Efficacy and tolerability of ixazomib, daratumumab and low dose dexamethasone (IDd) followed by ixazomib and daratumumab maintenance therapy until progression for a maximum of 2 years in unfit and frail newly diagnosed multiple myeloma patients; an open-label phase II trial

Takeda study number #X16086

Janssen study 54767414MMY2013

PROTOCOL

Principal Investigator	**:**	S. Zweegman (HOVON)
Sponsor	**:**	HOVON
EudraCT number	**:**	2016-002600-90

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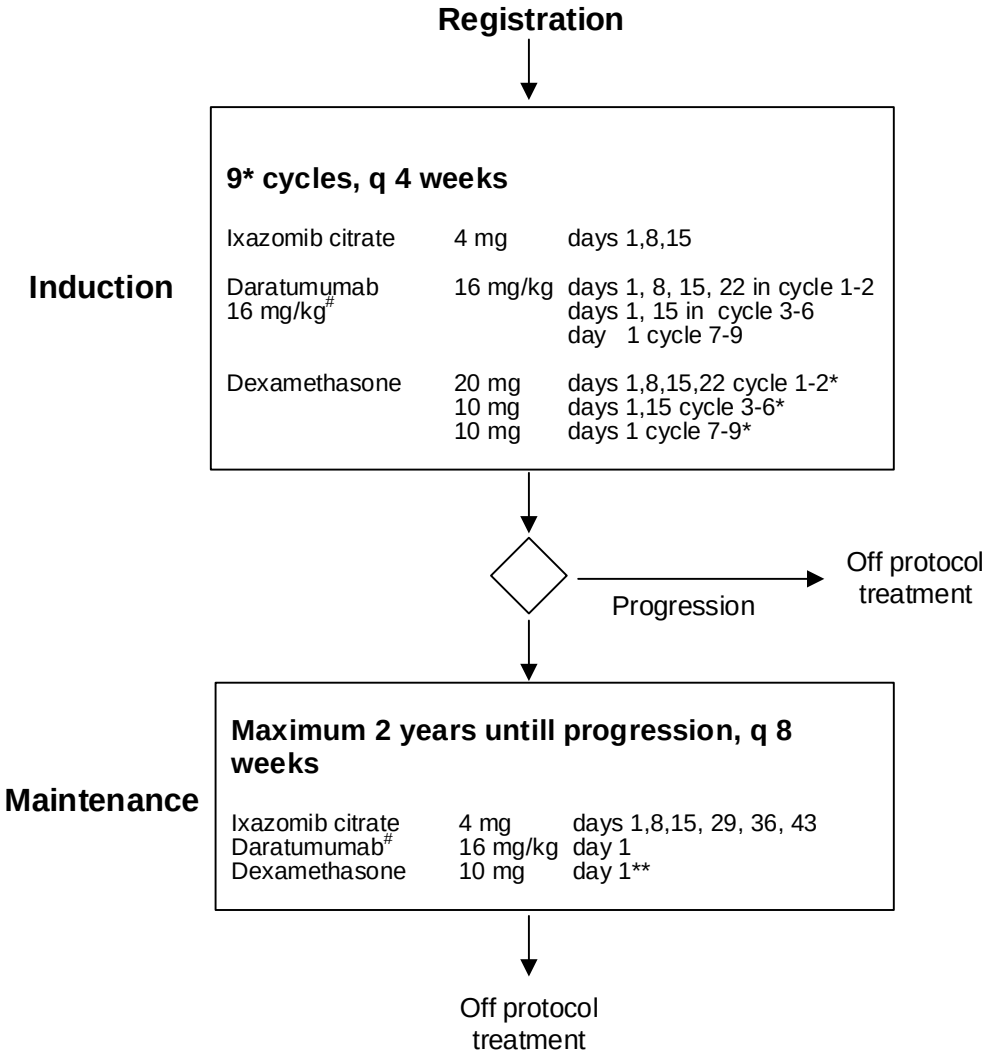
By my signature, I agree to personally supervise the conduct of this study in my affiliation and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice (2001-20-EG),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

1 Scheme of study

Previously untreated frail and unfit patients with MM ineligible for high dose therapy and peripheral stem cell transplantation



* in case either ixazomib or daratumumab is delayed or withhold dexamethasone should be administered, either i.v. or p.o.

** in case daratumumab is delayed or withhold dexamethasone is NOT administered.

after implementation of study protocol version 5.1 dd 16JUL2020 it is also possible to administer daratumumab subcutaneously (fixed dose 1800 mg)

Post-daratumumab infusion medication, consisting of 4 mg of dexamethasone will be routinely administered in the first 4 cycles, not during subsequent cycles, provided there were no IRRs during previous cycles. In case of infusion related reactions in the first 2 cycles dexamethasone dose should maintained at 20 mg and additional measures described in paragraph J 2 should be taken. In case of infusion related reactions during lowering the dose to 10 mg of dexamethasone or lowering the dose because of side effects to 4 mg of

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3 Synopsis

Rationale	Data from both clinical trials and population based registries indicate that elderly patients also benefit from novel therapies. The challenge is to identify a relatively non-toxic strategy for unfit and frail patient enabling effective treatment with novel agents. With the availability of the oral proteasome inhibitor ixazomib, that induces only minimal grade 3 and 4 neuropathy, the way is paved for oral proteasome-inhibitor based therapy, also in the maintenance setting. The implementation of daratumumab in novel treatment regimens for unfit and frail patients is obvious, being a novel class of drugs with promising efficacy and mild and mainly infusion-related side effects, which was manageable also in the elderly patients. Therefore, the efficacy and feasibility of 9 cycles of ixazomib, daratumumab and low dose dexamethasone followed by ixazomib and daratumumab maintenance until progression for a maximum of 2 years will be investigated in unfit and frail newly diagnosed multiple myeloma (NDMM) patients.
Study objectives	Primary objective – To determine the efficacy, defined as overall response rate (ORR; ≥ partial response (PR)), of 9 cycles of ixazomib, daratumumab and low dose dexamethasone Secondary objectives – To determine the tolerability, defined as discontinuation rate due to treatment related toxicity, of 9 cycles of ixazomib, daratumumab and low dose dexamethasone – To determine adverse events of CTCAE grade 2-4 – To determine complete response (CR) and very good partial response (VGPR) after 9 induction cycles – To determine complete response (CR) and very good partial response (VGPR) on protocol

	– To determine immunophenotypic complete response after 9 induction cycles – To determine immunophenotypic complete response on protocol – To determine the flow Minimal Residual Disease negative complete remission – To determine the imaging plus flow MRD negative complete remission – To determine progression free survival (PFS) – To determine overall survival (OS) – To determine efficacy of therapy determined as time to response and the time to best response – To determine the effect of maintenance therapy with ixazomib and daratumumab in terms of improvement of response during maintenance – To determine the tolerability of maintenance therapy, defined as discontinuation rate due to treatment related toxicity of ixazomib and daratumumab – To determine time to next treatment – To determine PFS2 – To evaluate quality of life (QoL) Exploratory objectives – To identify geriatric assessment outcomes that predict feasibility and the toxicity of treatment – To identify biological markers; sarcopenia and senescence markers, that reflect biological age and that predict feasibility and the toxicity of treatment – To identify immunological and molecular prognostic markers that predict outcome and toxicity – To identify biomarkers for response – To investigate the prognostic value of Minimal Residual Disease – To investigate the prognostic value of FDG-PET-CT at diagnostics and in follow up
Study design	Investigator-initiated, multicenter, non-randomized, open label, phase II clinical trial

Patient population	Previously untreated symptomatic patients with MM who are unfit or frail according to the criteria of the International Myeloma Working Group
Intervention	Induction therapy with 9 cycles of ixazomib, daratumumab and dexamethasone, followed by maintenance therapy with ixazomib and daratumumab until progression for a maximum period of two years
Duration of treatment	Expected duration of induction treatment: 9 months Maintenance therapy with ixazomib and daratumumab until progression for a maximum period of two years All patients will be followed 5 years after registration
Target number of patients	66 unfit and 66 frail patients for a total of 132 patients enrolled into the study. See section 14.1 for detailed information.
Expected duration of accrual	18-24 months
Main study endpoint	Overall response rate (ORR, i.e. ≥ PR) on induction therapy
Benefit and nature and extent of the burden and risks associated with participation	The benefit will be that unfit and frail patients can be treated with an oral proteasome inhibitor ixazomib instead of the currently available subcutaneous proteasome inhibitor bortezomib, with considerably less polyneuropathy. Bortezomib is currently standard of care. Secondly, patients will be treated with daratumumab, a novel class drug, with pronounced effectivity in heavily pretreated patients with limited toxicity only. When studied as single agent in this heavily pretreated patients not only response rate was high; 36%, in addition 65% of patients who responded were still in remission after one year[35]. Moreover, the data of the addition of daratumumab to bortezomib/dexamethasone, after 1 to 3 prior lines of therapy resulted in a 61% reduction of progression[41], which has not been reached with other novel drugs. Therefore, this regimen is expected to result in efficacy. Secondly, less side effects as compared to the standard of care “Melphalan, Bortezomib, Prednisone - MPV” is expected, which we recently investigated in the HOVON 123 study. Preliminary data showed that 49% of

	frail patients and 29% of unfit patients had to discontinue therapy because of toxicity. The burden will be that 1. Patients will receive intranvenous or subcutaneous daratumumab instead of a combined oral/sc regimen of MPV of an oral regimen with lenalidomide/dexamethasone. 2. Following induction therapy, maintenance therapy will be given until progression for a maximum of two years. Although a benefit with respect to prolongation of PFS is expected, the extent is currently unknown. Patients may suffer from side effects, although these are generally mild with ixazomib and daratumumab and also the combination of a proteasome inhibitor with daratumumab was found to be safe with limited additional side effects to a proteasome inhibitor only. There are additional procedures required as compared to standard care because of biological assessment of frailty, such as a CT scan to determine the presence of sarcopenia, geriatric assessments and a skin biopsy for senescence markers. Patients have to participate in QoL studies, and in case of a complete therapy response undergo a PET-CT scan and an additional BM aspirate.
Planned interim analysis and DSMB	Two interim analyses are planned. One safety monitoring analysis is planned primarily to describe the adverse events of induction therapy with ixazomib – daratumumab – low dose dexamethasone. This will be done separately for frail and unfit patients, when the first 10 registered frail and 10 registered unfit patients have available data regarding induction cycles 1-4. In addition, one interim analysis is planned to describe the efficacy, defined as response observed during the ixazomib - daratumumab - low dose dexamethasone induction therapy. This will be done separately for frail and unfit patients, when of the first 23 registered frail patients and first 23 unfit patients data of response after 9 induction cycles are available. Results of the interim analyses will be presented to a DSMB and to the PI.

4 Investigators and study administrative structure

This is an investigator-initiated trial sponsored by HOVON, which means that HOVON holds all sponsor responsibilities unless it is explicitly stated in this protocol that a sponsor responsibility is delegated to another party.

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Serious Adverse Events (SAEs) notification	HOVON Data Center	Erasmus MC Cancer Institute Clinical Trial Center

5 Introduction and rationale

5.1 Treatment of elderly multiple myeloma patients

MM is a disease of the elderly reflected by a median age at diagnosis of approximately 70 years, with 35-40% of patients being older than 75 years.[1] The introduction of the immunomodulatory agents (IMiDs; thalidomide, lenalidomide and pomalidomide) and the proteasome inhibitors (PIs; bortezomib and carfilzomib) has not only greatly improved the prognosis of younger patients with MM, also in elderly MM patients aged 65 years or over. Currently, therapy with MP-bortezomib (MPV) or MP-thalidomide (MPT) is standard of care in Europe. However, the use of MPT will decrease because of the FIRST trial showing superiority of lenalidomide/dexamethasone over MPT, both for PFS and OS. Although in the original paper only lenalidomide/dexamethasone until progression was found to result in a superior OS over MPT, subsequent analysis after longer follow up also showed a superior survival of lenalidomide/dexamethasone for 18 months over MPT. Moreover, given the fact that lenalidomide/dexamethasone is an oral regimen versus subcutaneous bortezomib administration, it is to be expected that the majority of patients will be treated with lenalidomide/dexamethasone.

However, 32.4% of patients over 75 years had to discontinue lenalidomide/dexamethasone due to toxicity.[2] In the MM015 trial the improvement in PFS that was obtained by addition of lenalidomide to melphalan/prednisone in induction- and a maintenance therapy was even lacking in patients over 75 years due to a high discontinuation rate of 22% as opposed to 12% in patients <75 years and due to a lower dose intensity in patients >75 years (PFS MP 15 months versus MPR-R 19 months).[3] In the VISTA trial the discontinuation rate was 34% during therapy with MPV, mainly due to peripheral neuropathy.[4]

Therefore, despite the fact that the novel agents greatly improved outcome, also in the elderly non-transplant eligible patients, there is an urgent need for less toxic regimens in the most elderly patients with MM.

5.1.1 Benefit of treatment with novel agents in elderly multiple myeloma patients

In addition, when considering the outcome as described in population-based registries (PBR), reflecting real-life situations, the elderly patients appear to benefit less. Recently, in the Italian and Dutch population-based registries, the overall survival (OS) of very old patients (≥75 years of age) was found to be similar over time, without any improvement in OS after the introduction of novel agents in 2006.[5] The limited benefit of novel agents in the elderly, as described in the PBR, might be explained by the fact that the majority of elderly patients are not being included in (randomized) clinical trials (RCT) because they do not fulfill the eligibility criteria due to co-morbidities. Usually, in these patients, treatment is either not given, or is given but without the addition of novel agents or with

a lower dose of novel agents. This fact at least partly explains the difference in outcome between RCT and PBR. This is supported by several observations showing that if novel therapy is given to the elderly outside of RCT, there is an increase in OS, even in the oldest patients. Data analysis of elderly patients actually receiving lenalidomide and/or bortezomib from the Mayo Clinic (89% of all patients used novel agents during the time period 2006-2010 *vs.* 29% in the period 2001-2005) showed an increase in OS over time, specifically in those aged over 65 years (median OS 5 *vs.* 3.2 years). Improved survival was seen among patients up to 75 years of age and also in those over 75 years of age.[6] In addition, Liwing *et al.* reported that 1127 patients receiving at least 2 lines of therapy with bortezomib, thalidomide or lenalidomide had a superior OS (63% at 5 years) compared to those treated with conventional drugs (22% at 5 years).[7] Lastly, the IFM presented registry data at ASH 2012 showing an increase in PFS in elderly patients, mainly being treated with thalidomide, irrespective of age.[8] Of course, such data analyses are biased by the fact that the reasons for either treatment or no treatment are unknown.

However, these data do indicate that also a subgroup of elderly patients do benefit from novel therapies. The challenge, therefore, is to identify a non-toxic strategy for unfit and frail patient enabling treatment with novel agents. In addition, more precise identification of those patients who will benefit is urgently needed.

5.1.2 Geriatric assessment to predict toxicity

It is known from general practice that calendar age is insufficient to predict biological age and thereby toxicity of therapy. Recently, Palumbo *et al.* showed that the frailty score, based on age (<75, 75-80, >80 years: score 0, 1, 2 respectively), Charlson comorbidity index (CCI ≤1 or ≥2: score 0 or 1) and (instrumental) Activities Daily Life score (ADL >4 or ≤4: score 0 or 1; iADL>5 or ≤5: score 0 or 1), predicted non-hematologic toxicity in 869 patients aged 65 years or over uniformly treated within 3 randomized clinical trials. Frail patients (total score ≥2) had a 1.8 times higher discontinuation rate compared to fit patients (score 0). Importantly, no difference was found in hematologic toxicity. In a multivariate analysis frailty (HR 1.64, 95% CI 1.24-2.17), ISS 3 (HR 1.49, 1.17-1.89) and high-risk fluorescence *in situ* hybridization (FISH) (HR 1.75, 1.38-2.22) equally predicted PFS, whereas for OS the HR was highest for the frailty score (HR 3.11, 1.97-4.90) *versus* (1.77, 1.26-2.63, ISS) and (1.83, 1.26-2.63, high-risk FISH). Importantly, of the patients aged under 75 years 9% were found to be frail, so geriatric assessments were of value not only in those aged 75 years or over but also in those aged 65 years or over. Importantly, of patients aged 75 years or over only 57% were found to be frail.[9] These data underscore the importance of geriatric assessments as well as the need for prospective validation in uniformly treated patient populations. This is currently being performed in the Dutch HOVON 123 study (EudraCT 2013-000320-33) that has also implemented objectively measured criteria (physical function such as gait speed and handgrip strength, cognitive function and

sarcopenia) and exploring the value of biomarkers reflecting biological age, such as the senescence marker p16INK4a and sarcopenia. Hopefully, these biomarkers will be even more precise in predicting treatment associated toxicity than geriatric assessment.[10]

5.1.3 Possibilities to improve treatment in the elderly

Besides more precise identification of elderly patients who might benefit from treatment, novel agents with an improved side effect profile need to be explored in the most elderly unfit and frail patients.

With the availability of the oral boron proteasome inhibitor ixazomib, known to induce limited grade III only and no grade IV neuropathy, the way is paved for oral proteasome-inhibitor based therapy. Importantly, it appeared that maintenance therapy with single agent ixazomib following induction therapy with ixazomib/lenalidomide/dexamathasone resulted in an improvement in response in 48% of patients. Only 8% of patients discontinued therapy because of toxicity and none of these patients discontinued therapy due to toxicity during maintenance .[11] Although, approximately 20% of patients over 75 were included in the study there was no separate analysis according to age. Moreover, an ECOG performance status of 0-2 was required in order to be included in the study. In view of the paradigm of continuous therapy in multiple myeloma the demonstrated efficacy and feasibility of ixazomib maintenance therapy will support the implementation of ixazomib maintenance in clinical practice.

The implementation of daratumumab in novel treatment regimens for unfit and frail patients is obvious. First, it is a novel class of drugs with promising efficacy. Moreover, toxicity appeared to be mainly infusion-related, with only 4.7% grade III and no grade IV, which was manageable also in the elderly patients. Less than 5% of patients had to discontinue therapy because of adverse events.[12;13] Therefore, it is to be expected that addition of daratumumab to ixazomib and low dose dexamethasone will be feasible in elderly unfit and frail patients also.

Although there is no data on the combination of ixazomib and daratumumab yet, there is information on the combination of the subcutaneously administered proteasome inhibitor, bortezomib and daratumumab; the Castor study[41]. In this study bortezomib/dexamethasone was compared to bortezomib/dexamethasone/daratumumab in a randomized controlled clinical trial. There was no increase in side effects with the addition of daratumumab, except for the limited infusion related side effects as described above and 10% increase in haematological toxicity. Therefore, the combination or the oral proteasome inhibitor ixazomib and daratumumab is not expected to result in unforeseen toxicity. We recently investigated the standard treatment for elderly unfit and frail patients; Melphalan/Bortezomib/Prednisone in the HOVON 123 study, and showed a high discontinuation rate due to toxicity of approximately 50% even when lowering the dose. So therefore, there is an urgent need for non-toxic therapeutic regimens in the unfit and frail patients.

It is advised to use a two-drug regimen in unfit and frail NDMM patients, however this is based on expert opinion only, as randomized clinical trials in frail patients are lacking. Moreover, this might hamper efficacy as in general a two-drug regimen is less effective as compared to a three-drug regimen.[14;15] And even four-drug regimens have been explored in non-transplant eligible patients, although not in frail patients. In view of this, it is interesting to explore the efficacy and feasibility of a three-drug regimen.

5.2 Rationale of the study

In view of above described considerations, we aim to determine the efficacy and feasibility of 9 cycles of ixazomib, daratumumab and low dose dexamethasone followed by ixazomib and daratumumab maintenance until progression for a maximum of 2 years in unfit and frail NDMM patients.

5.3 Ixazomib

Bortezomib, the first-in-class, small-molecule proteasome inhibitor, validated the proteasome as a therapeutic target. Recognizing that proteasome inhibition is an effective anticancer therapeutic approach, ixazomib (formerly known as MLN9708) has been developed with the aim of improving the pharmacology of the agent, building on the efficacy seen with bortezomib in MM and other hematologic malignancies, and improving safety and convenience of drug administration. Ixazomib is the first oral proteasome inhibitor approved by the U.S. Food and Drug Administration (FDA) for multiple myeloma therapy. In addition, ixazomib also recently received the European Medicines Agency's (EMA) conditional marketing authorization.

For the pharmacology, pharmacokinetics, pharmacodynamics, (pre)clinical experience and safety profile of Ixazomib please be referred to appendix F, section F1.

5.4 Daratumumab

Daratumumab is a human IgG1 anti-CD38 monoclonal antibody. CD38 is highly and uniformly expressed on all MM cells. Daratumumab received "breakthrough therapy" designation for relapsed/refractory MM by the US FDA in May 2013 [16;17]. In November 2015, the FDA granted accelerated approval for daratumumab to treat MM patients who have received at least 3 prior treatments including a proteasome inhibitor and an IMiD or who are double-refractory to a proteasome inhibitor and an IMiD. On May 23rd 2016 the EMA approved daratumumab monotherapy in Europe to treat MM patients whose prior therapy included a proteasome inhibitor and an immunomodulatory agent and who have demonstrated disease progression on the last therapy. In addition, on April 28th 2017 EMA also granted a marketing authorization for daratumumab in

combination with lenalidomide and dexamethasone, or bortezomib and dexamethasone, for the treatment of adult patients with multiple myeloma who have received at least one prior therapy.

Daratumumab has been approved in combination with bortezomib, melphalan and prednisone for the treatment of adult patients with newly diagnosed multiple myeloma who are inelegible for autolous stem cell transplant.

Moreover, the subcutaneous injection of Daratumumab has been reviewed and approved, since several trials have shown non-inferiority to intravenous Daratumumab administration with regards to PK concentrations and efficacy as well as an improved safety profile (Mateos et al, The Lancet Haematology 2020: https://doi.org/10.1016/S2352-3026(20)30070-3; San-Miguel et al, Haematologica 2020: Doi:10.3324/haematol.2019.243790).

For the pharmacology, pharmacokinetics, pharmacodynamics, (pre)clinical experience and safety profile of Daratumumab please be referred to appendix 0, section F2.

6 Study objectives

Primary objective

- To determine the efficacy, defined as overall response rate* (ORR), of 9 cycles of ixazomib, daratumumab and low dose dexamethasone
  * overall response will be defined as (stringent) complete response ((s)CR), very good partial (VGPR) response and partial response (PR) (appendix 0)

Secondary objectives

- To determine the tolerability, defined as discontinuation rate due to treatment related toxicity, of 9 cycles of ixazomib, daratumumab and low dose dexamethasone
- To determine adverse events of CTCAE grade 2-4
- To determine complete response (CR) and very good partial response (VGPR) after 9 induction cycles
- To determine complete response (CR) and very good partial response (VGPR) on protocol
- To determine immunophenotypic complete response after 9 induction cycles
- To determine immunophenotypic complete response on protocol
- To determine the flow Minimal Residual Disease negative complete remission
- To determine the imaging plus flow MRD negative complete remission
- To determine progression free survival (PFS)
- To determine overall survival (OS)

- To determine efficacy of therapy determined as time to response and the time to best response
- To determine the effect of maintenance therapy with ixazomib and daratumumab in terms of improvement of response during maintenance
- To determine the tolerability of maintenance therapy, defined as discontinuation rate due to treatment related toxicity of ixazomib and daratumumab
- To determine time to next treatment
- To determine PFS2
- To evaluate quality of life (QoL)

Exploratory objectives

- To identify geriatric assessment outcomes that predict feasibility and the toxicity of treatment
- To identify biological markers; sarcopenia and senescence markers, that reflect biological age and that predict feasibility and the toxicity of treatment
- To identify immunological and molecular prognostic markers that predict outcome and toxicity
- To identify biomarkers for response
- To investigate the prognostic value of Minimal Residual Disease
- To investigate the prognostic value of FDG-PET-CT at diagnostics and in follow up

7 Study design

This is a multicenter phase II clinical trial, with upfront registration. Details of all treatments (dose and schedule) are given in section 9.

1. Patients will be registered, and then they will be treated with 9 cycles of induction therapy with ixazomib - daratumumab – low dose dexamethasone;
2. If no progression after induction, patients will be treated with maintenance treatment with ixazomib and daratumumab until progression for a maximum of 2 years.

8 Study population

All eligible patients have to be registered before start of treatment.

8.1 Eligibility for registration

All patients must meet all of the following eligibility criteria.

8.1.1 Inclusion criteria

- Previously untreated patients with a confirmed diagnosis of multiple myeloma according to IMWG criteria (see appendix 0);
- Measurable disease according to the IMWG criteria (see appendix A);
  (If plasmacytoma is the only measurable parameter, the patient is not allowed to be included in the study, because of difficult response evaluation)
- Patients who are either unfit or frail according to the IMWG criteria (see appendix 0);
- Age 18 years or older;
- Absolute neutrophil count (ANC) ≥ 1.0 $x10^9$/l and platelet count ≥ $75x10^9$/l;
  Platelet transfusions and G-CSF to help patients meet eligibility criteria are not allowed;
- Written informed consent, including consent for additional bone marrow and blood sampling and a skin biopsy (with the understanding that consent may be withdrawn by the patient at any time without consequences to future medical care);
- Patient is capable of giving informed consent;
- Negative pregnancy test at study entry (only for women of childbearing potential);
- Male patients and female patients of childbearing potential must agree to use adequate contraception from the time of signing the informed consent form through 90 days after the last dose of study drug (see section 9.4 for details).

8.1.2 Exclusion criteria

- Non-secretory MM;
- Plasma cell leukemia;
- Systemic Amyloid Light-chain (AL) amyloidosis;
- Central nervous system involvement;
- Known allergy to any of the study medications, their analogues, or excipients in the various formulations of any agent;
- Neuropathy, grade 1 with pain or grade ≥ 2;
- Severe cardiac dysfunction (NYHA classification III-IV, appendix 0);
- Screening 12-lead ECG showing a baseline QT interval as corrected by Fridericia's formula (QTcF) >470 msec;
- Chronic obstructive pulmonary disease (COPD) with an Forced Expiratory Volume in 1 second (FEV1) < 50% of predicted normal. Note that FEV1 testing is required for patients suspected of having COPD and subjects must be excluded if FEV1 <50% of predicted normal;

- Moderate or severe persistent asthma within the past 2 years or currently uncontrolled asthma of any classification. (Note that subjects who currently have controlled intermittent asthma or controlled mild persistent asthma are allowed in the study);
- Significant hepatic dysfunction (total bilirubin ≥ 3 x ULN or transaminases ≥ 5 times normal level) except patients with Gilbert's syndrome as defined by > 80% unconjugated bilirubin;
- Creatinine clearance <20 ml/min or Calculated Glomerular Filtration Rate [ml/min/1.73m^2] <20;
- Patients with active, uncontrolled infections;
- Patients known to be Human Immunodeficiency Virus (HIV)-positive;
- Patients seropositive for hepatitis B, defined by a positive test for hepatitis B surface antigen [HBsAg]. Patients with resolved infection (ie, subjects who are HBsAg negative but positive for antibodies to hepatitis B core antigen [anti-HBc] and/or antibodies to hepatitis B surface antigen [anti-HBs]) must be screened using real-time polymerase chain reaction (PCR) measurement of hepatitis B virus (HBV) DNA levels. Those who are PCR positive will be excluded. EXCEPTION: Subjects with serologic findings suggestive of HBV vaccination (anti-HBs positivity as the only serologic marker) AND a known history of prior HBV vaccination, do not need to be tested for HBV DNA by PCR.
- Patients seropositive for hepatitis C (except in the setting of a sustained virologic response [SVR], defined as aviremia at least 12 weeks after completion of antiviral therapy).
- Known GI disease or GI procedure that could interfere with the oral absorption or tolerance of ixazomib including difficulty swallowing;
- Active malignancy other than MM requiring treatment or a malignancy that has been treated with chemotherapy currently affecting bone marrow capacity;
- Systemic treatment, within 14 days before the first dose of ixazomib, with strong CYP3A inducers (rifampin, rifapentine, rifabutin, carbamazepine, phenytoin, phenobarbital), or use of St. John's wort;
- Pre-treatment with cytostatic drug, immunomodulatory drugs (IMiDs) or proteasome inhibitors. Radiotherapy (provided the involved field is small and there are ≥ 7 days between radiotherapy and administration of ixazomib) or a short course of steroids (e.g. 4 day treatment of dexamethasone 40 mg/day or equivalent) are allowed;
- Major surgery within 14 days before enrollment;
- Any serious medical or psychiatric illness, or familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule;
- Participation in other clinical trials, including those with other investigational agents not included in this trial, within 30 days of the start of this trial and throughout the duration of this trial;
- Female patients who are lactating.

9 Treatment

9.1 Induction treatment with ixazomib/daratumumab/low dose dexamethasone

9.1.1 Treatment schedule

Induction therapy should start within 4 weeks after patient registration. Patients will receive 9 cycles of ixazomib, daratumumab and low-dose dexamethasone according to the schedule below.
Every next cycle will start at day 29.

Agent	**Dose**	**Route of administration**	**Days of a 28-day cycle**
Ixazomib	4 mg*	p.o	1,8 and 15
Daratumumab**	16 mg/kg or 1800 mg	i.v. s.c.	Cycle 1-2: 1, 8, 15 and 22 Cycle 3-6: 1 and 15 Cycle 7-9: 1
Dexamethasone***	20 mg 10 mg	i.v.	Cycle 1-2 : 1, 8, 15 and 22 Cycle 3-6: 1 and 15 Cycle 7-9: 1

* adapt the dose to 3 mg in case of creatine clearance 20-30 ml/min or in case total bilirubin ≥ 1.5 - < 3 x ULN or transaminases ≥ 2 and < 5 times normal level.
** for additional pre and post-treatment medication to prevent infusion reaction see appendix 0 - J2
*** in case daratumumab dose is withhold, dexamethasone may be administered orally (p.o.) instead of i.v. Additionally, when no grade >1 IRRs occurred during the first induction cycle, dexamethasone may be taken orally at home before administration of daratumumab from cycle 2 onwards

After cycle 1, 2, 3, 5, 7 and 9 evaluation will take place. In case of progressive disease after cycle 3, 5, 7 or 9 patients will go off protocol treatment.

9.1.2 Dose adjustments during treatment with ixazomib/daratumumab/low dose dexamethasone

During the first cycle, the first dose of ixazomib and daratumumab will always be administered in a 100% dose (apart from ixazomib for impaired renal or liver function as described in 9.1.1.), independently of blood cell counts.

For next cycles to begin the patient must meet the following criteria:

- ANC ≥ $1.0x10^9$/L
- platetelets ≥ $75x10^9$/L
- other non-hematological toxicities (except alopecia) ≤ CTCAE grade 2 or the patient's baseline condition.

If the patient fails to meet the above-cited criteria for initiation of the next cycle of treatment, dosing should be delayed for 1 week. At the end of that time, the patient should be re-evaluated to determine whether the criteria have been met. If the patient continues to fail to meet the above-cited criteria, delay therapy and continue to re-evaluate. The maximum delay before treatment should be discontinued is 6 weeks.

For specific details see the flow chart in appendix 0 and tables in appendix 0 (for ixazomib) and flow chart in appendix 0 and tables in appendix 0 (for daratumumab).

During a cycle for ixazomib, in case platelets are ≤ $25x10^9$/L and/or neutrophils are ≤ $0.5x10^9$/L at day 8, 15, 22 and/or 29, doses of ixazomib will be adjusted according to the flow sheet in appendix 0 and the table in appendix 0.

During a cycle for daratumumab, in case platelets are ≤ $50x10^9$/L with bleeding or platelets are ≤ $25x10^9$/L, in case of febrile neutropenia or in case neutrophils are ≤ $0.5x10^9$/L, the dose of daratumumab will not be adjusted, but delayed according to the flow sheet in appendix 0 and the table in appendix 0.

Instructions for intake/infusion are given in appendix 0 (ixazomib) and 0 (daratumumab).

Instructions for dose adjustments for dexamethasone are given in appendix 0.

9.1.3 Supportive care during induction therapy

- All patients will receive Herpes Zoster prophylaxis with valacyclovir at a dose of 500 mg two times daily during treatment with ixazomib until 6 weeks after discontinuation of ixazomib and/or daratumumab.
- Patients will receive prophylactic therapy with antibiotics during the induction phase (cotrimoxazole 480 mg/day, or in case of allergy levofloxacin 500 mg/day) and thereafter at the discretion of the treating physician.
- Patients will receive prophylactic antifungal therapy according to local policy by example fluconazole 50 mg.
- All patients need to receive proton pump inhibitior (esomeprazole 40 mg or pantoprazole 40 mg) at least once daily for the prevention of gastro-esophageal disease.
- Treatment with bisphosphonates, either pamidronate 30 mg/month or zoledronate 4 mg/month is advised and will be continued for at least 2 years or longer at the discretion of the treating physician.

- Myeloid growth factor (eg, granulocyte colony-stimulating factor [G-CSF]) is permitted, according to institutional practice.

9.2 Maintenance treatment with ixazomib and daratumumab

9.2.1 Treatment schedule

After the response evaluation of the last ixazomib-daratumumab-low dose dexamethasone cycle patients will start maintenance therapy. Maintenance treatment with ixazomib and daratumumab will be started within a maximum of 12 weeks after start of the last induction ixazomib-daratumumab-low dose dexamethasone cycle according to the schedule below.
Maintenance cycles will be repeated at 56-days intervals until progression for a maximum of 2 years or when a medical condition occurs that requires discontinuation of the treatment.

Agent	Dose	Route	Days of a 56-day cycle
Ixazomib	4 mg*	p.o	1, 8, 15, 29, 36 and 43
Daratumumab	16 mg/kg** or 1800 mg	i.v. s.c.	1
Dexamethasone	10 mg	i.v./ p.o.***	1

* or at the dose level during induction therapy in case dose reductions were required during induction therapy
** for additional pre- and post-treatment medication to prevent infusion reaction see appendix 0 - J2.

*** in case daratumumab is withhold discontinue dexamethasone. When during the 1st induction cycle no IRRs grade >1 have occurred, dexamethasone may be taken orally at home before administration of daratumumab.

Response evaluation will take place as described in section 10.3.

9.2.2 Dose adjustments during maintenance treatment with ixazomib and daratumumab

Dose delay and reduction instructions for ixazomib are given in appendix 0 (flow sheet) and 0 (tables) and dose delay instructions for daratumumab are given in appendix 0 (flow sheet) and 0 (tables).

9.2.3 Supportive care during maintenance therapy

Similar to induction therapy – see paragraph 9.1.3

9.3 Excluded Concomitant Medications and Procedures

The following medications and procedures are prohibited during the study:
Systemic treatment with any of the following metabolizing enzyme inducers should be avoided unless there is no appropriate alternative medication for the patient to use. (Rationale: If there were to be a drug-drug interaction with an inducer, ixazomib exposure would be decreased):

- Strong CYP3A inducers: rifampin, rifapentine, rifabutin, carbamazepine, phenytoin and phenobarbital
- Excluded foods and dietaty supplement include Sr. John's wort
- Any antineoplastic treatment with activity against MM except for drugs in this treatment regimen.
- Radiation therapy (the requirement for local radiation therapy generally indicates disease progression).
- Platelet transfusions and G-CSF to help patients meet eligibility criteria for inclusion in the study are not allowed, when on protocol G-CSF is allowed to meet the criteria for start of a new cycle and both platelet transfusions and G-CSF are allowed when clinically indicated.

9.4 Precautions and Restrictions

9.4.1 Prevention and treatment of Infusion-Related Reactions

Guidelines for the prevention of infusion related reactions (IRR) during daratumumab infusion are given in appendix 0.

9.4.2 NSAID

Nonsteroidal anti-inflammatory drugs (NSAIDs) should be avoided in patients with impaired renal function given reported NSAID-induced renal failure in these patients. Fluid deficit should be corrected before initiation of treatment and during treatment.

9.4.3 Pregnancy

It is not known what effects ixazomib has on human pregnancy or development of the embryo or fetus. Therefore, female patients participating in this study should avoid becoming pregnant, and male patients should avoid impregnating a female partner. Nonsterilized female patients of reproductive age group and male patients should use effective methods of contraception through defined periods during and after study treatment as specified below.

Female patients must meet 1 of the following:

- Postmenopausal for at least 1 year before the screening visit, or
- Surgically sterile, or
- If they are of childbearing potential, agree to practice 2 effective methods of contraception from the time of signing of the informed consent form through 90 days after the last dose of study drug, or

- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject (periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods] and withdrawal are not acceptable methods of contraception).

Male patients, even if surgically sterilized (ie, status postvasectomy) must agree to 1 of the following:

- Practice effective barrier contraception during the entire study treatment period and through 90 days after the last dose of study drug, or
- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject (periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods for the female partner] and withdrawal are not acceptable methods of contraception).

9.4.4 Daratumumab Interference with Indirect Antiglobulin Test (IAT) results

Daratumumab interferes with the Indirect Antiglobulin Test (IAT), which is a routine pre-transfusion test performed to identify a patient's antibodies to minor antigens so that suitable donor blood can be given for transfusion. Daratumumab does not interfere with ABO/RhD typing. CD38 is expressed at very low levels on erythrocytes. Daratumumab binds to the CD38 on erythrocytes, which results in a positive IAT (Indirect Coombs Test). This positive result masks the detection of antibodies to minor antigens and may prevent or delay blood banks from issuing donor blood for transfusion. This effect occurs during daratumumab treatment and for up to 6 months after treatment ends.

Prior to enrolling the patient in the study, you should notify the blood transfusion sevices of the fact that your patient will participate in a study with Daratumumab. Information such as the expected date of starting treatment and recent transfusion history should be provided.
Prior to starting the Daratumumab therapy, material should be sent to the blood blank for determination of the ABO, Rh, extensive phenotyping and IAT, according to the protocol that will be provided by HOVON the blood transfusion laboratories.

Subjects will receive a patient identification wallet card for the study that includes the blood profile (ABO, Rh, extensive phenotyping and IAT along with information on the IAT interference for healthcare providers/blood banks. Subjects are to carry this card throughout the treatment period and for at least 6 months after treatment ends.
Possible methods for blood banks to provide safe RBCs for transfusion to subjects receiving daratumumab include:

a) Providing ABO/RhD compatible, phenotypically or genotypically matched units in case a complete phenotyping/genotyping of the patient is available;
b) In case this information is not present ABO/RhD compatible, K-negative units after ruling out or identifying alloantibodies using dithiothreitol (DTT)-treated reagent RBCs can be provided.

Uncrossmatched, ABO/RhD compatible RBC units should be administered if transfusion is needed emergently as per local blood bank practice.

The laboratory manuals describing the procedures for pre-treatment bloodtyping and for eliminating the daratumumab IAT interference by treating reagent RBCs with dithiothreitol (DTT) will be provided. Of note; despite daratumumab binding to CD38 on erythrocytes, no indication of clinically significant hemolysis has been observed in daratumumab studies.

9.5 Investigational Medicinal Product: Ixazomib

Also see appendix 0 – section F1.

9.5.1 Summary of known and potential risks

The emerging safety profile indicates that ixazomib is generally well tolerated with manageable and reversible AEs with both the IV and PO formulations. Ixazomib may be harmful to the unborn child.
The most frequent (at least 20%) treatment-emergent adverse events (TEAEs) reported with the PO formulation pooled from single-agent studies (n = 201) irrespective of causality to ixazomib, include nausea (53%), fatigue (51%), diarrhea (44%), thrombocytopenia (34%), vomiting (38%), decreased appetite (32%), fever (21%), and anemia (21%). Overall rash of all grades is reported in approximately 50% of patients and is more common when ixazomib is given in combination with lenalidomide where rash is an overlapping toxicity. For al full overview of toxicity reported with ixazomib treatment we refer to the Investigator's Brochure.

9.5.2 Preparation and labeling

Ixazomib will be labeled as an Investigational Medicinal Product.
The ixazomib capsules will be provided by Takeda. The study drug will be labeled. Packaging labels will fulfill all requirements specified by governing regulations.
The ixazomib capsule formulation consists of drug substance, microcrystalline cellulose, talc, and magnesium stearate. The capsules are individually packaged in cold form foil-foil blisters. The 2.3-, 3.0-, and 4.0 mg capsules used in this trial are supplied as a 1 x 3 blister card in a child-resistant cardboard wallet. Each capsule strength has a unique color. Dosage strength is stated as the active boronic acid.

9.5.3 Storage and handling

Ixazomib is an anticancer drug and as with other potentially toxic compounds caution should be exercised when handling ixazomib capsules.

Upon receipt at the investigative site, ixazomib should remain in the blister and carton provided until use or until drug is dispensed. The container should be stored at the investigative site temperature controlled (2°C to 30°C, 36°F to 86°F). Ensure that the drug is used before the retest expiry date provided by Takeda. Expiry extensions will be communicated accordingly with updated documentation to support the extended shelf life.

In countries where local regulations permit, ixazomib capsules dispensed to the patient for take-home dosing should remain in the blister packaging and temperature controlled as noted above until the point of use. The investigative site is responsible for providing the medication to the patient in the correct daily dose configurations. Comprehensive instructions should be provided to the patient in order to ensure compliance with dosing procedures. Patients who are receiving take-home medication should be given only 1 cycle of medication at a time. Patients should be instructed to store the medication at room temperature (36°F to 86°F, 2°C to 30°C) for the duration of each cycle. Any extreme in temperature should be reported as an excursion and should be dealt with on a case-by-case basis.

Because ixazomib is an investigational agent, it should be handled with due care. Patients should be instructed not to chew, break, or open capsules. In case of contact with broken capsules, raising dust should be avoided during the clean-up operation. The product may be harmful by inhalation, ingestion, or skin absorption. Gloves and protective clothing should be worn during clean-up and return of broken capsules and powder to minimize skin contact.

The area should be ventilated and the site washed with soap and water after material pick-up is complete. The material should be disposed of as hazardous medical waste in compliance with federal, state, and local regulations.

In case of contact with the powder (eg, from a broken capsule), skin should be washed immediately with soap and copious amounts of water for at least 15 minutes. In case of contact with the eyes, copious amounts of water should be used to flush the eyes for at least 15 minutes. Medical personnel should be notified. Patients are to be instructed on proper storage, accountability, and administration of ixazomib, including that ixazomib is to be taken as intact capsules.

9.5.4 Study drug supply

The sponsor will arrange delivery of ixazomib 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.

9.5.5 Drug accountability

When ixazomib capsules are dispensed to the patient for take-home dosing, patients should be instructed to return their empty blister packs to the trial site, rather than discarding them. Reconciliation will occur accordingly when the patient returns for their next cycle of take-home medication.
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). Study drugs will be administered or dispensed only to eligible patients under the supervision of the investigator or identified sub-investigator(s). 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.

9.5.6 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 the trial or after expiry of the product unused investigational medicinal product should be destroyed by the trial site according to site's standard procedures. Destruction should be documented.

9.6 Investigational Medicinal Product: Daratumumab

Also see appendix 0, section F2.

9.6.1 Summary of known and potential risks

The emerging safety profile indicates that apart from infusion-related reactions, which occur mostly during the first infusion, daratumumab is generally well tolerated with maneagable and reversible AEs. Daratumumab may be harmful to the unborn child.

The following very common (≥10%) and common (≥1% to <10%) Adverse Drug Reactions (ADRs) have been identified in MM patients treated with daratumumab 16mg/kg: (For a complete overview we refer to the current version of the Investigators Brochure/Addendum.)
Very common: Infusion related reactions, bronchitis, neutropenia, thrombocytopenia, anemia, lymphopenia, leukopenia, decrease apetite, peripheral sensory neuropathy, headache, paresthesia,

hypertension, cough, dyspnoea, constipation, diarrhoea, nausea, vomiting, muscle spams, oedema peripheral, fatigue, pyrexia, asthenia, back pain, pneumonia
Common: Infusion related reaction, Upper respiratory tract infection, Influenza, Lower respiratory tract infection, Sepsis, Atrial fibrillation, Shingles (Herpes zoster), hyperglycaemia, hypocalcaemia, dehydratation, bronchospasm, hypoxia, pulmonary oedema, pneumonitis, laryngeal oedema, chills.

Daratumumab subcutaneous injections had an improved safety profile as compared to the intravenous administration (Mateos et al Lancet Hematol 2020;7:370-80). In the randomized controlled COLUMBA trial, that compared daratumumab subcutaneous versus daratumumab intravenously in patients with relapsed refractory MM, only neutropenia grade ≥3 was observed more often in the subcutaneous arm: 13% vs 8%. In contrast, with intravenous daratumumab, infusion-related reactions led to dose interruptions in 31% of patients, decreases in infusion rate in 10% of patients and <1% discontinuation whereas no reactions with daratumumab subcutaneous led to interruptions, incomplete dose administration or discontinuation. Injection-site reactions, all grade 1-2 were seen in 7% of patients.
The following very common (≥10%) and common (≥1% to <10%) Adverse Drug Reactions (ADRs) that have been observed with flat dose of 1800mg daratumumab subcutaneous co-formulated with recombinant human hyaluronidase PH20 (rHuPH20) 2000 U/ml:
Very common: anemia, neutropenia, thrombocytopenia.
Common: leukopenia, lymphopenia, febrile neutropenia, hypertension, hyponatremia, bone pain, pneumonia, back pain, lower respiratory tract infection, acute kidney injury, diarrhoea, fatigue, cough, dyspnoea, nausea, chills, hypokalaemia, upper respiratory infection, pyrexia, general physical health deterioration.

9.6.2 Preparation of daratumumab

9.6.2.1. Daratumumab intravenously

Infusion solution will be prepared as a dilution of daratumumab in sterile, pyrogen-free 0.9% NaCl. Preparation of infusion bags should be done on the day of the planned infusion. Daratumumab must be administered as an IV infusion given through a well-functioning IV catheter by using an infusion pump or syringe pump. The study drug must be filtered by using an inline filter (0.2 µM) during the infusion. Please refer to the SmPC for detailed descriptions for preparation and administration of daratumumab and Appendix J1. Furthermore, a 90-minute administration of intravenous daratumumab has been studied in a single center trial in the US that enrolled 28subjects. All subjects received a rapid infusion of daratumumab on the third or higher administration with no episode of a Grade 3 or higher IRR (Barr et al. Blood 2017 130: 1889). Additionally, this rapid infusion has been implemented in Janssen's sponsored trials in the US, Griffin, MMY2004 and multiple investigator-initiated trials. Hence and presently, also in the

HOVON143 trial, for patients without an history of infusion related reactions after the third daratumumab dose, the duration of infusion may be shortened to a 90-minute infusion starting in cycle 2 onwards.

9.6.2.2. Daratumumab subcutaneously

Daratumumab s.c. is supplied as a single use, sterile, liquid product in a glass vial. Each vial contains approximately 16 ml of solution which includes 120 mg/ml of daratumumab, 10 mM Histidine, 300 mM sorbitol, 1 mg/ml Methionine, 0.04% PS20, 2000 U/ml of RhuPH20 at pH 5.6. Filtration of daratumumab 1800 mg during preparation of the dosing syringe is mandatory.

9.6.3 Storage and handling

Daratumumab (i.v. and s.c.) should be stored refrigerated (2°C to 8°C, 36°F to 46°F) and handled in accordance with the instructions in the summary of product characteristics. The investigational medicinal product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.

9.6.4 Study drug supply

Janssen will supply daratumumab. The sponsor will arrange delivery of daratumumab 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.

9.6.5 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.

9.6.6 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)]
HBV serology and HBV DNA test	x[15)]	x[16)]	x[16)]	x[16)]

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 3-6 every 2 weeks, cycle 7-9 every 4 weeks, more often in case of dose modification/delay.
3) In case of ≥VGPR and in patients with an **IgG kappa M-protein** only 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 0, 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.
15) **Local testing for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (Anti-HBs) and hepatitis B core antibody (Anti-HBc).** At screening, subjects with active hepatitis B virus (HBV) infection (HBsAg positive) are excluded from the study. In addition, patients with serologic evidence of resolved HBV infection (i.e., positive anti-HBc with or without positive anti-HBs) but with a positive HBV DNA PCR are also excluded from the study. For details see HBV serology testing below.
16) **Local testing for HBV DNA:** Subjects on protocol treatment with serologic evidence of resolved HBV infection (i.e., positive anti-HBc with or without positive anti-HBs), the serum value of the ALAT should be monitored every cycle and HBV DNA testing by PCR must be performed Q12W during treatment, at the End of Treatment Visit, and Q12W for up to 6 months after the last dose of study treatment. Antiviral therapy must be started in subjects with active HBV infection (HBsAg positive and/or HBV DNA present). For more details see HBV DNA testing below and appendix J5 for information on Management of HBV reactivation.

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.

HBV serology

Within 3 months before registration, all patients will be screened locally for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (Anti-HBs) and hepatitis B core antibody (Anti-HBc). Additionally, subjects ongoing in the treatment phase who are within 6 months of starting study treatment when protocol version 5.0 amendment is implemented will be required to have HBV serology performed locally upon signing the updated informed consent.

Interpretation overview of the of common HBV test profiles:

HBsAg	anti HB-core	anti-HBs	HBV-DNA	
-	-	+	-	HBV vaccination
-	+	+/-	-	resolved HBV infection
+	+	-/+	+	Active HBV infection

Subjects who are candidates for study inclusion, but with an active HBV infection (HBsAg positive) or with with serologic evidence of resolved HBV infection (anti-HBc positive) but with a positive HBV-DNA PCR are excluded from the study.

HBV serology is not required 1) at screening or within 6 months of treatment when this was already performed as part of standard of care within 3 months prior to first dose, or 2) when subject received prior HBV vaccination (history of vaccination in combination with a positive anti-HBs without positive HBsAg and anti-HBc).

Antiviral therapy should be started in patients who are HBsAg positive with presence of HBV DNA. See appendix J5 for information on Management of Hepatitis B Virus reactivation.

HBV DNA testing

Subjects with serologic findings suggestive of HBV vaccination (Anti-HBs positivity as the only serologic marker) and a known history of prior HBV vaccination do not need to be tested for HBV DNA by PCR.

During and following study treatment subjects who have history of HBV infection or who are positive for anti-HBc or Anti-HBs will be closely monitored for clinical and laboratory signs of reactivation of HBV as follow:

- Serum ALAT levels before start treatment cycle (Q4W during induction and Q8W during maintenance). If ALAT levels are increased also preform HBV DNA testing by PCR;
- HBV DNA testing by PCR:
    - Q12W during treatment
    - At the End of Treatment Visit
    - Q12W for up to 6 months after the last dose of study treatment.

If HBV DNA is present, then antiviral therapy must be started and study treatment needs to be interrupted. See appendix J5 for information on Management of Hepatitis B Virus reactivation. Where required by local law, the results of HBV testing may be reported to the local health authorities.

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 patients with an IgG kappa M-protein and 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 0 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 0
- Questionnaires about QoL and neurotoxicity, geriatric assessments and CCI; see Hovon website and appendices 0 and, 0.

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 0.

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 0.

10.4 Quality of Life and geriatric assessments

All questionnaires can be downloaded from the HOVON website.

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

Questionnaire	**To be completed by**	**Before 1st dose Daratumumab SC**	**After 1st dose daratumumab SC**	**After 2nd dose daratumumab SC**	**6 months after 1st dose Daratumumab SQ**
Patient preference survey daratumumab IV vs SC	Patient	X	X	X	X

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 0).

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.

10.4.5 Patient preference survey Daratumumab IV versus SC administration

In case a patient switches daratumumab from intravenously to subcutaneous administration, the patient preference survey needs to be filled a maximum of four times: before switch, after 1 dose, after 2 doses and after 6 months from switching.

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

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can also decide to withdraw a patient from protocol treatment for other reasons than the criteria described above. Examples of such reasons for withdrawal from protocol treatment are:

- ♦ Excessive toxicity
- ♦ Refusal of patient to continue protocol treatment

- No compliance of the patient: patient is unable or unwilling to adhere to the treatment schedule and/or procedures required by the protocol

Patients who are withdrawn from protocol treatment will receive medical care according to local practice

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 section 10.2 for follow up. (S)AE information will be collected as described in section 12.2 and 12.3.

However, for patients who are withdrawn from treatment because in hindsight they did not fulfill the eligibility criteria (see section 8.1) at time of enrollment, data will be collected until 30 days after the last protocol treatment given. (S)AE information will be collected as described in section 12.2 and 12.3.

11.3 Premature termination of the study

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

- 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 enrollment of more patients; for example insufficient enrollment 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 administered a medicinal product and which 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 considered related to the medicinal (investigational) product. This includes any newly occurring event, or a previous condition that has increased in severity or frequency since the administration of study drug.

Serious adverse event (SAE)

A serious adverse event is defined as any untoward medical occurrence or effect 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. It does not refer to an event which hypothetically might have caused death if it were more severe)
- Inpatient **hospitalization or prolongation of an existing hospitalization** (see clarification in the paragraph below on planned hospitalizations).
- Significant / persistent disability or incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- 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 above characteristics/consequences, including suspected transmission of infectious agents by a medicinal product).

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 events

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 0).
Pre-existing conditions will be collected on the baseline concomitant diseases CRF, i.e. active (symptomatic) diseases of *CTCAE grade ≥ 2 (*however ***neuropathy grade 1 also has to be reported),*** diseases under treatment, chronic diseases and long term effects of past events as present at the time of baseline assessment.

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

- A pre-existing condition that does not increase in severity; the pre-existing condition should be reported on the baseline concomitant diseases CRF
- AE's of CTCAE grade 1, however ***neuropathy grade 1 has to be reported***
- An abnormal laboratory value that does not lead to discontinuation or delay in treatment, or dose modification, or therapeutic intervention, and is not considered by the investigator to be a clinically significant change from baseline
- Relapse/progression of the disease under study; complications as a result of disease progression remain reportable Adverse Events

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. Only follow up information of adverse events that led to discontinuation of protocol treatment and neuropathy should be reported on the AE CRF until recovery or until 6 months after the last dose of IMP, whichever comes first.
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. 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:

- Relapse/progression of the disease under study; complications as a result of disease progression remain reportable Serious Adverse Events
- Hospitalization for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- 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.
- Prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- Hospitalization for a procedure that was planned prior to study participation (i.e. prior to registration). 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, to Takeda Pharmacovigilance (or designee) and to Janssen Pharmaceuticals.
The HDC safety desk will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR).
The IB *w*ill be used as a reference document for expectedness assessment.
Where reporting of SAE's to the Ethics Committee is required by national laws or regulations or by the procedures of the Ethics Committee, the HOVON Data Center will report those SAE's by means of a six-monthly SAE line listing.

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), Takeda Oncology, Janssen Pharmaceuticals and the investigators in compliance with applicable laws and regulations, and in accordance with any trial specific agreements between the sponsor and Takeda Oncology or Janssen Pharmaceuticals

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 Reporting Reporting special situations

Overdose, abuse, misuse, medication error or occupational exposure are special reporting situations and must be reported to HOVON Data Center immediately.

Please inform HOVON Data Center of these events within 24 hours after the event was known to the investigator by email (hdc@erasmusmc.nl). Note that these special reporting situations in and of themselves are not AEs. If a special reporting situation results in an SAE, an SAE form should be completed and sent to HOVON Data Center (see section **Error! Reference source not found.**).

12.6 Pregnancies

Pregnancies of a female subject or the female partner of a male subject, occurring while the subject is on protocol treatment or within 90 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.

If the subject is on study drug, the study drug is to be discontinued immediately and the subject instructed to return any unused portion of the study drug to the Investigator. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Sponsor who will inform the manufacturer immediately. All details should be documented on the pregnancy form CRF. The patient should be referred to an obstetrician/gynaecologist experienced in reproductive toxicity for further evaluation and counseling.

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 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. The HOVON Data Center will forward all pregnancy reports within 24 hours of receipt to the Principal Investigator and the manufacturer of the investigational medicinal products Takeda Oncology and Janssen Pharmaceuticals.

12.7 Second Primary Malignancies

Second primary malignancies (SPM) will be monitored as events of interest and must be reported as serious adverse events. This includes any second primary malignancy, regardless of causal relationship to any study drug, occurring at any time for the duration of the study, from the time of signing informed consent until a minimum of three years after the last dose of the investigational product.
Events of second primary malignancy are to be reported using the SAE report form and must be considered an “Important Medical Event” even if no other serious criteria apply. Documentation on the diagnosis of the second primary malignancy must be provided at the time of reporting as a serious adverse event (e.g. pathology report).
SPM must also be documented in the other appropriate page(s) of the CRF (e.g. Adverse Event Form and Follow up Form).

For each case of SPM occurring during treatment, contact the Principal Investigator to discuss if treatment needs to be discontinued.

12.8 Reporting of safety issues

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities 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 local investigator will inform the patients and local ethics or review committees according to hospital policy. The sponsor will inform any other parties that are involved in the trial.

12.9 Annual safety report

The sponsor will submit once a year a safety report to the Ethics Committees and Competent Authorities of the concerned Member States. The first report is sent one year after the first approval date of the trial. Subsequent reports are sent annually until end of trial. 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'.*

12.10 Data Safety and Monitoring Board

A data and safety monitoring board will be installed before start of the study.
The DSMB will advise the Principal Investigator, co-investigators and the chair of the working group in writing about the continuation of the trial. The DSMB will review the general progress and feasibility of the trial, the quality and completeness of the data, adverse events and safety. The DSMB will consider if there is any concern regarding the safety and well-being of trial subjects or regarding the scientific validity of the trial results. The DSMB will base its advice on the reports provided by the statistician. The DSMB is free to take into consideration external information, such as the (interim) results of other trials or literature reports.
The DSMB consists of at least three members, with at least one statistician and two physicians. Details of the DSMB constitution and tasks are documented in the trial specific DSMB charter.

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

- Interim analyses reports (as described in 14.3)
- Annual safety data listing the incidence of (serious) adverse events, (serious) adverse reactions and SUSAR
- Annual progress data listing the number of enrolled patients and the status of data collection

12.11 Product Complaints

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product.

Individuals who identify a potential product complaint situation concerning ixazomib or daratumumab should immediately contact Takeda or Janssen (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Takeda Quality representative.

For Product Complaints IXAZOMIB
• Phone: 1-844-N1-POINT (1-844-617-6468) • E-mail: GlobalOncologyMedinfo@takeda.com • FAX: 1-800-881-6092 • Hours: Mon-Fri, 9 a.m. – 7 p.m. ET (US)
For Product Complaints DARATUMUMAB
• E-mail to the Global Trial Manager: nminoves@its.jnj.com A back-up contact wil be provided in case of absence

Please also inform the HOVON Data Center of your complaint by fax (+31 (0)10 704 1028) or email (hdc@erasmusmc.nl). Note that product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to HOVON Data Center see section 12.3.1).

13 Endpoints

13.1 Primary endpoint

- Overall response rate (at least PR) on induction therapy.

13.2 Secondary endpoints

- Discontinuation rate due to toxicity of maintenance therapy with Ixazomib and daratumumab.
- Safety and toxicity as defined by type, frequency and severity of adverse events as defined by the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4
- Complete Response and Very Good Partial Response rate after 9 induction cycles and on protocol
- Immunophenotypic Complete Response after 9 induction cycles and on protocol
- Minimal Residual Disease negative flow cytometry of bone marrow on protocol
- PET-CT negative, defined as disappearance of increased tracer uptake at entry, or decrease to less than mediastinal blood pool SUV or decrease to less than surrounding normal tissue
- Progression free survival, defined as time from registration to progression or death from any cause, whichever comes first

- Overall survival, measured from date of registration to death from any cause. Patients alive at the date last contact, will be censored
- Time to (maximum) response
- Improvement in response from the start of maintenance therapy
- Discontinuation rate due to toxicity of 9 cycles of Ixazomib, daratumumab and low-dose dexamethasone.
- Time to next treatment
- PFS2, defined as the time from registration to the date of objective disease progression or death from any cause after second line therapy
- Quality of life as defined by the EORTC QLQ-C30, QLQ-MY20 and EQ-5D-5L definitions

13.3 Exploratory endpoints

- Geriatric assessments (both questionnaires and physical assessments), senescence markers in fibroblasts obtained by skin biopsy and sarcopenia as determined by CT-scan that reflect biological age and predict feasibility and the toxicity of treatment
- Identification of immunological and molecular prognostic markers that predict feasibility and the toxicity of treatment
- Identification of biomarkers for response

14 Statistical considerations

14.1 Patient numbers and power considerations

In this phase II trial we will evaluate the efficacy of 9 induction cycles of ixazomib-daratumumab-low dose dexamethason, followed by ixazomib and daratumumab maintenance until progression for a maximum of 2 years, separately in unfit and frail patients with NDMM.

The overall response rate on induction treatment will be considered as the primary endpoint for the sample size calculation. The optimal Simon 2-stage design will be applied.[18] The efficacy of treatment will be determined in unfit and frail patients separately; results will not be formally compared between these two subgroups.

In elderly trials the percentage of patients reaching PR or better depends on the regimen used, but also on age. This last observation is supported by the data of the IFM trails comparing MPT versus MP. The ORR was 76% in patients aged 65-75, versus 62% in patients over 75 years of age, in whom a lower dose of thalidomide was used. In the First trial, in which 35% of patients were >75, ORR with Rd18 was 77% in patients ≤75 years and 66% in patients >75 years.[2;19;20]. In the VISTA trial ORR was 71%, no

separate data on patients >75 years patients are available.[4] In none of these trials separate data on unfit or frail patients were available.

It is anticipated that the addition of daratumumab to ixazomib-low dose dexamethasone will improve outcome, as compared to the two drug regimen lenalidomide-low dose dexamethasone. Therefore the following parameters and decision rules are used:

for both the unfit and frail population:

- Let P_0 be the largest ORR which, if true, implies that the therapeutic activity is too low and therefore does not warrant further investigation in this patient population. In the present trial, P_0 has been taken as 50%.
- Let P_1 be the smallest ORR rate, which, if true, implies that the regimen is sufficiently active and warrants further investigation. In the present trial, P_1 has been taken as 65%.

Statistical errors will be:

- Let α be the accepted probability of recommending this regimen for further investigation when the true ORR is equal to or lower than P_0. In the present trial, α has been taken as 0.10.

- Let β be the accepted probability of rejecting this regimen from further trials when the true ORR is at least equal to P_1. In the present trial, β has been taken as 0.20

For unfit and frail patients separately, these design parameters imply that a maximum of 60 (eligible) patients will be registered, with an interim analysis after the response data of the first 23 registered patients, who took at least one dose of the drug, are available and have been evaluated:

- If after the first 23 registered patients finish the induction treatment, there are only 12 or less ORR patients on induction treatment, the trial will be closed with the conclusion that the current regimen is not sufficiently active in this patient population, and should not be further investigated. Otherwise entry will be extended to 60 patients.
- If after 60 patients ≤ 34 patients have achieved a PR or better, the conclusion will be that the regimen is not sufficiently active, and should not be further investigated in this patient population.
- Otherwise, the trial will conclude that the regimen is active, and warrants further investigation in this patient population.

Consider 10% drop off rate due to ineligiblilty, 66 unfit and 66 frail patients will be included in this trial. With an expected accrual rate of 6-8 patients per month, entry will be completed in about 18-24 months. While awaiting the results of the interim analyses, accrual will continue.

14.2 Statistical analysis

The data will only be analyzed separately per patient population. Any formal comparison between unfit and frail patients will not be performed, nor will the results of both populations be pooled together. Patients that were initially registered but considered ineligible afterwards based on information that should have been available before registration, will be excluded from all analyses.

The main endpoint for each regimen is the ORR on induction treatment. Patients who achieve a PR or better during or after completion of induction therapy (but before initiation of maintenance therapy or second-line therapy, whichever comes first) will be considered as a success. This includes patients with an early PR or better who progress later while they are still on induction treatment. All other patients will be considered as a failure, including patients without any response evaluation whatever the cause. The numbers of successes at the interim analysis and final analysis will be compared with the critical numbers as provided by the respective Simon 2-stage designs, see before. In addition, the proportion of successes will be calculated along with an exact 95% confidence interval (CI).

14.2.1 Efficacy analysis

The primary efficacy analysis will be based on intention to treat (ITT) population. Estimate of ORR rates for unfit and frail patients of their best responses during induction will be presented with 2-sided 95% exact binomial confidence intervals. Efficacy endpoints will be response rate (sCR, CR, VGPR or PR), time to maximum response, PFS, OS and PFS2. PFS, OS and PFS2 will be estimated using the Kaplan-Meier method and 95% CIs will be constructed. PFS, OS and PFS2 will first be presented at the final analysis of the primary endpoint. In addition, PFS, OS and PFS2 will be updated at the end of maintenance and when the follow up for all patients still alive is 5 years from registration. The proportion of patients that start with maintenance therapy will be calculated. The time to discontinuation of maintenance therapy will be determined, as well as the reasons for discontinuation.

14.2.2 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of adverse events CTCAE grade 2 or more (CTCAE grade 1 events, with the exeption for neuropathy, will not be collected nor tabulated) by patient and for all cycles together. Data from all subjects who receive any study drug will be included in the safety analyses. .

14.2.3 Additional analyses

To investigate the predictive value of geriatric assessments and biological biomarkers with respect to discontinuation rate, we intend to build a multivariable logistic regression model. The model will be validated by applying internal cross-validation technique. The predictive value of the cross-validated model will be assessed in terms of sensitivity, specificity, and positive and negative predictive values.

14.2.4 Statistical analysis plan (SAP)

Before the final analysis, a SAP will be prepared by the trial statistician and approved by the principal investigator. It will describe in detail the analyses to be performed. Deviations from the analyses as specified in par. 14.2.1-14.2.3 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. All analyses except the primary analysis should be considered as hypothesis-generating only.

14.3 Interim analysis

Two interim analyses are planned. One safety interim analysis is planned primarily to describe the adverse events of induction therapy with ixazomib – daratumumab – low dose dexamethasone. This will be done separately for frail and unfit patients, when the first 10 frail and 10 unfit patients have available data regarding cycles 1-4. The accrual will not be discontinued while waiting for these data. Results of the safety interim analysis, separately for frail and unfit patients, will be presented to a DSMB and the PI. The DSMB is free in its public recommendations to the PI and (optionally confidential recommendations) to the study statistician. For the safety interim analysis a detailed report will be generated. It will include the number of entered patients and at that time evaluable patients, treatment given, and incidence of SAE's and other adverse events and infections by grade. Adverse events will be described by summary table broken by site, CTCAE grade and relation to trial treatment. 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, one efficacy interim analysis is planned to describe response observed during the ixazomib - daratumumab - low dose dexamethasone induction therapy. This will be done when of the first 23 registered patients per subgroup the response data of the induction treatment are available. Results of the interim analyses will be presented to a DSMB and the PI.

14.4 Statistical analysis of the quality of life assessement

All patients with the baseline and at least one follow-up QoL questionnaire, separately for QLQ-C30, QLQ-MY20 and EQ-5D-5L, will be included in the analysis. The main purpose will be to describe QoL during induction chemotherapy and maintenance. QoL after start maintenance will also be summarized separately for both subgroups. For patients who start with maintenance, the QoL after the last induction chemotherapy will be considered as baseline.

15 Registration

15.1 Regulatory Documentation

Required regulatory and administrative documents must be provided to the HOVON Data Center before shipment of study drug and 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

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 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
- Sex
- Age at date of registration
- Year of birth
- Date written informed consent
- Specific items patient gives consent for (see ICF)
- Eligibility criteria

All eligibility criteria will be checked with a checklist.
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.

16 Data collection and quality assurance

16.1 Case Report Forms

Data will be collected on electronic 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 trial number, and a combination of patient study number (assigned at registration) and hospital identification.
The e-CRF will be completed on site by the local investigator or sub-investigator or an authorized staff member. All CRF entries must be based on source documents.
. Access to the e-CRF will be provided byHOVON Data Center only to authorized site staff members who have received instructions on the use of the e-CRF. An instruction manual will be provided by HOVON Data Center.

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

This trial is part of the HOVON Site Evaluation Visit program. Site evaluation visits will be performed for HOVON trials to review the quality of the site and not specifically the quality of a certain trial. It will enable HOVON to collect quality data and facilitate improvement of the participating sites. Data cleaning or monitoring of the performance of specific trials is not the goal of the site evaluation visits. Site evaluation visits will be performed according to the site evaluation visit plan.
The HOVON site evaluation visit plan applies to sites in the Netherlands only. Monitoring of the quality of trial conduct in participating sites from other countries will be organized by the coordinating investigator or co-sponsor. The frequency and content of the site visits in other countries will be at least equal to the specifications of the site evaluation visit plan, and are described in a monitoring plan provided by HOVON.

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 site 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

In accordance with regulatory guidelines, audits may be carried out for this study. The investigator is required to facilitate an audit by means of a site visit.
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 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

An accredited Ethics Committee 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 (2013, www.wma.net)), 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 investigator should take into consideration if the patient is capable of giving informed consent. Before informed consent may be obtained, the investigator should provide the patient ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. (At least one week, if medically possible). 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 investigator 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.

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.

The benefit will be that unfit and frail patients can be treated with an oral proteasome inhibitor ixazomib instead of the currently available subcutaneous proteasome inhibitor bortezomib, with considerably less polyneuropathy. Secondly, patients will be treated with daratumumab, a novel class drug, which pronounced effectivity in heavily pretreated patients with limited toxicity only. The burden will be that patients will receive intranvenous daratumumab and dexamethasone in combination with oral Ixazomib instead of a combined oral/sc regimen. Secondly, following induction therapy, maintenance therapy will be given until progression for a maximum of two years. Although a benefit with respect to prolongation of PFS is expected, the extent is currently unknown. Patients may suffer from side effects, although these are generally mild with ixazomib and daratumumab. There are additional procedures required as compared to standard care because of biological assessment of frailty, such as an CT scan to determine the presence of sarcopenia, geriatric assessmentsand a skin biopsy for senescence markers. Patients will undergo an extra BM aspirate for MRD evaluation, a FDG-PET-CT scan for determining prognostic purposes and also participate in Quality of Life 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.
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. The first report is sent one year after the first approval date of the trial. Subsequent reports are sent annually until end of trial. 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 trial, the sponsor will submit an end of 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

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.

All and any publications of (interim) trial results are subject to the HOVON Publication Policy, according to the version of this policy that is effective at the time of publication. The HOVON Publication Policy is available on the HOVON website and a copy can be requested from the HOVON Data Center.

Glossary of abbreviations

(in alphabetical order)

5-HT_3	5-hydroxytryptamine 3 serotonin receptor
ADL	Activity of Daily Life
AE	adverse event
AL	Amyloid Light-chain
ANC	absolute neutrophil count
aPTT	activated partial thromboplastin time
ASCO	American Society of Clinical Oncology
AUC	area under the plasma concentration versus time curve
$AUC_{0\text{-last}}$	area under the plasma concentration versus time curve from zero to `last time'
BCRP	breast cancer resistance protein
BJ	Bence Jones
BM	bone marrow
BSA	body surface area
Ca	Calcium
CA	Competent Authority
CBC	complete blood count
CCI	Charlson Comorbidity Index
CFR	Code of Federal Regulations
CGA	Comprehensive Geriatric Assessment
C_{max}	single-dose maximum (peak) concentration
CR	complete remission
CRAB	Calcium elevation, Renal insufficiency, Anemia and Bone abnormalities
CRF	Case Report Form
CRP	C-reactive protein
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DDI	drug-drug interaction
DFS	Disease Free Survival
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DSMB	Data Safety and Monitoring Board
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay

ESRD	Ontbreekt
FISH	Fluorescence In Situ Hybridisation
FLC	Free Light Chain
GCP	Good Clinical Practice
G-CSF	granulocyte colony stimulating factor
Hb	Hemoglobin
HIV	human immunodeficiency virus
HOVON	Dutch-Belgian Hematology-Oncology Cooperative Group
IADL	Instrumental Activities of Daily Living
IAT	Indirect Antiglobulin Test
IB	Investigator's Brochure
IC_{50}	concentration producing 50% inhibition
ICF	informed consent form
ICH	International Conference on Harmonisation
IFE	Immunofixation Electrophoresis
IFM	Intergroup Français de Myelome
IMP	Investigational Medicinal Product
IRB	institutional review board
IRR	Infusion Related Reaction
ISS	International Staging System
ITT	intent-to-treat
IV	intravenous; intravenously
K_i	inhibition constant
LDH	lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MM	Multiple Myeloma
MMSE	Mini Mental State Examination
MNA	Mini Nutritional Assessment
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI	National Cancer Institute
NYHA	New York Heart Association
ORR	Overall Response Rate
OS	Overall Survival
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PD	progressive disease (disease progression)
PFS	Progression Free Survival
PI	Proteasome Inhibitor

PK	pharmacokinetic(s)
PO	*per os*; by mouth (orally)
PR	Partial response
QOL	quality of life
QTc	rate-corrected QT interval (millisec) of electrocardiograph
SAE	serious adverse event
SC	Subcutaneous
SD	stable disease
SPEP	Serum Protein Electro-Phoresis
SPM	Second Primary Malignancy
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Events
$t_{1/2}$	terminal disposition half-life
T_{max}	single-dose time to maximum (peak) plasma concentration
ULN	upper limit of the normal range
UPEP	Urine Protein Electro-Phoresis
WHO	World Health Organization

19 References

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Criteria for MM and measurable disease

S.V. Rajkumar et al. (The Lancet Oncology, 2014: 15; e538-e548)

Criteria for MM

Clonal bone marrow plasma cells ≥10% or biopsy-proven bony or extramedullary plasmacytoma[1] AND any one or more of the following myeloma defining events:

Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:

- Hypercalcemia: corrected serum calcium >0.25 mmol/L (>1 mg/dL) higher than ULN or >2.75 mmol/l

  (>11 mg/dL)
- Renal insufficiency: creatinine clearance[2] < 40mL/min or serum creatinine >177 µmol/L (>2 mg/dL)
- Anemia: hemoglobin >2 g/dL (1.2 mmol/L) below the lower limit of normal or hemoglobin <10 g/dL (6.2 mmol\L)
- Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT[3]

or one or more of the following biomarkers of malignancy:

- Clonal bone marrow plasma cell percentage[1] ≥60%
- Involved: uninvolved serum free light chain ratio[4] ≥100
- >1 focal lesion[5] on MRI studies

Footnotes:

1. Clonality should be established by showing light-chain restriction on flow cytometry, immunohistochemistry, or immunofluorescence. Bone marrow plasma cell percentage should preferably be estimated from a core biopsy specimen; in case of a disparity between the aspirate and the core biopsy, the highest value should be used.

2. Measured or estimated by validated equations.

3. If bone marrow has less than 10% clonal plasma cells, more than one bone lesion is required to distinguish from solitary plasmacytoma with minimal marrow involvement. Osteolytic lesions must be 5 mm or more in size.

4. These values are based on the serum Freelite assay (The Binding Site Group, Birmingham UK). The involved free light chain must be ≥100mg/L.

5. At least 2 focal lesions must be 5 mm or more in size.

Criteria for measurable disease

Serum M-protein ≥ 10 g/l **or**

Urine M-protein ≥ 200 mg/24 hours **or**

Abnormal serum FLC ratio with involved free light chain (FLC) > 100 mg/l **or**

Proven plasmacytoma by biopsy *

* If plasmacytoma is the only measurable parameter, the patient is not allowed to be included in the study, because of difficult response evaluation.

International Staging System for Multiple Myeloma (ISS stage)

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

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

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

Frailty score of the International Myeloma Working Group

VARIABLE		SCORE
AGE	≤ 75 years	0
	76 – 80 years	1
	> 80 years	2
Charlson Comorbidity Index	≤ 1	0
	≥ 2	1
Activities of Daily Living	> 4 (= ≥ 5)	0
	≤ 4	1
Instrumental Activities of Daily Living	> 5 (= ≥ 6)	0
	≤ 5	1

Definitions

FIT 0 points
UNFIT 1 point
FRAIL ≥ 2 points

For specific instructions on the CCI, ADL and iADL see CRFs and the document “Instructions Frailty score of the International Myeloma Working Group on the HOVON website”.

Response criteria for Multiple Myeloma

Based on International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma Kumar et al. Lancet Oncol 2016; 17: e328–46

Response criteria

IMWG MRD criteria§ (requires a complete response as defined below)	
Response subcategory	*Response criteria*
Sustained MRD-negative	*MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)†*
Flow MRD-negative	*Absence of phenotypically aberrant clonal plasma cells by NGF‡ on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher*
Sequencing MRD-negative	*Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT (Sequenta) platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher*
Imaging plus MRD-negative (Imaging should be performed once MRD negativity is determined)	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue¶
Relapse from MRD negative (to be used only if the end point is disease-free survival)	Any one or more of the following criteria: ▪ Loss of MRD negative state (evidence of clonal plasma cells on NGF or NGS, or positive imaging study for recurrence of myeloma); ▪ Reappearance of serum or urine M-protein by immunofixation or electrophoresis; ▪ Development of ≥5% clonal plasma cells in the bone marrow; ▪ Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcaemia)

MRD=minimal residual disease; NGF=next-generation flow; NGS=next-generation sequencing; FLC=free light chain; M-protein=myeloma protein; SPD=sum of the products of the maximal perpendicular diameters of measured lesions; CRAB features=calcium elevation, renal failure, anaemia, lytic bone lesions; FCM=fl ow cytometry; SUVmax= maximum standardised uptake value; MFC=multiparameter flow cytometry; 1^8F-FDG PET=1^8F-fluorodeoxyglucose PET.
§ MRD tests should be initiated only at the time of suspected complete response. For MRD assessment, the first bone marrow aspirate should be sent to MRD. All response categories require two consecutive assessments made any time before starting any new therapy; for MRD there is no need for two consecutive assessments, but information on MRD after each treatment stage is recommended (eg, after induction, high-dose therapy/ASCT, consolidation, maintenance). MRD require no known evidence of progressive or new bone lesions if radiographic studies were performed. However,

radiographic studies are not required to satisfy these response requirements except for the requirement of FDG PET if imaging MRD-negative status is reported.

† Sustained MRD negativity when reported should also annotate the method used (eg, sustained flow MRD-negative, sustained sequencing MRD-negative).

‡ Bone marrow MFC should follow NGF guidelines. 5 million cells should be assessed. The FCM method employed should have a sensitivity of detection of at least 1 in 10^5 plasma cells.

¶ Criteria used by Zamagni and colleagues, and expert panel (IMPetUs; Italian Myeloma criteria for PET Use). Baseline positive lesions were identified by presence of focal areas of increased uptake within bones, with or without any underlying lesion identifi ed by CT and present on at least two consecutive slices. Alternatively, an SUVmax=2x5 within osteolytic CT areas >1 cm in size, or SUVmax=1x5 within osteolytic CT areas ≤1 cm in size were considered positive.

Standard IMWG response criteria	
Response subcategory[a]	*Response criteria*
Stringent complete response	Complete response as defined below plus ▪ Normal FLC ratio (0.26-1.65)[b] and ▪ Absence of clonal cells in bone marrow[c] by immunohistochemistry or immunophenotyping[d]
Complete response	▪ Negative immunofixation of serum and urine and ▪ Disappearance of any soft tissue plasmacytomas and ▪ < 5% plasma cells in bone marrow aspirates
Very good partial response	▪ Serum and urine M-protein detectable by immunofixation.but not on electrophoresis or ▪ ≥ 90% reduction in serum M-protein plus urine M-protein level < 100 mg per 24 h (0.1 g/ 24 h)
Partial response	▪ ≥ 50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥ 90% or to < 200 mg per 24 h (0.2 g/ 24h) ▪ When the only method to measure disease is by serum FLC levels: PR is defined as a ≥ 50% decrease in the difference between involved and uninvolved sFLC levels ▪ In addition to the these criteria, if present at baseline, a ≥ 50% reduction in the size of soft tissue plasmacytomas (SPD) is also required[e] ▪ If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥ 50% reduction in bone marrow plasma cells is required in place of M-protein, provided baseline plasma-cell percentage was ≥ 30%
Minimal response	▪ ≥25% but ≤ 49% reduction of serum M-protein and reduction in 24 h urine M-protein by 50–89%. ▪ In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size of soft tissue plasmacytomas is also required[e]
Stable disease[f]	▪ Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease[g,h]	Any one or more of the following criteria:

	▪ Increase of 25% from lowest confirmed response value in one or more of the following criteria: ▪ Serum M-protein (absolute increase must be ≥0·5 g/dL); ▪ Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; ▪ Urine M-protein (absolute increase must be ≥200 mg/24 h); ▪ In patients without measurable serum and urine M-protein levels, the diff erence between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); ▪ In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); ▪ Appearance of a new lesion(s), ≥50% increase from nadir in SPD[e] of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; ▪ ≥50% increase in circulating plasma cells (minimum of 200 cells per µL) if this is the only measure of disease
Clinical relapse	Clinical relapse requires one or more of the following criteria: ▪ Direct indicators of increasing disease and/or end organ dysfunction (CRAB features) related to the underlying clonal plasma-cell proliferative disorder. It is not used in calculation of time to progression or progression-free survival but is listed as something that can be reported optionally or for use in clinical practice; ▪ Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fractures do not constitute progression); ▪ Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and ≥1 cm) increase as measured serially by the SPD[e] of the measurable lesion; ▪ Hypercalcaemia (>11 mg/dL); ▪ Decrease in haemoglobin of ≥2 g/dL not related to therapy or other non-myeloma-related conditions; ▪ Rise in serum creatinine by 2 mg/dL or more from the start of the therapy and attributable to myeloma; ▪ Hyperviscosity related to serum paraprotein
Relapse from CR (to be used only if the end point is disease-free survival)	Any one or more of the following criteria: ▪ Reappearance of serum or urine M-protein by immunofixation or electrophoresis; ▪ Development of ≥5% plasma cells in the bone marrow; ▪ Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, or hypercalcaemia see above)

[a] All response categories require two consecutive assessments made any time before starting any new therapy; All categories of response require no known evidence of progressive disease or new bone lesions if radiographic studies were performed. However, radiographic studies are not required to satisfy these response requirements .

[b] All recommendations regarding clinical uses relating to serum FLC levels or FLC ratio are based on results obtained with the validated Freelite test (Binding Site, Birmingham, UK).

[c] Confirmation with repeat bone marrow examination not needed.

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

[e] Plasmacytoma measurements should be taken from the CT portion of the PET/CT, or MRI scans, or dedicated CT scans where applicable. For patients with only skin involvement, skin lesions should be measured with a ruler. Measurement of tumour size will be determined by the SPD.

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

[g] Positive immunofixation alone in a patient previously classified as achieving a complete response will not be considered progression. For purposes of calculating time to progression and progression-free survival, patients who have achieved a complete response and are MRD-negative should be evaluated using criteria listed for progressive disease. Criteria for relapse from a complete response or relapse from MRD should be used only when calculating disease-free survival.

[h] In the case where a value is felt to be a spurious result per physician discretion (eg, a possible laboratory error), that value will not be considered when determining the lowest value.

NOTES:

- **Parameters that are considered measurable at baseline (serum and urine, FLC serum if both serum and urine are not measurable) should be performed at each assessment**
- **Urine M-protein is not needed to document partial response or minor response if baseline urine M-protein was not measurable; however, it is still required very good partial and complete response**
- **Once (s)CR is established, response remains (s)CR until relapse or progression is documented.**
- **Patients will continue in the last confirmed response category until there is confirmation of progression or improvement to a higher response status; patients cannot move to a lower response category.**
- **Any soft tissue plasmacytoma documented at baseline must undergo serial monitoring; otherwise the patient is classified as inevaluable until the size of the plasmacytoma is measured again.**
- **Patients will be considered to have progressive disease if they meet the criteria for progression by a variable that was not considered measurable at baseline; however, for patients who had a measurable serum or urine M-spike at baseline, progression cannot be defined by increases in serum FLC alone.**
- **When the only method to measure disease is by serum FLC levels: complete response can be defined as a normal serum FLC ratio of 0·26 to 1·65 in addition to the complete response criteria listed above.**
- **When the only method to measure disease is by serum FLC levels: VGPR is defined as a ≥ 90% decrease in the difference between involved and uninvolved serum FLC levels**
- **To achieve VGPR, if present at baseline, a ≥ 90% reduction in the size of soft tissue plasmacytomas (SPD) is also required**

ZUBROD-ECOG-WHO Performance Status Scale and New York Heart Association classification

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

5 Death

New York Heart Association classification

1 Cardiac disease, but no symptoms and no limitation in ordinary physical activity, e.g. no shortness ofbreath when walking, climbing stairs etc

2 Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity

3 Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). Comfortable only at rest.

4 Severe limitations. Experiences symptoms even while *at rest*. Mostly bedbound patients

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)

Investigational Medicinal Products Ixazomib and Daratumumab

F1 Ixazomib

Ixazomib (MLN9708): pharmacology, pharmacokinetics and pharmacodynamics

Like bortezomib, ixazomib is a modified peptide boronic acid analog. Ixazomib is the citrate ester of the biologically active dipeptide boronic acid, MLN2238. Ixazomib was formulated to improve the chemical properties of MLN2238 for clinical delivery. Ixazomib rapidly hydrolyzes to MLN2238 upon contact with either plasma or aqueous solutions. MLN2238 is the active form that potently, reversibly, and selectively inhibits the proteasome. In contrast to bortezomib, MLN2238 demonstrates a faster dissociation rate from the proteasome that may result in enhanced tumor penetration, exhibits antitumor activity in a broader range of tumor xenografts, and has more prolonged tissue penetration. MLN2238 preferentially binds the β5 site of the 20S proteasome; at higher concentrations, it also inhibits the activity of the β1 and β2 sites. MLN2238 was selective for the proteasome over a panel of several proteases (IC_{50} values between 20 and100 µM), 103 kinases (IC_{50} values > 10 µM), and receptors (IC_{50} values > 10 µM). MLN2238 and bortezomib have different β5 proteasome dissociation half-lives ($t_{1/2}$), reflecting differences in their on-off binding kinetics (the β5 proteasome dissociation [$t_{1/2}$] for MLN2238 and bortezomib are 18 and 110 minutes, respectively).

Pharmacokinetics and Drug Metabolism

After oral dosing, absorption of ixazomib is rapid with a median first time to maximum observed plasma concentration (T_{max}) of approximately 1 hour postdose. The plasma exposure (AUC) of ixazomib increases in a dose-proportional manner over a dose range of 0.2 to 10.6 mg based on population PK analysis. The absolute oral bioavailability (F) of ixazomib is estimated to be 58% based on population PK analysis. A high-fat meal reduced ixazomib C_{max} by 69% and AUC_{0-216} by 28%. This indicates that a high-fat meal decreases both the rate and extent of absorption of ixazomib. Therefore, ixazomib should be dosed at least 2 hours after food or 1 hour before food.

The steady-state volume of distribution of ixazomib is large and is estimated to be 543 L based on a population PK model. Based on in vitro plasma protein binding measurements on samples from clinical studies (Studies C16015 and C16018), ixazomib is highly bound to plasma proteins (99%). Ixazomib concentrations are higher in whole blood than in plasma, indicating extensive partitioning of ixazomib into red blood cells, which are known to contain high concentrations of the 20S proteasome.

Metabolism appears to be the major route of elimination for ixazomib. In vitro studies indicate that ixazomib is metabolized by multiple cytochrome P450 (CYP) and non-CYP proteins. At concentrations exceeding those observed clinically (10 µM), ixazomib was metabolized by multiple CYP isoforms with estimated relative contributions of 3A4 (42.3%), 1A2 (26.1%), 2B6 (16.0%), 2C8 (6.0%), 2D6 (4.8%), 2C19 (4.8%), and 2C9 (<1%). At 0.1 and 0.5 µM substrate concentrations, which are closer to clinical concentrations of ixazomib following oral administration of 4 mg ixazomib, non-CYP mediated clearance was observed and seemed to play a major role in ixazomib clearance in vitro. These data indicate that at clinically relevant concentrations of ixazomib, non-CYP proteins contribute to the clearance of ixazomib and no specific CYP isozyme predominantly contributes to the

clearance of ixazomib. Therefore, at clinically relevant concentrations of ixazomib, minimal CYP-mediated DDIs with a selective CYP inhibitor would be expected.

Ixazomib is neither a time-dependent inhibitor nor a reversible inhibitor of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4/5. Ixazomib did not induce CYPs 1A2, 2B6, and 3A4/5 activity or corresponding immunoreactive protein levels. Thus, the potential for ixazomib to produce DDIs via CYP isozyme induction or inhibition is low.

Ixazomib is not a substrate of BCRP, MRP2 and OATPs. Ixazomib is not an inhibitor of P-gp, BCRP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K. Ixazomib is unlikely to cause or be susceptible to clinical DDIs with substrates or inhibitors of clinically relevant drug transporters.

The geometric mean terminal half-life ($t_{1/2}$) of ixazomib is 9.5 days based on population PK analysis. For both IV and oral dosing, there is an approximately average 3-fold accumulation (based on AUC) following the Day 11 dose for the twice-weekly schedule and a 2-fold accumulation (based on AUC) following the Day 15 dose for the once-weekly schedule.

Mean plasma clearance (CL) of ixazomib is 1.86 L/hr based on the results of a population PK analysis. Taken together with the blood-to-plasma AUC ratio of approximately 10, it can be inferred that ixazomib is a low clearance drug. Using the absolute oral bioavailability (F) estimate of 58% (also from a population PK model), this translates to an apparent oral plasma clearance (CL/F) of 3.21 L/hr. The geometric mean renal clearance for ixazomib is 0.119 L/hr, which is 3.7% of CL/F and 6.4% of CL estimated in a population PK analysis. Therefore, renal clearance does not meaningfully contribute to ixazomib clearance in humans. Approximately 62% of the administered radioactivity in the ADME study (Study C16016) was recovered in the urine and 22% of the total radioactivity was recovered in the feces after oral administration. Only 3.2% of the administered ixazomib dose was recovered in the urine as unchanged ixazomib up to 168 hours after oral dosing, suggesting that most of the total radioactivity in urine was attributable to metabolites.

The PK of ixazomib was similar with and without co-administration of clarithromycin, a strong CYP3A inhibitor, and hence no dose adjustment is necessary when ixazomib is administered with strong CYP3A inhibitors. Consistently, in a population PK analysis, co-administration of strong CYP1A2 inhibitors did not affect ixazomib clearance. Therefore, no dose adjustment is required for patients receiving strong inhibitors of CYP1A2. Based on information from the clinical rifampin DDI study, ixazomib C_{max} and $AUC_{0\text{-}last}$ were reduced in the presence of rifampin by approximately 54% and 74%, respectively. Therefore, the co-administration of strong CYP3A inducers with ixazomib is not recommended.

Mild or moderate renal impairment (CrCL ≥ 30 mL/min) did not alter the PK of ixazomib based on the results from a population PK analysis. As a result, no dose adjustment is required for patients with mild or moderate renal impairment. In a dedicated renal impairment study (C16015), unbound $AUC_{0\text{-}last}$ was 38% higher in patients with severe renal impairment or ESRD patients requiring dialysis as compared to patients with normal renal function. Accordingly, a reduced starting dose of ixazomib is appropriate in patients with severe renal impairment or ESRD requiring dialysis. Pre- and post-dialyzer concentrations of ixazomib measured during the hemodialysis session were similar,

suggesting that ixazomib is not readily dialyzable, consistent with its high plasma protein binding (99%).

The PK of ixazomib is similar in patients with normal hepatic function and in patients with mild hepatic impairment, as defined by the National Cancer Institute Organ Dysfunction Working Group (total bilirubin <1.5 times the upper limit of normal [ULN]), based on the results from a population PK analysis. Consequently, no dose adjustment is required for patients with mild hepatic impairment. In a dedicated PK study in patients with moderate (total bilirubin >1.5 to 3 times the ULN) or severe (total bilirubin >3 times the ULN) hepatic impairment (Study C16018), unbound dose-normalized $AUC_{0\text{-last}}$ was 27% higher in patients with moderate or severe hepatic impairment as compared to patients with normal hepatic function. Therefore, a reduced starting dose of ixazomib is appropriate in patients with moderate or severe hepatic impairment.

There was no statistically significant effect of age (23-91 years), sex, body surface area (1.2-2.7 m^2), or race on the clearance of ixazomib based on the results from a population PK analysis.

Further details on these studies are provided in the IB.

Preclinical experience

Please be refered to the current MLN9708 Investigator's Brochure (IB).

Clinical Experience

Ixazomib has been evaluated as an oral single agent in phase 1 studies that have included patients with advanced solid tumors, lymphoma, relapse/refractory MM (RRMM), and relapsed or refractory light-chain (AL) amyloidosis and demonstrated early signs of activity. Ongoing studies continue to investigate both single-agent ixazomib and ixazomib in combination with standard treatments. Based on encouraging preliminary data observed in patients with MM requiring systemic treatment, 2 phase 3 trials in newly diagnosed MM (NDMM) (C16014) and RRMM (C16010) patient populations are currently evaluating ixazomib in combination with Revlimid and Dexamethasone (RevDex) versus placebo/RevDex. Both trials are combining ixazomib at a weekly dose of 4.0 mg on Days 1, 8, and 15 in a 28-day cycle to a standard dose of lenalidomide with a weekly dexamethasone dose of 40 mg. In addition, clinical pharmacology studies have evaluated drug-drug interactions with ketoconazole, clarithromycin, and rifampin, as well as the effect of food, renal impairment, and hepatic impairment on the PK of ixazomib. Studies evaluating the safety and pharmacokinetics (PK) of ixazomib alone (in Japanese patients) and in combination with lenalidomide and dexamethasone in Asian adult patients (including Japanese patients) with a diagnosis of RRMM are ongoing.

As of 27 March 2013, preliminary clinical data is available for a total of 653 patients across 13 studies. The emerging safety profile indicates that ixazomib is generally well tolerated. The adverse events (AEs) are consistent with the class-based effects of proteasome inhibition and are similar to what has been previously reported with VELCADE though the severity of some, for example peripheral neuropathy, is less. While some of these potential toxicities may be severe, they can be managed by

clinical monitoring and standard medical intervention, or, as needed, dose modification or discontinuation.

Fatigue was the most common AE reported among 384 patients treated in the oral (PO) studies (47%). Other common AEs reported in the pooled intravenous (IV) and PO safety populations include nausea, thrombocytopenia, diarrhea, and vomiting. Rash is also a commonly reported treatment-emergent event; however, there is some variety in its characterization and causality resulting in different preferred terms to describe it. A high-level term outline of rash events includes rashes, eruptions and exanthems NEC; pruritus NEC; erythemas; papulosquamous conditions; and exfoliative conditions. The dose escalation phases of most trials reported in the IB have now completed enrollment, and gastrointestinal (GI) symptoms were the common dose-limiting toxicities (DLTs) when the use of prophylactic anti-emetics was not permitted per protocol. In the expansion cohorts or phase 2 cohorts (as per each study), the incidence and severity of GI symptoms was mitigated by the use of the lower maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) (as per each study) and standard clinical usage of anti-emetics and/or antidiarrheal medications as deemed appropriate. Prophylactic use of anti-emetics has not been required as with other agents but (as outlined in Section 6.7) has been used according to standard practice and are effective.

The most frequent (at least 20%) treatment-emergent adverse events (TEAEs) reported with the PO formulation pooled from single-agent studies (n = 201) irrespective of causality to ixazomib, include nausea (53%), fatigue (51%), diarrhea (44%), thrombocytopenia (34%), vomiting (38%), decreased appetite (32%), fever (21%), and anemia (21%). The most frequent (at least 20%) TEAEs reported with the PO formulation pooled from combination trials (irrespective of the combination) (n = 173), irrespective of causality to ixazomib, include diarrhea (47%), fatigue (44%), nausea (38%), peripheral edema (35%), constipation (33%), insomnia (29%), thrombocytopenia (28%), anemia (26%), vomiting (26%), neutropenia (25%), back pain (24%), pyrexia (23%), peripheral edema (21%, each), fever (20%), cough (20%), hypokalemia (20%), neutropenia (20%), and upper respiratory tract infection (20%). Overall rash of all grades is reported in approximately 50% of patients and is more common when ixazomib is given in combination with lenalidomide where rash is an overlapping toxicity.

Additional detailed information regarding the clinical experience of ixazomib may be found in the IB, including information on the IV formulation.

Clinical Trial Experience Using the Oral Formulation of MLN9708

As of 27 March 2013, a total of 507 patients with differing malignancies (multiple myeloma, AL amyloidosis, nonhematologic cancers, and lymphoma) have been treated in studies evaluating the oral ixazomib formulation. These patients have been treated with different doses of ixazomib either as a single-agent treatment (in 201 patients) or in combination with currently clinically available treatments (in 306 patients). Information regarding the ongoing studies, patient populations, and doses investigated are included in Table 5-1.

Table 5-1 Clinical Studies of Oral Ixazomib

Trial/ Population	Description	Doses Investigated
C16003 RRMM N = 60	PO, TW, single agent	0.24-2.23 mg/m^2 TW MTD: 2.0 mg/m^2 DLT: rash, thrombocytopenia Closed to enrollment
C16004 RRMM N = 60	PO, W, single agent	0.24-3.95 mg/m^2 W MTD: 2.97 mg/m^2 DLT: rash, nausea, vomiting, diarrhea Closed to enrollment
C16005 NDMM N = 65	PO, W, combination with LenDex 28-day cycle	1.68-3.95 mg/m^2 W MTD: 2.97 mg/m^2 DLT: nausea, vomiting, diarrhea, syncope RP2D[a]: 4.0 mg fixed (switched to fixed dosing in phase 2, equivalent to 2.23mg/m^2) Closed to enrollment
C16006 NDMM N = 20	PO, TW (Arm A- 42 day cycle) and W (Arm B- 28 day cycle), combination with Melphalan and Prednisone	Arm A[a]: 3-3.7-mg fixed dose TW DLT: rash, thrombocytopenia, subileus Arm B[a]: 3-5.5-mg fixed dose, W DLT: Esophageal ulcer nausea, vomiting, hematemesis, thrombocytopenia, ileus, neurogenic bladder MTD = 3.0 mg
C16007 RRAL N = 27	PO, W, single agent	4-5.5-mg fixed dose[a] W DLT: thrombocytopenia, diarrhea, dyspnea, acute rise in creatinine, cardiac arrest MTD: 4.0 mg W
C16008 NDMM N = 64	PO, TW, combination with LenDex 21-day cycle	3.0-3.7-mg fixed dose[a] W MTD: 3.0 mg Closed to enrollment
C16009 Solid tumors, Lymphomas N = 54	PO, W, single agent	5.5-mg fixed dose[a] W
C16010 RRMM N = 200	PO, W, with LenDex versus placebo-LenDex	4.0 mg W
C16011 RRAL N = 4	PO, W, with Dex versus physician's choice of a Dex-based regimen	4.0 mg W
C16013 RRMM N = 9	PO, W, with LenDex	4.0 mg W
C16014 Symptomatic MM N=701	PO, combination with LenDex	ixazomib 4.0 mg or matching placebo on Days 1, 8, and 15, plus Len 25 mg on Days 1-21 (10 mg if low creatinine clearance, with escalation to 15 mg if tolerated) and Dex 40 mg (or 20 mg if >75 years old) on Days 1, 8, 15, and 22
C16015	PO, combination with Dex	Part A: ixazomib 3.0 mg on Day 1 Part B: ixazomib 4.0 mg on Days 1, 8, and 15, plus Dex 40 mg (or 20 mg if >75 years old) on Days 1, 8, 15 and 22 of a 28-day cycle

Table 5-1 Clinical Studies of Oral Ixazomib

Trial/ Population	Description	Doses Investigated
Symptomatic MM with normal renal function or severe renal impairment N=28		
C16017 RR follicular lymphoma N=58	PO, W	4.0, 5.3, and 7.0 mg, W Treatment at RP2D once determined.
C16018 Advanced solid tumors or hematologic malignancies with varying degrees of liver dysfunction N=45	Part A: PO, **Day 1 of 15-day cycle** **Part B:** PO, W	**1.5 mg (severe hepatic impairment), 2.3 mg (moderate hepatic impairment), or 4.0 mg (normal hepatic function)**
TB-MC010034 RRMM N = 10	PO, W	4.0 mg, W Single agent: 4.0 mg Combination with Rd

Abbreviations: RRAL = Relapsed and/or refractory Primary systemic light chain (AL) amyloidosis; BSA = body surface area; Dex=dexamethasone; DLT = dose-limiting toxicity; IV = intravenously; LenDex = lenalidomide plus dexamethasone; MTD = maximum tolerated dose; NDMM = newly diagnosed multiple myeloma; PO = orally; RR= relapsed and/or refractory; RRAL= relapsed and/or refractory systemic light chain amyloidosis RRMM = relapsed and/or refractory multiple myeloma; TBD = to be determined; TW = twice weekly; W = weekly; RP2D= recommended phase 2 dose.

Note that blinded data from pivotal Studies C16010 and C16011 are not included.

a Approximate BSA and fixed dosing equivalence: 3 mg~ equivalent to 1.68 mg/m^2 BSA dosing; 4.0 mg ~ equivalent to 2.23 mg/m^2 BSA dosing; and 5.5 mg~ equivalent to 2.97 mg/m^2 BSA dosing.

Overview of the Oral Formulation of MLN9708

The emerging safety profile indicates that ixazomib is generally well tolerated. The adverse events (AEs) are consistent with the class-based effects of proteasome inhibition and are similar to what has been previously reported with VELCADE though the severity of some, for example peripheral neuropathy, is less. While some of these potential toxicities may be severe, they can be managed by clinical monitoring and standard medical intervention, or, as needed, dose modification or discontinuation.

In the 4 ongoing studies (C16003, C16004, C16007, and C16009) investigating single-agent oral ixazomib in patients with differing malignancies (multiple myeloma, AL amyloidosis, nonhematologic cancers, and lymphoma), a total of 201 patients have been treated as of 27 March 2013. These patients have been treated with different doses of ixazomib as they are all phase 1 trials. An overview of the most frequent (at least 10%) AEs occurring in the pooled safety population from single-agent oral ixazomib Studies (C16003, C16004, C16007, and C16009) is shown the table below.

Table 5-2 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Single-Agent Studies

Primary System Organ Class Preferred Term	Oral Single Agent Total n = 201 n (%)
Subjects with at Least One Adverse Event	197 (98)
Gastrointestinal disorders	160 (80)
Nausea	106 (53)
Diarrhoea	88 (44)
Vomiting	77 (38)
Constipation	46 (23)
Abdominal pain	33 (16)
General disorders and administration site conditions	151 (75)
Fatigue	103 (51)
Pyrexia	51 (25)
Oedema peripheral	27 (13)
Asthenia	31 (15)
Nervous system disorders	92 (46)
Headache	29 (14)
Dizziness	26 (13)
Neuropathy peripheral	21 (10)
Metabolism and nutrition disorders	107 (53)
Decreased appetite	64 (32)
Dehydration	37 (18)
Blood and lymphatic system disorders	98 (49)
Thrombocytopenia	68 (34)
Anaemia	42 (21)
Neutropenia	29 (14)
Lymphopenia	20 (10)
Skin and subcutaneous tissue disorders	90 (45)
Rash macular[a]	23 (11)
Musculoskeletal and connective tissue disorders	93 (46)
Back pain	24 (12)
Arthralgia	28 (14)
Respiratory, thoracic and mediastinal disorders	78 (39)
Cough	28 (14)
Dyspnoea	30 (15)
Infections and infestations	89 (44)
Upper respiratory tract infection	31 (15)

Source: Ixazomib Investigator's Brochure Edition 7

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities, version 15.0.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

a Note that rash maculopapular and rash macular represent the 2 most common terms used to describe rash.

As of 27 March 2013, there are 5 studies actively enrolling patients with multiple myeloma to investigate oral ixazomib in combination with standard combination regimens.

The most frequent (at least 10%) AEs occurring in the pooled safety population from Studies C16005, C16006, C16008, and C16013 are shown for all grades (Table 5-3). Note that in combination trials, related is defined as related to any study drug in the combination regimen.

Table 5-3 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Combination Studies

Primary System Organ Class Preferred Term	Total Oral Combo Agent (5/6/8/13) n = 173 n (%)
Subjects with at Least One Adverse Event	163 (94)
Gastrointestinal disorders	139 (80)
Nausea	65 (38)
Diarrhoea	81 (47)
Vomiting	51 (29)
Constipation	57 (33)
General disorders and administration site conditions	132 (76)
Fatigue	76 (44)
Pyrexia	39 (23)
Oedema peripheral	61 (35)
Asthenia	20 (12)
Nervous system disorders	115 (66)
Headache	28 (16)
Dizziness	34 (20)
Neuropathy peripheral	45 (26)
Metabolism and nutrition disorders	91 (53)
Decreased appetite	25 (14)
Hypokalaemia	34 (20)
Blood and lymphatic system disorders	88 (51)
Thrombocytopenia	49 (28)
Anaemia	45 (26)
Neutropenia	43 (25)
Lymphopenia	20 (12)
Skin and subcutaneous tissue disorders	102 (59)
Rash maculopapular[a]	29 (17)
Rash macular[a]	22 (13)
Musculoskeletal and connective tissue disorders	99 (57)
Back pain	42 (24)
Pain in extremity	31 (18)
Arthralgia	22 (13)

Table 5-3 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Combination Studies

Primary System Organ Class Preferred Term	Total Oral Combo Agent (5/6/8/13) n = 173 n (%)
Respiratory, thoracic and mediastinal disorders	80 (46)
Cough	36 (21)
Dyspnoea	26 (15)
Infections and infestations	92 (53)
Upper respiratory tract infection	35 (20)
Psychiatric disorders	73 (42)
Insomnia	50 (29)

Source: Ixazomib Investigator's Brochure Edition 7

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities, version 15.0.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

Data from ongoing blinded pivotal trials (C16010) are not included.

a Note that rash maculopapular and rash macular represent the 2 most common terms used to describe rash..

The clinical experience with MLN9708 also shows early signs of antitumor activity as evidenced by at least a 50% reduction in disease burden in some patients and prolonged disease stabilization in others across all ongoing trials. The antitumor activity has been seen with single-agent MLN9708, when combined with established therapies, and across the malignancies studied (advanced solid tumors[21], non-Hodgkin's disease, Hodgkin's disease[22], relapsed and/or refractory multiple myeloma [RRMM; [23-25]], relapsed or refractory systemic light chain amyloidosis [RRAL], and newly diagnosed multiple myeloma [NDMM; [26-28]]) to date.

Though additional data are needed to characterize the clinical benefit of this drug, the emerging data supports the ongoing development of MLN9708.

Of particular relevance to this study (C16011) is the clinical experience from Studies C16004 and C16007 in which single-agent MLN9708 is administered weekly in patients with RRMM or RRAL, respectively.

Relapsed and/or Refractory Multiple Myeloma

The early development of ixazomib in patients with RRMM involves 2 studies (C16003 and C16004) with similar objectives, but each investigated 1 of the 2 dosing schedules commonly used with the first-in-class proteasome inhibitor, VELCADE.

Study C16003 is an open-label, dose escalation, phase 1 study of ixazomib dosing on a twice-weekly schedule on Days 1, 4, 8, and 11 of a 21-day cycle in adult patients with RRMM.(11, 12) Study C16004 is an open-label, dose escalation, phase 1 study of ixazomib dosing on a weekly schedule on Days 1, 8, and 15 of a 28-day cycle in adults patients with RRMM.(13, 14, 15) Both studies have now completed enrollment. The DLTs in Study C16003 were rash macular and thrombocytopenia and the DLTs in C16004 were nausea, diarrhea, vomiting, and erythema multiforme.

In the dose escalation component of both studies, patients had multiple myeloma that had relapsed following at least 2 lines of therapy that must have included bortezomib, thalidomide (or lenalidomide), and corticosteroids. In both studies, when the MTD was established, cohorts of patients representing the heterogeneous patient population currently seen in clinical practice were to be enrolled into 1 of 4 expansion cohorts, including a relapsed and refractory cohort, a carfilzomib cohort, a proteasome inhibitor-naïve cohort, and a VELCADE-relapsed cohort.

Final study results are currently being analyzed, but preliminary data suggest that ixazomib has anti-tumor activity in heavily pretreated MM patients, with durable responses/disease control, and is generally well tolerated. Please refer to the ixazomib IB further information.

Dose reductions required were due to AEs that included rash, neutropenia, thrombocytopenia, diarrhea, nausea, vomiting, dehydration, hypotension, increase in serum creatinine, abdominal pain, ileus, fatigue, and pneumonia. The AEs reported for the 5 patients who were required to discontinue treatment included Grade 2 MLN9708-related nausea/vomiting in 1 patient treated above the MTD, Grade 3 MLN9708-related diarrhea in a second patient, related Grade 3 thrombocytopenia, related Grade 2 dyspnea, and not related Grade 4 elevation in creatinine (1 patient each). There were no on-study deaths.
Study C16007 is evaluating single agent weekly , Day 1, 8, and 15 of a 28-day cycle, oral dosing in patients with RRAL after at least 1 prior therapy. The objectives of this study are to determine the safety, tolerability, and MTD, as well as to determine hematologic and organ response rates in this patient population. The starting dose level was selected from Study C16004 as previously described. In Study C16007 the dose was switched from the BSA-based dosing to the fixed dose, thereby the 4.0 mg fixed starting dose in Study C16007 corresponds to the 2.23 mg/m2 dose (one dose level below MTD) from Study C16004. This study is currently enrolling patients in the dose-expansion portion of the trial.
As of 30 April 2012, 14 patients have been treated in this study. At the first dose level of 4.0 mg, 1 of 6 patients experienced a protocol-defined DLT (that is, thrombocytopenia that lasted more than 2 weeks, which met the definition of a DLT due to the delay in starting Cycle 2). As per protocol, the dose was escalated to 5.5 mg for the next cohort of patients where 2 of 5 patients experienced a DLT (Grade 3 diarrhea, n=1; and Grade 2 dyspnea, Grade 2 acute rise in serum creatinine, and Grade 4 cardiac arrest, n=1). The latter patient did not appear to have cardiac AL amyloidosis by echocardiogram on study entry, but did have substantial renal involvement. After the occurrence of this DLT, diagnoses included cardiac involvement and CHF. The MTD of weekly oral MLN9708 was determined to be 4.0 mg. Following the establishment of the MTD, patients are currently being enrolled in to 1 of 2 cohorts: proteasome inhibitor naïve or proteasome inhibitor exposed.
As of the 30 April 2012 data cut, the patients enrolled in the study are considered heavily pretreated, as evidenced by a median number of 3 prior lines of therapy (range 1–7), with 38% and 46% of patients having been previously treated with bortezomib and lenalidomide, respectively. To be eligible for the study, patients must have amyloid involvement of the heart, kidney, or both; at the data cut the organ involvement distribution was 6, 4, and 4 patients, respectively. Patients have received a median of 2.5 cycles of therapy (range, 1-12). Eight patients remain on treatment. Early signs of activity have been reported. There were 11 patients who have received at least 1 cycle of therapy with completed

response assessments (9 in the 4.0 mg [MTD] cohort and 2 in the 5.5 mg cohort). The overall hematologic response rate at MTD is 56% (5 patients achieved a hematologic response [4 VGPR and 1 PR]; 3 patients showed no change, and 1 patient had an early progression.
A summary of the safety profile of patients treated in Study C16007 is outlined in Table 5-5. Overall, 86% of patients experienced a TEAE of any grade and of any cause.

Table 5-5 Study C16007, Oral MLN9708, Single Agent Given Weekly Most Common TEAEs as of 30April 12 (N = 14)

Most Common (> 20%) Any Grade and Irrespective of Cause	Nausea (50%) Fatigue (36%) Thrombocytopenia (29%) Diarrhea (29%) Decreased Appetite (21%) Peripheral Edema (21%) Dyspnea (21%) Abdominal pain (21%)
Drug-Related Grade ≥ 3 in more than 3 Patients	Thrombocytopenia 5 patients, rash 3 patients, dehydration 2 patients, fatigue 2 patients

Source: MLN9708 Investigator's Brochure Edition 6

One patient discontinued study drug administration due to a TEAE (patient with DLT of acute rise in serum creatinine, dyspnea, and cardiac arrest treated at 5.5 mg, as noted above). No death has been reported.
The potential risks reported with MLN9708 use, pooled from all studies using the oral formulations, were anticipated based on preclinical data and previous experience with VELCADE and are noted in the MLN9708 IB, SMA, and ICF documents. Regardless of whether MLN9708 is administered on the once weekly or twice weekly dosing schedule, there is consistency among the type of TEAEs reported, despite some differences in the frequency and severity of the reported events. While the predominant potential toxicities may be severe in some cases, they are largely reversible, and can be managed by routine clinical monitoring and standard medical interventions, which may include dose reductions and supportive care. Please refer to the MLN9708 IB and SMA for further information.

Newly Diagnosed Multiple Myeloma (NDMM)

Multiple research paths are being explored in patients with NDMM with a focus on evaluating ixazomib in combination with agents commonly used across treatment settings. The development of ixazomib in combination with lenalidomide with dexamethasone (LenDex) in patients with NDMM who are transplant eligible or ineligible involves 2 studies (C16005 and C16008) with similar study designs except for a few key differences, namely the schedules of ixazomib and dexamethasone. Ixazomib is also being evaluated in combination with melphalan and prednisone (MP) for patients who are not transplant eligible due to age or coexisting morbidity (in Study C16006).

All 3 studies are phase 1/2, with phase 1 focusing on safety and phase 2 on efficacy (and further characterization of safety). Please refer to the ixazomib IB for further information.

Potential Risks and Benefits

Please refer to the current ixazomib IB.

The clinical benefit of ixazomib continues to be studied in a comprehensive and global development plan that involves studies sponsored by Takeda. Ixazomib appears to show early signs of anti-tumor activity as evidenced by at least 50% reduction in disease burden in some patients, including patients that have been heavily pretreated as well as those with newly diagnosed MM, and prolongs stabilization of the underlying disease in other patients across all ongoing trials.The preliminary findings are favorable when considering historical and currently available therapies for the patient populations evaluated. Though additional data are needed to characterize the clinical benefit of this drug, the emerging data supports expanded development of ixazomib for the treatment of patients with advanced malignancy.

F2 Daratumumab

Pharmacology, pharmacokinetics.

Please refer to the investigators brochure for information on pharmacology and pharmacokinetics.

Preclinical experience

One of the most promising novel drugs is the human IgG1 anti-CD38 monoclonal antibody daratumumab, which received “breakthrough therapy” designation for relapsed/refractory MM by the US FDA in May 2013 [16;17]. In November 2015, the FDA granted accelerated approval for daratumumab to treat MM patients who have received at least 3 prior treatments including a proteasome inhibitor and an IMiD or who are double-refractory to a proteasome inhibitor and an IMiD. On May the 23rd 2016 the EMA approved daratumumab as monotherapy in Europe to treat MM patients whose prior therapy included a proteasome inhibitor and an immunomodulatory agent and who have demonstrated disease progression on the last therapy. Daratumumab has not been approved yet for first line treatment of MM.

CD38 is highly and uniformly expressed on all MM cells. Under normal conditions CD38 is expressed at relatively low levels on lymphoid or myeloid cells and in some tissues of non-hematopoietic origin. CD38 is a type II transmembrane glycoprotein with ectoenzymatic activity involved in the catabolism of extracellular nucleotides. Other functions ascribed to CD38 include receptor-mediated adhesion by interacting with CD31 or hyaluronic acid, regulation of migration, and signaling events[29-31].
Daratumumab induces killing of MM cells via the activation of potent cytotoxic immune effector functions, including antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC)[32;33]. Another mechanism of action is induction of apoptosis upon secondary crosslinking. Daratumumab has potent antimyeloma activity in mouse xenograft models, and more importantly, also in a humanized mouse model[34]. In

addition, daratumumab has activity against other CD38-positive tumors including follicular lymphoma, mantle cell lymphoma, and chronic lymphocytic leukemia[33].

Clinical Experience: Daratumumab as single agent for the treatment of myeloma

Two single-agent studies with daratumumab intravenously have been initiated and enrollment is completed (Studies GEN501 and MMY2002; Table 1). Seventy-seven (77) subjects have been treated in Parts 1 and 2 of Study GEN501 with doses ranging from ≤1 mg/kg to 24 mg/kg, and 124 subjects have been treated in Study MMY2002.

Among the 32 subjects treated in Part 1 of Study GEN501, the maximum tolerated dose (MTD) was not reached following intravenous (IV) infusions up to 24 mg/kg. Two subjects experienced dose-limiting toxicities (DLTs) in the lower dose cohorts (a subject in the 0.1-mg/kg group had Grade 3 anemia and Grade 4 thrombocytopenia, and a subject in the 1.0-mg/kg group had Grade 3 aspartate aminotransferase increased).

Table 5.6.

Study Number	**Study Design**	**Number of subjects Treated/ Treatment Regimen**	
GEN501	Open-label, Phase 1/2, first-in-human, single-agent study in subjects with multiple myeloma whose disease is relapsed or refractory to at least 2 prior lines of therapies Population was heavily treated with prior treatment, including ASCT, chemotherapy based regimens, IMiDs, and PIs	<u>Part 1</u> n=32 total treated with daratumumab weekly 0.005-1 mg/kg (n=17) 2 mg/kg (n=3) 4 mg/kg (n=3) 8 mg/kg (n=3) 16 mg/kg (n=3) 24 mg/kg (n=3)	
		<u>Part 2</u> n=51 total treated	
		8 mg/kg (n=30) First dose, followed by a 3-week resting period, followed by weekly doses for 7 weeks, then q2w for an additional 14 weeks, and monthly, until the subject experiences disease progression or unmanageable toxicity	16 mg/kg (n=21) Weekly for 8 weeks, followed by q2w for an additional 16 weeks, and monthly, for up to 96 weeks, or until the subject experiences disease progression or unmanageable toxicity
MMY2002	Open-label, multicenter, 2-stage, Phase 2 study of daratumumab for the treatment of subjects with multiple myeloma who have received at least 3 prior lines of therapy including a PI and an IMiD	Part 1, Stage 1 (18 subjects randomized to 8mg/kg and 16 subjects randomized to 16mg/kg): total of 34 subjects	

	or whose disease is double refractory to both a PI and an IMiD	Part 1, Stage 2 (16mg/kg): total of 25 subjects Stage 2 (16mg/kg): total of 65 subjects
ASCT=autologous stem cell transplant; IMiD= immunomodulatory agent; PI=proteasome inhibitor		

Among the 51 subjects treated in Part 2 of Study GEN501, serious adverse events (SAEs) were reported in 37% of subjects (43% of subjects in the 8-mg/kg group and 29% of subjects in the 16 mg/kg-group). The most frequently reported SAEs were pneumonia (6% subjects), and pyrexia (4% of subjects).

Among the 34 subjects treated in Stage 1 of Study MMY2002, SAEs were reported in 27% of subjects (33% of subjects in the 8 mg/kg group, and 19% of subjects in the 16 mg/kg group). The most frequently reported SAE was renal failure acute (6% of subjects).

Preliminary results from these studies show that daratumumab is well tolerated and that in the 16 mg/kg cohort at least a partial response can be achieved in 35% of the patients including CR in 10%[35;36]. Virtually all patients with PR or CR, achieved 50% reduction in tumor load within 3 months after start of therapy.

Clinical experience: Daratumumab combined with other agents for the treatment of myeloma

Based on preclinical evidence showing potential benefit of combining daratumumab i.v. with lenalidomide[37;38], another phase 1/2 study is currently evaluating the combination of daratumumab plus lenalidomide and dexamethasone in relapsed/refractory MM (GEN503; NCT01615029). Preliminary safety data show a manageable toxicity profile and high efficacy of this three-drug regimen[39]. Two phase 3 clinical trials in the relapse setting following 1 to 3 prior lines of therapy did evaluate the value of adding daratumumab to lenalidomide-dexamethasone (Pollux trial) or bortezomib-dexamethasone (Castor trial). The results of the Pollux and the Castor trials have recently been published [40;41]. 569 patients were randomized between lenalidomide and dexamethasone versus daratumumab, lenalidomide and dexamethasone. The latter was found to be superior; 83.2% of patients were found to be progression free at 12 months versus 60.1% of patients treated with lenalidomide and dexamethasone. In addition, response rates were higher with daratumumab, lenalidomide and dexamethasone; 92.9% ORR versus 76.4% in the control arm. Comparable superiority was shown in a study of 498 patients randomized between daratumumab in combination with bortezomib/dexamethasone and bortezomib/dexamethasone; 60.7% of patients were progression free at 12 months versus 26.9% in the control arm. The response rate was higher with daratumumab, bortezomib and dexamethasone; 82.8 ORR versus 63.2 % in the control arm.
Importantly, daratumumab will also be incorporated in front-line regimens. For instance, the French/Dutch/Belgian IFM/HOVON intergroup study will evaluate VTD with or without daratumumab in induction as well as consolidation after autologous stem cell transplantation. Furthermore, daratumumab will be combined with two standards of care for newly diagnosed non-transplant eligible elderly myeloma patients: velcade-melphalan-prednisone and lenalidomide-dexamethasone.

Clinical experience: Daratumumab combined with other agents for the treatment of myeloma

At the time of the implementation of HOVON143 study protocol version 5.1 , daratumumab i.v. has been approved in combination with other therapies: lenalidomide-dexamethasone (MAIA trial and Pollux trial in NNDMM and RRMM respectively), bortezomib-melphalan-prednisone (ALCYONE trial in NDMM), bortezomib-thalidomide-dexamethasone (CASSIOPEIA trial in NDMM) and bortezomib-dexamethasone (Castror trial in RRMM). Due to the long infusion time of approximately 7 hours for the first infusion and 3-4 hours thereafter, attempts have been made to shorten the infusion time. This led to the development of the subcutaneous formulation of daratumumab (1800 mg co-formulated with 2000 U/ml recombinant human hyaluronidase PH20).

In a randomized trial (COLUMBA trial), daratumumab intravenously was compared to daratumumab subcutaneously in RRMM patients who had received at least 3 prior treatment lines. After a median follow-up of 7.5 months (IQR 6.5-9.3), 43% of patients were still receiving treatment. Overall responses were seen in 41% of patients in the subcutaneous group and 37% in the intravenous group, meeting the non-inferiority criterion. Median time to first response was 1 month in both groups. Median PFS was 5.6 months in the subcutaneous group and 6.1 months in the intravenous group (HR 0.99). OS data are not yet mature.

Adverse events of special interest

Infusion-Related Reaction Adverse Events with daratumumab i.v. monotherapy and daratumumab in combination with lenalidomide/dexamethasone or bortezomib/dexamethasone

Of the 820 subjects who received daratumumab in the monotherapy and combination Studies MMY1001, MMY1002, MMY2002, MMY3003, MMY3004, GEN501, and GEN503, infusion related Reactions (IRRs) were reported in approximately half (48%) of subjects. The most common IRRs were cough (10%), dyspnoea (9%), chills (6%), throat irritation (6%), nasal congestion (5%), bronchospasm (5%), nausea (5%), and vomiting (5%). Grade 3 IRRs were reported in 6% of subjects. No Grade 4 IRRs were reported. For the majority of subjects (378 of 392; 96%) an IRR occurred with the first infusion . In the Pollux and the Castor trial daratumumab in combination with lenalidomide/dexamethasone and bortezomib/dexamethasone respectively, IRR were mild. In the Pollux trial daratumumab-associated infusion-related reactions occurred in 47.7% of the patients and were mostly of grade 1 or 2. Only 5.3% had Grade 3 reactions and only 1 patient had to discontinue daratumumab, but did completely recover and could continue lenalidomide. In the Castor trial IRRs that were associated with daratumumab treatment were reported in 45.3% of the patients in the daratumumab group; these reactions were mostly grade 1 or 2 (grade 3 in 8.6% of the patients), and in 98.2% of these patients, they occurred during the first infusion. Two patients discontinued treatment because of infusion-related reactions: bronchospasm in 1 patient and bronchospasm, laryngeal edema, and rash in the other patient.

Infections and Infestations with daratumumab monotherapy and daratumumab in combination with lenalidomide/dexamethasone or bortezomib/dexamethasone

In the integrated analysis of 3 studies of daratumumab administered as monotherapy to subjects with relapsed and refractory multiple myeloma (MMY2002, GEN501, and MMY1002), 59% of subjects who received 16 mg/kg daratumumab had an AE of infection or infestation. The most frequently reported

were upper respiratory tract infection (22%), nasopharyngitis (15%), pneumonia (9%), sinusitis (7%), and urinary tract infection (6%). Grade 3 and 4 TEAEs of infections or infestations were reported in 10% and 1% of subjects, respectively. The most common SAE of infections or infestations was pneumonia (6% of subjects). Two subjects discontinued study due to infections or infestations (H1N1 infection and pneumonia).

The incidence of infection and infestation TEAEs is consistent with the underlying disease and with the known side effects of the background therapies (bortezomib, lenalidomide, pomalidomide, dexamethasone, melphalan, prednisone, and thalidomide). The data in this section is based on Studies GEN503, MMY3003, MMY3004, and the D-pom-dex cohort of Study MMY1001 only.
In Studies GEN503 and MMY3003, 87% of subjects receiving DRd treatment had an TEAE of infection or infestation. The most frequently reported were upper respiratory tract infection (32%), nasopharyngitis (27%), bronchitis (17%), pneumonia (15%), and respiratory tract infection (11%). Grade 3 or 4 TEAEs classified in the Infections or Infestations SOC were reported in 30% of subjects. Among subjects receiving Rd treatment 74% had an AE of infection or infestation. The most frequently reported were upper respiratory tract infection (23%), nasopharyngitis (17%), bronchitis (14%), and pneumonia (13%). Grade 3 or 4 TEAEs classified in the Infections or Infestations SOC were reported in 24% of subjects.
In Study MMY3004, 73% of subjects receiving DVd treatment had an AE of infection or infestation. The most frequently reported were upper respiratory tract infection (30%), pneumonia (14%), and bronchitis (13%). Grade 3 or 4 TEAEs classified in the Infections or Infestations SOC were reported in 26% of subjects. Among subjects receiving Vd treatment, 54% had an AE of infection or infestation. The most frequently reported were upper respiratory tract infection (18%) and pneumonia (12%). Grade 3 or 4 TEAEs classified in the Infections or Infestations SOC were reported in 19% of subjects.
In Study MMY1001, 72% of subjects receiving D-Pom-dex had an AE of infection or infestation. The most frequently reported were upper respiratory tract infection (28%), pneumonia (15%), sinusitis (14%), and bronchitis (12%). Grade 3 or 4 TEAEs classified in the Infections or Infestations SOC were reported in 32% of subjects.

.

In the Pollux trial the rate of infection of grade 3 or 4 was slightly higher in the daratumumab group than in the control group (28.3% and 22.8%, respectively); the most common infection of grade 3 or 4 was pneumonia, which occurred at similar rates in the two groups. The percentage of patients with adverse events leading to the discontinuation of treatment was similar in the two groups: 6.7% in the daratumumab group and 7.8% in the control group. The most common adverse events (in ≥1% of the patients in either group) that led to the discontinuation of treatment included pneumonia (in 1.1% of the patients in the daratumumab group and in 0.7% of those in the control group).
In the Castor trial the rates of grade 3 or 4 infections and infestations were similar in the two groups (21.4% and 19.0%, respectively). The percentage of patients who discontinued treatment because of at least one adverse event was similar in the daratumumab group and the control group (7.4% and 9.3%, respectively). The most common adverse events (occurring in at least 1% of patients in either group) that led to treatment discontinuation were peripheral sensory neuropathy (0.4% and 2.5%, respectively) and pneumonia (1.2% and 0.4%, respectively).

Second Primary Malignancies with daratumumab i.v. monotherapy

None of the 156 subjects treated with 16 mg/kg daratumumab monotherapy in Studies MMY2002, GEN501, or MMY1002 developed second primary malignancies.

In the Pollux trial low rates of secondary primary cancer were reported in the two groups (in 2.8% of the patients in the daratumumab group and in 3.6% of those in the control group); 10 of 18 patients (5 patients in each group) with secondary primary cancer had noninvasive, cutaneous secondary primary cancer such as squamous-cell carcinoma or basal-cell carcinoma.

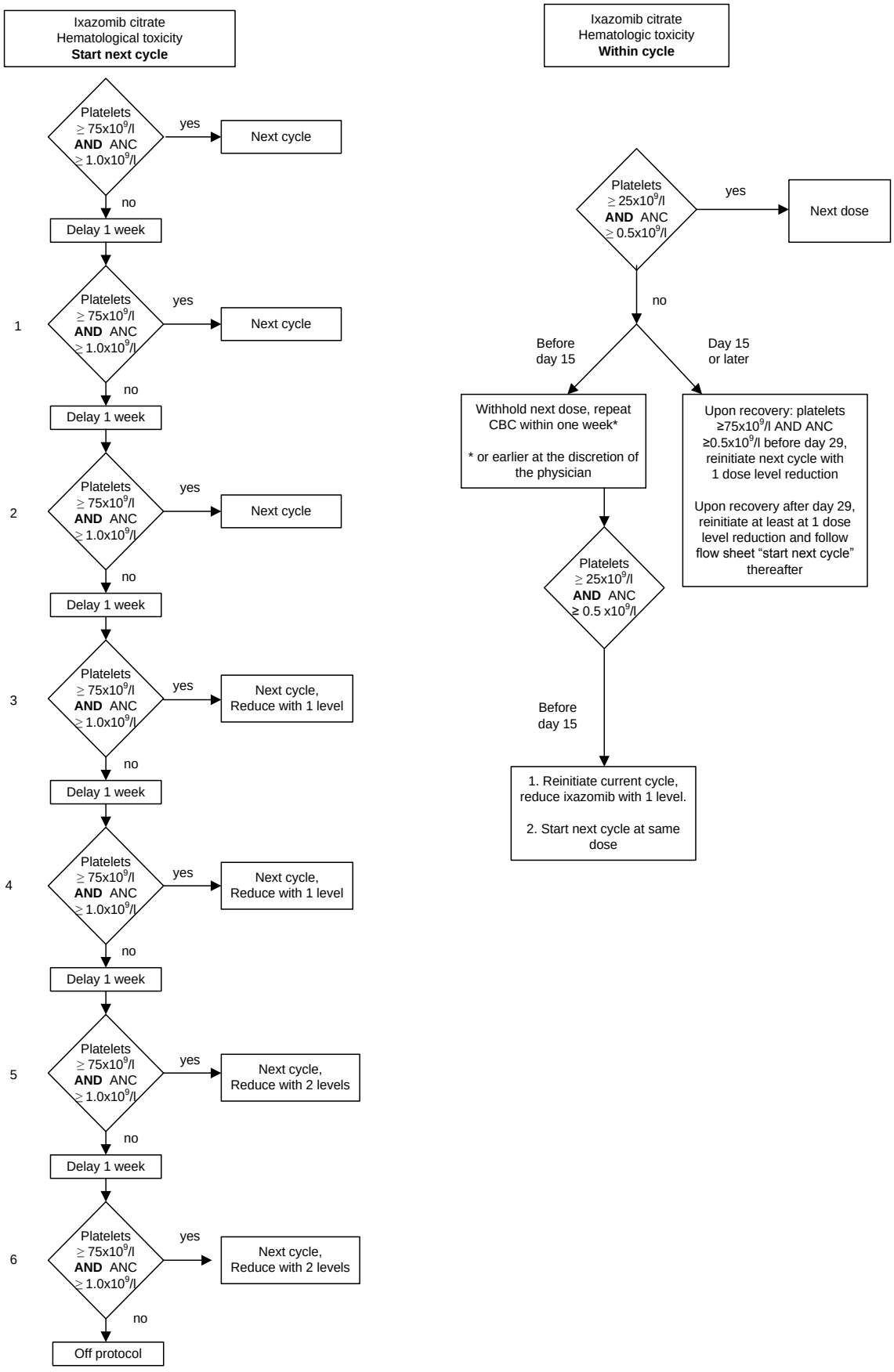
In the Castor trial the rates of secondary primary cancers were 2.5% in the daratumumab arm and 0.4% in the control group; a majority of these cancers had developed within 6 months after the initiation of trial treatment and occurred in patients who had previous exposure to immunomodulatory drugs and alkylating agents

For full overview of toxicity reported with intravenous daratumumab treatment we refer to the Investigator's brochure.

Adverse Events with daratumumab s.c. versus daratumumab i.v. monotherapy

The most common infusion-related reactions (subcutaneous group vs intravenous group) were chills (5% vs 12%), pyrexia (5% vs 3%), and dyspnoea (1% vs 7%). Median time to onset for IRRs after administration of the first dose was longer in the subcutaneous group (3·4 h, IQR 1·5–4·4, range 1–47·8) than the intravenous group (1·5 h, 1–1·8, 0–24·5). Most infusion-related reactions in both treatment groups occurred during or shortly after the first administration of daratumumab. One/263 patient in the subcutaneous group and 3/259 patients in the intravenous group had an IRR on the second or subsequent administrations. No patients had an IRR following the fourth or later administrations. One patient in the subcutaneous group and two in the intravenous group had at least one IRR on non-treatment days (delayed-onset IRRs). All delayed-onset IRRs were grade 1 or grade 2 and non-serious.With intravenous daratumumab, IRRs led to dose interruptions for 79 (31%) patients, one instance of a terminated infusion, decreases in infusion rate in 26 (10%) patients, and two treatment discontinuations. No IRRs with subcutaneous daratumumab led to treatment discontinuation, dose interruption, or incomplete dose administration. Injection-site reactions, all grade1 and grade 2, were seen in 18 (7%) patients in the subcutaneous group, with no treatment discontinuations. The only injection-site reaction in more than two patients of this group was erythema (four [2%] patients).

Ixazomib: flow sheet dose delay due to hematological toxicity at the start of a new cycle and within a cycle



Intake of ixazomib and management of ixazomib toxicity during an induction cycle and maintenance therapy

Ixazomib Administration

All protocol-specific criteria for administration of study drug must be met and documented before drug administration. Study drug will be administered or dispensed only to eligible patients under the supervision of the investigator or identified subinvestigator(s). Patients should be monitored for toxicity, as necessary, and doses of ixazomib should be modified as needed to accommodate patient tolerance to treatment; this may include symptomatic treatment, dose interruptions, and adjustments of ixazomib dose.

Ixazomib will be given as a single, oral dose of 4.0 mg weekly (Days 1, 8, and 15) for 3 weeks, followed by 1 week without study drug in a 28-day cycle. Patients should be instructed to swallow ixazomib capsules whole with water and not to break, chew, or open the capsules. Ixazomib should be taken on an empty stomach, at least 1 hour before or no sooner than 2 hours after a meal. The capsule should be swallowed with a sip of water. A total of approximately 150 mL of water should be taken with the capsules. Missed doses can be taken as soon as the patient remembers as long as the next scheduled dose is 72 hours or more away. A double dose should not be taken to make up for a missed dose. If the patient vomits after taking a dose, the patient should not repeat the dose but should resume dosing at the time of the next scheduled dose.

H 1 Dose levels

Dose Levels for ixazomib during Induction and Maintenance Therapy

Dose Levels	Ixazomib
Starting Dose	4 mg once weekly on days 1,8 and 15 every 28 days
Dose Level -1	3 mg once weekly on days 1,8 and 15 every 28 days
Dose Level -2	2,3 mg once weekly on days 1,8 and 15 every 28 days
Dose Level -3	discontinue Ixazomib treatment

H2 Dose adjustments instructions for Ixazomib (and Daratumumab) for hematological toxicities, see also flow chart in appendix 0 (For Ixazomib) and 0 (for Daratumumab)

Criteria	Action Ixazomib	Action Daratumumab*
Start of a cycle		
– If at start of a cycle ANC < 1.0*10^9/L and/or platelets < 75 x 10^9/L – Growth factor support is strongly recommended if ANC < 1.0*10^9/L Platelet transfusion support is not recommended in order to meet the required platelet count ≥ 75x10^9/L.	– Ixazomib dose should be delayed Complete blood count (CBC) with differential should be repeated within one week or earlier at the discretion of the physician until the ANC ≥ 1.0x10^9/L and/or platelet counts ≥ 75x10^9/L.	– Daratumumab dose should be delayed Complete blood count (CBC) with differential should be repeated within one week or earlier at the discretion of the physician until the ANC ≥ 1.0x10^9/L and/or platelet counts ≥ 75x10^9/L.

Criteria	Action Ixazomib	Action Daratumumab*
– Treatment should restart within 6 weeks.	– Follow instructions as depicted in Appendix 0 (start next cycle).	– Follow instructions as depicted in Appendix I1 (start next cycle)
Within-Cycle Dose Modifications		
– On a ixazomib dosing day (other than day 1) if platelet count < 25 × 10^9/L and/or ANC < 0.5 × 10^9/L – On a daratumumab dosing day (other than day 1) if platelet count < 25 × 10^9/L or if bleeding and platelet count < 50 × 10^9/L and/or ANC ≤ 0.5 × 10^9/L – Treatment should restart within 6 weeks.	– Ixazomib dose should be withheld. – Follow instructions as depicted in Appendix 0 (within a cycle)	– Daratumumab dose should be withheld. – Follow instructions as depicted in Appendix 0 (different flow sheets for Cycle 1-2 (02), Cycles 3-6 (03) and Cycle 7-9 (04))
Dose Modifications for Subsequent Treatment Cycles		
– All hematologic toxicities	– For hematologic toxicity that occurs during a cycle but recover in time for the start of the next cycle: – If Ixazomib dose was reduced within the cycle, start the next cycle at that same dose. – If due to toxicity timing, ie, after day 15 dosing a dose reduction was not required at that point in the cycle, reduce ixazomib by 1 dose level at the start of that cycleDo not reduce the Ixazomib dose both within a cycle and at the start of the cycle for the same most severe toxicity.	– Upon recovery Daratumumab may be reinitiated (no dose reductions apply for daratumumab)

***** There will be no dose reductions for daratumumab – please see paragraph I for details

H3 Dose modification instructions for ixazomib for non-hematological toxicity during a cycle and during maintenance therapy

Ixazomib Treatment Modification (Delays, Reductions, and Discontinuations) Due to Adverse Events (Non-Hematologic Toxicities)

Adverse Event (Severity)	Action on Study Drug	Further Considerations
Peripheral Neuropathy:		
Grade 1 peripheral neuropathy	No action	Grade 1 signs and symptoms: asymptomatic; without pain or loss of function; clinical or diagnostic observations only [14]

Ixazomib Treatment Modification (Delays, Reductions, and Discontinuations) Due to Adverse Events (Non-Hematologic Toxicities)

Adverse Event (Severity)	Action on Study Drug	Further Considerations
New or worsening Grade 1 peripheral neuropathy with pain or Grade 2	Hold study drug until resolution to Grade ≤ 1 or baseline	Grade 2 signs and symptoms: Moderate symptoms; limiting instrumental activities of daily living (ADL) [14]
New or worsening Grade 2 peripheral neuropathy with pain or Grade 3	Hold study drug until resolution to Grade ≤ 1 or baseline Reduce study drug to next lower dose upon recovery	Grade 3 signs and symptoms: severe symptoms; limiting self-care ADL; assistive device indicated [14]
New or worsening Grade 4 peripheral neuropathy	Discontinue study drug	
Grade 2 Rash	Symptomatic recommendations as described below	The investigator and project clinician may discuss considerations for dose modifications and symptom management.
Grade 2 Bullous Rash	Consider permanently discontinuing study drug	Exceptions are cases in which the investigator determines the patient is obtaining a clinical benefit
Grade 3 Stevens-Johnson Syndrome	Consider permanently discontinuing study drug	Exceptions are cases in which the investigator determines the patient is obtaining a clinical benefit
Grade 3 nonhematologic toxicity judged to be related to study drug	Hold study drug until resolution to Grade ≤ 1 or baseline	Symptomatic recommendations noted below
If not recovered to ≤ Grade 1 or baseline within 4 weeks	Reduce study drug 1 to next lower dose upon return to ≤ Grade 1 or baseline	
Subsequent recurrence Grade 3 that does not recover to ≤ Grade 1 or baseline within 4 weeks	Hold study drug until resolution to ≤Grade 1 or baseline Reduce study drug to next lower dose	Monitor closely, take appropriate medical precautions, and provide appropriate symptomatic care
Grade 4 nonhematologic toxicities judged to be related to study drug	Consider permanently discontinuing study drug	Exceptions are cases in which the investigator determines the patient is obtaining a clinical benefit . However, in case of grade 4 cutaneous reactions Ixazomib should be definitely discontinuous.

Once Ixazomib is reduced for any toxicity, the dose may not be re-escalated

Management of Clinical Events

Adverse drug reactions such as thrombocytopenia, diarrhea, fatigue, nausea, vomiting, and rash have been associated with ixazomib treatment. Management guidelines regarding these events are

outlined below. Further details of management of ixazomib AEs are described in Section 6 of the ixazomib IB. For hematological toxicity see flow sheet in appendix 0.

Nausea and/or Vomiting

Standard anti-emetics, including 5-HT_3 antagonists, are recommended for emesis occurring upon treatment initiation; prophylactic anti-emetics may also be considered. Dexamethasone should not be administered as an anti-emetic. Fluid deficits should be corrected before initiation of study drug and during treatment.

Diarrhea

Diarrhea should be managed according to clinical practice, including the administration of antidiarrheals once infectious causes are excluded. Fluid intake should be maintained to avoid dehydration. Fluid deficits should be corrected before initiation of treatment and during treatment. Prophylactic antidiarrheals are not generally recommended.

Erythematous Rash With or Without Pruritus

As with bortezomib, rash with or without pruritus has been reported with ixazomib, primarily at the higher doses tested and when given with agents where rash is an overlapping toxicity. The rash may range from limited erythematous areas, macular and/or small papular bumps that may or may not be pruritic over a few areas of the body, to a more generalized eruption that is predominately on the trunk or extremities. Rash has been most commonly characterized as maculopapular or macular. To date, when it does occur, rash is most commonly reported within the first 3 cycles of therapy. The rash is often transient, self-limiting, and is typically Grade 1 to 2 in severity.

Symptomatic measures such as antihistamines or corticosteroids (oral or topical)) have been successfully used to manage rash and have been used prophylactically in subsequent cycles. The use of a topical, IV, or oral steroid (eg, prednisone ≤ 10 mg per day or equivalent) is permitted. Management of a Grade 3 rash may require intravenous antihistamines or corticosteroids. Administration of ixazomib (and/or other causative agent if given in combination) should be modified per protocol and re-initiated at a reduced level from where rash was noted (also, per protocol).

In line with clinical practice, dermatology consult and biopsy of Grade 3 or higher rash or any SAE involving rash is recommended. Prophylactic measures should also be considered if a patient has previously developed a rash (eg, using a thick, alcohol-free emollient cream on dry areas of the body or oral or topical antihistamines). A rare risk is Stevens--Johnson Syndrome, a severe and potentially life-threateningrash with skin peeling and mouth sores, which should be managed symptomatically according to standard medical practice. Punch biopsies for histopathological analysis are encouraged at the discretion of the investigator.

Fluid Deficit

Dehydration should be avoided because ixazomibmay cause vomiting, diarrhea, and dehydration. Acute renal failure has been reported with ixazomib citrate. Fluid deficits should be corrected before initiation of study drug and during treatment and as needed during therapy. Until further information is available, intake of NSAIDs while on this protocol should be avoided.

Hypotension

Symptomatic hypotension and orthostatic hypotension have been reported with ixazomib citrate. Blood pressure should be closely monitored while the patient is on study treatment and fluid deficit should be corrected as needed, especially in the setting of concomitant symptoms such as nausea,

vomiting, diarrhea, or anorexia. Patients taking medications and/or diuretics to manage their blood pressure (for either hypo- or hypertension) should be managed according to standard clinical practice, including considerations for dose adjustments of their concomitant medications during the course of the trial.

Posterior Reversible Encephalopathy Syndrome

One case of posterior reversible encephalopathy syndrome (PRES) has been reported with ixazomib citrate. While this case ultimately resolved, PRES has also been reported rarely with another proteasome inhibitor, VELCADE. PRES is characterized by headache, seizures and visual loss, as well as abrupt increase in blood pressure. Prompt diagnosis and initiation of antihypertensive and anticonvulsant therapy are important to prevent irreversible end-organ damage.

Thrombocytopenia

Blood counts should be monitored regularly as outlined in the protocol with additional testing obtained according to standard clinical practice. Thrombocytopenia may be severe but has been manageable with platelet transfusions according to standard clinical practice. Ixazomib administration should be modified as noted as per dose modification recommendations in the protocol when thrombocytopenia occurs (see Table H2). Therapy can be reinitiated at a reduced level upon recovery of platelet counts. A rare risk is thrombotic thrombocytopenic purpura (TTP), a rare blood disorder where blood clots form in small blood vessels throughout the body characterized by thrombocytopenia, petechiae, fever, or possibly more serious signs and symptoms. TTP should be managed symptomatically according to standard medical practice.

Neutropenia

Blood counts should be monitored regularly as outlined in the protocol with additional testing obtained according to standard clinical practice. Neutropenia may be severe but has been manageable. Growth factor support is not required but may be considered according to standard clinical practice. Ixazomib administration should be modified as noted as per dose modification recommendations in the protocol when neutropenia occurs (see Table H2). Therapy can be reinitiated at a reduced level upon recovery of ANCs.

Transverse Myelitis

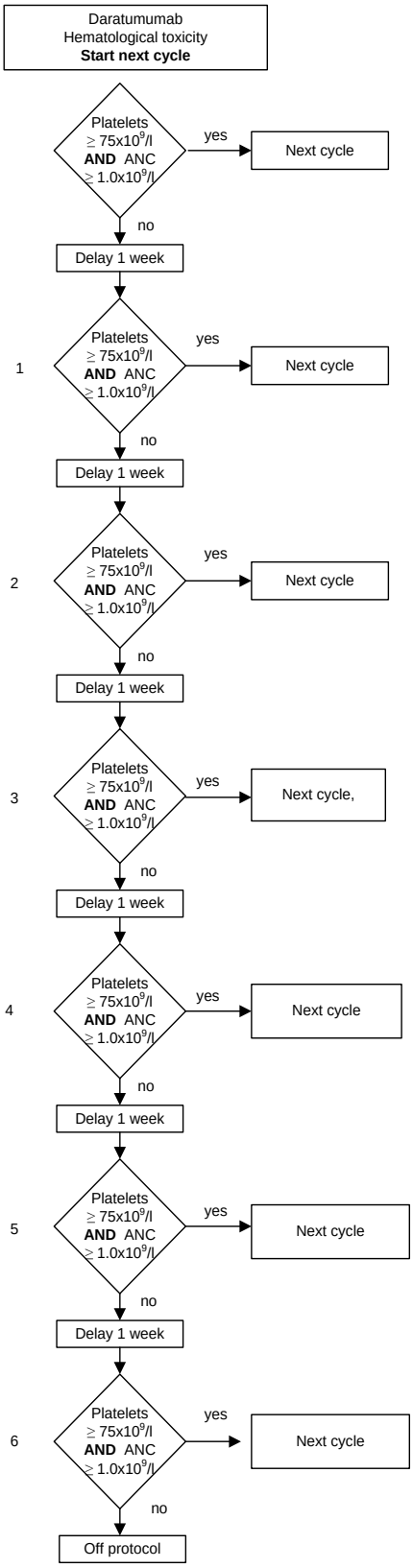
Transverse myelitis has also been reported with ixazomib. It is not known if ixazomib causes transverse myelitis; however, because it happened to a patient receiving ixazomib, the possibility that ixazomib may have contributed to transverse myelitis cannot be excluded.

Overdose

An overdose is defined as a known deliberate or accidental administration of investigational drug, to or by a study subject, at a dose above that which is assigned to that individual subject according to the study protocol. If overdose occurs, consider close observation including hospitalization for hemodynamic support. Gastric lavage may be considered if instituted within 1 hour of ingestion of ixazomib overdose

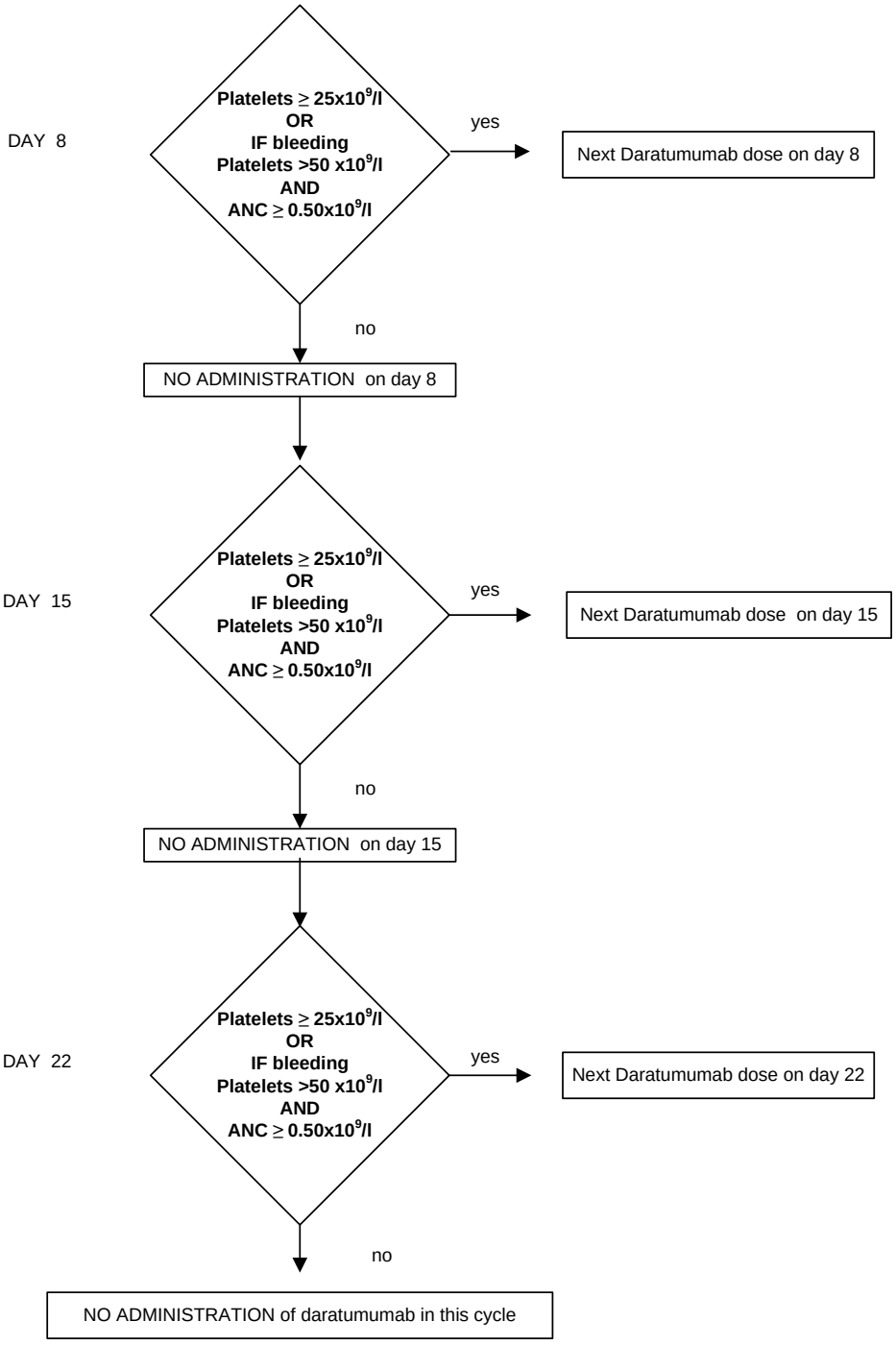
Daratumumab: dose delay due to hematological toxicity at the start of a new cycle and within a cycle

Flowsheet I1: Daratumumab dose delay at start of new cycle



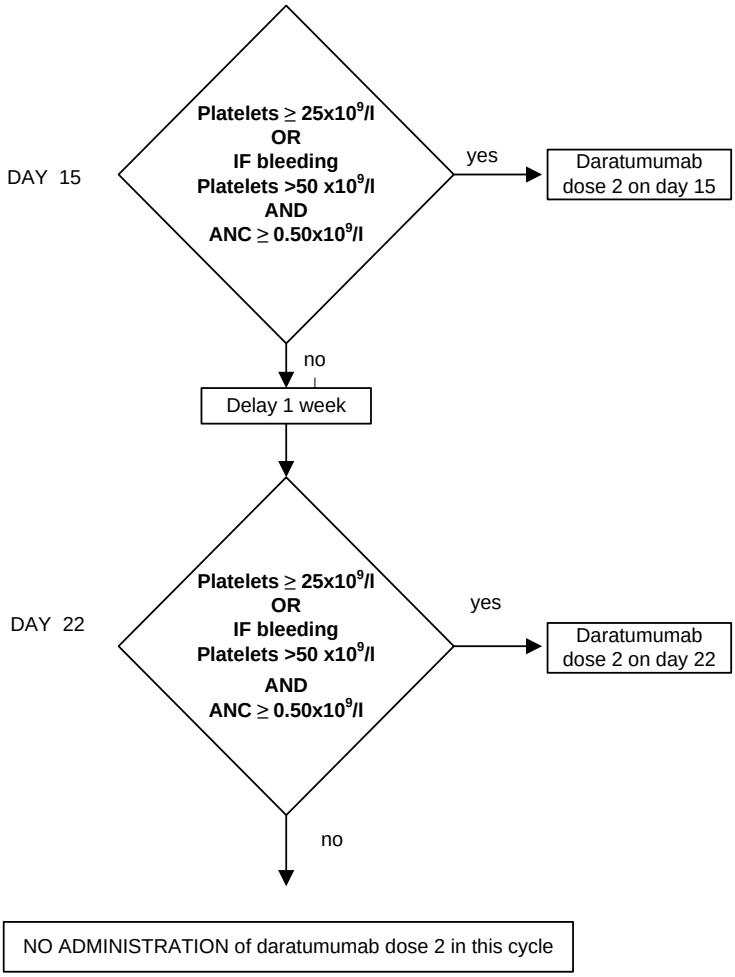
Flowsheet I2 Daratumumab dose delay within Cycles 1-2
[daratumumab every week]

AT DAY 1 PLATELETS SHOULD BE ≥75X10^9/L **AND** ANC SHOULD BE ≥1.0X10^9/L,
SEE FLOW SHEET I1 START OF A NEW CYCLE
In case the requirements for a daratumumab dose are not met that daratumumab dose will not be administered.
A missed dose will not be made up.



Flowsheet I3 Daratumumab dose delay within Cycles 3-6 [daratumumab every 2 weeks]

AT DAY 1 PLATELETS SHOULD BE ≥75X10^9/L **AND** ANC SHOULD BE ≥1.0X10^9/L, SEE FLOWSHEET I1 START OF A NEW CYCLE
In case the requirements for a daratumumab dose are not met that daratumumab dose will not be administered.



Flowsheet I4 Daratumumab dose delay within Cycles 7-9 [daratumumab once every 4 weeks]

AT DAY 1 PLATELETS SHOULD BE ≥75X10^9/L **AND** ANC SHOULD BE ≥1.0X10^9/L, SEE FLOWSHEET I1 START OF A NEW CYCLE
In case the requirements for a daratumumab dose are not met that daratumumab dose will not be administered.
A missed dose will not be made up.

Infusion of daratumumab and management of daratumumab toxicity during an induction cycle and maintenance therapy

J 1. Administration of daratumumab

Please note: Before the first administration of daratumumab an extensive red blood cell phenotyping and an indirect antiglobulin test (IAT) should be performed as daratumumab is known to interfere with the IAT. In case the patients received a transfusion of donor erythrocytes within 3 months previously the blood group should be perfroemd by molecular techniques (genotyping). Subjects should be provided with a wallet card with their blood type information, which they will carry with them throughout the treatment period. Protocols will be distributed to the blood transfusion laboratories.

Daratumumab will be administered as an i.v. or s.c. infusion. For the i.v. formulation, each subject's dose will be calculated based on the subject's weight rounded to the nearest kilogram. Daratumumab dosage must be adjusted at weight changes of more than 10% from baseline. All infusions may be performed as outpatient visits. For the s.c. formulation, daratumumab will be administered at a fixed dose of 1800 mg.

On Cycle 1 Day 1 vital signs (blood pressure, temperature and pulse) should be measured immediately before the start of dara infusion; at 0.5, 1, 1.5, 2, 3.5 hrs after the start of the infusion; at end of infusion; and 0.5, 1, 2 hr after end of infusion.
For all other i.v. infusions, vital signs will be measured immediately before infusion start and at end of dara infusion.
If a subject experiences any significant medical event, then the investigator should assess whether the subject should stay overnight for observation.

Instructions for administration of daratumumab i.v.
- first infusion: daratumumab will be diluted in 1000 ml NaCl 0,9% and administered at initial speed of 50ml/hour. In case no IRRs occur, infusion rate will be increased to a maximum infusion speed of 200 ml/hour (every hour increase with 50 ml/hour). Duration first infusion: ±6.30 hours.
- second infusion: when no IRRs > grade 1 occurred during the first 3 hours of the first infusion, the second infusion will be diluted in 500 ml NaCl 0.9% and infusion speed will be increased every hour with 50 ml/hour to 200 ml/hour. In case of an IRR > grade 1 during the first infusion, the infusion instructions for the first instruction will be used. Duration second infusion: ±4 hours.
- third and fourth infusion: when no IRRs > grade 1 occurred during the previous 2 infusions, daratumumab will be diluted in 500 ml NaCl 0.9% and initial infusion speed will be 100 ml/hour with a further increase every hour with 50 ml/hour to 200 ml/hour. In case of an IRR > grade 1 during the second infusion, the infusion instructions for the second instruction will be used. Duration first infusion: ±3.15 hours.

- subsequent infusion: when no IRRs > grae 1 occurred during the previous 4 infusions, initial infusion speed may be 200 ml/your and increased to 400 ml/hour after 30 minutes (Leukemia . 2018 Nov;32(11):2495-2518. doi: 10.1038/s41375-018-0120-2). Duration ≥ fifth infusion: ±1.5 hour.

Instructions for administration of daratumumab s.c.
Daratumumab will be administered by s.c. injection around the abdominal area between the bottom of the ribs and the waistline, approximately 7.5 cm to the right or left of the navel in an area free of tenderness, bruising, tattoo-swelling or hardened skin. The injection site should be alternated between individual doses.
The dose of 1800 mg (15 ml syringe) by manual injection in approximately 3.5 minutes.

J 2. Guidelines for the prevention and management of infusion reactions

Preinfusion medication

Preinfusion medications given one hour before the start of the administration of daratumumab include the following:

- Montelkulast 10 mg PO one hour **before first daratumumab** infusion
- Dexamethasone 20 mg i.v. during cycle 1 and 2, 10 mg i.v. only during cycle 3 to 9 and during maintenance, provided there were no IRRs during the previous cycles; In case of IRRs in the first 2 cycles the dose should maintained at 20 mg and additional measures described in this paragraph should be taken; In case of IRRs with decreased doses of dexamethasone, increase the dose to the previous dose during which no IRRs were observed.
    - Dexamethasone may be taken orally at home before administration of daratumumab when during the 1st induction cycle no IRRs grade >1 have occurred.
    - During the induction phase, in case daratumumab is not given dexamethasone will be administered as anti-MM therapy. This might then be administered p.o. instead of i.v.
    - During the maintenance phase, in case daratumumab is not given dexamethasone will not be administered.

- Paracetamol 1000 mg PO; and
- Clemastine (tavegyl ®) 2 mg IV.

Postinfusion medication

Postinfusion medication, consisting of 4 mg of dexamethasone will be routinely administered in the first 4 induction cycles, not during subsequent cycles, provided there were no IRRs during previous cycles. In case of a infusion reaction after discontinuation of the postinfusion dexamethasone, restart dexamethasone at the original postinfusion dose of 4 mg. In high risk patients for infusion reactions in all cycles postinfusion medication will be administered as described below.

Postinfusion medication as prophylaxis for infusion reactions in high-risk subjects

For subjects with a higher risk of respiratory complications (ie, subjects who have an FEV1 <75%), the following postinfusion medications should be considered:

- Dexamethasone 10 mg p.o. on the first and second days after all infusions
- Clemastine (tavegyl ®) 2 mg IV on the first and second days after all infusions
- Short-acting β2 adrenergic receptor agonist such as salbutamol aerosol
- Control medications for lung disease (eg, inhaled corticosteroids ± long-acting β2 adrenergic receptor agonists for subjects with asthma; long-acting bronchodilators such as tiotropium or salmeterol ± inhaled corticosteroids for subjects with chronic obstructive pulmonary disease)

In addition, these at-risk subjects may be hospitalized for monitoring for up to 2 nights after an infusion. If these at-risk subjects are hospitalized, then their FEV1 should be measured before discharge. If these subjects are not hospitalized, then a follow-up telephone call should be made to monitor their condition within 48 hours after all infusions. If the subject has not experienced a significant medical event but is hospitalized overnight only for observation, then the hospitalization should not be reported as a serious adverse event. Investigators may prescribe bronchodilators, antihistamines, and corticosteroids that are deemed necessary to provide adequate supportive care in the event a bronchospasm occurs after subjects are released from the hospital/clinic. If an at-risk subject experiences no major infusion-related reactions, then these postinfusion medications may be waived after 4 doses at the investigator's discretion.

Management of infusion-related reactions

Subjects should be carefully observed during daratumumab infusions. Trained study staff at the clinic should be prepared to intervene in case of any infusion reactions, and resources necessary for resuscitation (eg, agents such as epinephrine and aerosolized bronchodilator, also medical equipment such as oxygen tanks, tracheostomy equipment, and a defibrillator) must be available at bedside. Attention to staffing should be considered when multiple subjects will be dosed at the same time.

Interrupt the daratumumab infusion for infusion-related reactions of any severity. Institute medical management/supportive treatment for infusion-related reactions as needed. Reduce the infusion rate by half when re-starting the infusion. If tolerated, the infusion rate can be increased again per investigator discretion.

Subjects who experience adverse events during the infusion must be treated according to the investigator's judgment and best clinical practice. Subjects should be treated with acetaminophen, antihistamine, or corticosteroids as needed.

Intravenous saline may be indicated. For bronchospasm, urticaria, or dyspnea, subjects may

require antihistamines, oxygen, corticosteroids, or bronchodilators. For hypotension, subjects may require vasopressors.

In the event of a life-threatening infusion-related reaction (which may include pulmonary or cardiac events) or anaphylactic reaction, daratumumab should be discontinued and no additional daratumumab should be administered to the subject. Aggressive symptomatic treatment should be applied.

If an infusion is interrupted, then a longer-than-anticipated infusion time may occur. Overnight stays at the hospital because of slow infusion times should not be reported as serious adverse events. However, if the underlying cause of the delayed infusion time is an adverse event or serious adverse event, then that should be reported as such.

For patients without an history of infusion related reactions after the third daratumumab dose, the duration of infusion may be shortened to a 90-minute infusion starting in cycle 2 onwards (see section 9.6.2 for more details). Furthermore, premedication with dexamethasone may be administered orally at home (see section 9.1 for more details).

Infusion-related reactions of grade 1 or grade 2

If the investigator assesses an adverse event to be related to the infusion, then the infusion should be interrupted. When the subject's condition is stable, the infusion may be restarted at the investigator's discretion. Upon restart, the infusion rate should be half of that employed before the interruption. Subsequently, the infusion rate may be increased in following the recommendations from the SmPC.

If the subject experiences a Grade 2 or higher event of laryngeal edema, or a Grade 2 or higher event of bronchospasm that does not respond to systemic therapy and does not resolve within 6 hours from onset, then the subject must be withdrawn from daratumumab treatment.

Infusion-related reactions of grade 3 or higher

For infusion-related adverse events that are Grade 4, the infusion should be stopped and the subject withdrawn from daratumumab treatment, however will continue with ixazomib and dexamethasone treatment during induction therapy or ixazomib during maintenance therapy.

For infusion-related adverse events that are Grade 3, the infusion must be stopped and the subject must be observed carefully until resolution of the adverse event or until the intensity of the event decreases to Grade 1, at which point the infusion may be restarted at the investigator's discretion. Upon restart, the infusion rate should be half of that employed before the interruption. Subsequently, the infusion rate may be increased in following the recommendations from the SmPC. If the intensity

of the adverse event returns to Grade 3 after restart of the infusion, then the procedure described in this section should be repeated, or the subject may be withdrawn from treatment. Should the intensity of the adverse event increase to Grade 3 for a third time, then the subject must be withdrawn from daratumumab treatment.

J 3 Dose adjustments for hematological toxicities

Hematological requirements for initiation of a new cycle, also see flow chart appendix 01

In order to initiate a new infusion of daratumumab treatment cycle, the following parameters must be met:

- Platelet count ≥ 75 x 10^9/L
- ANC ≥ 1.0 x 10^9/L (growth factor support is strongly recommended if ANC < 1.0 x 10^9/L)

If those parameters are not satisfied, then delay the start of treatment until toxicity is resolved (follow instructions in Appendix 0 and table J1 below). The next cycle may be held for a maximum of 42 days until recovery to the specified levels.

Table J 1 Dose adjustments instructions for Daratumumab (and Ixazomib) for hematological toxicities, see also flow chart in appendix 0 (For Ixazomib) and 0 (for Daratumumab)

Criteria	Action Ixazomib	Action Daratumumab*
Start of a cycle		
– If at start of a cycle ANC < 1.0*10^9/L and/or platelets < 75 x 10^9/L – Growth factor support is strongly recommended if ANC < 1.0*10^9/L Platelet transfusion support is not recommended in order to meet the required platelet count ≥ 75x10^9/L. – Treatment should restart within 6 weeks.	– Ixazomib dose should be delayed Complete blood count (CBC) with differential should be repeated within one week or earlier at the discretion of the physician until the ANC ≥ 1.0x10^9/L and/or platelet counts ≥ 75x10^9/L. – Follow instructions as depicted in Appendix 0 (start next cycle).	– Daratumumab dose should be delayed Complete blood count (CBC) with differential should be repeated within one week or earlier at the discretion of the physician until the ANC ≥ 1.0x10^9/L and/or platelet counts ≥ 75x10^9/L. – Follow instructions as depicted in Appendix 01 (start next cycle)
Within-Cycle Dose Modifications		
– On a ixazomib dosing day (other than day 1) if platelet count < 25 × 10^9/L and/or ANC < 0.5 × 10^9/L – On a daratumumab dosing day (other than day 1) if platelet count < 25 × 10^9/L or if bleeding and platelet count < 50 × 10^9/L and/or ANC ≤ 0.5 × 10^9/L – Treatment should restart within 6 weeks.	– Ixazomib dose should be withheld. – Follow instructions as depicted in Appendix 0 (within a cycle)	– Daratumumab dose should be withheld. – Follow instructions as depicted in Appendix 0 (different flow sheets for Cycle 1-2 (02), Cycles 3-6 (03) and Cycle 7-9 (04))
Dose Modifications for Subsequent Treatment Cycles		

Criteria	Action Ixazomib	Action Daratumumab*
– All hematologic toxicities	– For hematologic toxicity that occurs during a cycle but recover in time for the start of the next cycle: – If Ixazomib dose was reduced within the cycle, start the next cycle at that same dose. – If due to toxicity timing, ie, after day 15 dosing a dose reduction was not required at that point in the cycle, reduce ixazomib by 1 dose level at the start of that cycleDo not reduce the Ixazomib dose both within a cycle and at the start of the cycle for the same most severe toxicity.	– Upon recovery Daratumumab may be reinitiated (no dose reductions apply for daratumumab)

Hematological toxicity management during a cycle, also see flow chart Appendix 02-04

The daratumumab dose must be held if any of the following criteria below are met, to allow for recovery from toxicity related to the study drug. The criteria for a dose delay are:

- Grade 4 hematologic toxicity (platelets <25 x 10^9 /l or neutrophils <0.5 x 10^9 /l), or
- Grade 3 or higher thrombocytopenia with bleeding (platelets ≥25 -<50 x 10^9 /l)

Please be referred for dose delay in cycle 1-2, 3-6 and 7-9 to flowsheet 02, 03 and 04 respectively and the Table 0 1 above.

0 4 Withholding daratumumab for non-hematological toxicities

The dose of daratumumab has to be withhold in case of CTCAE grade 3 or higher non-hematologic toxicities with the following exceptions:

- Grade 3 nausea or Grade 3 vomiting that responds to antiemetic treatment,
- Grade 3 diarrhea that responds to antidiarrheal treatment,
- Grade 3 fatigue or asthenia that lasts for <7 days after the last administration of daratumumab.

Daratumumab treatment should only be resumed when the toxicity has resolved to ≤ Grade 2.

J 5 Management of HBV reactivation

Antiviral therapy should be started in patients with an active HBV infection (HBsAg positive and/or HBV DNA, see table “Interpretation overview of common HBV test profiles” below). Two HBV

inhibitors are eligible: Tenofovir (245 mg 1dd) or Entecavir (0,5 mg 1dd). It is to be advised to consult a liver disease specialist before the start of treatment.

Where required by local law, the results of HBV testing may be reported to the local health authorities.

Interpretation overview of the of common HBV test profiles:

HBsAg	anti HB-core	anti-HBs	HBV-DNA	
-	-	+	-	HBV vaccination
-	+	+/-	-	resolved HBV infection
+	+	+/-	+/-	Active HBV infection

For subjects who are diagnosed with HBV reactivation/infection while on treatment, study treatment should be interrupted until the infection is adequately controlled. If the benefits outweigh the risks, study treatment may be resumed with concomitant antiviral prophylaxis as per local standard of care. Consult a liver disease specialist when clinically indicated.

Intake of dexamethasone and management of dexamethasone related toxicity

Dexamethasone will be given intravenously as described in the study scheme, in paragraph 9.1.1 and J2, before the administration of daratumumab. Dexamethasone may be taken orally at home before administration of daratumumab when during the 1st induction cycle no IRRs grade >1 have occurred.

Postinfusion medication, consisting of 4 mg of dexamethasone will be routinely administered in the first 4 cycles, not during subsequent cycles, provided there were no IRRs during previous cycles. During the induction phase, in case daratumumab is not given dexamethasone will be administered as anti- MM therapy. This might then be administered p.o. instead of i.v.
During the maintenance phase, in case daratumumab is not given dexamethasone will not be administered.

Dose Levels for Dexamethasone during Induction Therapy

Dose Level	Dexamethasone
Starting Dose	20 mg
Dose Level -1	10 mg
Dose Level -2	4 mg minimum dose required before daratumumab infusion (and following daratumumab in the first 4 cycles

Dose Modification Instructions for Dexamethasone during a Cycle

Toxicity	Dexamethasone**Dose Modification**
Dyspepsia = Grade 1-2	Maintain dose and treat with histamine (H2) blockers or proton pump inhibitors. Decrease by one dose level if symptoms persist.
Dyspepsia ≥ Grade 3	Hold dose until symptoms are controlled. Add H2 blocker or proton pump inhibitors and decrease one dose level when dose restarted.
Edema ≥ Grade 3	Use diuretics as needed and decrease dose by one dose level.
Confusion or mood alteration ≥ Grade 2	Hold dose until symptoms resolve. When dose restarted decrease dose by one dose level.
Muscle weakness (steroid myopathy) > Grade 2 Interfering with function	Decrease by one dose level. If weakness persist despite these measures, decrease dose by one dose level. Discontinue dexamethasone and do not resume if symptoms persist
Hyperglycaemia ≥ Grade 3	Treat with insulin or oral hypoglycaemic agents as needed. If uncontrolled despite these measures decrease dose by one dose level until levels are satisfactory
Acute pancreatitis	Discontinue dexamethasone and discontinue subject from the study.

Dexamethasone is not reduced for hematologic toxicity

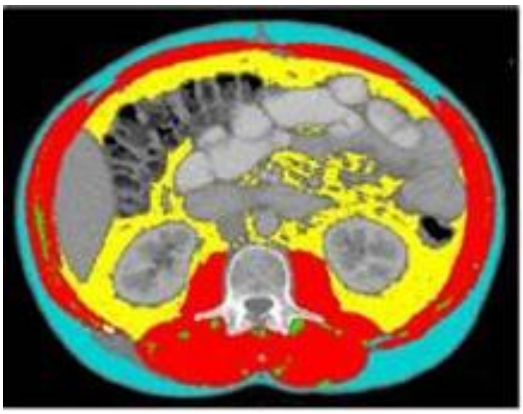
Geriatric assessments – muscle strength, gait speed, sit and stand, sarcopenia assessment

It is known that chronological age is an inferior measure of biological age. This has been supported by the data on the frailty score as described in the “Introduction”. However, functional assessments are lacking in this score. We aim to assess the predictive value of geriatric assessments, that might reflect biological age more precisely. So therefore, in addition to the questionnaires and the Charlson Comorbidity Index (Appendix 0) additional geriatric assessments, as described below will be performed.

Firstly, functional assessments: muscle strength, gait speed and sit and stand will be performed. For details and instruction video’s see the HOVON website.

Secondly, previous data on the presence of sarcopenia in renal and colon cancer patients show that sarcopenia, assessed using computed tomography (CT) scans (see Figure below), was associated with treatment related toxicity, with odds ratio’s varying from 2.5 to 4, affecting dose limiting toxicity and being translated into inferior survival.[42-44] However, data in patients with hematological malignancies is lacking.

Sarcopenia as assessed by CT scan



Fat – blue subcutaneous, yellow visceral, green skeletal
Muscle – Red

Skeletal muscle area (square centimeters) will be measured with SliceOmatic software (version 5.0; Tomovision, Magog, Quebec, Canada) using routine CT scans conducted for diagnostic purposes (see for technical details 10.4.3). The third lumbar vertebra (L3) will be used as a standard landmark, because this correlates best with whole-body muscle mass; the first image extending from L3 to the iliac crest was chosen to measure total muscle cross-sectional area. The L3 region contains psoas, paraspinal muscles, and the abdominal wall muscles. These muscles will be identified on the basis of their anatomic features by two trained researchers. The structures of those specific muscles will be quantified on the basis of pre-established thresholds of Hounsfield units (−29 to 150) of skeletal

muscle tissue. Cross-sectional areas (square centimeters) of the sum of all these muscles will be computed by adding tissue pixels and multiplying by the pixel surface area for each patient at each time point.

The CT scans for sarcopenia must be performed according to the "HOVON143 Imaging guidelines CT-scan abdomen".

Charlson Comorbidity Index

Clinical Condition	Weight
Myocardial infarction	1
Congestive heart failure	1
Peripheral vascular disease	1
Dementia	1
Cerebrovascular disease	1
Chronic pulmonary disease	1
Connective tissue disease	1
Ulcer disease	1
Mild liver disease	1
Slight diabetes without complications	1
Hemiplegia	2
Moderate or severe renal disease	2
Diabetes with end organ damage	2
Tumors	2
Leukemia	2
Lymphoma	2
Moderate or severe liver disease	3
Metastatis solid tumor	6
Acquired immunodeficiency syndrome	6

Definitions of clinical conditions for this H143 study:

1. Myocardial infarction: history of proven/probable infarction (chest pain with ECG abnormalities or increased heart enzymes)

- NO points when ECG abnormalities only, without clinical myocardial infarction

2. Congestive heart failure: complaints (eg. orthopnoea) that requires medication

3. Peripheral vascular disease: intermittent claudication, bypass graft, gangrene, acute arterial insufficiency, AAA

4. Dementia: chronic cognitive dysfunction, irrespective of etiology

5. CVA/TIA: without or with minimal symptoms only (off note: when hemiplegia (see 11), only score hemiplegia)

6. Chronic pulmonary disease: asthma, chronic lung disease (chronic bronchitis, emphysema) that requires medication

7. Connective tissue disease: eg. rhematoid artritis, systemic lupus erythematous, vasculitis, that requires medication

8. Ulcer disease: current (bleeding) ulcer

- NO points when treated for previous ulcer, or when on proton pump inhibition

9. Mild liver disease: chronic hepatitis (B or C) or cirrhosis without portal hypertension

10. Diabetes mellitus without complications (see also 13) but does require (oral/subcutaneous) anti-diabetic medication

11. Hemiplegia: or paraplegia, irrespective of etiology

12. Moderate to severe renal disease: defined as a creatinine clearance of < 30ml/min

13. Diabetes mellitus with end organ damage: retinopathy, nephropathy, neuropathy

14. Malignancies: all, but:

- NO points in case of more than 5 years previously and in case of basal- or squameous cell skin cancer, or carcinoma in situ

15. Leukemia: all acute or chronic leukemias (NO points when occurence > 5 years and cured)

16. Lymphoma: all, eg. Hodgkin, lymphosarcoma, Waldenstrom (NO points when occurence > 5 years and cured)

17. Moderate or severe liver disease: cirrhosis with portal hypertension, or ascites, jaundice, or variceal bleeding

18. Metastatic solid tumor: eg. breast, lung, colon, other tumors

19. Acquired immunodeficiency syndrome: hiv, aids

M. Charlson, T.P. Szatrowski, J. Peterson, et al. Validation of a combined comorbidity index
J Clin Epidemiol, 47 (1994), pp. 1245–1251

Correlative studies

(See lab manual at HOVON website for practical guidance and protocols)

A biobank including bone marrow cells and peripheral blood cells, bone marrow slides and peripheral plasma which is frozen and stored according to biobank laws in the separate countries. Bone marrow samples and peripheral blood cells and plasma will be collected at entry and in case bone marrow samples are taken to confirm either a complete remission or progressive disease/relapse. Peripheral blood will also be collected before the start of cycle 2 and 4, before the start of maintenance (after the last induction cycle) and at end of treatment. This material will be used for additional investigations in order to determine prognostic factors.This will include:

B. Senescence biomarkers in fibroblast obtained by skin biopsies

Skin biopsies (4mm) will be taken 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 and fixed in formalin overnight (18-24 hours), dehydrated and embedded in paraffin wax. To detect senescent cells, mouse monoclonal antibody clone E6H4 (CINtec Histology Kit, MTM Laboratories) raised against human p16INK4a protein will be used, which has been previously validated as specific to the p16INK4a protein in human tissue samples. For dermal fibroblast counts, cell identification and counting will be carried out in the papillary and upper to mid reticular areas. For positive p16INK4a cells in the epidermis, positive cells will be counted along the full length of the 4 mm epidermis, this number will be corrected for the length of the epidermal-dermal junction as all cells are located in or immediately above the basal membrane. The association between the presence of p16INK4a positive cells and the discontinuation rate, toxicity, response and progression-free survival will be investigated. In addition, additional and future markers indicating senescence, such as senescence associated beta galactosidase (pH 6), p16INK4a, p19ARF, p53, p21, p15INK4b, Dec1 and DcR2 might be incorporated in these analyses.

C. FISH analysis

Sending bone marrow for FISH analysis is mandatory in order to be included in the trial. FISH analysis will be performed on isolated plasma cells or by double-labelled identified myeloma cells slides according to EMN FISH recommendations[45] for chromosome 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 standardized by the HOVON Cytogenetic Working Party.

D. Gene expression and Genomic profiling

Genomic profiling is focused on identifying the mutations in myeloma which most often occur, and which have been connected to treatment efficacy. These include recurrent mutations such as KRAS, NRAS and DIS3, as well as mutations in proteasome subunits such as PSMG2 and PSMB5.[46;47] Targeted sequencing will be done using custom capture of specific genomic regions using SeqCap EZ choice library (Nimblegen) combined with the KAPAc HT library prep kit. Paired end sequencing will be performed on the HiSeq 2500 Illumina SBS sequencer, using barcodes to allow multiple samples to be analysed together. The expected number of samples with sufficient material and purity will be 88 (2/3 of patients included (n=132)). Running germ line DNA as a required control and DNA from purified tumor cells will then amount to 186 samples. A combination of 8 lane flow cells and Rapid run 2 lane flow cells will be used to meet the expected target. The Illumina HiSeq 2500 sequencer is located at the Rotterdam biobanking lab. A similar approach of targeted sequencing has been shown to be effective in identifying recurrent and actionable mutations in a large set of Myeloma patients.[48;49] The clonality analyses can be extended to include relapse samples within this study, of which 30 can be expected.

In addition to targeted DNA sequencing, we aim to perform RNA-seq using TruSeq Stranded Total RNA library prep kit (Illumina), with RiboZero to deplenish ribosomal RNA. This will allow analysis of both coding and noncoding RNAs. By this means, gene expression analysis is augmented by analysis of novel splicing events, expression of novel transcripts and the aforementioned noncoding RNAs. Similar to the DNA-seq approach, 214 expected samples will be run using the Illumina HiSeq 2500 machine. This will be performed using 6 samples per lane, using again a combination of 8 lane and 2 lane flow cells. Described in several papers Sonneveld Myeloma lab/Hematology department.[50-53] Moreover, in view of the possible emergence of resistant clones during maintenance therapy, as suggested by the differential effect of thalidomide maintenance in molecular defined subgroups of MM patients, proteasome subunit expression analysis will be performed in order to unravel proteasome inhibitor resistance. In vitro prolonged exposure to proteasome inhibitors have been found to result in overexpression of the proteasome beta5 subunit followed by mutations. Therefore, both at diagnosis and at relapse proteasome subunit expression will be determined. PSMB5 mutations will be addressed using the DNA-seq approach given above, both at diagnosis and relapse.

E. MRD

The importance of MRD in the outcome of MM has become clear in patients being treated with PIs and IMiDs. However, the prognostic value of MRD in patients receiving ixazomib and daratumumab both in induction and maintenance is unknown. Moreover, the optimal duration of maintenance is unknown. In order to design randomized clinical maintenance trials based on MRD, the number of patients reaching MRD and the prognostic value of reaching MRD should be known. Therefore, MRD will be investigated by performing flow cytometric analysis of patients who reach (s)CR (see for definition appendix C).

F. Biomarkers to predict response

Selection of patients that would benefit from addition of proteasome inhibitors, is expedient for future clinical studies, hence identification of biomarkers that can predict response is warranted. In our previous studies in acute myeloid and lymphoid leukemia we found that the ratio of immuno- over constitutive proteasome subunit levels at diagnosis can predict the sensitivity to proteasome inhibitors. Total proteasome levels in acute lymphoblastic leukemia and acute myeloid leukemia cells did not differ significantly, however, the ratio of immuno over constitutive proteasome was markedly higher in acute lymphoblastic leukemia cells over acute myeloid leukemia cells. In addition, in both acute lymphoblastic leukemia and acute myeloid leukemia, increased ratios of β5i/β5, β1i/β1 and β2i/β2 correlated with increased sensitivity to bortezomib and new generation proteasome inhibitors. [54-58] Besides subunit composition we also found indications that resistance to proteasome inhibitors is related to secretion of accumulating aggrosomes of ubiquinated proteins, which commonly induce apoptosis in sensitive cells. This extrusion of ubiquitinated proteins is mediated via vesicles which are associated with MARCKS proteins. To establish potential biomarkers of response to proteasome inhibition, in the current study analyses of proteasome subunit compostion and MARCKS expression at diagnosis will be performed at the VU University Medical Center, Cancer Center Amsterdam, using the centrally collected samples.

For these experiments proteins will be isolated by resuspending the pellet of 2.5 x10^6 CD138 positive cells in 100 µl lysis buffer (PBS containing 1% Igepal CA-630 (Sigma-Aldrich Zwijndrecht, NL) and Complete™ protease inhibitor cocktail (Boehringer Mannheim, Almere, NL) for 45 min at 4°C. Lysates will be clarified by microcentrifugation at 14 000g during 5 min.

To one aliquot of the lysed cell solution Laemli's sample buffer (Biorad, Veenendaal, NL) supplemented with 50 µl of β-mercaptoethanol/1 ml will be added (lysis buffer: Laemli buffer =2:1)

and used for Western blotting including antibodies against the different subunits and MARCKS protein. Protein bands will be quantified by Odyssey software, corrected for background, and normalized with β-actin. Subunit expression between patient samples on different gels will be normalized using subunit expression in the leukemic T-ALL cell line CCRF-CEM.

Another aliquot of the lysed cells will be further diluted to 200 µg/mL in 5 mM EDTA at pH 8.0 for the subunit activity assays, which will be performed at 37°C in a final volume of 200 µL using 96-well black opaque plates (Greiner bio-one, Germany). Diluted protein extract aliquots (50 µL) will be dispensed per well, giving 10 µg of protein extract per reaction. Reactions will be initiated by addition of 150 µL of 133 µM peptide-AMC substrate in 20 mM N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid] (HEPES), pH 7.4, containing 0.5 mM EDTA. Peptidase activity will be measured by kinetic monitoring of 7-amino-4-methylcoumarin (AMC) production over two hours with a Biotek plate reader (Winooski, VT, USA) and analyzed by GraphPad Prism software (La Jolla, CA, USA) with linear regression analysis.

G. Immune monitoring and evaluation of CD38 and CIP expression levels by flow cytometry

Evaluation of immune-status during therapy with ixazomib, daratumumab and dexamethasone will be done by means of assessment of cellular subsets (T cells, NK T cells, Treg cells) and functional analyses, including the induction of myeloma reactive T cell clones during follow-up of the patients. Changes in the immune-status will be correlated with response.

The immune phenotyping of MM cells and other cell subsets in BM and in the peripheral blood will be performed by 8-14 color flow cytometry. Briefly. BM localized MM cells will be identified and analyzed by staining 1x106 freshly isolated BM cells after a cell counting with an automated cell counter. Cells will be labeled with appropriately chosen fluorescent labeled antibodies against CD38, CD138, CD56, CD45, CD19 and with HuMax-003 FITC (this CD38 specific antibody binds to an epitope distinct from the epitope bound by daratumumab; and will be provided by Genmab/Janssen Pharmaceuticals),combined with cytoplasmic staining for immunoglobulin light chains using monoclonal anti-kappa and polyclonal anti-lambda. MM cells that are identified with this multicolor staining panel will be additionally analyzed for CD46, CD55 and CD59 and HLA class I, HLA class II expression by staining of the BM cells with fluorescent labeled antibodies against CD46, CD59, CD55. All MM phenotyping will be done without ficoll separation after lysing of Red blood cells. Separate multicolor panels will be used to identify the immune cell subsets such as T cells, NK cells Tregs and MDSCs in the BM as described below.). Remaining cells will be subjected to ficoll density separation and the isolated BM-MNC will be cryopreserved for further analyses.

The phenotyping of the immune cell subsets present in the peripheral blood and in BM will also be executed by 8 to 14 color flow cytometry using carefully designed multicolor identification panels In these analyses we will use antibodies against CD3, CD14, CD19, CD56, CD16, to determine the frequencies of T, Mo, B, and NK cells respectively. Antibodies against CD4 and CD8 will be in addition used to determine the main T cell subsets. These cell subsets will be further analyzed for naïve, effector memory and central memory compartments using antibodies against CCR7 and CD45RA. Resting vs activated T cells will be discriminated additional labeling of CD4+ and CD8+ T cells with antibodies against T cell activation related molecules such as CD25, CD69 and HLA-DR. HLA class I expression on Monocytes, B cells and lineage negative dendritic cells will be analysed by additional labeling with pan HLA-Calss I antibodies. CD25hiCD127dim/- T regulatory cell subsets within the CD4+ T cell subset will be determined by additional staining of CD3+CD4+ cells with CD25 and CD127 antibodies. Granulocytic and monocytic MDSCs will be analyzed in separate flow panels by staining the cells with CD33, CD11b, CD14, CD15 HLA-DR Regulatory Bregs will be identified as CD19, CD24, CD38+ B cells.

All cell subsets will be analyzed by additional labeling with CD38-specific humax-003-fitc antibody to determine the levels of CD38. The main lineage analyses will be executed using freshly isolated peripheral blood after lysing the erythrocytes. The detailed subtype analyses will be performed in PBMC cryopreserved after ficoll-density separation.

All flow cytometry analyses will be performed either using a 3-laser Canto II flow cytometer or using a 5-laser Fortessa flow cytometer (Both Becton Dickinson, Mountain view, CA). Fluorescent labeled beads (CS&T beads, Becton Dickinson) will be used to monitor the performance of the flow cytometers and verify optical path and stream flow. This procedure enables controlled standardized results and allows the determination of long-term drifts and incidental changes within the flow cytometer. Compensation beads will be used to determine spectral overlap, compensation will be automatically calculated using Diva software. PBMC cryopreserved during the course of treatment will also be functionally analyzed in standard MLR reactions for reactivity against allo PBMC and in standard antigen-specific T cell stimulation assays for reactivity against recall antigens (CEF: Cytomegalovirus, Epstein Barr virus, Influenza virus) by using commercially available HLA class I and HLA class II binding peptide pools. After stimulation of patient PBMC with alloPBMC or with CEF peptides for 5 days, T cell reactivity will be determined by measuring the antigen-specific release of IFN-γ in the cell free supernatants using commercially available Elisa kits. These assays will allow us to estimate the impact of treatment on the preexisting T cell responses. A well designed peptide pool of MM related antigens may also be used to determine the T cell reactivity against MM-associated tumor antigens. Whenever sufficient MM cells are available, NK cells and T cells within the isolated PBMC will also be separated and tested for reactivity against autologous MM cells to estimate the impact of therapy on the susceptibility of MM cells against these cytotoxic immune cells.
Bone marrow aspirates and blood samples may also be used to examine MM cell expression of other proteins presumed to be associated with immune mediated ADCC/CDC/ADCP or other mechanisms of action of or resistance to daratumumab as described in earlier publications (37).

The collection of material for these evaluations should be done at the following time points:
Peripheral blood should be obtained at day 1 of cycles 1, 2 and 4 (before start of therapy). Also at the time of progression peripheral blood should be collected.
Bone marrow aspirates should be obtained prior to start of therapy (entry), in case of CR, and at the time of progression.

H. Future analyses to be determined

Other analyses may appear to be relevant at a later stage and the biobank is left open to interested groups related to HOVON. The procedure and what analyses to be performed will be decided later.
In addition to cryopreserved bone marrow cells and DNA of peripheral blood cells, peripheral blood plasma will be stored.

Required material and logistics

Bone marrow samples and peripheral blood cells and plasma will be collected at entry and in case bone marrow samples are taken to confirm either a complete remission or progressive disease/relapse. Peripheral blood will also be collected before the start of maintenance treatment (after the last induction cycle) and at end of treatment. For further details on logistics and laboratory procedures see lab manual at the HOVON website.