A multicenter, prospective study of bortezomib and dexamethasone as induction treatment followed by high dose melphalan (HDM) and autologous stem cell transplantation (SCT) in patients with *de novo* amyloid light chain (AL) amyloidosis.

PROTOCOL

Short title: HO104

Principal Investigator	**:**	**Dr M.C. Minnema**
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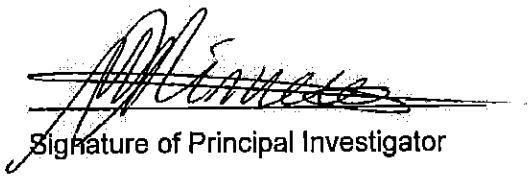
SPONSOR AND PRINCIPAL INVESTIGATOR SIGNATURE PAGE

The present trial protocol was subject to critical review and has been approved in the present version by the persons undersigned. The information contained is consistent with:

- the current risk-benefit assessment of the investigational medicinal product
- the moral, ethical, and scientific principles governing clinical research as set out in the applicable version of Declaration of Helsinki and the principles of GCP.

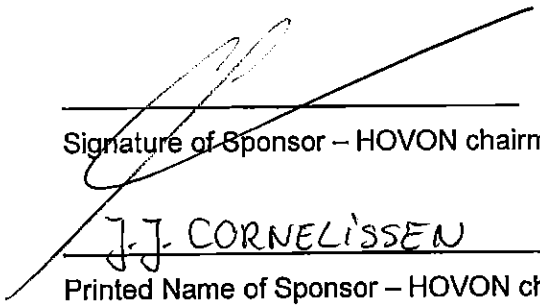
The local investigator will be supplied by the Sponsor with details of any significant or new finding including AEs relating to treatment with the investigational medicinal product.



Signature of Principal Investigator

12 SEPT 2014
Date

M.C. MINNEMA
Printed Name of Principal Investigator



Signature of Sponsor – HOVON chairman

15/9 - 2014
Date

J.J. CORNELISSEN
Printed Name of Sponsor – HOVON chairman

LOCAL INVESTIGATOR SIGNATURE PAGE

Local site name: _____

Local Investigator:

_____ _____

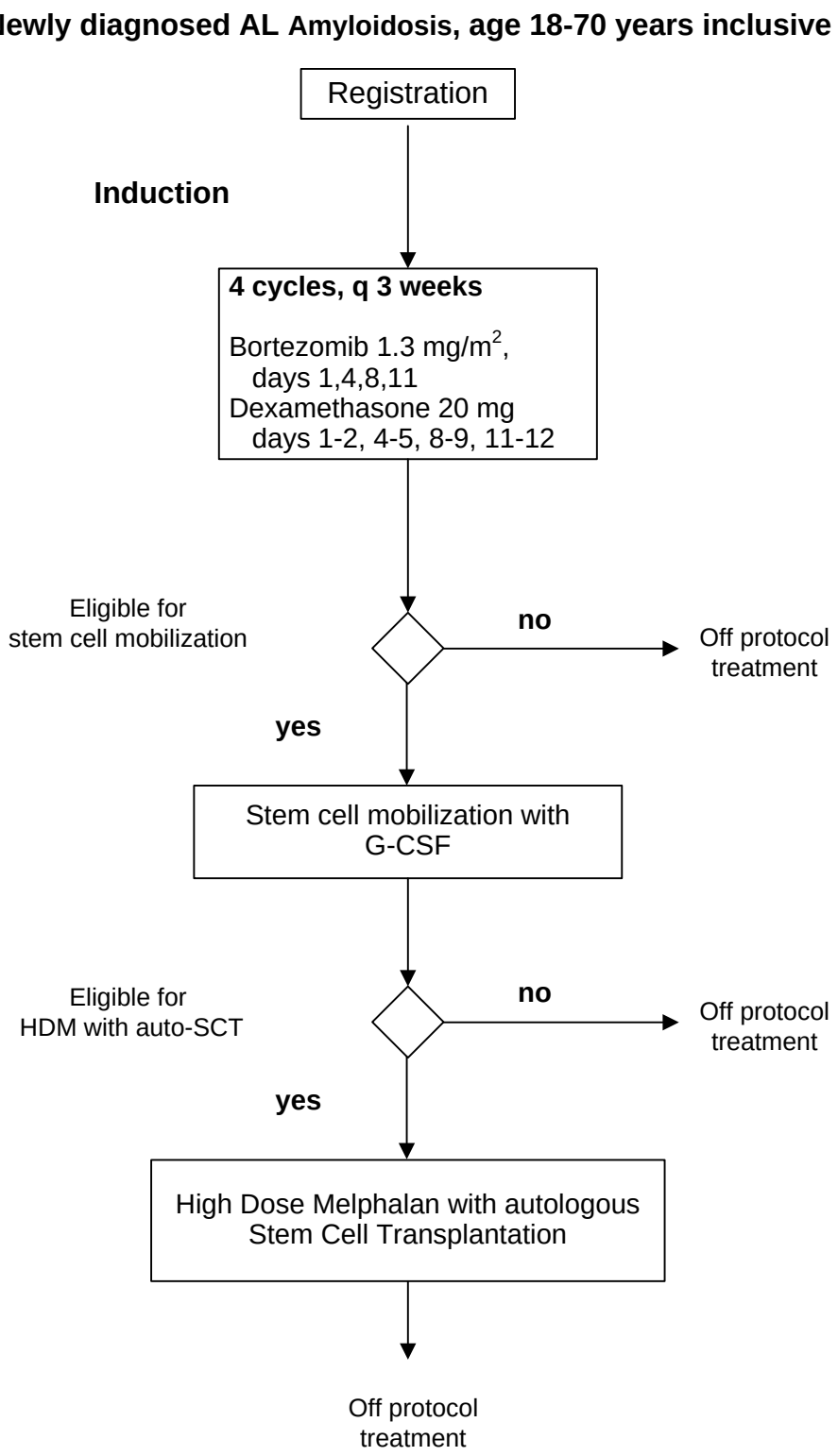
Signature of Local Investigator Date

_____

Printed Name of Local Investigator

By my signature, I agree to personally supervise the conduct of this study in my affiliation and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the current version of 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



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3 Synopsis

Rationale	The aim of the current study is to investigate the efficacy of an induction regimen with bortezomib and dexamethason followed by HDM and autologous SCT (auto-SCT) in improving the hematological response rate of patients with *de novo* AL amyloidosis. With the use of induction therapy the treatment related mortality (TRM) of the auto-SCT procedure is < 5% and the hematological response after the treatment is long-lasting. Because hematological response rate is closely related with survival in this patient population a better response rate will translate into better overall survival
Study objectives	**Primary:** To determine the efficacy of bortezomib plus dexamethasone followed by HDM and auto-SCT in patients with newly diagnosed AL amyloidosis who are 18-70 years inclusive. **Secondary:** To asses the safety of bortezomib plus dexamethasone in the induction regimen followed by HDM and autologous SCT in patients with newly diagnosed AL amyloidosis who are 18-70 years inclusive.
Study design	An international multi-center, open label, prospective 1 arm study.
Patient population	Newly diagnosed systemic AL amyloidosis, age between 18-70 years inclusive
Intervention	The treatment consists of bortezomib and dexamethasone followed by stem cell mobilization, HDM and auto-SCT.
Duration of treatment	Expected duration of induction and intensification is in total 4-5 months. All patients will be followed until 5 years after registration.
Target number of patients	50
Expected duration of accrual	4 years

Main study endpoints	**Primary:** • Hematological CR rate 6 months after autologous SCT **Secondary:** • OS • PFS • Hematological response rate after induction therapy • Response rate, hematological and organ • Time to response, hematological and organ • Duration of hematological and organ response • Time to next AL amyloidosis therapy • Safety (type, frequency, and severity of adverse events (AE) and relationship of AE to study drug • Evaluation of prognostic factors for surival **Exploratory** • assessment of multiparameter flow cytometry quantification of bone marrow plasma cells • amyloid deposition in abdominal fat aspiration samples.
Benefit and nature and extent of the burden and risks associated with participation	Patients are exposed to bortezomib and therefore to the possible side effects of the drug such as peripheral polyneuropathy, gastro-intestinal complaints and thrombocytopenia. If informed consent is given for participation in the experimental studies patients will undergo 4 additional abdominal fat aspiration procedures.
Planned interim analysis and DSMB (if applicable)	One interim analysis is planned, primarily to guard against unfavourably low efficacy results of the treatment. Results of the interim analysis will be presented confidentially to a DSMB. Only if the DSMB recommends that the study should be stopped or modified the results will be made public to the principal investigators for further decisions. The interim analysis is planned as soon as the hematological response data at 6 months post-transplant of the first 50% of the patients are available, which is the primary endpoint for this analysis.

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Data management	HOVON Data Center	Erasmus MC - Clinical Trial Center
Serious Adverse Events (SAEs) notification	HOVON Data Center	Erasmus MC - Clinical Trial Center

5 Introduction and rationale

5.1 AL Amyloidosis and current treatment

Disease

AL amyloidosis is caused by a small monoclonal population of plasma cells in the bone marrow, synthesizing monoclonal light chains that undergo conformational modifications and aggregate into amyloid fibrils that form extracellular deposits in one or more vital organs, most frequently the kidney, heart, liver and peripheral and autonomic nervous system.

The disease is rare with an estimated incidence of 0.8 per 100,000 person years. The median age is 60 years with a male predominance (57%). If untreated, AL amyloidosis is a progressive disease and is usually fatal within 2 years.[1]

Patients can present with nephrotic syndrome, renal failure, hepatosplenomegaly, congestive heart failure, arrythmias, postural hypotension, syncope, and pseudo-obstruction of the gastro-intestinal tract. Highly suggestive physical findings are macroglossia and periorbital purpura. Approximately 70% of patients present with 2 or more organs involved.

Diagnosis & Prognosis

The diagnosis of AL amyloidosis is based on a clinical suspicion and confirmed by a tissue biopsy, usually of an involved organ. The type of amyloid must then be determined. Patients with AL amyloidosis will have a clonal plasma cell dyscrasia.

Since the extent of cardiac involvement is the most important determinant of clinical outcome, a staging system to evaluate the cardiac injury has been developed.[2] This system uses the serum markers N-terminal fragment of pro-brain natriuretic peptide (NT-ProBNP) and troponin T or I. NT-ProBNP is produced when there is increased wall stress of the heart and the cardiac tropinins are highly specific sensitive markers of cardiac injury.

A very helpful tool in the diagnosis and response assessment is the serum free light chain (FLC) immunoassay which consists of a polyclonal latex-conjugated anti-free light chain antibody with high specificity and affinity for serum free light chains. This assay has improved the diagnosis and monitoring of AL Amyloidosis patients. M-proteins can be detected in the serum or urine in only 50% of patients, whereas the FLC assay can detect and quantify light chains in about 90% of patients.

Many studies have demonstrated that achieving a hematological response is closely related with survival. This is not only demonstrated after high dose therapy[3;4] but also after standard dose therapy.[5;6]

The quality of hematological response is also very important; patients achieving a complete hematological remission (CHR) having the best survival and therefore hematological response is a valid endpoint in clinical trials.[7]

Treatment

The aim of treatment is to reduce rapidly the supply of the amyloidogenic monoclonal light chains and to preserve and possibly restore organ function.

High dose melphalan (HDM) followed by autologous SCT (auto-SCT) is considered the most effective treatment for selected patients with AL amyloidosis. A recent phase III trial demonstrated that a melphalan-dexamethasone regimen is a valuable alternative to upfront HDM with auto-SCT.[6] However, a major critique on the study was the poor performance of the HDM arm, possibly related to treatment of these patients in inexperienced centers, and the treatment delay in the HDM arm of this study.

In contrast to the IFM study, 2 other studies demonstrated that treatment related mortality (TRM) of HDM and auto-SCT is lower after dexamethasone-based induction chemotherapy and patients have an excellent survival afterwards.[8;9] Dexamethasone is believed to be the most active component of this induction regimen and indeed it was shown that induction with dexamethasone alone followed by HDM and auto-SCT was equally effective as VAD induction.[10] The response rate after VAD was 48% with 13% CR and after dexamethasone 58% with 17% CR. However, in order to further improve survival there is a need for more effective induction chemotherapy resulting in more and better hematological responses.

5.2 Investigational Medicinal Product

Bortezomib (Velcade®), a proteasome inhibitor frequently used in first line and relapse treatment in Multiple Myeloma (MM) patients, has been given to newly diagnosed and relapsed AL amyloidosis patients in both retrospective and prospective studies.[11-16] Bortezomib demonstrated an excellent hematological response rate varying from 50 to 70%, including 25 to 47% complete remissions, which occurred rapidly, especially when dosed bi-weekly. Common toxicities included thrombocytopenia, peripheral sensory neuropathy, neuropathic pain, hypotension/orthostasis and peripheral edema.

In the only published prospective analysis dizziness was reported more often (26%) compared to reports in MM patients (<10%) and 2 out of 13 patients (6%) reported CTC AE grade 3 congestive cardiac failure which led to treatment discontinuation in one.[14] In MM patients subcutaneous (sc) and intravenous (iv) administration of bortezomib are equally effective, however the sc administration has less CTC AE grade 3 and 4 side effects, especially less induction of polyneuropathy.[17] Fifty-seven percent of patients experienced mild redness at the injection site. Considering the potent effect in relapsed AL amyloidosis patients and the improvement in response rates achieved when used as the first line treatment of MM patients, it is expected that the use of bortezomib will also improve the response rate in first line treatment of AL amyloidosis patients and this may lead to improved survival.

5.3 Rationale of the study

The aim of the current study is to investigate the efficacy of induction treatment consisting of bortezomib and dexamethasone followed by HDM and auto-SCT to improve the hematological response rate and especially the CHR of patients with *de novo* AL amyloidosis. With the use of induction therapy the TRM of the auto-SCT procedure is < 5% and the hematological response after the treatment is long-lasting. Because hematological response rate is closely related with survival in this patient population a better response rate will translate into better overall survival.

6 Study objectives

- To determine the efficacy of bortezomib plus dexamethasone induction therapy followed by HDM and auto-SCT in patients with newly diagnosed AL amyloidosis who are 18-70 years inclusive.
- To asses the safety of bortezomib plus dexamethasone as induction treatment followed by HDM and auto-SCT in patients with newly diagnosed AL amyloidosis who are 18-70 years inclusive.

7 Study design

This is an international multi-center, open label, prospective 1 arm, study. The primary study design was a phase III study with randomization. However due to slow inclusion the expectation was that the primary endpoint could not be reached with already 23 patients included. Of those 23 patients, 16 patients where randomized to bortezomib induction. No safety issues were identified. Because the scientific value of the study was still present and medical important information was collected it was considered unethical to stop the study. Therefore it was decided to close the control arm of the study and continue as a 1 arm study.

Potential patients will sign an informed consent prior to any study related procedure. Treatment consists of bortezomib and dexamethasone followed by stem cell mobilization, HDM and auto-SCT. The duration of treatment (including induction and intensification) is in total 4-5 months. Bortezomib will be given sc.

Subsequently patients will be followed for response, progression, overall survival and subsequent AL amyloidosis treatment until all subjects in the study have been followed for at least 5 years from registration.

Details of all treatments (dose and schedule) are given in paragraph 9. Study visits and serial measurements of efficacy and safety will be performed as outlined in paragraph 10.2.

8 Study population

8.1 Eligibility for registration/randomization

Newly diagnosed systemic AL amyloidosis patients can be eligible for the trial. All patients must be registered and randomized before start of treatment and must meet all of the following eligibility criteria.

8.1.1 Inclusion criteria

- Biopsy proven, systemic, untreated AL amyloidosis requiring systemic chemotherapy,
- Age 18 -70 years inclusive at the time of signing the informed consent form,
- Measurable plasma cell dyscrasia, defined as a detectable M-protein with serum electrophoresis and/or level of involved FLC > 50 mg/L,
- Life expectancy > 3 months,
- WHO performance status 0-2,
- NYHA stage 1-2,
- Negative pregnancy test at inclusion for women of childbearing potential,
- Written informed consent.

8.1.2 Exclusion criteria

- Multiple Myeloma stage II and III (Durie and Salmon, see appendix K),
- Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from signing the informed consent form,
- Any psychological, familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule,
- Previous treatment for plasma cell dyscrasia,
- Pregnant or breast feeding females,
- Presence of other active malignancy or a history of active malignancy during the past 5 years, with the exception of nonmelanoma skin cancer, stage 0 cervical carcinoma, or treated early-stage prostate cancer provided that prostate-specific antigen is within normal limits,
- Hypersensitivity to boron or mannitol,
- Uncontrolled infection,

- Symptomatic orthostatic hypotension defined as a decrease in systolic blood pressure on standing of >20 mmHg combined with symptoms like dizziness, cerebral and/or cardial ischemia,
- Symptomatic effusions, defined as pleural effusion or ascites needing drainage therapy,
- NT pro BNP level > 5000 pg/ml and Troponin T> 0.06 microgram/l (not high senstitivity assay) or NT proBNP level > 5000 pg/ml and Troponin I > 2 times ULN
- Positive for HIV or infectious hepatitis, B or C (screening obligatory),
- Bilirubin > 2x upper limit of normal,
- Creatinine clearance < 30 ml/min (after rehydration),
- Absolute neutrophil count < 1.0×10^9/L,
- NCI CTCAE grade peripheral sensory neuropathy > grade 2,
- NCI CTCAE grade peripheral sensory neuropathy > grade 1 in the presence of neuropathic pain,
- NCI CTCAE grade peripheral motor neuropathy > grade 2
- Concurrent diagnosis of B-cell NHL or B-CLL,
- Previous organ transplantation,
- Unwilling or unable to use adequate contraception

8.2 Eligibility criteria for stem cell mobilization and HDM with auto-SCT

8.2.1 Inclusion criteria

- Life expectancy > 3 months,
- WHO performance status 0-2,
- NYHA stage 1-2,
- Cardiac ejection fraction > 45% (only before HDM),
- Negative pregnancy test in female patients of childbearing potential.

8.2.2 Exclusion criteria

- Symptomatic effusions, defined as pleural effusion or ascites needing drainage therapy,
- Uncontrolled infection,
- Symptomatic orthostatic hypotension defined as a decrease in systolic blood pressure on standing of >20 mm Hg combined with symptoms like dizziness, cerebral and/or cardial ischemia. Anti-hypotensive medication is allowed,
- Absolute neutrophil count < 1.0×10^9/L,
- Bilirubin > 2x upper limit of normal.

- Stem cell mobilization started > 12 weeks after the start of the last course of bortezomib and dexamethasone

9 Treatment

9.1 Induction therapy

9.1.1 Treatment schedule

Patients will be treated with 4 cycles of bortezomib and dexamethasone (q 3 weeks). Dexamethasone is taken at home and provided by the local pharmacy. Bortezomib is given subcutaneously in the hospital on an outpatient basis. If hematological response analysis after cycle 2 or cycle 3 demonstrates progression (see appendix B) cycle 3 or cycle 4 is not given or is stopped if already started. Patients stay on protocol treatment and stem cell mobilization is planned according to protocol (see 9.2) for patients fulfilling the inclusion criteria for stem cell mobilization and HDM with auto-SCT. Patients with pre-existing sensory or motor PNP grade 2 without neuropathic pain or with sensory PNP grade 1 with neuropathic pain at study entry start with a 25% dose reduction of bortezomib, i.e.1.0 mg/m^2 and once per week schedule on days 1, 8, 15 and 22.If bortezomib is given once a week then the dexamethasone must also be rescheduled and given on days 1-2, 8-9, 15-16 and 22-23 thus also resulting in a 35 days schedule.

Hepatic dysfunction

In patients with moderate (bilirubin > 1.5x-3x ULN) or severe (bilirubin> 3x ULN) hepatic dysfunction with any increase in ASAT at study entry or during study the (starting) dose of bortezomib will be reduced to 0.7 mg/m^2 during the first or subsequent cycle. Depending on the tolerance an increase to 1.0 mg/m^2 or reduction to 0.5 mg/m^2 for the following cycles should be considered.

Agent	**Dose/day**	**Route of administration**	**Days**
Dexamethasone	20 mg/day	orally	1,2,4,5,8,9,11,12
Bortezomib	1.3 mg/m^2	subcutaneously	1,4,8,11 in a 21 day cycle

9.1.2 Dose adjustment of bortezomib and dexamethasone

Treatment with bortezomib and dexamethasone should be adjusted as described below if the patient experiences toxicities.

If toxicities related to bortezomib do not resolve after dosing has been withheld for two weeks or after using reduced doses to the last prescribed dose adjustment, patients must stop induction treatment. .

If dexamethasone cannot be given anymore due to toxicity, patients must continue with bortezomib monotherapy according to schedule. Patients who stop induction treatment due to toxicities must proceed to stem cell mobilization and HDM with auto-SCT if they fulfill the inclusion criteria, see paragraph 8.2. Patients that can not proceed to stem cell mobilization go off treatment

Bortezomib

Before each bortezomib dose, the patient will be asked for possible toxicities that may have occurred after the previous dose(s). Laboratory investigation will be performed on indication. All previously established or new toxicities observed any time, *with the exception of neuropathic pain and peripheral sensory neuropathy for which separate guidelines are defined in Appendix G and H,* 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 3 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

If ANC < 0.75 x 10^9/l and platelets < 30 x 10^9/l at the day of the planned bortezomib dose, bortezomib is to be withheld for up to 2 weeks until the following values are reached: hemoglobin > 4.4 mmol/l or 7.0 g/dl, ANC ≥ 1.0 x 10^9/l, **and** platelet count ≥ 70 x 10^9/l. Hb, ANC and platelets levels should be evaluated at the remaining days of bortezomib treatment of that cycle or at start of the next cycle to evaluate if bortezomib can be re-started.

Dose interruption or treatment discontinuation is not required for lymphopenia of any grade.

Hepatic imparment

If during treatment bilirubin levels increase > 2x upper limit of normal (moderate impaiment) or > 3x upper limit of normal (severe impairment) combined with any increase in ASAT, bortezomib should be decreased to 0.7 mg/m^2. Consider dose escalation to 1.0 mg/m^2 or reduction to 0.5 mg/m^2 in subsequent cycles based on patient tolerability.

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. In case of symptomatic hypotension first adequate volume

repletion and start of anti-hypotensive drugs like fludrocortisone or midodrine is advised. Chapter 9.4.1 describes all known side effects in bortezomib, in AL Amyloidosis patients hypotension, congestive cardiac failure and peripheral edema may occur more frequently.

Dose adjustments after withholding bortezomib dosing for toxicities, except Neuropathic pain and/or peripheral sensory neuropathy

If withholding the bortezomib dosing results in resolution of the toxicity, bortezomib may be restarted at a dose reduced by 25%, as follows:

- If the patient was receiving 1.3 mg/m^2, reduce the dose to 1.0 mg/m^2.
- If the patient was receiving 1.0 mg/m^2, reduce the dose to 0.7 mg/m^2.
- If the patient was receiving 0.7 mg/m^2, then the bortezomib must be discontinued, except in case of hepatic impairment, the dose can be lowered to 0.5 mg/m^2.

Neuropathic pain and/or peripheral neuropathy

Amyloidosis patients are prone to develop neuropathic pain and peripheral neuropathy. Therefore special quidelines are implemented using a management tool for the detection and grading of these complications and special dose reductions.
Appendix H is a management tool developed to relate PNP complaints with CTC grading. It is advised to use this screening list before start of therapy, before each cycle of induction therapy and at all other evaluation moments to correctly grade patient's symptoms and to adequately reduce bortezomib dosing if necessary.

Patients with already sensory or motor PNP grade 2 without neuropathic pain or with sensory PNP grade 1 with neuropathic pain at study entry start with a 25% dose reduction of bortezomib, i.e.1.0 mg/m^2 and once per week schedule on days 1,8, 15 and 22. If bortezomib is given once a week then the dexamethasone must also be adjusted and given on days 1-2, 8-9, 15-16 and 22-23 thus also resulting in a 35 days schedule.

Patients who experience bortezomib related neuropathic pain and/or peripheral sensory-motor neuropathy are to be managed as presented in the table in Appendix H. The first dose lowering step is a schedule change from twice per week to once per week, secondary steps are dose reductions. 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 given once per week and reduced by 50%.

Dexamethasone

Known side effects are fluid retention, hypertension, stomach ulcer, hyperglycemia, psychological disturbances like sleeplessness, mood changes and psychosis. If side effects occur related to dexamethasone which cannot be managed with other interventions the dose must be reduced to 10 mg (12 mg is allowed).The dose must also be reduced in case of signs of cardiac failure defined as:

- 4% weight gain (of last known weight)
- 1 upgrade on NYHA scoring list (appendix E)

No further dose adjustments are allowed.

9.1.3 Supportive care during bortezomib and dexamethasone

- Antibiotic prophylaxis is mandatory; the advice is to use ciprofloxacin 500 mg bid starting on day 1 of treatment until 2 weeks after the last intake of treatment medication. Advice is NOT to use co-trimoxazol profylaxis because of renal complications in AL amyloidosis patients.
- Gastric acid production inhibition is mandatory, advise is to use a protonpump inhibitor
- Herpes zoster prophylaxis is mandatory only when bortezomib is given, the advice is to use Valacyclovir 500 mg bid or Acyclovir 200 mg 3 dd, starting on day 1 of bortezomib until 4 weeks after the last administration
- Anti-fungal therapy is advised, advice is to use fluconazol 200 mg daily starting at the first day of treatment until 2 weeks after the last intake of treatment medication
- If symptomatic hypotension occurs during treatment it is advised to ensure adequate volume repletion and start of anti-hypotensive drugs like fludrocortisone or midodrine (see also 9.1.3, non-hematological toxicities).

9.2 Stem cell mobilization

After 4 courses of bortezomib and dexamethasone all patients who meet the eligibility criteria for stem cell collection and HDM with auto-SCT (see 8.2), continue with stem cell mobilization.
Patients who do not meet the eligibility criteria will go off-protocol treatment

Stem cell mobilization will start within 4-6 weeks after the start of last course of bortezomib and dexamethasone. Stem cell collection will be performed with G-CSF 10 μg/kg divided in 2 doses, given for 5 days. Stem cell collection will be performed starting from day 4 or 5 (or longer depending on the CD34+ count). A minimum of 2.0×10^6 CD34+cells/kg are required. Administration of plerixafor is allowed if the target CD34+ cells are not reached on the first day of apheresis. A second attempt of stem cell mobilization is allowed with the use of cyclofosfamide in mono- or in combination therapy

according to local quidelines when the first stem cell collection does not yield ≥ 2.0×10^6 CD34+cells/kg.
HDM with auto-SCT is performed within 2-6 weeks after stem cell collection.
Delays for the first attempt for stem cell mobilization are acceptable within 12 weeks after the start of the last course of bortezomib and dexamethasone, otherwise patients will go off-protocol treatment.

9.3 High dose melphalan followed by auto-SCT

9.3.1 Treatment schedule

Before start of HDM patients must fulfill the inclusion criteria (see 8.2).

Agent	Dose/day	Route	Days
Melphalan	100 mg/m^2	i.v.	-3,-2
Stem cell infusion			0

Melphalan infusion will start between 2-6 weeks after stem cell collection. The dose will be 100 mg/m^2 melphalan given as rapid infusion on days -3 and -2, with a total dose of 200 mg/m^2.

On day 0 stem cells are thawed at the bedside and infused according to local standard protocols.

9.3.2 Dose adjustment of melphalan

- Patients with a creatinine clearance ≤ 40 m/min will receive a total dose of 100 mg/m^2 of melphalan on day -2 only.

9.3.3 Special precautions and supportive care during melphalan treatment

- A hydration regimen will be started 30 minutes before administration of HDM according to local hospital rules.
- Diuretics must be administered if necessary.
- Placement of a central venous catheter is advised because neuropathy can involve the enteric plexus which may result in atony, persistent posttransplantation nausea, and need for prolonged nutritional support.
- Gastrointestinal toxicity may be severe with higher rates of edema, mucositis and bleeding compared with MM patients due to amyloid infiltrates in the intestinal tract.

- The use of proton-pump inhibitors is mandatory for prophylaxis.
- Menstruating premenopausal females will be started on anovulatory drugs according to local hospital protocols.
- Antibacterial and antifungal prophylaxis should be given according to local protocols.
- Engraftment syndrome may occur in up to 10% of patients which consists of constellation of symptoms and signs including fever, erythrodermatous skin rash, and noncardiogenic pulmonary edema and should be treated with prednisone.

9.4 Investigational Medicinal Product: Bortezomib

9.4.1 Summary of known and potential risks

Details on the potential risks of bortezomib (VELCADE®) may be found in the current Investigator Brochure and SmPC.

Most common side effects (ie, incidence ≥30%) observed in subjects are thrombocytopenia and anemia; gastrointestinal effects such as constipation, diarrhea, nausea, and vomiting; fatigue, pyrexia, and peripheral neuropathy.

Very common side effects (ie, incidence 10%–29%) observed in subjects are neutropenia, abdominal pain (excluding abdominal pain arising from oral and throat gastrointestinal disorders), chills, peripheral edema, asthenia, upper respiratory tract infection, nasopharyngitis, pneumonia, Herpes zoster, decreased appetite, anorexia, dehydration, bone pain, myalgia, arthralgia, paresthesia, dizziness excluding vertigo, headache, anxiety, insomnia, cough, dyspnea, and rash.

Common side effects (ie, incidence 1%–9%) observed in subjects are lymphopenia, pancytopenia, leucopenia, febrile neutropenia, tachycardia, atrial fibrillation, palpitations, cardiac failure congestive, blurred vision, conjunctivitis, conjunctival hemorrhage, dyspepsia, pharyngolaryngeal pain, gastroesophageal reflux, abdominal distension, gastritis, stomatitis, mouth ulceration, dysphagia, gastrointestinal hemorrhage, lower gastrointestinal hemorrhage ± rectal hemorrhage, neuralgia, lethargy, malaise, chest pain, mucosal inflammation, lower respiratory tract infection, sinusitis, pharyngitis, oral candidiasis, urinary tract infection, sepsis, bacteremia, cellulitis, Herpes simplex, bronchitis, gastroenteritis, decreased weight, increased ALT, increased AST, increased blood alkaline phosphatase, abnormal liver function test, increased blood creatinine, hyperglycemia, hypoglycemia, hyponatremia, hypokalemia, hypercalcemia, polyneuropathy, syncope, dysesthesia, dysgeusia, postherpetic neuralgia, confusional state, renal impairment, renal failure, hematuria, epistaxis, exertional dyspnea, pleural effusion, rhinorrhea, hypoxia, pulmonary edema, pruritic rash, erythematous rash, urticaria, petechiae, hypotension, and orthostatic hypotension, muscular weakness .

Uncommon side effects (ie, incidence <1%) observed in subjects are cardiogenic shock, atrial flutter, cardiac tamponade, bradycardia, atrioventricular block complete, arrhythmia, cardiac arrest, cardiac failure, arrhythmia, pericardial effusion, pericarditis, pericardial disease, cardiopulmonary failure, deafness, hearing impaired, eructation, gastrointestinal pain, tongue ulceration, retching, upper gastrointestinal haemorrhage, haematemesis, oral mucosal petechiae, ileus paralytic, ileus, odynophagia, enteritis, colitis, oesophagitis, enterocolitis, diarrhoea haemorrhagic, acute pancreatitis, intestinal obstruction, injection site pain, injection site irritation, injection site phlebitis, general physical health deterioration, catheter-related complication, hyperbilirubinaemia, hepatitis, drug hypersensitivity, angioedema, septic shock, catheter-related infection, skin infection, disseminated Herpes zoster, lung infection, infusion site cellulitis, catheter site cellulitis, infusion site infection, urosepsis, aspergillosis, tinea infection, ophthalmic Herpes zoster, ophthalmic Herpes simplex, meningoencephalitis herpetic, varicella, empyema, fungal esophagitis, subdural haematoma, increased gamma-glutamyltransferase, decreased oxygen saturation, decrease blood albumin, decreased ejection fraction, limb discomfort, tumor lysis syndrome, convulsion, loss of consciousness, ageusia, encephalopathy, paralysis, autonomic neuropathy, posterior reversible encephalopathy syndrome, delirium, micturition disorder, hemoptysis, acute respiratory distress syndrome, respiratory failure, pneumonitis, lung infiltration, pulmonary alveolar hemorrhage, interstitial lung disease, pulmonary hypertension, pleurisy, pleuritic pain, cutaneous vasculitis, leukocytoclastic vasculitis, cerebral hemorrhage, optic neuropathy, blindness and progressive multifocal leukoencephalopathy. Complications arising from these VELCADE® toxicities may result in death. The effect of VELCADE® on reproduction and its safety in pregnancy are unknown. Laboratory tests show that VELCADE® may damage DNA therefore it is possible that VELCADE® may cause infertility in men and women.

9.4.2 Preparation and labeling

Bortezomib will be shipped to trial sites in containers labeled as an Investigational Medicinal Product. Bortezomib will be prepared and labeled in compliance with GMP and other applicable regulatory requirements.

9.4.3 Storage and handling

Bortezomib should be stored and handled in accordance with the instructions in the summary of product characteristics or package insert.
The investigational medicinal product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.

9.4.4 Study drug supply

The sponsor will arrange delivery of bortezomib to trial sites. No investigational medicinal product will be shipped until the sponsor has verified that all regulatory required documents and approvals for the site are available.

9.4.5 Drug accountability

The investigator, or a pharmacist or other appropriate individual who is designated by the investigator, should maintain records of the product's delivery to the trial site, the inventory at the site, the use by each patient, and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s) and trial patients (if applicable). Investigators should maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor.

9.4.6 Study drug return and destruction

Partially used investigational medicinal product should not be redispensed to either the same or another patient after it has been returned.
The trial site should destroy used or partially used study drug containers after drug accountability records have been completed. Destruction should be documented.
Unused investigational medicinal product should be retained until the sponsor has instructed the investigator on the return or destruction of the product.

10 Study procedures

10.1 Time of clinical evaluations

- At entry: within 2 weeks before registration and before start of treatment
  Importantly, bone marrow aspirates and biopsies taken within 4 weeks before inclusion are valid as entry evaluations and do not have to be repeated. This also applies to echocardiography and X-ray or low dose CT / MRI of the whole body but not for other required evaluations. A separate informed consent is available in which a patient can consent to the extra bone marrow aspirate volume of 3.5 ml needed for the MRD study. This aspirate has to be sent to a central lab (see appendix I)

If a patient consents to the experimental fat aspiration side study this has to be performed after informed consent and before start of study medication or a patient can consent to take extra material during diagnostic work up with the separate infomed consent and send this to a central lab (see appendix J). All evaluations have to be done before start of treatment.

- After each induction cycle.
- After stem cell mobilisation and before HDM
- At 3 months after date of auto-SCT
- At 6 months after date of auto-SCT. This evaluation is the primary endpoint of the study and has to be combined with bone marrow examination (+ MRD analysis), echocardiography and ECG analysis. However if a bone marrow examination was performed earlier in the treatment schedule because a CHR was suspected and confirmed, and no signs of relapse (Appendix B) are established a patient is considered to be in continious CHR and a bone marrow examination does not have to be repeated at 6 months.
- During follow up every 3 months, calculated from the date of auto-SCT (or date off protocol treatment).
  The follow up evaluation may be done between 2 weeks before or 2 weeks after the 3-monthly evaluation date.
- Suspected CHR: If serum and urine IF becomes negative and FLC ratio normal a bone marrow examination has to be performed to establish CHR.

All patients will be followed until 5 years after registration.

10.2 Required investigations

Required investigations at entry, during treatment and during follow up

	Screening	**Treatment period**			**Follow-up**
Assessments and Procedures	At entry	After each cycle of induction	After GCSF prior HDM	At 3 months after auto-SCT	Follow up, every 3 months
Informed consent	x				
Medical history	x	x	x	x	x
Physical examination	x	x	x	x	x
In-/Exclusion criteria	x				
Pregnancy test	x[1]		x[1]		
Virology	x				
Evaluation of gammopathy					
Serum	x	x	x	x	x
24-hours urine	x	x	x	x	x
Bone marrow					
Bone marrow aspirate	x	x[2]	x[2]	x[2]	x[3]
Bone marrow biopsy	x	x[2]	x[2]	x[2]	x[3]
Hematology	x	x	x	x	x
Blood chemistry	x	x	x	x	x
Additional					
Chest X-thorax	x				x[4]
ECG	x		x		x[5]
Echocardiography	x		X[8]		x[5]
Ultrasound abdomen	x				x[4]
Imaging	x				
Spirometry *(advised)*	x				
MRD side study	x				x[6]
Fat aspiration side study	x				x[7]

1. for female patients of childbearing potential, before stem cell apheresis and HDM
2. In case of suspected CHR a bone marrow examination has to be performed.
3. At 6 months after auto-SCT (only if CHR was not established before) and in case of suspected CHR.
4. If abnormal at inclusion possible due to involvement of AL Amyloidosis, repeat every year after inclusion (5 years)
5. At 6 months after auto/SCT and therafter once a year after inclusion (2,3,4,5 years after inclusion)
6. At 6 months (or in case of suspected CHR before 6 months) after autoSCT bone marrow examination combined with MFC
7. At 1, 3 and 5 years after inclusion
8. Before start of GCSF!

Medical history

Standard medical history, with special attention for:

- WHO performance status (appendix C)
- NYHA heart classification (appendix E)
- Concomitant medication
- Autonomic neuropathy (like syncope, sweating, diarrhea, impotence), use appendix G for grading
- Sensory-motor peripheral neuropathy and neuropathic pain, use appendix G for grading
- Bleeding tendency
- Constipation/diarrhea/nausea/weight loss

Additional only at entry:

- Prior and present other diseases
- Antecedent hematological or oncological diseases
- Previous chemotherapy or radiotherapy
- Carpal tunnel syndrome
- Claudication of temporal artery
- Family history for amyloidosis related symptoms

Physical examination

Standard physical examination, with special attention for:

- Skin and soft tissue abnormalities (peri-orbital purpura, other purpura, macroglossia, shoulder pads, skeletal muscle pseudohypertrophy)
- Hepatosplenomegaly
- Lymphadenopathy
- Orthostatic hypotension defined as a decrease in systolic blood pressure on standing of >20 mm Hg
- Edema
- Weight

Additional only at entry:

- Height

Hematology

- Hemoglobin, leukocyte count, differential count, platelets
- Presence of Howell-Jolly bodies (only at entry)

Blood chemistry

required

- Creatinine, bilirubin, alkaline phosphatase, ASAT/SGOT, ALAT/SGPT, albumin, GFR (measured by MDRD formula (needed parameter: serum creatinine, age, sex, ethnicity))
- Troponin-T (not high senstivity) or Troponin-I, NT-pro-BNP
- Only at study entry: total protein, uric acid, β_2-microglobulin, aPTT, PT, TT, Factor X
- Serum (or urine) pregnancy test if applicable: at study entry, before stem cell collection and before HDM with auto-SCT for female patients of childbearing potential

advised

Na, K, gamma-GT, ureum

Virology

Serology tests for antibodies against HIV, hepatitis B and C only at study entry

- Antibodies against HIV-1 and/or HIV-2
- IgTotal anti HBcAg
- HbsAg
- IgG anti-HCV

Monoclonal gammopathy

- Serum electrophoresis (SPEP) with quantification of M-protein
- Immunofixation in case of first sample or negative SPEP during therapy
- Free light chain kappa, lambda, ratio and difference between involved and uninvolved FLC (dFLC)

24-hours urine

- Total protein, (albumin)
- Urine electrophoresis (UPEP) with quantification of free kappa or lambda chain
- Immunofixation in case of first sample or negative UPEP during therapy

Bone marrow

Bone marrow biopsy at entry, to confirm complete response (in case of suspected CHR) and at 6 months after auto-SCT:

- Morphology and percentage of clonal plasmacells (kappa lambda labeling)
- Assessment of the presence of amyloidosis

Bone marrow aspirate at entry, to confirm complete response (in case of suspected CHR) and at 6 months after auto-SCT:

- Percentage of plasma cells

- Flow cytometry (at study entry and if consented for MRD analysis)

If patients refuse a biopsy a bone marrow aspiration only is acceptable for all the follow up examinations.

The evaluation of CHR at 6 months, calculated from the date of auto-SCT, is the primary endpoint of the study. Therefore it is important to perform a bone marrow examination at this time point and if a CHR is suspected (see appendix B). However, in case a CHR was established at an earlier time point and no relapse is establised at 6 months after auto-SCT a CHR is also assumed to exist at this time point and bone marrow examination does not have to be repeated. In case CHR is not established at 6 months after auto-SCT but later suspected in the FU period patients will also need a bone marrow examination to confirm CHR.

Additional investigations

- Chest X-ray: interstitial radiographic abnormalities
- Ultrasound abdomen: total liver span
- Imaging ; X-ray, or low dose whole body CT or whole body MRI to exclude MM Durie and Salmon stage II and III
- ECG
- Echocardiography: intraventricular septum thickness, left ventricular wall thickness measured at posterior wall, diastolic dysfunction (grade I to III) and infiltrative cardiomyopathy (defined as presence of suspected pattern or not). Technical description and measurements of echocardiography are described in appendix F. A central review is installed for all Dutch and Belgian centers and this review can be used for additional analysis.

advised:

- Pulmonary function test/Spirometry

10.3 Response evaluation

The hematological response will be evaluated with serum and 24-hours urine examinations after each cycle of induction chemotherapy, before HDM and auto-SCT, 3 months after auto-SCT (or after going off protocol treatment) and every 3 months thereafter. Importantly, if CHR is suspected a bone marrow examination has to be performed. If patients refuse a biopsy only aspiration is acceptable.If the bone marrow contains more than 5% plasmacells there is no CHR and bone marrow examination has to be repeated at the next response evalution if urine and serum test are still compatible with CHR.

The organ response will be assessed at the same time points as hematological response, except for the echocardiography that will be done at entry, before HDM (before GCSF) and auto-SCT and at the primary endpoint at 6 months. Thereafter the echocardiography will be performed at 2,3 4 and 5 years

after date of inclusion, also if the previous echocardiogram was normal.. Responses will be evaluated according to appendix B.

10.4 Side study

MRD side study

Multiflow cytometry may be an important prognostic tool. Paiva et al demonstrated that MRD assesment after auto-SCT was the most important prognostic parameter for survival in MM patients but this is unknown for AL amyloidosis[15]. In this study 58% of the 295 patients still had detectable abnormal plasma cells on day 100 after auto-SCT; the other 42% had only normal plasma cells in the bone marrow. MRD negative patients had a better PFS and OS with an estimated 5 years PFS of 60% vs 22% and 5 years OS of 82% vs 60%. In multivariate analysis MRD status was an independant risk factor for PFS and OS with relative risk of 3.6 and 2.0.
For MRD analysis 3 ml bone marrow in heparin-coated tubes has to be collected. Samples are send to the immunology laboratory of the UMC Utrecht and will be analyzed as described in appendix I. See the laboratory manual for details.

Patients are MRD negative if no abnormal plasmacells can be demonstrated with a sensitivity of 0.01%, It is essential that a qualitive good bone marrow aspirate is used that must contain normal plasma cells.[18]

Fat aspiration side study

Subcutaneous abdominal fat tissue is diagnostic of amyloid in most patients with AL amyloidosis and can easily be obtained for analysis at regular intervals to monitor the actual course of amyloid deposition at tissue level. Monitoring of amyloid in fat tissue may be useful in AL amyloidosis because fat tissue in this disease is not actively involved in the production of the precursor protein. A small study in a selected group of patients with AL amyloidosis showed significant histological regression of amyloid deposition in fat tissue exclusively after normalization of serum free light chain. This side study wants to improve the monitoring of the amyloid regression and possibly organ function and relate those to prognosis. In patients who consent 4 additional fat aspirations are perfomed: at inclusion and 1,3 and 5 years afterwards. See appendix H for further instruction and website www.amyloid.nl
(instructions available in Dutch and English)

11 Withdrawal of patients or premature termination of the study

11.1 Specific criteria for withdrawal of individual patients

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a patient from the study for urgent medical reasons. Specific criteria for withdrawal are:

- Completion of the treatment defined as 3 months after autologous SCT. Notion: the primary end-point evaluation is performed at 6 months after autologous SCT
- Toxicity (including toxic death) that precludes further continuation of treatment
- If a patient cannot receive the planned study protocol treatment and if a patient is not eligible for stem cell collection and HDM. Please not that patients with hematological progression/relapse during induction treatment, stay on protocol treatment if they can proceed with stemcell apheresis and HDM with auto-SCT directly
- Intercurrent death
- No compliance of the patient (especially refusal to continue treatment)

11.2 Follow up of patients withdrawn from treatment

Patients who are withdrawn from treatment for other reasons than death will be followed as described in 10.2 for follow up.
For patients who are withdrawn from treatment because in hindsight they did not fulfil the eligibility criteria (see 8.1.) at time of enrolment, data will be collected until 30 days after the last protocol treatment given. SAE information will be collected as described in 12.3
No further information will be collected for patients who have withdrawn their consent. If a patient withdraws consent please consult HOVON Data Center.
Patients who are withdrawn from protocol treatment will receive medical care according to local practice.

11.3 Premature termination of the study

The sponsor may decide to terminate the study prematurely based on the following criteria:

- One of the stopping rules has been reached (see 14.4);
- There is evidence of an unacceptable risk for study patients (i.e. safety issue);

- There is reason to conclude that it will not be possible to collect the data necessary to reach the study objectives and it is therefore not ethical to continue enrolment of more patients; for example insufficient enrolment that cannot be improved.
- The DSMB recommends to end the trial based on viable arguments other than described above

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of the decision to terminate the study. The sponsor will provide information regarding the time lines of study termination and instructions regarding treatment and data collection of enrolled patients.

12 Safety

12.1 Definitions

Adverse event (AE)

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

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, including suspected transmission of infectious agents by a medicinal product.)

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.

Suspected unexpected serious adverse reaction (SUSAR)

All **suspected** Adverse Reactions which occur in the trial and that are both **unexpected** and **serious**. Suspected 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. Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorised medicinal product).

12.2 Adverse event

12.2.1 Reporting of adverse events

Adverse events will be reported from the first study-related procedure until 30 days following the last dose of any drug from the protocol treatment schedule 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 at least possibly related to the investigational medicinal product by the investigator.

Adverse Events have to be reported on the Adverse Events CRF. Adverse Events will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 4.0 (see appendix D).

Pre-existing conditions will be collected on the baseline concomitant diseases CRF, i.e. active (symptomatic) diseases of CTCAE grade ≥ 2, diseases under treatment, chronic diseases and long term effects of past events as present at the time of baseline assessment.

Neuropathic pain and peripheral neuropathy must always and especially be reported as AE (including grade 1) starting from baseline until end of AE reporting period as stated above.

All Adverse Events have to be reported, with the exception of:

1. A pre-existing condition that does not increase in severity; the pre-existing condition should be reported on the baseline concomitant diseases CRF
2. AE's of CTCAE grade 1 (except neuropathic pain and peripheral neuropathy see above)
3. Abnormal laboratory values that have been recorded as being not clinically significant by the investigator in the source documents
4. Progression of the disease under study; complaints and complications as a result of disease progression remain reportable Adverse Events
5. Nausea/vomiting of CTCAE grade 3 or less occurring during or within 14 days after high dose melphalan treatment

6. Hematological toxicity of CTCAE grade 3 or less occurring during or within 14 days after high dose melphalan treatment

12.2.2 Follow up of adverse events

All adverse events will be followed clinically until they have been resolved, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
On the AE CRF only the incidence of adverse events is recorded. Any ongoing adverse event that increases in severity is to be reported as a new adverse event on the CRF. Other follow up information is not collected on the CRF.

12.3 Serious Adverse Events

12.3.1 Reporting of serious adverse events

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

SAEs must be reported to the HOVON Data Center by fax **within 24 hours** after the event was known to the investigator, using the SAE report form provided. This initial report should contain a minimum amount of information regarding the event, associated treatment and patient identification, as described in the detail in the instructions for the SAE report form. Complete detailed information should be provided in a follow-up report within a further 2 business days, if necessary.
The following events are not considered to be a Serious Adverse Event:

1. Hospitalization for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
2. Hospitalization for diagnostic investigations (e.g., scans, endoscopy, sampling for laboratory tests, bone marrow sampling) that are not related to an adverse event. Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
3. Prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
4. Hospitalization for a procedure that was planned prior to study participation (i.e. prior to registration or randomization). This should be recorded in the source documents. Prolonged

hospitalization for a complication of such procedures remains a reportable serious adverse event.

12.3.2 Causality assessment of Serious Adverse Events

The investigator will decide whether the serious adverse event is related to trial medication, i.e. any of the products from the protocol treatment schedule. The decision will be recorded on the serious adverse event report. 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 judgment of the causal relationship.

12.3.3 Follow up of Serious Adverse Events

All serious adverse events will be followed clinically until they are resolved or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
Follow up information on SAE's should be reported monthly until recovery or until a stable situation has been reached. The final outcome of the SAE should be reported on a final SAE report.

12.3.4 Processing of serious adverse event reports

The HOVON Data Center will forward all SAE reports within 24 hours of receipt to the Principal Investigator, and the manufacturer of the investigational medicinal product(s).
The HDC safety desk will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR). The IB will be used as a reference document for expectedness assessment.
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.
Once every 3 months a safety report listing serious adverse events, serious adverse reactions and SUSARS, and a tabulation of all adverse events CTCAE grade ≥ 2 will be sent to the DSMB.

12.4 Reporting Suspected Unexpected Serious Adverse Reactions

The HDC Safety Desk, on behalf of the sponsor, will ensure the reporting of any SUSARs to the Ethics Committees (EC), the Competent Authorities (CA), the manufacturer of the investigational product and the investigators in compliance with applicable laws and regulations, and in accordance with any trial specific agreements between the sponsor and a co-sponsor or the manufacturer.

Expedited reporting of SUSARs will occur no later than 15 days after the HOVON Data Center had first knowledge of the serious adverse event. For fatal or life-threatening cases this will be no later than 7 days for a preliminary report, with another 8 days for a complete report.
The manner of SUSAR reporting will be in compliance with the procedures of the Ethics Committees and Health Authorities involved.

12.5 Pregnancies

Pregnancies of a female subject or the female partner of a male subject, occurring while the subject is on protocol treatment or within 30 days following the last dose of any drug from the protocol treatment schedule, should be reported to the sponsor. Pregnancies must be reported to the HOVON Data Center by fax within 24 hours after the event was known to the investigator, using the pregnancy report form provided. The HOVON Data Center will forward all pregnancy reports to the manufacturer of the investigational product.

The investigator will follow the female subject until completion of the pregnancy, and must notify the sponsor of the outcome of the pregnancy within 5 days or as specified below. 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 or therapeutic abortion,

stillbirth, neonatal death, or congenital anomaly – including that in an aborted fetus), the investigator should follow the procedures for reporting SAEs. In the case of a live "normal" birth, the sponsor should be informed as soon as the information is available. All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the investigator suspects is related to the *in utero* exposure to the investigational medicinal product(s) should also be reported.
The investigator is encouraged to provide outcome information of the pregnancy of the female partner of a male subject, if this information is available to the investigator and the female partner gives her permission.

12.6 Reporting of safety issues

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s), the regulatory authorities and the manufacturer of the investigational product of findings that could affect adversely the safety of patients, impact the conduct of the trial, increase the risk of participation or otherwise alter the EC's approval to continue the trial.
In the occurrence of such an event the sponsor and the investigators will take appropriate urgent safety measures to protect the patients against any immediate hazard. The accredited Ethics Committee will suspend the study pending further review, except insofar as suspension would jeopardize the patient's health. The local investigator will inform the patients.

12.7 Annual safety report

The sponsor will submit, once a year throughout the clinical trial, a safety report to the Ethics Committees and Competent Authorities of the concerned Member States. The content of the annual safety report will be according to the EU guidance document '*Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use'.*

12.8 Data Safety and Monitoring Board

The Data and Safety Monitoring Board will advise the chair of the HOVON working group, the Principal Investigator and the Co-investigator(s) about the continuation of the study. The DSMB will evaluate the general progress and the feasibility of the study, the quality and completeness of the data, side effects and safety.

The DSMB consists of at least 3 members, among whom (at least) one statistician and minimally two physicians. The members of the DSMB are invited on personal title on the basis of their expert

knowledge of the disease involved or the research methodology. Members of the DSMB will have ample experience with clinical trials.
The members of the DSMB will not be involved in the study, work at the HOVON Data Center, be a member of the HOVON board, or work in a hospital department participating in the study. The members will not have a conflict of interest due to ties with a company involved in the study.

The DSMB members are:

a. Ashutosh Wechalekar, MD, MRCP, FRCPath
   Senior Lecturer/Hon. Consultant in Amyloidosis and Haematology, UCL Medical School (Royal Free Campus)
b. Cees G. M. Kallenberg, prof. dr., Professor of Clinical Immunology
   Head Department of Reumatology and Clinical Immunology, University of Groningen
c. Thomas Hielscher, statistician, division biostatistics of the German Cancer Research Center, Heidelberg,Germany

The DSMB reports their written recommendations to the trial statistician. The report may consist of a confidential and a public part, where the confidential part contains references to unblinded data. The trial statistician forwards the public part of the DSMB recommendation to the Principal Investigator, the co-investigator(s) and the chair of the HOVON working group involved. The DSMB recommendations are not binding.

The DSMB will receive at least the following reports from the trial statistician for review:

a. Interim analysis report (as described in 14.3)
b. Once every 3 months a safety report listing serious adverse events, serious adverse reactions and SUSARs, and a tabulation of all adverse events CTC AE grade ≥ 2.

The DSMB will meet on receipt of these reports, evaluate the data and report to the trial statistician.

13 Endpoints

13.1 Primary endpoint

- Hematological CR rate 6 months after auto-SCT. Patients are considered a success if they received HDM and auto-SCT and are in CHR at 6 months after auto-SCT; all other patients are considered a failure.

13.2 Secondary endpoints

- Overall survival measured from the time of registration. Patients still alive or lost to follow up are censored at the day they were last known to be alive
- Progression Free Survival, (hematological), i.e. time from registration until hematological progression, relapse or death, whichever occurs first.
- Hematological response rate after induction therapy
- Response rate, hematological and organ
- Time to response, hematological and organ
- Duration of response, hematological and organ
- Time to next AL amyloidosis therapy
- Safety (type, frequency, and severity of adverse events (AE) and relationship of AE to study drug
- Exploratory assessment of multiparameter flow cytometry quantification of bone marrow plasma cells and change in amyloid deposition in abdominal fat aspiration samples
- Evaluation of prognostic factors for survival included in the hematological and organ response criteria

14 Statistical considerations

14.1 Patient numbers and power considerations

The primary objective of the trial is to evaluate the efficacy of bortezomib induction treatment followed by HDM and autologous SCT measured as proportion of patients with a complete hematological response (CHR) at 6 months after HDM and auto-SCT (CHR_{6mo}). For the sample size calculation the following assumptions are made.

- Let P_0 be the largest CHR_{6mo} which, if true, implies that the therapeutic activity is too low. In the present trial P0 has been taken as 30%.
- Let P_1 be the smallest CHR_{6mo} which, if true, implies that the therapeutic activity is sufficiently high to warrant further investigation in clinical trials. In the present trial P_1 has been taken as 50%.

In order to reject the null hypothesis H_0: $P=P_0$ in favor of the alternative hypothesis H_1: $P=P_1$ with 80% power (two-sided significance level $\alpha = 0.05$), 44 eligible patients are required. In order to overcome possible dropouts due to ineligiblity, 50 patients will be registered. With 16 patients already in the bortezomib arm of the primary phase III study, and with an expected accrual of at least 17 patients per year, the remaining required number of 34 patients would be achieved in about two years.

14.2 Statistical analysis

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

14.2.1 Efficacy analysis

The CHR_{6mo} rate will be determined with a 95% confidence interval (CI). 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 0.3.
Patients with a CHR at six months (± 2 weeks) after auto-SCT will be considered a success. In case a CHR is available before 5.5 months after auto-SCT and the next CHR only after 6.5 months post-SCT, then it will be assumed that the response at 6 months after auto-SCT was also CHR, and such a patient will also be considered as a success. All other patients will be considered a failure.

Other secondary efficacy endpoints include the best hematological response during protocol, and survival endpoints. The actuarial curves for PFS and OS from randomization will be computed using the Kaplan-Meier method and 95% Cis will be constructed.

Organ response will be tabulated for the different time points as described in the protocol.

14.2.2 Toxicity analysis

The analysis of toxicity will be done primarily by tabulation of the incidence of adverse events with CTCAE grade 2 or more, after each induction cycle separately, for the collective induction cycles per patient, and after HDM + auto-SCT.

14.2.3 Additional analyses

Additional analyses may be added. However, these analyses should be regarded as exploratory, and therefore only as hypothesis-generating.

14.2.4 Statistical analysis plan (optional)

A detailed statistical analysis plan (SAP) will be made for the final analysis. It will be discussed with the study coordinators and can only affect the exploratory analyses, but not the primary (confirmatory) analysis on which the sample size is based.

14.3 Interim analysis

One interim analysis is planned, primarily to guard against unfavourable results of the bortezomib induction treatment. Results of the interim analysis will be presented confidentially to a DSMB. Only if the DSMB recommends that the study should be stopped or modified the results will be made public to the principal investigators for further decisions. The interim analysis is planned when the CHR data at 6 months post-transplant of the first 50% of the patients are available, which is the primary endpoint for this analysis.

At this interim analysis a detailed report will be generated and presented to the DSMB. The report includes the number of entered patients and at that time evaluable patients, treatment given, CHR rate, the number of events on the actuarial (secondary) endpoints, actuarial estimates for those endpoints and incidence of SAE's and other adverse events by grade. Adverse events will be described by summary tables broken by site, grade and relation to trial treatment.
The DSMB is free in her public recommendations to the study coordinators and the confidential recommendations to the study statistician. Unfavourably lower CHR rate than P_0 (30%) with a P-value < 0.1 is a good reason to recommend the stopping of the trial or recommendations for modifications. The study will be closely and sequentially monitored before the interim analysis. Monitoring will be based on the reported SAE's, which are not subjected to data delay. In addition, a separate report on the incidence of SAE's and other adverse events and infections, as described before, will be sent to the DSMB once every 3 months. Again, the DSMB is free in her public recommendations to the study coordinators and the confidential recommendations to the study statistician.

14.4 Stopping rules

The sponsor may decide to terminate the study prematurely in case

1. The number of patients that does proceed to HDM and stem cell transplantation is below 35% *or*
2. The number of deaths during induction treatment is above 30% *or*
3. The number of deaths during stem cell mobilization and HDM with auto-SCT is above 20%

15 Registration and Randomization

15.1 Regulatory Documentation

Required regulatory and administrative documents must be provided to the HOVON Data Center before enrolment of the first patient. This will always include an Ethics Committee approval for the investigational site. The HOVON Data Center will provide each investigator with an overview of the required documents. Each investigational site will be notified when all requirements are met and enrolment can start.

15.2 Registration and Randomization

Eligible patients should be registered before start of treatment. Patients need to be registered at the HOVON Data Center by one of the following options:

- Trial Online Process (TOP, https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.
- By faxing the completed registration/randomization CRF +31.10.7041028 Monday through Friday, from 09:00 to 17:00 CET
- By phone +31.10.7041560 Monday through Friday, from 09:00 to 17:00 CET

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 and “Age in years”
- Date written informed consent
- Specific items patient gives consent for (see ICF)
- Eligibility criteria

All eligibility criteria will be checked with a checklist.

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Each patient will be given a unique patient study number (a sequence number by order of enrolment in the trial). Patient study number will be given immediately by TOP or phone and confirmed by fax or email.

Local Patient Code is a code assigned to the patient by the investigational site for local administrative purposes. The code may be up to 8 characters long (letters and numbers allowed). The code should

be in compliance with privacy regulations. It should not contain identifying data, such as patient initials or the complete hospital record number. The local code will be visible in the confirmation messages sent by TOP to local participants after registration of the patient. The key to this local patient code should only be accessible by the local investigator and the local trial staff. Using or entering a local patient code is not obligatory.

16 Data collection and quality assurance

16.1 Case Report Forms

Data will be collected on paper-based 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;
- Baseline concomitant diseases and 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 combination of patient study number (assigned at registration), hospital name 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.
Country specific addition for GERMANY:
On the study specific case report forms the complete birth date will be asked for. In Germany only the entry of the year of birth is allowed according to data protection. Therefore spaces for day and month on the CRF-pages will be crossed out and only the year will be entered.

16.2 Data quality assurance

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study centers, review of protocol procedures with the investigator before the study, and site visits by the sponsor.
Data collected on the CRF will be verified for accuracy. If necessary, queries will be sent to the investigational site to clarify the data on the CRF. The investigator should answer data queries within the specified time line.

16.3 Monitoring

The sponsor will perform on-site monitoring visits to verify that the rights and well-being of patients are protected, the reported trial data are accurate, complete, and verifiable from source documents and the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement(s). Monitoring visits will take place according to the study specific monitoring plan.
Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. The sponsor expects that during monitoring visits the relevant investigational staff will be available, the source documentation will be available and a suitable environment will be provided for review of study-related documents.

16.4 Audits and inspections

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

17 Ethics

17.1 Accredited ethics committee or Institutional Review Board

An accredited Ethics Committee or Institutional Review Board will approve the study protocol and any substantial amendment.

17.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki in the current version, the ICH-GCP Guidelines, the EU Clinical Trial Directive (2001/20/EG), and applicable regulatory requirements. The local investigator is responsible for the proper conduct of the study at the study site.

17.3 Patient information and consent

Written informed consent of patients is required before enrolment in the trial and before any study related procedure takes place.
The investigator will follow ICH-GCP and other applicable regulations in informing the patient and obtaining consent. The informed consent procedure will be performed by a physician* without a treatment relationship with the patient. Before informed consent may be obtained, the patient should have ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the patient.
There is no set time limit for the patient to make a decision. The physician should inform each patient if there is a specific reason why he/she must decide within a limited time frame, for example if patients condition necessitates start of treatment or if the trial is scheduled to close for enrolment.
(*country specific adaption for GERMANY: The informed consent procedure will be performed by an investigator (approved by the EC) without a treatment relationship with the patient.)

The content of the patient information letter, informed consent form and any other written information to be provided to patients will be in compliance with ICH-GCP and other applicable regulations and should be approved by the Ethics Committee in advance of use.
The patient information letter, informed consent form and any other written information to be provided to patients will be revised whenever important new information becomes available that may be relevant to the patient's consent. Any revised informed consent form and written information should be approved by the Ethics Committee in advance of use. The patient should be informed in a timely manner if new information becomes available that might be relevant to the patient's willingness to continue participation in the trial. The communication of this information should be documented.

17.4 Benefits and risks assessment.

Most analyses in the trial are considered normal patient care and therefore the burden of participation is minimal. Patients participating in the trial will undergo 3 additional 24 hour collection of urine

analysis, additional 2 (in total 7) echocardiographies and 1 additional bone marrow analysis 6 months after auto-SCT. The risks associated with the investigational product mostly consist of: non-hematological toxicity like development of peripheral sensory neuropathy with or without neuropathic pain, orthostatic hypotension, peripheral edema, hyopnatriemia, worsening of congestive heart failure, fatique, constipation or diarrhea and nausea.[11;14] Hematological toxicity is mainly reversible thrombocytopenia.
The benefits for participating in the trial is close monitoring of the patients condition which is very important in these fragile patients and access to a drug not registered for this condition but with very promising results in clinical studies.

17.5 Trial insurance

Prior to the start of the trial, the sponsor will ensure that adequate insurance for patients is in place covering losses due to death or injury resulting from the trial, in accordance with applicable laws and regulations in each country where the trial is conducted. The sponsor will take out an insurance policy or delegate this responsibility to a national co-sponsor. Proof of insurance will be submitted to the Ethics Committee.
In addition, the sponsor will ensure that adequate insurance is in place for both investigator(s) and sponsor to cover liability pertaining to death or injury resulting from the trial.

18 Administrative aspects and publication

18.1 Handling and storage of data and documents

18.1.1 Patient confidentiality

Each patient is assigned a unique patient study number at enrolment. In trial documents the patient's identity is coded by patient study number as assigned at enrolment. In some cases date of birth is also listed.
The local investigator will keep a subject enrolment and identification log that contains the key to the code, i.e. a record of the personal identification data linked to each patient study number. This record is filed at the investigational site and should only be accessed by the investigator and the supporting site staff, and by representatives of the sponsor or a regulatory agency for the purpose of monitoring visits or audits and inspections.

18.1.2 Filing of essential documents

Essential Documents are those documents that permit evaluation of the conduct of a trial and the quality of the data produced. The essential documents may be subject to, and should be available for, audit by the sponsor's auditor and inspection by the regulatory authority(ies)
The investigator should file all essential documents relevant to the conduct of the trial on site. The sponsor will file all essential documents relevant to the overall conduct of the trial. Essential documents should be filed in such a manner that they are protected from accidental loss and can be easily retrieved for review.

18.1.3 Record retention

Essential documents should be retained for 15 years after the end of the trial. They should be destroyed after this time.
Source documents (i.e. medical records) of patients should be retained for at least 15 years after the end of the trial. Record retention and destruction after this time is subject to the site's guidelines regarding medical records.

18.1.4 Storage of samples

Biological samples should only be stored for the purpose of additional research if the patient has given consent. If no informed consent was obtained, samples should be destroyed after the patient has completed all protocol treatment and procedures.
Storage of biological samples on site is subject to the site's guidelines; samples may be labeled with the patients identifying information (e.g. name, hospital record number)
Samples that are shipped to another facility (e.g. a central laboratory) for a purpose as described in this protocol or for additional scientific research, should be stripped from any identifying information and labeled with a code (trial name or number and patient study number as assigned at enrolment).

18.2 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the Ethics Committee application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the patients of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be submitted to the Ethics Committee and to the Competent Authority.

Non-substantial amendments will not be submitted, but will be recorded and filed by the sponsor.

18.3 Annual progress report

The sponsor will submit a summary of the progress of the trial to the accredited Ethics Committee once a year. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

18.4 End of study report

The sponsor will notify the accredited Ethics Committee and the Competent Authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the sponsor will notify the accredited Ethics Committee and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited Ethics Committee and the Competent Authority.

18.5 Publication policy

Final publication of trial results

Trial results will always be submitted for publication in a peer reviewed scientific journal regardless of the outcome of the trial – unless the trial was terminated prematurely and did not yield sufficient data for a publication. The trial was designed by the Principal Investigator and the German Coordinating investigator on equal base and this will be reflected in all publications of the trial.

The final publication of the trial results will be written by the Principal Investigator and the German Coordinating investigator on equal base, with the other coordinating investigator , Co-investigators and the trial statistician on the basis of the statistical analysis performed by the trial statistician. A draft manuscript will be submitted for review to:

- All co-authors

- The chair of the relevant HOVON working group, who is entitled to share and discuss the manuscript with working group members
- An industry partner if so agreed in the contract between HOVON and company

After revision the final manuscript is submitted to the HOVON secretary for review of compliance with this policy. After approval by the HOVON board the manuscript will be sent to a peer reviewed scientific journal.

Authorship

Authors of the main manuscript will include the Principal Investigator, the German Coordinating investigator, the Co-investigators, investigators who have included more than 5% of the evaluable patients in the trial (by order of inclusion rate), the trial statistician and the trial manager. If a substantial part of the publication is based on centrally reviewed data (e.g. MFC, pathology, echocardiography), the central reviewer will be included as author. Others who have made a significant contribution to the trial may also be included as author, or otherwise will be included in the acknowledgement.

Authors of correlative manuscripts (e.g. results of side studies) will include the Principal Investigator, the German Coordinating investigator, the Co-investigators, and those persons who have made a significant contribution to the published results.

The Principal Investigator should discuss and decide on the matter of authorship of the main manuscript prior to the start of the trial – with the exception of authors included on account of inclusion rate. The Principal Investigator is urged to use the maximum number of authors allowed by the journal to the full extent.

Interim and partial publications

Interim publications, abstracts or presentations of the study may include demographic data, overall results and prognostic factor analyses, results for secondary endpoints, but no results for the primary endpoint may be made publicly available before the recruitment is discontinued.

Investigators participating in the trial have a right to publish results from data they collected for the study. The Principal Investigator, the Co-investigator(s) and the trial statistician must approve any such publication, abstract or presentation based on patients included in this study. This is applicable to any individual patient or any subgroup of the trial patients. Such a publication cannot include an analysis of any of the study endpoints unless the final results of the trial have already been published.

Abstracts and presentations

Abstracts and presentations at public meetings will represent the trial as a project under HOVON affiliation and the Amyloidosis Centre of Heidelberg University. The abstract or presentation should not be represented under affiliation of the working group or a specific hospital.

Slides will be designed using both the HOVON style template together with the Amyloidosis Centre of Heidelberg University logo and any other presentation materials will show the HOVON and Amyloidosis Centre of Heidelberg University logo.

If the trial is conducted in partnership with a co-sponsor (e.g. intergroup trial), the abstract and presentation should represent the co-sponsor contribution and slides may show the co-sponsor logo in addition to the HOVON logo.

Prior to its public use, the abstract or presentation is submitted to the HOVON secretary for review of compliance with this policy.

19 Glossary of abbreviations

(in alphabetical order)

AE	Adverse Event
AL	Amyloid Light-chain
ALAT/ ALT	Alanine aminotransferase
ANC	Absolute Neutrophil Count
ASAT/ AST	Aspartate aminotransferase
B-CLL	B-cell chronic lymphatic leukemia
BJ	Bence Jones
BM	Bone Marrow
Ca	Calcium
CA	Competent Authority
CHR	Complete Hematological Remission
CKS	Commissie voor Klinische Studies
CR	Complete Remission
CRF	Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
dFLC	Difference between involved and uninvolved Free Light Chain
DSMB	Data Safety and Monitoring Board
EC	Ethical Committee
ECG	Electrocardiogram
EBMT	European Group for Blood and Marrow Transplantation
FLC	Free Light Chain
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GFR	Glomerular Filtration Rate
GI	Gastro-intestinal
Hb	Hemoglobin
HBcAg	Hepatitis B core Antigen
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HDM	High dose melphalan
HIV	Human Immunodeficiency Virus
HOVON	Dutch-Belgian Hematology-Oncology Cooperative Group

ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
iFLC	Involved Free Light Chain
IFM	Intergroup Français de Myelome
IMP	Investigational Medicinal Product
ISS	International Staging System
ITT	Intention To Treat
IU	International Units
KCl	Potassium chloride
LDH	Lactate Dehydrogenase
MDRD	Modification of Diet in Renal Disease
METC	Medical Ethical Review Committee
MM	Multiple Myeloma
NaCl	Sodium Chloride
NCI	National Cancer Institute
NHL	Non Hodkin Lymphoma
NT-ProBNP	N-terminal fragment of pro-brain natriuretic peptide
NYHA	New York Heart Association
OS	Overall Survival
PB	Peripheral Blood
PD	Progressive Disease
PFS	Progression Free Survival
PNP	Polyneuropathy
PO	Per Os
PR	Partial Response
SAE	Serious Adverse Event
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvate transaminase
PT	Prothrombin Time
SC	Subcutaneously
SCT	Stem cell Transplantation
SD	Stable Disease
SPEP	Serum protein electro-phoresis
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNI	Troponin I
TNT	Troponin T

TRM	Treatment related mortality
TT	Thrombin Time
ULN	Upper Limit
UPEP	Urine protein electro-phoresis
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen

20 References

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A. Criteria for diagnosis of systemic AL amyloidosis

In most cases systemic AL amyloidosis can be diagnosed without any problem. However, because of prognostic and therapeutic consequences, the diagnosis should be indisputable and firmly based on 3 elements:

1. A histological proof of amyloid in a biopsy specimen
2. The systemic nature of the amyloidosis should be established
3. The amyloidosis should be characterised as the AL type

Comments:

1. A biopsy specimen with amyloid should be positively stained with Congo red dye, showing apple-green birefringence in polarised light. If there is any doubt, another specimen should be stained or an experienced pathologist should review the original biopsy specimen.

2. The systemic nature of the amyloidosis can be established in different ways:
   a. Some sites of the body (such as eyelid, cornea, joints, thyroid, parathyroid glands, pituitary gland, skin, aorta, lung, pleura, trachea-bronchial tree, larynx, pharynx, ureter, bladder, urethra, pancreas, prostate, seminal vesicles and cerebrum) are sites where localised amyloid can be found. In these cases amyloid must be detected somewhere else in the body to exclude localised amyloidosis.
   b. Some sites are exclusively involved in systemic amyloidosis, such as subcutaneous abdominal fat, kidneys, liver, nerves, and spleen. If such a site is positive for amyloid (by histology) systemic involvement may be assumed.
   c. All other sites (such as bone marrow, heart, bowel) are often involved in systemic amyloidosis. In this case it is recommended to show amyloid to be present in two different organs or tissues. It is however sufficient to have histological proof at one site (such as bone marrow or rectum) and typical clinical involvement (such as nephrotic syndrome, hepatomegaly, macroglossia, or restrictive cardiomyopathy) at the other site.

3. Amyloidosis should be characterised as AL type by the following guidelines.
   a. An underlying monoclonal plasma cell dyscrasia is a prerequisite for the diagnosis and has to be present (sometimes hard to detect) in serum, urine, or bone marrow.
   b. When a chronic inflammatory disease is present or in case amyloid is clinically restricted to the kidneys or gastrointestinal tract, it is recommended to exclude AA amyloidosis by immunohistology or immunohistochemistry.

c. In case amyloid is clinically restricted to kidneys only and enlarged glomeruli filled with amyloid are detected in the kidney biopsy, it is recommended to exclude AFib amyloidosis by fibrinogen gene DNA analysis. In case amyloid is clinically restricted to heart, nerves, and/or eyes, it is recommended to exclude ATTR amyloidosis by immunohistology and by screening of the serum for variants of transthyretin, but preferably by transthyretin gene DNA analysis.

When a family history for amyloidosis is present gene analysis is obligatory.

In The Netherlands genetic analysis is performed via the clinical genetic centers and in Germany via the Institute for Human Genetics at the University Hospital Heidelberg

After diagnosis it is important to screen which organs are involved[18].

ORGAN	MEASUREMENT
Kidney	24-hr (non-Bence Jones) urinary protein > 0.5 g/day, predominantly albumin
Heart	Echo: mean left ventrical wall thickness > 12 mm, no other cause (mean from septum and posterior wall) ***or*** NT-pro-BNP > 332 ng/L (caution, renal failure can increase the NT-pro-BNP level)
Liver	Total liver span > 15 cm in the absent of heart failure ***or*** Alkaline phosphatase > 1.5 times ULN
Nerve	Peripheral: symmetric lower extremity sensorimotor neuropathy Autonomic: gastric emptying disorder, pseudo obstruction, voiding dysfunction not related to organ infiltration
GI tract	Direct biopsy verification with symptoms. Vascular amyloid deposits only are **not** evidence of GI involvement
Lung	Direct biopsy verification with symptoms Interstitial radiographic pattern
Soft tissue	Macroglossia Claudication (temporal artery) Skin abnormalities (purpura) Myopathy by biopsy Lymphadenopathy Carpal tunnel syndrome Skeletal muscle pseudohypertrophy

B. Response criteria of hematological and organ response

Hematologic (clonal) response criteria, based on Gertz et al with some modifications

Complete response	Serum and urine negative for a monoclonal protein by immunofixation ***and*** Free light chain ratio normal and normal absolute value involved serum free light chain (iFLC normalisation only needed in patients with normal renal function, , i.e. GFR ≥ 60 ml/min) ***and*** Bone marrow < 5% plasma cells
Very good partial response	dFLC < 40 mg/l (difference between involved and uninvolved FLC)
Partial response	If serum M protein > 5 g/L, a ≥ 50% reduction ***and*** If urinary M-protein > 100 mg/day, a 50% reduction ***and*** If involved serum free light chain > 50 mg/L, a ≥ 50% dFLC reduction
Stable disease	No CR, no PR, no progression
Progression[a]	*From CR*, detectable original monoclonal protein ***or*** increase of involved serum free light chain (absolute value of light chain must at least double) *From start of therapy, PR or stable response*, ≥ 50% increase in serum M protein to > 5 g/L,***or*** ≥ 50% increase in urinary M protein to > 200 mg/day ***or*** Involved serum free light chain increase of ≥ 50% to > 50 mg/L All values calculated from baseline if from start of start of therapy, and other from lowest value reached

[a] All progression categories require two consecutive assessments made at anytime before classification as progression and/or the institution of any new therapy. 1st assessment date remains date of progression.

Organ Response

ORGAN	
Kidney	≥ 50% decrease of 24-hr urinary protein (urinary protein must be > 0.5 g/day pre-treatment) in the absence of ≥ 25% increase over baseline serum creatinine **and** 25% decrease in creatinin clearance
Heart	Mean left ventricular wall thickness decreased by ≥ 2 mm compared with baseline ***or*** ≥ 20% improvement in ejection fraction ***or*** improvement by at least 2 NYHA classes without an increase in diuretic use and no increase in wall thickness ***or*** Reduction of NT-proBNP of ≥ 30% and >300 ng/L over the starting value if starting value > 650 ng/L. Evaluation of NT-pro-BNP response only in the absence of ≥ 25% increase over baseline serum creatinine **and** 25% decrease in creatinin clearance
Liver	≥ 50% decrease of abnormal alkaline phosphatase value

Organ disease progression

ORGAN	
Kidney	≥ 50% increase (at least 1 g/day) of urinary protein over the lowest value ***or*** ≥ 25% increase of serum creatinine or 25% decrease in creatinin clearance over the lowest value.
Heart	Mean left ventricular wall thickness increased by ≥ 2 mm compared with the lowest value ***or*** ≥ 20% decrease of ejection fraction ***or*** Increase of NT-proBNP of ≥ 30% and >300 ng/L over the starting value (only applicable in the absence of ≥ 25% increase over baseline serum creatinine **and** 25% decrease in creatinin clearance)
Liver	≥ 50% increase of alkaline phosphatase above the lowest value

C. 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

D. Common Terminology Criteria for adverse events

The grading of adverse events will be done using the NCI Common Terminology Criteria for Adverse Events, CTCAE version 4.0. A complete document may be downloaded from the HOVON website:

http://www.hovon.nl (under Trials > General information about studies)

E. NYHA scoring list

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

Grade 1 No breathlessness

Grade 2 Breathlessness on severe exertion

Grade 3 Breathlessness on mild exertion

Grade 4 Breathlessness at rest

F. Echocardiography

Left ventricular dimensions, systolic function, valvular function:

- Parasternal long axis: Enddiastolic septal and posterior wall thickness, endsystolic and enddiastolic LV dimension, left atrial dimension,
- Apical 4 chamber view: Left atrial volume using 2d trace method
- Peak systolic velocity Left ventricular outflow tract (pulsed wave LV V1 max) and aortic valve (continuous wave Ao V2 max)
- Apical 4 chamber view: Tissue Doppler: Peak systolic velocity intraventricular septum and lateral wall.
- Grading of concomitant left sided valvular disease as absent, mild, moderate or severe. (mitral regurgitation or stenosis, aortic regurgitation or stenosis)
- LV ejection fraction, if possible with Simpson bi-plane method

Right ventricular function:

- Recording of TAPSE in apical 4 chamer view (M-mode)
- Peak velocity tricuspid regurgitation (TR max)
- Recording of inferior v.cava for estimation of right atrial pressure.
- Pulmonay artery acceleration time (PAacc)
- Apical 4 chamber view: Tissue Doppler peak systolic speed tricuspid annulus
- Right ventricular wall thickness, recorded in subcostal or parasternal long axis view

LV diastolic function:

- Mitral inflow: Peak A en E top, A/E ratio, deceleration time E top
- Long vene flow: Peak S and D
- Apical 4 chamber Tissue Doppler imaging: peak E' and A' of intra ventricular septum and lateral wall

Results to be given in conclusion;

- ejection fraction in %
- intraventricular septum thickness
- left ventricular wall thickness measured at posterior wall
- mean left ventricular wall thickness
- diastolic dysfunction no or yes. If yes grade I to III,as per European/American recommendations, see next page
- infiltrative cardiomyopathy (defined as presence of typical speckled myocardium, yes or no).

Dutch and Belgian Centers only:

For central review, echocardiograms (DICOM readable) and reports have to be sent to:

UMC Utrecht

tav HOVON 104 AL amyloidosis echocardiography (dr. Broeyer of dr. De Jonge)

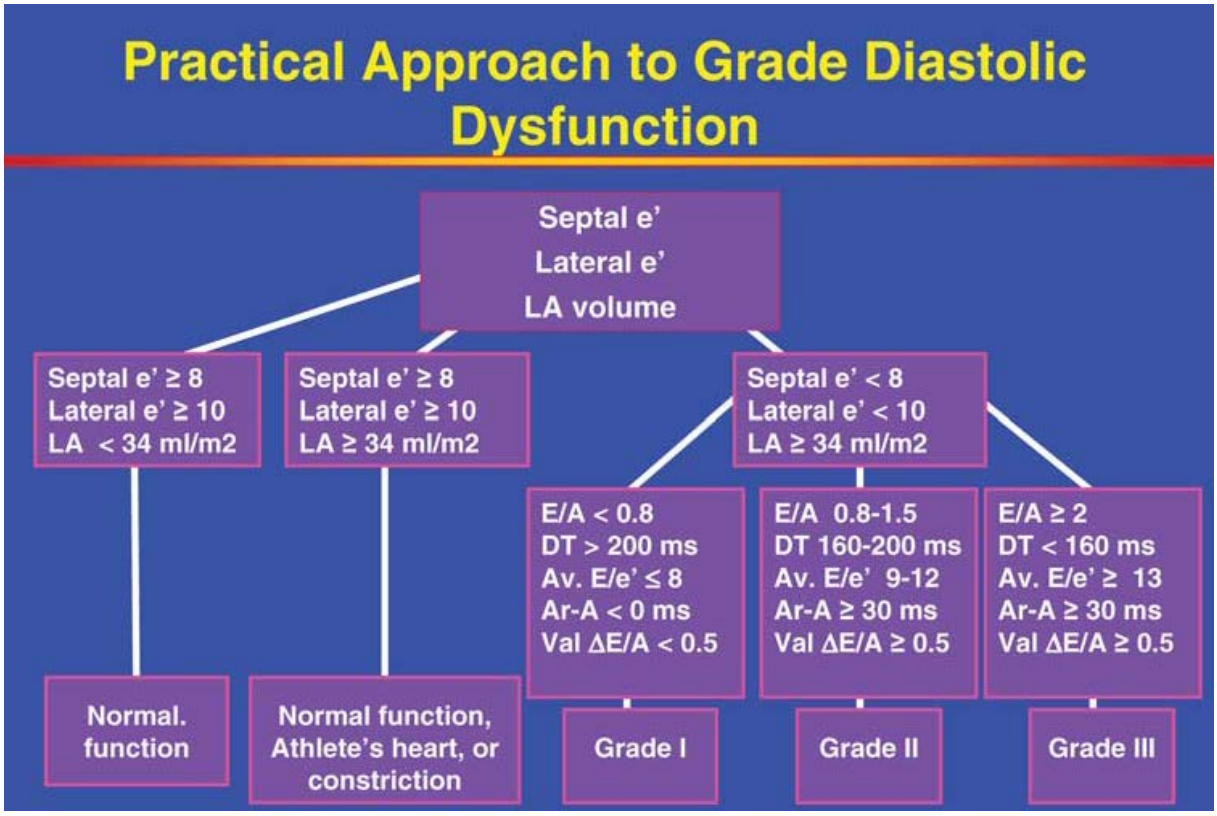
Huispostnummer E03.511

Afdeling hartfalen en harttransplantatie

Postbus 85500

3508 GA Utrecht

Grading of diastolic dysfunction:



Source:

Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography.

American Society Echocardiography/European Association echocardiography.

European Journal of Echocardiography (2009) 10, 165–193. Nagueh et al.

G. Indicatie CTC-gradering Perifere Neuropathie

This appendix is only to be used locally at the hospital.

Naam/Patiëntnr.:
Datum:

Indicatie CTC-gradering Perifere Neuropathie

Verandering in gevoel
(T.o.v. het begin van de ziekte en/of de behandeling)

		JA
• Bij aanraking		☐
• Doof gevoel		☐
- in tenen	☐	
- in voeten	☐	
- in onderbenen	☐	
- in vingers	☐	
- in handen	☐	
- in onderarmen	☐	
• Tintelend gevoel		☐
- in tenen	☐	
- in voeten	☐	
- in onderbenen	☐	
- in vingers	☐	
- in handen	☐	
- in onderarmen	☐	
• Verandering in warmte- en koudegewaarwording		☐
- in tenen	☐	
- in voeten	☐	
- in onderbenen	☐	
- in vingers	☐	
- in handen	☐	
- in onderarmen	☐	

Indien patiënt verandering in gevoel ondervindt => minimaal Graad 1

		JA
• Pijn (bijv. brandend, schietend, stekend of krampend)		☐
- in tenen	☐	
- in voeten	☐	
- in onderbenen	☐	
- in vingers	☐	
- in handen	☐	
- in onderarmen	☐	

• Is de pijn continu aanwezig?
• Is de pijn met tussenpozen aanwezig?

Indien patiënt ook pijn ondervindt => minimaal Graad 1 met pijn

Veranderingen in autonome functies
(Alleen scoren indien geen andere oorzaak wordt aangetoond)

	JA
• Frequent plassen	□
• Moeite met ophouden of uitplassen	□
• Vrijen:	
- impotentie	□
- verminderde opwinding en vochtigheid	□
• Obstipatie	□
• Duizeligheid bij opstaan	□
• Hartkloppingen	□
• Meer of minder zweetsecretie	□

Krachtverlies

Indien patiënt veranderingen ondervindt bij autonome functies en/of kracht => minimaal Graad 2

Functionele vragen om indruk te krijgen van ADL* afhankelijkheid (Heeft de patiënt moeite met onderstaande situaties t.o.v. voor de behandeling?)

	JA
• Zelfstandigheid	
- zelf aankleden	□
- zelf wassen, haren wassen en kammen	□
- zelf bestek vasthouden	□
- toetsenbord computer bedienen	□
- knoopjes open-/dichtdoen	□
- deuren open-/dichtdoen	□
- zelfstandig lopen/stappen	□
- autorijden	□
- veters strikken/knopen	□
- schrijven	□
• Maakt de patiënt gebruik van hulpmiddelen bij het zelfstandig lopen/stappen? (bijv. krukken, rollator, etc.)	□
• Pijn	
- matige pijn	□
- ernstige pijn	□
- pijnmedicatie nodig om ADL te kunnen uitvoeren	□

Indien patiënt t.g.v. neuropathische verschijnselen of pijn beperkingen ondervindt bij ADL
=> Graad 3

Indien patiënt voor ADL afhankelijk is van een ander
=> Graad 4

** ADL: algemene dagelijkse levensverrichtingen*

H. Management of patients with bortezomib (Velcade®)-related neuropathic pain and/or peripheral neuropathy

			Peripheral Neuropathy (NCI CTCAE Grade)			
		0	1	2	3	4
		Normal	Asymptomatic; loss of deep tendon reflexes or paresthesia (including tingling) but not interfering with function	Alterations or paresthesia (including tingling), interfering with function, but not interfering with ADL	Alterations or paresthesia interfering with ADL	Disabling
Neuropathic Pain (NCI CTCAE Grade) 0	None	No action	25% dose reduction	Reduce to once weekly and 25% dose reduction	Discontinue bortezomib	Discontinue bortezomib
1	Mild pain not interfering with function	25% dose reduction	Reduce to once weekly and 25% dose reduction	Reduce to once weekly and 50% dose reduction	Discontinue bortezomib	Discontinue bortezomib
2	Moderate pain: pain or analgesics interfering with function, but not daily activities	Reduce to once weekly and 25% dose reduction	Hold; Reduce to once weekly and 50% dose reduction	Hold; Reduce to once weekly and 50% dose reduction	Discontinue bortezomib	Discontinue bortezomib
3	Severe pain: pain or analgesics severely interfering with daily activities	Discontinue bortezomib	Discontinue bortezomib	Discontinue bortezomib	Discontinue bortezomib	Discontinue bortezomib
4	Disabling	Discontinue bortezomib	Discontinue bortezomib	Discontinue bortezomib	Discontinue bortezomib	Discontinue bortezomib

Key:

Reduce to once weekly: Schedule change from bortezomib twice per week (days 1, 4, 8 and 11) to once per week (days 1, 8, 15, and 22) required. The cycle will change to a 5 week- cycle. The dexamethasone is given on the bortezomib day and the day afterwards, i.e. days 1,2,8,9,15,16,22 and 23.

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.

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

I. Multiparameter flow cytometry protocol for MRD analysis

A first pull of minimal 3.5 ml bone aspirate is used for cytomorphology, maximal 4 smears, and for flow cytometry and put in a heparin coated tube. Bone marrow plasma cells analysis will be performed centrally (Dept of Medical Immunology, University Medical center Utrecht, the Netherlands) using 8-color multiparameter flowcytometry. Using this protocol (see below) clonal plasma cells can be detected with a sensitivity of 0.01-0.001%. Furthermore the immunophenotype of the clonal plasmacells will be assessed. MFC will be performed at diagnosis and follow up.

	PB	PO	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-eFluor780
1.	CD45	CD27	cytKappa	cytLambda	CD138	CD56	CD38	CD19
2.	CD20	CD45	CD28	cytkappa	CD138	CD33	CD38	CD117

See lab manual for details of procedure for sending material.

Reporting

1. The total percentage of plasma cells: given on the total of nucleated bone marrow cells.
2. Percentage of normal and malignant plasma cells: each given on the total of nucleated bone marrow cells. Percentages normal plus malignant plasma cells is percentage of total plasma cells.
3. Immunophenotype of malignant plasma cells is given as positive or negative.
4. Minimal residual disease (MRD) is negative if malignant plasma cells are NOT detected with a sensitivity of 0.01%. MRD is positive if malignant plasma cells are detected with a sensitivity of 0.01%. If this sensitivity is not reached or other (technical) problems are encountered the MRD is not assessable
5. Results will be reported back to the hospital using the same e-mail address which was used to notify the laboratory (see lab manual)

J. Subcutaneous abdominal fat aspiration

See www.amyloid.nl for training how to perform a subcutaneous abdominal fat aspiration

Fat aspiration procedure for the detection of amyloid

Aspiration of abdominal subcutaneous fat tissue

Aspiration of abdominal subcutaneous fat tissue is a simple outpatient procedure (1). It should be noticed, however, that it takes at least 10 - 15 minutes to avoid unnecessary pain and bruising and to get ample material. The patient should know that bruising might occur.

Equipment used for fat aspiration

See figure 1A for the equipment: Chloorhexidine solution for skin cleaning, a 5 ml syringe connected to a 22 Gauge needle for lidocaine anaesthesia (figure 1B), two 10 ml syringes connected by a valve system to 16 Gauge needles for fat aspiration (figure 1C), small band-aids, gauzes, and protective gloves.

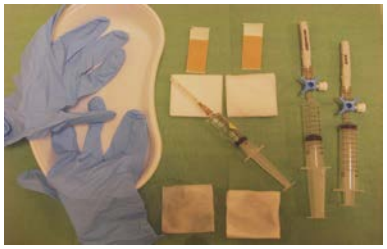


Fig 1A. Equipment for procedure

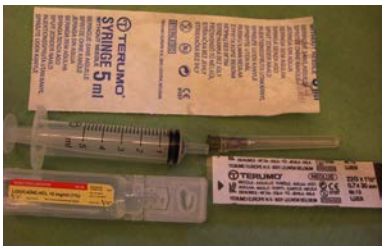


Fig 1B. Equipment for anaesthesia

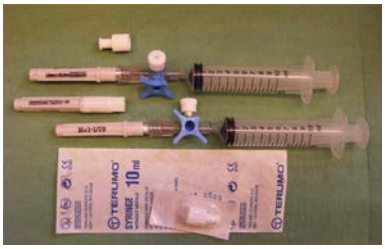


Fig 1C. Equipment for fat aspiration

Fat aspiration technique

A syringe of 10 ml is connected by a valve system to a needle of 16 Gauge (figure 2A). After closing the valve the plunger is pulled out, fixed with squeezed fingers, and the cap of the lidocaine needle can be reused elegantly by positioning it upside-down inside the plunger ("Tarek's trick") to fix firmly the position of the plunger and thus maintain negative pressure in the syringe during aspiration (see figure 2B and 2C).

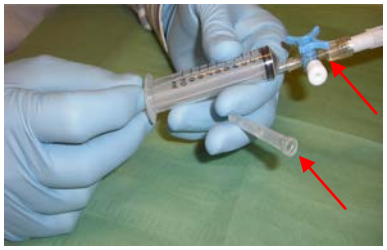


Fig 2A. Closed valve, reuse cap

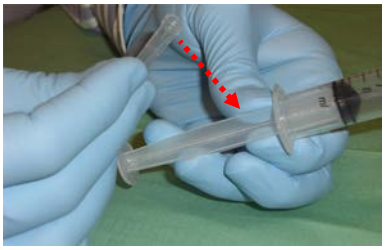


Fig 2B. Pull plunger, (Note cap) …

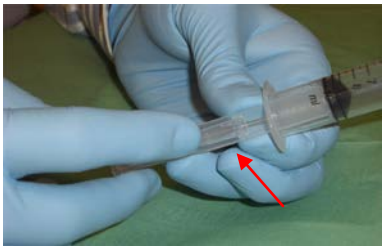


Fig 2C. … place cap, fix plunger.

The skin of the patient is marked (figure 3A) and cleaned (e.g. with chloorhexidine) at both sides of the umbilicus at about 7-10 cm distance. Skin and subcutaneous tissue (three directions, see below) are then anaesthetized with lidocaine (each side 2 ml=20 mg)(figure 3B). Check first that the patient is

not allergic to lidocaine. After inserting the needle beneath the skin the valve can be opened to start aspiration of fat tissue (figure 3C).

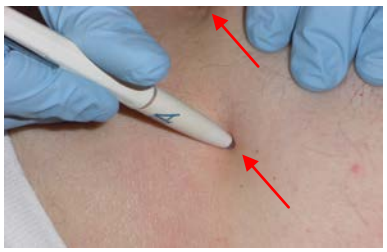


Fig 3A. Umbilicus, marking the skin

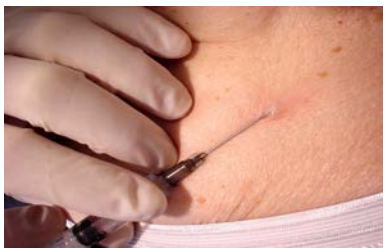


Fig 3B. Anaesthesia with lidocaine

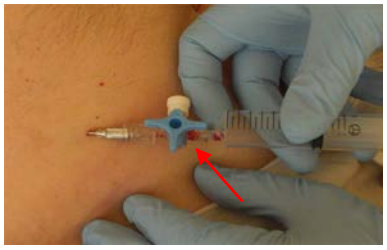


Fig 3C. Opened valve, aspiration

The needle can be moved into three directions (Northeast, East, and Southeast) at the left side of the abdomen and mirror-wise at the right side. The aspiration procedure should be performed slowly and gently into each of the three directions, going to and fro with some rotation, and one should realise that it takes some time before the needle will be filled with fat tissue and the first fat can be seen passing the valve and entering the top of the syringe. This can be continued until enough fat tissue has been collected (figure 4A). After finishing the procedure press the puncture site for a while and cover the puncture site with a band-aid (figure 4B). Aim of the procedure is to obtain an adequate quantity for microscopic analysis (30 mg) and further at least 30 mg of fat tissue for immunochemical quantification of SAP and the specific amyloid proteins. Aspiration can be done at both sides of the umbilicus in order to obtain at least 60 mg of fat tissue (figure 4C).

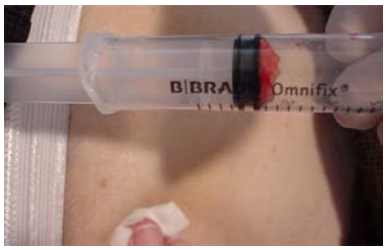


Fig 4A. Result in syringe

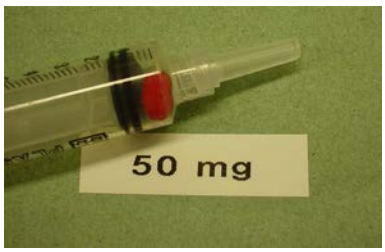


Fig 4B. Gross weight and size

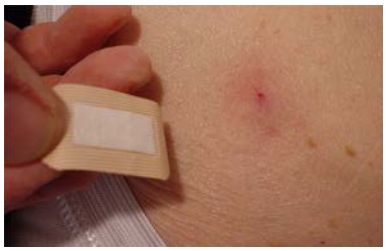


Fig 4C. Cover puncture site

When you are finished and have collected enough fat tissue, the easiest solution for you is:

Seal the syringes and ship them to Groningen for analysis at room temperature; see below ➤

Two technical problems can be encountered during aspiration: no tissue at all or much blood entering the syringe.

1. If no fat appears in the syringe or the aspiration has stopped completely, the needle may have become obstructed. The simplest way to check this is to remove the needle out of the patient. Normally, fat tissue present in the needle is then directly and audibly forced into the syringe because of negative pressure. If this is not the case and fat tissue obstructs the needle completely, tissue in the needle can be removed by using positive pressure in the syringe. This may lead to a rather explosive evacuation (“firing fat tissue”) and should therefore be carried out carefully. Tissue is evacuated into a clean container (e.g. sputum or urine) or empty syringe, while fixing the needle firmly to the syringe to prevent the needle leaving the syringe (“firing needles”).

2. If much arterial or venous blood enters the syringe by accident, the needle should be removed out of the body. The puncture site should be pressed for at least one minute, and the procedure can be repeated into a different direction or at a different site. Pain is infrequent, localised, and seldom a real problem necessitating the use of more lidocaine. If bruising is suspected to be present at the end of the procedure, the patient self may press the puncture site for a couple of minutes before rising from the supine position.

➤ **Address for shipping syringes for amyloid detection and precursor quantification:** (Please also see the labmanual and notify us by phone or e-mail before sending the material)

University Medical Center Groningen
Mr. Johan Bijzet, BSc
Lab. Rheumatology, T2.238
HPC EA41
Hanzeplein 1,
9713 GZ Groningen
The Netherlands
Tel: +31 50 361 3421
Fax: +31 50 361 3591
E-mail: j.bijzet@umcg.nl

K. Durie and Salmon STAGING OF MULTIPLE MYELOMA

Stage I– 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:

Patients who qualify for neither Stage I nor III

Stage III– 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 lytic bone lesions on skeletal survey

A	Normal renal function (creatinin < 177 μmol/l)
B	Renal insufficiency (creatinin ≥ 177 μmol/l)

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

L. Cardiac staging system based on TNT or TNI and NT-pro-BNP

- Stage I : TNT < 0.035 μg/L or TNI < 0.1 μg/L and NT-pro-BNP < 332 ng/L
- Stage II: not fulfilling the criteria for stage I or III
- Stage III: TNT ≥ 0.035 μg/L or TNI ≥ 0.1 μg/L and NT-pro-BNP ≥ 332 ng/L