A randomized phase III study on the effect of Bortezomib combined with Adriamycin, Dexamethasone (AD) for induction treatment, followed by High Dose Melphalan and Bortezomib alone during maintenance in patients with multiple myeloma

A HOVON AND GMMG PROTOCOL

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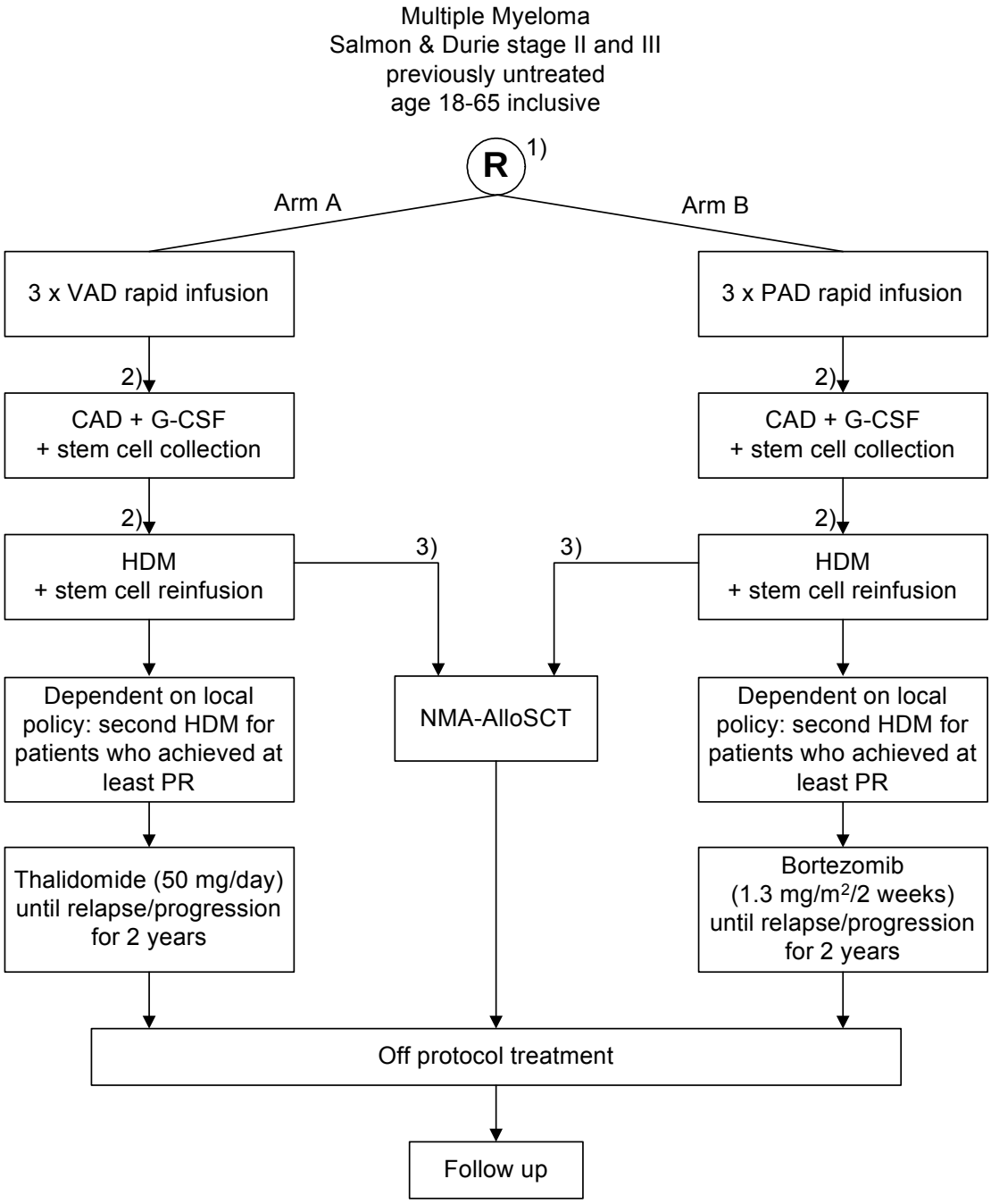
1 Scheme of the study



1) Inclusion of patients in the ongoing Zoledronate trials is strongly recommended.

2) Patients who do not meet the inclusion criteria for CAD or HDM but with a CR, PR or MR, may proceed with 3 more cycles of VAD (Arm A) or PAD (Arm B), followed by Thalidomide maintenance (Arm A) or Bortezomib maintenance (Arm B) for 2 years until progression.

3) Patients with an HLA-identical family donor who meet the egilibility criteria proceed with non-myeloablative allogeneic stem cell transplantation after the first course of HDM. It is strongly recommended to include patients in ongoing non-myeloablative AlloSCT trials.

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3 Synopsis

Study phase	Phase III
Study objectives	Evaluation of the effect of Bortezomib in addition to AD and High Dose Melphalan for induction and maintenance treatment
Patient population	Patients with multiple myeloma, previously untreated, Salmon & Durie stage II or III, age 18-65 years inclusive
Study design	Prospective, multicenter, randomized
Duration of treatment	Expected duration of induction, stem cell collection and intensification (with or without Bortezomib) is 6 - 7 months. Maintenance therapy with Bortezomib or Thalidomide will be given for 2 years
Number of patients	800 patients registered and randomized
Adverse events	Adverse events will be documented if observed, mentioned during open questioning, or when spontaneously reported.
Planned start and end of recruitment	Start of recruitment: II 2005 End of recruitment: II 2008

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5 Introduction

Multiple myeloma is a malignancy of the plasma cells. It represents the second most common hematological malignancy. The annual incidence rates in northern Europe are 4-5/100.000. Approximately 850 cases of multiple myeloma are diagnosed in the Netherlands each year. Multiple myeloma is uniformly fatal. As the disease progresses, morbidity and eventual mortality are caused by reduced immunoresistance to infections, significant skeletal destruction (with bone pain, pathological fractures, and hypercalcemia), anemia, renal failure, and, less commonly, neurological complications and hyper viscosity. Despite the use of high-dose chemotherapy and autologous stem cell transplantation, this cancer remains incurable. The 5-year survival rate for patients with multiple myeloma among patients treated with conventional chemotherapy is 25%, while with intensified therapy this may increase to 50 %. Novel agents are urgently needed to improve the treatment results of this disease.

5.1 Conventional therapy

Multiple myeloma is a hematological malignancy characterized by a proliferation of monoclonal plasma cells, which produce a homogeneous immunoglobulin (M-protein), which can be detected in the serum and/or the urine. It accounts for approximately 1% of all malignancies and 10% of hematological cancers[1]. For several decades intermittent Melphalan and prednisone has been the treatment of choice. Many trials with combination chemotherapy have been performed, but these did not result in an improved outcome as compared to Melphalan and prednisone[2,3]. Fifty to sixty percent of patients respond to conventional chemotherapy and only a minority (< 5%) of patients achieve a complete response[4]. Virtually all patients succumb to refractory disease and the median overall survival is less than 3 years. Even after having achieved a response, patients may remain symptomatic due to a considerable residual tumor load.

5.2 Intensive treatment

High dose chemotherapy for myeloma was introduced in 1983 showing for the first time that in a substantial percentage of patients complete remissions could be induced[5]. Morbidity and mortality however were high, but were strongly reduced later by the application of autologous stem cell rescue. Bone marrow was the source of stem cells in the first studies, peripheral blood stem cells (PBSC) are now routinely applied as autologous rescue[6].

In 1996 a randomized study has been published which showed that autologous transplantation was superior to conventional treatment regarding response rate, event-free and overall survival. In this study patients under 65 years were randomized at diagnosis to receive VBAP/VMCP or High Dose Melphalan 140 mg/m^2 and TBI 8 Gy supported with autologous bone marrow collected after 2 courses of

VBAP/VMCP[7]. Subsequently, a study from the UK again showed a survival benefit from high-dose therapy over standard therapy. In 1994 the Nordic Myeloma Study Group (NMSG) started a study with high-dose chemotherapy in newly diagnosed patients under 60 years[8]. After induction therapy with VAD followed by stem cell collection after Cyclophosphamide 4 g/m^2 and G-CSF, patients received Melphalan 200 mg/m^2 with stem cell rescue. Survival in the intensive group was significantly prolonged as compared to the control group. The control patients were selected from a historic population of 313 patients identified from 5 previous population-based Nordic studies. Of these, 274 fulfilled the eligibility criteria for the high dose therapy in the NMSG group.

5.3 Double transplantation

Attempts have been made to improve the outcome of myeloma by performing double transplants. The rationale of this approach was based on the observation that the achievement of CR after intensive therapy was a favorable prognostic factor for EFS and OS. The largest series of double transplants has been performed by the group led by Barlogie[9]. In previously untreated patients the CR rate increased from 26% after the first transplant to 41% after the second. Median OS and EFS durations were 68 months and 43 months, respectively. On multivariate analysis, superior EFS and OS were observed in the absence of unfavorable karyotypes (11q breakpoint abnormalities, -13, or 13-q) and with a low β_2-microglobulin at diagnosis. Using case-matched registry data as controls double transplants improved response rate, EFS and OS as compared to conventional treatment. In a recent update of results of tandem transplants in 1000 patients the adverse impact of chromosome 13 deletion was established.
In a recently completed randomized study by the “Intergroup Français de Myelom” (IFM), single versus double stem cell transplantation was compared in previously untreated patients. The results show that patients with a low β_2-microglobulin at diagnosis had a slightly better OS after double transplants[10]. However no improvement of outcome was found in patients with unfavorable prognostic factors like a high β_2-microglobulin and/or deletion of chromosome 13[11]. A retrospective EBMT registry study showed that double transplants, planned or unplanned at the time of the first transplant may be superior to single transplants. Cavo et al found in a small series of patients significantly improved CR rate and EFS in patients following double transplant[12].
In 1996 HOVON initiated a phase III study in which single intensive therapy without stem cell rescue was compared with double high-dose chemoradiotherapy including stem cell rescue. After induction therapy with VAD patients were randomized between arm A: High Dose Melphalan divided into 2 courses of i.v. Intermediate Dose Melphalan (IDM, Melphalan 70 mg/m^2) followed by maintenance therapy with α-Interferon and arm B: High Dose Melphalan divided into 2 courses of IDM followed by myelo-ablative treatment (Cyclophosphamide,120 mg/m^2 and TBI 8 Gy) with PBSCT followed by maintenance with α-Interferon. Stem cells were mobilized after VAD with Cyclophosphamide 4 g/m^2 and G-CSF. The study was closed April 1, 2000, after the inclusion of 453 patients. An interim analysis performed in March 2004 showed that there is an ongoing improvement of the response rate with every treatment step. In

addition, progression-free survival and event free survival were significantly superior in patients having received myeloablative treatment[13]. For definite firm conclusions about the effectiveness of tandem transplants further follow-up of the French randomized study seems warranted. In addition it is evident that the quality of the obtained response is generally better, with a significant number of complete responses, using standard response criteria[14]. Therefore, high-dose Melphalan will remain a standard treatment for younger patients with multiple myeloma.

5.4 Bortezomib

Bortezomib (VELCADE® ,PS-341, JNJ-26866138) is a small molecule proteasome inhibitor which is being developed through a joint collaboration between Millennium Pharmaceuticals, Inc. and Johnson & Johnson Pharmaceutical Research & Development. Bortezomib is a potent, reversible, and specific inhibitor of the proteasome and represents a first-in-class anti-neoplastic cytotoxic agent that is distinguished from conventional cytotoxic agents by a favorable side effect profile, including its lack of significant myelosuppression, hair loss and mucositis. Bortezomib is a modified dipeptidyl boronic acid derived from leucine and phenylalanine; its chemical name is N-pyrazinecarbonyl-L-phenylalanine-L-leucine boronic acid and has a molecular weight of 384.25 daltons.

Inhibitors of the 26S proteasome act through multiple mechanisms to suppress tumor survival pathways, arrest tumor growth, tumor spread, and angiogenesis. Unlike conventional chemotherapeutics, Bortezomib represents a novel class of anti-cancer agents because it has the ability to affect a combination of cellular regulatory mechanisms. This multiple mechanistic approach potentially represents a more effective anti-cancer strategy compared to the anti-tumor activity afforded by conventional chemotherapy.

The mechanisms of anti-tumor activity that have been established for Bortezomib involve many pathways thought to be integral to cancer treatment strategies. The following mechanisms have been demonstrated in *in-vitro* and *in-vivo* experiments:

Directly induces apoptosis of tumor cells

- Inhibits activation of NF-κB in cells and in tumor microenvironment
- Reduces adherence of myeloma cells to bone marrow stromal cells
- Blocks production and intracellular signaling of IL-6 in myeloma cells
- Blocks production and expression of pro-angiogenic mediators
- Overcomes defects in apoptotic regulators, such as Bcl-2 overexpression and alterations in tumor suppressor p53
- Activity is cell-cycle independent
- Stabilizes cell cycle regulatory proteins
- Unaffected by drug efflux pumps

- Active under hypoxic conditions

Nonclinical Experience

Bortezomib has been studied extensively for its effect on various cellular functions requiring the ubiquitin-proteasome pathway in both in vitro and in vitro systems. A complete review of the nonclinical data is provided in the Treating physician's Brochure.

Bortezomib's biological and antineoplastic activity has been demonstrated in multiple murine syngeneic and human xenograft models[15]. Weekly and twice weekly IV administration of Bortezomib can significantly reduce tumor volume and delay tumor growth in these animal models both alone and in combination with cytotoxic agents[16,17].

Clinical Pharmacology

The clinical pharmacology program has been designed and partially carried out to investigate the disposition characteristics, and the pharmacodynamics of Bortezomib. While additional studies are planned from Phase 1 combination studies and Phase 2 and 3 single agent trials, conclusions from the completed investigations are:

- Upon IV bolus administration, Bortezomib displays a rapid distribution phase ($t_{1/2\alpha}$ < 30 minutes) followed by a longer elimination phase ($t_{1/2\beta}$ > 10 hours) and a large volume of distribution, all consistent with a 2-compartment PK model.
- The high volume of distribution, rapid distribution phase, prolonged biological effect ($t_{1/2}$~24 hours), and high potency (Ki = 0.6nM with slow off rate), along with *in vitro* metabolic studies suggest that de-boronation and proteolytic cleavage of Bortezomib at the cellular level represent the majority of the catabolism of this compound. This conclusion is based on extensive preclinical evaluation of the disposition characteristics, pharmacokinetics and the pharmacodynamics of Bortezomib. Inhibition of 20S proteasome activity occurs in a dose-related manner. The maximum pharmacodynamic effect on circulating whole blood 20S activity occurs within 1 hour of dosing. The relationship between Bortezomib plasma concentrations and proteasome inhibition is well described by a simple E_{max} model.

Clinical Experience

Bortezomib has been extensively studied in Phase 1 and Phase 2 studies, and the efficacy in multiple myeloma has been evaluated in a randomized, open-label Phase 3 study, M34101-039 (data subject to evaluation).

Phase 1 Clinical Experience

Data from 4 Phase 1 studies designed to evaluate the maximum tolerated dose (MTD) of Bortezomib and dose limiting toxicities (DLTs) in a variety of dose and dose schedules have been evaluated. The MTD of Bortezomib determined in these studies, regardless of individual protocol definition, appeared to

be dependent on the treatment schedule employed and the patient population treated. The MTDs and DLTs in these studies were as follows:

- The MTD of Bortezomib administered twice per week for 2 weeks followed by a 10-day rest period to patients with advanced solid tumors was determined to be 1.3 mg/m^2. At this schedule, DLT (fatigue, diarrhea, and peripheral neuropathy) was observed at 1.56 mg/m^2/dose.
- The MTD of Bortezomib administered once per week for 4 weeks followed by a 14-day rest period to patients with solid tumors is 1.6 mg/m^2. This was the least dose-intensive schedule but had the highest individual doses administered.
- The MTD of Bortezomib administered twice per week for 4 weeks in 8 dose cycles followed by a 14-day rest period to patients with hematologic malignancies was determined to be 1.04 mg/m^2. At this schedule, DLT (hyponatremia) and more frequently Grade 3 thrombocytopenia was observed at Bortezomib 1.04 mg/m^{2}and 1.38 mg/m^2.

In the Phase 1 studies neurotoxicity was observed, particularly a painful sensory peripheral neuropathy, that was dose-related, and more prevalent among patients previously treated with neurotoxic agents (e.g., platinum, thalidomide, vincristine and taxane-containing regimens) and dose-limiting in patients with refractory solid tumors.
Notable in the Phase 1 setting was the relatively low incidence of significant myelosuppression, febrile neutropenia, infections and transfusion-dependent thrombocytopenia or anemia, mucositis or alopecia; this also has been borne out in the Phase 2 data evaluated to date. Effects on the liver, kidney, and heart were rarely observed. Decreases in platelet count have been observed on treatment during both Phase 1 and Phase 2 studies and appear to be related to dose. Clinically significant thrombocytopenia can occur and appears to be influenced by baseline platelet count. Platelet count tends to recover during the rest period. Patients should be carefully monitored throughout treatment with Bortezomib for hematological abnormalities.
Although demonstration of efficacy was not a primary objective in the Phase 1 clinical studies, anti-tumor activity was observed in patients with squamous cell carcinoma of the nasopharynx, bronchoalveolar carcinoma of the lung, renal cell carcinoma, prostate cancer, lymphoma, Waldenström's macroglobulinemia, and multiple myeloma.

Phase 2 Clinical Experience

The safety and efficacy of Bortezomib were evaluated in an open-label, single-arm, multicenter Phase 2 study of 202 patients with relapsed and refractory multiple myeloma who had received at least 2 prior lines of treatment and were progressing on most recent therapy (SUMMIT)[18]. Patients with relapsed and refractory myeloma have an expected survival of 6-9 months.
The 202 patients had multiple poor prognostic factors at study entry including elevated β_2-microglobulin, poor hematopoietic reserve, evidence of organ dysfunction, abnormal renal function and chromosomal abnormalities. The median number of prior lines of therapy was six.

An IV bolus injection of Bortezomib 1.3 mg/m^2/dose was administered twice weekly for 2 weeks without routine pre-medication, followed by a 10-day rest period (21 day treatment cycle) for a maximum of 8 treatment cycles. Patients who experienced benefit from Bortezomib treatment were allowed to continue treatment in an extension study. Patients who experienced progressive disease after at least two cycles, or had stable disease after at least 4 cycles with Bortezomib were allowed, at their physician's discretion, to have high dose dexamethasone (40mg) added to their Bortezomib treatment.

Response rates to Bortezomib alone were determined by an independent response committee (IRC) based on criteria published by Blade et al., 1998[14].

Complete remission required 100% reduction in M-protein and includes patients whose immunofixation was negative (IF-) and positive (IF+). All 202 patients were evaluable for time to event analyses. A total of 193 patients were evaluated for response (9 patients with nonmeasurable disease could not be evaluated for response by the IRC). In SUMMIT, Bortezomib demonstrated an overall response rate (CR+PR+MR) of 35% with 59% patients experiencing improved or stable disease. A total of 10% of patients experienced a complete remission (4%IF- and 6% IF+). The median time to response was 38 days.

The median survival of all patients enrolled in SUMMIT was 16 months. The response rate was independent of the number or type of previous therapies. In addition, the rate of response remained consistent regardless of the patient's gender, race, body surface area, performance status, myeloma type or chromosome 13 deletion status. The median time to progression for all 202 patients enrolled in SUMMIT was 7 months. Median time to progression on their last previous therapy was 3 months and when using patients from SUMMIT as their own controls, the median time to progression was twice as long on Bortezomib relative to last therapy.

Effects on serum and/or urine monoclonal paraprotein and plasma cells from bone marrow aspirate and biopsy were also evaluated. Overall 70% of patients had either reduction or stable serum and/or urine paraprotein levels. A total of 69% of patients included in the analysis for bone marrow biopsy results had a ≥50% decrease in plasma cells, thereby demonstrating that treatment with Bortezomib reduces the number of or clears myeloma cells from the bone marrow. Bone marrow aspirate results were consistent with those obtained by biopsy.

Responders (CR+PR) in SUMMIT also had an increase in mean hemoglobin and decreased overall transfusion requirements; stable renal function; stable or improved Karnofsky Performance Status (KPS) and increased mean non-myeloma immunoglobulin levels (IgM, IgA and IgG). The mean IgM returned to the normal range by the end of treatment; 28% (15/53) of patients had increases of ≥ 2 fold in one of their non-myeloma immunoglobulins. An association between response rate and improvement in quality of life was apparent. Patients who responded to treatment experienced an improvement in EORTC-C30 Global and Physical parameters including a decrease in disease symptoms, pain and fatigue.

In SUMMIT, 74 patients were administered dexamethasone in combination with Bortezomib and were assessed for response. Eighteen percent (13/74) of patients achieved an improved response (MR or PR) with combination treatment.

The CREST study was a randomized open-label,single-arm, multicenter study which enrolled 54 patients with multiple myeloma that progressed or relapsed on or after front-line therapy (CREST). Bortezomib was administered twice weekly for 2 weeks followed by a 10-day rest period for a maximum of 8 treatment cycles as second line therapy.

Patients were prospectively randomized to receive either 1.0 or 1.3 mg/m^2/dose. A total of 28 patients received 1.0 mg/m^2/dose and 26 patients were administered 1.3 mg/m^2/dose. Patients who experienced benefit from Bortezomib treatment were allowed to continue treatment in an extension study. Patients who experienced progressive disease after at least two cycles or stable disease after at least 4 cycles with Bortezomib alone were allowed, at their physician's discretion, to have high dose dexamethasone (40mg) added to their treatment.

Both dose groups were similar with regard to demographic and baseline characteristics. The median age for all patients was 63 years; 57% had a Karnofsky performance status score of 90 to 100 with only 13% of patients with a score of ≤70; 19% had a hemoglobin level <100 g/l and no patients had a platelet count < 50 x 10^9/l. The median duration of time between diagnosis of multiple myeloma and first dose of Bortezomib was 2.0 years and patients had received a median of one prior line of treatment (median of three prior therapies), including: 98% of patients who received prior steroids, 72% of patients who received prior alkylating agents, 54% of patients who received prior anthracyclines, 48% of patients who received prior stem cell transplant, and 30% of patients who received prior thalidomide. The median time to progression for all patients treated was 11 months. Eighty (80%) percent of patients were alive at one year.

In CREST, the combination of Bortezomib and dexamethasone was administered to 28 patients, 16 patients in the 1.0mg/m^2 group and 12 patients in the 1.3mg/m^2 group. A total of 9 patients (32%) had an improved response (CR, PR or MR) with combination treatment (4 of the 16 patients receiving 1.0 mg/m^2 and 5 of the 12 patients in the 1.3 mg/m^2 group). Two of these 9 patients achieved a CR while receiving dexamethasone in combination with Bortezomib therapy.

A total of 51 patients entered the extension study. The initial data indicate that Bortezomib can be administered to patients with relapsed or refractory multiple myeloma for longer than 6 months with tolerability similar to the first 6 months of treatment. Patients were able to maintain their response or had an improved response with additional cycles of Bortezomib therapy.

Adverse Events Profile from Phase 2 studies with Bortezomib

The data described below reflect exposure to Bortezomib in 256 patients with multiple myeloma who were administered Bortezomib 1.0 or 1.3 mg/m^2/dose twice weekly for 2 weeks followed by a 10 - day rest period (21 day treatment cycle length) for a maximum of 8 treatment cycles. The median total dose administered across all 256 patients was 46 mg, median duration of treatment was 131 days, and median number of doses administered was 22 (6 cycles).

The most commonly reported adverse events were nausea (62%), fatigue (54%), diarrhea (48%), constipation (41%), thrombocytopenia (41%), pyrexia (36%), vomiting (34%), and anorexia (30%).

Events reported as peripheral neuropathy, peripheral sensory neuropathy and peripheral neuropathy aggravated were reported in 35% of patients. Notably, infusion reactions, infusion site reactions, alopecia, mucositis, febrile neutropenia and sepsis were rarely reported. Acute development or exacerbation of congestive heart failure has been seen in subjects with risk factors for or existing heart disease.

Thirteen percent of patients experienced at least one episode of Grade 4 toxicity, with most common events being thrombocytopenia (3%) and neutropenia (2%).

A total of 124 (48%) of the 256 patients experienced serious adverse events during the studies. The most commonly reported serious events included pyrexia (7%), pneumonia (7%), diarrhea (5%), vomiting (5%), dehydration (5%) and nausea (4%).

Adverse events leading to discontinuation were reported in 28% of patients. The reasons for discontinuation were evenly distributed across the most common types of toxicity and included peripheral neuropathy (5%), thrombocytopenia (4%), disease progression (3%), diarrhea (2%), and fatigue (2%). The majority of patients discontinuing treatment due to adverse events were not responding to therapy.

The addition of dexamethasone did not appear to adversely affect the safety profile of Bortezomib.

Fatigue

Fatigue was reported as a treatment-emergent adverse event in 54% of patients and was predominantly reported as Grade 1 or 2 in intensity. The first onset of fatigue was most often reported during the 1st and 2nd cycles of therapy. The event was Grade 3 in intensity for 11% of patients; no Grade 4 fatigue was reported. Most patients were able to continue therapy despite fatigue with only 2% of patients discontinuing treatment due to fatigue.

Pyrexia

Pyrexia was reported as an adverse event for 36% of patients and was assessed as Grade 3 or 4 in intensity for 4%. However, there was a low incidence of clinically significant infections (13%) and the incidence of febrile neutropenia (<1%) was lower than expected for this patient population.

Gastrointestinal Events

The majority of patients experienced treatment-related gastrointestinal events during the studies including nausea, diarrhea, constipation, and vomiting. These events tended to occur early in treatment (Cycles 1 and 2) and persisted for several cycles. Grade 3 gastrointestinal events occurred in 19% of patients, were serious in 12% of patients, and in rare cases vomiting and diarrhea were of Grade 4 severity. A minority of patients (5%) discontinued due to gastrointestinal events, while most patients were able to continue Bortezomib treatment either with or without the addition of anti-emetic or anti-diarrheal supportive therapies. Anorexia was reported as an adverse event for 30% of patients. The incidence of Grade 3 anorexia was low (2%); no patient experienced Grade 4 anorexia during the studies.

Peripheral Sensory Neuropathy

Events reported as peripheral neuropathy, peripheral sensory neuropathy and peripheral neuropathy aggravated were reported in 35% of patients. Peripheral neuropathy was Grade 3 for 13% of patients and Grade 4 for <1% of patients. New onset or worsening of existing neuropathy was noted throughout the cycles of treatment. Five percent (5%) of patients discontinued Bortezomib due to neuropathy. Notably, more than 80% of patients had signs or symptoms of peripheral neuropathy at baseline evaluation. The incidence of Grade 3 neuropathy was low (2 of 60 patients, 3%) in patients without baseline neuropathy. Symptoms may improve in some patients upon discontinuation of Bortezomib; complete resolution of peripheral neuropathy has been reported.

Hypotension

Hypotension (including reports of orthostatic hypotension) was observed at an incidence of 11%, of which half was considered Bortezomib-related. Most events were Grade 1 or 2 in severity. Grade 3 hypotension occurred in 3% of patients; no patient experienced Grade 4 hypotension. Patients developing orthostatic hypotension did not have evidence of orthostatic hypotension at study entry, although half had pre-existing hypertension and one third had evidence of peripheral neuropathy. A minority of patients with orthostatic hypotension experienced syncopal events. Orthostatic hypotension was not acutely related to infusion of Bortezomib.

Thrombocytopenia

Transient and uncomplicated thrombocytopenia was reported during treatment with Bortezomib for 42% of patients. The thrombocytopenia observed was characterized by a dose related decrease in platelet count during the Bortezomib dosing period (Days 1 to 11) with a return to baseline in platelet count during the rest period (Days 12 to 21) in each treatment cycle. Thrombocytopenia was assessed as Bortezomib related for 38% of patients and Grade 3 or 4 in intensity for 29% of patients. Three percent (3%) of patients experienced Grade 4 thrombocytopenia. All episodes of Grade 4 events were Bortezomib related. Four percent (4%) of patients discontinued Bortezomib treatment due to thrombocytopenia of any grade.

5.5 Thalidomide

Thalidomide has a broad spectrum of pharmacological and immunological effects and is now used in a wide spectrum of diseases like erythema nodosum leprosum and refractory Becet's disease[19]. Thalidomide has substantial antitumor activity in patients with advanced myeloma[20]. In 84 previously treated patients oral Thalidomide at a dose of 200-800 mg/day had a total response rate of 32%. After 12 months of follow-up EFS and OS for all patients was 22% and 58% respectively. At least 30% of patients had mild to moderate side effects - constipation, somnolence, neuropathy, rash, weakness and fatigue- while 10% of patients had severe adverse effects. Side effects were most frequent in the group with the

higher Thalidomide dosis. The promising results of Thalidomide in refractory myeloma have been confirmed by several other groups[21-25].
In general the response rate varies from 40- 50 % with a median response duration of 20 months. Addition of dexamethasone significantly improves the response rate. However, for maintenance lower dosages of Thalidomide are generally used, from 50 – 200 mg daily in order to avoid serious side-effects. The efficacy of low dose Thalidomide as maintenance therapy has already been confirmed in clinical studies. The combination of Thalidomide with corticosteroids has not been seriously investigated during maintenance therapy.
The mechanisms of action of Thalidomide are still not clear. Bone marrow vascularization is strongly increased in myeloma and it may be that Thalidomide inhibits angiogenesis thereby inducing apoptosis of myeloma plasma cells[21]. The microvascular density of bone marrow however did not change in responding patients. Other possible mechanisms of action may include a direct apoptosis inducing effect on myeloma cells, or indirectly influencing the growth and survival of myeloma cells by modulating adhesion molecules or the secretion of cytokines.
The optimal dose of Thalidomide is not known. The maximum tolerated dose varies substantially among patients. However very few patients tolerate the higher doses of 600 - 800 mg. The observation that in most responding patients M-protein levels begin to drop within the first weeks of treatment suggests that dose escalation may not be necessary to induce responses in myeloma and in that way unnecessary side effects can be avoided. The efficacy of low dose Thalidomide as maintenance treatment has already been confirmed in clinical studies[26,27]. A recent analysis in 138 patients indicates that the duration of disease remissions increases with the achieved cumulative dose of Thalidomide (B. Barlogie, personal communication).
Recently the outcome of several studies with Thalidomide alone or combined with Dexamethasone or Doxorubicin were presented at the IXth Myeloma Workshop (May 2003, Salamance, Spain). Important is that Thalidomide, especially when it is combined with Dexamethasone and Doxorubicin, may increase the risk on Deep Venous Thrombosis (DVT).

5.6 Prognostic factors

Like in other hematological malignancies, several adverse prognostic factors can be identified in multiple myeloma at diagnosis. Conventional adverse prognostic factors include β2-microglobulin ≥ 3.5 µg/l, serum albumin < 35 g/l, stage II/III according to the Salmon/Durie staging system, serum creatinin > 187 umol/l and low hemoglobin. Recently, a new staging system for multiple myeloma (International Staging System, ISS), which is based on serum β2-microglobulin and serum albumin, has been published [P. Greipp, 2005, J Clin Oncol; 23:3412-3420].
Recent studies indicate that specific chromosomal abnormalities have prognostic significance in multiple myeloma. Using conventional cytogenetics, partial or complete deletions of chromosome 13 and abnormalities of chromosome 11 are present in 20-25% of untreated myeloma. Other cytogenetic

abnormalities observed in multiple myeloma include t(4;14)(*mmset/fgfr3)*, t(14;16)(*c-maf*) and chromosome1 abnormalities, which are strong adverse prognostic factors. Using interphase FISH with specific probes for the retinoblastoma gene (rb-1) the frequency -of chromosome 13 deletions is much higher than found with metaphase analysis and varies from 33% up to 80% in different studies[28-34]. Despite the fact that FISH reveals 13q14 deletions in a much higher frequency than metaphase analysis, the presence of abnormal chromosome 13 remains the single most significant adverse prognostic factor. It is obvious that in every prospective myeloma trial "classic" metaphase analysis and interphase FISH to detect abnormal chromosomes 11 and 13 should be part of the pretreatment staging of patients.

5.7 Rationale of the study

This is a phase III study to test the efficacy and feasibility of Bortezomib combined with intensive treatment and Bortezomib as maintenance treatment as compared to standard intensive treatment and intensive maintenance therapy, which is nowadays one of the worldwide used therapies in younger patients with multiple myeloma. Finally, the overall efficacy of Bortezomib in relation to clinical and molecular prognostic factors in multiple myeloma will be tested.

The rationale for combining Bortezomib with *induction* chemotherapy is based on the different mechanisms of actions and the potential synergism of Bortezomib with cytostatics and/or Dexamethasone.
These assumptions were confirmed in a phase I/II study in 19 patients who were previously untreated which showed that Bortezomib (1.3 mg/m^2) can be safely combined with standard doxorubicin and dexamethason (PAD) and has greater antitumor activity than VAD chemotherapy alone (J Cavanagh, unpublished results[35]). In that study, 12/19 patients received 4 cycles of PAD, while 16 received > 1 cycle. Hematologic recovery was normal. The response rate after 4 PAD in 18 evaluable patients was 100 % PR/CR (69 % PR/CR after cycle 1). The median harvest of hemopoietic stem cell collection using a high dose cyclophosphamide mobilization was 3.4x10^6 CD34+/kg. The median recovery to neutrophils > 0.5 in 7 patients transplanted was 18 days. The major adverse events were sensory neuropathy grade 1 (35%) or grade 2 (5%) and painfull neuropathy (44%, 35% grade 1, 9% grade 2), which both recovered after the induction phase. These data indicate that PAD is feasible and effective. It should be noted that in these studies a rapid bolus administration of Adriamycin was used, based on the published schedule of rapid bolus administration of VAD instead of the conventional continuous infusion[36]. This schedule has now been widely accepted as standard administration of VAD, since it combines convenience with equal response results.

The rationale for Bortezomib *maintenance* treatment is based on the experience from the prospective MLN 039 study, where a weekly schedule of Bortezomib was well tolerated during maintenance proved effective in prolonging the response duration (P. Richardson, P. Sonneveld and D. Schenkein, abstract

submitted to EHA 2004[37]). The rationale for “low dose“ Thalidomide is based on the observation that refractory myeloma patients may be sensitive to low doses given over a prolonged period of time and the lack of evidence for a dose-response relation[26]. Novel data on the strong prognostic impact of chromosomal aberrations in patients with multiple myeloma make it possible to divide these patients into different prognostic groups. Therefore the potential benefit of Bortezomib can be evaluated in standard-risk patients (serum β_2-microglobulin $\leq$ 3.5 μg/l and normal chromosome 13 as determined by FISH) and high-risk patients (β_2-microglobulin > 3.5 μg/l and/or abnormal chromosome 13 as determined by FISH).

The protocol allows the choice to apply two courses of intensified treatment with stem cell rescue. Based on the recently published study by the French IMF group, it is advised to restrict a second intensive treatment with High-dose Melphalan to patients who did achieve at least a partial response (PR) after the first high-dose treatment.

6 Study objectives

- To assess the efficacy of Bortezomib combined with intensive chemotherapy and in maintenance therapy in comparison with intensive therapy with Vincristine followed by thalidomide maintenance in patients with previously untreated multiple myeloma, as measured by the progression free survival as defined in chapter 14.
- To evaluate the overall response rate and CR + VGPR (complete and very good partial response) both after induction therapy and after autologous transplant.
- To evaluate overall survival.
- To assess the safety and toxicity of Bortezomib combined with intensive chemotherapy and in maintenance therapy.
- To assess the prognostic value of risk factors at diagnosis, including β_2-microglobulin, karyotypic abnormalities of chromosomes 9, 11 and 13 as analyzed in bone marrow plasma cells by karyotyping and FISH, with respect to progression free survival.
- To analyze the prognostic value of myeloma gene expression profiles on the overall response on induction of all patients and of patients treated with Bortezomib separately.

7 Study design

Details of all treatments (dose and schedule) are given in 9.1-9.8.
Patients with multiple myeloma, meeting all eligibility criteria (see 8.1) will be randomized on entry between:

Arm A: Standard Vincristine, Adriamycin and Dexamethasone (VAD) induction, followed by intensive chemotherapy with High-dose Melphalan, followed by maintenance therapy with Thalidomide

or

Arm B: induction chemotherapy with Bortezomib, Adriamycin and Dexamethasone (PAD) followed by intensive chemotherapy with High-dose Melphalan, followed by maintenance with Bortezomib

7.1 Induction chemotherapy with Vincristine, Adriamycin and Dexamethasone (VAD) or with Bortezomib, Adriamycin and Dexamethasone (PAD)

All patients will be given 3 cycles of induction chemotherapy. Dosages of (Vincristine), Adriamycin and Dexamethasone are according to the original VAD scheme (Vincristine, Adriamycin, Dexamethasone), with the exception that the dosage of Dexamethason is the same in all three cycles. Vincristine is omitted in the Bortezomib arm because of the increased risk of polyneuropathy with the combination. Instead, Bortezomib will be administered according to a twice weekly intravenous schedule for 2 weeks followed by two rest weeks, at each cycle.
Patients will be evaluated for response after cycle 3 at 3 months.
Patients who meet the inclusion criteria for CAD and stem cell collection, will continue with CAD.
Patients who do not meet these inclusion criteria but who are in CR, VGPR, PR, MR or NC are strongly recommended to be treated as described in section 7.7. Otherwise they go off protocol treatment.

7.2 Stem cell mobilization and collection

In all eligible patients (see 9.3.1) stem cell collection will be performed after CAD (Cyclophosphamide, Adriamycin, Dexamethasone) chemotherapy and G-CSF.
Patients will be evaluated for response after stem cell collection.

7.3 High Dose Melphalan

All patients who meet the eligibility criteria (see 9.3.1) for intensification will be treated with High Dose Melphalan 200 mg/m² total (given in two days) followed by autologous stem cell reinfusion.
Patients in a hospital with a policy of double intensification will receive the second course of High Dose Melphalan between 2 and 3 months after the first course when the patient achieved at least PR.
Patients will be evaluated for response after each course of High Dose Melphalan.

7.4 Maintenance therapy with Thalidomide

In patients randomized to arm A (standard arm), maintenance will start at 4 weeks after the last course of High Dose Melphalan.
Thalidomide is continued for 2 years or until progression. It is also stopped when a patient has not achieved at least MR 6 months after start of maintenance. When Thalidomide maintenance after HDM is

interrupted for more than 6 weeks, it is regarded as end of Thalidomide maintenance and the patient will go off protocol treatment.

7.5 Maintenance therapy with Bortezomib

In patients randomized to arm B who meet the inclusion criteria for Bortezomib, maintenance will start at 4 weeks after the last course of High Dose Melphalan if ANC $\geq$ 0.5 x 10^9/l and platelets > 20 x 10^9/l. Bortezomib is administered once every other week for 2 years or until progression. It is also stopped when a patient has not achieved at least MR 6 months after start of maintenance. When Bortezomib maintenance after HDM is interrupted for more than 6 weeks, it is regarded as end of Bortezomib maintenance and the patient will go off protocol treatment.

7.6 Non-Myeloablative allogeneic transplantation

Patients who are $\leq$ 65 years at diagnosis with an HLA-identical sibling donor may proceed to non-myeloablative allogeneic stem cell transplantation after induction therapy and HDM.
Maintenance therapy (Bortezomib or Thalidomide) will not be applied after non-myeloablative AlloSCT.

7.7 Treatment of patients in CR, VGPR, PR,MR or NC who do not meet the inclusion criteria for stem cell mobilization or intensification

For patients who do not meet the inclusion criteria for either stem cell mobilization or intensification but who achieved a CR, VGPR, PR, MR or NC, it is strongly recommended to continue the treatment with 3 more cycles of VAD or PAD according to their randomization arm, followed by maintenance with Thalidomide (arm A) or Bortezomib (arm B) for 2 years. Maintenance therapy with Bortezomib or Thalidomide will be stopped when a patient has not achieved at least a MR 6 months after start of maintenance.

8 Study population

8.1 Eligibility for registration

All eligible patients have to be registered and randomized before start of treatment. If platelets are < 25 x 10^9/l at screening, platelet transfusion before start of treatment is obligatory. In these patients, careful platelet monitoring during the study is necessary.

8.1.1 Inclusion criteria

- Patients with a confirmed diagnosis of multiple myeloma stage II or III according to the Salmon & Durie criteria (see appendix A);
- Age 18-65 years inclusive;
- WHO performance status 0-3 (WHO=3 is allowed only when caused by MM and not by co-morbid conditions) (see appendix D);
- Negative pregnancy test at inclusion if applicable;
- Written informed consent.

8.1.2 Exclusion criteria

- Known intolerance of Thalidomide or Boron;
- Systemic AL amyloidosis;
- Non-secretory MM
- Previous chemotherapy or radiotherapy except 2 cycles of Melphalan/Prednisone or local radiotherapy in case of local myeloma progression;
- Severe cardiac dysfunction (NYHA classification II-IV, see appendix E);
- Significant hepatic dysfunction (serum bilirubin ≥ 30 μmol/l or transaminases ≥ 2.5 times normal level), unless related to myeloma;
- Patients known to be HIV-positive;
- Patients with active, uncontrolled infections;
- Patients with neuropathy, CTC grade 2 or higher
- Patients with a history of active malignancy during the past 5 years with the exception of basal carcinoma of the skin or stage 0 cervical carcinoma;
- Patients who are not willing or capable to use adequate contraception during the therapy (all men, all pre-menopausal women);
- Patients ≤ 65 years with an HLA-identical sibling who will undergo **non-myeloablative** AlloSCT;
- Lactating women.

9 Treatments

All men and pre-menopausal women should use adequate contraception during the study. Sperm should be frozen from men with child wish before start of treatment.
All patients will receive 3 cycles of VAD (arm A) or PAD (arm B) by rapid infusion.

9.1 Arm A: VAD induction phase

Agent	Dose/day	Route	Days
Vincristine	0.4 mg	i.v. rapid infusion	all cycles: 1, 2, 3, 4
Doxorubicin	9 mg/m^2	i.v. rapid infusion	all cycles: 1, 2, 3, 4
Dexamethasone	40 mg	p.o.	all cycles: 1, 2, 3, 4, 9, 10, 11, 12, 17, 18, 19, 20

Cycle 2 will start at day 29, cycle 3 will start at day 57.

Assessment of response after cycle 3 is described in appendix B.
All patients who meet the inclusion criteria for CAD and stem cell collection (see 9.3.1) will continue with CAD. This also holds for patients with progressive disease after VAD.
Patients who do not meet the inclusion criteria for CAD and stem cell collection and with progressive disease will go off protocol treatment. Patients who do not meet these inclusion criteria but who are in CR, VGPR, PR, MR or NC are strongly recommended to be treated as described in section 7.7. Otherwise they go off protocol treatment.
It should be noted that progressive disease after VAD or PAD by itself is not a reason to go off protocol treatment.

9.1.1 Special management orders in conjunction with VAD

It is strongly recommended to give prophylactic treatment for pneumococcus infections and anti-fungal prophylaxis according to local protocols.

9.2 Arm B: Bortezomib induction phase

Bortezomib will be administered only to patients randomized to arm B.

Agent	Dose/day	Route	Days
Bortezomib	1.3 mg/m^2	i.v. rapid infusion	all cycles: Days 1,4,8,11
Doxorubicin	9 mg/m^2	i.v. rapid infusion	all cycles: 1, 2, 3, 4
Dexamethasone	40 mg	p.o.	all cycles: 1, 2, 3, 4, 9, 10, 11, 12, 17, 18, 19, 20

Cycle 2 will start at day 29, cycle 3 will start at day 57.

Assessment of response after cycle 3 is described in appendix B.
All patients who meet the inclusion criteria for CAD and stem cell collection (see 9.3.1) will continue with CAD. This also holds for patients with progressive disease after PAD.
Patients who do not meet the inclusion criteria for CAD and stem cell collection and with progressive disease will go off protocol treatment. Patients who do not meet these inclusion criteria but who are in CR, VGPR, PR, MR or NC are strongly recommended to be treated as described in section 7.7. Otherwise they go off protocol treatment.
It should be noted that progressive disease after VAD or PAD by itself is not a reason to go off protocol treatment.

9.2.1 Special management in conjunction with Bortezomib therapy

Patients may be treated on an outpatient basis. The appropriate amount of Bortezomib will be drawn from the injection vial and administered as an IV push over 3 to 5 seconds followed by a standard saline flush or through a running IV line. Vials are for single use administration. The patient should be considered clinically stable by their physician before discharge.
Before each Bortezomib dose, the patient will be evaluated for possible toxicities that may have occurred after the previous dose(s). All previously established or new toxicities observed any time, *with the exception of neuropathic pain and peripheral sensory neuropathy for which separate guidelines are defined in Appendix F*, are to be managed as follows:

Bortezomib doses should be withheld if the following events occur and are thought to be related to Bortezomib:

- febrile neutropenia;
- grade 4 hematological toxicity;
- grade ≥ 3 non-hematological toxicity

The detailed management instructions for handling Bortezomib are described in section 9.5.3.
It is strongly recommended to give prophylactic treatment for pneumococcus infections and anti-fungal prophylaxis according to local protocols.

9.3 Stem cell mobilization and collection

All eligible patients will be given CAD chemotherapy followed by G-CSF for stem cell collection. CAD will start 4-6 weeks after start of the third PAD or VAD cycle.

9.3.1 Eligibility criteria for CAD and stem cell collection

- WHO performance 0-2
- Absence of severe pulmonary, neurologic, or psychiatric disease
- Bilirubin and transaminases of less than 2.5 times the upper limit of normal values

9.3.2 Stem cell mobilization with CAD

Agent	Dose/day	Route	Days
Cyclophosphamide	1000 mg/m^2	i.v.	1
Doxorubicin	15 mg/m^2	i.v. rapid infusion	1, 2, 3, 4
Dexamethasone	40 mg	p.o.	1, 2, 3, 4
G-CSF (filgrastim)	10 μg/kg (divided in 2 gifts daily, according to local rules)	s.c.	day 5 until last pheresis*

* GMMG-centers are allowed to start with G-CSF treatment 5 days after CAD treatment (day 9).

9.3.2.1 Special management orders in conjunction with CAD

Selective gut decontamination should be performed according to local protocols.

9.3.3 Stem cell collection

Stem cell collection will be performed as soon as $CD34^+$ cells are present in peripheral blood, which is usually between 9-14 days after first day of CAD. In case double intensification is planned (immediately or a second course at relapse) a minimum of 5 x 10^6 $CD34^+$ cells/kg is required. Otherwise 2.5 x 10^6

$CD34^+$ cells/kg are sufficient. In case insufficient stem cells are collected the procedure may be repeated (possibly after the use of cyclophosphamide priming (4000 mg/m^2)) or alternatively bone marrow stem cell collection may be performed.
Assessment of response after stem cell collection is described in appendix B.
All patients who meet the inclusion criteria for intensification (see 9.4.1) will continue with High Dose Melphalan.
Patients who do not meet the eligibility criteria for intensificationwill go off protocol treatment.
It should be noted that no response or progressive disease by itself is not a reason to go off protocol treatment.

9.4 Intensification

All eligible patients will be given High Dose Melphalan between 6 and 8 weeks after stem cell collection. GMMG-centers are allowed to start with Intensification treatment 3-5 weeks after stem cell collection.

9.4.1 Eligibility criteria for intensification

- WHO performance 0-2
- Absence of severe pulmonary, neurologic, or psychiatric disease
- Bilirubin and transaminases of less than 2.5 times the upper limit of normal values
- A suitable stem cell graft containing at least 2.5 x 10^6 $CD34^+$ cells/kg

9.4.2 High Dose Melphalan followed by stem cell reinfusion

Agent	Dose/day	Route	Days
Melphalan	100 mg/m²	i.v. rapid infusion	-3, -2*
Stem cell infusion	2.5 x 10^6 $CD34^+$ cells/kg		0

* Patients with renal insuffency 100 mg/m² only at day –3.

Although Melphalan pharmacokinetics are not adversely affected by impaired renal function, the general toxicity of Melphalan 200 mg/m^2 total may be increased in patients with a creatinin clearance ≤ 40 ml/min. For patients with a creatinin clearance ≤ 40 ml/min, Melphalan dose should be reduced to 100 mg/m^2 total, given only at day -3.
Assessment of response after each course of High Dose Melphalan is described in section 11 and appendix B.

9.4.2.1 Special management orders with Melphalan 200 mg/m² total and stem cell reinfusion

A hydration regimen will be started 30 minutes before administration of Melphalan and consists of 500 ml NaCl 0.9 % and 40 mmol KCl over 1 hour. Diuretics must be administered when needed.
On day 0 the stem cells are thawed at the bedside and infused without washing steps. The procedure will be performed according to the local standard protocols.

9.4.2.2 Supportive care during Melphalan 200 mg/m² total aplasia

- Placement of an indwelling central venous catheter;
- Anovulatory drugs for menstruating females;
- Antibacterial and antifungal prophylaxis;
- Antistreptococcus prophylaxis is recommended from day +4 until day +14.

9.4.3 Second course of Melphalan 200 mg/m^2 total followed by stem cell reinfusion

A second course of High Dose Melphalan may be administered between 2 and 3 months after the first course when the patient achieved at least PR. This second course of HDM is optional. Patients have to meet the criteria as described under 9.4.1 before starting the second course. This applies to HOVON centers only: GMMG-centers will perform a double HDM procedure for all patients regardless of response after first HDM.

9.5 Maintenance therapy with Bortezomib

Patients randomized to arm B will continue with Bortezomib after the last course of HDM. The dose of maintenance Bortezomib is 1.3 mg/m^2 every 2 weeks.

9.5.1 Eligibility criteria for Bortezomib

All patients randomized in arm B, who have achieved hematological recovery (neutrophils ≥ 0.5 x 10^9/l, platelets > 20 x 10^9/l) after intensification therapy, are eligible for maintenance therapy with Bortezomib.

9.5.2 Administration of Bortezomib

Bortezomib will be given at a dose of 1.3 mg/m^2/2 weeks. Maintenance therapy will be initiated 4 weeks after HDM. If Bortezomib has not started within 8 weeks after HDM, the patient will go off protocol treatment. Bortezomib maintenance will be stopped after progression and also in patients who have not achieved at least a MR 6 months after start of maintenance. When Bortezomib maintenance after HDM is interrupted for more than 6 weeks, it is regarded as end of Bortezomib maintenance and the patient will go off protocol treatment. Assessment of response during Bortezomib maintenance is described in appendix B.

Agent	Dose	Route	Days
Bortezomib	1.3 mg/m^2	i.v. rapid infusion	Start 4 weeks after HDM if neutrophils ≥ 0.5 x 10^9/l and platelets > 20 x 10^9/l. One dose/two weeks for 2 years. Stop after progression, 6 weeks interruption or when not at least in MR 6 months after start of maintenance.

9.5.3 Dose adjustment of Bortezomib

Before each Bortezomib dose, the patient will be evaluated for possible toxicities that may have occurred after the previous dose(s). All previously established or new toxicities observed any time, *with the exception of neuropathic pain and peripheral sensory neuropathy for which separate guidelines are defined in Appendix F*, are to be managed as follows:
Bortezomib doses should be withheld if the following events occur and are thought to be related to Bortezomib:

- febrile neutropenia;
- grade 4 hematological toxicity;
- grade ≥ 3 non-hematological toxicity.

Febrile neutropenia
Bortezomib should be withheld until resolution of this condition, according to the judgement of the threating physician.

Hematological toxicities
For grade 4 hematological toxicities, Bortezomib is to be withheld for up to 2 weeks until the following values are reached: hemoglobin ≥7.0 g/dl (4.4 mmol/l), ANC ≥ 0.5 x 10^9/l, **and** platelet count ≥ 50 x 10^9/l.
Dose interruption or treatment discontinuation is not required for lymphopenia of any grade.
Non-hematological toxicities

For any grade ≥ 3 non-hematological toxicities, Bortezomib is to be withheld for up to 2 weeks until the toxicity returns to at least grade 2.
If the toxicity does not resolve after dosing has been withheld for two weeks, then the patient must be discontinued from treatment.

Dose adjustments after withholding Bortezomib dosing for toxicities
If withholding the Bortezomib dosing results in resolution of the toxicity, Bortezomib may be restarted at a dose reduced by 25%, as follows:

- If the patient was receiving 1.3 mg/m^2, reduce the dose to 1.0 mg/m^2.
- If the patient was receiving 1.0 mg/m^2, reduce the dose to 0.7 mg/m^2.
- If the patient was receiving 0.7 mg/m^2, then the Bortezomib must be discontinued.

Neuropathic pain and/or peripheral sensory neuropathy
Patients who experience Bortezomib related neuropathic pain and/or peripheral sensory neuropathy are to be managed as presented in the table in Appendix F.
According to that scheme, for example, if a patient had peripheral sensory neuropathy with objective sensory loss or paresthesia that interfered with function but not ADLs (grade 2) and mild neuropathic pain not interfering with function (grade 1), then the Bortezomib dose is to be reduced by 25%.

9.6 Maintenance therapy with Thalidomide

Thalidomide will be administered only to patients randomized to arm A. Thalidomide will be given at a dose of 50 mg/day. Maintenance therapy will be initiated 4 weeks after HDM. If Thalidomide has not started within 8 weeks after HDM, the patient will go off protocol treatment. Thalidomide will be stopped after progression and also in patients who have not achieved at least a MR 6 months after start of maintenance. When Thalidomide maintenance after HDM is interrupted for more than 6 weeks, it is regarded as end of Thalidomide maintenance and the patient will go off protocol treatment. Assessment of response during Thalidomide maintenance is described in appendix B.

Agent	Dose/day	Route	Days
Thalidomide	50 mg	p.o.	Start 4 weeks after HDM . Stop after progression, 6 weeks interruption or when not at least in MR 6 months after start of maintenance.

9.7 Non-Myeloablative allogeneic stem cell transplantation

Patients who have an HLA identical sibling donor are eligible for allogeneic stem cell transplantation. It is strongly recommended to include these patients in ongoing non-myeloablative AlloSCT trials. Once they are allocated to non-myeloablative AlloSCT, they will be treated with 3 courses of PAD or VAD, followed by CAD and stemcell apheresis. Next these patients will receive 1 course of High Dose Melphalan followed by peripheral blood stem cell reinfusion before proceeding to non-myeloablative AlloSCT between 2 and 6 months after HDM. Maintenance will not be applied after non-myeloablative AlloSCT.

9.8 Bisphosphonates

It is strongly recommended to start treatment with i.v. bisphosphonates at diagnosis and to continue this treatment every 4-6 weeks for at least 2 years. A commonly used regimen consists of zoledronate 4 mg or pamidronate (APD) 90 mg i.v. once every 4-6 weeks.

9.9 Concomitant medication

9.9.1 Guidelines for platelet transfusions

Thrombocytopenia can occur as a consequence of bone marrow infiltration by myeloma cells or may be related to study drug administration. The clinical significance of thrombocytopenia experienced by a patient should be assessed in light of its etiology (bortezomib or disease or both), the state of the underlying myeloma (stable versus worsening disease), and whether the patient is bleeding or being prepared for a surgical procedure.

The use of any platelet product should be considered in the following circumstances:

- As preparation for an invasive surgical procedure, transfuse in order to maintain a platelet count > 50 x 10^9/l to prevent bleeding.
- If the patient has an active infection, high fever, rapid decrease in platelet count to ≤ 20 x 10^9/l and/or coagulopathy, transfuse to maintain a platelet count to > 20 x 10^9/l as prophylaxis for spontaneous bleeding.
- If the patient is actively bleeding or has a platelet count below 10 x 10^9/l, transfuse in order to maintain a platelet count > 10 x 10^9/l.

9.9.2 Guidelines for red cell transfusions

The use of any red cell product should be considered in the following circumstances:

- If the patient has a hemoglobin < 4.3 mmol/l, transfuse to maintain a hemoglobin > 5.0 mmol/l in order to reduce the risk of inadequate oxygenation.
- If the patient is asymptomatic and has a hemoglobin between ≥ 4.3 and ≤ 5.0 mmol/l, the investigator may consider transfusion on a per-patient basis in order to maintain a hemoglobin > 5.0 mmol/l.
- If the patient is actively bleeding or has symptomatic cardiac or pulmonary disease or other extenuating circumstances where oxygenation is impaired, the investigator may elect to transfuse on a per-patient basis. In these instances, the trigger hemoglobin value may be > 5.0 mmol/l.
- The use of erythropoeitin (e.g. Eprex®/Erypo®) is allowed.

9.9.3 Forbidden concomitant medication during the study

- The use of steroids, other than < 10 mg prednisone or equivalent, is not allowed.
- The use of antineoplastic therapy, other than protocol-specified study medication, is not allowed until progressive disease is established.

9.10 Study drug information

9.10.1 Physical description of study drug

Bortezomib for injection is an antineoplastic agent available for i.v. use only. Each single dose vial contains 3.5 mg bortezomib as a sterile lyophilized powder. Inactive ingredient: 35 mg mannitol.

9.10.2 Packaging

Bortezomib will be supplied as single-use vials containing 3.5 mg bortezomib and 35 mg mannitol. All study medication will be dispensed in child-resistant packaging.

9.10.3 Labeling

Study drug labels will contain information to meet the applicable regulatory requirements.

9.10.4 Preparation and handling

Bortezomib for Injection drug product was found to be stable for at least 18 months under storage conditions from 2°C to 25°C with excursions permitted up to 30°C. The reconstituted product is preservative free and is chemically and physically stable for up to 8 hours when it is stored at 25 °C.

Stability studies are ongoing, and MPI will notify the investigator should this information be revised during the conduct of the protocol.
The drug product is supplied in vials containing 3.5 mg of bortezomib. The pharmacist must prepare the study drug under aseptic conditions. Each vial of Bortezomib for Injection should be reconstituted within 8 hours before dosing with 3.5 mL of normal (0.9%) saline, Sodium Chloride Injection USP, so that the reconstituted solution contains bortezomib at a concentration of 1 mg/mL. The reconstituted solution is clear and colorless, essentially free from particles or foreign matter, and the pH of the reconstituted solution is approximately 4 to 7.
Reconstituted Bortezomib for Injection should be administered promptly and in no case administered more than eight hours after preparation. The reconstituted material may be stored in the original vial and/or the syringe prior to administration. The product may be stored for up to three hours in a syringe, however total storage time for the reconstituted material must not exceed eight hours when exposed to normal indoor lighting.
Bortezomib for Injection drug product is a cytotoxic anticancer drug and, as with other potentially toxic compounds, caution should be exercised when handling and preparing Bortezomib for Injection. Refer to published guidelines regarding the proper handling and disposal of anticancer agents. The pharmacist should prepare Bortezomib for Injection using a vertical laminar flow biological cabinet (hood) and proper aseptic techniques. It is recommended that gloves and protective garments be worn during preparation.
If Bortezomib for Injection reconstituted solution contacts the skin, wash the skin immediately and thoroughly with soap and water. If Bortezomib for Injection reconstituted solution contacts the mucous membranes, flush thoroughly with water.

9.10.5 Drug accountability

The local investigator is responsible for ensuring that all study drug received at the site is inventoried and accounted for throughout the study. The dispensing of study drug to the subject, and the return of study drug from the subject (if applicable), must be documented.
Study drug must be handled strictly in accordance with the protocol and the container label and will be stored under appropriate environmental conditions. Contents of the study drug containers must not be combined.
The return of used study drug will be documented. Unused study drug and returned used study drug will be destroyed at the investigational site. Vials should be discarded in a safe manner. Destruction must be documented.
Study drug should be dispensed under the supervision of the investigator, a qualified member of the investigational staff, or by a hospital/clinic pharmacist. Study drug will be supplied only to subjects participating in the study. Returned study drug must not be dispensed again, even to the same subject. Study drug may not be relabeled or reassigned for use by other subjects. The investigator agrees neither to dispense the study drug from, nor store it at, any site other than the study sites agreed upon with the sponsor.

10 End of protocol treatment

Reasons for going off protocol treatment are:

- Not eligible for CAD and stem cell collection and response is not CR, VGPR, PR, MR or NC
- Not eligible for intensification with HDM
- Not eligible for Bortezomib maintenance
- Not at least MR 6 months after start Thalidomide maintenance (arm A)
- Not at least MR 6 months after start of Bortezomib maintenance (arm B)
- Excessive toxicity (including toxic death)
- Progression / relapse (after HDM or during maintanance)
- Non-myeloablative AlloSCT
- Intercurrent death
- No compliance of the patient (especially refusal to continue treatment)
- Pregnancy (of female patient)
- Completion of protocol treatment

11 Required clinical evaluations

Aim of the clinical evaluation at entry is to know in which stage of disease according to Salmon & Durie (see appendix A) the patients are classified and to determine the presence of adverse prognostic factors and establish a baseline for response evaluations. Aim of the clinical evaluation during treatment and follow up is to determine response, toxicities and eligibility for further treatment. Before start of each treatment cycle, routine investigations like blood cell count and renal function will be performed according to local policy.

11.1 Time of clinical evaluations

- At entry: before start of treatment (results from diagnostic tests may be used, provided that they are no older than 4 weeks prior to randomization)
- After PAD/VAD III: 2 weeks after the third PAD/VAD cycle
- After stem cell collection: 4 weeks after start CAD
- After each HDM: 6-8 weeks after each course of HDM
- Maintenance and follow up: every 2 months

11.2 Required investigations at entry, during treatment and during follow up

	At entry	After each VAD/PAD	After stem cell collection	After each HDM	Maintenance and follow up
Medical history	X	X	X	X	X
Physical examination	X	X	X	X	X
Hematology	X	X	X	X	X[6)]
PB cryopreservation	X				
Blood chemistry	X	X	X	X	X[6)]
Immunochemistry	X	X	X	X	X
Bone marrow					
Bone marrow aspirate	X	X[4)]		X	X[2)]
Bone marrow biopsy	X				
BM cryopreservation[3)]	X				
Specific investigations					
β_2-microglobulin	X		X		
Creatinin clearance	o.i.	o.i.	o.i.	o.i.	
Skeletal survey[5)]	X	o.i.	o.i.	X	X[1)]
X-thorax	X				
ECG	X			X	
Cardiac ejection	X[7)]	o.i.	X[7)]	o.i.	
Additional investigations	o.i.	o.i.	o.i.	o.i.	o.i.
Cytogenetic analysis	X				

o.i. on indication

1) once a year

2) once a year

3) for microarray analysis

4) only after PAD/VAD cycle III

5) in case of extramedullary plasmacytoma, the skeletal surveys should be repeated at all evaluation moments

6) during maintenance: haematology and blood chemistry tested every two weeks in the first month, then every four weeks

7) recommended for all patients at entry and during pre-transplantation screening prior to HDM

11.2.1 Medical history

Standard medical history, with special attention for:

- WHO performance status
- Bone pain
- Infections
- Bleeding tendency
- Obstipation
- Polyneuropathy

Only at entry:

- Occupational history
- Prior and present other diseases
- Antecedent hematological or oncological diseases
- Previous chemotherapy or radiotherapy
- HLA typing of patient and family
- Ethnicity

11.2.2 Physical examination

Standard physical examination including body weight and height, with special attention for:

- Macroglossia
- Kyphoscoliosis
- Orthostatic hypotension
- Carpal tunnel syndrome
- Polyneuropathy or other neurologic symptoms
- Edema
- Infections
- Bleeding tendency

11.2.3 Hematology

- Hemoglobin
- Leukocyte count, differential count
- Platelets

At entry: PB cryopreservation for SNP analysis (see paragraph 11.2.11)

11.2.4 Blood chemistry

- BUN
- Creatinin
- Liver enzymes
- Total bilirubin
- Alkaline phosphatase
- Total proteins
- Albumin
- LDH
- CRP
- Calcium
- Phosphate
- Sodium
- Potassium
- Uric acid

11.2.5 Immunochemistry

- Quantitative serum M-protein, including immunofixation to confirm CR
- Quantitative urine M-protein in 24 hrs urine, including immunofixation to confirm CR

Only at entry:

- Qualitative serum M-protein
- Qualitative urine M-protein (Bence Jones)

11.2.6 Bone marrow

- Bone marrow biopsy
- Bone marrow aspirate at entry for:
  - Morphology, immunophenotyping
  - Labeling Index (by BRDU) or KI-67
  - Cytogenetic analysis (see 11.2.9)
  - Molecular analysis (Plasma cell purification and cryopreservation for DNA microarray analysis, see for collecting and handling of samples for DNA microarray analysis Appendix G)
- Bone marrow aspirate during treatment and follow up for:
  - Morphology

11.2.7 Specific investigations

- Serum β_2-microglobulin
- Creatinin clearance if increased serum creatinin
- Radiographic skeletal survey including skull, pelvis, vertebral column and long bones
- X-Thorax
- ECG
- Cardiac ejection by scintigraphy or cardiac echo; it is advised to perform a Left Ventricular Ejection Fraction (LVEF) in all patients at entry (before start of doxorubicin treatment). In addition it is recommended to repeat the LVEF after stem cell collection, as part of the pre-transplantation screening prior to HDM.

11.2.8 Additional investigations

Only on clinical indication:

- Survey for exclusion of AL amyloidosis
- Bleeding time
- Cryoglobulins, cold agglutins
- Serum viscosity, funduscopy
- Spirometry

11.2.9 Cytogenetic analysis

Conventional cytogenetic analysis should be performed in all patients at diagnosis. Additional FISH analysis is required for chromosome 13q deletions. Additional FISH is recommended for numerical aberrations for chromosome 9 or 11 (to detect hyperdiploidy) and for 14q32 abnormalities. The following cytogenetic abnormalities will be evaluated as prognostic variables: 1p/q, t(4;14)(p16;q32), t(14;16)(q32;q23), del(13q), 13q-, numerical abnormalities 9 or 11 (i.e. hyperdiploidy), complex cytogenetic abnormalities. Conditions for FISH will be standardized by the HOVON Cytogenetic Working Party.

Each cytogeneticist, responsible for the cytogenetic analysis of the patients in a hospital will be notified automatically by email of the registration of a patient from that hospital in the study. A filled out cytogenetic form together with 2 representative karyotypes and a copy of the original cytogenetic report is requested to be sent within 3 months to the HOVON Data Center for central review.

11.2.10 Microarray analysis

Using microarray analysis the distinct patterns of gene expression, which are involved in proliferation and growth of MM cells will be investigated. Bone marrow samples will be taken before start of treatment of the Dutch patients in one of the academic centres (AMC, UMCG, AZM, Erasmus MC, LUMC, UMC St. Radboud, UMCU, VUMC), HagaZiekenhuis - Leyenburg, St. Antonius hospital or Medisch Spectrum Twente. Bone marrow samples from German patients and patients from Belgium will be collected in the academic centers. Patients diagnosed and/or treated in other hospitals should visit before the start of their treatment one of these centers for collecting bone marrow for micro array analysis. At these centers the bone marrow samples are handeled according the procedure described in Appendix G. The laboratory kit for pharmacogenetic bone marrow handling will be sent to those laboratories after initiation of the hospital.

11.2.11 Single Nucleotide Polymorphism (SNP) analysis

The involvement of specific genes in the drug metabolism and anti-tumor effect of Bortezomib and Thalidomide will be investigated, using Taqman and PCR-RFLP analysis of DNA isolated from blood. The presence of inherited genotype polymorphisms will be correlated to response and toxicity.
Blood samples will be taken before start of treatment and shipped to the Erasmus MC hematology lab, where analysis of all samples will take place. Procedures for collecting and handling samples are described in appendix H.
Since there are inter-ethnic differences in frequency of SNPs, it is necessary to document the ethnicity of patients included in the trial. This will allow us to perform multivariate analysis to find whether a certain SNP is an independent prognostic factor.

11.3 Evaluation of response

Response will be evaluated according to EBMT, IBMTR and ABMT criteria (see appendix B).
Time points are after the third PAD or VAD course, after stem cell collection, and after each course of HDM. During maintenance, disease status will be evaluated every 2 months. According to the response criteria a response should be confirmed after 6 weeks. However, in general this cannot be applied to the response measurements after PAD III or VAD III, after stem cell collection and between the 2 courses of HDM as the treatment intervals are too short.

12 Toxicities

All the chemotherapeutic agents used in the protocol cause pancytopenia and can induce septic or hemorrhagic complications.

Side effects of Thalidomide are constipation, somnolence, neuropathy, rash, weakness and fatigue, which are more frequent with higher doses (400 mg and more). Thalidomide, especially when it is combined with Dexamethasone and Doxorubicin, may increase the risk on Deep Venous Thrombosis (DVT). **DVT is considered a Serious Adverse Event (SAE), and accordingly any DVT must be reported to the HOVON Data Center within 24 hours of the initial observation of DVT** (see chapter 13).

Most common side effects of bortezomib (ie, incidence ≥ 30%) observed in patients are weakness, fatigue, and general discomfort; gastrointestinal (GI) effects such as constipation, diarrhea, nausea, vomiting and anorexia, which may result in dehydration and/or weight loss; fever; peripheral neuropathy (including painful sensations or numbness and tingling in hands and feet that may not get better after discontinuation of bortezomib); thrombocytopenia that may increase the risk of bleeding, and anemia.
Very common side effects of bortezomib (ie, incidence 10–29%) observed in patients are neutropenia that may increase the risk of infection; abdominal pain; dyspepsia; nasopharyngitis; arthralgias; myalgias; skin rash that can be erythematous, pruritic and display leukocytoclastic vasculitis at biopsy; rigors; hypotension; dizziness; fluid retention; pain in limbs and bones; paresthesia; dysesthesia; dyspnea; cough; epistaxis; headache; blurred vision; changed sense of taste; insomnia; anxiety; herpes zoster, and lower respiratory/lung infections including pneumonia.
Common side effects of bortezomib (ie, incidence 1–9%) observed in patients are lymphopenia; pancytopenia; palpitations; tachycardia; atrial fibrillation; angina pectoris; acute onset of congestive heart failure including pulmonary edema (patients with risk factors for, or existing, heart disease should be closely monitored); pleural effusion; tinnitus; conjunctivitis; abdominal distension; oral and esophageal mucositis; oral candidiasis; upper and lower GI bleeding; bronchitis; sinusitis; urinary tract infection; gastroenteritis; sepsis; hyponatremia; hyperglycemia; hypoglycemia (Patients on oral antidiabetic agents may require close monitoring of their blood sugar levels.); dehydration; orthostatic hypotension; syncope; convulsions; renal failure; hematuria; depression; confusion; increases in serum AST, ALT, GGT and alkaline phosphatase.
Uncommon side effects of bortezomib (ie, incidence <1%) observed in patients are febrile neutropenia; atrial flutter; bradycardia; new onset of decreased left ventricular ejection fraction; cardiogenic shock; hearing impairment; ileus paralytic/small bowel obstruction; upper gastrointestinal hemorrhage; oral mucosal petechiae; liver injury including abnormal liver function tests, hyperbilirubinemia, hepatitis, and liver failure (reported in patients receiving multiple concomitant medications and with serious underlying medical conditions); drug hypersensitivity; injection site reaction; aspergillosis; pulmonary embolism;

hemoptysis; cerebral hemorrhage; and tumor lysis syndrome. Isolated cases of QT-interval prolongation have been reported, but are not thought to be related to bortezomib treatment.
Complications arising from these bortezomib toxicities may result in death.
The effect of bortezomib on reproduction and its safety in pregnancy are unknown. Laboratory tests show that bortezomib may damage DNA therefore it is possible that bortezomib may cause infertility in men and women.

Further details on the potential risks of bortezomib may be found in the Investigator Brochure.

Toxicities will be scored according to the NCI Common Toxicity Criteria, version 3.0 (Appendix C).

13 Safety evaluations and adverse events reporting

13.1 Definitions

Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject during protocol treatment. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Adverse reaction (AR)

Adverse reactions (AR) are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected.

Serious adverse event (SAE)

A serious adverse event is defined as any untoward medical occurrence that at any dose results in:

- death
- a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- hospitalization or prolongation of hospitalization
- significant / persistent disability
- a congenital anomaly / birth defect
- any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above)

Note that ANY death, whether due to side effects of the treatment or due to progressive disease or due to other causes is considered as a serious adverse event.

Unexpected SAE

Unexpected Serious Adverse Events are those SAE's of which the nature or severity is not consistent with information in the relevant source documents. For a medicinal product not yet approved for marketing in a country, a company's Investigator's Brochure will serve as a source document in that country.

Suspected unexpected serious adverse reaction (SUSAR)

All suspected ARs which occur in the trial and that are both unexpected and serious.

Protocol treatment period

The protocol treatment period is defined as the period from registration until 30 days after stopping of the protocol treatment.

13.2 Reporting of (serious) adverse events

Adverse event

All AEs have to be reported on the Adverse events and/or Infection form and sent to the HOVON Data Center as soon as possible.

All adverse events, with the exception of progression of multiple myeloma, will be reported from the first study-related procedure until 30 days following the last dose of study drug or until the start of subsequent systemic antimyeloma therapy, if earlier. Resolution information after 30 days should also be provided. Adverse events occurring after 30 days should also be reported if considered related to study drug.

All Grade 3 or 4 adverse events considered related to study drug must be followed until recovery to Grade 0 or 1. Neuropathic and cardiac adverse events of Grade 2 or higher will be followed until improvement to Grade 0 or 1. The unresolved aforementioned events will be followed for a maximum of 6 months.

SAE and Unexpected serious adverse event

During protocol treatment all SAEs must be reported to the HOVON Data Center by fax **within 24 hours of the initial observation of the event**, except:

- neutropenia or leukopenia CTC grade 4. Any complication from neutropenia or leukopenia, including (prolongation of) hospitalization or unexpected duration of the event, remains reportable as a Serious Adverse Event.
- hospitalizations for progression of multiple myeloma. Hospitalization or prolonged hospitalization for a complication of progression of multiple myeloma remains a reportable Serious Adverse Event.
- hospitalizations for a standard procedure for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- hospitalizations for the administration of blood or platelet transfusion. Hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable serious adverse event.
- hospitalizations for a procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
- prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- hospitalizations for a procedure that is planned (i.e., planned prior to starting of treatment on study; must be documented in the CRF). Prolonged hospitalization for a complication considered to be at least possibly related to the study drug remains a reportable serious adverse event.

All details should be documented on the **Serious Adverse Event Report**. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information. Initial reports must be followed-up by a complete report within a further 2 working days and sent to the HOVON Data Center. All SAE Reports must be dated and signed by the responsible investigator or one of his/her authorized staff members.

At any time after the protocol treatment period, *unexpected* Serious Adverse Events that are considered to be at least suspected to be related to protocol treatment must also be reported to the HOVON Data Center using the same procedure, **within 24 hours after the SAE was known to the investigator**.

The investigator will decide whether the serious adverse event is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the serious adverse event form. The assessment of causality is made by the investigator using the following:

RELATIONSHIP	DESCRIPTION
UNRELATED	There is no evidence of any causal relationship to the protocol treatment (also include pre-existing conditions)
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

13.3 Actions of the HOVON Data Center upon receipt of (S)AE reports

AE

The HOVON Data Center will notify Johnson & Johnson Pharmaceutical Research and Development (J&JPRD) of all adverse events, in report format, at the end of the study.

SAE and unexpected SAE

The HOVON Data Center will forward all reports within 24 hours of receipt to the study coordinator, the study central datamanager and J&JPRD. The report of an SAE will be the signal for the central datamanager to ask the investigator or the responsible local datamanager to complete and send as soon as possible all relevant CRF's for the involved patient with details of treatment and outcome. Patients without a report of an SAE are implicitly considered alive without SAE. This information will be used in monitoring the incidence of SAE's, the estimation of overall survival and safety monitoring.

In addition, the HOVON Data Center will send copies of any relevant correspondence with regulatory authorities regarding serious adverse events, irrespective of association with the Study Drug(s) in the course of the Clinical Trial, within one working day of such report or correspondence being sent to applicable regulatory authorities. Copies will be faxed within one working day to J&JPRD.

13.4 SUSAR reporting

J&JPRD will notify the HOVON Data Center of any new information, which becomes available during the course of the study, which may affect the overall safety profile of Bortezomib.

Any suspected unexpected serious adverse reactions (SUSARs), from any source, which are considered by J&JPRD to be reportable to investigators, Health Authorities and Ethics Committees will be sent to the HOVON Data Center within 12 calendar days of J&JPRD personnel first becoming aware of such events, or 5 calendar days for fatal or life-threatening reports. The HOVON Data Center will have responsibility for reporting such events to all applicable Health Authorities and the Ethics Committee which approved the study, within the required timelines. Additionally, the HOVON Data Center will report all such events within the required timelines to co-investigators (for multi-centre studies). Co-investigators will report all such events to their Ethics Committees, where required.
J&JPRD has contracted with Millennium Pharmaceuticals, Cambridge, MA, USA, the safety database holder, and Pharmaceutical Research Associates, Mannheim, Germany, to process adverse events on its behalf. Reports will come back directly to the HOVON Data Center from either of these organizations, acting with and on behalf of J&JPRD. The HOVON Data Center will receive, via the J&JPRD Operating Company Representative, a quarterly "frequency" listing of the serious adverse event reports received for site reconciliation.

14 Endpoints

Primary:

- Progression free survival (i.e. time from registration to progression or death from any cause whichever occurs first).
  - Note 1: Patients with progression before HDM but obtain at least PR on HDM are not counted as an event for PFS.
  - Note 2: Patients who receive an allogeneic transplantation will be censored for PFS at the date of transplantation.

Secondary :

- Response (PR, VGPR and CR)
- Overall survival measured from the time of registration. Patients still alive or lost to follow up are censored at the date they were last known to be alive.
- Toxicity
- Progression free survival from HDM (i.e. time from last HDM treatment to progression or death from any cause whichever occurs first for patients who received at least PR on HDM)
- Progression free survival analysed as primary endpoint, but patients with an allogeneic transplant not censored. This primarily to check whether censoring has a major impact.

15 Registration and randomization

15.1 Registration and randomization

The patient should be registered immediately after diagnosis, and before the start of chemotherapy. Patients need to be registered at the HOVON Data Center of the Erasmus MC Rotterdam – location Daniel by phone call: +31.10.4391568 or fax +31.10.4391028 Monday through Friday, from 09:00 to 17:00 or via the Internet via TOP (Trial Online Process; http://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.

The following information will be requested at registration:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Patient's initials or code
- Patient's hospital record number (not obligatory)
- Sex
- Date of birth
- Serum β_2-microglobulin value

- Serum albumin value
- Eligibility criteria

All eligibility criteria will be checked with a checklist. ISS stage will be calculated from the provided serum β_2-microglobulin value and serum albumin value.
Each patient will be given a unique patient study number. Patients will be randomized, stratified by center and ISS stage (I vs. II vs. III) , using a minimization procedure with a random element incorporated (as recommended by ICH-E9), ensuring balance within each stratum and overall balance. Patient study number and result of randomization will be given immediately by TOP or phone and confirmed by fax or email.

15.2 Regulatory Documentation

The following documents must be provided to the HOVON Data Center before shipment of study drug to the investigational site and before enrollment of the first patient.
By the principal investigator or study coordinator for all sites within their country:

- name and address of the (central) Ethical Committee including a current list of the members and their function;
- any other documentation required by local regulations.

By the local investigator for each investigational site:

- HDC Hospital Registration Form, signed and dated by the local investigator;
- Investigator Agreement, signed and dated by the local investigator;
- a copy of the dated and signed (central) Ethical Committee approval of the protocol, any amendments and informed consent form for the investigational site. This approval must clearly identify the specific protocol by title, number and version date and must be signed by the chairman or authorized designee. The approval must also clearly identify the site(s) the approval applies to;
- a copy of the approved local version of the Patient Information and Informed Consent form;
- approval of participation by site's Board of Directors, if required by local regulations;
- CV of local investigator;
- any other documentation required by local regulations.

16 Forms and procedures for collecting data

16.1 CRF's and schedule for completion

LIST OF FORMS

Form nr	Nr of pages	Title
1	2	Registration & Randomization Form
2	4	On Study Form
3	2	Cytogenetics Form
4	3	FISH Form
5	2	VAD/PAD Treatment Form
6	2	CAD Mobilization & Stem Cell Collection Form
7	2	HDM & Stem Cell Reinfusion Form
8	1	Thalidomide Maintenance Form
9	1	Bortezomib Maintenance Form
10	3	Response Evaluation Form
11	2	Off Treatment Form
12	2	Follow Up Form
13	1	Adverse Event Form
14	1	Infection Form
15	1	Bisphosphonates Form
16	1	Pregnancy Form[1)]
17	1	General Comments Form
	2	SAE form (for all HOVON studies, not specific HOVON 65)

[1)] the pregnancy form needs to be send in in case of pregnancy of female patient during treatment and pregnancy of the partner of the male patient

Table for filling out forms

	Forms														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Registration & randomization	X														
On study		X	X[1]	X[1]											(X)
Induction Treatment PAD or VAD					X					X			(X)	(X)	(X)
Stem Cell Mobilization						X				X			(X)	(X)	(X)
HDM & Stem Cell Reinfusion							X			X			(X)	(X)	(X)
Thalidomide Maintenance								X		X			(X)	(X)	(X)
Bortezomib Maintenance									X	X			(X)	(X)	(X)
End of treatment											X	X			(X)
Follow up										X		X			(X)

(x) fill out if necessary, see instructions

[1] by local cytogeneticist

Instructions for completion and sending in of the forms are specified in a separate document together with the forms.

16.2 Monitoring

The sponsor will perform on-site monitoring visits. At these visits, the monitor will compare the data entered into the CRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the CRF are known to the sponsor and investigational staff and are accessible for verification by the sponsor site contact. At a minimum, source documentation must be available to substantiate: subject identification, eligibility and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; records of safety and efficacy parameters; adequate reporting and follow-up of adverse events; administration of concomitant medication; drug receipt/dispensing/return records; study drug administration information; date of subject completion, discontinuation from treatment, or withdrawal from the study, and the reason if appropriate. Specific items required as source documents will be reviewed with the investigator before the study.

Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. The sponsor expects that,

during monitoring visits, the relevant investigational staff will be available, the source documentation will be available, and a suitable environment will be provided for review of study-related documents.

16.3 Data quality assurance

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study centers, review of protocol procedures with the investigator and associated personnel before the study, and monitoring visits by the sponsor. CRF completion guidelines will be provided. The data will be entered into the clinical study database and verified for accuracy.

16.4 On-site audits

The local investigator/institution will permit site-visits to carry out an audit of the study in compliance with regulatory guidelines. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Subject privacy must, however, be respected.
Similar auditing procedures may also be conducted by agents of any regulatory body reviewing the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

17 Statistical considerations

17.1 Patient numbers and power considerations

For the calculation of the required number of patients to be entered in the study, the progression free survival (PFS), as defined in chapter 14, will be considered as primary endpoint. Patients who receive an allogeneic transplantation will be censored for PFS at the date of transplantation. It is expected that the PFS at three years of patients in the standard arm is 50%. It is expected that the PFS at three years will increase to 60% for patients in the experimental arm. This corresponds with a relative hazard rate of 0.74 for the experimental arm. The number of events needed to detect this difference with a power of 80% and α = 0.049 (two-sided and adjusted for one interim analysis at a significance level of 0.001) is 356. Assuming that 10% of the patients will receive an allogeneic transplantation, this number of events is expected to be reached with the inclusion of 800 patients in three years and an additional follow up of 2 years.

17.2 Statistical analysis

All analyses will be according the intention to treat principle.

17.2.1 Efficacy analysis

Main endpoint for the comparison of the two induction treatment arms will be PFS from registration as defined in chapter 14. Formal test for the difference in PFS between the two treatment arms will be done with a multivariate Cox regression analysis with adjustment for the stratification factor ISS stage. We also perform a non-modeling based stratified logrank test for difference in PFS between the two treatment arms, but this analysis should be regarded as a secondary analysis.
Secondary efficacy endpoints are response rate and overall survival from registration. The incidence of patients with progressive disease before HDM will be tabulated by treatment arm. An additional efficacy analysis, with PFS as endpoint, will be performed without censoring of the patients who received an allogeneic transplantation. Under the same conditions as stated in section 17.1 the power of this analysis will be 84%.
Progression free survival from HDM as defined in chapter 14 will be estimated for both treatment arms. Although effects of induction treatment on this endpoint cannot be ruled out this can give some more insight in the effect of maintenance treatment.

An preliminary efficacy analysis on response (CR or VGPR) after induction therapy is planned immediately after end of recruitment. This will be restricted to the first 600 patients, because not all responses will be available at that moment. No conclusions will be drawn based on this analysis.

17.2.2 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of adverse events and infections (Appendix C) by treatment arm and cycle.

17.2.3 Additional analyses

Additional analyses involve the analysis of prognostic factors, especially β_2-microglobulin, chromosome 13 deletion, albumin, age, LDH level and Salmon & Durie stage with respect to response rate, PFS, and OS from registration. Logistic and Cox regression analysis will be used for this purpose. β_2-microglobulin and albumin (used for stratification factor ISS stage) are required covariates at randomization and will therefore have no missing values. From other covariates it is expected that the number of missing values is limited i.e. < 10 %. To include all patients in the multivariate analysis, a single conditional mean imputation algorithm will be used to impute those missing covariate values. To account for multiple testing, the significance level for those analyses will be 1%. In addition, an exploratory analysis

evaluating the prognostic value of gene expression profiles on overall response will be performed. At the time of analysis of this microarray data an appropriate tool will be used to overcome the problem of overfitting. This will also be done on the subgroup of patients treated with Bortezomib.
Furthermore we have planned to evaluate treatment-covariate interaction for the covariates β_2-microglobulin, albumine and chromosome 13 deletion.
Devations of the analysis plan will be discussed with the study coordinator and can only affect this additional (exploratory) analysis, but not the primary (confirmatory) analysis on which the sample size is based.

17.3 Interim analyses and safety monitoring

One interim analysis is planned, primarily to guard against unfavorable results in the Bortezomib arm. Results of the interim analysis will be presented confidentially to an independent data and safety monitoring board (DSMB). Only if the DSMB recommends that the study should be stopped or modified the results will be made public to the principal investigators for further decisions. The interim analysis is planned after 75 events with regard to PFS as defined in chapter 14, which is the primary endpoint for this analysis. Under the assumption of uniform accrual for 3 years, immediate reporting of events for PFS, and 10% of the patients being censored due to an allogeneic transplantation, the interim analysis will take place after about 500 patients have been randomized.

At this interim analysis a detailed report will be generated and presented to the DSMB. The report includes by treatment arm the number of entered patients and at that time evaluable patients, treatment given, response rate, the number of events on the actuarial endpoints, actuarial estimates for those endpoints and incidence of SAE's and other adverse events and infections by grade. Adverse events will be described by summary tables broken by site, grade and relation to trial treatment.
The DSMB is free in her public recommendations to the study coordinators and the confidential recommendations to the study statistician. A lower PFS in the experimental arm with a P-value < 0.1 (logrank-test) is a good reason to recommend the stopping of the trial or recommendations for modifications.
A benefit in terms of PFS in the experimental arm is in general no reason to recommend early stopping of the study, unless the associated P-value is very extreme (P < 0.001, logrank).
The study will be closely and sequentially monitored before the interim analysis. Monitoring will be based on the reported SAE's, which are not subjected to data delay. The difference in the number of patients with an SAE in both arms and the difference in the number of deaths in both arms are tested using the logrank test. We repeatedly test whether those incidences in the experimental arm is higher at a significance level of 0.05, adjusted for multiple testing. If one of both incidences is significantly higher in the experimental arm an early report will be presented to the DSMB.
In addition, a separate report on the incidence of SAE's and other adverse events and infections, as described before, will be sent to the DSMB once a year. Again, the DSMB is free in her public

recommendations to the study coordinators and the confidential recommendations to the study statistician.

17.4 Data and safety monitoring board

A data and safety monitoring board will be installed before start of the study.

18 Ethics

18.1 Independent ethics committee or Institutional review board

The study protocol and any amendment that is not solely of an administrative nature will be approved by an Independent Ethics Committee or Institutional Review Board.

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki (Edinburgh, Scotland, 2000) and the ICH-GCP Guidelines of 17 January 1997. The local investigator is responsible for ensuring that the study will be conducted in accordance with the protocol, the ethical principles of the Declaration of Helsinki, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory requirements.

18.3 Patient information and consent

Written Informed consent of patients is required before randomization. The procedure and the risks and the opinions for induction therapy in multiple myeloma will be explained to the patient.

19 Trial insurance

The HOVON insurance program covers all patients from participating centers in the Netherlands according to Dutch law (WMO). The WMO insurance statement can be viewed on the HOVON Web site www.hovon.nl.

Individual participating centers from outside the Netherlands have to inform the HOVON about the national laws regarding the risk insurance of patients participating in a study
The HOVON insurance program does not cover the risk insurance of patients from centers participating within another cooperative group taking part in an intergroup study. The other participating groups will cover the insurance of patients registered/randomized through their offices. HOVON will ensure that insurance is in place for all participating sites.

20 Publication policy

The final publication of the trial results will be written by the Study Coordinator(s) on the basis of the statistical analysis performed at the HOVON Data Center. A draft manuscript will be submitted to the Data Center and all co-authors and Johnson & Johnson PRD/Millennium for review. After revision by the Data Center, the other co-authors and Johnson & Johnson PRD/Millennium, the manuscript will be sent to a peer reviewed scientific journal.

Authors of the manuscript will include the study coordinator(s), the lead investigators of the major groups (in case of intergroup studies), investigators who have included more than 5% of the evaluable patients in the trial (by order of inclusion), the statistician(s) and the HOVON datamanager in charge of the trial, and others who have made significant scientific contributions.

Interim publications or presentations of the study may include demographic data, overall results and prognostic factor analyses, but no comparisons between randomized treatment arms may be made publicly available before recruitment is discontinued.

Any publication, abstract or presentation based on patients included in this study must be approved by the study coordinator(s). This is applicable to any individual patient randomized in the trial, or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study end-points unless the final results of the trial have already been published.

21 Glossary of abbreviations

(in alphabetical order)

AD	Doxorubicin (Adriamycin), Dexamethasone
AE	Adverse Event
AL	Amyloid Light-chain
ANC	Absolute Neutrophil Count
BJ	Bence Jones
BM	Bone Marrow
BMT	Bone Marrow Transplant
BRDU	Bromo Deoxy Uridine
BUN	Blood Urea Nitrogen
Ca	Calcium
CAD	Cyclophosphamide, Doxorubicin (Adriamycin), Dexamethasone
CKTO	`Commissie voor Klinisch Toegepast Onderzoek'
CR	Complete Remission
CRF	Case Report Form
CRP	C-Reactive Protein
CTC	Common Toxicity Criteria
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EBMT	European Group for Blood and Marrow Transplantation
EFS	Event Free Survival
EORTC	European Organization for Research and Treatment of Cancer
FISH	Fluorescence In Situ Hybridisation
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GI	Gastro-intestinal
GMMG	German-speaking myeloma multicenter group
HB	Hemoglobin
HDM	High Dose Melphalan
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte histocompatibility Antigen
HOVON	Dutch-Belgian Hematology-Oncology Cooperative Group
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IFM	Intergroup Français de Myelom

ISS	International Staging System
ITT	Intention To Treat
IU	International Units
KCl	Potassium chloride
LDH	Lactate Dehydrogenase
METC	Medical Ethical review committee
MM	Multiple Myeloma
NaCl	Sodium Chloride
NCI	National Cancer Institute
NMSG	Nordic Myeloma Study group
NYHA	New York Heart Association
OS	Overall Survival
PAD	Bortezomib, Doxorubicin (Adriamycin), Dexamethasone
PB	Peripheral Blood
PBSC	Peripheral Blood Stem Cell(s)
PD	Progressive Disease
PO	Per Os
PR	Partial Response
SAE	Serious Adverse Event
SC	Subcutaneous
SCT	Stem Cell Transplantation
SD	Stable Disease
SNP	Single Nucleotide Polymorphism
TBI	Total Body Irradiation
ULN	Upper Limit of Normal
VAD	Vincristine, Doxorubicin (Adriamycin), Dexamethasone
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen

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A. Diagnostic Criteria Multiple Myeloma

DIAGNOSIS OF MULTIPLE MYELOMA

Major criteria:

1. plasmacytoma (tissue biopsy)
2. > 30% plasma cells in bone marrow
3. monoclonal serum M-protein IgG > 35 g/l; IgA >20 g/l, or urine M-protein >1 g/24 hrs in the absence of amyloidosis

Minor criteria:

a. plasma cells in bone marrow > 10% but ≤ 30%

b. monoclonal serum M-protein IgG ≤ 35 g/l, IgA ≤ 20 g/l, urine M-protein ≤ 1 g/24 hrs

c. lytic bone lesions

d. normal IgG <6 g/l or IgM <0.5 g/l or IgA <0.2 g/l

Multiple Myeloma is diagnosed in case one of the following combinations of criteria is present:

1 + b **or** 1 + c **or** 1 + d

2 + b **or** 2 + c **or** 2 + d

3 + a **or** 3 + c **or** 3 + d

a + b + c **or** a + b + d

STAGING OF MULTIPLE MYELOMA

Staging according to Salmon & Durie criteria

Stage I	Low Tumor Mass – all of the following:
	Hemoglobin > 6.2 mmol/l
	Ca^{2+} < 2.65 mmol/l *
	IgG < 50 g/l
	IgA < 30 g/l
	Urine M-protein < 4 g/24 hrs
	Normal skeletal assessment or solitary plasmacytoma
Stage II	Intermediate Tumor Mass:
	Patients who qualify for neither Stage I nor III
Stage III	High Tumor Mass – Any one of the following:
	Hemoglobin < 5.3 mmol/l
	Ca^{2+} > 2.65 mmol/l *
	IgG > 70 g/l
	IgA > 50 g/l
	Urine M-protein > 12 g/24 hrs
	≥ 3 lytic bone lesions on skeletal survey (bone scans are not acceptable)
A	Normal renal function (creatinin < 177 μmol/l)
B	Renal insufficiency (creatinin ≥ 177 μmol/l)

* Correct the serum Ca^{2+} by adding 0.02 mmol/l for every g/l albumin below 40 g/l

Staging according to ISS criteria

Stage I:	Serum β_2-microglobulin < 3.5 mg/l AND Serum albumin ≥ 3.5 g/dl (≥ 35 g/l)
Stage II:	Patients who qualify for neither Stage I nor III
Stage III:	Serum β_2-microglobulin ≥ 5.5 mg/l

B. Response Criteria for Multiple Myeloma

Based on EBMT, IBMTR and ABMT criteria (British J. Haemat. 102: 1115-1123, 1998)

Complete response (CR) requires *all* of the following:

1. Absence of the original monoclonal paraprotein (M-Protein) in serum and (10 x concentrated) urine by immunofixation, maintained for at least 6 weeks.
2. < 5% plasma cells in a representative bone marrow aspirate or otherwise in a bone marrow biopsy. Only in patients with non-secretory myeloma, bone marrow investigation must be repeated after an interval of 6 weeks to confirm CR.
3. No increase in size or number of lytic bone lesions (development of compression fractures does not exclude CR)
4. Disappearance of any soft tissue plasmacytoma.

Patients in whom some, but not all, criteria for CR are fulfilled are classified as PR or VGPR, providing the remaining criteria satisfy the requirements for PR / VGPR. This includes patients in whom routine electrophoresis is negative but in whom immunofixation has not been performed.

Very good partial response (VGPR) requires all of the following:

1. Meeting the criteria for partial response but show a 90% reduction of serum M-protein concentration for at least 6 weeks.

Partial response (PR) requires *all* of the following:

1. 50% reduction of serum M-protein concentration maintained for at least 6 weeks.
2. Reduction in 24 hrs urine M-protein either by ≥ 90% or to < 200 mg, maintained for at least 6 weeks.
3. In patients with non-secretory myeloma, ≥ 50% reduction in plasma cells in a representative bone marrow aspirate, or otherwise bone marrow biopsy, maintained for at least 6 weeks.
4. 50% reduction in size of soft tissue plasmacytoma.
5. No increase in size or number of lytic bone lesions (development of compression fractures does not exclude PR).

Patients in whom some, but not all, criteria for PR are fulfilled are classified as MR, providing the remaining criteria satisfy the requirements for PR.

Minimal response (MR) requires *all* of the following:

1. 25% reduction of serum M-protein concentration maintained for at least 6 weeks.
2. 50% reduction in 24 hrs urine M-protein, maintained for at least 6 weeks.
3. In patients with non-secretory myeloma, ≥ 25% reduction in plasma cells in a representative bone marrow aspirate, or otherwise bone marrow biopsy, maintained for at least 6 weeks.
4. 25% reduction in size of soft tissue plasmacytoma.
5. No increase in size or number of lytic bone lesions (development of compression fractures does not exclude MR).

No change (NC)

1. Not meeting the criteria of either minimal response or progressive disease.

Progressive disease (for patients without prior response) requires one or more of the following:

1. 25% increase in serum M-protein level, which must also be an absolute increase of at least 5 g/l and confirmed at least once.
2. 25% increase in 24 hrs urine M-protein, which must also be an absolute increase of at least 200 mg/24 hrs and confirmed at least once.
3. 25% increase in plasma cells in a representative bone marrow aspirate or bone marrow biopsy
4. Definite increase in the size of existing bone lesions or soft tissue plasmacytomas.
5. Development of new bone lesions or soft tissue plasmacytomas (development of compression fractures does not exclude continued response and may not indicate progression).
6. Development of hypercalcaemia (corrected serum calcium > 2.80 mmol/l) not attributable to any other cause.

Plateau

1. Stable values (within 25% above or below value at the time response is assessed) maintained for at least 3 months.

Relapse from CR requires at least one of the following:

1. Reappearance of serum or urine M-protein on immunofixation or routine electrophoresis, confirmed by at least one further investigation and excluding oligoclonal immune reconstitution.
2. 5% plasma cells in a representative bone marrow aspirate or bone marrow biopsy
3. Development of new lytic bone lesions or soft tissue plasmacytomas or definite increase in the size of residual bone lesions (development of compression fractures does not exclude continued response and may not indicate relapse).
4. Development of hypercalcaemia (corrected serum calcium > 2.80 mmol/l) not attributable to any other cause.

Progression after PR / MR requires one or more of the following:

1. 25% increase in serum M-protein level compared to nadir, which must also be an absolute increase of at least 5 g/l and confirmed at least once.
2. 25% increase in 24 hrs urine M-protein compared to nadir, which must also be an absolute increase of at least 200 mg/24 hrs and confirmed at least once.
3. 25% increase in plasma cells in a representative bone marrow aspirate or bone marrow biopsy compared to nadir.
4. Definite increase in the size of existing bone lesions or soft tissue plasmacytomas.
5. Development of new bone lesions or soft tissue plasmacytomas (development of compression fractures does not exclude continued response and may not indicate progression).
6. Development of hypercalcaemia (corrected serum calcium > 2.80 mmol/l) not attributable to any other cause.

C. Common Toxicity Criteria

The grading of toxicity and adverse events will be done using the NCI Common Terminology Criteria for Adverse events, CTCAE version 3.0, revised Dec 12, 2003. A complete document may be downloaded from the following sites:

http://ctep.cancer.gov/reporting/ctc.html
http://www.eortc.be/Services/Doc/CTC
http://www.hovon.nl

A hardcopy may be obtained from the HOVON Data Center on request.

D. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

2 Some bed time, but to be in bed less than 50% of normal daytime

3 Needs to be in bed more than 50% of normal daytime

4 Unable to get out of bed

E. NYHA* scoring list

Grade 1 No breathlessness

Grade 2 Breathlessness on severe exertion

Grade 3 Breathlessness on mild exertion

Grade 4 Breathlessness at rest

The *New York Heart Association functional and therapeutic classification applied to dyspnoea

F. Management of patients with Bortezomib (Velcade®)-related neuropathic pain and/or peripheral sensory neuropathy

			Peripheral Sensory Neuropathy (NCI CTC Grade)				
			0	1	2	3	4
			Normal	Asymptomatic; loss of deep tendon reflexes or paresthesia (including tingling) but not interfering with function	Sensory alteration or paresthesia (including tingling), interfering with function, but not interfering with ADL	Sensory alteration or paresthesia interfering with ADL	Disabling
Neuropathic Pain(NCI CTC Grade)	0	None	No action	No action	25% dose reduction	Hold; 50% dose reduction; Schedule Δ required	Discontinue Bortezomib
	1	Mild pain not interfering with function	No action	No action	25% dose reduction	Hold; 50% dose reduction; Schedule Δ required	Discontinue Bortezomib
	2	Moderate pain: pain or analgesics interfering with function, but not daily activities	25% dose reduction	50% dose reduction	Hold; 50% dose reduction	Hold; 50% dose reduction; schedule Δ required	Discontinue Bortezomib
	3	Severe pain: pain or analgesics severely interfering with daily activities	Hold; 50% dose reduction; Schedule Δ required	Hold; 50% dose reduction; schedule Δ required	Hold; 50% dose reduction; schedule Δ required	Discontinue Bortezomib	Discontinue Bortezomib
	4	Disabling	Discontinue Bortezomib	Discontinue Bortezomib	Discontinue Bortezomib	Discontinue Bortezomib	Discontinue Bortezomib

Key:

Hold: Interrupt Bortezomib for up to 2 weeks until the toxicity returns to Grade 1 or better.

25% Dose reduction: Bortezomib dose reduction from 1.3 to 1.0 mg/m^2/dose.

50% Dose reduction: Bortezomib dose reduction from 1.3 to 0.7 mg/m^2/dose.

Schedule Δ Required: Schedule change from Bortezomib twice per week (Days 1, 4, 8 and 11) to once per week (Days 1, 8, 15, and 22) required. If the patient is already on a once weekly schedule, then the drug will be given every other week (e.g. Day 1, Day 15).

G. Management and handling DNA samples for microarray

Pharmacogenomic analysis

Whole genome gene expression profiling

Whole genome transcriptional profiling will be used to establish the level of over 47,000 transcripts, representing 38,500 genes. Aim of this exploratory analysis is to develop a molecular classification of multiple myeloma patients, validation of prognostic markers identified in previous studies and identification of novel candidate markers that predict patients response to the specific treatment used in the current study (Bortezomib versus standard treatment in previously untreated myeloma) by correlations with clinical outcome.

Bone marrow samples for whole genome transcriptional profiling will be collected at entry in regional centers (section 11.2.10) where plasma cells will be purified using CD138 Magnetic Cell Sorting (MACS) selection. Performance of the purification will be monitored using FACS analysis of the original bone marrow sample and the final plasma cell fraction with CD38 and CD138 antibodies. Purified plasma cells will be stored at -80°C at the centers where they were isolated and will be collected batch-wise by the central laboratory at the Erasmus MC, Rotterdam where they will be further processed and analyzed as outlined below.

Total RNA will be isolated using the RNeasy kit (Qiagen). RNA levels, and quality will be assessed with the RNA6000 Nano assay on the Agilent 2100 Bioanalyzer. Samples in which the ratio between 28S and 18S RNA is less then 2 will be rejected from analysis.

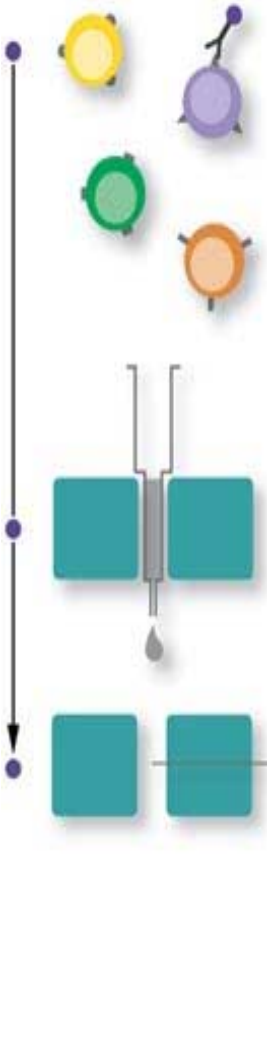
Total RNA will be used to prepare antisense biotinylated RNA using the Two-Cycle Target Labeling kit (Affymetrix). The biotinylated RNA will be hybridized to the Affymetrix U133 Plus 2.0 array. Staining, washing and scanning procedures, as well as hybridization controls provided by Affymetrix will be used and GeneChips will be visually inspected for irregularities.

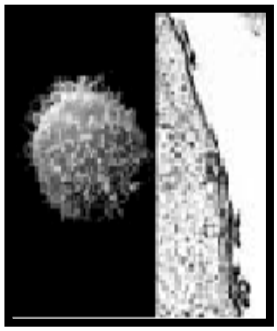
The global method of normalization will be used and the mean difference between all GeneChips will be used as indicator of assay-quality. In addition, the variations in percentage of genes present, the ratio of action 3’ to 5’ and the ratio of GAPH 3’ to 5’ will be assessed to monitor sample and assay quality.

The Omniviz package will be used to perform and visualize the results of unsupervised cluster analysis, whereas all supervised analyses will be performed using SAM software. For supervised class-prediction analyses, PAM software in R will be applied.

Magnetic Cell Separation (MACS)

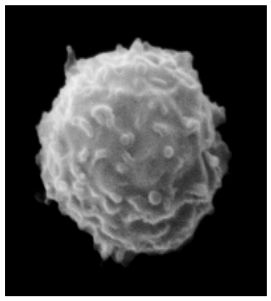
Ficoll separated target cells (plasma cells) are magnetically labeled with CD138 MACS MicroBeads



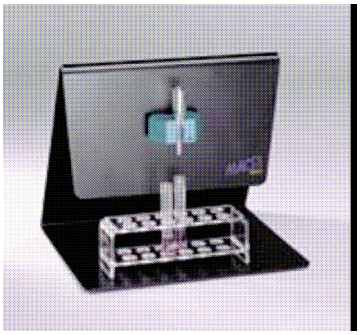


B-Cells labeled with MicroBeads

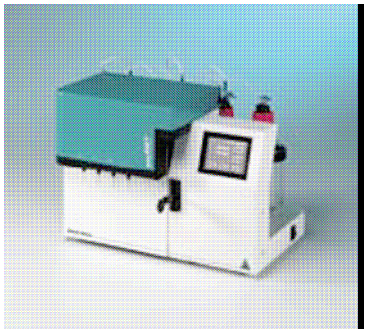
Cells labeled with MicroBeads are retained on the MACS Column in a high-gradient magnetic field, while the unlabeled cells pass through



The retained cells are eluted as the enriched, positively selected cell fraction. Plasma cell purity will be analyzed using the Fluorescent Activated Cell Sorter (FACS)



MiniMACS™ Separator



AutoMACS™ Separator

H. Single Nucleotide Polymorphisme (SNP) analysis in myeloma patients

Thalidomide and Bortezomib have a remarkable effect in patients with relapsed or refractory multiple myeloma with 30-40% response rates (1). However, 30% of the patients have to stop prematurely because of intolerable side effect.(2-3) The toxicity profile consists of painful neuropathy (Bortezomib / Thalidomide), neutropenia, thrombocytopenia (Bortezomib) and gastro-intestinal symptoms (Bortezomib / Thalidomide). The proportion of patients experiencing these side effects in trials ranged from 10 to 50%. The most likely explanation for the inter-individual variation in response and toxicity may be found in the genetic heterogeneity of genes involved in detoxification processes, DNA repair, myeloma biology and neuropathy.

This explanation is substantiated by retrospective analysis that has been done in the Erasmus MC. We observed that patients with multiple myeloma who were treated in a phase III trial with conventional vs. high-dose regimens and who have a variant polymorphism genotype of a gene involved in drug metabolism, CypP450 3A5, have a better overall survival compared to patients with a wild-type genotype of this gene (4, figure 1). It is known that such single nucleotide polymorphisms are observed in many genes that are important for multiple myeloma biology and/ or are involved in metabolism of anti-cancer drugs. Furthermore, it is anticipated that these SNP's play an important role in outcome (OS and DFS) and toxicity in patients treated with conventional agents, while nothing is known about their role for the effects of novel agents.
The novel agents Bortezomib and Thalidomide are now moving from relapse treatment to up-front therapy of multiple myeloma. Therefore it is of critical importance to investigate which gene(s) are involved in the drug metabolism and anti-tumor effect of these agents.

The presence and involvement of specific genes in the drug metabolism and anti-tumor effect of Bortezomib and Thalidomide will be investigated, using Taqman analysis of DNA isolated from blood. The presence of inherited genotype polymorphisms will be correlated to response and toxicity.
Blood samples will be taken before start of treatment. About 2 ml of EDTA or Citrate blood is needed to obtain a reasonable amount of DNA, necessary for the analyses.
Blood samples will be stored at 4-12 °C. The samples should be sent to the laboratory of the Erasmus MC at room temperature within one week after sampling to maintain a good quality of DNA. The centers in the Netherlands will be provided with special envelopes (according to Dutch post office directives) for the sending of diagnostic samples. Centers from other participating countries will be contacted directly by the Erasmus MC laboratory to make arrangements for shipping of samples.
SNP analysis will be performed in a high trough-put system with assays developed by ABI.

We have set up a list with genes of relevance on condition that the variant allele frequency is more then 10 %. Genes to be studied:
Xenobiotic metabolism: CYP 2C9, 2C19, 2D6, 3A4, 3A5, GSTP1, GSTM, GSTT1, MDR1, C-MOAT, Glucocorticoid receptor
DNA repair (tumor protection): XRCC4, XRCC3, ERCC1, ERCC2, RAD51, BRCA1 en 2, FANCA, FANCD2, FANCG, NBS, ATR, ATM
Bone disease : Dickkopf1, ACP5, RANKL, RANK, MIP1a
Neuropathy: APOE, FASN, NGFR
Venous Thrombosis: PT, FV, FVIII, FXIII, TAFI, PAI
Pro-inflammatory and cytokine networks : IGF1-R, IL6-R, TNFa, TNFb, VEGF, IL-1B
Myeloma survival: CCDN1, CCDN2, CCDN3, MAF, FGFR3, RelA/NFkB, IKK, JNK

References

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Figure 1:Unadjusted Kaplan-Meier plots of OS for CYP3A5*3 polymorphism.