (25 of 26 patients) in the 1.3mg/m^2 dose group.

Chromosome abnormalities were assessed in 24 patients in the 1.0mg/m^2 dose group and 23 patients in the 1.3mg/m^2 dose group from their screening bone marrow plasma cells. The cytogenetics analysis was abnormal in 7 (29%) of 24 patients in the 1.0mg/m^2 dose group and in 11 (48%) of 23 patients in the 1.3mg/m^2 dose group. Chromosome 13 deletions were noted in 5 (11%) of the 47 patients with samples obtained, including 8% (2/24) of patients in the 1.0mg/m^2 dose group and 13% (3 of 23) of patients in the 1.3mg/m^2 dose group.

More patients (5 of 28 patients; 18%) in the 1.0mg/m^2 dose group had received blood products over the 6 months prior to study entry than in the 1.3mg/m^2 dose group (1 of 26 patients; 4%). No patients in either dose group were reported by the investigator to be transfusion dependent at study entry.

Mean hemoglobin level at baseline (Cycle 1, Day 1) was 111.9g/L in the 1.0mg/m^2 dose group and 114.5g/L in the 1.3mg/m^2 dose group; 21% of patients in the 1.0mg/m^2 dose group and 17% in the 1.3mg/m^2 dose group had a hemoglobin level <100g/L, with 1 patient in the 1.0mg/m^2 dose group having NCI CTC Grade 3 anemia (hemoglobin <80g/L) at study entry; no patients had NCI CTC Grade 4 anemia. Mean platelet count at baseline was 166.6 and 208.3 x 109/L in the 1.0mg/m^2 and 1.3mg/m^2 dose groups, respectively. No patients in either dose group entered the study with NCI CTC Grade 3 or 4 thrombocytopenia (platelet count < 50 x 109/L).

In study M34100-025, the two study cohorts were similar with regard to demographic characteristics. The majority of the 202 patients treated in this study were male (121 patients; 60%) and Caucasian (164 patients; 81%). The mean age of patients overall and within each cohort was 60 years with a range of 34 to 84 years. Baseline Karnofsky performance status score was determined by the investigator to be 70 for 40 (20%) of the 196 patients with data available, including 19 (25%) of the 76 patients in Cohort 1 and 21 (18%) of the 120 patients in Cohort 2. Serum M protein was of the IgG subtype in the majority (60%) of the 202 patients, with 24% of patients having IgA subtype. Fourteen percent (14%) of patients had light chain disease, 1 (<1%) patient had IgM myeloma, and 2 (<1%) patients had IgD myeloma. Oligo-secretory myeloma (serum IgG M spike <1g/dL, IgA M spike <0.5g/dL, or urine M spike <200mg/24hrs) was observed in 17 (8%) of the 202 patients and 2 (1%) patients had non-secretory myeloma at study entry.

Approximately three-fourths (133 of 185 patients, 72%) of patients had advanced stage disease (Durie-

Salmon stage IIIA or IIIB) at diagnosis. Median duration between diagnosis of multiple myeloma and first dose of bortezomib was 4.0 years in both Cohorts, with mean durations since diagnosis of 4.3 and 4.7 years, respectively.

Among the 103 patients with IgG multiple myeloma and data available for assessment, mean serum IgG was 36.6g/L at screening. Among the 43 patients with IgA multiple myeloma and data available, mean serum IgA was 30.4g/L.

Mean and median â$_2$-microglobulin at study entry were 3.6 and 2.8mg/dL in Cohort 1; in approximately 32% of patients this measure of disease severity was 4 mg/L at baseline and in 18%, â$_2$-microglobulin was 6mg/L. In Cohort 2, mean and median baseline â$_2$-microglobulin were higher at 6.8 and 4.0mg/dL, respectively, and a higher proportion of patients in this cohort had â$_2$-microglobulin 4mg/L (50%) and 6mg/L (31%) compared with Cohort 1 (32% and 18%, respectively).

Renal function also appeared to be more compromised in Cohort 2 compared with Cohort 1. This may be related to an amendment to the protocol (Amendment 6) allowing for patients with more compromised renal function to be enrolled in the study. Four (5%) of the 78 patients in Cohort 1 had significant renal impairment (creatinine 2 mg/dL) compared to 11 (9%) of 123 patients in Cohort 2 with data available at baseline; serum creatinine was 1.5mg/dL in 13% and 28% of patients in Cohorts 1 and 2, respectively. In addition, mean and median creatinine clearance were lower in patients in Cohort 2 than patients in Cohort 1, with mean and median creatinine clearance values of 83.8 and 79.6mL/min, respectively, in Cohort 1 and 77.7 and 70.4mL/min, respectively, in Cohort 2.

Over 95% of patients with data available had an abnormal skeletal survey at the screening visit. A total of 15 (7%) of the 202 patients had extramedullary plasmacytomas identified at the screening visit including 2 patients (3%) in Cohort 1 and 13 (10%) in Cohort 2. The extramedullary plasmacytomas were most commonly located in soft tissue or were paraspinal; two patients had plasmacytomas identified in the skull, one patient had renal and adrenal plasmacytomas identified, and one had a plasmacytoma identified in the maxillary sinus. Chromosomal abnormalities were assessed in 172 patients who had cytogenetics analysis done on their screening bone marrow cells. The cytogenetics analysis was abnormal in 60 (35%) of these 172 patients. The most common abnormality reported was the deletion of chromosome 13 observed in 26 (15%) of 172 patients.

Mean haemoglobin level at baseline (Day 1, Cycle 1) was 105g/L (10.5g/dL) in Cohort 1 and 101g/L (10.1g/dL) in Cohort 2. In Cohorts 1 and 2, 35% and 49% of patients, respectively, had a baseline haemoglobin level <10g/dL, with 5% and 6% of patients, respectively, having NCI CTC Grade 3 or 4 anemia (haemoglobin <8.0g/dL). Mean platelet count was 175 and 154.109/L in Cohorts 1 and 2, respectively. In Cohorts 1 and 2, 12% and 15% of patients, respectively, had Grade 3 thrombocytopenia (platelet count <50.109/L) at baseline. Note that there were no restrictions on transfusion support that could be given prior to study entry; therefore, baseline haematologic values may reflect recent transfusion.

Overall, 65 (32%) of 202 patients had received blood products during the six months prior to study entry, including 21 (27%) of the 78 patients in Cohort 1 and 44 (35%) of the 202 patients in Cohort 2. Fifty-two (26%) of the 202 patients had received red blood cell transfusions during the 6 months prior to study entry; the number of red cell transfusion events (i.e., received at least one transfusion on a given day) ranged from 1 to 12 events with a mean of 2. Platelet transfusions were administered during the six months prior to study entry in 32 (16%) of the 202 patients; the number of platelet transfusion events ranged from 1 to 6 with a mean of 2. Twenty-four (12%) of the 202 patients were reported by the investigator to be transfusion-dependent at study entry, 7 (9%) of 78 patients in Cohort 1 and 17 (14%) of 124 patients in Cohort 2.

- **Outcomes and estimation**

In Study M34100-024, the overall response rate to treatment with bortezomib alone was higher, at 50%, in the 1.3mg/m^2 dose group with a 90% lower confidence bound of 32.7%, compared with 33% in the 1.0mg/m^2 dose group with a 90% lower confidence bound of 18.6%. The rate of CR+PR to bortezomib alone was 38% in the 1.3mg/m^2 dose group and 30% in the 1.0mg/m^2 dose group. Overall, 57% of patients in the 1.0mg/mg^2 dose group and 46% of patients in the 1.3mg/m^2 dose groups received combination treatment with bortezomib and dexamethasone. The ORR to treatment with

bortezomib alone or in combination with dexamethasone was 62% (16 of 26 patients) with a two-sided 90% CI lower bound of 43.6% for the 1.3mg/m^2 dose group and was 44% (12 of 27 patients) with two-sided 90% CI lower bound of 28.0% in the 1.0mg/m^2 dose group. The rates of response for patients achieving either a CR or PR were also higher in the 1.3mg/m^2 dose group (50%) compared with the 1.0mg/m^2 dose group (37%).

In Study M34100-025, the overall response rate to treatment with bortezomib alone was 33% with a lower 90% confidence bound of 24% in Cohort 1; this result was confirmed in Cohort 2 with an overall response rate of 36% and a lower 90% confidence bound of 28.5. Calculation of 95% bounds yielded minimal difference in the lower bound. The overall response rate of the 193 patients included in the ITT population was 35% (lower 95% bound 28.0%). A total of 53 (27%) of the 193 patients had a CR or PR to treatment with bortezomib alone. The complete remission rate to bortezomib alone (CRBlade and CRIF+) was 10% (12% in Cohort 1 and 9% in Cohort 2) in Study M34100- 025. CRBlade were 7 (4%).

The CR rate was similar in Study M34100-024 with a combined CR rate of 7.5% and CRBlade of 4%.

	Study M34100-025 (1.3 mg/m^2) by Cohort and Overall			Study M34100-024 by Dose Group	
Confirmed Response Category[a]	**Cohort 1 (n=76)**	**Cohort 2 (n=117)**	**Total (n=193)**	**1.0 mg/m^2 (n = 27)**	**1.3 mg/m^2 (n = 26)**
Overall Response (CR+PR+MR)	25 (33)	42 (36)	67 (35)	9 (33)	13 (50)
CR + PR	21 (28)	32 (27)	53 (27)	8 (30)	10 (38)
CR[b]	9 (12)	10 (9)	19 (10)	3 (11)	1 (4)
CR^{Blade}	3 (4)	4 (3)	7 (4)	1 (4)	1 (4)
CR^{IF+}	6 (8)	6 (5)	12 (6)	2 (7)	--
PR	12 (16)	22 (19)	34 (18)	5 (19)	9 (35)
MR	4 (5)	10 (9)	14 (7)	1 (4)	3 (12)
NC	21 (28)	25 (21)	46 (24)	7 (26)	5 (19)
PD (Non-responder)	16 (21)	22 (19)	38 (20)	8 (30)	5 (19)
NE (Non-responder)	14 (18)	28 (24)	42 (22)	3 (11)	3 (12)

Source: M34100-025, section 14.2, Table 14.2.1A and M34100-024, section 14.2.1, Table 14.2.1A.

Note: CR = complete remission, MR = minimal response, NC = no change, PD = progressive disease, PR = partial response, NE = not evaluable for response by the IRC.

a Response to treatment while patients were receiving PS-341 alone.

b $CR^{Blade} + CR^{IF+}$.

In both studies, patients with either no change in disease status or progression of disease could continue in the study and add dexamethasone 40mg to each dose of bortezomib at the investigator's discretion. A total of 74 and 28 patients included in the ITT population in studies M34100-025 and M34100-024, respectively, went on to combination therapy. A higher proportion of patients in study M34100-024 with a sub optimal response to bortezomib experienced an improved response on combination therapy with dexamethasone [11 (39%) of 28 patients] compared to patients in study M34100-025 [18 (24%) of 74 patients]. Two patients in study M34100-024 achieved a complete remission (IF+) on the combination.

The response data to bortezomib alone (CR + PR) were subjected to both univariate and multivariate analyses to examine the effect of standard prognostic markers on response and to examine the response in subgroups of patients. CR + PR rate to bortezomib was not adversely affected by gender, type of myeloma (e.g., heavy vs light chain), or type or number of prior therapies. Patients >65 years of age had a lower response rate than those <65 years (19% vs 32%, p=0.06). In addition, patients with >50% plasma cells at the screening bone marrow assessment had a statistically significantly lower response rate (20%) than those with <50% plasma cells in the bone marrow (35%; p = 0.030). There was no difference in CR + PR rate for patients with chromosome 13 abnormalities as detected by conventional methodology (24%), a known marker of poor prognosis, compared to those without abnormalities (28%).

While the CR + PR rate was slightly lower in patients with an elevated □2-microglobulin in the univariate analysis (p=0.071) it did not remain a factor in the multivariate examination. In the multivariate analysis, categorical variables age and percent plasma cells in the bone marrow were both statistically significant at p <0.05.

- **Ancillary analyses**

In addition to the primary endpoint of overall response rate, study M34100-025 data were analysed for parameters that describe time to critical events, which included time to response, duration of response, time to disease progression and survival. These analyses were conducted using standard Kaplan-Meier methodology.

Response to bortezomib was rapid (38 days) and consistent across different response categories. In addition, it was durable in this relapsed and refractory population, with a median duration of response of 12 months for patients with CR and 8 months for patients with PR. The time to progression was 7 months for all patients, 13 months for patients with CR and 9 months for patients with PR. The median survival for all patients was 16 months and has not yet been reached for responding patients (CR, PR or MR) while the survival for non-responding patients was 8 months. The expected survival from literature data is 6 to 9 months while the expected time to progression (based on review of prior therapy data collected in study M34100-025) was 3 months.

Analysis of the pharmacodynamic marker (inhibition of 20S proteasome activity) analysed on whole blood samples revealed 60% mean inhibition at 1 hour and no relationship between level of 20S proteasome activity and response to treatment.

As of follow-up information updated during the procedure, a total of 81 of the 202 patients had died, including 1 (5%) of 19 patients with CR, 10 (26%) of 39 patients with PR, 5 (25%) of 20 patients with MR, 10 (27%) of 37 patients with NC and 55 (63%) of 87 non-responding patients. In the updated data, the median survival for all 202 patients enrolled in Study M34100-025 increased to 533 days (~17.5 months) from 478 days (~16 months) in the submission. For the subset of 58 patients with CR or PR, the median survival has still not been reached despite a median follow-up in this patient group of ~15 months. For the 87 non-responders, the median survival including the updated follow-up data was 219 days (~7 months).

Table Q50I: Updated Summary of Survival on Bortezomib Alone or in Combination with Dexamethasone in Study M34100-025 Including Data from the Extension Study M34100-029 (Days) (All Patients Treated)

Response Category	N	Median OS Reported in Submission[a]	Updated Time to Event Data				
			Median OS	95% CI	% Censored	Minimum	Maximum
All Patients	202	478	533	(419, NE)	60	9	612+
CR + PR	58	NE	NE	(NE, NE)	81	135	612+
CR	19	NE	NE	(NE, NE)	95	155+	612+
CR^{Blade}	7	NE	NE	(NE, NE)	100	266+	581+
CR^{IF+}	12	NE	NE	(NE, NE)	92	155+	612+
PR	39	NE	NE	(518, NE)	74	135	575+
CR^{SWOG}	37	NE	NE	(NE, NE)	86	155+	612+
MR	20	NE	NE	(NE, NE)	75	164	548+
NC	37	471	NE	(471, NE)	73	49	596+
Non-responders[a]	87	244	219	(160, 304)	37	9	584+

Note: + denotes censored value, NE = not estimated.

a From Module 5, Section 5.3.5.2.5, M34100-025 CSR, Section 11.3.1.8, Table 11-16, page 149.

b Includes patients with IRC response of progressive disease and non-evaluable.

Response rate was chosen as the primary endpoint for these studies on the assumption that it is an appropriate surrogate for benefit (survival) in patients with relapsed and refractory multiple myeloma. In order to verify this assumption, the Kaplan Meier survival curves based on the updated survival data for the CR and PR patients were compared statistically to the curves for the non-responding patients; results are presented in Figure Q50B and Figure Q50C, respectively. As shown, the survival curves for both patients with CR and with PR were significantly prolonged ($p < 0.0001$; log-rank test) as compared with non-responding patients. This has occurred in the absence of any prognostic factors that would clearly suggest a baseline difference in prognosis or pace of disease between the two groups of patients at baseline (such as type of multiple myeloma, type of prior therapy, time from diagnosis, type or number of prior therapies, etc.).

Figure Q50B: Kaplan-Meier Curve: Survival for CR Patients Compared to Non-responders (Study M34100-025)

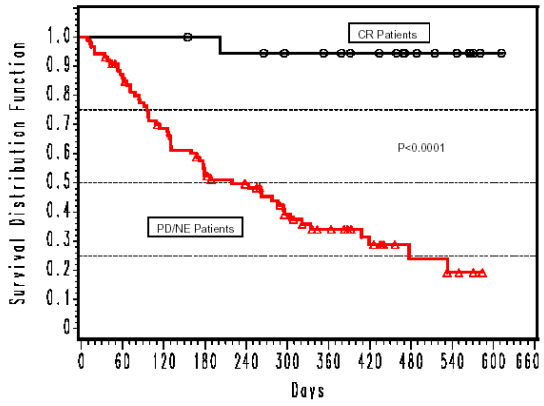
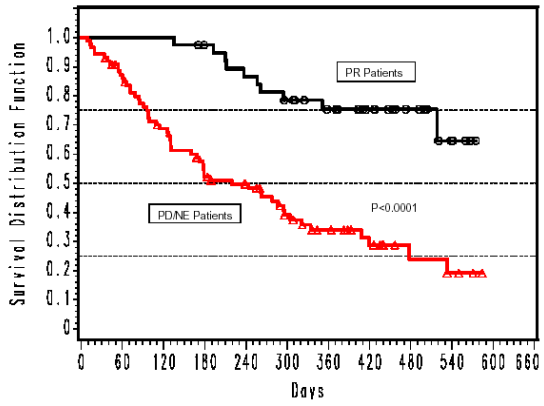


Figure Q50C: Kaplan-Meier Curve: Survival for PR Patients Compared to Non-responders (Study M34100-025)



A landmark analysis of survival was provided in the submission in the clinical study report for M34100-025. The results of the landmark analysis have also been revised based on the updated survival information from Study M34100-025. These results were consistent with those presented in the submission and indicate that there is a significant association between responder status and ultimate survival (p=0.0055), although response rate cannot be regarded as a surrogate or an independent predictor, but only as a prognostic factor

Supportive studies

Further evidence was considered, supporting response to treatment in patients with relapsed and refractory myeloma as reasonably likely predictor of survival. Since published evidence suggests that patients experience lesser rates of response and shorter durations of response to each successive regimen of therapy, an unplanned analysis was performed comparing patients' time to progression on bortezomib alone to the time to progression on their last prior therapy. The time to progression on last therapy for all patients was short at a median of 93 days, reflecting the refractory nature of this patient population. On-study analysis of the same patients revealed a median TTP of 213 days in response to treatment with bortezomib alone, reflecting a more than two fold increase in the time to progression.

Further findings supporting the prognostic value of response rate was derived by the literature. An analysis of all published randomised studies in multiple myeloma conducted between 1966 and 2001 in patients with relapsed myeloma was sponsored by the applicant. Ten randomised studies of salvage therapy for myeloma were identified, enrolling a total of 808 patients. Nine of the 10 published trials provided information regarding both response and median survival in the populations studied. Results from linear regression analysis demonstrate an R2 value of 0.704, indicating a strong correlation between overall response and survival in these studies.

Discussion on clinical efficacy

A novel pharmacodynamic assay (inhibition of 20S proteasome activity) has been developed as a

guide to dose-finding studies. However, this assay has some limitations, in that its variability was reportedly low in the presence of high level of inhibition and high when the level of inhibition is low. Based on two of the Phase I studies with dosing over 4 weeks, the twice weekly dosing for two weeks, followed by a 10-day rest period, appeared to be better tolerated than that with dosing over 4 weeks
Phase III randomised comparative studies are generally required for marketing authorisation. The minimum requirement is generally one controlled study with statistically compelling and clinically relevant results. As there is a general demand for replication of scientific results, it is usually recommended to plan for more than one study in the Phase III program.

In the case of bortezomib, no adequate and well-controlled clinical trials have been presented. The demonstration of efficacy of bortezomib basically relies on anti-tumour activity results (response rate) from the M34100-025 single arm Phase II study, in which 2 cohorts of patients (78 and 124) were evaluated, for a total of 202 cases. Supportive data about the biological activity of bortezomib come from the other Phase II single arm trial (54 cases) testing two different doses of bortezomib (M34100-024).

The patient characteristics in the two Phase II trials were different. As compared with study M34100-024, in study M34100-025 patients were heavily pretreated. More than 99% of patients had received prior treatment with steroids, 92% had received prior alkylating agent therapy, 81% had received prior anthracyclines and 83% had received prior therapy with thalidomide; 64% had received prior high dose therapy including bone marrow transplant. These patients had multiple poor prognostic factors, an expected survival of 6 to 9 months.

The data provided by the Applicant are of good quality. Selected sites have been audited, including the highest enrolling sites. These internal audits were performed for 8 of the 14 sites for study M34100-025 and for 2 of the 10 sites for study M34100-024 and confirm the integrity of the data collected. The CPMP also requested a GCP inspection. As acknowledged by the Applicant, a number of GCP violations and critical findings were found. However, the CPMP concluded that the violations did not appear to invalidate the evaluation of treatment response. The results can be considered reliable and robust enough to consider study M34100-025 as a pivotal trial for the MAA.

Directive 75/318/EEC provides for the possibility of granting marketing authorisation, in exceptional circumstances, among others, when in the present state of scientific knowledge, comprehensive information on the efficacy and safety under normal conditions of use cannot be provided. In this situation, should an Applicant consider that Phase II studies have unequivocally established outstanding benefits of the new agent for the target patients, this position must be fully justified by the Applicant before authorisation can be granted. In addition, in such circumstances, a further trial programme must be agreed to, together with any other experimental studies judged necessary on the basis of observed adverse drug reactions. The CPMP Note for Guidance “Evaluation of anti-cancer medicinal products in man” (CPMP/EWP/205/95 rev.2, 19 September 2002), presents guidelines on the requirements for authorisation for documentation for applications in exceptional circumstances where only non-comparator studies are available (Panel 1).

Panel 1. CPMP guidance on regulatory requirements for anticancer agents within applications under exceptional circumstances (Evaluation of anti-cancer medicinal products in man" CPMP/EWP/205/95 rev.2, 19 September 2002)

Non-comparator studies may be acceptable in this subgroup (previously treated patients – no existing established regimen) only in the following circumstances:
a) proven outstanding anticancer activity (relative to the given clinical situation and based on response rate and duration of response) in patients strictly defined as resistant to relevant first-line therapies **and**
b) the new agent has an acceptable and extensively documented toxicity profile **and**
c) tumour response is a justified surrogate marker for clinical benefit **and**
d) the overall results indicate a positive risk/benefit assessment for this treatment in this clinically identifiable group of patients

a) As for the required outstanding activity, the results show that bortezomib given monotherapy induced a complete remission rate up to 10% and an overall response rate up to about 30% in a population of relapsed and refractory patients. The inclusion of a "minimal response" (MR) as part of the responder definition in the primary efficacy variable is debatable. However, CR+PR and CR according to three sets of criteria were also assessed. The contribution of MR in the ORR was minor. The CR according to "complete Blade" criteria was only 4% (100% reduction in serum and urine M-protein by both electrophoresis and immunofixation), whereas the SWOG CR criteria (at least 75% reduction in paraprotein levels confirmed at 6 week interval along with stable bone disease and normocalcemia), gave an encouraging 18% CR rate. An independent evaluation by the US FDA identified 188 eligible cases in study M34100-025 and only 5 (Blade IF-) CR, which represents a proportion of CR (2.7%, 95% CI: 1-6%) lower than claimed. This figure could even have been lower since a systematic protocol violation might have lead to overestimate the proportion of patients experiencing CR. Indeed, clinical investigators did not always consider one of the criteria required by the protocol to establish CR (the x-ray confirmation of a stable bone disease (no increase in the size or number of lytic lesions)). However, these violations do not seem to have lead to overestimation of the reported CR rate, as it was shown that the response rate was similar in patients who underwent per protocol X-rays compared to overall population. Secondary endpoints evaluating patients' clinical benefit as quality of life, incidence of bone fractures, evolution of anaemia, etc give no reliable information due to the uncontrolled and open setting they are drawn from. A total of 74 patients with a sub optimal response to bortezomib went on to combination therapy (dexamethasone 40mg added to each dose of bortezomib): 18 (24%) of these patients experienced an improved response. Thus, it can be concluded that outstanding anticancer activity has been shown.

b) the toxicity profile seems to be acceptable as documented in the Safety section below.

c) tumour response cannot be regarded as a surrogate marker for clinical benefit; however the Applicant has provided evidence that clinical response has a prognostic value with respect to clinical outcome in MM patients.

d) the population enrolled in the pivotal trial can hardly be considered homogeneous according to the entry criteria. Though heavily treated, patients enrolled in study M34100-025 were not necessarily refractory to multiple treatments. The mean number of prior regimens administered was 6 with a range of 2 to 15. Among all 202 patients, 98% had received at least 2 of 4 types of chemotherapeutic agents, 92% had received 3 of these 4 agents, and 66% had received all 4 chemotherapeutics. These data indicate that in reality randomisation to alternative active comparator would have been possible, since several patients had not shown to be refractory to all alternative treatments at the time they were enrolled in the study. However, approximately 2/3 of the patient population may be considered homogeneous, at least with regard to prior therapies received. An independent expert evaluation produced similar results to those provided by the Applicant in its application: the majority of the 19 patients (89.4%) who achieved CR were judged refractory to their last treatment. In addition, a sensitivity analysis (response according to types of previous treatment and number of previous treatments) suggests that the overall study population is homogeneous with respect to the apparent clinical outcome analyses, in that the percentage of responders to bortezomib (CR/PR) was similar regardless of study cohort or the number or types of treatments the patients had received prior to entering the study.

In conclusion. The results are encouraging. However, results from comparative trials are required in

order to better understand the association between response and duration of survival and the real clinical benefit of bortezomib.

Clinical safety

Safety data derive from three Phase I studies comprising 123 patients with advanced haematological and solid tumours and from two Phase II studies (M34100-024 and M34100-025) overall enrolling 256 patients with myeloma. Table 4 summarises exposure to bortezomib by dose and over all 256 multiple myeloma patients treated in studies M34100-024 and M34100-025. The mean total dose of bortezomib administered across all 256 patients was 43.6mg, with a range of 2.1 to 86.6mg; mean duration of treatment was 112.2 days (~4 months), with a range of 1 to 278 days (~9 months). The mean total number of bortezomib doses received was 19.9, with a range of 1 to 32 doses.

Table -4 Total Exposure to bortezomib in Studies M34100-024 and M34100-025 (All Patients Treated; N=256)

	Bortezomib Dose		
Parameter:	**1.0 mg/m^2 (n = 28)**	**1.3 mg/m^2 (n = 228)**	**Total (n = 256)**
Bortezomib Dose (mg/m^2)			
Mean ± SD	26.7 ± 9.54	23.3 ± 12.61	23.7 ± 12.34
Median	30.5	24.7	26.0
Minimum, Maximum	1.0, 41.6	1.3, 43.5	1.0, 43.5
Bortezomib Dose (mg)			
Mean ± SD	48.4 ± 17.46	43.0 ± 23.52	43.6 ± 22.97
Median	53.9	43.7	45.8
Minimum, Maximum	2.1, 73.0	2.2, 86.6	2.1, 86.6
Duration of bortezomib Treatment (days)[a]			
Mean ± SD	142.6 ± 51.00	108.5 ± 61.47	112.2 ± 61.26
Median	158.0	116.0	130.5
Minimum, Maximum	1.0, 228.0	1.0, 278.0	1.0, 278.0
Number of bortezomib Doses			
Mean ± SD	26.5 ± 9.07	19.1 ± 10.29	19.9 ± 10.40
Median	31.0	20.0	22.0
Minimum, Maximum	1.0, 32.0	1.0, 32.0	1.0, 32.0

a Duration from first to last dose.

The actual mean total bortezomib dose administered across all 256 patients was 77.6% of expected dose in each treatment cycle. The maximum mean total bortezomib dose within a cycle of 8.7mg, which was ~91% of the total mean bortezomib dose expected to be administered, was administered in Cycle 1. The mean total bortezomib dose administered decreased sequentially from Cycle 1 to Cycles 7 and 8; the minimum mean total bortezomib dose of 7.2mg, which was ~78% of the mean total dose expected to be administered, was administered in both Cycle 7 and Cycle 8.

There were no apparent differences in exposure by patient subgroups for gender, race or myeloma type. Exposure to bortezomib, including number of doses, total dose, and duration of treatment did not appear to decrease with increasing age. While the maximum number of treatment cycles in the Phase II studies was 8, patients who achieved benefit and entered in the extension study MS34100-029 received additional cycles of therapy (45 patients).

Adverse events

Commonly Reported Adverse Events in the Phase I Studies

All (100%) 123 patients in the Phase I studies experienced at least one treatment-emergent adverse event. An apparent dose-relationship was noted with regard to the incidence of several gastrointestinal symptoms, including nausea, vomiting, and constipation. Although an apparent dose-relationship was not necessarily seen across all three dose groups, the incidence of fatigue, diarrhoea, headache, arthralgia, weakness, catheter-related complications, and abdominal distention was notably higher in the highest dose group, >1.3mg/m^2, than in the 2 lower dose groups. A similar incidence of these events was seen in the <0.7mg/m^2 and 0.7 to 1.3mg/m^2 groups.

Commonly Reported Adverse Events in the Phase II Studies

Two hundred and fifty six (256) patients received bortezomib for the treatment of multiple myeloma, either as a single agent, or in combination with dexamethasone. Of these, 97% of patients (n=248) experienced an adverse drug reaction (ADR) to bortezomib. ADRs leading to discontinuation were reported in 17% (n=44) of patients.

The most commonly reported events were nausea (62%), fatigue (54%), diarrhoea NOS (48%), constipation (41%), thrombocytopenia (41%), pyrexia (36%), vomiting (34%), and anorexia (30%). Peripheral neuropathy, including reports of peripheral neuropathy NOS, peripheral sensory neuropathy, and peripheral neuropathy aggravated, was reported in 35% of patients. Gastrointestinal events and peripheral neuropathy showed an apparent trend towards increasing incidence with dose. Sixty-eight percent (68%) of patients experienced at least one episode of Grade 3 toxicity, the most common being thrombocytopenia (27%), neutropenia (12%), and fatigue and peripheral neuropathy NOS (11% each). Thirteen percent (13%) of patients experienced at least one episode of Grade 4 toxicity, the most common being thrombocytopenia (3%) and neutropenia (2%).

Comparison of adverse event incidence rates across the two dose groups is made difficult by the small number of patients in the 1.0mg/m^2 dose group, relative to the 1.3mg/m^2 dose group. However, gastrointestinal events (including reports of nausea, diarrhoea, and vomiting), associated events of anorexia and dehydration and peripheral neuropathy (including sensory neuropathy and neuropathy aggravated) appeared to occur more frequently in patients at the 1.3mg/m^2 dose of bortezomib compared with the 1.0mg/m^2 dose.

Table 5 presents the most commonly reported events over all 256 patients and includes those commonly reported events that were Grade 3 or Grade 4 in severity. All 256 patients experienced at least one adverse event during the studies; in 68% of patients at least one event was reported by the investigator to be Grade 3 in severity and in 13% at least one Grade 4 event was reported. The most commonly reported Grade 3 events were thrombocytopenia (27%), neutropenia (12%) and fatigue and peripheral neuropathy NOS (11% each). The most commonly reported Grade 4 events were thrombocytopenia (3%) and neutropenia (2%); all other Grade 4 events were reported in <1% of patients.

Table –5 Most Commonly Reported (≥10% Overall) Adverse Events in Studies M34100-024 and M34100-025 Overall and by Intensity (All Patients Treated; N=256)

	All Patients (N = 256) [n (%)]		
MedDRA Preferred Term	**All Events**	**Grade 3 Events**	**Grade 4 Events**
Patients with at least 1 adverse event	256 (100)	174 (68)	33 (13)
Nausea	158 (62)	13 (5)	0
Fatigue	137 (54)	28 (11)	0
Diarrhoea NOS	123 (48)	16 (6)	2 (<1)
Constipation	106 (41)	5 (2)	0
Thrombocytopenia	105 (41)	68 (27)	7 (3)
Pyrexia	91 (36)	10 (4)	0
Vomiting NOS	86 (34)	16 (6)	1 (<1)
Anorexia	77 (30)	5 (2)	0
Anaemia NOS	74 (29)	21 (8)	0
Arthralgia	73 (29)	13 (5)	0
Insomnia	73 (29)	4 (2)	0
Peripheral neuropathy NOS	73 (29)	27 (11)	1 (<1)
Headache NOS	71 (28)	8 (3)	0
Pain in limb	66 (26)	19 (7)	0
Dyspnoea NOS	57 (22)	9 (4)	1 (<1)
Neutropenia	55 (21)	31 (12)	6 (2)
Dizziness (excl vertigo)	54 (21)	4 (2)	0
Rash NOS	53 (21)	1 (<1)	0
Weakness	51 (20)	15 (6)	1 (<1)
Upper respiratory tract infection NOS	50 (20)	2 (<1)	0
Cough	44 (17)	1 (<1)	0
Dehydration	42 (16)	15 (6)	0
Bone pain	40 (16)	5 (2)	0
Back pain	38 (15)	10 (4)	0
Anxiety NEC	36 (14)	1 (<1)	0
Muscle cramps	35 (14)	2 (<1)	0
Myalgia	34 (13)	6 (2)	0
Appetite decreased NOS	32 (13)	1 (<1)	0
Dyspepsia	32 (13)	0	0
Abdominal pain NOS	31 (12)	5 (2)	0
Dysgeusia	31 (12)	1 (<1)	0
Rigors	31 (12)	3 (1)	0
Hypoaesthesia	30 (12)	1 (<1)	0
Oedema peripheral	30 (12)	0	0
Herpes zoster	29 (11)	2 (<1)	0
Paraesthesia	29 (11)	4 (2)	0
Oedema lower limb	28 (11)	2 (<1)	0
Pruritus NOS	28 (11)	0	0
Depression NOS	27 (11)	1 (<1)	0
Malaise	27 (11)	2 (<1)	0
Vision blurred	27 (11)	1 (<1)	0
Pain NOS	26 (10)	3 (1)	0

Serious adverse events and deaths

Overall, approximately one-third of patients (45 of 123; 37%) experienced at least one serious adverse event in the Phase I studies. The most common serious adverse events reported across all 123 patients included pyrexia (6 patients; 5%), diarrhoea and dyspnea (5 patients; 4%), and abdominal pain, back pain, and hypotension NOS (3 patients; 2%). All other serious adverse events were reported for 2 patients or fewer. 19 (15%) of 123 patients experienced at least one serious adverse event that was considered by the investigator to be study drug-related. An apparent dose-relationship was seen with regard to the incidence of study drug-related serious adverse events, with 0%, 16% (8 of 51 patients), and 26% (11 of 43 patients) in the <0.7mg/m^2, 0.7 to 1.3mg/m^2, and >1.3mg/m^2 groups, respectively, experiencing at least one study drug-related serious adverse event. The most commonly reported study

drug-related serious adverse event was diarrhoea (5 patients; 4%). All other study drug-related serious adverse events were reported for 2 patients or fewer.

The most commonly reported serious adverse events in the Phase II studies included pyrexia, pneumonia, diarrhoea, vomiting, dehydration, and nausea. Patients who experienced a CR or PR to treatment were less likely to experience serious adverse events (32%) compared with patients who achieved a minimal response (MR) or had no change in disease status (53%) and with patients with progressive disease or who were not evaluable for response (58%).

Overall, approximately half (48%) of patients experienced at least one serious adverse event in the Phase II studies. Not unexpectedly, serious adverse events were most commonly reported in the infections and infestations SOC, with 13% of patients experiencing at least one serious adverse event within this SOC. Commonly reported serious adverse events within this SOC included pneumonia NOS (7%) and sepsis NOS (2%). Other MedDRA SOCs in which serious adverse events were commonly reported included general disorders and administration site conditions (13%), gastrointestinal disorders (12%), and nervous system disorders (9%). Overall, the most commonly reported serious adverse events, other than pneumonia NOS, included pyrexia (7%), diarrhoea (5%), vomiting and dehydration (5% each), and nausea (4%).

Forty-four percent (44%) of patients experienced at least one serious adverse event that met the serious criteria because hospitalisation was required or prolonged. Similarly to the incidences of serious adverse events overall, the most commonly reported serious adverse events requiring or prolonging in-patient hospitalisation were pyrexia (7%), pneumonia NOS (7%), vomiting, and diarrhoea (5%), and nausea (4%).

Of the 18 patients who had pyrexia reported as a serious adverse event, pyrexia occurred concurrently with another event that met the serious criteria for 12 patients. Pyrexia occurred concurrently with a serious infection, either pneumonia or URI, for two of these patients.

Of the 17 patients who had pneumonia NOS reported as a serious adverse event, pneumonia was assessed as study drug-related for only two patients, both of whom received bortezomib 1.3mg/m^2.
Patients who experienced a CR or PR to treatment were less likely to experience serious adverse events (32%) compared with patients who achieved an MR or had no change in disease status (53%) and to patients with progressive disease or who were not evaluable for response (58%).

The estimated probability of onset of a serious adverse event among all bortezomib-treated showed an approximately linear, gradual rise within the first ~32mg/m^2 administered, corresponding to approximately 6 treatment cycles, and levelled off thereafter. The probability of the first onset of a serious adverse event increases from approximately 50% to approximately 60% in an additional increase in cumulative dose from 27mg/m^2 to 40mg/m^2 (approximately 8 treatment cycles).

Deaths

In Phase I studies, 3 (2%) of 123 patients died within 30 days after the last study drug dose. For all three patients, the cause of death was reported as progressive disease and was considered by the investigator to be unrelated to study drug. In the Phase II studies, a total of 12 (5%) patients died within 20 days after the last study drug dose or died of a cause considered to be study drug-related at any time after the last study drug dose. Most deaths were due to disease progression unrelated to treatment. Two deaths were considered study drug related. One patient received 4 doses and died of a cardiopulmonary arrest 9 days after the last dose of study drug. The other received 6 doses and died of respiratory failure secondary to diaphragmatic failure, considered possibly due to myopathy, 39 days after the last dose of study drug.

All the 12 (5%) patients who died within 20 days after the last study drug dose or died of a cause considered to be study drug-related at any time after the last study drug dose received bortezomib 1.3mg/m^2. Additionally, 62 patients died >20 days after the last study drug dose; for all 62 patients, the cause of death was considered to be unrelated to study drug.

1.2 Laboratory findings

Episodic thrombocytopenia was seen, with a general progressive decrease in platelet count during the bortezomib dosing period (Days 1 to 11) and a return to baseline in platelet count during the rest period (Days 12 to 21) in each treatment cycle. An apparent trend towards an increase in hemoglobin and ANC (Absolute neutrophil count) across treatment cycles was noted. A trend towards decrease in ALC was noted across the 8 treatment cycles; however, no trend was noted by cycle. No significant effects were noted on renal or hepatic function. No apparent trend towards increase in glucose within or across treatment cycles was seen. No effects on electrolytes and calcium were noted.

1.3 Safety in special populations

The association between adverse events and intrinsic factors, including age, gender, race, body surface area, renal and liver function, KPS, proteasome inhibition, and myeloma subtype were explored. Whites experienced slightly higher incidence of fatigue, diarrhoea, pyrexia, and myalgia than non-whites. No apparent differences in Grade 3 or 4 events or adverse events leading to discontinuation were noted in patients with impaired renal function. The incidence of serious adverse events increased with decreasing renal function with most common events in renal and urinary disorders SOC. Neutropenia increased in incidence with decreasing renal function. No differences were noted in the types or severity of events in patients with creatinine clearance <30mL/day relative to the general population of patients treated. Limited data were available in patients with impairment of liver function. Higher rates of vomiting, blurred vision, anorexia, and weakness were seen among patients with lower KPS scores compared with those with higher KPS scores. Furthermore, higher rates of serious adverse events were noted in patients with lower KPS scores compared with patients with higher KPS scores. Incidence rates of Grade 3 or 4 adverse events, serious adverse events and discontinuations due to adverse events were not higher among patients with higher levels of inhibition of 20S proteasome activity compared to those with lower levels of inhibition of 20S proteasome activity. Patients with IgA myeloma appeared more likely to experience serious adverse events or adverse events leading to discontinuation than patients with IgG or other types of myeloma; however, review of specific serious adverse events and adverse events leading to discontinuation did not reveal any specific event that occurred with an increased incidence among patients with IgA myeloma relative to other subgroups.

Concerning extrinisic factors, increased rate of some adverse effects with prior exposure to thalidomide was reported (although this is not strictly a drug-drug interaction).

1.4 Immunological events

One patient in the Phase I studies, who had a primary diagnosis of multiple myeloma and received bortezomib 1.04mg/m^2, experienced an immunocomplex-mediated hypersensitivity reaction, described by the investigator as a serum sickness reaction, concurrent with maculopapular rash and dyspnoea after receiving 14 bortezomib doses. Study drug was discontinued because of these events. Serum sickness/hypersensitivity reaction and maculopapular rash each resolved within one week after treatment with prednisone. These events were assessed as Grade 3 in intensity and study drug related. These events also were considered by the investigator to be medically significant and therefore were reported as serious adverse events. No other immune-mediated reactions were reported in the Phase I studies. However, two additional cases of potential immunocomplex-mediated reactions were reported in the overall safety database (c.f. Discussion of clinical safety).

1.5 Safety related to drug-drug interactions and other interactions

No formal drug interaction studies have been conducted. The incidence of Grade 3 or 4 adverse events, serious adverse events, and adverse events leading to discontinuation were evaluated comparing patients who did and did not take the following classes of drugs: drugs affecting mineralization, growth factors, anti-hypertensives, narcotics, corticosteroids, anti-diabetic agents, substrates and inhibitors of both 2D6 and 3A4. Slight differences were noted in the rates of serious events for those using anti-hypertensives, anti-diabetic agents, and narcotics. During clinical trials, hypoglycemia and hyperglycemia were reported in diabetic patients receiving oral hypoglycemics. No unique safety issues account for these differences, and interpretation of the data may be confounded by the indication for which the concomitant medication was used. A higher rate of discontinuation in

patients taking corticosteroids may reflect progression of disease in these patients.

1.6 Discontinuation due to AES

Adverse Events Leading to Bortezomib Discontinuation in the Phase I Studies
Overall, 26 (21%) of 123 patients discontinued bortezomib because of an adverse event/toxicity in the Phase I studies. An apparent dose-relationship was seen with regard to the incidence of adverse events leading to bortezomib discontinuation, with 7% (2 of 29 patients), 16% (8 of 51 patients), and 37% (16 of 43 patients) of patients in the <0.7mg/m^2, 0.7 to 1.3mg/m^2, and >1.3mg/m^2 groups, respectively, discontinuing bortezomib because of an adverse event. Overall, the most commonly reported adverse events leading to bortezomib discontinuation were fatigue (4 patients; 3%) and dyspnoea (3 patients; 2%). All other adverse events leading to bortezomib discontinuation were reported for two patients or fewer.

At least one adverse event leading to bortezomib discontinuation was considered by the investigator to be study drug-related for 15 (12%) of 123 patients; 0% (0 of 29 patients), 8% (4 of 51 patients), and 26% (11 of 43 patients) in the <0.7mg/m^2, 0.7 to 1.3mg/m^2, and >1.3mg/m^2 groups, respectively. All study drug-related adverse events leading to bortezomib discontinuation were reported for two patients or fewer.

Adverse Events Leading to Bortezomib Discontinuation in the Phase II Studies
Overall, 28% of patients discontinued bortezomib because of an adverse event. Adverse events leading to bortezomib discontinuation most commonly reported included nervous system disorders (8%), general disorders and administration site conditions (6%), and blood and lymphatic system disorders (5%). The most commonly reported adverse events leading to bortezomib discontinuation included peripheral neuropathy NOS (5%), thrombocytopenia (4%), disease progression (3%), diarrhea (2%), and fatigue (2%). At least one adverse event leading to bortezomib discontinuation was considered by the investigator to be study drug-related for 17% of patients. Commonly reported adverse events leading to bortezomib discontinuation that were considered study drug-related included peripheral neuropathy NOS and thrombocytopenia (each 4%). Of the 71 patients who discontinued bortezomib because of an adverse event, the majority (62 of 71 patients; 87%) had not experienced a response (CR or PR) to treatment.

Updated clinical safety summary

Over 2,000 patients have been treated with bortezomib in Millennium-sponsored trials, in trials conducted by the National Cancer Institute, in investigator-sponsored trials and with commercially available medicinal product. The safety database for the Millennium-sponsored trials includes ~1000 patients who have received treatment for multiple myeloma, solid tumors, and other hematologic malignancies. The Applicant has reviewed the safety data from more than 700 patients with multiple myeloma, including 256 patients who participated in two Phase II studies of bortezomib (M34100-024 and M34100-025) (as presented in the CTD) and for the 475 patients who are participating or have participated in ongoing clinical studies of bortezomib in patients with multiple myeloma (Studies M34101-029, M34101-039, and M34101-040). In addition, safety data from over 200 patients who have received bortezomib in clinical studies sponsored by Millennium in indications other than multiple myeloma have been reviewed. The ability to detect rare events has therefore increased.

Acute infusion-related life-threatening complications have not been observed and intensive monitoring is not required after the bolus injection.

A cumulative dose related peripheral sensory neuropathy occurs and is more prominent in patients with a baseline condition. Severe myelosuppression is uncommon compared with traditional chemotherapy agents. This is reflected in the low incidence of Grade 4 neutropenia and thrombocytopenia and their attendant complications of bleeding and febrile neutropenia with sepsis. The absence of alopecia and mucositis (despite other GI side effects) also distinguishes this novel cytotoxic agent.

The adverse event profile emerging in the Phase III study to date appears consistent with that seen in the Phase II studies. Overall, the most commonly reported adverse events were nausea (31%), diarrhea NOS (27%), fatigue (26%), constipation (16%), and headache NOS (15%). Although the adverse

event profile was similar to that seen in the Phase II studies, the incidence rates of commonly reported adverse events generally were lower in Study M34101-039 than in the Phase II studies, which may be reflective of the less advanced disease stage among patients in this study.

The Applicant has identified potentially concerning adverse events that may be associated with bortezomib treatment and that have occurred at low incidences in the clinical program and continues to closely monitor new occurrences of such events. Such events include serious bleeding events, orthostatic hypotension, seizure activity, mental status changes/confusion and other serious psychiatric disorders, immune-mediated reactions and other systemic allergic reactions, tumour lysis syndrome, pulmonary hypertension, cardiac/cardiopulmonary arrest, pancreatitis, amyloidosis, and liver function test abnormalities. The incidence of such events was generally more common among patients with advanced disease participating in Study M34101-040 than in those with less advanced disease.

Discussion on clinical safety

Peripheral neuropathy is clearly among the most important undesirable effects of bortezomib and of several other treatments multiple myeloma patients may be prescribed. In the Phase II main study, approximately one third (34%) of patients experienced treatment-emergent peripheral sensory neuropathy. The incidence of Grade 3 peripheral neuropathy was 12%. It appears that patients with pre-existing peripheral neuropathy or symptoms of peripheral neuropathy (80% in this study) are likely to experience worsening of these symptoms during treatment with bortezomib. Reversibility was reported in 15% of patients. However, the data on the reversibility are limited and at short-term. In the Phase II studies, no cases classified as motor neuropathy were reported, but this possibility has not been excluded by systematic studies. The possible development of autonomic neuropathy, which may responsible for the observed cases of postural/orthostatic hypotension and syncope, should be closely monitored.

Very common Grade 3 toxicity included thrombocytopenia and neutropenia. Thrombocytopenia and neutropenia were also the most common Grade 4 adverse events. The onset of adverse events in the course of treatment was variable. Typically, gastrointestinal adverse events and fatigue had an early onset during the first treatment cycles and persisted for several cycles. Thrombocytopenia was typically episodic, with progressive decrease in platelet counts during the treatment cycle, and return to baseline during the rest period. Serious haemorrhagic complications were reported in the current database. They are an obvious, but to a large extent preventable and manageable risk. Neutropenia of any grade was common, but febrile neutropenia was reported in <1% of patients. Importantly, the data do not suggest cumulative bone marrow toxicity.

The safety update provided by the Applicant in its Response document identifies a number of potentially concerning adverse events reported during the clinical development that may be associated with bortezomib. These include serious bleeding events, orthostatic hypotension, seizures, mental status changes/confusion and other serious psychiatric disorders, immune-mediated reactions and other systemic allergic reactions, tumour lysis syndrome, pulmonary hypertension, cardiac/cardiopulmonary arrest, pancreatitis, amyloidosis, and liver function test abnormalities. New concerns not reported in the original MAA submission are cases of hepatitis and seizures. These undesirable effects will remain under close surveillance. A warning has been included in section 4.4 of the SPC about the possibility of developing moderate to severe thrombocytopenia and bleeding and the suggestion that patients with risk factors for bleeding should be monitored carefully and prophylactic platelet transfusions should be considered in thrombocytopenic patients with high risk of bleeding. In the presence of severe thrombocytopenia (<50,000/µL) and any major risk factors for bleeding, a careful evaluation of the risk/benefit ratio of continued therapy with bortezomib should be performed, as proposed for patients who have experienced life-threatening bleeding during treatment with bortezomib. Predisposing factors to haemorrhage include thrombocytopenia and, in some cases, the presence of extramedullary disease in sites such as the liver and CNS.

No clear signal for CNS toxicities had been noted at the time off the MAA, based on review of possible CNS toxicities in the Phase II studies. However, neurological and psychiatric disorders continue to be reported and must be closely monitored in view of the possibility of proteasome inhibition-related neurodegenerative disorders. It is even possible that these reactions may have a prolonged lag time before becoming clinically identifiable. Seizure activity has been reported. Seizure and psychiatric disorders might be related to confounding risk factors or concomitant diseases.

Preliminary data from study –039 suggested that cardiovascular reactions and seizures may be more frequent in the bortezomib group compared to dexamethasone. Patients with risk factors for seizure activity e.g., hyponatremia, need to be monitored for this event.

Although not commonly reported as a serious adverse event, bortezomib treatment can cause orthostatic/postural hypotension. These events are observed throughout therapy and are not acute reactions associated with bortezomib administration. Caution should be used when treating patients with a history of syncope, patients receiving medications known to be associated with hypotension, and patients who are dehydrated. The management of orthostatic/postural hypotension may include adjustment of antihypertensive medications, hydration, or administration of mineralocorticoids. The Applicant has highlighted the rare cases of tumour lysis syndrome, since they illustrate specific management principles in patients at risk. Bortezomib is cytotoxic and in patients with a high tumour burden, additional hydration, allopurinol, and close observation are advised. Asymptomatic increases in amylase has been reported rarely. The Applicant has recently reviewed the safety database and has found no evidence of any signal for pancreatitis. Careful monitoring of blood amylase levels will continue. Hepatitis (elevation in liver enzymes) has been recently reported in a small number of patients; the Applicant will continue to closely monitor for this event to evaluate potential risk factors or etiology.

The Applicant argues that no clear relationship was found between the level of proteasome inhibition and the incidence of adverse events. This is not totally unexpected, since proteasome inhibition was measured one hour after bortezomib administration. An analysis of relationship between trough level of inhibition and adverse events would have been more appropriate. A correlation with cumulative dose and many of the key adverse events was found.

During the update period hypoglycaemia has been reported (1%) and has been included in the SPC. Two of the patients were diabetics who had a history of stable control prior to bortezomib and required dose reduction of antidiabetic medication. A cautionary sentence was added in Section 4.4 of the SPC regarding concomitant use with oral hypoglycaemics, and appropriate information in section 4.5. It is unclear if this is a pharmacodynamic and/or pharmacokinetic interaction.

Bortezomib was not studied in children and in patients with impaired renal or hepatic function. Only few data are available from post-hoc analyses in patients with kidney or liver function impairment. No formal (pre-planned) PD and PK studies were undertaken on special populations, to evaluate the effect of age, gender, race or organ function (such as liver or kidney). The assessment of safety in special populations rests on such sub-group analyses, which are of limited value due to the small number of patients studied. This lack of data is of concern especially in patients with renal insufficiency, a condition frequently present in MM patients. Available data are only based on 10 patients with creatinine clearance $\leq$30mL/min. These reportedly had the same type or severity of adverse events reported in the overall study population, in which however the incidence of serious adverse events, cardiac disorders, and neutropenia increased with decreasing renal function. Due to lack of experience, severe renal impairment should be listed as a contraindication. The same applies to liver impairment, which is expected to have a major impact on pharmacokinetics.

Bortezomib treatment may be associated with immunological events (e.g. immunocomplex-mediated reactions). Three individual reactions described may represent immunocomplex-mediated reactions. It should be noted that in 2 of the 3 patients, treatment with bortezomib was continued after symptom resolution with no recurrence of these symptoms. One patient, who had received 1.04mg/m^2 in a Phase I study, experienced an immunocomplex-mediated hypersensitivity reaction, described as a serum sickness reaction and classified as severe, with maculopapular rash and dyspnea, which resolved within one week after treatment with prednisone. Another patient experienced rapidly progressive acute renal failure with proliferative glomerulonephritis (as determined by renal biopsy) with no obvious precipitating cause after completion of treatment in Cycle 3. Renal failure resolved after treatment with IV methylprednisolone followed by oral prednisone. The third patient experienced diffuse polyarthritis concurrent with an erythematous rash after completion of Cycle 3. The patient received treatment with prednisone, and the event resolved.

No drug-drug interactions studies were conducted.
Reports of haemolytic anaemia, deafness/hypoacusis and inappropriate ADH secretion have been received, and the applicant has committed to discuss causality in the first periodic safety update report

(PSUR). In study 039, the DMC noted that cardiovascular reactions and seizures may be more frequent in the bortezomib group compared with dexamethasone. The applicant has included these ADRs in the SPC.

A Phase I/II trial of single-agent bortezomib patients with primary amyloidosis is planned. This trial should provide some exploratory insight into the efficacy and safety in this disease. Once the results from this trial will be available, the applicant will consider the feasibility of conducting a randomized controlled trial in patients with amyloidosis to rule out the possibility that secondary to proteasome inhibition bortezomib treatment may increase the risk of amyloidosis and/or may have an adverse impact on its progression and organ manifestations.

The Applicant has committed to carry out a separate study to look into the possibility of autonomic neuropathy. The Applicant has committed to adopt a pharmacovigilance plan covering the major safety concerns identified by the CPMP, namely amyloidosis, cardiovascular, neurological, psychiatric and immunological reactions and tumour lysis syndrome. The Applicant will continue to undertake proactive pharmacovigilance and close monitoring of AEs of concern for bortezomib.

In summary, the applicant has shown that fatigue and gastrointestinal side effects are the most common undesirable effects but do not require premedication in all patients. The most troublesome side effect for patients is a cumulative dose-related peripheral sensory neuropathy, which is more prominent in patients with baseline neuropathy. Early detection and appropriate dose reduction is the approach to management of this side effect. Severe myelosuppression and complications of myelosuppression are uncommon compared with traditional chemotherapy agents. This is reflected in the low incidence of Grade 4 neutropenia and thrombocytopenia and the attendant febrile neutropenic infections and bleeding. Acute infusion-related life-threatening complications have not been observed and intensive monitoring is not required after the bolus injection. The absence of alopecia also distinguishes this novel cytotoxic agent. Further experience is accumulating concerning the use of bortezomib in combination with other chemotherapy agents, radiation therapy and biological agents under ongoing clinical trials sponsored by Millennium and by the NCI. At this time, additive and/or synergistic toxicities have not been observed with the agents studied.

5 Overall conclusions, benefit/risk assessment and recommendation

Quality

Benefit Risk

The overall quality of both the drug substance bortezomib and the drug product VELCADE is sufficiently characterized.

During the development one of the main problems was related to the purity specifications of drug substance and, consequently , drug product. Many impurities, including chiral ones, arise from the synthetic pathway of bortezomib, which involves stereoisomeric starting materials. Furthermore several degradation impurities have been reported as possible contaminants.

A thorough discussion has taken place on identification and quantification of impurities in the drug substance as well as their origin and reflection on the specifications.

The initial specifications for assay and related substances have been revised during the registration procedure and lower limits have been introduced according to batch analysis of more recently produced batches. Moreover the applicant has made efforts to identify the related substances and to synthesise them independently. Although in most cases the applicant succeeded in identifying the related substances, impurities O and M not in all cases it has ben posscould not beible to obtained the impurities in sufficient amount and suitable purity for analytical validation. The applicant has committed to revaluate drug substance specifications for both assay and related substances once data from the first ten commercial lots will be available. They have also committed to improve the separation method for impurities so as to re-evaluate the combined reporting of three impurities (M, N and F).

Qualification of impurities was correctly done using batches prepared for toxicological studies. These batches contained the same levels of impurities as those contained in commercial lots. The purity specifications are therefore acceptable from the toxicological point of view.

Presently the drug substance specifications include appearance, identity, assay, chiral purity, specific rotation, related substances, residual solvents, heavy metals, endotoxins and bioburden and are assessed by either pharmacopeial methods or validated methods. They are acceptable.

Finally the drug substance is very instable and has to be stored at –20°C -- 5°C protected from light. A retest period of 9 months only has been presently granted.

Concerning the development of the drug product, it had to be taken into account that bortezomib is unstable in solution, thermolabile, photosensitive and sensitive to gamma irradiation. The finished product required was a sterile, pyrogen free solution to be injected intravenously.

The problem of stability encountered with the liquid formulations used in clinical trials has been overcome by developing a lyophilised dosage form, in which mannitol is used as the only excipient acting as a bulking as well as a stabilizing agent. During lyophylisation the drug substance forms a 1:1 mannitol boronic ester, which stabilises the boronic acid. During reconstitution of the lyophilised cake in saline solution and even during subsequent intravenous administration an equilibrium between the mannitol ester and the boronic acid (bortezomib) is established which results into the complete hydrolyzation of the ester to the active substance bortezomib.

The sterilization method using sterile filtration and aseptic processing is justified (accordingly the specific guidelines CPMP/QWP/054/98) by the intended lyophilized dosage form.

The suitability of the container closure system has been demonstrated by stability studies. The compatibility of the product with the reconstitution solvent , normal saline, has been demonstrated in stability studies.

Since the overall manufacturing process of the finished product is considered a non-standard process, full process validation data for three consecutive batches at production scale have been requested and provided. All acceptance criteria foreseen by the validation scheme were met and drug product manufacturing process is considered validated. A point was raised during the evaluation of the manufacturing process concerning the bioburden of the solution before sterile filtration that should comply with the limits posed by the EU guidelines on the manufacturing of finished product. The company has declared that the action limit for bioburden of bulk solution before aseptic filtration has been revised to 10 CFU's/100 mL accordingly the EU guidelines.

The finished product specifications include appearance, identification, assay, impurities, moisture, residual solvents, content uniformity, sterility, and bacterial endotoxins. Additional specifications for the reconstituted vial include: reconstitution time, colour/clarity, and particulate matter. Methods are validated when necessary. Presently the specifications are acceptable. However, with regard to assay and purity, it has to be reminded that the commitment to re-evaluate specifications for both assay and related substances, once data from the ten commercial lots will be available, also applies to the finished product. New stability studies have allowed a shelf life of 24 months to be granted.

Non-clinical pharmacology and toxicology

Codering the proposed clinical indication, the pre-clinical toxicological profile is sufficiently characterised. Bortezomib was positive for clastogenic activity (structural chromosomal aberrations) in the *in vitro* chromosomal aberration assay using Chinese hamster ovary cells at concentrations as low as 3.125µg/ml, which was the lowest concentration evaluated. Bortezomib was not genotoxic when tested in the *in vitro* mutagenicity assay (Ames assay) and *in vivo* micronucleus assay in mice. Positive genotoxicity results are common for cytotoxic agents, and could be considered acceptable in the overall evaluation of the risk/benefit ratio for a product intended to treat multiple myeloma.

Developmental toxicity studies in the rat and rabbit have shown embryo-fetal lethality at maternally toxic dosages, but no direct embryo-foetal toxicity below maternally toxic dosages. Fertility studies were not performed but evaluation of reproductive tissues has been performed in the general toxicity

studies. In the 6-month rat study, degenerative effects in both the testes and the ovary have been observed. It is, therefore, likely that bortezomib could have a potential effect on either male or female fertility. Peri- and postnatal development studies were not conducted.

In multi-cycle general toxicity studies conducted in the rat and monkey, the principal target organs included the gastrointestinal tract resulting in vomiting and/or diarrhea, hematopoietic and lymphatic tissues resulting in peripheral blood cytopenias and lymphoid tissue atrophy and hematopoietic bone marrow hypocellularity, peripheral neuropathy (observed in monkeys, mice and dogs) involving sensory nerve axons, and mild changes in the kidneys. All these target organs have shown partial to full recovery following discontinuation of treatment.

Based on animal studies, the penetration of bortezomib through the blood-brain barrier appears to be limited, if any and the relevance to humans is unknown. However, the neurological dysfunctions reported in dogs and the neurotoxicity reported in humans made the issue about the potential penetration of bortezomib in the nervous system of some concern. The applicant has provided additional reassuring information on the penetration of bortezomib into the CNS but some concerns remain on this topic, which the applicant committed to carefully monitor in man. Studies are ongoing to clarify the mechanism responsible for the peripheral neuropathy observed in monkeys, mice and dogs, believed to be due to a dose-related disruption of the normal homeostasis of some of the cytosolic proteins involved in axonal transport.

Efficacy

The efficacy of bortezomib was evaluated at the recommended dose in an open-label, single-arm, multi-centre study of 202 patients with relapsed and refractory multiple myeloma, who had received at least 2 prior lines of treatment and who were progressing on their most recent treatment.

Median age of the patients in this trial was 59 (range: 34 to 84 years), 21% had platelet counts <75 x 109/l and 44% had haemoglobin <100g/l at study entry. 60% were diagnosed with IgG myeloma, 24% with IgA myeloma and 14% with light chain myeloma. 15% had a known tumour chromosome 13 deletion. Median B2 microglobulin was 3.5mg/l. These patients received a median number of six lines of prior therapies including steroids, alkylating agents, anthracyclines, thalidomide and stem cell transplants.

Bortezomib induced a complete remission in 10% and an overall response in about 30% of relapsed and refractory MM patients, where an overall response to treatment of greater than 10% and any complete remission are unprecedented. Data are further supported by the consistency of results of the two independent cohorts of patients, each reviewed by an independent response committee. Results for response rate, TTP, survival, showed consistent benefit. Although these results are clearly encouraging, comparative trials are required in order to better understand the association between response and duration of survival and the clinical benefit of bortezomib in the target population.

The outstanding nature of the benefit claimed for bortezomib needs to be confirmed under two points of view: the size of the benefit (the proportion of patients with CR) and the kind of patients who benefit (how homogeneous was the study population).

Concerning the size of the benefit, the response rates reported above are encouraging and can be considered outstanding in an advanced and refractory MM population. However, an independent evaluation by the FDA identified only 5 (Blade IF-) CR among 188 eligible cases, which represents a proportion of CR (2.7%, 95% CI: 1-6%) much lower than claimed.

The lack of x-ray confirmation of a stable bone disease does not seem to have lead to overestimation of the reported CR rate as it was shown that the response rate was similar in patients who underwent per protocol X-rays compared to overall population

As for the homogeneity issue, the overall study population enrolled in the pivotal trial can hardly be considered homogeneous with regards to the availability or unavailability of “last-line” treatment options. However, approximately 2/3 of the patient population may be considered homogeneous at least with regard to prior therapies received and the refractory status. In the subset of study –025 patients who had received prior anthracyclines, alkylating agents and steroids, and who were

refractory to their last treatment and who had skeletal follow-up as per protocol (n=64), the applicant has convincingly shown that the CR+PR rate was outstanding. There were 9 patients with CR (14.0%) and 25 CR + PR (39.0%). Moreover, sensitivity analyses showed that the percentage of CR/PR was similar regardless of the number and types of treatments received by patients prior to entering the study. The mean number of prior regimens administered was 6 with a range of 2 to 15. The lack of standard entry criteria yielded heterogeneity, which indicates that in reality randomisation to alternative active comparators would have been possible. The majority of patients (91%) were determined to be refractory to their previous treatment and the CR + PR rate in this subgroup of patients was virtually identical to the intention-to-treat population.

Safety

Fatigue and gastrointestinal effects are the most common undesirable events. The most troublesome adverse effect for patients is a cumulative dose-related peripheral neuropathy, mainly sensory but possibly including autonomic neuropathy. SAE include: bleeding, orthostatic hypotension, psychiatric disorders, immunocomplex-mediated reactions and other systemic allergic reactions, tumour lysis syndrome, pulmonary hypertension, cardiac arrest, pancreatitis, amyloidosis, and liver function test abnormalities. Seizure and hepatitis have been identified more recently, along with potential interaction with oral antidiabetic medications. A recent safety update on 981 patients has confirmed issues like neurotoxicity, and bleeding. Thrombocytopenia is a very common undesirable effect of bortezomib with potential important clinical sequelae.

Acute, infusion-related, life-threatening complications have not been observed and intensive monitoring is not required after the bolus injection. Severe myelosuppression and complications of myelosuppression are uncommon compared with traditional chemotherapy agents. The absence of alopecia also distinguishes this novel cytotoxic agent.

Benefit/risk assessment

Depending on the different evaluation criteria adopted, bortezomib induced a complete remission in 2.7-10% and an overall response in up to about 30% of relapsed and refractory MM patients, where an overall response to treatment of greater than 10% and any complete remission are unprecedented. Although these results are encouraging and are considered outstanding in advanced and refractory MM, randomised comparative trials are required in order to better understand the association between response and duration of survival and the clinical benefit of bortezomib in the target population.

The recently terminated Phase III study testing bortezomib ($1.3 mg/m^2$) versus high dose dexamethasone in patients with relapse or refractory MM will provide additional information about the benefit of the drug under evaluation in terms of tumour response, TTP and other measures of clinical benefit in a slightly different population of patients with less advanced MM compared to study -025. Following the interim analysis in December 2003, the Data Monitoring Committee recommended the trial be stopped, since the efficacy analysis showed that the main endpoint (time to progression) had crossed the pre-defined boundary for early termination and was highly significant ($P<0.001$) to the advantage of bortezomib. The applicant has committed to provide a full report on the results of this study.
The toxicity profile appears acceptable and within the usual range of other standard and experimental cytotoxic therapies. Further experience is accumulating concerning the use of bortezomib in combination with other chemotherapy agents, radiation therapy and biological agents under ongoing clinical trials. So far, additive and/or synergistic toxicities have not been observed with the agents studied.

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered that the benefit/risk ratio of bortezomib in the treatment of patients with multiple myeloma who have received at least two prior therapies and have demonstrated disease progression on the last therapy, was favourable and therefore recommended the granting of the marketing authorisation under exceptional circumstances.