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.

9.6.6 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.
At the end of study, when all patients have stopped protocol treatment, for daratumumab, complete drug reconciliation per batch should be available at the site for verification by HOVON (as appropriate) in order to allow drug destruction or return procedure. Both the unused and expired daratumumab must be destroyed, upon authorization of the sponsor, according to local regulations and procedures.

10 Study procedures

10.1 Time of clinical evaluations

- At entry: before start of treatment (peripheral blood values within 2 weeks, serum and urine M-protein within 4 weeks prior to start, bone marrow, whole body PET-CT and CT for sarcopenia within 8 weeks).
- During induction therapy after 1, 2, 3, 5 ,7 and 9 cycles (just before start of the next cycle).
- Before start of maintenance treatment (peripheral blood and urine lab values within 4 weeks prior to start).
- During maintenance therapy after every maintenance cycle, every 8 weeks.
- When patient is taken off protocol treatment.
- During follow up every 8 weeks until progression until second prgoression and every 6 months thereafter.

All patients will be followed until 5 years after registration.

10.2 Required investigations

Required investigations at entry, during treatment and during follow up

	At entry	After cycles 1,2, 3,5,7,9 (just before start next cycle)	During maintenance after every cycle; and during follow up every 8 weeks[1)]	When going off protocol treatment
Medical history	x	x	x	x
Physical examination	x	x	x[1)]	x
Hematology	x	x[2)]	x	x
Extensive erythrocyte typing and indirect antiglobulin test (section 9.4.4)	x			
Blood chemistry	x	x	x	x
Immunochemistry	x	x	x	x
DIRA test (central lab)[3)]		(x)[3)]	(x)[3)]	
Bone marrow				
Bone marrow aspirate	x	x[4)]	x[4)]	x[4)]
Bone marrow biopsy	x[5)]	x[5)]	x[5)]	x[5)]
Cytogenetic analysis[6)]	x			
Molecular profiling (central lab)	x[7)]			
MRD analysis (central lab)		(x) [7)]	(x) [7)]	
Biological studies (central lab)		x[8)]	x[8)]	x[8)]
Specific investigations				
ISS β_2-microglobulin and albumin	x			
Creatinine clearance	x			
Whole body low dose FDG-PET-CT	x	(x)[9)]	(x)[9)]	
ECG	x			
Additional correlative studies				
Peripheral blood (central lab)	x[10)]	x[10)]	x[10)]	x[10)]
Quality of Life	x[11)]	x[11)]	x[11)]	x[11)]
Geriatric assessments and biomarkers for biological age				
CT-abdomen (muscle mass)	x[12)]	x[12)]		x[12)]
Skin biopsy	x[13)]			
Gait speed/Grip strength	x[14)]	x[14)]	x[14)]	x[14)]
Chair rise test	x[14)]	x[14)]	x[14)]	x[14)]
Questionnaires	x[14)]	x[14)]	x[14)]	x[14)]

1) During maintenance therapy out clinic visits, hematology, blood chemistry and immunochemistry will be performed every cycle, physical examination will be done every 2 cycles. After discontinuation of maintenance therapy during follow up visits hematology, blood chemistry and immunochemistry will be performed every eight weeks (+/- 2 weeks) or at shorter intervals at the discretion of the treating physician until *second* progression and every 6 months (+/- 8 weeks) thereafter.
2) Hematology first 2 cycles every week, cycle 3-9 every 2 weeks, more often in case of dose modification/delay.
3) In case of ≥VGPR PB has to be sent to the central laboratory for Daratumumab IFE reflex assay (DIRA) to correct for the presence of daratumumab in the peripheral blood and to correctly determine CR. In case the DIRA test indicates CR, it should not be repeated.
4) In case of confirming CR, at the moment of complete disappearance of serum/urine M-component by immunofixation, or at progression, a bone marrow aspirate and/or bone marrow biopsy is indicated. To confirm stringent CR, either kappa/lambda labeling of a bone marrow biopsy or immunophenotyping of the BM aspirate has to be performed. Also at progression a bone marrow aspirate and/or bone marrow is indicated (optional).

5) A bone marrow biopsy is optional. In case of first diagnosis or confirming stringent CR at the moment of complete disappearance of serum/urine M-component by immunofixation, either a kappa/lambda labeling of a bone marrow biopsy or immunophenotyping of a BM aspirate has to be performed.
6) Cytogenetic analysis will be performed in the cytogenetic reference labs for each local site
7) **Bone marrow will be sent to the central laboratory at entry for molecular profiling. Moreover, in case (s)CR is reached, bone marrow has to be sent to the central laboratory to determine the MRD status by use of flow cytometry. At that time also a whole body FDG-PET-CT must be performed (see 9).**
8) **Send material for biological studies to the central laboratory at the time of progression.** See lab manual at the HOVON website for procedures for collecting and handling of the samples.
9) A whole body low dose FDG-PET-CT will be repeated when clinically indicated **and in all patients reaching (s)CR. The FDG-PET-CT must be performed according to the HOVON Imaging FDG-PET-CT protocol.**
10 **Peripheral blood for additional research will be sent to the central laboratory:** for SNP analysis at entry and at the time of progression, for immunological studies at entry, after cycle 1, after cycle 3, at the start of maintenance, and at the time of progression. See lab manual at the HOVON website for procedures for collecting and handling of the samples.
11) Quality of life questionnaires at entry, after cycle 3 and 9 (or earlier in case of prematurely discontinuation of induction treatment), after 6 and 12 months of maintenance and at discontinuation of (maintenance) therapy. For details see table 10.4.
12) In order to determine body composition and muscle mass a plain CT scan without contrast will be performed at entry, after completion of induction therapy and when going off protocol. See 10.4.3 for details on CT-scan requirements.
13) A skin biopsy (4mm) will be taken for senescence analysis and future biological studies. See appendix N, part B and the lab manual at the HOVON website for procedures for collecting and handling samples.
14) Questionnaires and functional assessments at entry, after cycle 3 and 9 (or earlier in case of prematurely discontinuation of induction treatment), after 12 months of maintenance and at discontinuation of maintenance therapy. For details see table 10.4.

Medical history

Standard medical history, with special attention for adverse events, WHO performance status, bone pain, infections, bleeding tendency and polyneuropathy.

Only at entry occupational history, prior and present other diseases, antecedent hematological or oncological diseases, previous chemotherapy or radiotherapy.

Physical examination

Standard physical examination including body weight and height, with special attention for macroglossia, kyphoscoliosis, orthostatic hypotension, carpal tunnel syndrome, polyneuropathy or other neurological symptoms , edema, infections and bleeding tendency.

Hematology

Hemoglobin, Leukocyte count, Neutrophil count, Platelets. Moreover, as daratumumab is known to interfere with the Indirect Antiglobulin Test (IAT) extensive erythrocyte phenotyping and an IAT have

to be performed before the start of therapy. In addition, the patient identification wallet card must be completed and handed over to the patient. Please see section 12.6 for more detailed information.

Blood chemistry

Creatinine, ASAT, ALAT, Total bilirubin, Total proteins, Albumin, LDH, Calcium.

Immunochemistry

- At entry: Qualitative and Quantitative serum and urine (24 hrs urine) M-protein, including immunofixation and serum FLC ratio.
- Evaluation: Qualitative and Quantitative serum and urine (24 hrs urine) M-protein, including immunofixation to confirm CR. Serum FLC ratio only to confirm (s)CR or when serum FLC ratio is the only measurable parameter.
- In case of ≥VGPR, PB has to be sent to the central laboratory for the Daratumumab IFE reflex assay (DIRA) to correct for the presence of daratumumab in the peripheral blood and to correctly determine (s)CR.

Quantitative M-protein in serum and urine by gel electrophoresis preferably. Nephelometry or turbidometry are allowed, see appendix C for instructions.
Qualitative M-protein in serum and urine by immunofixation.
Immunofixation and serum FLC ratio to determine the achievement of CR and sCR respectively.

Bone marrow

Bone marrow aspiration (obligatory) and biopsy (optional) at entry, including (molecular) cytogenetic evaluation.
Repeated bone marrow aspiration (biopsy is optional) in case the decline in M-protein suggest achievement of CR or sCR (see Appendix C for response criteria) and at progressive disease (optional at progressive disease only).

- Bone marrow **aspirate:**

- **at entry** for:
  - Morphology
  - FISH analysis: see section 10.5
  - Immunophenotyping has to be performed at entry in case no BM biopsy is performed in order to determine the presence of monoclonal plasma cells.
- **at response evaluation** for confirmation of (s)CR, flowcytometry analysis to determine MRD and molecular and immunological studies.
- **at progressive disease** for confirmation of progression and molecular and immunological studies.

- Bone marrow **biopsy** (optional)

  at entry and **to confirm (stringent) complete response**, including kappa lambda labeling. In case no BM biopsy is performed the presence of monoclonal plasmacells at entry or a stringent CR has to be confirmed by kappa/lambda labeling using immunophenotyping of the BM aspirate. After a CR repeated sampling of bone marrow aspirate is no longer necessary.

Radiographic assessment with low dose whole body FDG-PET-CT before start treatment and after reaching (s)CR.

Specific investigations

- Serum ß2-microglobulin
- Creatinine and calculated glomerular filtration rate
- ECG
- CT scan abdomen (plain CT scan without contrast; Th12 until L4; 5 mm slices) see section 10.4.3 and appendix L
- Questionnaires about QoL and neurotoxicity, geriatric assessments and CCI; see Hovon website and appendices L and, M.

Skin biopsy

A skin biopsy (4mm) from the area of the bone marrow aspirate where the patient received a local anesthetic or from the sun-protected side of the inner upper arm; see appendix N.

Peripheral blood At entry, day 1 cycle 2, day 1 cycle 4, before the start of maintenance and at progressive disease for immunomonitoring, molecular, and future analyses. Peripheral blood sampling for immunomonitoring can be combined with local peripheral blood tests. A time window of -3 days is allowed (PB may be collected from day 26 of a cycle up to and including day 1 of the next cycle). The samples should be sent to the central laboratory (Erasmus MC and VUmc) at room temperature. See lab manual at the HOVON website for procedures for collecting and handling of the samples.

Additional investigations

Only on clinical indication:

- Survey for exclusion of AL amyloidosis, by biopsy of either subcutaneous fat or organ suspected of amyloid deposition
- aPTT, PT (INR).
- In case of prolonged aPTT and/or PT(INR) a factor X activity has to be determined

- Cryoglobulins, cold agglutinins
- Fundoscopy
- Spirometry

10.3 Response evaluation

The response will be evaluated after the induction cycles 1, 2, 3, 5, 7 and 9; after every maintenance cycle. Response evaluation should also be done when taken off protocol treatment and during follow up every 8 weeks until *second* progression and every 6 months or more extensive at the discretion of the treating physician, thereafter. Response will be evaluated according to appendix C.

10.4 Quality of Life and geriatric assessments

Questionnaire	To be completed by	At entry	After cycle 3	After cycle 9 or premature discontinuation of induction therapy	6 months after the start of maintenance therapy	12 months after the start of maintenance therapy	After completion or discontinuation of maintenance therapy
EORTC QLQ-C30	Patient	X	X	X	X	X	X
EORTC QLQ-MY20	Patient	X	X	X	X	X	X
EQ 5D 5L	Patient	X	X	X	X	X	X
Geriatric Depression Scale 15 (GDS15)	Patient	X	X	X		X	X
Neurotoxicity questionnaire	Study/site coordinator	X	X	X		X	X
Katz scale for basal activity of daily life (ADL)	Study/site coordinator	X	X	X		X	X
Instrumental Activities of Daily Living (IADL)	Study/site coordinator	X	X	X		X	X
Mini Mental State Examination (MMSE)	Study/site coordinator	X	X	X		X	X
Mini Nutritional Assessment (MNA)	Study/site coordinator	X	X	X		X	X
Grip Strength	Study/site coordinator	X	X	X		X	X
Gait Speed	Study/site coordinator	X	X	X		X	X
Chair rise test	Study/site coordinator	X	X	X		X	X
CT-abdomen	Radiologist	X		X			X
Skin biopsy	Hematologist	X					
Charlson Comorbidity Index (CCI)	Hematologist	X	X	X		X	X

All questionnaires can be downloaded from the HOVON website.

10.4.1 Quality of Life

Quality of life (QoL) will be assessed by means of the following questionnaires:

- EORTC QLQ-C30 questionnaire

The QLQ-C30 is a multidimensional, cancer-specific quality-of-life questionnaire developed by the European Organization for Research and Treatment of Cancer (EORTC) Study Group on Quality of Life for use in international clinical trial settings. The questionnaire is designed for use with a wide range of cancer patient populations, irrespective of specific diagnosis. The QLQ-C30 includes 5 functional scales (physical, role, emotional, social and cognitive functioning), 3 symptom scales (fatigue, pain, and nausea and vomiting), a global health status/quality of life scale and a number of single items assessing additional symptoms (dyspnoea, sleep disturbance, constipation and diarrhea) and perceived financial impact.

- EORTC QLQ-MY20

QoL will also be measured with the EORTC-QLQ-MY20. This questionnaire contains 20 items, and is a reliable and valid instrument recommended for use in myeloma patients. The questionnaire contains the following scales: pain, side effects of treatment, social support, body image, and future prospectives

- EQ-5D-5L

The EQ-5D-5L is a generic prefrence-based HRQoL. The EQ-5D-5L measures generic quality of life and can be converted into a “health utility” score, ranging from 0.0 (death) to 1.0 (“perfect health”). The 5 dimensions of the self-classifier are mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, with 5 levels of severity.

- GDS15

The GDS15 is a self-report assessment used to identify depression in the elderly. It consists of 15 questions about presence of symptoms of depression the past week that can be answered ‘yes’ or ‘no’. A score of 6 or more indicates a possible depression.

Collection of the QoL questionnaires will be performed in the following manner:
A QoL coordinator will be assigned in each participating center. The QoL questionnaire collection is left to the responsibility of the QoL coordinator.
As soon as a patient is registered at the HOVON Data Center (HDC) the QoL coordinator is notified by email. Patient study number, (partial) date of birth and date of registration are mentioned in this mail.

The baseline questionnaire will be handed or sent to the patient by the QoL coordinator. At the time points mentioned in the beginning of this section, the coordinator will hand over the questionnaire at the correct date.

The QoL coordinator will collect the questionnaire from the patient and will and send it to HDC. If a QoL questionnaire has not been received by HOVON Data Center at the expected date, a reminder/request will be sent to the local QoL coordinator to collect and send in the questionnaire.

10.4.2 Geriatric assessment analysis

To explore the predictive value of geriatric assessments with respect to discontinuation rate, and efficacy, interviews will be taken by a study nurse/coordinator using the following:

- ADL
- IADL
- MNA
- MMSE*

*A MMSE of less than 15 points indicates severe dementia. In case the score is less than 15 the treating hematologist will be informed in order to reconsider treatment.

In addition, physical factors will be determined to provide a broader overall understanding of individual characteristics that may affect the feasibility of therapy and life expectancy. These factors are muscle strength, gait speed, chair rise and muscle mass. Grip strength is used as a proxy of muscle strength and measured by three maximal squeezes with both the left and right hand applied at a hand-held dynamometer. Gait speed will be investigated by determination of the time needed to walk a distance of 4 meters. Chair rise time test will measure the time to needed to stand up straight as quickly as the patient can 5 times, without stopping in between, with arms folded across the chest. Muscle mass and body composition will be determined by CT-scan.

The treating hematologist will determine the Charison Comorbidity Index (Appendix M).

10.4.3 Neurotoxicity questionnaire

The neurotoxicity questionnaire is a tool to assess the CTCAE grade of neurotoxicity, and should be filled out during a study visit by the doctor or study nurse, together with the patient. Please note that signs such as ‘frequent urination’ should only be ascribed to neurotoxicity if there is no other explanation for this complaint, such as urinary tract infection.

10.4.4 CT abdomen for determination of lean body mass

In order to determine body composition and muscle mass a plain CT scan or spiral CT scan (axial scans) without contrast will be performed at entry, after completion of induction therapy and when going off protocol. A normal tube voltage of 120 kV is sufficient (Other scanparameter: mAs 70.00). A thickness of 3 to 5 mm slices is preferred (‘Raw views’ of 0.9 mm can not be analyzed).

L3 should be present on the slices. To be sure that L3 is present, the area from Th12 until L4 (beginning) should be scanned. The skin should be present on the scan in order to measure subcutaneous fat. A supine position of the patient is recommended. Please take care that the patient is not turned on his or her side.

Local radiologist review of the CT scan is not necessary, since there is a central review. CDs should be anonymized (scan as well as CD-label). HOVON 143 study number (e.g. HOVON 143 pt 1, and date of assessment dd-mm-yyyy) are sufficient to mark the CD.

10.5 Cytogenetic analysis

FISH analysis is required in all patients at diagnosis/start of study. The following cytogenetic abnormalities will be evaluated as prognostic variables: del1p, gain 1q, t(4;14)(p16;q32), t(14;16)(q32;q23), t(11;14)(q13;q32), del13q/13-, del17p and hyperdiploidy (at least 2 of the chromosomes 5, 9, 11 and 15 should be analyzed). Conditions for FISH will be according to the EMN guidelines (Ross et al., Haematologica 97, 1272-1277 (2012)).

10.6 Correlative studies

All correlative studies are described in appendix N in detail.

The correlative studies consist of

- Senescence markers in skin fibroblasts;
- Gene expression and genomic profiling;
- MRD measurements by the use of multicolour flowcytometry;
- Biomarkers to predict response;
- Immune monitoring and evaluation of CD38 and complement-inhibitory proteins expression levels by flow cytometry.

11 Withdrawal of patients or premature termination of the study

11.1 Withdrawal of individual patients from protocol treatment

Patients should be withdrawn from protocol treatment if any of the following criteria for withdrawal are met:

- ♦ Death
- ♦ Patient not eligible in hindsight
- ♦ Progression during treatment