Phase I/II trial of Lenalidomide plus Bortezomib combined with Dexamethasone in patients in 1st relapse or primary refractory after first line therapy for Multiple Myeloma

PROTOCOL

Principal Investigator:		P. Sonneveld
Study Coordinators	:	E. Vellenga
		H.M. Lokhorst
Sponsor	:	HOVON
		VU Medisch Centrum,
		P.O.Box 7057
		1007 MB Amsterdam
Statistician	:	B. van der Holt
Datamanagement	:	HOVON Data Center
Registration	:	HOVON Data Center
		Erasmus MC - Daniel den Hoed
		P.O.Box 5201
		3008 AE ROTTERDAM
		The Netherlands
		tel. +31.10.7041560
		fax +31.10.7041028
		https://www.hdc.hovon.nl/top

EudraCT number		2007-002533-37
First version	:	December 11, 2006
Final version	:	January 30, 2008
Date of activation	:	September 15, 2008
Approved	:	CKTO 2007-4221, 21 November 2007
		METC Erasmus MC 2008-115, 02 June 2008
Amendment 1	:	October 07, 2009
Amendment 2	:	October 11, 2010

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Principal Investigator:

Signature of Investigator

25/10/2010

Date

P Sonneveld

Printed Name of Investigator

LOCAL INVESTIGATOR SIGNATURE PAGE

Local site name: _____

Local Investigator:

_____ _____

Signature of Investigator Date

_____

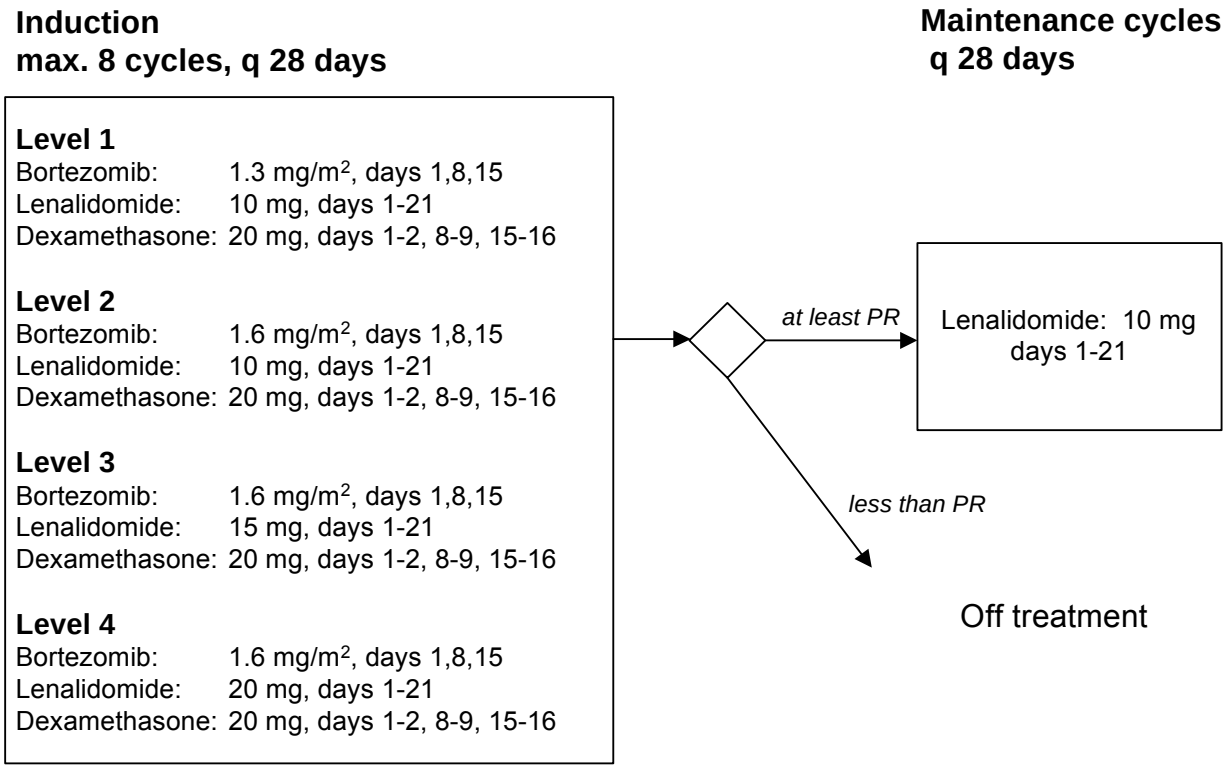
Printed Name of Investigator

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice (2001-20-EG), and local regulations governing the conduct of clinical studies.

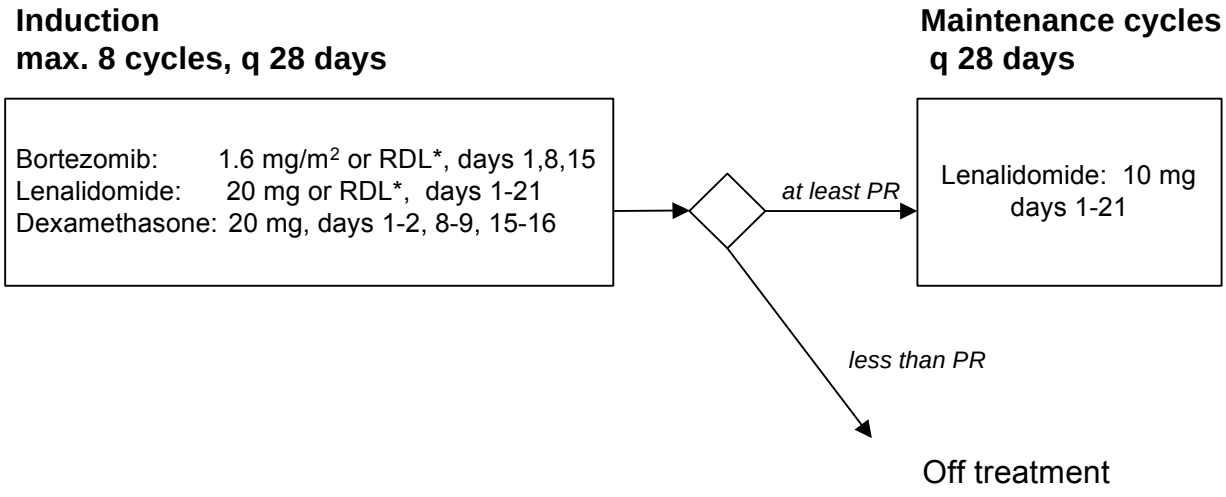
1 Scheme of study

Please note: in **Phase II** we are including patients according to **level 2** (1.6 mg/m^2 Bortezomib and 10 mg Lenalidomide, 20 mg Dexamethasone)

Phase I



Phase II



* Recommended dose level (RDL) will be determined during phase I

2 Table of contents

Page

3 Synopsis

Study phase	Phase I-II
Study objectives	Evaluation of the effect of Bortezomib combined with Lenalidomide in addition to Dexamethasone for induction and the effect of Lenalidomide alone for maintenance treatment
Patient population	Patients with multiple myeloma, in 1st relapse or refractory after first line therapy, Salmon & Durie stage II or III, age >= 18 years inclusive
Study design	Prospective, multicenter
Duration of treatment	Expected duration of induction is 6 - 7 months. Maintenance therapy with Lenalidomide will be given until relapse/progression. All patients will be followed until 5 years after registration or, for patiens who are still on maintenance at that moment, until completion of maintenance therapy.
Number of patients	72-84 patients registered
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: I 2008 End of recruitment: IV 2012

4 Investigators and study administrative structure

Responsibility	Name	Affiliation/Address
Study Coordinators	P. Sonneveld	Erasmus MC, Rotterdam
	H.M. Lokhorst	Utrecht Medical Center, Utrecht
	E. Vellenga	University Medical Center Groningen
Representative of	P.C. Huijgens, chairman	VUMC, Amsterdam
Sponsor (HOVON)	P. Sonneveld, treasurer	Erasmus MC, Rotterdam
	L.F. Verdonck, secretary general	Utrecht Medical Center, Utrecht
Registration	HOVON Data Center	Erasmus MC - Daniel den Hoed P.O.Box 5201 3008 AE ROTTERDAM The Netherlands tel. +31.10.7041568 fax +31.10.7041028 https://www.hdc.hovon.nl/top
Monitoring	HOVON Data Center	Erasmus MC - Daniel den Hoed
Writing Committee	J.W. Baars	A. van Leeuwenhoek Hospital, Amsterdam
	R. Willemze	LUMC, Leiden
	G.M.J. Bos	University Hospital Maastricht
	J.J. Cornelissen	Erasmus MC, Rotterdam
	A.J. Croockewit	Radboud MC, Nijmegen
	R. Raymakers	Radboud MC, Nijmegen
	P.C. Huijgens	VUMC, Amsterdam
	P. Joosten	Medical Center, Leeuwarden
	H.M. Lokhorst	Utrecht Medical Center, Utrecht
	M. van Marwijk Kooy	Isala Clinics – Sophia, Zwolle
	M.H.J. van Oers	Academic Medical Center, Amsterdam
	M.R. Schaafsma	Medical Spectrum Twente, Enschede
	P. Sonneveld	Erasmus MC, Rotterdam
	E. Vellenga	University Medical Center Groningen
	G.E.G. Verhoef	University Hospital Leuven
	O. de Weerd	St. Antonius Hospital, Nieuwegein
	P.W. Wijermans	Haga Hospital, The Hague
	S. Wittebol	Meander MC, Amersfoort
	P.Ypma	Haga Hospital, The Hague
	S. Zweegman	VUMC, Amsterdam
Cytogenetics review	H.B. Beverloo	Erasmus MC, Rotterdam
Statistician	B. van der Holt	HOVON Data Center, Rotterdam

Datamanagement	HOVON Data Center	Erasmus MC – Daniel den Hoed
Serious Adverse Events (SAEs) notification	HOVON Data Center	Erasmus MC – Daniel den Hoed Fax: +31.10.7041028

5 Introduction

5.1 Multiple Myeloma

Multiple myeloma (MM) 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 MM among patients treated with conventional chemotherapy is 25%, while with intensified therapy this may increase to 50 %[1, 2]. Novel agents are urgently needed to improve the treatment results of this disease.

5.2 Treatment of elderly patients with Multiple Myeloma

The results of therapy of MM in elderly patients are less favourable due to several factors including the presence of concomitant diseases and increased toxicity and poor tolerability of intensified treatment regimens. Higher age has been identified as a risk factor in many clinical trials. In addition, elderly patients with MM frequently fail to complete rescue treatment at first or later relapse of MM. The standard treatment of elderly patients with MM has been melphalan plus prednisone, melphalan alone, dexamethasone alone or melphalan plus dexamethasone[3-6]. None of these regimens has been shown to be clearly superior to each other or to combination chemotherapy, while toxicity may differ[7, 8]. While dose-escalation of Melphalan has been demonstrated to be superior in selected elderly patients, its routine use is restricted to highly specialized centers where frequent monitoring is standard[9, 10]. Recent improvements in the first-line treatment of MM of the elderly patient include the addition of Thalidomide to melphalan/prednisone or to dexamethasone [11, 12]. These new combinations have resulted in increased overall and complete response rates and a prolonged disease-free survival. However, ultimately patients continue to relapse and many patients suffer from debilitating side-effects such as irreversible peripheral polyneuropathy.
Treatment options anno 2006 for elderly patients with first or later relapse of MM include Thalidomide, Bortezomib and Lenalidomide as single agent or combined with Dexamethason[13-17]. Recently, two randomized clinical trials showed a superior effect of Lenalidomide plus Dexamethasone over Dexamethasone alone (Weber, ASCO 2005; Dimopoulos, ASH 2005).

5.3 Bortezomib

Bortezomib (VELCADE®) 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 pyrazinecarbonyl L phenylalanine L leucine boronic acid and has a molecular weight of 384.25 daltons. The mechanisms of anti-tumor activity that have been established for Bortezomib involve many pathways including direct induction of apoptosis of tumor cells through inhibition of activation of NF-κB in cells and in tumor microenvironment; reducing adherence of myeloma cells to bone marrow stromal cells; blocking production and intracellular signaling of IL-6 in myeloma cells; blocking production and expression of pro-angiogenic mediators; overcoming defects in apoptotic regulators, such as Bcl-2 overexpression and alterations in tumor suppressor p53[18-20]

5.3.1 Clinical Pharmacology

Upon IV bolus administration, Bortezomib displays a rapid distribution phase (t½α < 30 minutes) followed by a longer elimination phase (t½β > 10 hours) and a large volume of distribution, all consistent with a 2-compartment PK model. 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 [21].

5.3.2 Clinical Experience

Bortezomib has been extensively studied in Phase 1, Phase 2 and Phase 3 studies. The Maximum Tolerated Dose (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 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.
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 low incidence of significant myelosuppression, febrile neutropenia, infections and transfusion dependent thrombocytopenia or anemia, mucositis or alopecia. Decreases in platelet count have been observed on treatment 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. 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)[15]. The 202 patients had a median number of 6 prior lines of therapy.

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 were allowed to have high dose dexamethasone (40mg) added to their Bortezomib treatment.

Bortezomib demonstrated an overall response rate (CR+PR+MR) of 35% with 59% patients experiencing improved or stable disease. The median time to response was 38 days and the median survival of all patients enrolled was 16 months. Responders (CR+PR) 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). In another study 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[22].

Patients were prospectively randomized to receive either 1.0 or 1.3 mg/m^2/dose. The median time to progression for all patients treated was 11 months. Eighty (80%) percent of patients were alive at one year.

The most commonly reported adverse events of Bortezomib 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.
Recently, the results of a randomized Phase 3 trial comparing Bortezomib with high-dose Dexamethasone in patients with a first or later relapse of MM were published[16]. Bortezomib had a significant higher overall and complete response rate, a longer time to progression and a longer overall survival. The observed toxicities were identical to those registered in the Phase 2 trials.
The traditional administration schedule for Bortezomib has been bi-weekly gifts of 1.3 mg/m^2/dose in a 21-days cycle. A potential disadvantage if this administration schedule is the frequency of visits to the outpatient clinic, which may be hard on elderly patients. More recently, a weekly schedule of Bortezomib has been explored in patients with relapse/refractory MM or indolent non-Hodgkin's lymphoma. Weekly Bortezomib (1.3 mg/m^2/dose) with or without steroids was observed to be an effective and convenient therapy in 30 previously treated patients with MM(Suvannasantha et al, ASH #2562, 2005). In a trial in 81 patients with indolent non-Hodgkin lymphoma, Bortezomib could be safely combined with Rituximab at a weekly dose of 1.3 mg/m^2 or1.6 mg/m^2 (de Vos et al, ASH #17, 2005).

In a multicenter, community-based phase II trial, Greco et al (ASCO abstract # 7547, 2006) evaluated the feasibility, toxicity, and efficacy of weekly bortezomib in patiens with previously treated multiple myeloma. Eligibility criteria included a diagnosis of multiple myeloma treated with 1 or 2 previous systemic regimens (only 1 if first-line therapy included high-dose therapy); ECOG PS 0-2; creatinine < 2.0 mg/dL; WBC > 3.0x10^9/l; ANC > 1.0x10^9/l; platelets > 50x10^9/l; no peripheral neuropathy > grade 1; informed consent. All patients received Bortezomib 1.6mg/m^2 IV on days 1, 8, 15, and 22 of each 5-week cycle. Patients were reevaluated at 10-week intervals; treatment continued for 8 cycles (40 weeks) or until myeloma progression. 37 patients entered this trial. Patient characteristics included: median age 70 years; male/female, 20/17; 24 pts (65%) had received 2 previous regimens (previous high dose therapy, 2 patients); elevated β-2 microglobulin, 27 pts (73%). Of 26 patients evaluated, 13 patients (50%) had major responses, 11 patients (42%) stable disease, and 2 patients (8%) had progression. After a median follow-up of 7 months, projected median PFS is 9.6 months; overall survival at 1 year is 81%. Weekly bortezomib was well tolerated. Grade 3/4 toxicities included fatigue (21%), diarrhea (11%), neutropenia (7%), thrombocytopenia (4%), all others < 5%. No grade 3/4 neuropathy occurred. Only 1 patient required Bortezomib dose reduction during treatment, and 2 patients discontinued treatment because of toxicity (myelosuppression, 1; fatigue/dehydration, 1). The authors concluded that weekly Bortezomib is a convenient, well tolerated treatment for previously treated multiple myeloma. Overall response rates with this schedule are similar to those previously reported with the standard twice-weekly schedule.

5.4 Lenalidomide

Lenalidomide (Revlimid®) is a member of a class of pharmaceutical compounds known as immunomodulatory drugs (IMiDs). It is derived from Thalidomide and it offers potential benefit over this first generation IMiD in terms of safety and efficacy in human subjects[23]. The key to its therapeutic potential lies in the fact that it has multiple mechanisms of action, which act to produce both anti-inflammatory and anti-tumor effects. These effects are thought to be multi-factorial in that they depend on both the cell type and the triggering stimulus. To date, Lenalidomide has been associated with TNF-α inhibitory, T cell co-stimulatory and anti-angiogenic activities. Lenalidomide is a 50.000 times more potent inhibitor of TNF-α than Thalidomide, it augments IL-2 and IFN-gamma production and it inhibits IL-6 and VEGF production[24].

5.4.1 Clinical experience

In a Phase I trial of Lenalidomide in relapse/refractory MM 25 patients were treated with Lenalidomide 5-50 mg/day during 28 days. The dose-limiting toxicity was myelosuppression in 13/13 patients treated with the 50 mg/day dose. Other adverse events included fatigue (35 %), leg cramps (40%) and skin rash (40%). In 71% of patients a response was observed, including a PR in 29% [25].
In another Phase I trial in 15 patients who had a relapse after high-dose chemotherapy 20% experienced a PR at the 25 mg or 50 mg doses, and 6 patients continued on an extension study. In a Phase 2 trial in 101 patients with relapsed/refractory MM, a randomization was performed between Lenalidomide 15 mg during 3 weeks for 6 28 day cycles and Lenalidomide 30 mg in the same schedule, to which Dexamethasone was added if no response was achieved. This trial showed a 38% overall response rate including 6% CR and an additive effect of Dexamethasone in some patients [25].
Recently, the results of 2 large randomized , double-blind, placebo-controlled Phase 3 trials were presented, which compared Lenalidomide plus Dexamethasone with Placebo plus Dexamethasone in > 600 patients with first or later relapse of MM [26]. In the MM-010 trial, 351 patients were enrolled of whom 67% had received ≥ 2 prior treatments and 55% had received a prior regimen with high-dose chemotherapy with stem cell support. The overall response rate was 50% for Lenalidomide Plus Dexamethasone, including 17% (near)CR and 24% for Placebo plus Dexamethasone with 4% (near)CR. The median Time to Progression was 11.3 months vs 4.7 months ($p<0.001$). Clinically relevant adverse events were Grade 3-4 neutropenia (27% vs 2%), Grade 3-4 thrombocytopenia (10% vs 6%) and constipation (41% vs 22%). The median overall survival had not been reached yet. While no anticoagulant had been used, the rate of deep venous thrombosis was 18% with Lenalidomide Plus Dexamethasone as compared with 7% with Placebo plus Dexamethasone in those

patients receiving erythropoietin. Lenalidomide plus Dexamethasone has also shown high effectivity in previously untreated MM.[17]

5.5 Bortezomib plus Lenalidomide

The combination of Bortezomib plus Lenalidomide has been explored in the Dana Farber Cancer Institute in a phase 1 trial in relapsed and/or refractory patients with MM, in which the dose of Bortezomib and Lenalidomide were escalated. The MTD was Bortezomib 1.0 mg/m^2 per gift, days 1,4,8,11 with Lenalidomide 15 mg/day for 21 days (P. Richardson ASH abstract # 365,2005; personal communication). The dose limiting toxicity was neutropenia. In 17 heavily pretreated patients this combination was well tolerated and a CR + PR was achieved in 59% even in patients who had been exposed to either agent alone.

5.6 Rationale of the study

The current treatment strategies for MM in elderly patients are focusing on first line therapy. However, with the availability of new targeted agents, second-line treatment for patients who have a relapse or who were not responding, is a realistic option with a high probability of response and disease-free survival longer than 18 months. It is imperative that second-line treatment can be administered within the planned dose and schedule. Toxicity should be minimized and overlapping toxicity profiles of drug combinations should be avoided. Bortezomib and Lenalidomide are both effective anti-myeloma agents which have complementary modes of action and which do not share an overlapping toxicity profile. The combination of these drugs appears to be a viable opportunity for treatment of elderly patients with MM. The feasibility and effectivity of this drug combination will be investigated, using a more convenient once weekly administration of Bortezomib with a standard schedule of Lenalidomide and Dexamethasone. In order to reduce the risk of relapse, Lenalidomide alone will be used as maintenance treatment.

6 Study objectives

The goal of this trial is to investigate the feasibility (phase I) and efficacy (phase II) of a combination of newly developed anti-myeloma drugs, i.e. Bortezomib and Lenalidomide in elderly patients with Multiple Myeloma who have relapsed after having obtained a previous response with first line therapy or who failed first-line therapy and who are not candidates for high-dose therapy.

6.1 Phase I

Primary objective

- To determine the maximum tolerated dose (MTD) and recommended phase II dose level (RDL) of Bortezomib administered once weekly, and of Lenalidomide administered for 3 weeks when combined with Dexamethasone in a 28-days schedule. See paragraph 14 for definitions of MTD and RDL

Secondary objectives

- To evaluate toxicity, especially myelosuppression, polyneuropathy and thrombosis

6.2 Phase II

Primary objective

- To investigate the efficacy of a maximum of 8 cycles of Bortezomib plus Lenalidomide with Dexamethasone at the RDL, as determined by the (s)CR+VGPR rate

Secondary objectives

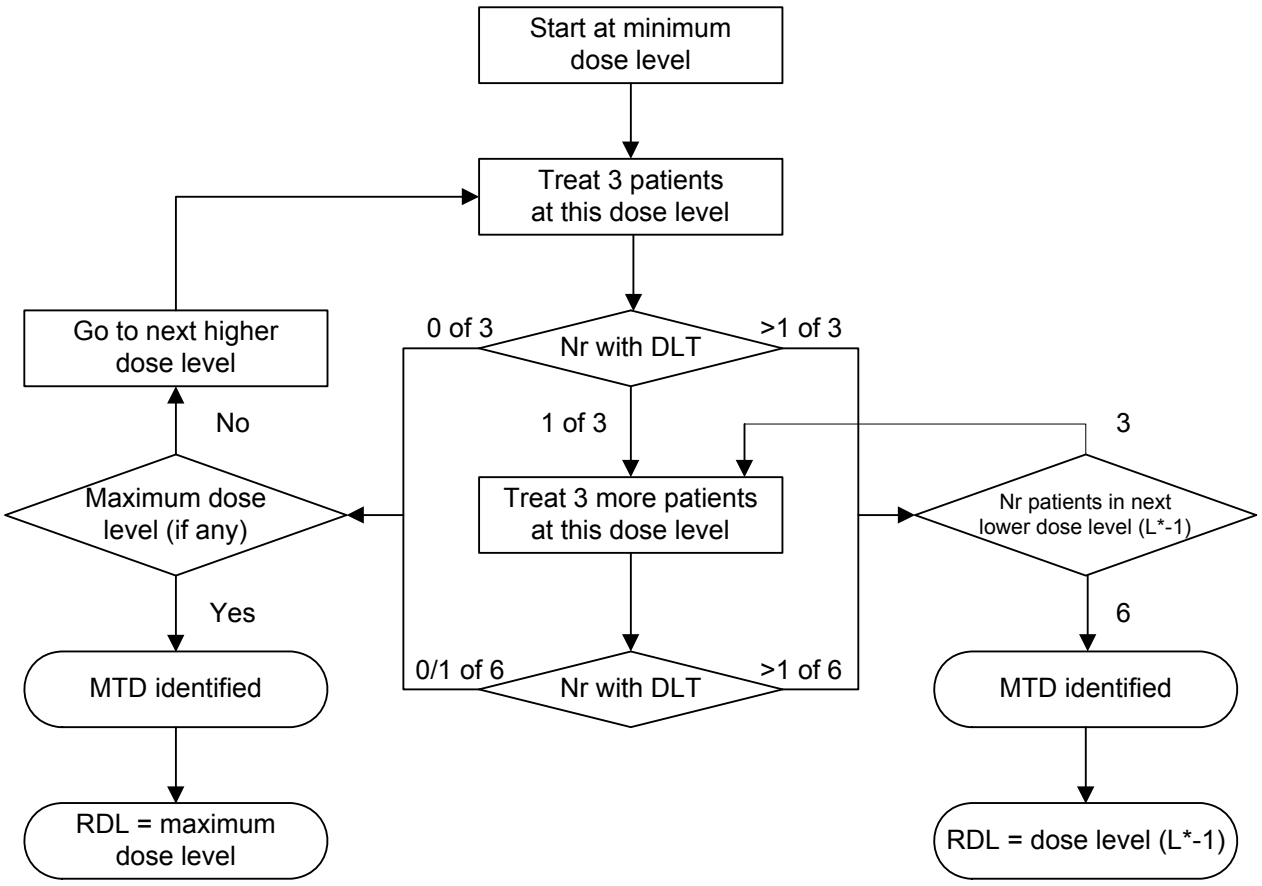
- To evaluate the effect of maintenance treatment with Lenalidomide in patients who have achieved any response (sCR, CR, VGPR, or PR) upon treatment with the combination of Bortezomib plus Lenalidomide with Dexamethasone
- To evaluate toxicity, especially myelosuppression, polyneuropathy and thrombosis
- To evaluate progression free survival
- To evaluate overall survival

7 Study design

This is a prospective, open label, phase I/II study.

7.1 Phase I

During the phase I part of the study, the MTD and RDL of Bortezomib and Lenalidomide with Dexamethasone will be determined according to a slightly modified `3 + 3' dose-escalation scheme, as illustrated in the figure below. A maximum of 4 dose levels will be evaluated.



Enrollment at each dose level will consist of a minimum of 3 patients and a maximum of 6 patients. When 3 or 6 patients have been entered, inclusion will be discontinued until dose limiting toxicity (DLT) has been determined at day 22 after start of cycle I for these patients (see paragraph 14 for definition of DLT). Patients who die of myeloma within 21 days after start of cycle I, but without a DLT, will be considered not-evaluable, and will be replaced by other patients.

The dose escalation stops as soon as at least two patients experience a DLT, either in the first cohort of three patients treated at that dose level, or in the two cohorts of three patients treated at that dose level. Before opening the next higher dose level the dose limiting toxicity information at the preceding dose level will be reviewed and expansion or escalation will be undertaken as appropriate.

The RDL for the phase II part is defined as the highest dose level with 0 or 1 DLT's observed among 6 patients. Therefore, if in level *n* (with *n* > 1), 2 or more DLT's have been observed, and only 3 patients have been included in level *n-1*, then another 3 patients will have to be included in level *n-1*, to ensure that there are fewer than two DLTs among 6 patients at that dose level.

Details of all dose levels (dose and schedule) are given in paragraph 9.

All patients who have achieved at least a PR after 6-8 induction/consolidation cycles, will receive maintenance treatment with Lenalidomide. Dose and schedule of Lenalidomide maintenance are given in paragraph 9.

7.2 Phase II

When the phase I part has established the RDL of Bortezomib and Lenalidomide for the phase II study, all further included patients will be treated with Bortezomib and Lenalidomide at the RDL, combined with Dexamethasone.

Details of the dose and schedule are given in paragraph 9.

All patients who have achieved at least a PR after 6-8 induction/consolidation cycles, will receive maintenance treatment with Lenalidomide. Dose and schedule of Lenalidomide maintenance are given in paragraph 9.

8 Study population

8.1 Eligibility for registration

All eligible patients have to be registered before start of treatment

8.1.1 Inclusion criteria

- Multiple Myeloma Salmon/Durie stage II/III A+B
- Primary refractory to or first relapse after previous objective response (PR, VGPR, CR) on standard first-line treatment
- Not a candidate for high-dose therapy
- Age >= 18 years
- Measurable disease, i.e., serum M-component (>10 g/l), or urinary light-chain excretion (>200mg/24h),or abnormal FLC ratio with involved free light chain (FLC) > 100 mg/l or proven plasmacytoma by biopsy
- Able and/or willing to use adequate contraceptives (especially male patients)
- Written informed consent

8.1.2 Exclusion criteria

- Patient is unable or unwilling to adhere to the requirements of the Lenalidomide Pregnancy Prevention Risk Management Plan.

- Prior therapy with Bortezomib or Lenalidomide
- History of allergic reaction attributable to compounds containing boron or mannitol.
- Peripheral neuropathy or neuropathic pain Grade 2 or higher as defined by NCI CTCAE version 3.
- AL amyloidosis
- Uncontrolled or severe cardiovascular disease:
  - New York Heart Association (NYHA) Class III or IV heart failure
  - Myocardial infarction within the last 6 months of study entry
  - Reduced left ventricular function with an ejection fraction $\leq$ 50% as measured by MUGA scan or echocardiogram (another method for measuring cardiac function is acceptable)
  - Unstable angina
  - Unstable cardiac arrhythmias
  - Clinically significant pericardial disease
- Impaired hepatic or renal function as defined by:
  - ALT and/or AST > 3 x normal value
  - Bilirubin > 3 x normal value
  - Serum creatinin > 3 x normal value (after adequate hydration)
- Concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes, infection, hypertension, etc.)
- Known HIV positivity
- History of active malignancy during the past 5 years, except basal carcinoma of the skin or stage 0 cervical carcinoma

9 Treatment schedule

9.1 Phase I

During induction treatment, Bortezomib will be administered once weekly by rapid intravenous administration at a starting dose of 1.3 mg/m^2, on days 1, 8, 15 followed by a wash-out. During the Phase I part of the trial the dose of Bortezomib will be escalated to 1.6 mg/m^2. Lenalidomide will be administered as an oral formulation at a starting dose of 10 mg/day, days 1-21, followed by a one week interval. During the Phase I part of the trial the dose of Lenalidomide will be escalated to dose of 20 mg/day.
Dexamethasone will be added orally at a dose of 20 mg on days 1,2, 8,9,15, and 16.
Induction cycles will be repeated at 28-days intervals until patients have achieved a (s)CR or until a maximum of 6 induction cycles have been given.

In patients who have achieved at least VGPR after a maximum of 6 cycles treatment will be continued with 2 more cycles as consolidation. In patients who did not achieve at least VGPR after 6 cycles, no consolidation cycles will be given.

For patients who achieve at least PR after induction/consolidation treatment maintenance treatment with Lenalidomide will be started at day 29 after start of the last cycle at a dose of 10 mg days 1-21. Maintenance cycles will be repeated at 28-days intervals until relapse, progression or when a medical condition occurs that requires stopping the treatment.

Induction/consolidation cycles

Agent	Dose/day	Route	Days
Dose level 1			
Bortezomib	1.3 mg/m^2	i.v.	1,8,15
Lenalidomide	10 mg	p.o	1-21
Dexamethasone	20 mg	p.o	1,2, 8,9,15,16
Dose level 2			
Bortezomib	1.6 mg/m^2	i.v.	1,8,15
Lenalidomide	10 mg	p.o	1-21
Dexamethasone	20 mg	p.o	1,2, 8,9,15,16
Dose level 3			
Bortezomib	1.6 mg/m^2	i.v.	1,8,15
Lenalidomide	15 mg	p.o	1-21
Dexamethasone	20 mg	p.o	1,2, 8,9,15,16
Dose level 4			
Bortezomib	1.6 mg/m^2	i.v.	1,8,15
Lenalidomide	20 mg	p.o	1-21
Dexamethasone	20 mg	p.o	1,2, 8,9,15,16

Maintenance

Agent	Dose/day	Route	Days
All dose levels			
Lenalidomide	10 mg	p.o.	1-21

9.1.1 Dose modification

If a patient developes DLT during cycle 1 in dose level 1 the patient will go off protocol treatment. In dose level 2-4, dose reduction for the next cycles by one dose level is permitted for patients who develop DLT.
Guidelines for managing Bortezomib or Lenalidomide related toxicity can be found in appendices E and F.
In case an event occurs witin 22 days after start of cycle 1 which does not qualify as DLT but requires dose modification please contact the principal investigator P.Sonneveld.

9.2 Phase II

The Phase II part of the trial will be performed at the MTD level of Bortezomib and Lenalidomide. Below the expected MTD's are listed, based on the above mentioned literature. If the MTD's will deviate from the expected, the protocol will be amended accordingly.
During induction treatment, Bortezomib will be administered once weekly by rapid intravenous administration at the MTD dose or if no MTD is reached, at the dose of 1.6 mg/m^2, on days 1, 8, 15 followed by a wash-out.
Lenalidomide will be administered as an oral formulation at the MTD dose or highest dose if MTD is not reached, days 1-21, followed by a one week interval.
Dexamethasone will be added orally at a dose of 20 mg on days 1,2, 8,9,15 and 16.
Induction cycles will be repeated at 28-days intervals until patients have achieved a (s)CR or until a maximum of 6 induction cycles have been given. In patients who have achieved at least VGPR after a maximum of 6 cycles treatment will be continued with 2 more cycles as consolidation. In patients who did not achieve at least VGPR after 6 cycles, no consolidation cycles will be given.

For patients who achieve at least PR maintenance treatment with Lenalidomide will be started at day 29 after start of the last cycle, at a dose of 10 mg days 1-21.
Maintenance cycles will be repeated at 28-days intervals until relapse, progression or when a medical condition occurs that requires stopping the treatment.

Induction /consolidation cycles

Agent	Dose/day	Route	Days
Bortezomib	1.6 mg/m^2 or MTD	i.v.	1,8,15
Lenalidomide	10 mg or MTD	p.o	1-21
Dexamethasone	20 mg	p.o	1,2, 8,9,15,16

established RDL from phase I

Maintenance

Agent	Dose/day	Route	Days
Lenalidomide	10 mg	p.o	1-21

9.2.1 Dose modification

Guidelines for managing Bortezomib or Lenalidomide related toxicity can be found in appendices E and F.

9.3 Lenalidomide information

Lenalidomide is available in 5 mg and 10 mg capsules for oral administration. Each capsule contains lenalidomide as the active ingredient and the following inactive ingredients: lactose anhydrous, microcrystalline cellulose, croscarmellose sodium, and magnesium stearate. The 5 mg capsule shell contains gelatin, titanium dioxide and black ink. The 10 mg capsule shell contains gelatin, FD&Cblue #2, yellow iron oxide, titanium dioxide and black ink.

In the Netherlands Celgene Corporation will supply Revlimid®, lenalidomide (CC-5013) to be used for the induction cycles, maintenance treatment with lenalidomide will be on prescription. Medication labels will comply with the legal requirements of each country and will be printed in the local language. The storage conditions for study drug will be described on the medication label. Bottles must be stored in a safe, secure location. The investigator must maintain an overall drug accountability log for the study, as well as individual records for each patient. The drug formulation, dose, number of bottles/capsules dispensed, received and returned must be recorded for each patient.

Patients must comply with the Lenalidomide (Revlimid®) Risk Management Program.

9.4 Prophylaxis

During treatment with Bortezomib, patients will receive antiviral prophylaxis to prevent Herpes Zoster, consisting of Valacyclovir (Zelitrex®) 2 x 500 mg daily. During treatment with Lenalidomide patients will use Aspirin 1 x 100 mg daily to prevent thrombo-embolic events.

9.5 Concomitant medication

9.5.1 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.

9.5.2 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.

9.5.3 Forbidden concomitant medication during the study

- The use of antineoplastic therapy, other than protocol-specified study medication, is not allowed until progressive disease is established.

10 End of protocol treatment

Reasons for going off protocol treatment are:

- DLT in dose level 1
- Response is not (s)CR, VGPR, or PR at the end of induction treatment
- Excessive toxicity (including toxic death)
- Progression / relapse (during induction or during maintanance)
- Intercurrent death
- No compliance of the patient (especially refusal to continue treatment)
- Pregnancy (of female patient)
- Intercurrent disease leading to inadequate dosing and/or treatment

11 Required clinical evaluations

11.1 Time of clinical evaluations

- At entry
- After each induction cycle
- During maintenance and follow up: every 2 months

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

11.2.1 Prior to treatment at entry

- Prior history of multiple myeloma, diagnostic results, prognostic factors (cytogenetics / FISH), prior treatment, prior response to treatment and toxicities
- Physical examination including body weight, height, signs of extramedullary myeloma
- WHO performance status
- Hematology
- Blood chemistry
- β_2- microglobuline
- Immunochemistry
- Chest X-ray and skeletal X-ray
- Cardiac ejection fraction, measured by MUGA or echocardiogram
- ECG
- Bone marrow aspiration and biopsy
- Peripheral blood to obtain germ-line DNA for pharmacogenomics

11.2.2 During induction treatment

- History and physical examination before start of each treatment cycle
- Hematolgy at each Bortezomib administration
- Blood chemistry after each induction cycle (before start of the next cycle)
- β_2- microglobuline at the end of induction treatment
- Immunochemistry after each cycle (before start of the next cycle)
- Bone marrow examination for % plasma cells at the moment of complete disappearance of serum/urine M-component and/or at 4 weeks after last treatment.
- Skeletal radiography at the moment of complete disappearance of serum/urine M-component and/or at 4 weeks after last treatment.

11.2.3 During maintenance treatment and follow up

All patients will be followed until 5 years after registration or, for patiens who are still on maintenance at that moment, until completion of maintenance therapy.

- History and physical examination once monthly
- Hematology at each outpatient visit
- Blood chemistry once monthly
- β_2- microglobuline
- Immunochemistry
- Bone marrow examination for % plasma cells at the moment of complete disappearance of serum/urine M-component and/or once every 6 months and at the time of progression.
- Skeletal radiography at the moment of complete disappearance of serum/urine M-component and/or once every 12 months and at the time of progression.

.

Required investigations at entry, during treatment and during follow up

	At entry	After each Bor/Len/Dex	At end of induction	Maintenance and follow up
Medical history	X	X	X	X
Physical examination	X	X	X	X
Hematology	X	X	X	X[4)]
PB cryopreservation	X			
Blood chemistry	X	X	X	X[4)]
Immunochemistry	X	X	X	X
Blood for SNP analysis	X			
Bone marrow				

Bone marrow aspirate	X	X[5]	X	X[6]
Bone marrow biopsy	X			
BM cryopreservation[2)]	X			
Specific investigations				
β_2-microglobulin	X		X	
Creatinin clearance	o.i.	o.i.	o.i.	
Skeletal survey[3)]	X		X	X[1)]
X-thorax	X			
ECG	X			
Cardiac ejection	X		X	
Additional investigations	o.i.	o.i.	o.i.	o.i.
Cytogenetic analysis	X			

o.i. on indication

1) once a year

2) for micro-array analysis

3) Radiographic skeletal survey is preferred.
in case of extramedullary plasmacytoma, the skeletal surveys should be repeated at all evaluation moments

4) during maintenance: haematology and blood chemistry tested every four weeks

5) at the moment of complete disappearance of serum/urine M-component

6) at the moment of complete disappearance of serum/urine M-component, and/or every 6 months and at time of progression

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

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

Hematology

- Hemoglobin
- Leukocyte count, differential count
- Platelets
- At entry: PB cryopreservation for SNP analysis

Blood chemistry

- Creatinin
- Liver enzymes
- Total bilirubin
- Alkaline phosphatase
- Total proteins
- Albumin
- LDH
- Calcium
- Potassium
- Uric acid

Immunochemistry

- Qualitative and Quantitative serum M-protein, including immunofixation to confirm CR
- Free light chain at entry and at best reponse
- Qualitative and Quantitative urine M-protein in 24 hrs urine, including immunofixation to confirm CR

Bone marrow

Bone marrow biopsy

Bone marrow aspirate at entry for:

- Morphology
- Labeling Index (by BRDU) or KI-67
- Cytogenetic analysis, see 11.2.4 (if not already done in a previous HOVON trial)
- Molecular analysis (Plasma cell purification and cryopreservation for DNA microarray analysis, see for collecting and handling of samples for DNA microarray analysis Appendix G) (if not already done in HO65)
- Bone marrow aspirate at response evaluation for confirmation of response (CR):
- Morphology including immunofixation to confirm CR

Specific investigations

- Serum b2-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

Additional investigations

Only on clinical indication:

- Survey for exclusion of AL amyloidosis
- Bleeding time
- Cryoglobulins, cold agglutinins
- Serum viscosity, fundoscopy
- Spirometry

11.2.4 Cytogenetic analysis

Conventional cytogenetic analysis should have been already performed in all patients at diagnosis. For this study additional FISH analysis is required for chromosome 13q deletions and 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 FISH 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 representative FISH results and a copy of the original cytogenetic/FISH report is requested to be sent within 3 months to the HOVON Data Center for central review.

11.2.5 Micro-array analysis

Using micro-array 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 Medical Center, LUMC, UMC St. Radboud, UMCU, VUMC, HagaZiekenhuis - Leyenburg, St. Antonius hospital or Medisch Spectrum Twente). 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 I

11.2.6 Single Nucleotide Polymorphism (SNP) analysis

The involvement of specific genes in the drug metabolism and anti-tumor effect of Bortezomib and Lenalidomide will be investigated, using the Genome-Wide Human SNP 6.0 array (Affymetrix) for polymorphism 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 Hematology laboratory of the Erasmus Medical Center, where analysis of all samples will take place. Procedures for collecting and handling samples are described in appendix I.

11.3 Response evaluation

The response to re-induction treatment will be evaluated after each cycle and at 2 months intervals during maintenance treatment. Response will be evaluated according to appendix B. Progression-free survival will be calculated from the start of therapy until progression or death.

12 Toxicity assessment

- During and following each cycle, toxicity has to be carefully examined and evaluated. During the treatment 4-weekly assessments of toxicities will be performed or more frequently when indicated. The toxicity assessment must include the following:
- Complete history of symptoms and complaints
- Complete physical examination, with special emphasis on neurological symptoms
- Blood cell count, quantitative platelets, WBC count and differential
- Electrocardiography when indicated
- Electromyography (EMG) and N.Suralis biopsy when indicated at the discretion of the neurologist
- All toxicities will be graded according to the NCI-CTC version 3 toxicity criteria

13 Reporting serious adverse events and SUSARS

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 a clinically significant 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, a company's Investigator's Brochure will serve as a source document.

Suspected unexpected serious adverse reaction (SUSAR)

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

13.2 Reporting of (serious) adverse events

Adverse event

All AEs of CTCAE grade 2 or higher and polyneuropathy grade ≥ 1, with the exception of alopecia, nausea/vomiting and progression of the disease under study, have to be reported on the Adverse Events CRF.

Adverse events will be reported from the first study-related procedure until 30 days following the last protocol treatment or until the start of subsequent systemic therapy for the disease under study, if earlier.
Adverse events occurring after 30 days should also be reported if considered related to study drug.
Grade 3 or 4 adverse events considered related to study drug, *and* neuropathic and cardiac adverse events of Grade 2, must be followed until recovery or until 6 months after the last protocol treatment, whichever comes first.
All other adverse events must be followed until recovery or until 30 days after the last protocol treatment, whichever comes first.

SAE and Unexpected serious adverse event

Serious Adverse Events (SAEs) will be reported from the first study-related procedure until 30 days following the last protocol treatment or until the start of subsequent systemic therapy for the disease under study, if earlier.
Adverse events occurring after 30 days should also be reported if considered to be at least suspected to be related to the study drug.

All SAEs must be reported to the HOVON Data Center by fax **within 24 hours of the initial observation of the event**, except 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.
- the administration of blood or platelet transfusion. Hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable serious adverse event.
- 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.
- a procedure that is planned (i.e., planned prior to starting of treatment on study; must be documented in the source document and 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.

Foetal exposure to lenalidomide

Female patients in this study will not be of childbearing potential. Female partners of males taking investigational product should be advised to call their healthcare provider immediately if they get pregnant and male subjects should notify their doctors as well. The pregnancy should be reported to the Sponsor and to the manufacturers for follow-up as necessary. All details should be documented on the pregnancy form CRF.

Causality assessment

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
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 Processing of serious adverse event reports

The HOVON Data Center will forward all SAE reports within 24 hours of receipt to the prinicipal investigator, the product manufacturers, the study central datamanager and to the safety desk. The safety desk will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR).
The HOVON Data Center will ensure that a six-monthly line listing of all reported SAE's is provided to the Ethics Committee(s) if this is required by national laws or regulations or by the procedures of the Ethics Committee.
Any suspected unexpected serious adverse reactions (SUSARs) arising from this trial will be reported expedited by HOVON to the investigators, to Johnson & Johnson /Millennium and Celgene and to all applicable Ethics Committees and Health Authorities within the timelines required by the EU Clinical Trial Directive.
The manner of SUSAR reporting will be in compliance with the procedures of the Ethics Committees and Health Authorities involved.

13.4 Pregnancies

Pregnancies occurring while subjects are on study drug or within 4 weeks after a subject's last dose of study drug are considered events to be reported immediately to the sponsor. If the subject is on study drug the study drug is to be discontinued immediately and the subject is to be instructed to return any unused portion of the study drug to the investigator. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the sponsor using an SAE Report Form. The pregnancy must be reported to the sponsor within 24 hours of the investigator's knowledge of the pregnancy by phone and facsimile using the SAE Form. All details should be documented on the pregnancy form CRF.
The patient should be referred to an obstetrician/gynaecologist experienced in reproductive toxicity for further evaluation and counselling. The investigator will follow the subject until completion of the pregnancy, and must notify the sponsor of the outcome (including notification of false-positive tests) within 24 hours of having knowledge of the event. The investigator will provide this information as a follow-up to the initial pregnancy report.
If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., spontaneous abortion [any congenital anomaly detected in an aborted foetus is to be documented], stillbirth, neonatal death, or congenital anomaly), the investigator should follow the procedures for reporting SAEs. All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the investigator suspects is related to the in utero exposure to the study drug should also be reported.

In the case of a live “normal” birth, the sponsor should be informed as soon as the information is available.

Any suspected foetal exposure to lenalidomide must be reported to the sponsor within 24 hours of being made aware of the event.

Male Subject:
Female partners of males taking investigational product should be advised to call their healthcare provider immediately if they get pregnant. The male subject should notify the investigator of his partner’s pregnancy and her healthcare provider information. The investigator will then provide this information to the sponsor for follow-up as necessary. All details should be documented on the pregnancy form CRF.

The sponsor will forward any information regarding pregnancies and foetal exposure described above, to Celgene immediately by phone at + 31 (0)30 28 44 525 then by email at DrugSafety-netherlands@Celgene.com or by fax +31 (0)30 28 44 511.

Contact details for Drug Safety Celgene B.V.:
Fax: +31 (0)30 28 44 511

Celgene B.V. (The Netherlands)
P.O. Box 2507
3500 GM Utrecht
The Netherlands
Tel: +31 (0)30 28 44 525
Email: DrugSafety-netherlands@Celgene.com

If the female is found not to be pregnant, any determination regarding the subject’s continued participation in the study will be determined by the investigator(s).

14 Endpoints

Term	Abbreviation	Definition
Dose-limiting toxicity	DLT	Adverse event of severity or consequence that may limit dose escalation. In this trial, DLT is defined as: - grade $\geq$ 3 non-hematological toxicity - grade 4 neutropenia lasting $\geq$ 5 days - neutropenic fever - platelet count $\leq 10 \times 10^9$/L on 2 or more occasions despite transfusion - death whatever the cause, except death due to myeloma any of which must occur before day 22 of cycle I.
Maximum tolerated dose	MTD	Maximum safe dose, i.e. the maximum dose at which only 0/1 of 6 patients exhibit DLT
Recommended phase II dose	RDL	Dose (level) recommended for further study in phase II part of the study. In this trial, RDL equals MTD

Ref: Eisenhauer E.A., Twelves C., Buyse M. Phase I cancer clinical trials. Oxford, UK: Oxford University Press; 2006.

14.1 Phase I

Primary endpoint

- Dose-limiting toxicity (DLT), maximum tolerated dose (MTD) and recommended phase II dose (RDL) of Bortezomib and of Lenalidomide when combined with Dexamethasone.

Secondary endpoints

- Toxicity, especially myelosuppression, polyneuropathy and thrombosis

14.2 Phase II

Primary endpoint

- (s)CR+VGPR rate. In order for patients to be considered as a success for the primary endpoint, a VGPR or (s)CR must be documented according to criteria in appendix B. All other patients will be considered as not having achieved at least a VGPR. In this analysis we will consider the best response obtained during induction/consolidation chemotherapy.

Secondary endpoints

- Overall Response
- Improvement of response due to maintenance treatment
- Toxicity, especially myelosuppression, polyneuropathy and thrombosis
- Progression free survival (PFS; i.e. time from registration to progression or death from any cause, whichever comes first)
- Overall survival measured from registration. Patients still alive or lost to follow up are censored at the date they were last known to be alive
- PFS calculated from start of maintenance treatment
- OS calculated from start of maintenance treatment

15 Registration

15.1 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 (dated and signed) (if not recently provided);
- ♦ signed local investigator signature page ;
- ♦ local lab accreditation and list of local lab normal values (if not recently provided);
- ♦ any other documentation required by local regulations.

15.2 Registration

The patient should be registered immediately after diagnosis of relapse, and before the start of protocol treatment. Patients need to be registered at the HOVON Data Center of the Erasmus MC Rotterdam – location Daniel via the Internet via TOP (Trial Online Process; https://www.hdc.hovon.nl/top) or by phone call: +31.10.7041568 or fax +31.10.7041028 Monday through Friday, from 09:00 to 17:00 CET. 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
Local patient code (optional)
Sex
Date of birth
Date written informed consent
Eligibility criteria

All eligibility criteria will be checked with a checklist.
Each patient will be given a unique patient study number. Patient study number and result of treatment cohort (Phase I) will be given immediately by TOP or phone and confirmed by fax or email.

16 Data collection

16.1 Reporting of DLT

Dose limiting toxicity must be reported by fax within 24 hours after occurrence, up to 7 days after completion of the first cycle. In order to closely monitor the occurrence of untoward events it is important that the DLT report form is sent immediately by fax if any DLT occurs. If no DLT occurs fax the DLT form at day 22 after start of cycle 1. Please FAX the DLT report form first and then send it by regular mail.

16.2 CRF's

Data will be collected on Case Report Forms (CRF) to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected on the CRF are derived from the protocol and will include at least:

- inclusion and exclusion criteria;
- baseline status of patient including medical history and stage of disease;
- timing and dosage of protocol treatment;
- adverse events;
- parameters for response evaluation;
- any other parameters necessary to evaluate the study endpoints;
- survival status of patient;
- reason for end of protocol treatment.

Each CRF page will be identified by a pre-printed trial number, and a unique combination of patient study number (assigned at registration), hospital and patient name code (as documented at registration) to be filled out before completing the form.
The CRF will be completed on site by the local investigator or an authorized staff member. Each page must be dated and signed by the local investigator upon completion. All CRF entries must be based on source documents. The CRF and written instructions for completing the CRF will be provided by the HOVON Data Center.
Copies of the CRF will be kept on site. The original CRF pages must be sent to the HOVON Data Center at the requested time points. How and when to send in forms is described in detail in the CRF header and the CRF instructions.
All data from the CRF will be entered into the study database by the HOVON Data Center.

17 Statistical considerations

17.1 Patient numbers and power considerations

17.1.1 Phase I

Due to the 3+3 dose-escalation scheme, a maximum of 24 evaluable patients will be entered in the phase I part.
Patients who die of myeloma within 21 days after start cycle I without having obtained a DLT, will be considered as not-evaluable for DLT, and will be replaced.

17.1.2 Phase II

The phase II part is designed to determine whether reinduction treatment with Bortezomib and Lenalidomide at the RDL in combination with Dexamethasone warrants further investigation in clinical trials. The (s)CR+VGPR rate will be considered as primary endpoint for the sample size calculation.

- Let P_0 be the largest (s)CR+VGPR rate which, if true, implies that the therapeutic activity is too low and therefore does not warrant further investigation. In the present trial, P_0 has been taken as 15%.
- Let P_1 be the smallest (s)CR+VGPR rate which, if true, implies that the therapeutic activity is sufficiently high and therefore the RDL warrants further investigation in clinical trials. In the present trial, P_1 has been taken as 30%.

In order to reject the null hypothesis H_0: $P = P_0$ in favor of the alternative hypothesis H_1: $P = P_1$ with power $1 - \beta = 0.80$ (2-sided significance level $\alpha = 0.05$), 53 eligible patients are required [Stata-command `sampsi 0.15 0.30, power(0.8) onesample`]. However, in order to overcome dropout, 60 patients will be included in the phase II part of the trial.

17.2 Statistical analysis

All analyses will be according the intention to treat principle, restricted to eligible patients.

17.2.1 Efficacy analysis

The main endpoint of the phase II part is the proportion of patients who obtain a sCR, CR, nCR or VGPR during induction chemotherapy. A 95% confidence interval (CI) will be constructed, and the null hypothesis H_0: $P = P_0$ will be rejected in favor of the alternative hypothesis H_1: $P = P_1$ if the lower bound of the 95% CI is larger than 0.15.

Secondary efficacy endpoints concern (improvement of) response, and survival endpoints. Response rates will be described as percentages with 95% CI. Actuarial survival curves for all time-to-event endpoints will be computed using the Kaplan-Meier method, and 95% CI will be constructed.

17.2.2 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of adverse events and infections by treatment cohort and cycle.
The incidence of the adverse events defined as DLT will be reported by treatment cohort and cycle.

17.2.3 Additional analyses

Additional analyses may involve the analysis of prognostic factors, e.g. β2-microglobulin, chromosome 13 deletion, FISH results, albumin, age, LDH level and ISS stage with respect to response rate, PFS, and OS from registration. Logistic and Cox regression analysis could be used for this purpose.
Before any additional analysis will be performed, a separate analysis plan will be discussed with the principal investigator. Any such analysis should, however, be considered as exploratory, i.e. hypothesis generating, and not confirmatory.

18 Ethics

18.1 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 (Tokyo, 2004), the ICH-GCP Guidelines and EU directive Good Clinical Practice (2001-20-EG). 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), EU directive Good Clinical Practice (2001-20-EG), and applicable regulatory requirements.

18.3 Patient information and consent

Written Informed consent of patients is required before registration. 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.

20 Publication policy

The final publication of the trial results will be written by the Principal Investigator and 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 /Millennium and Celgene for review. After revision by the Data Center, the other co-authors and Johnson & Johnson /Millennium and Celgene, the manuscript will be sent to a peer reviewed scientific journal.

Authors of the manuscript will include the study coordinator(s), 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.

Any publication, abstract or presentation based on patients included in this study must be approved by the Principal Investigator and Study Coordinator(s). This is applicable to any individual patient or any subgroup of the trial patients. Such a publication cannot include any of the study end-points unless the final results of the trial have already been published.

21 Glossary of abbreviations

(in alphabetical order)

AE	Adverse Event
AL	Amyloid Light-chain
ANC	Absolute Neutrophil Count
BJ	Bence Jones
BM	Bone Marrow
Ca	Calcium
CKTO	Commissie voor Klinisch Toegepast Onderzoek'
CR	Complete Remission
CRF	Case Report Form
CRP	C-Reactive Protein
CTC	Common Toxicity Criteria
DLT	Dose limiting toxicity
ECG	Electrocardiogram
EBMT	European Group for Blood and Marrow Transplantation
FISH	Fluorescence In Situ Hybridisation
FLC	Free Light Chain
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GI	Gastro-intestinal
HB	Hemoglobin
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 Myelome
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
MTD	Maximum tolerated dose
NaCl	Sodium Chloride
NCI	National Cancer Institute
NYHA	New York Heart Association
OS	Overall Survival
PB	Peripheral Blood
PD	Progressive Disease
PFS	Progression Free Survival
PO	Per Os
PR	Partial Response
RDL	Recommended dose level
SAE	Serious Adverse Event
SC	Subcutaneous
sCR	Stingent Complete Remission
SD	Stable Disease
SNP	Single Nucleotide Polymorphism
SPEP	Serum protein electro-phoresis
ULN	Upper Limit of Normal
UPEP	Urine protein electro-phoresis
VAD	Vincristine, Doxorubicin (Adriamycin), Dexamethasone
VGPR	Very good partial remission
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen

22 References

1. Attal, M., et al., A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. N Engl J Med, 1996. 335(2): p. 91-7.
2. Kyle, R.A., Management of patients with multiple myeloma: emphasizing the role of high-dose therapy. Clin Lymphoma, 2001. 2(1): p. 21-8.
3. Alexanian, R., et al., Melphalan therapy for plasma cell myeloma. Blood, 1968. 31(1): p. 1-10.
4. Boccadoro, M., et al., Multiple myeloma: VMCP/VBAP alternating combination chemotherapy is not superior to melphalan and prednisone even in high-risk patients. J Clin Oncol, 1991. 9(3): p. 444-8.
5. Cavo, M., et al., Melphalan-prednisone versus alternating combination VAD/MP or VND/MP as primary therapy for multiple myeloma: final analysis of a randomized clinical study. Haematologica, 2002. 87(9): p. 934-42.
6. Mellstedt, H., M. Bjorkholm, and G. Holm, Intermittent melphalan and prednisolone therapy in plasma cell myeloma. Acta Med Scand, 1977. 202(1-2): p. 5-9.
7. Gregory, W.M., M.A. Richards, and J.S. Malpas, Combination chemotherapy versus melphalan and prednisolone in the treatment of multiple myeloma: an overview of published trials. J Clin Oncol, 1992. 10(2): p. 334-42.
8. Facon, T., et al., Dexamethasone-based regimens versus melphalan-prednisone for elderly multiple myeloma patients ineligible for high-dose therapy. Blood, 2006. 107(4): p. 1292-8.
9. Palumbo, A., et al., Dose-intensive melphalan with stem cell support (MEL100) is superior to standard treatment in elderly myeloma patients. Blood, 1999. 94(4): p. 1248-53.
10. Palumbo, A., et al., Intermediate-dose melphalan improves survival of myeloma patients aged 50 to 70: results of a randomized controlled trial. Blood, 2004. 104(10): p. 3052-7.
11. Palumbo, A., et al., Oral melphalan and prednisone chemotherapy plus thalidomide compared with melphalan and prednisone alone in elderly patients with multiple myeloma: randomised controlled trial. Lancet, 2006. 367(9513): p. 825-31.
12. Rajkumar, S.V., et al., Combination therapy with thalidomide plus dexamethasone for newly diagnosed myeloma. J Clin Oncol, 2002. 20(21): p. 4319-23.
13. Palumbo, A., et al., Low-dose thalidomide plus dexamethasone is an effective salvage therapy for advanced myeloma. Haematologica, 2001. 86(4): p. 399-403.
14. Barlogie, B., et al., Extended survival in advanced and refractory multiple myeloma after single-agent thalidomide: identification of prognostic factors in a phase 2 study of 169 patients. Blood, 2001. 98(2): p. 492-4.

15. Richardson, P.G., et al., A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med, 2003. 348(26): p. 2609-17.
16. Richardson, P.G., et al., Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med, 2005. 352(24): p. 2487-98.
17. Rajkumar, S.V., et al., Combination therapy with lenalidomide plus dexamethasone (Rev/Dex) for newly diagnosed myeloma. Blood, 2005. 106(13): p. 4050-3.
18. Adams, J. and M. Kauffman, Development of the proteasome inhibitor Velcade (Bortezomib). Cancer Invest, 2004. 22(2): p. 304-11.
19. Mitsiades, N., et al., Molecular sequelae of proteasome inhibition in human multiple myeloma cells. Proc Natl Acad Sci U S A, 2002. 99(22): p. 14374-9.
20. Mitsiades, N., et al., The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. Blood, 2003. 101(6): p. 2377-80.
21. Adams, J., Proteasome inhibition in cancer: development of PS-341. Semin Oncol, 2001. 28(6): p. 613-9.
22. Jagannath, S., et al., A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol, 2004. 127(2): p. 165-72.
23. Anderson, K.C., Lenalidomide and thalidomide: mechanisms of action--similarities and differences. Semin Hematol, 2005. 42(4 Suppl 4): p. S3-8.
24. Knight, R., IMiDs: a novel class of immunomodulators. Semin Oncol, 2005. 32(4 Suppl 5): p. S24-30.
25. Bartlett, J.B., et al., Recent clinical studies of the immunomodulatory drug (IMiD) lenalidomide. Br J Cancer, 2005. 93(6): p. 613-9.
26. CC-5013 MM 0017: a multicenter, randomized, parallel-group, double-blind, placebo-controlled study of CC-5013 plus dexamethasone versus dexamethasone alone in previously treated subjects with multiple myeloma. Clin Adv Hematol Oncol, 2003. 1(3): p. 189-90.

A. Criteria for staging of Multiple Myeloma

According to Salmon & Durie

Stage I	Low Tumor Mass – all of the following:
	Hemoglobin > 6.2 mmol/l
	Ca2+ < 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
	Ca2+ > 2.65 mmol/l *
	IgG > 70 g/l
	IgA > 50 g/l
	Urine M-protein > 12 g/24 hrs
	³ 3 lytic bone lesions on skeletal survey (bone scans are not acceptable)
A	Normal renal function (creatinin < 177 mmol/l)
B	Renal insufficiency (creatinin ≥ 177 mmol/l)

* Correct the serum Ca2+ by adding 0.02 mmol/l for every g/l albumin below 40 g/l

Based on the criteria of the International Staging System for Multiple Myeloma of the International Myeloma Working Group (J Clin Oncol 2005: 23; 3412-3420).

Stage	Criteria
I	Serum β_2-microglobulin < 3.5 mg/L
	Serum albumin ≥ 3.5 g/dL
II	Neither stage I nor stage III*
III	Serum β_2-microglobulin ≥ 5.5 mg/L

* There are two categories for stage II: serum β_2-microglobulin < 3.5 mg/L but serum albumin < 3.5 g/dL; or serum β_2-microglobulin 3.5 to < 5.5 mg/L irrespective of the serum albumin level.

B. Response criteria for Multiple Myeloma

Based on EBMT, IBMTR and ABMT criteria (Br J Haemat, 1998: 102; 1115-1123) and adapted to B.G.M. Durie et al. (Leukemia, 2006: 20; 1467-1473)

RESPONSE CRITERIA

Response subcategory	*Response criteria*[a]
sCR	CR as defined below plus ▪ Normal FLC ratio and ▪ Absence of clonal cells in bone marrow[b] by immunohistochemistry or immunofluorescence[c]
CR	▪ Negative immunofixation on the serum and urine and ▪ Disappearance of any soft tissue plasmacytomas and ▪ ≤ 5% plasma cells in bone marrow[b]
VGPR	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or 90% or greater reduction in serum M-protein plus urine M-protein level < 100 mg per 24 h
PR	▪ ≥ 50% reduction of serum M-protein and reduction in 24-h urinary M-protein by ≥ 90% or to < 200 mg per 24 h ▪ In addition to the above listed criteria, if present at baseline, a ≥ 50% reduction in the size of soft tissue plasmacytomas is also required
SD[e]	Not meeting criteria for CR, VGPR, PR or progressive disease

Abbreviations: CR, complete response; FLC, free light chain; PR, partial response; SD, stable disease; sCR, stringent complete response; VGPR, very good partial response.

[a] All response categories require two consecutive assessments made at anytime before the institution of any new therapy; all categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements.

[b] Confirmation with repeat bone marrow biopsy not needed.

[c] Presence/absence of clonal cells is based upon the κ/λ ratio. An abnormal k/l ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is κ/λ of > 4:1 or < 1:2.

[d] See below for definitions of measurable disease.

[e] not recommended for use as an indicator of response; stability of disease is best described by providing the time to progression estimates

NOTE: Once (s)CR is established, response remains (s)CR until relapse is documented.

RELAPSE CRITERIA

Relapse subcategory	*Relapse criteria*
Progressive disease[a] To be used for calculation of time to progression and progression-free survival end points for all patients including those in CR (includes primary progressive disease and disease progression on or off therapy)	Progressive Disease: requires any one or more of the following: Increase of ≥ 25% from baseline/**nadir** in ▪ Serum M-component (the absolute increase must be ≥ 0.5 g/dl)[b] and/or ▪ Urine M-component (the absolute increase must be≥ 200 mg/24 h) and/or ▪ Bone marrow plasma cell percentage: the absolute % must be ≥10%[c] ▪ Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas ▪ Development of hypercalcemia (corrected serum calcium > 11.5 mg/dl or 2.65 mmol/l) that can be attributed solely to the plasma cell proliferative disorder
Clinical relapse[a]	Clinical relapse requires one or more of: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features)[b]. It is not used in calculation of time to progression or progression-free survival but is listed here as something that can be reported optionally or for use in clinical practice 1. Development of new soft tissue plasmacytomas or bone lesions 2. Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and at least 1 cm) increase as measured serially by the sum of the products of the cross-diameters of the measurable lesion 3. Hypercalcemia (> 2.65 mmol/l) [11.5 mg/dl] 4. Decrease in hemoglobin of ≥ 1.25 mmol/l [2 g/dl] 5. Rise in serum creatinine by 177 µmol/l or more [2 mg/dl or more]
Relapse from CR[a] (To be used only if the end point studied is DFS)[d]	Any one or more of the following: ▪ Reappearance of serum or urine M-protein by immunofixation or electrophoresis ▪ Development of ≥ 5% plasma cells in the bone marrow[c] ▪ Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, or hypercalcemia see above)

Abbreviations: CR, complete response; DFS, disease-free survival.

[a] All relapse categories require two consecutive assessments made at anytime before classification as relapse or disease progression and/or the institution of any new therapy.

[b] For progressive disease, serum M-component increases of ≥ 10 g/l are sufficient to define relapse if starting M-component is ≥ 50 g/l.

[c] Relapse from CR has the 5% cutoff versus 10% for other categories of relapse.

[d] For purposes of calculating time to progression and progression-free survival, CR patients should also be evaluated using criteria listed above for progressive disease

PRACTICAL DETAILS OF RESPONSE EVALUATION

Laboratory tests for measurement of M-protein

- Serum M-protein level is quantitated using densitometry on SPEP except in cases where the SPEP is felt to be unreliable such as in patients with IgA monoclonal proteins migrating in the beta region. If SPEP is not available or felt to be unreliable (e.g., in some cases of IgA myeloma) for routine M-protein quantitation during therapy, then quantitative immunoglobulin levels on nephelometry or turbidometry can be accepted. However, this must be explicitly reported, and only nephelometry can be used for that patient to assess response and SPEP and nephelometric values cannot be used interchangeably.
- Urine M-protein measurement is estimated using 24-h UPEP only. Random or 24 h urine tests measuring kappa and lambda light chain levels are not reliable and are not recommended

Definitions of measurable disease

- Response criteria for all categories and subcategories of response except CR are applicable only to patients who have 'measurable' disease defined by at least one of the following three measurements:
    - Serum M-protein ≥ 1 g/dl (10 g/l)
    - Urine M-protein ≥ 200 mg/24 h
- Response criteria for CR are applicable for patients who have abnormalities on one of the three measurements. Note that patients who do not meet any of the criteria for measurable disease as listed above can only be assessed for stringent CR, and cannot be assessed for any of the other response categories

Follow-up to meet criteria for PR or SD

- It is recommended that patients undergoing therapy be tracked monthly for the first year of new therapy and every other month thereafter
- Patients with 'measurable disease' as defined above need to be followed by both SPEP and UPEP for response assessment and categorization
- Except for assessment of CR, patients with measurable disease restricted to the SPEP will need to be followed only by SPEP; correspondingly, patients with measurable disease restricted to the UPEP will need to be followed only by UPEP[a]
- Patients with measurable disease in either SPEP or UPEP or both will be assessed for response only based on these two tests and not by the FLC assay. FLC response criteria are only applicable to patients without measurable disease in the serum or urine, and to fulfill the requirements of the category of stringent CR
- To be considered CR, both serum and urine immunofixation must be carried out and be negative regardless of the size of baseline M-protein in the serum or urine; patients with negative UPEP values pretreatment still require UPEP testing to confirm CR and exclude light chain or Bence–Jones escape
- Skeletal survey is not required for assessment of response unless clinically indicated, but is recommended once a year in clinical practice; bone marrow is required only for categorization of CR, and for patients with non-secretory disease

Abbreviations: CR, complete response; FLC, free light chain; PR, partial response; SD, stable disease; SPEP, serum protein electro-phoresis; UPEP, urine protein electrophoresis.

[a] For good clinical practice patients should be periodically screened for light chain escape with UPEP or serum FLC assay.

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

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. Management of Bortezomib associated toxicity

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 below and, 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 treating 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 109/l, **and** platelet count ≥ 50 x 109/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 1.6 mg/m^2, reduce the dose to 1.3 mg/m^2.
- 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 below.
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%.

Peripheral Sensory Neuropathy (NCI CTC Grade)

Neuropathic Pain (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
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: (see next page)

- **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 once a week to every other week (e.g. Day 1, Day 15).

F. Management of Lenalidomide associated toxicity

Hematological toxicity

In published studies, 11.6% of patients treated with lenalidomide/dexamethasone (Len/Dex) had NCI grade 3 neutropenia and 3.3% grade 4 neutropenia (Chen et al., Blood 2006; 108:3556). This was the most frequent reason for discontinuation of therapy and dose reduction. The rate of grade 4 febrile neutropenia was <1%. Thrombocytopenia occurred in 11.1%. Risk factors for cytopenia during Len/Dex include low counts at baseline, previous chemotherapy and response to treatment. Patients with renal insufficiency were reported to suffer from more severe thrombocytopenia, but age has not been reported to be a risk factor. Recommendations regarding monitoring and management of cytopenia during treatment with Len/Dex for relapsed/refractory MM have been developed. In case of a normal baseline full blood count (FBC), biweekly monitoring is recommended. If baseline FBC is abnormal because of MM infiltration, treatment should still be pursued with a full dose and at least weekly monitoring. Standard dose-reduction strategies should be followed for all other causes of abnormal baseline and follow-up FBC. As a general rule, G-CSF can be used in neutropenic patients.

In case of neutrophils $<1.0x10^{9,}$/l G-CSF was recommended to prevent dose-reduction and febrile neutropenia aiming at $>0.5x10^{9,}$/l neutrophils.

If neutrophils fall $<0.5x10^{9,}$/l, Lenalidomide should be interrupted and restarted at a lower dose once neutrophils $>0.5x10^{9,}$/l.

Similarly, if platelets fall $<50x10^{9,}$/l, anticoagulation should be stopped

In case of thrombocytopenia $<10x10^{9,}$/l, Lenalidomide should be interrupted and restarted at a lower dose once platelets $>10x10^{9,}$/l. (*30 veranderd in 10 in verband met DLT defenitie)*

Also, antibiotic prophylaxis with cotrimoxazole should be applied if patients receive Len with high dose Dex and the patient should receive clear instructions to seek medical care within 3 hours if febrile while neutropenic

None hematological toxicity

Dose adjustments of Lenalidomide

Table 1: Lenalidomide Dose Reduction Steps	
Starting Dose:	10 mg daily for 21 days every 28 days
Dose Level 1:	5 mg daily for 21 days every 28 days

Table 2: Dose Modification for lenalidomide (Based on CC-5013-Related Toxicity Observed on Days 2-28)		
CTC AE Grade	Day 2-14 of Cycle	≥ Day 15 of Cycle
Non-blistering rash Grade 3 Grade 4	If Grade 3 hold (interrupt) dose. Follow weekly. If the toxicity resolves to ≤ grade 2 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21. Discontinue lenalidomide study drug.	Omit lenalomide for remainder of cycle. Discontinue lenalidomide study drug.
Desquamating (blistering) rash- any Grade	Discontinue lenalidomide study drug.	Discontinue lenalidomide study drug.
Erythema multiforme ≥ Grade 3	Discontinue lenalidomide study drug.	Discontinue lenalidomide study drug.
Neuropathy Grade 3 Grade 4	If Grade 3 hold (interrupt) dose. Follow weekly. If the toxicity resolves to ≤ grade 2 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21. Discontinue lenalidomide study drug.	Omit lenalidomide for remainder of cycle. Discontinue lenalidomide study drug.
Sinus bradycardia/ other cardiac arrhythmia Grade 2 ≥ Grade 3	Hold (interrupt) dose. Follow at least weekly. If the toxicity resolves to ≤ grade 1 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21. Discontinue lenalidomide study drug.	Omit lenalidomide for the remainder of the cycle. Discontinue lenalidomide study drug.
Allergic reaction or hypersensitivity Grade 2-3 Grade 4	Hold (interrupt) dose. Follow at least weekly. If the toxicity resolves to ≤ grade 1 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21. Discontinue lenalidomide study drug.	Omit lenalidomide for the remainder of the cycle. Discontinue lenalidomide study drug
Constipation Grade 1-2 ≥ Grade 3	Initiate bowel regimen and maintain dose level. Hold (interrupt) dose. If the toxicity resolves to ≤ grade 2 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21.	Initiate bowel regimen and maintain dose level. Omit lenalidomide for the remainder of the cycle.
Venous thrombosis/embolism ≥ Grade 3	Hold (interrupt) dose and start anticoagulation; restart at investigator's discretion (maintain dose level).	Omit lenalidomide for remainder of cycle and start anticoagulation.
Other non-hematologic toxicity assessed as lenalidomide-related ≥ Grade 3	Hold (interrupt) dose. Follow at least weekly. If the toxicity resolves to ≤ grade 2 prior to Day 21 restart at next lower dose level and continue the cycle until Day 21.	Omit lenalidomide for remainder of cycle.
Hyperthyroidism or hypothyroidism	Omit lenalidomide for remainder of cycle, evaluate etiology, and initiate appropriate therapy. Restart lenalidomide next cycle (decrease dose by one dose level).	Omit lenalidomide for remainder of cycle, evaluate etiology, and initiate appropriate therapy. Restart lenalidomide next cycle (decrease dose by one dose level).

A new course of treatment may begin on the scheduled Day 1 of a new cycle if:

- The ANC is $\geq 1.0 \times 10^9$/l;
- The platelet count $\geq 50 \times 10^9$/l;
- Any lenalidomide-related allergic reaction/hypersensitivity or sinus bradycardia/ other cardiac arrhythmia adverse event that may have occurred has resolved to ≤ grade 1 severity;
- Any other lenalidomide-related adverse event that may have occurred has resolved to ≤ grade 2 severity.

If these conditions are not met on Day 1 of a new cycle, the subject will be evaluated weekly and a new cycle of lenalidomide will not be initiated until the toxicity has resolved as described above. If lenalidomide dosing was halted during the previous cycle and was restarted with a one-level dose reduction without requiring an interruption for the remainder of the cycle, then that reduced dose level will be initiated on Day 1 of the new cycle. If lenalidomide dosing was omitted for the remainder of the previous cycle or if the new cycle is delayed due to toxicity newly encountered on the scheduled Day 1, then the new cycle will be started with a one-level dose reduction. There is only one-level dose reduction, if the subject experiences side-effects that prevent dosing at 5 mg/day the study treatment has to be discontinuated.

G. Approved risk section for Velcade protocols

Known Anticipated Risks of VELCADE

The known anticipated risks of VELCADE are presented in Table 1. Adverse events are grouped according to the combined frequency observed in a pooled analysis of studies as of 27 March 2006 evaluating single agent VELCADE at a dose of 1.3 mg/m2, administered on days 1, 4, 8, and 11 of a 21-day schedule. The analysis included the phase 3 study (M34101 039)(1) and the phase 2 studies (M34100 024, M34100 025, M34101 040, and M34103 053).(2; 3; 4; 4) Additional events reported since the pooled analysis was completed are reviewed as part of the ongoing safety review of VELCADE. These additional events have been incorporated into Table 1 as of 04 December 2007.

Table -1 Known Anticipated Risks of VELCADE by MedDRA System Organ Class, Observed Incidence, and Preferred Term

System Organ Class Observed Incidence	Preferred Term
Blood and Lymphatic System Disorders	
Most common	Thrombocytopenia that may increase the risk of bleeding*, anemia*
Very common	Neutropenia*
Common	Lymphopenia, pancytopenia*, leukopenia*
Uncommon	Febrile neutropenia
Cardiac Disorders	
Common	Arrhythmias including tachycardia, atrial fibrillation, and palpitations; acute development or exacerbation of cardiac failure, including congestive heart failure*; pulmonary edema*
Uncommon	Cardiogenic shock*, new onset of decreased left ventricular fraction*, atrial flutter, cardiac tamponade*, bradycardia, atrioventricular block (complete), cardiac arrest, cardiopulmonary failure
Ear and Labyrinth Disorders	
Uncommon	Deafness, hearing impairment
Eye Disorders	
Common	Blurred vision, conjunctival infection and irritation
Uncommon	Conjunctival hemorrhage
Gastrointestinal Disorders	
Most common	Constipation, diarrhea*, nausea, vomiting*
Very common	Gastrointestinal and abdominal pain, excluding oral and throat
Common	Dyspepsia, pharyngolaryngeal pain, gastroesophageal reflux, abdominal distension, stomatitis and mouth ulceration, dysphagia, gastrointestinal hemorrhage (upper and lower gastrointestinal tract)*, rectal hemorrhage (includes hemorrhagic diarrhea)
Uncommon	Eructation, tongue ulceration, retching, upper gastrointestinal hemorrhage*, hematemesis*, oral mucosal petechiae, ileus paralytic*, odynophagia, enteritis, colitis, oesphagitis, fungal oesphagitis, enterocolitis, acute pancreatitis*, gastritis

System Organ Class Observed Incidence	Preferred Term
General Disorders and Administration Site Conditions	
Most common	Asthenic conditions, including weakness, fatigue, lethargy, and malaise; pyrexia
Very common	Rigors, edema of the lower limbs
Common	Neuralgia, chest pain, mucosal inflammation*
Uncommon	Injection site pain and irritation, injection site phlebitis, general physical health deterioration*, injection site cellulitis, catheter site cellulitis, injection site infection
Hepatobiliary Disorders	
Common	Abnormal liver function tests
Uncommon	Hyperbilirubinemia, hepatitis*
Immune System Disorders	
Uncommon	Drug hypersensitivity, angioedema
Infections and Infestations	
Very common	Upper respiratory tract infection, nasopharyngitis, lower respiratory tract and lung infections*, pneumonia*, Herpes zoster*
Common	Herpes zoster disseminated,*, postherpetic neuralgia, sinusitis, pharyngitis, oral candidiasis, urinary tract infection*, catheter-related infection*, sepsis and bacteremia*, cellulitis and other skin infections*, Herpes simplex
Uncommon	Bronchitis, gastroenteritis*, septic shock*, urosepsis*, aspergillosis*, tinea infections, Herpes zoster ophthalmic, Herpes simplex ophthalmic, meningoencephalitis herpetic, varicella, empyema
Injury, Poisoning, and Procedural Complications	
Common	Catheter-related complication
Uncommon	Subdural haematoma
Investigations	
Common	Increased ALT, increased AST, increased alkaline phosphatase
Uncommon	Increased GGT, oxygen saturation decreased*, blood albumin decreased
Metabolism and Nutritional Disorders	
Most common	Decreased appetite and anorexia, which may result in dehydration and/or weight loss
Very common	Dehydration*
Common	Hyperglycemia, hypoglycemia, hyponatremia, hypokalemia, hypercalcemia*
Musculoskeletal and Connective Tissue Disorders	
Very common	Bone pain, pain in limb, myalgia, arthralgia
Nervous System Disorders	
Most common	Peripheral neuropathy (including all preferred terms under the MedDRA high-level term peripheral neuropathy NEC)
Very common	Paresthesia and dysesthesia; dizziness, excluding vertigo; headache
Common	Polyneuropathy, syncope, dysgeusia
Uncommon	Convulsions, loss of consciousness, ageusia, encephalopathy,

System Organ Class Observed Incidence	Preferred Term
	paralysis*, reversible posterior leukoencephalopathy syndrome, autonomic neuropathy
Neoplasms, Benign, Malignant, and Unspecified (including cysts and polyps)	
Uncommon	Tumor lysis syndrome*
Psychiatric Disorders	
Very common	Anxiety
Common	Confusion, insomnia
Uncommon	Delirium
Renal and Urinary Disorders	
Common	Renal impairment, including renal failure and increased serum creatinine*; hematuria
Uncommon	Difficulty in micturition
Respiratory, Thoracic, and Mediastinal Disorders	
Very common	Cough, dyspnea
Common	Epistaxis, exertional dyspnea, pleural effusion*, rhinorrhea, hypoxia*
Uncommon	Hemoptysis*, acute respiratory distress*, respiratory failure*, pneumonitis*, lung infiltrates, pulmonary alveolar hemorrhage*, interstitial lung disease*, pulmonary hypertension*, pleurisy, pleuritic pain
Skin and Subcutaneous Tissue Disorders	
Very common	Skin rash, which can be pruritic, erythematous, and can include evidence of leukocytoclastic vasculitis
Common	Urticaria
Uncommon	Leukocytoclastic vasculitis
Vascular Disorders	
Very common	Hypotension*
Common	Orthostatic/postural hypotension, petechiae
Uncommon	Cerebral hemorrhage*

Most common = ≥ 30%, Very common = 10% to 29%, Common=1% to 9%, Uncommon= < 1%,

* Fatal outcomes have been reported

Abbreviations: ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma glutamyl transferase

Medical Events from Postmarketing Experience

Adverse drug reactions, as listed in the postmarketing section of the VELCADE PI(5) or SPC(6) are listed in Table 2.

Table -2 **Reports of Adverse Reactions from Postmarketing Experience**

System Organ Class Preferred Term	Observed Incidence[a]
Blood and lymphatic system disorders	
Disseminated intravascular coagulation	Rare
Cardiac Disorders	
Atrioventricular block complete	Rare
Cardiac tamponade	Rare
Ear and labyrinth disorders	
Deafness bilateral	Rare
Eye Disorders	
Ophthalmic herpes	Rare
Gastrointestinal Disorders	
Acute pancreatitis	Rare
Ischemic colitis	Rare
Hepatobiliary disorders	
Hepatitis	Uncommon
Liver failure	Unknown
Infections and infestations	
Herpes meningoencephalitis	Rare
Immune System Disorders	
Angioedema	Rare
Nervous System Disorders	
Autonomic neuropathy	Rare
Dysautonomia	Unknown
Encephalopathy	Rare
Respiratory, thoracic and mediastinal disorders:	
Acute diffuse infiltrative pulmonary disease	Rare
Acute respiratory distress syndrome (ARDS)	Unknown
Interstitial pneumonia	Unknown
Pneumonitis	Unknown
Skin and subcutaneous system disorders	
Toxic epidermal necrolysis	Unknown

a Incidence is assigned using the following convention: very common (>1/10); common (>1/100 and <1/10); uncommon (>1/1000 and <1/100); rare (>1/10,000 and <1/1000); very rare (<1/10,000, including isolated reports).

REFERENCES

- Currie MA, Balser B, Porter J. An International, Multi-Center, Randomized, Open-Label Study of PS-341 Versus High-Dose Dexamethasone in Patients with Relapsed or Refractory Multiple Myeloma: Study M34101-039 Final Clinical Study Report. Cambridge, MA: Millennium Pharmaceuticals, Inc.; 2004. Report No. M341-CSR-003.
- Richardson PG, Barlogie B, Berenson J, Singhal S, Jagannath S, Irwin D et al. A Phase 2 Study of Bortezomib in Relapsed, Refractory Myeloma. N Engl J Med 2003; 348 (26):2609-17.
- Jagannath S, Barlogie B, Berenson J, Siegel D, Irwin D, Richardson PG et al. A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol 2004; 127 (2):165-72.
- Currie MA. An International, Non Comparative, Open Label Study of PS 341 Administered to Patients with Multiple Myeloma Who Received High dose Dexamethasone in Millennium Protocol M34101 039 or Experienced Progressive Disease after Receiving at Least Four Previous Therapies: Study M34101-040 Final Clinical Study Report. Cambridge, MA: Millennium Pharmaceuticals, Inc.; 2005. Report No. M34101-040 CSR.
- VELCADE® (bortezomib) for Injection, October [package insert]. Cambridge, MA: Millennium Pharmaceuticals, Inc.; 2007.
- VELCADE® (bortezomib)[Summary of Product Characteristics]. Beerse, Belgium: JANSSEN-CILAG INTERNATIONAL NV; 2007.

.

H. VELCADE risk section for informed consent

version: 30 January 2009

DISCOMFORTS AND RISKS

VELCADE should not be taken if you have ever had a serious allergic reaction to bortezomib (VELCADE), boron, or mannitol. You face some risks or discomforts when you are treated with the study drug, VELCADE. You are at risk of having all, some, or none of these symptoms and they may vary in severity. The severity may be mild, moderate or severe, up to and including death. Any symptoms or conditions that you have before you start study drug may get worse. Also, there is always a chance that a risk that is rare or not yet known may occur. If any of these symptoms occur, you must tell your doctor who may give you other drugs to ease discomforts you have. Your doctor may lower or withhold the dose of VELCADE. Also, if you have a very bad reaction to the study drug, your doctor may permanently stop the study treatment for good.

Other drugs and supplements may affect the way VELCADE works. Tell your doctor about all drugs and supplements you are taking while you are in this study.

Most Common VELCADE Risks:

The most common risks are those that have occurred in greater than or equal to 30% of patients who have received VELCADE:

- Feeling weak, tired, and generally uncomfortable
- gastrointestinal effects such as constipation, diarrhea, nausea, vomiting, and loss of appetite. These may result in dehydration and/or weight loss
- fever commonly with shaking chills
- painful feelings or numbness and tingling in hands and feet. which may not get better after stopping VELCADE. Uncommonly, the nerves that control things like your heart rate, gut movement and urinary bladder may be affected
- lowered platelets; that may increase the chance of bleeding
- lowered red cells or anemia which may make you feel tired

Very Common VELCADE Risks:

The very common risks are those that have occurred in 10-29% of patients who have received VELCADE:

- lowered white blood cells called neutrophils that may increase your risk of infection and is uncommonly associated with fever; commonly you may have lowered white blood cells called lymphocytes or have lowered red blood cells, white blood cells and platelets at the same time
- flu-like symptoms and other upper respiratory tract infections, such as chills, sore throat, and runny nose and sinus and throat infections

- abdominal (belly) pain
- Aches and pains in muscles and joints pain in bones and in arms and legs
- swelling or fluid build up in the arms and legs, and feeling dizzy and weight gain. You should not drive or operate any dangerous tools or machines if you have these or any other symptoms
- cough, feeling short of breath, lung infections including pneumonia and commonly bronchitis
- headache
- skin rash with itching and redness. An uncommon risk is a severe, life-threatening or deadly rash with skin peeling and mouth sores.
- Herpes virus such as shingles (herpes zoster) that can sometimes cause local pain that does not go away for a while and herpes simplex virus. Shingles can sometimes spread over large parts of the body. Both may also affect the eyes or brain, but this is uncommon
- feeling anxious
- problems sleeping (insomnia)

Common VELCADE Risks:

Common risks are those that have occurred in 1-9% of patients who have received VELCADE:

- lowered blood pressure that can commonly cause you to feel light headed or faint when you stand up
- changes in heart rate and heart beat that can cause you to possibly feel light-headed, dizzy, faint, short of breath, and/or have chest pain. This may also cause you to feel confused. An uncommon risk is a possible life threatening abnormal heart beat
- new or worsening heart failure, that can show up as feeling short of breath, swelling in the legs, and/or chest pain, or decreased heart function and can uncommonly be severe. If you have heart failure or other diseases that put you at risk of getting heart failure, you should tell your doctor
- fluid build up around the lungs
- infection and/or inflammation of the eye or eyelids
- blurred vision
- painful sores of the mouth and/or throat, which may make swallowing difficult
- heartburn, acid reflux and stomach bloating
- severe bleeding, including bleeding in the stomach and intestines (gut) that may be linked with low platelet counts, and blood clotting changes. Uncommonly, this bleeding may cause bloody diarrhea and/or bloody vomit.
- nosebleeds
- kidney function that gets worse
- infections of the bladder, sinuses, throat, stomach and intestines (gut), skin and at the area of skin where your catheter is placed
- fungal infections in the mucous membrane such as the mouth and throat and uncommonly in the skin and nails
- life-threatening infections in the blood (sepsis)

- changes in blood sugar have been reported in a few diabetic patients who took oral antidiabetic medicine. If you are taking oral antidiabetic medicines you may need your blood sugar levels watched more closely.
- blood in the urine
- feeling confused
- changes in the way things taste
- abnormal liver tests and decreased protein in the blood
- lowered amount of potassium and sodium in your blood and increase in the amount of calcium in your blood

Uncommon VELCADE Risks:

Uncommon risks are those that have occurred in less than 1% of patients who have received VELCADE:

- inflammation and fluid build up in the lungs, or pus build up between the layers surrounding the lungs that may cause breathing problems, and can be life-threatening or lead to death. Increased blood pressure in the lungs, called pulmonary hypertension, has also been reported. This can cause breathing problems and can be life-threatening. If you have new or worsening breathing problems you should tell your doctor.
- Inflammation of the layers surrounding your heart or collection of fluid around the heart may cause chest pain or breathing problems and can be life-threatening or lead to death. If you have new or worsening chest pain or breathing problems you should tell your doctor.
- hepatitis, and liver failure (in patients who also got many drugs and had other serious medical problems).
- pain, redness, swelling and infection in the area of the skin where VELCADE is injected into the vein
- pain in the mouth and throat when swallowing
- loss of hearing
- intestinal obstruction (blockage in the gut) that may get better on its own and not need surgery and inflammation of the intestines, pancreas or stomach
- coughing up blood
- bleeding in the brain and subdural hematoma which is bleeding between the skull and your brain
- fast death of cancer cells that may let toxins into the blood and injure organs, such as the kidneys
- allergic reactions that may include skin swelling and/or swelling of the face or throat and could be severe or life threatening
- severe muscle weakness and paralysis (not being be able to move your arms and legs)
- changes to the brain that may cause convulsions and confusion
- reversible posterior leukoencephalopathy syndrome affects the brain and may cause headaches, changes in your vision, changes in your mental status, or seizures (fits), but is usually reversible

Study Procedures Risks:
In addition to the risks of VELCADE, routine needle sticks for blood samples may cause pain, bruising and rarely, infection at the site where blood is drawn.

Risk to the Unborn Child and Fertility (Men and Women):
Both men and women will be included in this study. Because the drugs in this study may affect an unborn baby, you should not become pregnant or father a baby while in this study. You must use a highly effective birth control method or a combination of 2 additionally effective birth control methods while in this study. Examples of highly effective birth control are a condom or a diaphragm, either with spermicidal jelly; oral, injectable, or implanted birth control; or abstinence. The effect of VELCADE on reproduction and its safety in pregnancy are unknown. If you are a woman capable of becoming pregnant [anyone who has not undergone a hysterectomy (removal of the womb), has not had both ovaries removed or has not been post-menopausal (stopped menstrual periods) for more than 24 months in a row], you must have a negative pregnancy test before beginning treatment. In addition, you must not be breastfeeding a baby during this study.

If you think that you have become pregnant or may have fathered a child while taking part in this study you must tell the study doctor immediately. The study doctor will advise you of the possible risks to your unborn baby and discuss options for managing the pregnancy with you. You should also notify the doctor managing your pregnancy that the mother/father received a study drug (name of study drug or drugs).

If you are a female study subject and you become pregnant during your participation in this study, your treatment with study drug will be stopped and you may be withdrawn from some of the study procedures but not from follow-up by your study doctor. The study doctor will ask for your permission to stay in contact with you throughout the length of the pregnancy.

If you are a male study subject and your partner becomes pregnant, the study doctor will ask for your partner's permission to collect information about her pregnancy and the health of the baby.

Laboratory tests show that VELCADE may damage DNA. Based on this information, it is possible that VELCADE may cause infertility in men and women (not being able to become pregnant or father a child).

I. Management and handling RNA samples for gene expression profiling

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 by correlations with clinical outcome.

Bone marrow samples for whole genome transcriptional profiling will be collected at the Erasmus Medical Center .(section 11.2.10) where plasma cells will be purified using positive selection kit (Stemcell technologies). 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. The viability of the cells will be measured using annexin and 7AAD.

Purified plasma cells will be stored at -80°C at the laboratory of the Erasmus Medical Center, 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 1,7 will be rejected from analysis. Also samples with a low RIN number (e.g. lower then 7,0) will be rejected from analysis.

Total RNA will be used to prepare antisense biotinylated RNA using the genechip ® 3”IVT express 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 3’/5’ ratio of Actine and the 3’/5’ ratio of GAPH will be assessed to monitor sample and assay the quality of the array.

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

J. Single Nucleotide Polymorphisme (SNP) analysis in multiple myeloma patients

Lenalidomide de 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), neutropenia (Lenalidomide), thrombocytopenia (Bortezomib) and gastro-intestinal symptoms (Bortezomib). 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 MEDICAL CENTER. 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 Lenalidomide 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 Lenalidomide will be investigated, using in a high trough-put system with a Genome-Wide Human SNP array 6.0 (Affymetrix) platform 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 6 ml of EDTA blood divided over two tubes, 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 MEDICAL CENTER at room temperature within one week after sampling to maintain a good quality of DNA.

Since there are inter-ethnic differences in frequency of SNP's, 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.

References

1. Barlogie B, Desikan R, Eddlemon P et al.: extended survival in advanced and refractory myeloma after single agent Thalidomide: identification of prognostic factors in a phase 2 study of 169 patients. Blood ; 98: 492-4, 2001

2. Wu KI, Helgason HH, Holt van der B, Sonneveld P: analysis of efficacy and toxiciy of Thalidomide in 122 patients with multiple myeloma: response of soft-tissue plasmacytomas. Leukemia. 19(1):143-5, 2005

3. Richardson PG, Sonneveld P, Schuster MW, et al.: Phase 3 randomized study of Bortezomib versus Dexamethasone in relapsed multiple myeloma. N Engl J Med 352:2487-2498, 2005

4. Schilthuizen C, Broyl A, van der Holt B, de Knegt Y, Lokhorst H, Sonneveld P: Influence of genetic polymorphisms in CYP3A4, CYP3A5, GSTP1, GSTM1, GSTT1 and MDR1 genes on survival and therapy-related toxicity in multiple myeloma. Haematologica; 92(2): 277-8, 2007

5. Mulligan G, Mitsiades C, Bryant B, Zhan F, Chng W, Roels S, Koenig E, Burrington B, Richardson P, Trepicchio W, Dalton W, Sonneveld P, Broyl A, Shaughnessy J, Bergsagel L, Schenkein D, Esseltine D, Boral A, Anderson K: Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. Blood in press 2007